PROCEEDINGS of the 12th ICP

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OP Opening Ceremony (Jubilee Session)

Abstract OP.1
THE INTERNATIONAL ASSOCIATION FOR PARATUBERCULOSIS: <1989 – 1996

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In 1983, Richard (Dick) Merkal, unequivocally the paratuberculosis "guru" of the time, sent out a little newsletter he called the "Paratuberculosis Newsletter". Shortly thereafter, Dick organized the First International Colloquium on Paratuberculosis held at the National Animal Disease Center in Ames, Iowa in June 1984. This was a small meeting, by invitation only, comprising a total attendance of only 26 participants. Despite the small group, the meeting was a great success and the general consensus was "we should do this again". Marie Thorel, prompted by Dick, agreed to organize and hold the second Colloquium in Alfort, France in September 1988. This meeting was also a great success but the prospects of future Colloquia and continuing the tradition looked rather dim. Then, one night in a hotel room in Alfort, the decision was made: it was time to organize a formal Association for Paratuberculosis. This had been Dick Merkal's dream for years.

In January 1989, the first official Paratuberculosis Newsletter was published announcing the organization of the International Association for Paratuberculosis and other Intestinal Mycobacterioses. The Association was formally registered and incorporated as The International Association for Paratuberculosis, Inc., in March 1989 with a complete Laws & By-Laws in compliance with US law. The Association had 3 members: Chiodini was self-appointed as President and Treasurer, Merkal as Vice-President and Editor-in-Chief, and Claus Buergelt as a Member of the Board of Directors.

Unfortunately, the response from the scientific community to the announcement of the Association was not very promising and it looked like the Association was not going to become a reality. Despite our best efforts, the Association had been successful in recruiting only 12 members, which included the original 3. Things looked rather dismal.

In an act of desperation, over 1,000 letters were sent out to all individuals involved in any way with paratuberculosis explaining that, without members, the Association would have to fold. The response was overwhelming – within weeks the Association had over 100 members representing 20 different counties – we had an Association. By the end of 1989, the Association had 139 members and a fiscal balance of $1,638.07 – we were off to a good start in 1990! We had succeeded in organizing an International Association which represented worldwide interest in paratuberculosis, with a diverse membership large enough to make a difference.

The first official meeting of the Association, called the Third International Colloquium on Paratuberculosis, was held in Orlando, Florida in September 1991 under the organizational skills of Claus Buergelt. This meeting brought together 95 participants from 18 countries, but unfortunately, Dick Merkal had passed away. The Proceedings from that meeting was dedicated to him and prompted the R. S. Merkal Memorial Fellowship to provide young investigators the opportunity to attend future Colloquia.

Over the years, the Association had its upsets, turmoils, and controversies, both internal and external, but was able to overcome these obstacles and not only survive, but to grow and prosper. As a founding father, it was my honor and privilege to guide the Association during its first baby steps and watch it grow into a vibrant and thriving organization. But, like any father, there comes a time to let go and cast the baby from its nest. In 1996, it was my honor to pass the torch off to Mike Collins to become the next President of the Association. I knew the Association was in good hands.

Abstract OP.2
THE INTERNATIONAL ASSOCIATION FOR PARATUBERCULOSIS: 1996 – 2007

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Dr. Rod Chiodini passed the presidential torch to me in 1996, at the conclusion of the 5th International Colloquium on Paratuberculosis (ICP) held in Madison, Wisconsin, USA. Rod made the International Association for Paratuberculosis (IAP) a strong organization. It had an effective constitution and by-laws, an active governing board, a regular newsletter, a membership of 160 people, and was in a healthy financial position. Over the years I was President, I strived to continue what Rod had so effectively started. During those years, the ICP was held in Melbourne, Australia (1999), Bilbao, Spain (2002), Copenhagen, Denmark (2005), and finally Tsukuba, Japan in 2007; our first meeting in Asia. By that time our organization had grown to 191 members representing 30 countries.

Consistent with our mission, the Association worked to engage researchers on paratuberculosis from around the world. Holding the 9th ICP in Japan was part of that effort. However, the IAP membership was over-represented by people from countries with well-developed economies, i.e. the USA, Canada, Australia, New Zealand, and European countries. To help rectify this, the IAP invested money to make it easier for researchers from lower income countries to attend the ICP by the creation of the "Helping Hand" scholarships. These scholarships facilitate participation in ICPs by paratuberculosis researchers in India, several countries in South America, and elsewhere.

Initially, the IAP was involved primarily with paratuberculosis as an animal health problem. Early meetings were dominated by veterinary concerns about paratuberculosis diagnosis and control. Because of the pioneering work of Dr. Rod Chiodini and other IAP members like Professor John Hermon-Taylor, it became evident that M. paratuberculosis might be a zoonotic agent and a possible cause of Crohn's disease. Gradually the IAP membership became populated with medical doctors and researchers primarily concerned with M. paratuberculosis as a potential human pathogen. A section of each ICP devoted to public health and food safety issues became regular parts of every meeting. At the 2005 ICP meeting held in Copenhagen, Dr. Tom Dow and Dr. Leonardo Sechi became acquainted leading to studies on the relationship between M. paratuberculosis infections and Type 1 Diabetes Mellitus illustrating how the Association, through its regular meetings, serves to foster pioneering paratuberculosis research.

The internet became vital to the IAP, as with every other organization in the world. During my tenure as President, the IAP, in collaboration with Mr. Alan Kennedy, built a stronger website. Through this website dues were collected (we began accepting credit cards), meeting abstracts were submitted, and ICP Proceedings were published. The 7th ICP (Bilbao, Spain) was the last time a print version of the ICP Proceedings was published. For anyone interested in those hard-to-find studies that only appeared in ICP Proceedings, I can send copies of Proceedings for the 3rd, 4th, 5th, 6th, and 7th ICPs for free! Only the cost of shipping is required.

The IAP is the only organization in the world devoted to M. paratuberculosis. It has had a profound impact on the caliber of science and extent of international collaboration. As the IAP continues to grow and mature, ideas on how to foster scientific investigations, increase collaboration, improve our regular meetings, and strengthen and broaden the IAP membership are solicited. Contact any officer or member of the Governing Board with your suggestions. It was an honor to serve as IAP President.

O-01 Pathogenomics and MAP Biology

Abstract O-01.1: INVITED SPEAKER
MODELS AND METHODS TO DISSECT MUCOSAL IMMUNE RESPONSES FOLLOWING MAP INFECTION

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The current view of Mycobacterium avium subspecies paratuberculosis (MAP) pathogenesis includes fecal-oral transmission in young calves with MAP invasion occurring in the terminal small intestine or ileum. MAP is thought to effectively evade innate immune defences when infecting mucosal macrophages and subsequently subvert acquired immunity to establish a persistent enteric infection. Extensive research has identified multiple mechanisms by which MAP circumvents both innate and acquired immune responses but these studies are often limited by the use of in vitro infection models or analysis of responses in immune compartments distant from the site of enteric infection. Numerous questions remain regarding host-pathogen interactions that occur at the initial site of infection and how these interactions determine whether an animal controls a persistent MAP infection or develops Johne`s disease. These questions are critical if we are to understand the dichotomy between infected animals that fail to shed or transmit MAP and animals that perpetuate the MAP life cycle through fecal shedding. Infected animals that never shed MAP may provide significant insight into mechanisms by which MAP infection is controlled despite its capacity to evade immune defences. The current presentation focuses on new approaches to explore host-pathogen interactions that may define the balance between disease resistance and susceptibility.

There is increasing evidence that the route of MAP infection may be much more variable then first confirmed by studies demonstrating efficient uptake by M-cells. Both respiratory and enteric routes of infection are possible and MAP invasion of both M-cells and mucosal epithelial cells has been reported. These observations raise the question how the portal of entry may alter host responses to MAP and the subsequent balance between control of infection and disease. The mucosal-associated lymphoid tissue (MALT) in the small intestine of young calves can be divided into two functionally distinct organs. The continuous or ileal Peyer`s patch (ilPP), is located in the terminal small intestine and was thought to be the primary site of MAP invasion. The ilPP, however, functions primarily as an antigen-independent site for generating the pre-immune B cell repertoire. In contrast, discrete or jejunal (jej)PPs are distributed throughout the small intestine and function as sites for the induction of mucosal effector responses, such as IgA plasma cells. Targeting MAP infection to the ilPP results in a failure to induce detectable MAP-specific B cell responses within one month post-infection. In contrast, infection targeted to a jejPP results in the induction of a robust and diverse MAP-specific IgA response. It remains to be determined how these differences in antibody responses are reflected in mucosal effector T cell responses. There are 25-30 jejPP located proximal to the oral cavity which would provide abundant opportunity for MAP uptake following fecal-oral transmission. Research is in progress to further characterize

immune responses following MAP invasion of ileal and jejunal PPs and determine whether portal of entry is a critical determinant of disease resistance versus susceptibility. These observations also raise the question whether a dichotomy in immune responses develops early after MAP infection.

The development of mucosal immune responses following MAP have been further characterized with kinome analysis. Kinome analysis provides a high throughput analysis of protein phosphorylation, a key post-translational modification regulating cell signaling. Bovine kinome arrays were developed and validated as a useful tool to analyze cell signaling events in bovine monocytes following MAP infection. Kinome arrays were then used to analyze mucosal responses following MAP infection of the terminal small intestine. Using a surgical model, it was possible to directly compare MAP infected and uninfected intestinal tissues collected from the same animal. This analysis determined that a significant dichotomy in cell signaling events was established within one month post-infection. Furthermore, this dichotomy in mucosal signaling segregated on the basis of individual animals developing either a predominantly cell-mediated or humoral immune response. Kinome analysis also provided insight into specific cell signaling pathways that defined this dichotomy in host responses. Further work to link specific cell signaling pathways to individual mucosal cell populations will provide greater insight into the effector cells that regulate these responses and may provide surrogate markers for evaluating potential vaccine candidates.

Another level of immune regulation that has recently become apparent is transcriptional regulation of gene families by micro(mi)RNAs. Specific miRNAs have been directly linked with the regulation of immune functions within individual leukocyte subpopulations. Furthermore, there is now evidence that pathogens can effectively exploit this level of immune regulation to circumvent host responses. We have begun to characterize miRNA expression in bovine intestinal tissues by constructing miRNA libraries and using RNA-Seq to profile transcripts. RNA-Seq analysis of tissues collected throughout the bovine gastro-intestinal tract revealed marked temporal changes in the pattern of miRNA expression during the first 6 weeks of life. Furthermore, there were significant regional differences in miRNA expression patterns throughout the small intestine, including miRNAs known to regulate immune functions. These analyses support the conclusion that regulation of mucosal immune responses following MAP invasion may vary significantly depending on both animal age and the site of infection. RNA-Seq analysis is now in progress with tissues from MAP infected animals with the objectives of identifying altered host miRNA expression patterns and determining whether MAP-specific long non-coding (lnc)RNAs are present. Pathogen production of lncRNAs that are released in host cell microsomes and then taken up uninfected cells is a novel mechanism to circumvent host immune defences. Furthermore, the capacity of miRNAs or lncRNAs to regulate gene transcription may depend very much on host or pathogen genetic polymorphisms. Understanding gene regulation at this level may inform future investigations when analyzing genetic variation among MAP strains or isolates or genetic variation in cattle that are either resistant or susceptible to MAP infection.

In conclusion, new infection models are being developed that enable us to direct MAP infections to specific sites in the small intestine and determine how the portal of entry alters the induction of immune responses. As genetically defined or manipulated MAP isolates become available, it may also be valuable to use these infection models to determine whether these genetic differences alter MAP pathogenicity, in terms of either invasion, replication, or immunogenicity. These models also facilitate a comparison of host responses in genetically matched samples from infected and uninfected sites in the small intestine. This is critical when using kinome analysis to identify specific cell signaling pathways that are either activated or inhibited following MAP infection. Each

animal has a unique kinotype that reflects the combined temporal, environmental, and genetic factors that combine to define phenotype. Therefore, kinome analysis is much more revealing when performed within the context of each individual`s kinotype. Similarly, profiling miRNAs expression patterns is more powerful when variation due to genetic variation is eliminated. Tools are now available that will facilitate an analysis of the complex host-pathogen interactions within the mucosal immune system that define the dichotomy between MAP resistance and Johne`s disease. The future challenge will be to determine if this dichotomy is established early after infection and then persists throughout life, unless perturbed by major changes in host metabolism or immunity.

Abstract O-01.2
ENVELOPE PROTEIN COMPLEXES OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS AND THEIR ANTIGENICITY

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Abstract text:
Mycobacterium avium subsp. paratuberculosis (MAP) is the causative agent of Johne's disease, a chronic enteric disease of ruminant animals. In the present study, blue native PAGE electrophoresis and 2D SDS-PAGE were used to separate MAP envelope protein complexes, followed by mass spectrometry (MS) to identify individual proteins within the complexes. Identity of individual proteins within complexes was further confirmed by MS upon excision of spots from 2D SDS-PAGE gels. Among the seven putative membrane complexes observed, major membrane protein (MAP2121c), a key MAP antigen involved in invasion of epithelial cells, was found to form a complex with cysteine desulfurase (MAP2120c). Other complexes found included those involved in energy metabolism (succinate dehydrogenase complex) as well as a complex formed by Cfp29, a characterized T cell antigen of M. tuberculosis. To determine antigenicity of proteins, Western blot was performed on replicate 2D SDS-PAGE gels with sera from noninfected control cows (n=9) and naturally infected cows in the subclinical (n = 10) and clinical (n=13) stages of infection. Clinical animals recognized MAP2121c in greater proportion than subclinical and control cows, whereas cysteine desulfurase recognition was not differentiated by infection status. To further characterize antigenicity, recombinant proteins were expressed for 10 of the proteins identified and evaluated in an interferon-gamma (IFN- γ) release assay as well as immuno-blots. This study reveals the presence of protein complexes in the cell envelope of MAP, suggesting protein interactions in the envelope of this pathogen. Furthermore the identification of antigenic proteins with potential as diagnostic targets were characterized.

Keywords:
Envelope Protein Complexes, Antigenicity, Proteomics

Abstract O-01.3

FECAL SHEDDING PATTERNS OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN JOHNE'S INFECTED DAIRY COWS

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Abstract text:

Fecal cultures are currently considered a standard diagnostic test for detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP), but long incubation times, costs, and intermittent MAP shedding hinder efficient screening programs. This study assessed how fecal shedding patterns of MAP may vary with lactation stage and season to improve the use of both culture and molecular methods for fecal detection and monitoring of MAP shedding. Fifty-one MAP-infectious cows from 7 Atlantic Canadian dairy farms had fecal samples collected monthly over a 12 month period for as long as the cows remained in the herds. Samples were analysed for MAP bacterial load via solid culture (Herrold's, Fisher Scientific), broth culture (*Para*-JEM®, Thermo Scientific), and direct real-time PCR (qPCR; VetAlert™, Tetracore®). For all fecal samples, 46% (95% CI: 40 to 51%; n=313) were positive with solid culture, 55% (50 to 60%; n=345) with broth culture, and 78% (73 to 82%; n=344) with qPCR. Sensitivity of qPCR was numerically higher for samples collected in the dry and postpartum (14 days post calving) periods. In addition, average qPCR cycle threshold (Ct) corresponded to culture-determined shedding levels, with mean Ct values of <35 indicating moderate shedding and <29 indicating heavy shedding. Less animals (85%) were identified with qPCR in summer and fall than in winter and spring. However, in summer, MAP-positive samples tended to have lower Ct values indicating moderate to heavy shedding. Solid cultures had more failure of the decontamination procedures in summer and fall. Direct fecal qPCR is a MAP detection method that is quick, less costly than culture techniques, and avoids the use of decontamination steps. This study indicates that for known positive cows, there was a high sensitivity of MAP detection with qPCR, thereby supporting the use of direct fecal qPCR as part of a Johne's herd control program.

Keywords:

feces, PCR, season

Abstract O-01.4

SERUM METABOLOMICS DETECTS MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS INFECTION IN CATTLE IN THE EARLY STAGES

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Abstract text:

The sensitivity of current diagnostics for Johne's disease is too low to reliably detect all infected animals in the subclinical stage. In this study, we aimed to discover individual metabolites or metabolite profiles that can be used as biomarkers of early MAP infection in cattle. In a monthly follow-up for 17 months, calves infected at 2 weeks of age were compared with aged-matched controls. Fecal cultures, antibody ELISAs and interferon-gamma release assays were performed routinely. Additionally, sera from all animals were analyzed and compared by 1H nuclear magnetic resonance spectrometry. Time series repeated measures ANOVA revealed many metabolite concentrations to change during the development of the calves, but also identified metabolite changes specific to MAP infection. The best separation by hierarchical multivariate statistical analysis was achieved between 300 and 400 days after infection. Therefore, a cross-sectional comparison between 1-year-old calves experimentally infected at different ages and with either a high or a low dose and age-matched non-infected controls was performed. Orthogonal Partial Least Squares Discriminant Analysis showed distinct separation of non-infected from infected animals regardless of dose and time (3, 6, 9 or 12 months) after infection. Receiver Operating Curves analysis demonstrated high quality of the constructed models. Several metabolites changes were in agreement between the longitudinal and cross-sectional analysis, and in general, the high and low dose animals behaved similarly. Differences in acetone, citrate, glycerol and iso-butyrate concentrations indicated energy shortages and increased fat metabolism in infected animals while changes in urea and several amino acids, including the branched chain AA indicated increased protein turnover. In conclusion, metabolomics is can detect MAP infection much sooner than current diagnostic methods, with individual metabolites significantly distinguishing infected from non-infected animals.

Keywords:

metabolomics, biomarker, energy shortage

Abstract O-01.5

WHOLE BLOOD GENE EXPRESSION PROFILING IDENTIFIES PUTATIVE BIOMARKERS FOR EARLY MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS INFECTION IN DAIRY CALVES

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Abstract text:
Current diagnostic tools for Johne's disease lack sensitivity for early detection of infection with Mycobacterium avium subsp. paratuberculosis (MAP). Hence, alternative diagnostic methods are desired. The aim of this study was to profile the gene expression of MAP infected calves at 3, 6 and 9 months after infection and identify potential biomarkers in the whole blood. Holstein-Friesian dairy steers were orally challenged with a clinical strain of Map at 2 weeks of age with either a high or low dose of MAP. Differential expression of transcripts in the whole blood was analysed between HD, LD and non-infected calves using Affymetrix® GeneChip® Bovine Genome Array at 3, 6 and 9 months after infection. Microarray data were analyzed using RMA and PLIER algorithms. The differential expression of a selection of genes was confirmed by qPCR. Results: 322, 287 and 80 transcripts were differentially expressed respectively at 3, 6 and 9 months after infection. The infectious dose influenced the levels of differentially expressed genes. Downstream pathway analysis pointed to inhibition of several defence mechanisms, including phagocytosis, antigen presentation, apoptosis, necrosis, leukocyte and lymphocyte trafficking. qPCR validation verified differential expression of a selection of genes: PARVB, MFAP3, ICOS, CTLA4, CD46, YARS, CEP350 and ZWINT at 3 months, ALOX15, ALOX5AP, GPR77, BOLA, BNBD9-Like and S100A9 at 6 months, and BOLA, IGSF6, IL4R, TEX261 and CCR7 at 9 months post infection. BOLA, BNBD9-Like and CD46 were longitudinally followed-up and found to be consistently differentially expressed in both LD and HD calves as early as 3 months after infection till 15 months after infection. Conclusions: Putative biomarkers of early MAP infection with roles in immune responses were identified and also the importance of dosage of infection on the discovery of biomarkers was revealed.

Keywords:
transcriptomics, microarray, biomarkers

Abstract O-01.6

MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS AS A STEALTH INVADER OF INTESTINAL EPITHELIAL CELL LAYERS IN-VITRO

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Abstract text:

The objective of this study was to determine the effects of in-vitro MAP infection on intestinal cell layer function. Although M-cells are known to facilitate MAP invasion in-vivo, direct invasion via enterocytes might also be possible. A bovine strain of MAP was added to in-vitro cultures of CACO-2 human intestinal epithelial cell layers on permeable membranes. Infection of the cells was confirmed by acid-fast staining, rt-PCR and culture examination of cell lysates. Barrier function was assessed by 14C-D-mannitol permeability and transepithelial electrical resistance. Short circuit current was used to assess sodium channel/pump function.

Cell layers were infected in a dose related fashion, with increasing MAP recovery from cell layers with increasing MAP CFU/ml added to the culture medium. Cells were more susceptible to infection when exposed to MAP just post-confluence, as opposed to when cell layer differentiation was more complete. Cells were also significantly more susceptible to MAP invasion from the apical surface, compared with the basal-lateral surface. Although there was no effect on transepithelial permeability, a small increase in short circuit current was observed. Neither cell morphology nor cell division rates were affected by MAP invasion.

These results suggest that MAP could invade through intestinal epithelial (enterocyte) cell layers independent of M-cells, but do not induce morphologic or permeability changes in the cell layers, in the acute stages of infection. Intestinal epithelial changes induced by MAP in-vivo likely are the result of MAP interaction with immune cells not present in pure cell culture, with cytokine feedback on the epithelium.

Keywords:

enterocyte, permeability, invasiveness

Abstract O-01.7

MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS INTERACTION WITH HOST CELLS REVEALS A NOVEL IRON ASSIMILATION MECHANISM LINKED TO NITRIC OXIDE STRESS DURING EARLY INFECTION

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Abstract text:

The initial interaction between host cell and pathogen sets the stage for the ensuing infection and ultimately determine the course of disease. However, there is limited knowledge of the transcripts utilized by host and pathogen and how they may impact one another during this critical step. The purpose of this study was to create a host-Mycobacterium avium subsp. paratuberculosis (MAP) interactome for early infection in an epithelium-macrophage co-culture system using RNA-seq. Establishment of the host-MAP interactome revealed a novel iron assimilation system for carboxymycobactin. Iron assimilation is linked to nitric oxide syntetase-2 production by the host and subsequent nitric oxide buildup. Iron limitation as well as nitric oxide is a prompt for MAP to enter into an iron sequestration program. This new iron sequestration program provides an explanation for mycobactin independence in some MAP strains grown in vitro as well as during infection within the host cell. Utilization of such a pathway is likely to aid MAP establishment and long-term survival within the host. The host-MAP interactome identified a number of metabolic, DNA repair and virulence genes worthy for consideration as novel drug targets as well as future pathogenesis studies. Reported interactome data may also be utilized to conduct focused, hypothesis-driven research. Co-culture of uninfected bovine epithelial cells (MAC-T) and primary bovine macrophages creates a tolerant genotype as demonstrated by downregulation of inflammatory pathways. This co-culture system may serve as a model to investigate other bovine enteric pathogens.

Keywords:

interactome, RNA-Seq, co-culture

Abstract O-01.8
FURA CONTRIBUTES TO THE OXIDATIVE STRESS RESPONSE REGULATION OF MYCOBACTERIUM AVIUM SSP. PARATUBERCULOSIS

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Abstract text:
Johne's disease (JD) is triggered by the ability of Mycobacterium avium ssp. paratuberculosis (MAP) to persist and replicate in the subepithelial macrophages of the intestine. Our previous works showed that MAP persistence is associated by metabolic adaptation of MAP to the gut environment. In the host MAP metabolism seems to be dominated by adaptation to antimicrobial reactions which was concluded from the enhanced expression of protecting enzymes such as SodA and KatG. This indicates that during infection MAP is persistently exposed to host cell defense mechanisms like oxidative stress.

The ferric uptake regulator FurA is known to be involved in iron homeostasis in many bacteria. In mycobacteria FurA is proposed to contribute to stress response regulation. Yet, a proof for this hypothesis is missing so far.

Our current study was conducted to elucidate the regulation and functional role of FurA in MAP. We constructed a furA deletion strain (MAPΔfurA) by specialized transduction and analyzed the FurA regulon by RNA deep sequencing. Among the 97 differentially expressed genes 79 could be associated to stress response or intracellular survival. No genes related to metal homeostasis were found to be affected by furA deletion. This suggested a minor role of FurA in iron metabolism. qRT-PCR analyses supported this assumption as regulation of furA was not iron dependent but affected by peroxide stress. Furthermore, repression of gene expression by FurA was iron dependent, whereas activation seemed to occur iron independently, most probably by the FurA apoform.

To address the role of FurA for intracellular survival we studied the viability of MAPΔfurA in J774 macrophages. The mutant exhibited enhanced survival rates compared to the wildtype. This indicates that the activation of the FurA regulon induces a better preparation of MAP to counteract the hostile environment of the macrophage phagosome.

Keywords:
Transcriptome analysis, FurA, Stress response

Abstract O-01.9

GUT MICROBIOTA PROFILING OF DAIRY CALVES INFECTED WITH MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS (MAP): IMPACTS OF INFECTION DOSE AND AGE AT THE TIME OF INFECTION

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Abstract text:

A metagenomic approach was used to investigate if the profile of the gut microbiota can be used as a biomarker for early detection of dairy calves infected with high or low doses of MAP (5.10^9 and 5.10^7 CFU) at different ages (2 weeks, 3, 6, 9 and 12 months). Control (n=6) and infected animals (n=60) were euthanized at 17 month of age. Ileum tissue, ileum digesta and fecal samples were collected. DNA was extracted and V4 region of bacterial 16S rRNA was amplified and subjected to Illumina paired-end sequencing. The paired-end reads were merged using PANDASeq assembler and analyzed using QIIME pipelines. The resulting operational taxonomic units were aligned to Greengenes database. The differences between microbial communities were tested using PERMANOVA. Partial least square discriminant analysis (PLS-DA) was applied to identify taxa that were most characteristic of the treatment groups. On average, 56,000 sequences per sample were generated resulting in classification of 800 genera. A total of 38, 36, and 19 phyla were identified in the ileum tissue, ileum digesta and fecal samples, respectively. The fecal microbiota profile was significantly different between control and MAP infected claves with greater difference observed with those exposed to pathogen at earlier stages of life. Dose of infection had no significant impact on microbiota profile. The PLS-DA analysis revealed that proportion of several taxa, including Bacteroides, CF231, Phascolarctobacterium and Planococcaceae were significantly higher in the feces of infected calves suggesting that composition of fecal microbiota can potentially be used as a diagnostic tool and may provide new insight into the pathogenesis of the disease.

Keywords:

MAP, Gut Microbiota, 16S rRNA Sequencing

Abstract O-01.10

THE USE OF BACTERIOPHAGE AS A TOOL TO UNDERSTAND MAP BIOLOGY

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Abstract text:

The Phage amplification assay (RapidMAP, PBD Biotech, UK) has been shown to be able to rapidly detect viable Mycobacterium avium subsp. paratuberculosis (MAP) in a range of matrixes such as milk, cheese and - most recently - in clinical blood samples. For the phage assay to perform efficiently, the phage host interactions needs to be fully understood to ensure efficient phage infection. The broad spectrum bacteriophage used in the RapidMAP assay (phage D29) was found to only infect actively growing MAP cells, but does not infect dormant cells. When dormancy was induced in MAP by limiting oxygen in the growth tube, or growing them on acidic agar (pH 5.5), the ability of phage D29 to infect MAP cells was abolished. However these cells could still be infected by another broad spectrum phage, TM4 indicating that these growth conditions result in a change in the cell surface so that the D29 receptor is not expressed. Interestingly when the MAP cells were grown on low pH agar and under limited oxygen conditions, pigmentation was observed in several cattle strains of MAP, including the reference strain K10. These pigmented cultures were also resistant to phage D29 infection but were still sensitive to phage TM4. The unexpected ability of the two different bacteriophage to rapidly distinguish between dormant and actively growing cells enables important questions to be asked about MAP cell biology when it is grown under different conditions. As well as dormancy, the novel observation of induction of pigment production by cattle strains in MAP could lead to a greater understanding of MAP biology through the use of these phage based assays.

Keywords:

Bacteriophage, Dormancy, Pigmentation

Abstract O-01.11: PERSPECTIVE

PATHOGENOMICS OF MAP INFECTION: POWERS OF TEN

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Paratuberculosis, caused by Mycobacterium avium subspecies paratuberculosis is a slow progressive infection of ruminants. Infection for example in calves which appear most susceptible is followed by a long latent period of several years. As the infection progresses intermittent shedding becomes more frequent and a detectable immune response to mycobacterial antigens becomes apparent. Ultimately animals will succumb to infection showing intractable diarrhea, decreasing milk production, weight loss and ultimately death.

Although the above description of the disease is the well known text book variant the reality in animals and populations is much more complex. Some animals do not get infected or can overcome and clear the infection, the majority of infected animals will be in a long protracted latent stage and do not progress during their lifetime. Only a minority of infected animals will progress to the typical clinical stage described above.

With the increasing use of high-throughput high density –omics technologies we are gathering exponentially increasing amount of data commonly on a limited number of animals from the population or even a limited number of cells from a single source. From a different perspective studies in large populations are conducted gathering relatively few data per animal at a single point in time. And as a third but minor variety of gathering large amounts of information there are longitudinal studies repeatedly sampling a limited amount of animals over time. Major challenges are no longer in the ability to acquire the data but to transform this data in information about the pathogenesis of the disease. And more so the different study designs yield different information about pathogenesis but studies combining these approaches are scarce.

Within the research field encompassed by pathogenomics micro-array based techniques are e.g. abundantly used to predominantly study the gene expression behaviour of monocyte derived macrophages upon infection with MAP. Newer technologies used within this topic are for instance RNAseq and kinomic approaches addressing gene expression and regulation upon infection. Considerably fewer examples exist in which cells or tissues are studied directly ex vivo and fewer still are the studies which have a longitudinal design. Nevertheless these studies have learned us a great deal on how MAP subverts macrophages to further its goal of replication by preventing apoptosis, inducing anti-inflammatory pathways and blocking pro-inflammatory pathways for instance.

On the other side of the spectrum a number of quantitative genetic studies are being performed and describe genetic variation in cattle large populations and invariably show significant genetic differences are present in the populations influencing resistance and susceptibility. In addition many candidate gene approaches targeting genes which are thought to be of biological significance also indicate that single nucleotide polymorphisms are present in these genes between animals and correlate with resistance or susceptibility to MAP. Only very recently studies are being done which also address the size of these effects and the potential use of for instant marker assisted breeding in the control of paratuberculosis.

The variation within and between MAP strains has been documented and has predominantly focused to differences in MAP strains isolated from different species of different geographical areas. Few studies address the possibilities of individuals of a single species in a single herd being infected with different strains of MAP as a source of the variation in outcome of infection next to variable such as dose and time of exposure. The population demographics and dynamics within cattle or sheep herds is a knowledge gap which deserves scientific attention.

Finally work has been done trying to find biomarkers of infection. These techniques such as serum proteomic approaches for instance represent an unbiased technique trying to identify any protein correlating with infection status of individual animals. And although these studies need extensive followup to not only correlate but also show causality between changing biomarkers and infection status these studies also may and will open up new avenues to explore in the pathogenesis of MAP infection. Recent data suggests that metabolic pathways appear to be changed during early infection. These approaches will broaden our understanding of the infection biology of paratuberculosis and may open up new ways to control the disease.

The review and prospective will therefore address some of the complexities of the techniques currently used as well as the complexities of paratuberculosis in an adventure in magnitudes. It will take you on a journey from the molecules to populations which we study in great detail. As a prospective part attention will be drawn to the knowledge gaps we currently face and which need to be addressed to further our understanding of the pathogenesis of paratuberculosis and MAP biology towards improved control.

P-01 Pathogenomics and Map Biology

Abstract P-01.1

MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS INFECTION IN NATURALLY INFECTED CATTLE INDUCES UPREGULATION OF LIPID METABOLISM GENE EXPRESSION

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Abstract text:

The immunopathogenicity of MAP remains unexplored despite numerous studies. In this study, microarray was conducted on RNA from PBMCs of four groups of naturally infected cattle, ELISA negative- fecal-PCR positive (NP), ELISA positive-PCR positive (PP), ELISA positive-PCR negative (PN) and negative for both (NN). Cluster analysis of microarray data with IPA database revealed 577 unique probes that were simultaneously regulated in all infected groups compared to control; only 412 probes represented 392 genes in each infected group. The most highly activated function with an activation z-score greater than 2 were genes of lipid metabolism. Furthermore, downstream analysis revealed a gradual increase in fold change of genes involved in lipid metabolism from the NP toward PP group. Functional annotation clustering tool which was performed by DAVID revealed a significant enrichment score of 1.24 to lipid metabolism genes. Results indicated a possibility of novel mechanisms by which MAP suppresses macrophages through the upregulation of lipid metabolism that lead to foam-like formation. The significantly upregulated nuclear receptors genes like PPARγ could be involved in the upregulation of the lipid build up in macrophages. In addition, the MAP anti-inflammatory strategies could be attributed to the upregulation of the APOE gene. The APOE upregulation sustains the MAP evasion mechanism by early modulation of the inflammatory response by downregulation of IL-12 production. It appears that APOE suppresses IL-12 by upregulating the NCf1 gene, which influences a series of responses such as downregulation of different TLRs. Our findings shed light on a novel mechanism underlying MAP pathogenesis, indicating the immense involvement of lipid metabolism genes in mediating the immune response and revealing a potential evasion mechanism used by MAP during the infection.

Keywords:

MAP, lipid metabolism, APOE

Abstract P-01.2

MYCOBACTERIUM AVIUM SUSBP. PARATUBERCULOSIS ISOLATES TRIGGER THE FORMATION OF IN VITRO GRANULOMA-LIKE AGGREGATES

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Abstract text:

Mycobacterium avium susbp. *paratuberculosis* (Map) can survive within host macrophages (Mfs) encased within an organized aggregate of immune host cells called granuloma. Within granulomas, activated Mfs differentiate into foamy Mfs, epithelioid cells and/or fuse together to form giant cells (GCs). T and B lymphocytes (Lys) surround the granuloma core and a tight coat of fibroblasts and collagen closes the structure. In this study we describe the development of an *in vitro* model of granuloma that mimics the conditions encountered by Map within natural granulomas. Bovine or ovine peripheral blood mononuclear cells (PBMCs) were added to an extracellular matrix (ECM) at 5 x 10^5 cells/50µl ECM/well of a 96-well plate. The ECM was composed of fibronectin and collagen, components of the surrounding tissue in which natural granulomas are anchored. Cells were infected by triplicate with the bovine K10 reference strain and with the ovine isolate of *Map* (2349/06-1) at MOIs (bacteria/cell) of 1:8, 1:16 and 1:33. After 3-5 days of incubation at 37 °C both isolates triggered the formation of microscopic, well-defined aggregates which size and number increased with time. Differences between the number of aggregates generated by both strains at MOI 1:8 and 1:33 were statistically significant at 10 days p. i. At this time point, the number of aggregates formed by both strains was not significantly different at any of the 3 assessed MOIs. The aggregates shared phenotypical characteristics of granulomas, such as the three-dimensional aggregation of activated Mfs and Lys. When granuloma sections were stained with Ziehl–Neelsen stain, Map could be observed residing within the granulomas. Uninfected PBMCs did not form granulomas indicating that aggregation occurs only in response to Map infection. I*n vitro* models of granuloma may be useful to understand what molecules play a role in granuloma formation and in its continued integrity. They could also provide a platform for testing vaccine and drug candidates against Map.

Keywords: Granuloma, *in vitro* model, Map-host interaction

Abstract P-01.3

NESTED PCR AS A DIAGNOSTIC AID IN THE DETECTION OF PARATUBERCULOSIS IN VACCINATED AND INFECTED CONTROL GROUP IN MURINE MODEL

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Abstract text:

Johne's disease (JD), also called paratuberculosis, is one of the most economically important diseases of dairy cattle, costing over $250 per cow in inventory per year in highly infected herds. Most of the diagnostic tools available for the early identification of infected animals are less than satisfactory, which limits disease detection. Faecal culture for agent detection is the most sensitive method to identify shedding animals, but it is still time-consuming and not suitable to use as screening diagnostic method for the whole herd. Early stage detection of Mycobacterium avium subsp. paratuberculosis (Map) infection would accelerate progress in control programmes. Therefore, alternative diagnostic method such as nested PCR is needed for rapid detection of infected animal. In the present study, nested PCR for detection of IS900 and f57 gene was employed for detection of Map in samples from mice vaccinated with Map killed vaccine adjuvanted with saponin (Gr I) and Freund's Incomplete adjuvant (Gr II) and also from Saponin control group (Gr III) and FIC control group (Gr IV). A total number of 72 samples were collected after challenged infection (1010 cfu) during the 11 months experimental period. The samples included faeces (n=52,) and organ tissues (n=20). Of the faecal samples, 29 (3 from Gr I, 5 from Gr II, 10 from Gr III and 11 from Gr IV) were identified as positive by nested PCR. Of the tissue samples, only 3 were identified as positive (1 from Gr III and 2 from Gr IV). The positive tissue samples recorded here as positive by Nested PCR were recorded as negative in prior analysis by Ziehl Neelsen test. These findings show the great potential of nested PCR as a useful tool for the rapid diagnosis of paratuberculosis in animals. The test results also depicts the efficacy of saponin adjuvanted Map vaccine over FIC.

Keywords:

Nested PCR, Saponin adjuvant, Freund's Incomplete adjuvant

Abstract P-01.4

THE MYCOBACTERIAL ADHESINS HEPARIN-BINDING HEMAGGLUTININ (HBHA) AND LAMININ-BINDING PROTEIN (LBP) ARE INVOLVED IN MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS ATTACHMENT TO EPITHELIAL CELLS

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Abstract text:

Background: The current model of biology of paratuberculose proposes that after ingestion into the host, Mycobacterium avium subsp. paratuberculosis (MAP) crosses the intestinal barrier via internalization by the M cells. However, MAP may also transcytose the intestinal wall via the enterocytes, but the mechanisms and the bacterial factors involved in this process remain poorly understood. Adhesins such as Heparin-Binding HemAgglutinin (HBHA) and Laminin-Binding Protein (LBP), have been characterized in MAP.

Objective: The aim of this study is to determine how these adhesins may promote the bacterial attachment to host cells. To achieve this, we examined the in vitro interaction between MAP and epithelial cells as well as the interaction between the adhesins and extracellular matrix molecules.

Methods: MAP cytoadherence assays were performed on epithelial cells A549 in presence or absence of inhibitors. The binding activity of recombinants HBHA and LBP was investigated both by heparin-Sepharose chromatography and by in vitro adherence assays.

Results: Cytoadherence assays revealed that pre-incubation of the bacteria in medium containing heparin inhibits the adhesion of MAP to A549 cells. In contrast, addition of laminin to the cell culture supernatant of the infected cells increased the percentage of bacterial adhesion. The in vitro assays confirmed the capacity of HBHA and LBP to promote the attachment of MAP to the extracellular matrix molecules of epithelial cells. Interestingly the adhesins HBHA and LBP expressed by the S and C types of MAP strains are different. This difference may be related to an adaptation to the host preference.

Conclusion: We demonstrated that HBHA and LBP express by MAP are involved in its adherence to epithelial cells by two different mechanisms. However, role of these adhesins on MAP entry and survival into the cells remain to be investigated.

Keywords:

Adhesin, Cytoadherence, Epithelial cells

Abstract P-01.5

WEIGHT DEVELOPMENT IN GOATS EXPERIMENTALLY INFECTED WITH MYCOBACTERIUM AVIUM SUBS. PARATUBERCULOSIS - A TWO YEARS ANALYSIS

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Abstract

Johne's disease has a long delayed onset of clinical symptoms. Associated with this come negative diagnostics leaving farmers almost unknown about the status of their animals. Nonspecific sign, symptoms or parameter might help to overcome this burden. Recently, we reported the changes in weight development and weight gain of infected goats during the first six months after infection. While the weight gain difference were only significant closely after infection, the overall weight development was significant throughout the six months of infection reported. In this report we present our finding for the following 18 months and demonstrate the weight development of the infected and uninfected goats for their first two years of life. During this reporting period we analyzed the monthly weight of ten experimentally infected goats in comparison to ten uninfected goats. During the reported period one of the infected goats became fecal culture positive, but was still negative for standard serology. Weight gain differences were only detected during the first weeks after infection. While the weight gains later were minimal and weight gain differences were not detectable, the overall weight development was still significantly different. The weight differences did not change over time and the infected goats were on average still significant lighter than the goats in the negative control group. Weight is still a good non-specific parameter in experimentally infected goats two years after infection.

Introduction

Johne's disease, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), is a chronic granulomatous enteritis in domestic and wild ruminants. Johne's disease poses a significant problem in animal health, which is underscored by its extremely high prevalence in US dairy herds, with 95% and 68.1% prevalence in large dairy herds and dairy operations respectively [1]. Infection usually occurs at birth or during the first months of life through ingestion of contaminated water, milk, or feed. MAP infection can occur in young animals by vertical transmission *in utero* [2]. Johne's disease has a long incubation period, estimated to be 6 months to two years (in some cases even more than five years), and so MAP-infected cattle often go undetected [3,4]. The bacterium replicates in macrophages of the intestinal wall and regional lymph nodes. After an extended incubation period the animal develops a granulomatous inflammation in the distal portion of the small intestine, but also could develop those in the jejunum, at least in small ruminants, that leads to malabsorption, diarrhea, and emaciation. There are four stages of Johne's disease: (1) silent infection, (2) subclinical infection, (3) clinical infection, and (4) advanced clinical infection [5].

Nielsen and Toft (2009) recently published a meta-analysis on studies focusing on the diagnosis of JD [6]. They classified the tested animals in three groups: (1) affected animals, that shed the pathogen and have clinical symptoms, (2) infectious animals in the subclinical stage, which could also shed bacteria, and (3) infected animals that are considered "latent" or in the silent stage. Infected animals have the least detection rate for current diagnostic tests. The key question for the management of this disease is can infected animals with the greatest potential to develop the

disease in the future be identified during the silent and subclinical stage. It seems important to have additional parameters to detect animals infected with the pathogen that are not specific for Johne's disease but could help to detect animals with a potentially harmful chronic infection. Several parameters could be used including weight and weight development. The scope of this study was to determine the effect of infection with *Mycobacterium avium* subsp. *paratuberculosis* on early weight gain and weight development during the early period of the silent phase of Johne's disease. Here we report the development of total body weight and weight gain for the first six months after experimental infection of goats and its potential use to detect goats with Johne's disease during the silent phase of the disease.

Methods

Animals. Twenty goat kids age two to five days were purchased from a local Johne's disease-free goat dairy farm section (CCI/Juniper Valley Products; Canon City, CO) and transferred to Colorado State University Campus immediately. The goat kids were housed on Colorado State University Campus (Johne's disease-free location prior experimental infection) in accordance with Colorado State University animal ethics regulations. There were nine Alpine goats with three different sub-breeds (two Alpine-Sundgau, two Alpine-Cou Blanc, five Alpine-Chamoise) and sub-breeds were distributed evenly between groups. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Colorado State University (#11-3120A).

All goats were housed in the same barn until the age of seven weeks and were then split into two groups (infected, uninfected). This barn was cleaned and disinfected before used. No animals with Johne's disease were housed in this barn before. Each group of ten goat kids was housed in non-adjacent corrals with open barns (fully covered, front wall open, all other walls closed) at the CSU Foothills Campus. All corrals at CSU campus are not attached to other corrals and have space in between the corrals. There were no other animals next to the infected goats and the cows next to the uninfected goats were obtained from Johne's disease-free herds and were tested for Johne's disease with negative serological and fecal culture results during this study. The corral in which the infected goats were housed, had only Johne's disease goats prior and during this study. Goats were fully milk fed for 2 months (three times a day). Whole cow milk was purchased from a local store (Walmart, Inc., Fort Collins, CO) in 1-gallon containers. Goats were fed with warm milk in individual feeding bottle with individual nipples used only for the individual goat assigned. Goat kids were fed individually by hand. Milk feeding was reduced to twice a day for another six weeks and once a day for additional 4 weeks. While weaning, alfalfa hay was introduced to supplement the goats' nutritional needs. From week 12 after infection, goats were fed with alfalfa hay.

Weights were obtained in pounds (lbs) with a commercially available scale until goats reached 50 pounds. The weight was determined by weighing the person holding the goat minus the weight of the person alone. After this period goat weights were determined with a hanging scale and a calf sling. Weights were obtained on a weekly basis. Weights in pounds were later converted into kg (1 kg = 2.20462 lbs).

Goat Infection and Inoculum Preparation

Mycobacterium avium subspecies *paratuberculosis* (MAP) strain K-10 is a bovine isolate from Nebraska that was provided by V. Kapur (University of Minnesota; now Pennsylvania State University). MAP was grown first on Middlebrook 7H11 agar plates supplemented with 10% OADC (oleic acid, albumin, dextrose, catalase) and mycobactin J (2 μg/ml). Bacteria were then transferred to a liquid culture of Middlebrook 7H9 supplemented with 10% OADC, mycobactin J (2 μg/ml) and 1% glycerol. Cells were washed with PBS (phosphate buffered saline) (pH7.2) and suspended in 20 ml whole cow milk to a final amount of 10^9 cfu per inoculum. Ten goat kids were inoculated with MAP orally for three consecutive days in compliance with the recommendation of

the international committee of Johne's disease researchers. The bacterial suspension was provided to the goats in a 20-ml syringe capped with the individual nipple of each goat kid. The sterile syringe was not modified for the attachment of the nipple. Goats ingested the whole amount of milk. The infection was performed when the goat kids were 7 weeks old. The negative control group received the same amount of normal milk but without the bacterial load.

Results

The weights and weight gains of the goats were determined to obtain correct amounts for milk feeding. Milk bottle-feeding was performed for almost four months including weaning off. Almost three months of bottle-feeding was performed after one group was inoculated with MAP. This allowed for better comparison since all goats (positive and negative groups) received the same amount of food. After weaning off the goats food supply was ad libitum with a maximum of half a bale of alfalfa hay per group of goats per day.

The only significant weight gain differences were obtained during the first few weeks after inoculation. After that there was no statistical significance, although differences were observed. Weight differences were significant throughout the whole time of this study. Inoculated goats were constantly lighter on average than the negative control group. When comparing the weight of individual goats, it should ne noted that all inoculated goats have a lower weight that eight of the ten uninfected goats. In addition, four inoculated goats had a lower weight than the uninfected goats with the lowest weight.

We also obtained weight differences between the breeds with the Anglo-Nubian breed the heaviest breed. Thus, it was no surprise that those goats are on the top of each group. However, even within this breed there was a strong difference in the weight development and the final weight at 2 years (uninfected Anglo-Nubian: 73.48 kg, 79.38 kg, and 92.99 kg versus infected Anglo-Nubian: 59.42 kg and 67.13 kg). Statistical significance could not be determined because of the low number of animals.

Conclusion

Weight development and weight gain during the first months – assuming infection occurs during first few weeks after birth – are excellent markers to detect animals suspected for Johne's disease. Further studies need to be performed to define the weight gain and weight development differences for specific goat breeds.

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Keywords:

Weight development, Goat model, diagnostics

Abstract P-01.6

IDENTIFYING LOCI ASSOCIATED WITH INHIBITION OF MYCOBACTERIAL GROWTH BY WHOLE GENOMIC (ILLUMINA®) DNA SEQUENCING

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Abstract text:

BACKGROUND

Vitamins A & D inhibit mycobacteria in culture. The purpose of these studies are to identify possible genomic loci where inhibition is mediated. Growth at moderately inhibitory doses is maintained until spontaneous genomic mutation occurs, resulting in loss of inhibition. The genome of the control and its mutated strain are then compared to identify possible targets of inhibition.

METHODS

Two isolates of M. avium subspecies paratuberculosis (MAP) isolated from patients with Crohn's disease ("Dominic" (ATCC 43545) and UCF-4) were repetitively passaged in Bactec® vials containing 3.2% DMSO and sub-cultured onto 7H10 plates impregnated with DMSO, MbJ. ± appropriate vitamin. Vitamin A or D dose was 4µg/ml. Single mutated colonies, obtained from 7H10 plates, were cultured, DNA organically (phenol/chloroform) extracted & purified to A260/280 ≥ 1.9. The entire genome of each control and mutated strain was sequenced (Ilumina®) and compared.

RESULTS

Loss of inhibition was observed between 6 and 18 months. By passage 12 months, vitamin D mutated Dominic actually had enhanced growth, compared to its DMSO control. Whole genome sequencing permitted identification of multiple mutations, which may be responsible for the observed resistance and possibly, subsequently, growth enhancement.

CONCLUSIONS:

Our preliminary data indicate that this experimental model may add a powerful tool to identify, pan-genomically, how inhibition of mycobacteria by multiple inhibitory agents occurs. The whole genomic sequencing data indicate that DNA mutation of MAP under serial inhibitory passage is genomically more multicentric than anticipated.

Keywords:

genome, inhibition, vitamins

Abstract P-01.7

GENETIC MARKERS AND PARATUBERCULOSIS FORMS IN HOLSTEIN-FRIESIAN CATTLE

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Abstract text:

Many genetic variations have been proposed to be involved in susceptibility to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infections in ruminants. However, histopathological variables have been rarely considered in previous works dealing with genetic factors in bovine paratuberculosis (PTB). The aim of this study was to investigate the association of selected polymorphisms and *latent* (*focal* lesions) and *patent* (*multifocal* and *diffuse* lesions) PTB forms in Holstein-Friesian cattle. A total of 406 controls (without lesions) and 366 cases (80.3% *latent* PTB and 19.7% *patent* PTB) were genotyped for twenty-four single-nucleotide polymorphisms (SNPs) in six immunity-related candidate genes (*Nucleotide-binding oligomerization domain 2* (*NOD2*), *Solute carrier family 11member A1* (*SLC11A1*), *Nuclear body protein SP110* (*SP110*), *Toll-like receptors* (*TLRs*) *2* and *4*, and *CD209* (also known as *DC-SIGN*, *Dendritic Cell-Specific ICAM3-Grabbing Non-integrin*)) by using TaqMan® OpenArray® technology. Logistic regression analysis confirmed a novel genotypic-phenotypic association between *CD209* gene and *latent* PTB. The minor allele (C) of the rs208222804 SNP was associated with a reduced likelihood of developing *latent* PTB (log-additive model: $P < 0.0034$ after permutation procedure; OR=0.64, 95% CI=0.48-0.86). Further studies are needed to assess the role of *CD209* gene in the pathogenesis of bovine PTB.

Keywords:

paratuberculosis, susceptibility, gene

Abstract P-01.8

COMPARATIVE PROTEOMIC ANALYSIS OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN M-CSF INDUCED BOVINE MACROPHAGES

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Abstract text:

Mycobacterium avium subsp. paratuberculosis (MAP) is known to reside in host macrophages for extensive periods, thus survival within macrophages is an important strategy of the organism. In this study, we characterised protein expression profiles of MAP phagocytised by bovine macrophages. Monocytes were isolated from blood collected from healthy cattle using a MACS column system and incubated with recombinant bovine macrophage colony-stimulating factor (M-CSF) to allow differentiation into macrophages. As M-CSF has been suggested to be an essential growth factor for development of intestinal macrophages, the intracellular environment of the macrophages induced by M-CSF was expected to simulate natural infection. The differentiated macrophages were infected with cattle (C) or sheep (S) strains of MAP, and then intracellular MAP was harvested from the macrophages after 6 days of incubation. The growth of the C strain of MAP collected from the infected macrophages was delayed compared to that of cultured bacteria in MGIT Para TB medium, while no differences in their growth were observed in the S strain. Proteins differentially regulated between intracellular MAP and cultured MAP were identified using two-dimensional (2-D) gel electrophoresis followed by mass spectrometry. Proteins up-regulated/expressed in intracellular MAP were related to fatty acid metabolism and translational elongation, while proteins related to amino acid synthesis and glycolysis were down-regulated. Further analysis of the proteins we found to be regulated in host macrophages may provide clues to bacterial pathogenesis and MAP survival responses within host cells.

Keywords:

proteome, macrophage, M-CSF

Abstract P-01.9

CHANGING CELLULAR COMPOSITION OF GRANULOMATOUS LESIONS IN EXPERIMENTALLY INFECTED GOATS DURING THE CLINICALLY INAPPARENT PHASE OF PARATUBERCULOSIS

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Abstract text:

The development of lesions during the clinically inapparent phase of paratuberculosis is not fully understood. Histopathological examination of granulomatous infiltrates in gut-associated lymphoid tissue and mesenteric lymph nodes induced by oral inoculation of goat kids with Mycobacterium avium subsp. paratuberculosis (MAP) revealed morphologically distinct lesions in clinically healthy animals sequentially necropsied between 3 and 12 months post inoculation (mpi). The objective of this study was to characterize the cellular composition of these lesions. Peyer's patches from jejunum and at the ileocecal entrance were collected from goats at 3 mpi (n=6), 9 mpi (n=5) and 12 mpi (n=6) and snap frozen. Consecutive frozen sections were labelled for CD4+, CD8+, γδ T lymphocytes, MHC II, B lymphocytes and MAP by the indirect immunperoxidase method. Distribution and number of the different cell types were evaluated. At 3 mpi, granulomatous infiltrates were characterized by high numbers of CD4+ and γδ T lymphocytes as well as epitheloid cells in 4 goats. At 9 and 12 mpi, mixed lymphoplasmacellular infiltrates with few epitheloid cells that did not contain MAP were seen in some (n=5) of the goats, whereas lesions consisted of numerous epitheloid cells containing MAP and few lymphocytes in the others (n=3). Small granulomatous foci of epitheloid cells and lymphocytes were found in goats at all times post inoculation. The changes in the composition of granulomatous lesions were interpreted as different stages of the interaction between MAP and the host's immune system. The finding of a marked diversity in reaction during the clinically inapparent phase of paratuberculosis underlines the importance of the early phase of infection for the further course of the disease.

Keywords:

lymphocyte subsets, experimental MAP infection, goats

Abstract P-01.10
AGE- AND DOSE-DEPENDENT FECAL SHEDDING IN DAIRY CALVES SOON AFTER EXPERIMENTAL INFECTION WITH MAP

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Abstract text:
Johne's disease control programs focus on interrupting the within-herd transmission of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) by implementing best-hygiene management practices. Fecal shedding is expected to start intermittently towards the end of the subclinical stage and continue persistently until the advanced clinical stage. However, the potential risk of calf-to-calf transmission is often overlooked.
In an infection trial to determine age- and dose-dependent susceptibility to MAP infection, shedding of MAP was observed in young calves. Fifty Holstein-Friesian calves were experimentally infected at 5 ages (2 wk, or 3, 6, 9, or 12 mo); within each age group, 5 calves were infected with a low and 5 with a high dose of MAP. All calves were euthanized at 17 months of age.
After exclusion of passive shedding, 64% of calves, representing all age and dose groups, shed MAP in the feces at least once during the follow-up period. Intermittent as well as continuous shedding patterns were observed. Calves inoculated at 2 wk or 3 mo shed MAP more frequently when inoculated with a high dose compared a low dose. When calves were inoculated at an older age (6, 9 or 12 months) no dose effect was observed. A peak in shedding was observed at 2 months after inoculation, clearly in absence of clinical signs. Finally, frequently shedding calves had more severe gross and histological lesions and more MAP culture-positive tissue locations. Calves inoculated up to 1 y of age with MAP can shed MAP in their feces and thus can infect pen-mates when kept in groups; calves up to 1 year of age should be considered both susceptible and infectious.

Keywords:
shedding, age-dependent, dose-dependent

Abstract P-01.11

ASSAY OF MYCOBACTINS FROM DIFFERENT MYCOBACTERIAL SPECIES BY ITS EFFECT ON GROWTH OF MYCOBACTERIUM AVIUM SUBSP PARATUBERCULOSIS

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Abstract text:

Genus Mycobacterium consists of pathogenic and saprophytic organism which meets the demand of iron by synthesizing mycobactins and exochelins. Mycobacterium avium subsp paratuberculosis (MAP) is unable to synthesize required amount of mycobactin and require exogenous mycobactin. Fast growers such as Mycobacterium fortuitum subsp fortuitum MTCC929, M phlei MTCC 1724, M smegmatis MTCC 940, M vaccae and slow growers such as M bovis AN5, M avium D4, M avium 1723, M microti MTCC 1727, M tuberculosis H37Rv and M.avium subsp paratuberculosis ATCC 19698 obtained from Biological products division, IVRI were used for study. For studying mycobactin production Dorset and Henley's media was prepared with iron at a concentration of 200µg/liter for mycobactin production. Fast growers were incubated for 3 weeks and slow growers were incubated for 8 weeks at 370c. After incubation mycobactin were extracted from cells by ethanol and chloroform extraction and purified by aluminium oxide chromatography. Among all the mycobacterial species studied, highest mycobactin production was noticed in M fortuitum followed by M tuberculosis, M smegmatis, and M phlei. When production of mycobactin is analyzed per gram of cells, efficient mycobactin producer was M smegmatis. MAP produced least amount of mycobactin in high iron media. MAP was unable to grow in medium and low iron medium. Assay of mycobactins was done by analyzing growth rate of MAP in iron deficient media for 2 months with extracted mycobactins. Out of all extracted mycobactins used for the growth assay, mycobactins extracted from M tuberculosis, M phlei and MAP had produced best growth of MAP & mycobactin extracted from M microti was least efficienct growth promoter. Mycobactin of M tuberculosis, M phlei and MAP is to be used for large scale production and for primary culturing of MAP.

Keywords:

Mycobacterium avium subsp paratuberculosis, Mycobactins, growth analysis

Abstract P-01.12

STATUS OF DIAGNOSTIC EFFICACY OF DIFFERENT TESTS FOR MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS INFECTION IN ENDEMICALLY INFECTED GOATS WITH RESPECT TO THE PATHOLOGICAL LESIONS

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Abstract text:

Diagnostic efficacy of histopathology, ZN staining, immunofluorescent test, PCR and culture of tissues were compared. Tests were performed on 74 paired tissues of intestine and mesenteric lymph nodes. Gross lesions were mainly seen at ileo-caecal valve and MLN. Histopathology of H/E stained tissues revealed variable grades of lesions of JD in 37 (50.0%) ilea and 21 (28.4%) MLN. In general, affected part of intestines revealed degeneration and partial to complete denudation of lining epithelial cells forming naked villi. These villi exhibited variable changes (dilated lacteals, villous distortion and thickening and fusion of villi). At places, villi were shortened, thin and atrophied. Histologically lesions were classified into four grades (Grade 1 - least severe to grade 4 - most severe) on the basis of types and density of cellular infiltrate (lymphocytes, macrophages and epithelioid cells). ZN staining, revealed presence of acid fast bacilli in the epithelioid cells indistinguishable to MAP in 16 (21.6%) and 10 (13.5%) tissues of intestine and MLN respectively. In fluorescent antibody test (FAT), 13 (17.5%) and 9 (12.1%) tissues of intestine and MLN were positive, respectively. Eleven (14.8%) and 7 (9.4%) tissues of intestine and MLN were positive by IS900 PCR. Culture was positive in 7 (9.5%) and 8 (10.8%) of intestine and MLN, respectively. Sensitivity of H&E staining was more as compared to ZN staining and IS900 PCR. Sensitivity of ZN staining was 100% as compared to PCR (69.2%). Sensitivity of FAT was 90.9% as compared to ZN staining (76.9%). Sensitivity of FAT was 100% in comparison to PCR (81.8%). Comparison of ZN staining and PCR with FAT revealed substantial agreement. No significant difference was found between sensitivity of ZN staining, FAT and PCR in grade II, III and grade IV lesions. In grade I lesions, histopathologic H&E staining based diagnosis was more sensitive followed by ZN staining.

Keywords:

Mycobacterium avium subspecies paratuberculosis, Histopathology, FAT

Abstract P-01.13

EVALUATION OF PATHOGENICITY OF 'INDIAN BISON TYPE' BIOTYPE, STRAIN 'S 5' OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBER-CULOSIS (A CANDIDATE FOR 'FIRST INDIGENOUS VACCINE' TO CONTROL JOHNE'S DISEASE IN DOMESTIC LIVESTOCK), AT 45TH PASSAGE LEVEL

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Abstract text:

Highly pathogenic strain 'S 5', a novel biotype (Indian Bison Type) of Mycobacterium avium subspecies paratuberculosis isolated in 1998-99, from a terminally ill goat with clinical Johne's disease (vaccine strain) is under continuous passage on HEYM medium with mycobactin J, since then. In the present study pathogenicity of the strain, which is at 45th passage level, was evaluated in homologous host. Twenty Barbari kids (>6 months) were infected with MAP 'S 5' (5x109 bacilli per kid), in 100 ml of milk by oral feeding. Kids were screened microscopically at 0 day and were negative for MAP in feces. Shedding of MAP bacilli was monitored in fecal samples at zero, 30, 60, 90 and 120 days post infection. Shedding started at 30 dpi and continued up to 120 DPI. At 90 and 120 dpi, majority of kids (80-100%) were shedding MAP and developed active disease. Using 'Indigenous ELISA kit', 20.0, 28.0, 33.3 and 80.0% kids were positive for MAP infection at 30, 60, 90 and 120 dpi, respectively. After infection, kids sero-converted 30 days onwards. Heavy presence of MAP bacilli on examination of tissues (intestines and MLN) after infection showed high rate of infection in experimental kids at 90 and 120 dpi. Necropsy of infected goats exhibited gross lesions typical of MAP infection. Gross lesions were thickening and corrugation of the intestinal mucosa, lymphatic cording and enlargement of mesentric lymph nodes and emaciation of carcass at 90 and 120 dpi. Histopathology revealed Infiltration of epitheloid, macrophages and lymphocyte cells in the mucosa of intestines and MLN. Gross and histo-pathological findings of the necropsied goats were consistent with paucibacillary paratuberculosis.

Keywords:

Mycobacterium avium subspecies paratuberculosis, Pathogenicity, Histopathology

Abstract P-01.14

COMPARISON OF THE ANTIMICROBIAL ACTIVITY OF GALLIUM NITRATE AND GALLIUM MALTOLATE AGAINST MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN VITRO

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Abstract text:
Gallium is an element with antimicrobial activity due to its inhibition of bacterial iron metabolism, and is proposed as a chemoprophylactic agent to prevent MAP infection in calves. This study compared the in-vitro antimicrobial activities of gallium nitrate (GaN) and gallium maltolate (GaM) against two field isolates of MAP. Previous studies demonstrated GaN efficacy against MAP both in-vitro and in-vivo. GaM, based on its superior lipid solubility, is expected to have greater activity against MAP than GaN.

The antimicrobial activities against two MAP field isolates were tested by use of broth culture with automated detection (Bactec MGIT). For each MAP isolate, a series of nine dilutions of GaN and GaM was tested. Growth inhibitions of 90% and 99% were determined by measuring the time to detection (TTD) relative to that for cultures of the MAP stock solution (without the addition of Ga) diluted 1:10 and 1:100, respectively.

Both Ga compounds showed dose-dependent antimicrobial efficacy against MAP. The concentrations that resulted in 99% growth inhibition of isolates 1 and 2 were, respectively, 636 µM and 183 µM for GaN, and 251 µM and 142 µM for GaM. TTD values were far higher for GaM than GaN: by 2.2 days for isolate 1 (95 CI 1.8-2.7 days; $P < 0.001$) and 35.6 days (95% CI 23.8-47.4 days; $P < 0.001$) for isolate 2, indicating superior MAP inhibition by GaM. These results suggest that GaM may be more potent than GaN as a chemoprophylactic agent in the prevention of MAP infection in calves.

Keywords:
chemoprophylaxis, gallium, antimicrobial

Abstract P-01.15

IN VITRO ACTIVITY OF THE NOVEL IN SILICO DEVELOPED ANTI-MICROBIAL PEPTIDE AMP2041 AGAINST MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS AND FAST-GROWING MYCOBACTERIA

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Abstract text:

Available vaccines and chemotherapeutic agents for the control of mycobacterial infections are of limited efficacy and fail to prevent the spread of mycobacterial diseases. Cationic antimicrobial peptides (AMPs) could be a valuable aid, either alone or in combination treatments, to treat mycobacterial infections, and have the advantage of a low likelihood of resistance development induction. The aim of this study is to evaluate the antimicrobial activity against M. avium subsp. paratuberculosis (MAP) and fast-growing mycobacteria, M. fortuitum and M. smegmatis, of an in silico developed AMP who previously showed a broad spectrum microbicidal activity against Gram-negative and Gram-positive bacteria. The tested synthetic AMP2041 was designed by an ad hoc screening software developed in house. AMP2041 activity was measured by resazurin assay. Middlebrook 7H9 broth and 7H10 agar, with or without mycobactin J, were used as growth media.

As already reported for M. tuberculosis, EDTA showed an inhibitory activity on the growth of mycobacteria tested. Therefore, the activity of AMP2041 was evaluated in presence of 2.68 mM EDTA, at a concentration of 100 ug/ml. AMP2041 was able to inhibit the growth of M. fortuitum and M. smegmatis and also that of MAP. In particular, the minimum inhibitory concentration (MIC) of AMP2041 against M. smegmatis was close to 25 ug/ml, while only an IC50 around 100 ug/ml was observed for MAP. The results so far obtained suggest an higher level of resistance of MAP compared to the fast-growing mycobacteria M. fortuitum and M. smegmatis. In conclusion, the resistance to AMP2041 of the tested mycobacteria was higher compared to other Gram-positive and Gram-negative bacteria (IC50 around 0.5-1 ug/ml).

Keywords:

Antimicrobial peptide, MAP, resistance

Abstract P-01.16

TRANSCRIPTIONAL PROFILING OF HOST RESPONSES TO MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS INFECTION AND IDENTIFYING BIOMARKER CANDIDATES IN CATTLE

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Abstract text:

Mycobacterium avium subsp. paratuberculosis (MAP) is the causative agent of Johne's disease, which is characterized by chronic granulomatous enteropathy, persistent diarrhea, progressive wasting, and can lead to death in ruminants. Paratuberculosis has a prolonged subclinical stage that can continue to excrete the infective bacteria in feces without showing outward symptoms. Because these fecal shedders might act as sources of infection to other animals, the development of a diagnostic method that is useful in the early stage is very important to control the disease. Therefore, strategies for control of MAP have been focused on diagnosis and removal of the animals during early stage of the disease, thus leading to prevent new infection. However the control of the disease has been interfered with a lack of sensitive techniques for detection of asymtomatic paratuberculosis. Here, we characterized the transcriptional profiles in blood of cattle, which were identified and grouped according to the results of MAP-specific antibody ELISA and fecal IS900-PCR. The cattles were divided into four groups: (1) ELSIA negative and fecal-PCR negative; (2) ELSIA negative and fecal-PCR positive; (3) ELSIA positive and fecal-PCR negative; (4) ELSIA positive and fecal-PCR positive. The differentially expressed genes, which were statistically significant with log2-fold change > |1.5| and $p < 0.05$, were characterized by functional, network, and pathway analysis using Ingeunity Pathway Analysis (IPA). Based on these results, we are also identifying biomarker candidates for MAP. The present study may be expected to discovery a potential biomarker candidate for blood diagnosis of MAP to improve traditional diagnostic methods. This study was supported by the Rural Development Administration (PJ00897) and the Research Institute for Veterinary Science, Seoul National University, Korea.

Keywords:

Transcriptomes, biomarkers, cattle

Abstract P-01.17

IDENTIFICATION OF TRANSCRIPTOMIC BIOMARKER CANDIDATES FOR MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS INFECTION IN MICE

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Abstract text:

Paratuberculosis (PTB), or Johne's disease, is a chronic granulomatous enteropathy of ruminants caused by infection with Mycobacterium avium subsp. paratuberculosis (MAP). A number of researches have proposed that the principal infective agent of Crohn's disease, a chronic enteropathy in humans, is MAP. PTB has very long latent periods and continue to excrete the infective bacteria in feces before development of the disease. These fecal shedders might act as sources of infection to other animals, the development of a diagnostic method that is useful in the early stage is very important to eradicate or control the disease. The identification of biomarkers in specific stage of PTB will provide prognostic information and advanced knowledge of pathogenesis and will enable especially their use in early diagnosis of slow-growing bacterial infection. In the present study, we tried to identify biomarker candidates which were shown early responses using translational profiles in MAP-infected mice. Transcriptional profiles were considered as early host response to MAP at 3 and 6 weeks post infection in mice infected with MAP ATCC 19698 (1□108 CFU/mouse). The genes were filtered with log2-fold change > | 2 | with $p < 0.05$ by Biomarker Filter and analyzed by Biomarker Discovery of Ingenuity Pathway Analysis (IPA) system. Based on these results, CCL4, CCL5, CD14, CD68, CHI3L1, CXCL9, CXCL10, ELANE, and IGF-1 were proposed as potential biomarkers of PTB. These genes are expected to be deliberated as possible candidates for biomarkers of MAP infection. This study was supported by the Rural Development Administration (PJ00897) and the Research Institute for Veterinary Science, Seoul National University, Korea.

Keywords:

transcriptomes, biomarker, MAP infection

Abstract P-01.18

TRANSCRIPTOMIC ANALYSIS OF HOST RESPONSES IN MICE AT EARLY STAGE OF INFECTION WITH MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS

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Abstract text:

Paratuberculosis, or Johne's disease, is one of the most serious chronic, debilitating diseases of ruminants worldwide by Mycobacterium avium subsp. paratuberculosis (MAP). MAP is an intracellular organism which has very long incubation periods that has difficulties in diagnosis and control of MAP infection. These features have been served limitations for development of vaccination and diagnostic methods against MAP using bovine model, as a natural host. Therefore, a murine challenge model for MAP has been used for early screening of vaccine candidates or preliminary analysis of pathogenesis. Although mice are not a natural host for MAP, the murine challenge model can be available for research of pathogenetic mechanisms due to well-developed immunological reagents, variable genetic background of mice, and possible association between MAP and Crohn's disease in humans. In the present study, mice were intraperitoneally infected with MAP ATCC 19698 and the status of MAP infection was investigated by analysis of pathological, immunological and genetic features. Based on these results, we focused on how MAP influences the subsequent disease process to investigate the host anti-mycobacterial immunopathology during MAP infection in spleen of mice. Transcriptomic profiles were characterized by functional, network, and pathway analysis in MAP-infected mice, which demonstrated that MAP-infected mice showed not only the initial innate immune reaction but also suppressor activity against T cells by M2 macrophages, resulting in mycobacterial persistence and lack of immune responsive in the advanced stages of MAP infection. These results may provide valuable information for diagnosis and prognosis. This study was supported by the Rural Development Administration (PJ00897) and the Research Institute for Veterinary Science, Seoul National University, Korea.

Keywords:

transcriptomes, host-responses, early stage infection

O-02 Diagnostics and Detection

Abstract O-02.1: INVITED SPEAKER
THE USE OF SEROLOGY IN THE CONTROL OF PARATUBERCULOSIS

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The serological assays

Shortly after the first "official" description of paratuberculosis or Johne's disease in Europe as a chronic enteritis in cattle (Johne and Frothingham, 1895), the first serological assay for the detection of the disease, the complement fixation test (CFT) was developed (Twort, 1912). Further optimization of the CFT (Bang and Anderson, 1913) allowed the assay to be used as a routine laboratory method for the detection of antibodies against Mycobacterium avium subsp. paratuberculosis (MAP) and allowing its use in the first attempts to control paratuberculosis. For most of the 20th century, the CFT remained one of the most prominent tools in the control of paratuberculosis. For example, earlier control programs for paratuberculosis in dairy cattle in the Netherlands relied on the CFT, either on its own or in combination with the skin test using Johnin or avian tuberculin to decide on the infection status of the cattle tested. However, due to a lack of results, attributed mainly to the inadequate diagnostics tools in combination with an insufficient adherence to the introduced management measures, these attempts were abandoned. Despite its commonly recognized shortcomings, in particular in sub-clinically infected animals, the CFT is still one of the recommended serological tests by the O.I.E. and in use for the export of (often young) animals (Anon., 2012).

One of the first alternatives for the CFT was the development of the agar gel immune diffusion assay (AGID) for the detection of antibodies against paratuberculosis, first in goats and later in cattle (Shermann et al., 1984). In particular in small ruminants the AGID offers a high specificity when compared with ELISAs (Sergeant et al., 2003) or histology (Hope et al., 2000).
However, since the first introduction of the enzyme-linked immunosorbent assay (ELISA) (Engvall and Perlmann, 1971) the use of this technique for the detection of antibodies has gained ground in the serodiagnosis of many infectious diseases. Since then, a wide variety of antigens have been used in a large number of different ELISA's for the detection of paratuberculosis, a non exhaustive list includes protoplasmic antigens, lipid fractions, Johnin (Griffin et al, 2005), and a variety of extracts including ethanol extracts (Wadhwa et al., 2013).

However, all these antigens used are "crude antigens' resulting often in false reactions in the ELISA and therefor require a careful interpretation of the test results by experienced operators. A major breakthrough in the use of the ELISA was the introduction of the absorbed ELISA by Yokomizo: pre-absorbing the serum to be tested with an extract of Mycobacterium phlei, a rapid growing mycobacterial species, resulted in an increased specificity of the ELISA based on the use of a protoplasmic antigen (Yokomizo et al., 1985). Moreover, the newly developed absorbed ELISA showed a better sensitivity and specificity over the CFT when compared in cattle (Yokomizo et al., 1991). This resulted in a rapid and worldwide uptake of the absorbed ELISA in the serodiagnosis of paratuberculosis.

At least as important as these reported test characteristics in the uptake of the assay was the simple fact that several companies used this approach to develop commercially available absorbed ELISAs. The ELISA format allows automation and a quantitative analyses of the results.

Given their availability as well as their price, many laboratories stopped using their own in-house developed ELISA based assays. This is caused the increased demands imposed on diagnostic assays by new ISO standards in recent years, which make it very costly to produce relatively small, but reproducible batches and maintain an adequate quality control system in place.

Furthermore, despite optimism at the time of the introduction of the molecular methods to characterize and produce more specific recombinant antigens of MAP to be used in immune assays, these approaches resulted thus far not in a viable competitor for the absorbed ELISA. Even though the use of most recombinants would result in an increased specificity, they lack a sufficient sensitivity when used individually. However, when combined to improve this sensitivity, these combinations are likely show a decrease of specificity. Moreover, claims made for the diagnostic characteristics of novel antigens, being recombinant or of other nature, are often overly optimistic and based on the use of small cohorts of serum samples. A wider introduction of standards and reporting guidelines as developed by the Johne's Disease Integrated Program (JDIP) will allow a better characterization of novel diagnostic assays for paratuberculosis and prevent such premature claims (Gardner et al., 2011).

Using (absorbed) ELISAs: some practical considerations

Even though there are reasons one could prefer organism detecting assays over the use of serological assays, mainly because of a better sensitivity and specificity (Collins et al., 2005). However there are highly practical reasons to prefer the use of serological assays as a basis for the design of paratuberculosis control programs. In the current situation, programs have to be voluntary and are therefore, in most schemes, to be paid for by the farmer, hence making costs a limiting factor for such a participation. For the same reason, the use of milk as the testing matrix is preferred over serum: milk samples are often routinely tested for a number of other factors and are therefor collected often already and the milk sampling can be done by the owners themselves. In recent years, for the same reasons a rapid shift from fecal culture took place to the use of the absorbed ELISA and is e.g. the use of IS900 based polymerase chain reaction (PCR) often limited to confirm ELISA positive animals and only when asked for by the owner of the animal. However, despite the fact that absorbed ELISA is suitable for use in a paratuberculosis control program, it is all but the perfect diagnostic assay for the detection of MAP and in order to use the absorbed prudently, acknowledging its limitations as well as its benefits is essential for a sustainable and long term success of any conceivable paratuberculosis control program. (Nielsen; Thesis, 2009).

Despite the robustness of the (commercially available) ELISAs, there are a number of inherent characteristics to take into account when using the assays in routine diagnostics. For example, even when using S/P ratios, test results can vary between laboratories (Adaska et al., 2002). Thus resulting in conflicting results when sending the same sample to different laboratories, even when they are using the same assay. Similarly, differences in results can occur within the same laboratory (e.g. Nielsen, 2002) and an adequate quality control needs to be in place to minimize the occurrence of such variable test results. The above could undermine the confidence of herd owners in the reliability of the assay. Moreover, even though confirmation of ELISA results by culture or PCR is often offered to the participants, these test results do not necessarily have to agree, again resulting in a potential loss of confidence (Nielsen, 2008). The above suggests that it would be wise to test individual herds using a single diagnostic assay performed by the same lab over time. On the other hand, by adjusting testing strategies for the ELISA, for example by testing colostrum or by taking into account the days-in-milk, which both could result in a more sensitive use of the assay (e.g. Zervens et al., 2013). All indicating that clear guidelines will have to be developed after careful analysis of the available strategies.

A crucial component of the Danish control program, as described in the aforementioned thesis, is the need for a frequent testing using the absorbed ELISA for the program to be successful. Routinely, milk samples are collected on participating 4 times per year, given the annual dry period of dairy cattle, effectively resulting in sampling 3 times per year per animal. The results of sequential ELISA tests are used in combination with milk production data to advise the farmer on the voluntary removal of test positive animals. Obviously, this frequency of testing adds to the cost of the program, but given the perceived low sensitivity of the absorbed ELISA (Nielsen and Toft, 2008) and the short interval between tests allows to detect animals before they become infectious to others (Lu et al., 2008; Nielsen, 2009), this frequency is essential to achieve the goals of this program.

For those acquainted with the control of bovine tuberculosis, using the (comparative) skin test with tuberculins (a test with significant better specifications than the absorbed ELISA) at more regular intervals, thus increasing the sensitivity, is regarded to be essential in the eradication of this disease from an infected herd ever since it was introduced by Bang (1908).

In addition to the Danish approach, several paratuberculosis programs, both national and regional, and based on the use of the (absorbed) ELISA have been developed and are ongoing. An example is the current program in the Netherlands, primarily aimed at the reduction the presence of MAP in milk and thus entering the food chain, by detecting and removing high shedders. Testing is done less frequent and a higher cut-off is used to detect just the high shedders posing the biggest risk for the contamination of the milk, but results in a less stringent control of the within herd MAP infection. Obviously, the reduced testing cost is an attractive component of the program to encourage their participation.

Ultimately, a detailed analysis, comparing the results of the ongoing programs will provide information on their respective benefits and where to adapt to optimize their outcome. However, given the globalization of animal trade, food and food components, harmonization of programs remains an essential goal.

Concluding thoughts

Not that many years have passed since the Colloquium in Melbourne (1999) where the mandatory slaughter of paratuberculosis infected herds was still being discussed. The development of the paratuberculosis control program in Denmark, currently without assigning an infection status to the participating farms is in stark contrast with this approach.

At present, the “eradication” of paratuberculosis is no longer the ultimate goal and achieving “control” of paratuberculosis is regarded as an enormous challenge already. Sweden might have a (near) zero herd prevalence and has very strict control measures in place to protect this status (Frössling et al., 2013). Several countries show less reticence when discussing their national paratuberculosis prevalence (Nielsen, 2009), despite the highly competitive nature of the global market for dairy products. In addition, the need for control of the disease is seen as an essential component for a sustainable dairy farming.

In the absence of a solid confirmation of the potential role of MAP in the aetiology of Crohn’s disease in humans, the same competitive nature of the global market in combination with farm economics are very important driving force in the ultimate design of paratuberculosis control programs. In general, the effect of a consumer demand for “healthy food from healthy animals” remains quite an unpredictable factor in the driving forces for control programs.

Hence, finding a way of using historical milk ELISA data and data relating to the removal of test-positive animals to develop a system of assigning a “paratuberculosis status” to a herd, a status

from which the herd owner can benefit financially is therefor an important challenge needed to ensure a large scale participation in the Danish paratuberculosis control programs (Nielsen, 2009).

Given the century long history of failed programs for the control of paratuberculosis, it is essential to state clear and realistic goals at the start in order to maintain a dedicated participation of all stakeholders involved during the long period any conceivable program will need to show progress with the currently available tools.

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Abstract O-02.3

USE OF THE RAPID, SENSITIVE AND SPECIFIC PMS-PHAGE ASSAY REVEALS THE TRUE PICTURE ABOUT NUMBERS OF VIABLE MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN RAW MILK

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Introduction

Culture methods traditionally used to detect viable *M. avium* subsp. *paratuberculosis* (Map) in milk are insensitive, non-specific, and slow, and involve chemical decontamination, protracted incubation and laborious confirmatory tests. Use of these methods has resulted in the accumulation of very poor information on the levels of Map in milk because any Map colonies recovered on HEYM slopes cannot easily be counted, and they represent only a fraction of the number of Map actually present in the milk sample if chemical decontamination has been applied (Dundee et al. 2001, Grant and Rowe 2004). With the advent of peptide-mediated magnetic separation (PMS, Foddai et al. 2010) and the optimised phage amplification assay (Foddai et al. 2009) for Map the situation has changed, because now the presence and number of viable Map in milk samples can be determined within 48-72 h using the optimised PMS-phage assay. Limited results are published to date in relation to application of the PMS-phage assay to test naturally infected milk (Foddai et al. 2011), so further raw milk testing was recently carried out and results are presented here.

Methods

The milk testing protocol adopted for this study incorporated recent findings by Foddai and Grant (oral presentation at 12ICP) in relation to milk processing before the PMS-phage assay. The optimised milk testing protocol involves frozen (-20 or -70^{o}C) storage of milk before testing (to maintain Map viability), testing of pellet fraction only (majority of Map sediment into this milk fraction upon centrifugation), and ultrasonication of resuspended pellet in an iced water bath (to disperse Map clumps and ensure accurate enumeration). Two types of raw milk sample were tested: (1) milk from 146 individual animals in a Johne's affected UK dairy herd for which parallel milk-ELISA results were also available, and (2) bulk tank milk from the same herd and 21 other dairy herds of known Johne's disease status, mostly located in Scotland, UK. All milk samples were frozen immediately after collection, stored at -20^{o}C at least overnight, before being transported by overnight courier to the laboratory at Queen's University Belfast. Each 50 ml milk sample was thawed overnight in fridge, brought to room temperature, centrifuged at 2500 x g for 15 min, milk pellet resuspended in 1 ml PBS-Tween 20, and then subjected to automated PMS using in-house prepared MyOne tosylactivated Dynabeads coated separately with N-terminally biotinylated aMp3 (Stratmann et al. 2002) and aMptD (Stratmann et al. 2006) peptides and a Dynal BeadRetriever instrument. The bead suspension after PMS was split equally three ways for the phage assay (PMS-phage), qPCR (PMS-qPCR) and broth culture (PMS-culture), so that results would be directly comparable for each milk sample.

Results

Individual milk samples: 46 (31.5%) of 146 individual milk samples from cattle in a single UK Johne's affected herd yielded plaques in the PMS-phage assay (mean plaque count of 228 PFU/50 ml, range 6-948 PFU/50 ml), 76.1% (35) of which were confirmed to contain Map by plaque PCR. In contrast, 6 (7.8%) individual milks tested PMS-qPCR positive, and 20 (13.6%) individual milks

tested PMS-culture positive. Significantly more individual milk samples tested positive by the PMS-phage assay than by the other two tests (P<0.0001). A single bulk tank milk sample from the same farm, tested at the same time, also tested PMS-phage assay positive with a viable Map count of 51 PFU/50 ml milk.
Bulk tank milks: 13 (59.1%) of 22 bulk tank milks from dairy herds of known Johne's disease status yielded plaques in the PMS-phage assay (mean plaque count of 146.9 PFU/50 ml, range 6-918 PFU/50 ml), 100% (13) of which were confirmed to contain Map by plaque PCR. In contrast, 10 (45.4%) bulk milks tested PMS-qPCR positive, and 12 (54.5%) bulk milks tested PMS-culture positive. In the case of the bulk tank milks, there was no significant difference between the detection rates by the three methods (P>0.05).

Irrespective of type of milk being tested, samples testing positive for Map presence by PMS-qPCR or PMS-culture also generally tested positive by PMS-phage assay, with only a few exceptions. The differences in detection rates observed between PMS-qPCR and the other two methods can partly be explained by differences in the amount of original milk sample actually processed through each test (PMS-phage assay equivalent of 16.7 ml, PMS-culture equivalent of 16.7 ml, and PMS-qPCR 5 µl of 50 µl DNA extracted from 16.7 ml so 1.67 ml per qPCR reaction, assuming no losses).

Whilst not the primary objective of our study, some attempt was made to relate milk testing outcomes to available information on infection status of supplying animal or herd. The milk-ELISA test result history of the 146 cows was obtained from the farm manager once PMS-phage assay results became available. When PMS-phage assay positive results (Map count in PFU/50 ml) were plotted against milk-ELISA reading it appeared that there was an inverse relationship between the two measures, i.e. high plaque (Map) counts when milk-ELISA reading low and vice versa. Also, plaque (Map) counts tended to be highest when a cow had never tested milk-ELISA positive. Some information was obtained about the Johne's status/history of the dairy herds providing the bulk tank milks, however it proved difficult to draw any firm conclusions about how Johne's status of herd impacted the presence of Map in bulk tank milk.

Conclusions

This study provides new information on the presence and levels of viable Map in raw milk at farm level. The PMS-phage assay results indicate that raw milk from infected herds may contain from 10-1,000 viable Map per 50 ml milk. The clear advantages of the PMS-phage assay over existing Map culture methods are that *quantitative results* can be obtained *relatively quickly* (48 h if PMS-phage assay negative result, 72 h if PCR confirmation of plaques is required for a PMS-phage assay positive sample). The main disadvantage is that no Map isolate is obtained. However, if Map isolation was desirable, for example for epidemiological tracking purposes, the PMS-phage assay could be used as an initial screening test and culture efforts subsequently focussed on PMS-phage assay positive milk samples only (using PMS not chemical decontamination as step preceding inoculation of broth or solid culture media). Clearly, if you wish to find out the real situation regarding presence and levels of viable Map in milk then you need to use the most appropriate test, which in our opinion is the PMS-phage assay.

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Keywords:
PMS-phage assay, viable MAP, milk

Abstract O-02.4

USE AND APPLICATION OF PHAGE FOR THE DETECTION OF M. PARATUBERCULOSIS: DEVELOPMENT OF PRACTICAL APPLICATIONS

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Abstract text:

We have been developing applications of a rapid, combined phage-PCR MAP detection method for a range of different sample types. To date the assay has been shown to be useful for the detection of MAP in milk, cheese and clinical blood samples. The milk assay has best evaluated and we have initiated a trial to model the impact of using this assay for the early detection of super shedders on management of Johne's disease. The major challenge for developing new methods has been to recover the MAP cells from the sample matrix to allow efficient phage infection, and we have used a variety of approaches including centrifugation, filtration and immune-magnetic separation. The latter has been used to detect very low number of MAP cells in clinical blood samples, but the limitation was found to be the efficiency of MAP capture by the beads. We have investigated alternative sample preparation methods and report here that the efficiency of detection is the same when using IMS to recover cells from whole blood or by simple isolation of buffy coat. This finding, combined with the development of a rapid, 5 h tube assay format that does not require overnight culture of agar plates to allow plaque formation, means that automated rapid detection of MAP in clinical blood samples is now possible. Since removing the need for plaque formation allows MAP cells to be specifically detected against a background level of high numbers of non-pathogenic environmental Mycobacterial spp., the new assay format is also being evaluated for the rapid detection of MAP in faecal samples. Here we will describe progress with developing these different applications towards practical tests that can be routinely applied for the study of Johne's disease.

Keywords:

phage-based assay, rapid detection, blood

Abstract O-02.5
LYMPHATIC FLUID FOR THE DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN COWS

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Abstract text:
The aim of the study was to challenge the hypothesis that Mycobacterium avium subsp. paratuberculosis (MAP) detected in lymphatic fluid collected from the bovine udder is of diagnostic value for the diagnosis of Johne's disease (JD) in cattle.

Lymphatic fluid from was collected from the udder of 122 cows by puncture and tested for the presence of MAP. Detection was performed by IS900 nested PCR using the primers P90 and P91 for the first and J1 and J2 for the second reaction, respectively.
Lymph PCR results were compared to ELISA results in blood and milk, achieved by 2 commercial test kits, as well as to fecal culture on Herrold's egg yolk medium.

MAP was detected by PCR in 27.1% of the lymph samples with a poor agreement between the different tests and sampling materials. Of the lymph positive cows 6.9% also were positive in all other tests applied and 69.0% of the animals with a positive lymph result had negative results in all other tests applied. Resampling of 25 cows after 8 to 12 months resulted in 20.0% lymph positive animals at the first, 5.5% at the second and 27.8% at the third sampling, respectively. Only one cow showed positive lymph-PCR results at more than one sampling date. Analysis of the herd records revealed that cows with positive lymph PCR had a 7.2 times greater likelihood of being culled within 8 to 12 months after sampling, compared to cows with a negative lymph result.

It can be concluded that lymphatic fluid might be promising for the detection of early MAP-infection in cows, but further studies are needed to evaluate this new diagnostic approach for JD in cattle.

Keywords:
Johne's disease, lymphatic fluid, udder

Abstract O-02.6

TIKA14 MEDIUM SIGNIFICANTLY IMPROVES MAP GROWTH AND PRIMARY ISOLATION FROM HUMAN BLOOD AND TISSUE

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Abstract text:

Introduction

The isolation of MAP, whilst being a gold standard method, is one of the most difficult challenges in detection studies because of its extreme slow growth and tendency to enter dormant. This is particularly acute in samples with a low infectious load. New methods are called for to recover MAP more efficiently, particularly from human samples where culture has often proved either restrictively long or not possible at all.

Methods

A novel liquid medium (TiKa14) was devised, using Middlebrooks base plus selected modified natural product supplements. Growth curves from a range of Type II MAP isolates from animals and humans, inoculum sizes and clinical samples were obtained and compared to parallel isolation/growth on conventional media.

Results

All strains of Type II MAP tested grew in TiKa14 medium with a significantly decreased lag phase ranging from 6-12 days depending on inoculum size. Furthermore, stationary phase was delayed, increasing overall yields. Serial dilutions of MAP cultures in TiKa14 medium grew from at least 2 logs greater than conventional media suggesting the new media suspends conversion at low concentrations to the dormant non-proliferative phenotype. TiKa14 medium grew human MAP strains from blood and gut biopsy samples from 70% of patients with Crohn's Disease within 4-6 weeks.

Conclusion

TiKa14 medium offers great improvements in the recovery and propagation of Type II MAP strains and could have significant impact on the sensitivity and rapidity of culture diagnosis in MAP diseases.

Keywords:

Culture medium, Human MAP, Improved growth

Abstract O-02.7

A NEW MOLECULAR DIAGNOSTIC TEST FOR JOHNE'S DISEASE; FROM LAB TO NATIONAL UPTAKE.

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Abstract text:

The High-throughput Johne's (HT-J) test for faeces was recently approved for use in control programs in Australia and New Zealand. The challenges faced in developing a highly sensitive and specific test that was cost-effective and suitable for the routine diagnostic environment were compounded by the subsequent challenges of national uptake of the test and its initial use in a large-scale investigation in a region previously believed to be disease-free. From its development and subsequent validation, the new test underwent a rigorous process prior to and immediately following its approval by the national regulatory body SCAHLS. The steps were i) application for test approval, ii) national training workshop and test role out, iii) initial testing in an extremely sensitive cross-jurisdictional political environment, iv) follow-up technical face-to-face meeting of diagnostic laboratory technicians to discuss issues with the test uptake and establish a dialogue. In hindsight, we identified a number of key issues for "new generation" molecular tests that are developed to work at diagnostic sensitivities that have been previously unachievable, and that challenge "gold standards" such as culture. This included stochastic subsample related variations in the distribution of the organism and DNA, standardisation and quality assurance between laboratories to ensure performance for low level detection. A secondary issue was the scope of the test, with the defined approval criteria (herd-level testing) stretched beyond what was validated in an attempt to get further information and outcomes from the test results. The ultimate outcomes have been positive, with successful use in many jurisdictions, modifications to address "real-world" challenges at the lab bench and open discussion between users. Plans are underway to further this dialogue and continue the education of researchers, test providers and end-users.

Keywords:

PCR, diagnostic test, HT-J

Abstract O-02.9

EVALUATION OF VIABLE MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN MILK USING PEPTIDE-MEDIATED SEPARATION AND PROPIDIUM MONOAZIDE QPCR.

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Abstract text:
The causative agent of Paratuberculosis in ruminants, Mycobacterium avium subsp. paratuberculosis (MAP), although still a matter of debate, has been linked with Crohn's and other human diseases. The availability of rapid methods for assessing the viability of MAP cells in food, in particular milk, could be of great use for risk management in food safety. MAP viability is generally assessed using culture techniques, which require prolonged incubation periods for the growth of MAP.

Aim of our work was the developing of new approaches to differentiate between viable and non-viable MAP cells in milk samples. For this purpose, present study explores the combination of two already described techniques: peptide magnetic bead separation for the capture of MAP cells, followed by Propidium Monoazide-qPCR.

The method was successful in the assessment of MAP cells viability in milk samples. Moreover, analysing the results obtained by spiking milk samples with mixture containing different percentages of viable/dead cells, an Ordinal Multinomial Logistic Regression model can determine the probability related to viability status of MAP cells in milk. Finally, this model was succesfully applied to artificially contaminated pasteurized milk to ascertain the efficacy of heat treatment in MAP cells killing. Hovewer, the limit of detection of the model was around 500 CFU/ml of milk, a concentration of MAP cells higher than that reported to be present in both individual and bulk tank milk.

In conclusion, the method herein reported can be used for direct detection of MAP cells viability status in milk; however, further studies are needed to improve the sensitivity of the assay.

Supported by Ministry of Health, RF-2009-1545765

Keywords:
MAP viability, Peptide Magnetic Separation, Propidium Monoazide

Abstract O-02.10

NOVEL MAP SECRETED PROTEINS AS DIAGNOSTIC BIOMARKERS FOR JOHNE'S DISEASE IN CATTLE

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Abstract text:

ELISAs fail to detect asymptomatic MAP-infected cattle due to the use of poorly-defined antigens. Moreover, our understanding of MAP components eliciting pathogen-specific immune responses is limited. We set out to (i) define a subset of proteins that contain putative B-cell antigens and (ii) screen these protein pools for immunogens relevant in serodetection. To this end, we captured MAP secreted proteins using a 2-step fractionation method to identify 162 unique proteins, of which 66 had not been previously observed in MAP culture-filtrate (CF). Screening of MAP secreted proteins revealed four antigens, of which one or more reacted on immunoblotting with individual serum from 35 MAP-infected cows. Moreover, these antigens reacted with sera from 6 low-MAP shedders, and 3 fecal-culture positive cows labeled as ELISA seronegative. The specificity of these antigens was demonstrated using negative control sera from uninfected calves (n=5) and uninfected cows (n=5), which did not react to any of these antigens by immunoblotting. Cloning and recombinant expression of these antigens in E.coli and M. smegmatis revealed their localization in whole-cell lysates and CF of E.coli, and exclusively in the CF of M.smegmatis. Polyclonal rat antiserum was generated against these proteins and successfully used to: detect the native MAP protein exclusively in CF, demonstrate that M.avium subsp. hominissuis (MAH) and M.smegmatis whole-cell lysates and CFs do not cross-react with these antisera, and that absorbing the antiserum with MAH whole-cell lysates does not result in the loss of immunoreactivity with native or recombinant MAP protein. Bioinformatic analyses of these proteins have identified homologs in MAH (99% protein identity) and Mycobacterium bovis (68-87% protein identity). Despite the high protein sequence identity, these data collectively suggest that these proteins contain MAP-specific epitopes. Ongoing work is focused on mapping the antigenic epitopes that confer specificity of these proteins.

Keywords:

Serodiagnosis, ELISA, Secretome

Abstract O-02.11

MOLECULAR DIAGNOSTIC TESTS FOR JOHNE'S DISEASE; TIME TO STANDARDISE

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Introduction: Polymerase chain reaction (PCR) has undoubtedly made a significant contribution to both research on and diagnostic applications for microbial pathogens including *Mycobacterium avium* subspecies *paratuberculosis* (MAP). However, its widespread use has led to problems with standardisation and harmonisation within and between laboratories, especially as the sensitivity of PCR-based tests has progressively increased. In 2009 an international collaboration was undertaken to establish the Minimum Information for the publication of real-time Quantitative PCR Experiments (MIQE) to assist researchers in the publication of more robust quantitative real-time PCR assays. More recently, the STAndards for the Reporting of Diagnostic accuracy studies (STARD, www.stard-statement.org/) initiative was introduced to improve the accuracy and completeness for reporting studies on diagnostic accuracy. These initiatives in conjunction with an increasing focus on measurement of uncertainty should result in diagnostic assays that generate highly reproducible results. However, are we using these tools to our advantage?

A review of the scientific literature on direct faecal-PCR for MAP in cattle from 2007 onwards, (summarized in Table 1) demonstrated very little if any standardisation of conventional or quantitative PCR and that the majority of these assays failed to meet the MIQE or STARD guidelines particularly in terms of the number of animals tested and both analytical and diagnostic sensitivity and specificity data. Furthermore, the diagnostic sensitivity values may have been biased (inflated) due to comparisons of PCR with a variety of culture techniques with differing (lower) analytical sensitivities. We believe it is time to establish an international initiative to assist in the development and validation of standardised diagnostic PCR protocols for paratuberculosis.

Table 1: Summary of findings following a review of direct-fecal PCR of bovine Johne's disease.

Number of samples	DNA extraction methods	Target gene and PCR type	PCR Type	Analytical Se/Sp	Diagnostic Se/Sp	Culture
<10 = 1 >10<100 = 4 >100<250 = 6 >250<1000 = 8 >1000 = 1	**Commercial Kits** Tetracore VetAlert™ kit, High Pure PCR Template Prep. Kit (Roche), Qbiogene's Fast DNA soil Spin Kit, SmartHelix™ First DNAid kit (Institute of Physical Biology, Slovenia), PSP Spin Stool DNA kit (Invitek), QIAamp DNA stool minikit (Qiagen), All-for-One (Qiagen), MagMax **In-House Including** Mechanical lysis, Boiling, Phenol/chloroform/ isoamyl alcohol CTAB/NaCl, Guanidine	IS*900* IS*MAP02* f57 IS*Mav2* locus 255 MAP276 MAP0865 *hspX* Single Multiplex Nested	**Conventional** Single Nested **qPCR** SYBR green Probe 2-Step 3-Step	**Sensitivity** fg pg µg Not reported **Specificity** 98-100% Unclear Not reported	**Sensitivity** 17.6-100% Unclear Not reported **Specificity** 100% Not reported	**Commercial** Trek MGIT **In-house** HEYM Lowenstein - Jensen BACTEC

Models on standardisation and harmonization.

High Throughput Johne's (HT-J) test in Australia: Following approval of the High Throughput Johne's (HT-J)[12] test in Australia for national use as a herd-level test, a technical working group was convened that included representatives from each of the Australian state laboratories that now offer the test (Queensland, New South Wales, Northern Territory, South Australia, Victoria and Western Australia), test developers and representatives from the Sub-Committee on Animal Health Laboratory Standards (SCAHLS, the governing body for veterinary diagnostic tests in Australia). Over the last year this group has successfully established the necessary requirements to standardise the use of the HT-J test in Australia and established a national quality assurance programme such that inter-laboratory results can be more easily and reliably compared. The success of a National Johne's Disease (JD) Program is largely attributable to the science behind diagnosis and epidemiology. Alignment of the policies with science is required when advances such as the HT-J test are made available. Improved epidemiological knowledge based on accurate prevalence data, underpinned by consistent standardised tests, will benefit policy decisions that support the aims and objectives of the control program over time.

Microbiological testing in the European Union: The European Union (EU) has strongly promoted the development of standardised laboratory practices for many bacterial diseases. Ahmed *et al.,* (2008) published an informative review on the harmonisation and distribution of pathogen detection and differentiation tools in support of the growing need for national and regional diagnostic laboratory networks to accurately monitor the threat to the European livestock industry from neighboring countries and trading partners (INCOME-Project)[1]. The key objectives of this project were to establish standardised and harmonised diagnostic tools and build an integrated disease control programme. According to the final report these objectives have been achieved[2]. Similarly, a study by the European Food Safety Authority (EFSA) identified the need for greater harmonisation and standardisation in order to collect meaningful data to monitor zoonoses, antimicrobial resistance and foodborne disease outbreaks[6]. Other European Union initiatives targeting microbiological pathogens include: The European Aspergillus PCR Initiative (EAPCRI), formed in 2006 to provide standardised protocols for the widespread evaluation of PCR for Invasive Aspergillus (IA) diagnosis. Although PCR had been used for the diagnosis of IA for sometime, studies had shown the lack of homogeneity and standardisation amongst laboratories had limited its acceptance and inclusion in disease-defining criteria[10,13]. A MIQE-compliant qPCR has now been developed and published for the diagnosis of IA[5].

A survey of the diagnostic tests to confirm pertussis throughout the EU identified disharmony and a significant heterogeneity amongst the reference laboratories across member states[4]. Consequently, evaluation of immunization programmes in Europe has been difficult. An EUVAC.NET study on serological diagnosis of pertussis found antigen coating practices of ELISA plates to be the major source of disharmony. Poor assay performance was linked to commercial kits when using WHO reference preparations[14]. Similarly, variance amongst Reference Laboratories within member states of the EU that regulate serological tests for the diagnosis of Brucellosis, was found to be the cause of disharmony for a number of tests and overall poor test performance[8]. An increasing number of participants in quality assurance trials indicates the need to for standardised diagnostic tests.

PulseNet, the global standardisation model: In an effort to address the global problem of foodborne diseases, diagnosticians and epidemiologists are moving away from in-house diagnostic and DNA-typing tests and protocols to internationally validated and standardised protocols that allow for immediate comparison of results within the international disease and surveillance community. Following the 1993 *E. coli O157* outbreak in the USA, that resulted in 726 sick and 4

dead, it became apparent that this outbreak was more widespread than originally thought. Health departments did not have data to determine which illnesses were linked by a common food source. Consequently, the United States of America Centre for Disease Control and Prevention (CDC) established the PulseNet disease surveillance network. PulseNet now includes over 80 laboratories throughout the United States (PulseNet USA) and 83 member countries from seven national and regional PulseNet networks throughout the world. PulseNet International uses standardised DNA fingerprinting techniques to monitor pathogens of foodborne diseases and track outbreaks at local, national and international levels. PulseNet has also provided a sound platform for international collaboration to transition the international foodborne disease surveillance network into Next Generation DNA fingerprinting. The integration of whole or partial genome sequencing will steadily grow as new databases become available via projects like the Global Microbial Identifier Initiative and the 100K Foodborne Pathogens Project (both projects include the PulseNet network as collaborators).

Conclusion: Standardisation and harmonisation of diagnostic testing is gaining momentum at many levels, from local to international, for a number of microbial pathogens[7,9]. Meaningful comparisons between results are becoming increasingly important to support our understanding of the epidemiology of disease, disease outbreaks and to meaningfully underpin trade agreements between countries. We believe it is time to establish an international initiative to evaluate the use of diagnostic tests and make recommendations on their use with paratuberculosis. An initiative such as this will directly support the level of precision and accuracy required as new technologies such as whole genome sequencing becomes a desktop reality in laboratories. At the time of preparing this paper the paratuberculosis (Johne's disease) chapter of the OIE terrestrial manual is dated 2008 and the development of this initiative may well coincide with an updated version of this chapter. It is critical now that new diagnostic assays and tests meet guidelines such as OIE, MIQE and STARD. This task has been made easier for the paratuberculosis research community through the efforts of a group of well established paratuberculosis researchers who have published STARD-based guidelines specifically for this disease and design criteria for diagnostic test validation studies[3,11].

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Abstract O-02.12: PERSPECTIVE
IS IT TIME TO CHANGE THE GOLD STANDARD IN MAP DETECTION?

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The culture method is widely considered by many as the gold standard for the diagnosis of paratuberculosis. However, this test does not allow accurate identification of infected animals because limitations of culture protocols like low sensitivity, slow growth of MAP (long time to detection) and overgrowing of MAP with contaminating microflora do not permit to reveal MAP infection in early stages. Moreover, due to the intermittent shedding of MAP, culture is very poor tool to identify positive animals with the high sensitivity in adequate time.

The majority of the disadvantages of culture can be solved by the application of the qPCR methods. They provide higher sensitivity and specificity and in connection with the appropriate DNA isolation protocols from wide spectrum of matrices it represents a powerful diagnostic tool for the control of MAP. However, the price for the analysis is still an issue that needs to be considered and also inability to determine viability is the reason why it is still used as a supplementary detection method.

Culture can be applied on different types of matrices; however, it is routinely applied mainly on faeces and tissue. Less common matrices for the detection of MAP like milk can be also cultured, however, there is a question about the percentage of the false negative results. For example, milk was referred to be very difficult template for the MAP culture and number of milk samples positive for MAP by qPCR was significantly higher than culture (Donaghy et al., 2008; Slana et al., 2008, 2009). Conversely, some studies reported higher percentage of positive samples by culture rather than conventional PCR (Shankar et al., 2010). When faeces are used as the template for the MAP detection, differences between culture and PCR results are not as high as in the case of milk. It was referred that PCR techniques for MAP detection from faeces are comparable with culture on solid or liquid media (Bogli-Stuber et al., 2005; Vansnick et al., 2007; 2009; Soumya et al., 2009). These differences would be reduced by standardization and harmonization PCR methods within and between laboratories worldwide.

The prevailing implementation of qPCR techniques can be documented by the records in the Web of Science database (Thomson Reuters). In last 14 years, there are 3085 articles about paratuberculosis in total. Under the keywords “paratuberculosis” AND “cultivation” 1104 records was found. The number of published papers about culture remains constant over selected period. On the other hand, the number of papers referring about the development and application of the PCR methods steadily grows and reached 795 in the year 2014. The same trend can be found in the application of the culture independent methods that can assess the viability of MAP.

These novel culture independent techniques for the determination of MAP viability still suffer from the certain imperfections, which prevent them in wider implementation to routine laboratory use. Theoretically they can be split into two trends. The first of them is based on the employment of the DNA dyes like propidium monoazide (PMA) that can selectively bound to the DNA of dead cells. Further determination of viability is carried out by qPCR comparing PMA treated and PMA untreated samples. The second trend represents improvement of the culture techniques either by the reformulations of culture media or by the usage of phage assays.

Although the current reference method is culture, the spectrum of available detection tools is much wider. It is impossible to select a single test that could be used universally. The application of the tests is dependent on factors such as age of the animal, stage of the disease or source of the material. The pros and cons of each approach will be presented.

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P-02 Diagnostics and Detection

Abstract P-02.1

A SENSITIVE AND SPECIFIC ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTING SERUM ANTIBODIES AGAINST *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* IN FALLOW DEER (*DAMA DAMA*)

Prieto M. [1], **Balseiro A.** [1], **Casais R.** [1], **Abendaño N.** [2], **Fitzgerald L.E.** [2], **Garrido J.M.** [2], **Juste R.A.** [2], **Alonso-Hearn M.** [2]

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Enzyme-linked immunosorbent assay (ELISA) is the diagnostic test most commonly used for the control of paratuberculosis in domestic ruminants. However, commercial ELISAs have not been validated for detecting antibodies against *M. avium* subsp. *paratuberculosis* in wild animals. In this study, we compared sensitivity and specificity of five ELISAs using individual serum samples collected from 41 fallow deer with or without histopathological lesions consistent with paratuberculosis. Two target antigenic preparations were selected: an ethanol-treated protoplasmic preparation obtained from a fallow deer *M. avium* subsp. *paratuberculosis* isolate (ELISAs A and B) or a paratuberculosis protoplasmic antigen (PPA3) (ELISAs C and D). Fallow deer antibodies bound to the immobilized antigens were detected by using a horseradish peroxidase (HRP)-conjugated anti-fallow deer IgG antibody (ELISAs A and C) or (HRP)-conjugated Protein-G (ELISAs B and D). A commercially available assay, ELISA-E, designed for the detection of *M. avium* subsp. *paratuberculosis* antibodies in cattle, sheep and goats was also tested. Although the ELISAs A, C and E had the same sensitivity (72 %), the ELISAs A and C were more specific (100 %) in detecting fallow deer with lesions consistent with paratuberculosis at necropsy than the ELISA-E (87.5 %). In addition, the ELISA-A was particularly sensitive at detecting fallow deer in latent stages of the infection (62.5 %). Antibody responses detected with the ELISA-A correlated with both severity of enteric lesions and presence of acid-fast bacteria in gut tissues. In summary, our study showed that the ELISA-A could be a cost effective diagnostic tool to prevent the spread of paratuberculosis among fallow deer populations.

Keywords:
ELISA, serum antibodies, fallow deer

Abstract P-02.2

PARATUBERCULOSIS IN ITALIAN MEDITERRANEAN BUFFALO: SEROLOGICAL SCREENING AND PREVALENCE STUDY IN A BREEDING FARM IN THE PROVINCE OF ROME (2009-2012)

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Abstract text:

Prior to the approval of a national voluntary paratuberculosis control plan (approved on 2013), the disease management in the Italian herds was carried out just on the farmers' initiative and sensitivity. The prevalence of paratuberculosis in buffalo herds is quite unknown in Italy. We studied a buffalo breeding farm in a lapse of four years (2009-2012), to assess the prevalence. The farm, placed in the province of Rome, counted roughly 350 animals. The buffaloes over 12 months were subject to serological examination (ELISA) yearly and positive animals were culled. The overall raw prevalence obtained was very low (1,2-0,0 %). Paratuberculosis in the Italian Mediterranean Buffalo often occurs with mild clinical signs, consequently it is difficult to suspect it. In addition, with regards to diagnostics, the detection of MAP infection in buffalo is challenging, further studies are necessary to better understand immune response, clinical signs and to improve the sensitivity of the laboratory diagnosis of paratuberculosis in buffalo species.

Keywords:

paratuberculosis, buffalo, seroprevalence

Abstract P-02.3

L5P: NEWS DEVELOPMENT FOR SPECIFIC SERO-DIAGNOSIS OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS INFECTION

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Abstract text:

Background: Unlike other MAC members, Mycobacterium avium subsp. paratuberculosis does not produce the GPL on the surface of the cell wall but a lipopentapeptide called L5P or LP01. The molecular and genetic characterization of this antigen demonstrated that L5P is specific to Mycobacterium avium subsp. paratuberculosis. L5P produced by chemical synthesis was used to show that this molecule is suitable to detect Mycobacterium avium subsp. paratuberculosis infected animals. Currently available diagnostic tests are based on the use of whole cell antigen that are mainly not Mycobacterium avium subsp. paratuberculosis specific and that require a pre-absorption step with antigen of Mycobacterium phlei. These diagnostics detect animals with a late infection but are not sensitive enough to detect early infection. We hypothesize that the pre-absorption step prevents detection of informative populations of immunoglobulins, especially in animals with“asymptomatic shedding” not detected by the current serology-based diagnostics.

Objective: Assess the potential of L5P and soluble variants for the serological diagnosis of Mycobacterium avium subsp. paratuberculosis infection using collections of sera from different contexts.

Method: In order to find the best compound for use in serology we chemically synthesized L5P and derivatives of L5P including water-soluble forms. These pure compounds were evaluated on three collections of serum, each well characterized from infected and non-infected cattle, goats and sheep.

Results: ROC analysis showed that L5P and also water-soluble derivatives are suitable for the development of a serological diagnostic test. Advantageously, these pure synthetic Mycobacterium avium subsp. paratuberculosis specific antigens can be produced at low cost.

Keywords:

antigen, serology, ELISA

Abstract P-02.4

EVALUATION AND COMPARISON OF TWO METHODS FOR THE EXTRACTION OF MYCOBACTERIUM AVIUM SSP. PARATUBERCULOSIS (MAP) SPECIFIC DNA FROM FECES FOR PCR

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Abstract text:
In this work, two different methods for the extraction of MAP-specific DNA from feces were evaluated and compared. To measure the yield of extracted MAP – DNA, two real time assays were also used. For an early diagnosis of Paratuberculosis it is essential to have a reliable method for the extraction of MAP- specific DNA from feces providing fast results within one day. We evaluated a new extraction protocol based on “Adiafilters” from Biomérieux (Adiagène). Results were compared to those generated with a standard extraction protocol which is used in our laboratory. All fecal samples were tested with an in house real-time PCR protocol according to Herthnek et al. (2006) and a part of the samples was also analyzed with the ADIAVET™ PARATB Real-time PCR Kit. Results were compared to those of the bacterial culture. 59 samples were tested in total; all of them were analyzed with the in house PCR protocol and 57 with the ADIAVET™ PARATB PCR. DNA was isolated from 55 of the fecal samples with the standard extraction method; they were all tested with the in house real-time PCR and 18 of them with the ADIAVET™ PARATB PCR.

In summary it could be shown that the use of Adiafilters with the DNA-extraction protocol of Biomérieux optimize the extraction of MAP- specific DNA and the real-time PCR results are improved. There is a high level of agreement between the bacterial culture and the real-time PCR results of feces with both PCR protocols.

In contrast, results of the real-time PCR with DNA extracted with the standard protocol showed a low agreement to the bacterial culture, some positive samples were not detected and Ct values of the positive samples were higher compared to the results of the new extraction method. Therefore, these preliminary results make it an excellent protocol for the efficient extraction of MAP-DNA from feces.

Keywords:
DNA extraction, feces, real-time PCR

Abstract P-02.5

CONVENTIONAL DETECTION AND MOLECULAR EPIDEMIOLOGY OF PARATUBERCULOSIS IN FARMER HERD'S OF DOMESTIC LIVESTOCK OF NORTH INDIA

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Abstract text:

Paratuberculosis caused by Mycobacterium avium subspecies paratuberculosis (MAP) has been emerged as most serious problem for livestock owners and dairy industries in India. Information about the status of MAP infection in farmer's herds of domestic livestock species is requisite for the formulation of National control strategies for paratuberculosis in the country. In present study, a total 184 fecal samples were collected from farmer's herds of different livestock species (113 cattle, 39 buffaloes and 32 goats) from North India and screened for the presence of MAP infection using microscopic examination, culture on HEY medium with mycobactin J and direct IS900 PCR. All the isolates recovered on HEY medium were subjected to molecular identification and genotyping using IS900, ISMav2 PCR and IS1311 PCR-REA, respectively. Of the 184 fecal samples, 46 (25.0%), 29 (15.7%) and 9 (4.8%) fecal samples were positive for the presence of MAP using microscopic examination, direct IS900 PCR and culture, respectively. Species wise, 20.3, 23.0, 43.7%; 15.9, 10.2, 21.8% and 3.5, 2.5, 12.5% samples were positive for the presence of MAP from cattle, buffaloes and goats, respectively. Isolates recovered on Hey medium were positive for IS900 and ISMav2 sequence and genotyped as Bison type using IS1311 PCR-REA. Present study reported moderate prevalence and interspecies transmission of 'Bison type' genotype of MAP in farmer's herds of domestic livestock species of north India. National surveillance and control program for paratuberculosis is urgently required to secure optimum productivity from domestic livestock of India.

Keywords:

Bison Type, IS1311PCR-REA, ISMav2

Abstract P-02.6

LONGITUDINAL STUDY OF MYCOBACTERIUM AVIUM SPP. PARA-TUBERCULOSIS ANTIBODIES KINETICS BETWEEN MILK AND SERUM IN DAIRY CATTLE

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Abstract text:

Johne's disease, chronic granulomatous enteric disease in dairy cattle, is caused by Mycobacterium avium sub paratuberculosis (MAP) infection. The prevalence of MAP in the US was approximately 68% of dairy farms in 2008. The disease caused decreased milk production and increased cow replacement costs, resulting to US dairy industry economic loss of $200 to $250 million. The milk enzyme-linked immunosorbent assay (ELISA), serum ELISA, fecal culture and fecal sample polymerase chain reaction are widely applied for diagnosis of the disease infection. The test of milk ELISA is less labor-intensive as samples can be easily collected from cattle to determine MAP infection. However, the levels of MAP antibodies are varied in terms of lactation stages, peripaturient period and parities. The kinetics of MAP antibodies levels both on milk and serum samples from same individual were evaluated for one (1) year among 10 dairy cows with different ages and parities. Although there was a fluctuation of MAP antibodies levels during the period, change of pattern on MAP antibodies level was similar both in milk and serum. On two cases, milk MAP antibodies levels were higher than that of serum during early lactation period for short period of time. However, levels of MAP antibodies were maintained mostly high in serum samples. In conclusion, although the sensitivity of milk MAP ELISA test was a bit low, it is still a feasible assay for MAP screening in dairy herd. Also, periodic Johne's disease screening is recommended due to fluctuation in MAP antibodies levels at the time of sample collection.

Keywords:

Mycobacterium avium sub paratuberculosis , Milk and serum ELISA, Kinetics of antibody

Abstract P-02.7
INTEREST OF INTERFERON GAMMA RELEASE ASSAY FOR THE DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS INFECTION

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Abstract text:
PCR, ELISA and fecal culture are used to detect Map (Mycobacterium avium subsp. paratuberculosis) infection. Interferon gamma (IFNg) release assay (IGRA) has been used since the 90's for bovine tuberculosis diagnostic. Up to now, there was no commercially available stimulation antigens for paratuberculosis. The aim of this study is to assess the specificity and sensitivity (in infected herds showing different infection profiles) of antigens developed by IDVET.

Specificity was evaluated on cattle herds with no history of paratuberculosis (n=1027, France and Belgium). Sensitivity was evaluated in infected herds of different age class and compared to fecal Q-PCR and serology. Match-paired antigens from purified Map and M. phlei extracts (IDVET)were used for whole blood simulation. IFNg production was assessed with the IDScreen® Ruminant IFNg assay (IDVET).

With Map and phlei antigens, all samples coming from Paratuberculosis-free herd were found negative, giving a specificity of 100% (IC95%: 99,6–100%). 8-10 months aged animals coming from infected herds did not show any IFNg response. With older animals from infected herds, most of the IFNg positive animals were found positive by at least one other technique.

For the first time, IGRA stimulation antigens are validated in field conditions on a large number of samples, using a calibrated and standardized IFNg ELISA. An excellent specificity was shown, indicating excellent matched potencies of antigens. In infected herds, no IFNg response was detected in young animals. Few animals, 18-months aged minimum, were detected only by this technique. If its sensitivity seemed lower compared to serology or Q-PCR, IFNg could be used as a new complementary technique.

We want to acknowledge Alain JOLY (GDS Bretagne, France), Sébasien GEOLLOT (GDS Finistère, FRANCE) and Laurent CACQUINEAU (LDA29, FRANCE) for their participation to this study.

Keywords:
interferon gamma , PPDj, paratuberculosis

Abstract P-02.8

EVALUATION OF AGREEMENT BETWEEN CULTURE, PCR, HISTO-PATHOLOGY FOR POST MORTEM DIAGNOSIS OF PARATUBERCULOSIS FROM FEACES AND TISSUES

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Abstract text:
The purpose of this study was to acquire a more detailed knowledge in the direct diagnosis of Paratuberculosis (PTB) by post mortem tests. Portions of intestine, gut-associated lymph nodes and feaces from 49 dairy cattle belonging to 2 PTB infected heards were collected at slaughter and processed for Mycobacterium avium subsp. paratuberculosis (MAP) detection (culture, PCR, histopathology). Strain isolation from feaces and tissues were carried out according to the NRC. PCR was performed by amplifying a portion of the IS900 specific for MAP. For histopathology, samples were fixed in formalin, embedded in paraffin, stained with hematoxylin-eosin and Ziehl-Neelsen stain.

Kappa index of Coehn and exact CI95% was calculated to evaluate the agreement between the tests at animal level and for each sampled organ. A head was classified "positive" if at least one organ resulted positive.

21 cows resulted negative and 28 positive. At animal level the highest agreement resulted between culture and histopathology (K=0.71, CI95%: 0.52-0.90). Considering only organs the highest agreement was found for lymph node between PCR and histopathology (K=0.67, CI95%: 0.46-0.89), for rectum between culture and histopathology (K=0.50, CI95%: 0.23-0.77), for small intestine between PCR and histopathology (K=0.49, CI95%: 0.21-0.76), for cecum between culture and PCR (K=0.48, CI95%: 0.20-0.75), and for ileocecal valve PCR and histopathology (K=0.43, CI95%: 0.14-0.71). The agreement for feaces between isolation and PCR was found to be excellent (k=0.73, CI95%: 0.50-0.93). Lymph node showed the highest sensitivity (Se=69.2; CI95%: 0.48-0.90), while rectum the lowest (Se=48,0; CI95%: 0.18-0.72).

Results confirmed good agreement between culture and histopathology at animal level and between PCR and histopathology for lymph node. Based on results, to improve the post mortem protocol it is advisable the sampling and analysis of all tissues considered, associated with feaces.

Keywords:
Post mortem diagnosis, Kappa agreement , Paratuberculosis

Abstract P-02.9
DETECTABILITY OF MYCOBACTERIUM AVIUM SSP. PARATUBERCULOSIS IN SUBCLINICALLY INFECTED BULLS

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Abstract text:
Little is known about the individual pathogenesis to Mycobacterium avium ssp. paratuberculosis (MAP) in subclinically infected bulls. In a longitudinal study with four naturally infected but asymptomatic bulls had shown that MAP to be intermittently detectable up to high concentrations (10^6 MAP-DNA equivalents/ml) in semen.

From one of the tested bulls, faecal, blood, and semen samples (n=139/139/82) were collected over a period of four years to characterize detectability of MAP. All samples were analysed by IS900 based semi-nested PCR, quantitative real-time PCR (qPCR), culture and ELISA. Data revealed that MAP was detectable intermittently in faecal, blood, and semen samples. MAP was not obvious in any of the three matrices in 89/141 sampling days and during intervals of up to nine weeks. MAP-DNA was detected most frequently (25%) and also at highest concentration (up to 10^6 MAP-DNA equivalents/g) in faeces by PCR. MAP was only cultivable in 2% of faecal and 1% of blood samples. An antibody response was not detectable.

Further 42 tissue samples were collected during necropsy of the bull to get a detailed overview concerning MAP distribution within an asymptomatic bovine host. MAP was widely spread, giving MAP-DNA positive results in tissues of the lymphatic system (7/15) and digestive tract (5/14). Most frequent MAP positive was tissue of the urogenital tract (5/9). No tissue of thorax organs (0/2) and nervous system (0/2) were tested positive. Using qPCR, only two tissue samples (lymph node lung and inguinales superficiales) could be quantified (10^6 MAP-DNA equivalents/g). No tissue culture gave a positive result.

The presented study points out once more the problematic detectability of MAP. The discrepancy between the results of PCR and culture indicated that the bacterium might exist in a metabolically reduced, viable but non-cultivable state (VBNC) in infected but asymptomatic hosts.

Keywords:
bull, pathogenesis, detection

Abstract P-02.10

ESTIMATING DIAGNOSTIC ACCURACY OF PARATUBERCULOSIS (PTB) DIAGNOSTIC TEST WITH LATENT CLASS MODELS

Vitale N.[1], Possidente R.[1], D'Errico V.[1], Dondo A.[1], Bergagna S.[1], Barbero R.[1], Meistro S.[1], Bozzetta E.[1], Peletto S.[1], Cerrutti F.[1], Romano A.[1], *Goria M.[1], Arrigoni N.[2], Chiavacci L.[1]**

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Abstract text:

A prospective longitudinal study was carried out on 2 dairy herds in order to evaluate diagnostic test for PTB. A cohort of 300 dairy cattle (from 1.5 to 4.5 years of age) were selected and tested 7 times from July 2010 to July 2012. At each sampling, every 4 months, serum ELISA, faecal culture (FC) and PCR on individual faeces were performed. Different latent class models were compared by the method of Maximum Likelihood and Bayesian inference. Maximum Likelihood (ML) analysis was performed by lEM (log-linear and event history analysis with missing data using the EM algorithm, J. K. Vermunt), using the Deviance Information Criterion (DIC), starting with the simplest model under conditional independence and gradually increasing the complexity by including conditional covariances between test pairs. Bayesian analysis was performed by WINBUGS, comparing different models: assuming conditional independence, including conditional dependence between pairs of tests (Elisa- FC, Elisa –PCR, FC-PCR). Priors for sensitivity and specificity of tests were based on data reported by literature. For ML analysis, according to the deviance information criterion, the best model was the one allowing a dependency between ELISA and FC. Also for Bayesian analysis, the best model accounted for conditional dependence between ELISA and FC. Results of ML analysis overlapped to Bayesian analysis. Estimated mean within-herd prevalence ranged from 21% to 44%. FC test highlighted the presence of 5 high shedders. ELISA overall sensitivity resulted 82.7% (CI95%:70.5%-91.7%), FC Se: 83.8% (CI95%: 69.7%-95.4%) and PCR Se: 91.8% (CI95%: 81.9%- 97.5%). ELISA overall Specificity resulted 97.7% (CI95%: 96.8%-98.4%); FC Sp: 99.8% (CI95%: 99.5% - 100%) and PCR Sp: 88.7% (CI95%: 86.9%-90.2%). Results from this study are similar to that reported in literature for high paratuberculosis within-herd prevalence. The dependence found between ELISA and FC was reported in literature to be correlated with the disease stage.

Keywords:

LATENT CLASS MODELS, SENSITIVITY, SPECIFICITY

Abstract P-02.11
MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS STRESSOME AND DIAGNOSTIC SIGNIFICANCE: A REVIEW AND META-ANALYSIS

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Abstract text:
Johne's disease in ruminants is caused by Mycobacterium avium subspecies paratuberculosis. The host-pathogen interaction is a complex interplay and both host and pathogen alter their gene expression levels to achieve a situation beneficial to self. Stress-regulated genes and encoded proteins of pathogen play critical role in evading defence mechanism and survival within the host. Knowledge of stress-regulated pathogen transcriptomes and proteomes provide important information for understanding disease pathogenesis and diagnosis. Several studies have investigated genes and encoded proteins that are differentially regulated by Mycobacterium avium subspecies paratuberculosis when exposed to in vitro and in vivo stress conditions. This study reviewed the findings from previous studies on stress-regulated transcriptomes and proteomes and their diagnostic significance. Comparison of stress-regulated transcriptomes identified dissimilar sets of genes regulated in different studies. Only a few genes were commonly identified as stress-regulated among different studies. The disagreement between the studies may be attributed to variance in the methodology and/or the analysis method employed by different studies. A stress-regulated proteomic response was more predictive of immunogenicity compared to the findings relating to the transcriptomic response. The majority of the stress-regulated proteins identified as immunogenic were reported to have shown an overall upregulated response. This association indicates that there is an opportunity to investigate more of these proteins to identify their diagnostic potential. A consolidated list of proteins that are not evaluated for their diagnostic potential but are found to be differentially regulated at gene and protein level was prepared. These proteins may be worth investigating for their diagnostic value.

Keywords:
Immunogenicity, Proteome, Transcriptome

Abstract P-02.12

L5P A SPECIFIC ANTIGEN SUITABLE TO DETECT MYCOBACTERIUM AVIUM SPP PARATUBERCULOSIS INFECTION BY CELL MEDIATED IMMUNE RESPONSE

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Abstract text:

Context: The livestock management needs an early diagnostic test to prevent dramatic rise of Mycobacterium avium spp paratuberculosis (Map) infection in cattle. Current serology based diagnostic tests available, detect animals in later stages when they are shedding huge amount of bacillus. Cell mediated immune (CMI) responses that can be detected by Interferon Gamma Release Assays, appear before or combined with antibody responses. Therefore, diagnostic tools based on IGRA could help to identify recently infected animals in order to prevent disease transmission. The sensitivity and specificity of these tests need to define Map-specific T-cell antigens. Map produces a specific cell-wall lipopentapeptide called L5P or LP-01. L5P is suitable to detect Map-infected animals by serodiagnostic. Moreover, L5P can be synthetized chemically at high purity, large scale, and low cost. .

Objectives: The purpose of this work was to assess the potential of L5P to detect Map-specific cell mediated immune responses to develop an IGRA test.

Methods: A panel of 36 cows were selected from two naturally infected herds where clinical paratuberculosis had occurred. We performed 1) serology with the commercial IDEXX diagnostic test and a house-made test using L5P antigen 2) IGRA with Purified Protein Derivative avian (PPD-A) or synthetic L5P antigen 3) microbiological analyses including isolation and identification of bacillus from faeces.

Results: In this study 47.2 % of cows were scored positive by the commercial IDEXX diagnostic test but only 22% developed anti-L5P antibodies. PPD-A induced CMI responses in 97 % of cows while 22.2% animals were L5P responders. Map was isolated in 25.7% of animals. We provide, for the first time, evidence that Map-specific L5P is a suitable antigen for serologic and IGRA diagnosis.

Perspectives: We will now carry out a longitudinal study to investigate the potential of L5P-based IGRA to predict clinical outcome of Map-infected 18-24 months cattle.

Keywords:

Interferon Gamma Release Assay IGRA, Lipopentapeptide L5P, Specific antigen

Abstract P-02.13

LONGITUDINAL STUDY FOLLOWING NATURAL PARATUBERCULOSIS INFECTION; DIAGNOSIS USING CLASSICAL SEROLOGY, FAECAL CULTURE AND INTERFERON GAMMA RELEASE ASSAYS IN RESPONSE TO PPDA AND THE NOVEL ANTIGENS MAP 0268C, MAP1365 & MAP3651C.

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Abstract text:
Studies in naturally infected sheep indicate that the proteome-determined Mycobacterium avium subsp paratuberculosis –specific antigens MAP 0268c, MAP1365 & MAP3651c were able to discriminate between infected and non-infected animals when incorporated into a gamma interferon release assay (IGRA). In order to determine if these antigens could be used in a broader context, they were tested in an experimental infection of cattle. Data will be presented to show that these antigens can also indicate infected cattle. Thus they may be able to indicate animals with early-stage infections, before the classical methods of detection by serology and faecal culture can diagnose infection.

In order to determine how these antigens performed in a natural setting, a beef cow herd in southern Scotland with a previous history of Johne's disease was selected. A longitudinal study comparing the classical methods of diagnosis of Mycobacterium avium subsp. Paratuberculosis (serological detection and faecal culture) with IGRA 's (using PPDA and the novel MAP antigens as stimulants) was undertaken. Beginning in March 2012, a cohort of 50 animals was tested at six monthly intervals. Data collected to-date for individual animals from the early stages of this longitudinal study are collated. The correlation of high IGRA scores with subsequent detection of Johne's disease by the classical diagnostic methods will be presented and the implications of these findings discussed.

Keywords:
MAP-Specific proteins, Interferon gamma release assay, longitudinal

Abstract P-02.14
A POTENTIAL ROLE FOR BULK MILK OR HERD MILK POOL ELISA TESTING IN A JOHNE'S DISEASE CONTROL PROGRAM

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Abstract text:
A voluntary Johne's Disease (JD) control program for dairy cattle launched in Ontario, Canada in 2010 (www.johnes.ca) and included an on-farm risk assessment(RA), funded milk/serum based test of cows in the herd and terminal removal of high test positive cows. Based on the RA and testing, herds were classified as high or low risk for JD. Periodic testing of herds to detect changes in JD risk is encouraged, however there is no consensus on frequency or type of testing. Options include fecal, serum or milk tests applied to individual or group (pooled) samples. In Ontario it is possible to access a bulk milk sample (quality/payment sample) from every farm every two days. Periodic testing of these samples is an attractive option for inexpensive herd screening. The objective was to evaluate the performance of a bulk milk ELISA to identify herds with milk test positive cows and fecal shedding cows.

Data from 575 herds that had tested all lactating cows with milk ELISA were available for analysis. At the same time as cow testing, bulk milk testing was done with the IDEXX MAP Antibody ELISA, using a modified protocol. Herds were classified as test positive (TP) if they had one or more positive (SP value >0.1) cows, and as high TP (HTP) if they had at least one high test positive (SP value >1.0, high risk fecal shedder) cow. A likelihood ratio (LR) approach over a range of cut-points was used for identifying positive herds. In a subset of herds, the accuracy of the ELISA test applied to a bulk tank sample was compared to testing a herd pool sample created by combining an equal volume of milk from all milking cows.

Herd pool testing had a higher specificity than bulk tank testing, resulting in significantly higher positive predictive values. Based on the LR's, a herd with a bulk tank test >0.14 was 2.6 times as likely to have at least one test positive cow; >0.20 was 7 times as likely to have at least one test positive cow; and >0.30 was 12 times as likely to have at least one fecal shedder.

Keywords:
Bulk milk, Pooled milk, ELISA

Abstract P-02.15

DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS VIABILITY IN FERMENTED MILK PRODUCTS USING PROPIDIUM MONOAZIDE

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Abstract text:
Mycobacterium avium subsp. paratuberculosis (MAP) is able to survive extreme conditions like low pH (e.g. in fermented products) and high or low temperature (during pasteurization or storage). Propidium monoazide (PMA) is a dye which is able to penetrate into dead cells, link with the DNA and thus allows to distinguish between live and dead cells.

We attempted to discriminate viable MAP from artificially spiked fermented milk products using peptide-mediated magnetic separation (PMS) following PMA treatment, a method successfully used for milk samples. However, different product weights, pH, liquid and solid MAP cultures revealed failure in the ability to clearly distinguish between live and dead cells when using PMS and PMA. A possible explanation could be the interference caused by the high concentration of lactic acid bacteria present in fermented milk products with the magnetic beads. This assumption was confirmed when the isolation of MAP using silica-based DNA purification kit was carried out. The viability of MAP during the fermentation of milk products was then successfully determined using PMA.

This work was supported by the project of the Ministry of the Agriculture of the Czech Republic no. QI9101A094.

Keywords:
fermented milk products, propidium monoazide, pH

Abstract P-02.16

DEVELOPMENT OF A METHOD FOR MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS DETECTION IN FAECES USING PEPTIDE-MEDIATED MAGNETIC SEPARATION AND PROPIDIUM MONOAZIDE

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Abstract text:
The aim of our study is to develop a detection method for Mycobacterium avium subsp. paratuberculosis (MAP) as a faster alternative to culture method. Peptide-mediated magnetic separation (PMS) selectively captures MAP cells while effectively removes the contaminating microorganisms present in the sample. Propidium monoazide (PMA) is a dye which can enter into dead cells, link with their DNA and thus allows to distinguish amongst live and dead cells. To implement this detection method into our laboratory, series of optimization experiments were performed. Differences between various kinds of buffers, load of magnetic beads, spiked faeces and naturally MAP-positive faeces samples and other parameters were tested. This detection method allows to determine MAP cells viability in faeces within couple of hours compare to time consuming 'gold standard' culture method.

This work was supported by the project of the Ministry of the Agriculture of the Czech Republic no. QI9101A094.

Keywords:
faeces, magnetic separation, propidium monoazide

Abstract P-02.17

IDENTIFICATION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP) VIA VOC ANALYSIS OVER IN VITRO CULTURES USING DIFFERENTIAL ION MOBILITY SPECTROMETRY (DMS)

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Abstract text:
Differential ion mobility spectrometry (DMS) is an analytical technique to detect volatile organic compounds (VOCs) in gaseous samples at very low concentration ranges from ppb to ppt. The aim of this study was to investigate whether VOC analysis using DMS is capable of identifying Mycobacterium avium subspecies paratuberculosis (MAP) in culture. Headspaces of in vitro cultures of two different MAP strains were analyzed with DMS at 1, 2, 3, 4 and 6 weeks after inoculation (each at two dilutions). Additionally, controls were characterized in parallel (consisting of (i) blank medium, (ii) medium inoculated with heat-inactivated MAP, and (iii) sterile-filtered colony material of MAP). Data analysis included peak detection, cluster analysis, identification of discriminating VOC features (Mann-Whitney U test) and different cross-validated discriminant analyses. VOC analysis involved 127 clusters, and revealed highly significant differences between MAP-samples (both strains) and controls at all points in time. A cross-validated discriminant analysis (using only 20 clusters) of 1 week old MAP-samples and controls showed a correct classification rate of 100%; thereby demonstrating that VOC analysis using DMS is able to identify in vitro MAP-samples after 1 week of growth. In addition, results also showed highly significant differences in VOC-patterns between the two different MAP-strains. This study provides strong evidence that DMS analysis of headspace of bacterial cultures has the potential to become a valuable method to identify positive samples much earlier than with current standard methods.

Keywords:
volatile organic compounds (VOC), differential ion mobility spectrometry (DMS), MAP culture

Abstract P-02.18
VALIDATION OF THE TRIPLEX QPCR SYSTEM FOR THE DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS

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Abstract text:
The aim of this study was to evaluate the prototype of the triplex qPCR for the simultaneous detection of IS900 and F57 elements in the genome of Mycobacterium avium subsp. paratuberculosis (MAP) in different materials. This approach should eliminate specificity issue concerning the existence of IS900-like sequence in non-MAP bacteria. The prototype qPCR system is supplemented with the internal amplification control in order to identify false positive results.

Altogether 150 culture positive and 150 negative faecal samples were examined by the triplex qPCR assay supplemented with magnetic beads based automated DNA isolation. Faecal samples were culture on HEYM medium supplemented with Mycobactin J and antibiotics with preceding decontamination in 0.75% hexadecylpyridinium chloride. Interpretation of the triplex qPCR examination was performed according to the manufacturer's recommendations.

The diagnostic specificity was determined to be 96%, however diagnostic specificity was found to be only 31.67%. These findings are in concordance with previous reports and low diagnostic specificity is caused by the low sensitivity of culture and therefore high number of "false positive" samples. This situation was reflected also by reduced positive predictive value, accuracy and positive likelihood ratio.

The presented data together with the previous findings should lead to the re-evaluation of culture as the gold standard for the determination of MAP. Comparison of two methods with high difference in sensitivity should be omitted. On the other hand, it is essential to determine and obey rules for the interpretation of the qPCR data as they cannot be accepted ultimately.

This work was supported by the LifeTechnologies Inc. and the project of the Ministry of the Agriculture of the Czech Republic no. QI9101A094.

Keywords:
Triplex qPCR, Validation, Specificity of culture

Abstract P-02.19

COMPARISON OF THE THREE ELISA KITS FOR THE DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS

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Abstract text:
The aim of this study was to compare three most common ELISA kits available on the market in their ability to detect antibodies against Mycobacterium avium subsp. paratuberculosis (MAP) in serum. Together 431 serum samples from 7 farms with known history of paratuberculosis were analysed. Animals, from which the sera samples were originating, were examined for the presence of MAP by faecal culture and/or qPCR according to the accredited protocols. In culled animals the intestinal tissue was examined for the presence of MAP by culture. The animal was considered to be positive if at least one test result confirmed presence of MAP. Using this criterion, the panel was composed from 196 positives and 235 negatives serum.

The ELISA examinations were performed according to the manufacturer's recommendation including the criteria for the interpretation of data.

The best diagnostic specificity, sensitivity, positive and negative predictive value and positive and negative likelihood ratio was reached by the LSIVet™ Ruminant Paratuberculosis Advanced - Serum ELISA Kit, the second best results was provided by IDEXX Paratuberculosis Screening Ab Test and the third position belonged to ID Screen Paratuberculosis Indirect ELISA kit.

This work was supported by LifeTechnologies Inc. and the project of the Ministry of the Agriculture of the Czech Republic no. QI9101A094.

Keywords:
ELISA, Comparison, qPCR

Abstract P-02.20

DETECTION OF MAP IN CAPTIVE WILD UNGULATES OF THE METROPOLITAN REGION, CHILE

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INTRODUCTION

Paratuberculosis is a chronic enteric disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (*MAP*) that affects primarily domestic and wild ruminants, but also some non-ruminant species and primates (Beard *et al* 2001, Motiwala *et al* 2004). The disease is worldwide distributed in free-ranging wildlife and captive animals, mainly causing detriment of body condition, diarrhea and death (Manning 2011). In Chile the disease was first reported in cattle more than fifty years ago (Grinbergs and Caorsi 1958), and thereafter it has been reported in sheep (Zamora *et al* 1975), goats (Kruze *et al* 2006), and deer (Paredes *et al* 2007). In addition, *MAP* has also been isolated from wildlife species without clinical signs of disease such as pudu (Salgado *et al* 2009[a]), guanacos (Salgado *et al* 2009[b]), hares (Salgado *et al* 2011), and alpacas (Salgado *et al* 2012). However no records of infection in captive wild animals have been reported so far. The aim of this study was to determine the status of *MAP* infection in a population of captive wild ungulates in the Metropolitan Region of Chile using individual fecal samples obtained directly from the rectum of each animal, and from environmental fecal samples collected from their enclosures areas.

MATERIALS AND METHODS

Fecal samples. Individual fecal samples were collected from all ungulate animals older than 18 mo. which were present at the day of sampling in the zoological park Buin Zoo of the Metropolitan Region of Chile. A total of 57 fecal samples from 12 different ungulates species were collected: 11 Thompson's gazelle (*Gacella thomsoni*), 10 fallow deer (*Dama dama*), 9 mouflons (*Ovis orientalis musimon*), 6 alpacas (*Lama pacos*), 6 pudu (*Pudu puda*), 4 llamas (*Lama glama*), 3 red deer (*Cervus elaphus*), 3 guanacos (*Lama guanicoe*), 2 camels (*Camelus bactrianus*), 1 wild boar (*Sus scrofa scrofa*), 1 nyala antelope (*Tragelaphus angasii*), and 1 sitatunga antelope (*Tragelaphus spekii*). Fecal samples were obtained via rectum with individual sterile disposable gloves, transferred into sterile 60 mL plastic bottles, and stored at -80°C until processed.

Environmental samples. A total of 41 environmental fecal samples were collected from two zoological parks of the Metropolitan Region, 28 from de zoological park Buin Zoo and 13 from the zoological park La Dehesa. Environmental samples were collected from the exhibition areas of different animal species following the procedures recommended by the USDA (2006) for environmental sampling in dairy herds. From each exhibition area two composite environmental fecal samples were collected from areas where animals congregate or from manure effluents. Each composite environmental fecal sample was composed of approximately 20 g of fecal material or soil from four different sites within each sampling location. Samples were collected using individual disposable latex gloves, placed into a 95 mL plastic bottle and stored at -80°C until processed.

Culture of fecal and environmental fecal samples: For *MAP* isolation from individual fecal samples, the conventional method on solid medium (Herrold Egg Yolk Medium with mycobactin J) was used, and the tubes incubated at 37°C for 4 months to promote the development of typical *MAP* colonies. For *MAP* isolation from environmental fecal samples 2 g of each subsample

collected from each environmental site were pooled into a 50 mL centrifuge tube, vortexed and treated as a single fecal sample. Prior to culture each sample was decontaminated with hexadecylpirydinium chloride (HPC) and an antibiotic brew (amphotericin B, vancomycin and nalidixic acid) following the procedure described by Soto *et al* (2002). Colonies resembling *MAP* and showing mycobactin dependence were considered presumptively positive for *MAP* and further tested by conventional PCR to detect the *MAP*-specific insertion element IS*900*. Simultaneously, all samples were cultured in a liquid medium (Middlebrook 7H9-OADC) and incubated at 37°C for 49 days in the BACTEC MGIT 960 automated system. All tubes signaled positive by the BACTEC system were confirmed by real-time PCR (IS*900*).

RESULTS

From the 57 fecal samples collected from different species of ungulates of the zoological park Buin Zoo, 13 (22.8%) were positive for *MAP* in liquid medium in the BACTEC system but negative on solid medium. The infection was detected in 6 different animal species: fallow deer, red deer, pudu, llamas, sitatunga antelope, and Thompson's gazelle (Table 1).

Table 1 *MAP* isolation from fecal samples of different captive wild ungulates of the zoological park Buin Zoo, Chile

		MAP positive samples		
Species	N° Sampled	HEYM*	BACTEC	qRT-PCR
Thompson's gazelle	11	0	2 (18.2%)	2
Falow deer	10	0	5 (50.0%)	5
Mouflon	9	0	0	0
Alpaca	6	0	0	0
Pudu	6	0	2 (33.3%)	2
Llama	4	0	1 (25.0%)	1
Red deer	3	0	2 (66.7%)	2
Guanaco	3	0	0	0
Camel	2	0	0	0
Nyala antelope	1	0	0	0
Sitatunga antelope	1	0	1 (100%)	1
Wild boar	1	0	0	0
Total	57	0	13 (22.8%)	13

*Herrold Egg Yolk Medium

From the 41 environmental samples collected only 5 (12.2%) were positive for *MAP*, 1 from the zoological park Buin Zoo and 4 from the zoological park La Dehesa. The positive sample of the Buin Zoo was obtained from the exhibition area of camels. The positive samples of the La Dehesa were obtained from the exhibition areas of lesser kudu (*Tragelaphus imberbis*), bongo antelope (*Tragelaphus euryceus*), nyala antelope (*Tragelaphus angasii*), and impala antelope (*Aepyceros melampus*).

CONCLUSION

According to these results, it is possible to conclude that both zoological parks examined are heavily infected with *MAP* either the animals or the environment of the exhibition halls. This study provides the first evidence in Chile of *MAP* infection in captive wild animals, which was suspected due to the high prevalence of this bacterium in the country. In addition, this is the first report of *MAP* infection in antelopes and llamas in Chile.

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Research supported by DID/UACH Project N°I-2011-06

Keywords:
MAP, Wild ungulates, Captive animals

Abstract P-02.21
PCR PROTOCOL OPTIMIZATION FOR THE DETECTION OF MYCOBACTERIUM AVIUM SUBSP PARATUBERCULOSIS

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Abstract text:
Introduction. Paratuberculosis (Johne's disease) is a disease of ruminants characterized by chronic enteritis, the etiological agent of which is Mycobacterium avium subsp paratuberculosis (MAP). A commonly used ante-mortem diagnostic test for the detection of MAP in faeces is liquid culture; however a major constraint is the 2-3 months incubation period. Recently, for the direct determination of the paratuberculosis pathogen, polymerase chain reaction (PCR) has been actively used.

The aim of the work was the optimization of a new PCR protocol for the detection of MAP.

Materials and methods: In this work primers selected to nucleotide sequence ISMav2: M_ISMav2-F - gacggtcgcgcaaatggaagc and M_ISMav2_r1 - cctcgaccggtggatgacaaaac were used. Optimization was performed in a temperature gradient of the magnesium concentration from 1.5 to 3,5 mM using DNA reference strains (MAP DSM No. 44133, Type strain, ATCC 19698, TMC 807; MAP DSM No. 44156, Type strain ATCC 25291, TMC 724).

Results: As a result, the optimal composition of the reaction mixture included primers 0,35 µM of each, 1 unit Taq DNA Polymerase (Fermentas); 0,2 mM each dNTP, 1-fold PCR buffer (Fermentas, 10 mM Tris-HCl (pH 8,8 at 25°C), 50 mM KCl, 0,08% (vol/vol) Nonidet P40), betaine 0,8 M, MgCl2 1,5 mM. Optimal temperature for primer annealing 62°C.

The limit of detection protocol composed 150 fg or about 30 genomic copies of MAP. In assessing the specificity of the developed PCR protocol for collection of DNA samples isolated from the main pathogens of farm livestock: specific amplification of mycobacterial strains including genetically close subspecies M. avium subsp avium (20 strains), Brucella, Listeria, Pasteurella, Salmonella, Campylobacter, Bacillus was detected only in MAP samples.

Discussion: Developed protocol has a high specificity and sensitivity of 30 genome equivalents (150 fg) and promising for further testing on clinical samples and introduction into clinical veterinary practice.

Keywords:
PCR, protocol, primers

Abstract P-02.22

CREATION OF POSITIVE CONTROL SAMPLE OF M. AVIUM SUBSP. PARATUBERCULOSIS

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Abstract text:

Introduction. Despite that PCR is used for paratuberculosis diagnosis in the Republic of Kazakhstan, PCR diagnosis is represented by foreign test-systems in Kazakhstani market, which are too expensive. For this reason the problem of creation of domestic analogues is important.

The aim of the work was the creation of positive control sample for its use in composition of PCR test-system.

Materials and methods: DNA of M. avium subsp. paratuberculosis (ATTC Catalog № BAA-968D-5) was used to amplify the target fragment. M_IS900, M_ISMav2, M_F57, M_hspX (Sigma) primers were used in this work. Extraction of specific PCR product from agarose gel was carried out using GeneJET Gel Extraction Kit (Thermo Scientific). Cloning of extracted fragments into the plasmid pGEM-T was done using Promega pGEM-T Easy cloning kit (Promega). pGEM-T and pGEM-T Easy Vector Systems (Promega) were used to carry out cloning. Selection of colonies that have target fragment of M. avium subsp. Paratuberculosis was performed by white-blue selection method. CEQ WellRED (CEQ Dye Terminator Cycle Sequencing kit) was used to carry out sequencing with subsequent separation of fragments on automatic genetic analyzer CEQ8000 (Beckman Coulter).

Results: Specific PCR product was obtained in the result of amplification of target fragment of M. avium subsp. Paratuberculosis, which was cloned into plasmid pGEM-T. Obtained ligase mixes were used for subsequent chemical transformation of competent cells of E. coli DH5α. 12 white colonies were selected for 3 primers (M_IS900, M_ISMav2, M_F57) by white-blue selection method. Only 3 plasmids obtained with the use of primers M_IS900 (2) and M_F57 (1) confirmed specificity by PCR.

Discussion: The result of nucleotide sequence analysis of the plasmid showed that 3 clones do not have nucleotide substitutions and cannot be used as the positive control sample in composition of PCR test-system.

Keywords:
PCR, positive control , plasmids

Abstract P-02.23

PATTERNS IN MILK ELISA RESULTS FOR DETECTING ANTIBODIES TO MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN JOHNE'S INFECTED DAIRY COWS

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Abstract text:

Milk ELISA for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection have benefits of low cost, quick processing, and ease of collection for large sample numbers; but have low sensitivity and specificity less than 100%, especially for cows in early-stage disease or shedding. Furthering the industry's knowledge of factors affecting the interpretation of results could enhance Johne's herd control programs and diagnostic protocols. This study compared milk ELISA sensitivity with fecal MAP detection and assessed how milk antibody detection varies across fecal MAP-shedding concentration, host age or parity, days in milk, and season. Forty-six MAP-infectious cows and 52 MAP test-negative control cows were selected from 7 Atlantic Canadian dairy farms for monthly milk and fecal sample collection over a 12 month period. Results from a commercial milk ELISA (Parachek®, Prionics) were compared to solid (Herrold's, Fisher Scientific) and broth (*Para*-JEM®, Thermo Scientific) fecal cultures and direct fecal real-time PCR (qPCR; VetAlert™, Tetracore®). Sensitivity of milk ELISA was 29.9% (95% CI: 24.8-35.1%; n=304), compared to 46.7% (40.7-52.7%; n=270), 55.0% (49.3-60.7%; n=298), and 78.4% (73.7-83.1%; n=297) with fecal solid culture, broth culture, and qPCR respectively. Milk ELISA sensitivity improved with increasing fecal shedding levels, as well as with increasing age and parity of the cows. Furthermore, milk ELISA sample-to-positive scores improved towards late lactation and in winter months as compared to summer. Knowledge of this information can facilitate decision-making of how to incorporate and interpret results of milk ELISAs for Johne's disease management programs in infected herds.

Keywords:

milk, ELISA, season

Abstract P-02.24

SEMI-NESTED PROBE-BASED REAL-TIME PCR FOR THE DETECTION OF MYCOBACTERIUM AVIUM SUBSP PARATUBERCULOSIS IN GOATS

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Abstract text:

Mycobacterium avium subsp. paratuberculosis (MAP) is the causative agent of paratuberculosis or Johne's disease that is manifested by progressive and fatal weight loss, significant decrease of milk production, infertility, oedema and diarrhoea. In Portugal paratuberculosis probably runs under-diagnosed with the evaluation of its prevalence in small ruminants limited to a few geographically-restricted studies. The aim of this work was to assess the presence of MAP in a dairy herd of 186 Saanen goats that presented clinical signs of cachexia and diarrhoea, from Azores (Portugal), by culture and an in-house direct semi-nested TaqMan probe-based real-time PCR (RTD-PCR). Sixteen faecal and five milk samples from seropositive animals and one pool of faecal and one pool of milk samples from seronegative animals were collected and analysed by RTD-PCR. The faecal samples were also cultured in HEYM with and without mycobactin and LJ with mycobactin at 37 °C for 6 months. Confirmation and identification of isolates was done by Auramine-Rhodamine staining and by IS900 and F57 real-time PCR. The total DNA of the samples was extracted using a commercial kit (Invisorb® Spin Tissue Mini Kit, Stratec Molecular) and an RTD-PCR was performed targeting a specific region of IS900. Fourteen faecal samples including the pool of feces were positive by RTD-PCR and culture. Two faecal samples were RTD-PCR negative and culture positive and 1 faecal sample was RTD-PCR positive and culture negative. From the six milk samples two, including the pool of milk were RTD-PCR positive. The RTD-PCR offers great advantages compared conventional PCR and culture, since it allows the detection of few organisms and avoids the extremely long incubation time. This is the first study reporting the identification and isolation of MAP from Azores samples, suggesting that the infection may be more widespread in Portugal than initially expected.

Keywords:

direct real-time PCR, faeces, detection

Abstract P-02.25

DEVELOPMENT OF PLASMID DNA MATERIAL FOR THE QUANTITATION OF MYCOBACTERIUM AVIUM SUBSP PARATUBERCULOSIS BY REAL TIME-PCR

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Abstract text:
Determining the exact number of Map cells in a sample by culture is complicated due to the long period required for Map growth as well as its tendency to clump. So many labs have begun using molecular techniques such as real time PCR as a quantitative method using plasmid as predetermined standards. The purpose of the study was to produce 3 plasmids: 2 plasmids with one insert, named pIS900 (IS900) and pF57 (F57), and a plasmid with 2 inserts, named pIS900-F57 (IS900 and F57). The two single plasmids were obtained by using the QIAGEN PCR Cloningplus Kit (QIAGEN SA, France) according to the manufacturer's recommendations. Plasmid DNA was extracted from white colonies cells by using the QIAprep Spin Miniprep Kit (QIAGEN SA, France).

The double plasmid was prepared by inserting the F57 sequence derived from the plasmid pF57 on pIS900 plasmid. Three protocols have been tested for ligation: the best result was obtained with 2 restriction enzymes (ApaI and HindIII). As all colonies were white it was not possible to distinguish single plasmids and double plasmids after transformation of competent cells. To multiply plasmids, white colonies were selected at random and cultivated straightaway or after a PCR in order to verify the presence of a plasmid with 2 inserts. Then plasmids were extracted and purified. The size of the plasmid and the presence of the 2 inserts were verified by electrophoresis after digestion by enzymes PstI and ClaI. Finally, the plasmid was sequenced.

The plasmid pIS900-F57 has been recognized by the three commercial tested RT-PCR kits. After quantitation by spectrophotometric measurement of the adsorption at 260 nm, plasmids DNA were ten-fold diluted to determine the analytical sensitivity (1 to 10 copies, according to the kits). The reliability and the stability of plasmids were tested.

This plasmid can be use in RT-PCR reactions to determine the amount of Map in the samples.

Keywords:
recombinant plasmid, PCR, quantitation

Abstract P-02.26

DIAGNOSIS OF PARATUBERCULOSIS BY MILK AND SERUM ELISA ON DAIRY CATTLE IN RIVER NILE STATE, SUDAN

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Abstract text:

The present study was constructed to compare between milk and serum ELISAs for the detection of specific antibodies against Mycobacterium avium subspecies paratuberculosis. Twenty milk and serum samples were collected concurrently from infected Butana local breed dairy herd located in River Nile state, Sudan. Both milk and serum samples were investigated for the specific antibodies against Mycobacterium avium subspecies paratuberculosis using pourquier ELISA kit and were assayed according to manufacturer's instructions (Institut pourquier, France). Kappa analysis was used to determine the level of agreement between milk and serum ELISA in antibody carriage at individual cow level. Agreement between milk and serum ELISA was high on the basis of weighted kappa value of 0.77. The relative sensitivity and specificity of milk ELISA, using serum ELISA as the reference, were 66.7 and 95% respectively. It is valuable to mentioning that, in view of the small number of animals studied, the relative sensitivity and specificity of milk ELISA using Serum ELISA as reference must be evaluated carefully. Further large scale study to determine the accuracy of milk ELISA for the diagnosis of paratuberculosis on dairy cattle at herd and cow level might elucidate those findings.

Keywords:

paratuberculosis, milk, serum

Abstract P-02.27
USE OF NESTED-POLYMERASE CHAIN REACTION IN FECAL CULTURE FOR SCREENING OF JOHNE'S DISEASE

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Abstract text:
Culture of bacilli from clinical samples is the confirmatory test for diagnosis of Johne's disease (JD). But, it require long incubation period which restricts its routine use. More than eight months of incubation is required to declare a sample to be negative. Use of molecular test such as PCR may shorten or overcome the long incubation. Three hundred fecal samples from cattle were screened by Ziehl-Neelsen (ZN) staining and Nested-PCR before and after culture on solid media. Fecal culture was performed on samples decontaminated with 0.75% Hexadecylpyridium chloride. Two species specific sequences viz; IS900 and f57 of Mycobacterium avium subspecies paratuberculosis (Map) were targeted in nested manner (Nested-PCR) separately as complementary to confirm the bacilli. A total 24 (8.00%) and 46 (15.33%) fecal samples were positive in Ziehl-Neelsen (ZN) staining and nested-PCR, respectively. However, after culture around four months on Herrold's Egg Yolk Medium with mycobactin, 75 (25.00%) samples were identified as acid-fast positive and 101 (33.67%) samples had Map bacilli (Nested-PCR). Culture on solid media along with Nested-PCR had increased the percentage detectable number of cases by 18.33%. In comparison to ZN staining, nested-PCR of cultured samples diagnosed and confirmed more number (26; 8.67%) of Map positive samples. Observation of visible colonies and its confirmation by ZN staining had missed a considerable number of samples. Further, it will reduce the time to monitor the inoculated samples for visible growth. In addition, shedding of undetectable level of Map bacilli had overcome by culture. Simultaneous use of specific primers in combination will verify and characterized a new isolates if any. Therefore from present study it was concluded that, culture on solid media and screening of growth targeted to specific sequences in nested approach had increased the sensitivity and specificity of diagnosis of JD.

Keywords:
Herrold's Egg Yolk Medium, Nested-PCR, Ziehl-Neelsen staining

Abstract P-02.28
IMUNOHISTOPATOLOGIC DIAGNOSIS OF SUBCLINIC BOVINE PARATU-BERCULOSIS IN RIO DE JANEIRO, BRAZIL

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Abstract text:
The early and specific diagnosis of paratuberculosis (PTB) remains a challenge due to the low sensitivity of the currently available laboratory tests and also because of variations in the immune response towards infection with Mycobacterium avium subsp. paratuberculosis. Cases of clinical PTB have been reported from numerous regions of Brazil and from a variety of ruminant species; yet, at present, there exists no official Brazilian program for control of this disease. The aim of this study was to characterize the anatomo-histopathological and immunohistochemical (IHC) findings in the bowel and mesenteric lymph nodes of assymptomatic cattle, derived from PTB positive herds located in state of Rio de Janeiro, Brazil. Macroscopic examination revealed nonspecific changes including reddening of the gut mucosa, increased volumes for the Peyer's patches and mesenteric lymph nodes and dilation and whitening of the mesenteric lymphatic vessel. Histopathology revealed granulomatous infiltration with the formation of giant cells in the jejunal and ileal mucosa or sub-mucosa, and/or in the cortical region of the mesenteric lymph nodes in 32 of the 52 cattle examined. Tissue sections from these animals were subjected to Ziehl-Neelsen staining, but the presence of acid-fast bacilli was not observed. Subsequent analysis, employing genus specific IHC for Mycobacterium, revealed immunoreactivity in sections prepared from a total of six animals. The results of this study indicate that IHC has potential become a valuable tool for the detection of subclinical PTB and may enhance conventional methods for histopathological confirmation of PTB. Furthermore, this methodology may be advantageous in situations where no material is available for bacteriological analysis and it provides a basis for retrospective diagnosis of archive material.

Keywords:
bovine, Immunohistochemistry, subclinical paratuberculosis

Abstract P-02.29

INTERFERENCE BETWEEN DIAGNOSTIC TEST FOR MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS AND MYCOBACTERIUM BOVIS IN IRELAND.

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Abstract text:
The purpose of this study was to determine whether infection with Mycobacterium avium subspecies paratuberculosis (MAP) interfered with the tuberculin test for Mycobacterium bovis and if infection with Mycobacterium bovis interfered with diagnostic test for MAP.

Good et al. (2009) carried out a simple random survey in Ireland during 2005 to estimate the ELISA-prevalence of paratuberculosis in the cattle population.

Three groups of animals were included in this analysis.

a) 200 animals positive on the 2005 seroprevalence survey
b) 40 positive on a faecal test submitted on clinical animals to the Regional Veterinary Laboratories in 2005.
c) Two control animals were matched with each positive animal. These animals were picked from herds that had been tested negative on the prevalence survey and were matched on enterprise type, herd size and age of animal.

72.5 % of group a, 59% of group b and 67% for group c were in herds restricted for tuberculosis in the period 2004 – 2012.

In the SICTT positive herds TB lesions were present in 63% of group a herds, 46% of group b and 59% of group c.

5 % of animals subsequently became SICTT reactors and a further 21% would have been positive on the single intradermal test in group a. The figures for group b were 2.5% and 20% and for group c 2.75% and 17%.

The figures for animals showing an increase on the avian tuberculin reading in TB tests in the three groups was 54%, 35% and 48%.

The routine SICTT performed at least once annually on every bovine is removing some animals that would if ELISA tested for MAP be ELISA positive. It is unclear if this indicates these animals are TB infected, MAP infected or showing a non-specific response to both tests.

Keywords:
mycobacterium avium subspecies paratuberculosis, mycobacterium bovis, interference with diagnostic tests

Abstract P-02.30

LONG TERM SURVIVAL OF ANIMALS POSITIVE TO MAP DIAGNOSTIC TESTS IN IRELAND.

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Abstract text:
To determine if whether being positive on an ELISA for Mycobacterium avium subspecies paratuberculosis decreased survival time on animals that participated in a serum prevalence survey the results of which were not reported to the owners.

Good et al. (2009) carried out a simple random survey in Ireland during 2005 to estimate the ELISA-prevalence of paratuberculosis in the cattle population.

Three groups of animals were included in this analysis.

a) Two hundred animals positive on the 2005 seroprevalence survey
b) Forty animals positive on a faecal test submitted on clinical animals to the Regional Veterinary Laboratories in 2005.
c) Two control animals were matched with each positive animal. These animals were picked from herds that had been tested negative on the prevalence survey and were matched on enterprise type, herd size and age of animal.

The average survival for animals in groups a), and b) were 1054 and 1364 days. Of the 40 animals positive on the faecal sample, the great majority were dead within 250 days of the sample being taken whereas 5.7% of the control animals were still alive five years later.

When the positive animals were broken in to four quartiles based on survival after positive test, the average S/P for the two quartiles with the longest survival was lower than for the two quartiles with the shortest survival.

When positive animals were broken into quartiles based on their age in animals under three years of age, the two groups with the highest S/P values had an average survival time twice that of those in the two lowest groups and that of the control animals. In animals from three to nine years survival was longer in the control animals than those with a positive result on the ELISA test, being more evident in the groups with the highest average S/P. In the quartile over nine years of age, the positive animals survived longer than the control animals.

It is concluded that there may be a higher number of false positives in the youngest and oldest age groups.

Keywords:
herd ELISA-prevalence, Mycobacterium avium subspecies paratuberculosis, Survival

Abstract P-02.31
EVALUATION OF SEROLOGICAL DIAGNOSIS BASED ON THE MAP-ECHA ANTIGEN FOR BOVINE JOHNE'S DISEASE

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Abstract text:
The objective of this study is to evaluate the Map-echA ELISA, which detects antibodies against recombinant enoyl-CoA hydratase (echA) protein of Mycobacterium avium subsp. paratuberculosis (Map), with commercially available ELISA kits in Japan. A total of 744 serum samples from Map experimental infection, 20 infected herds, 6 herds certified as free from Johne's disease (JD) and 12 herds with high proportions of false-positive serologic reactions were assessed by the Map-echA ELISA and 3 commercially available ELISA kits. Serum samples were divided into two groups of 274 positive and 470 negative sera based on a bacterial culture and fecal PCR as the reference standard tests. Receiver operating characteristic (ROC) curves analysis of the results yielded the area under the curves (AUC) value of 0.903 for the Map-echA ELISA, which was significantly higher than those of commercial ELISA kits ($P < 0.01$). At the cut-off point recommended by the ROC curve analysis, the sensitivity and specificity of the Map-echA ELISA were calculated to be 78.5% and 89.4%, respectively. The specificity of all ELISA demonstrated using 315 negative sera from 6 herds certified free of JD was nearly 100%. Additionally, the serological reactivity of cattle was evaluated with different shedding levels of Map, and antibody responses to the Map-echA antigen were higher than those of other ELISAs, especially in low and moderate shedder groups. Thus, the Map-echA ELISA showed the superior performance in comparison to the commercial ELISA kits, particularly in discriminating the ELISA false positive sera and detecting low shedders.

Keywords:
antigen, serological diagnosis, ROC

Abstract P-02.32
RETROSPECTIVE ANALYSIS OF PARACHEK® ELISA ON MILK SAMPLES IN MINNESOTA DAIRY HERDS

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Abstract text:
Purpose: Routine milk testing is a convenient and effective measure to control Johne's disease in dairy herds. The aim of this retrospective analysis was to assess the results of periodic testing with regard to Johne's disease herd prevalence in Minnesota dairy herds for a 7 year period from 2007 – 2013.

Methods: Milk samples were collected on the farm by DHIA field representatives according to the Uniform Data Collection Procedures developed and adopted under the direction of the National DHIA. On average, 2000 samples from around 100 herds per month were tested for Johne's disease at the Zumbrato DHIA Laboratory using the PARACHEK® ELISA (Prionics) following the manufacturers instructions for use. Average herd size is around 124 cows.

Results: Average herd prevalence for the years 2007 – 2013 was 4.01 % with a peak prevalence of 5.17% in 2008 and lowest prevalence of 3.19 % in 2013. Overall, a trend to reduced prevalence is seen over the seven year reporting period.

Conclusion: This retrospective study shows that the PARACHEK® Milk and Serum ELISA is a cost-effective and labor efficient tool to screen and detect Johne's disease in dairy operations. Screening results can be used by producers and their veterinarians to develop additional testing and management strategies for the control of Johne's disease.

Keywords:
Milk testing to control Johne's disease in dairy herds, Prevalence in Minnesota dairy herds from 2007-2013, Parachek milk and serum ELISA

Abstract P-02.33

GENERATION OF NOVEL PEPTIDE BINDERS AGAINST MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS BY PHAGE DISPLAY BIOPANNING

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Abstract text:

The aim of this study was to generate novel peptide binders against *Mycobacterium avium* subsp. *paratuberculosis* (MAP) with potential future application for magnetic separation of Map from bovine milk. Gamma irradiated whole cell antigens (WCA) and ethanol extracted antigens (EEA) of Map strain B4 (a Northern Ireland field isolate) were prepared and used as targets for a process known as phage display bio-panning. A commercially available phage display library (PhD12, New England Biolabs) of M13 bacteriophage, each pentavalently expressing different random 12-mer peptides on the N-terminal, was exposed to both WCA and EEA MAP antigens. Phage which bound to the target Map antigens were eluted off, amplified in *E. coli*, and used as the phage library for the following round of biopanning. With each successive biopanning round, the pool of potential Map-specific peptides should increase, but in order to maximise specificity for Map, 'subtraction' biopanning against irradiated antigens of *Mycobacterium avium* subsp. *avium* NCTC 13034 and *Mycobacterium bovis* AF2122/97 was carried out. After the fourth round of biopanning, phage were randomly selected, individually amplified and the phage DNA sequenced, in order to identify the expressed peptide sequences. In total, eight different 12-mer peptide sequences were identified for each of the biopanning targets (WCA and EEA). Phage binding ELISA was performed on the eight phage clones expressing the different peptides in order to identify which showed the greatest binding for whole cells of Map B4. Four peptide sequences generated using the WCA target demonstrated a low level of binding to Map. In contrast, a high level of binding to Map was observed for all eight peptides identified using the EEA target. Based on a combination of sequencing and phage binding ELISA results, peptides EEA 402, EEA 405 and EEA 421 were selected for further evaluation. The peptides have been chemically synthesised with a biotinylated N-terminus, and will be evaluated, along with a range of monoclonal antibodies, using magnetic separation protocols for their ability to specifically bind whole cells of Map over other *Mycobacterium* spp. and milk microflora. Ultimately, we hope to improve the current magnetic separation protocol for Map from milk which involves the peptides aMp3 and aMptD.

Keywords:

MAP, Magnetic separation, Peptide binders

Abstract P-02.34

SEROLOGICAL ASSAY FOLLOWED UP BY QUANTITATIVE PCR; A SYNERGISTIC COMBO FOR ANTE-MORTEM DIAGNOSIS OF JOHNE'S DISEASE IN FARMED NEW ZEALAND DEER.

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Abstract text:

New Zealand (NZ) is somewhat unique in that it has a population of more than 1.2m farmed red deer (Cervus elaphus), a farming practice not commonly employed internationally. Johne's disease (Jd) in deer manifests as a more acute infection with progression from infection to clinical disease occurring more rapidly than in cattle or sheep and with susceptible animals dying from the disease as early as 8 months of age. In the absence of an organised control programme for Jd in NZ, the full cost of diagnostic testing as well as losses from resultant culling must be borne by the farmer, placing extreme demands both on individuals attempting to mitigate losses and upon the diagnostic services employed to support them. Serological testing using a custom ELISA (Paralisa) developed specifically for red deer has for some years been a mainstay of diagnostic testing for Jd for the NZ deer industry. The specificity of serodiagnosis however may be compromised by common antigens shared by MAP and other environmental mycobacteria that evoke an immune response in non-infected animals whereas its sensitivity, particularly for subclinically infected animals, is temporally influenced by the dynamics of antibody production and the point at which a sample is assayed.

As ELISA retains the attributes of cheaper, higher throughput screening, an attempt was made to assess the sensitivity, specificity and positive/negative predictive values of Paralisa for estimating levels of faecal shedding of MAP as a basis for JD management in deer, with the option of secondary testing of antibody positive individuals via faecal PCR to confirm their shedding status. We describe here the application of sequential testing using quantitative faecal PCR to measure the bacterial load in cervine faecal samples as an adjunct to the existing standalone serodiagnostic test.

Keywords:

Deer, Diagnostics, qPCR

Abstract P-02.35

DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS DNA BY PCR IN RAW MILK OF BUFFALO

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Abstract text:

Paratuberculosis is a chronic granulomatous disease caused by Mycobacterium avium subsp. paratuberculosis (MAP), which affects domestic and wild ruminants. It is a chronic granulomatous disease characterized chronic and profuse diarrhea, refractory to treatment with antibiotics, and is responsible for major economic losses. The objective of this study was to verify the presence of MAP DNA by PCR technique in milk samples of buffaloes from a property of the State of Alagoas. 20 buffalo milk samples were collected and sent to the Bacteriosis Laboratory of the Federal Rural University of Pernambuco. DNA extractions of the samples were performed using commercial kit. After the extraction, the amplification reactions were performed in a final volume of 15 µL containing: 5µL of genomic DNA, 0.5 µL of specific primers for the IS900 at 20pM (DF: 5' - GACGACTCGACCGCTAATTG - 3 ' and DR - 1: 5' – CCGTAACCGTCATTGTCCAG - 3'), 2.75 µL of Milli - Q ultrapure water, and 6.25 µL of MasterMix according to the manufacturer's protocol. Sequencing was performed on one of the positive samples to validate the technique. Of 20 milk samples collected and analyzed, It was verified the presence of MAP DNA in 2 (10%) samples. The sequencing sample showed a 97% homology with the DNA of Mycobacterium avium subsp. paratuberculosis (MAP4, complete genome) deposited on BLAST. It was concluded that MAP DNA is present in buffalo milk on the property studied. More research and health control measures should be implemented, because this agent is responsible for great economic losses.

Keywords:

Molecular Biology, buffaloes, paratuberculosis

Abstract P-02.36

IMPROVING THE SENSITIVITY OF HERD SCREENS USING TARGETED SELECTION OF HIGH RISK COWS

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Abstract text:

Introduction

A national engagement program in UK dairy herds used a targeted 30 cow milk ELISA screen in combination with a structured risk assessment (www.myhealthyherd.com) to estimate herd prevalence of Johne's Disease (JD). A methodology, based on the results of a large retrospective study of herds undergoing quarterly testing for JD, has enhanced the process of selecting animals for screening.

Method

385 herds from the NMR Herdwise quarterly milk testing program were identified. The probability of animals being test positive was analysed with reference to possible risk factors: lactation number, current somatic cell count (SCC), history of high SCC, milk yield compared to herd average. Relative risks were calculated to estimate the strength of association between the test status of individual animals and the possible risk factors. A scoring system was devised to select cows automatically for targeted testing based on significant risk factors.

Summary

Probability of being test positive increased x1.5 for cows in parity 3+; x2 for cows with a SCC >200,000 cells/ml; x1.8 for cows with >2 SCC >200,000 cells/ml; and x2 for cows with milk yields more than 25% below their adjusted herd average.

The scoring system for targeted testing improved the herd-level sensitivity of correctly identifying positive herds. While 84% of positive herds were detected using random cow sampling, this increased to 95% of positive herds in a targeted screen. The effect was more marked in low prevalence herds (<4% positive cows) with targeted screening identifying infection in 83% of herds vs 56% using random selection.

Conclusion

Automatic selection of high risk animals for testing is a practical way of enhancing herd-level sensitivity for JD screening.

Keywords:

Johne's, Disease, Screening

Abstract P-02.37

DEVELOPMENT OF AN IMPROVED DETECTION METHOD OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN FECES OF CATTLE AND ITS APPLICATION TO KOREAN CATTLE

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Abstract text:

Paratuberculosis(PTB) caused by Mycobacterium avium subsp. paratuberculosis(MAP) is one of the most important disease in economically and clinically in cattle industry. Clinical onset of the disease is required long time after infection due to long incubation period. The infection of the disease can occur at early stage of life via oral route by ingestion of contaminated feed, water, milk, etc.. Therefore, prevention of the infection at early stage of calf is the most effective strategy to control the PTB. The most important infection source is the MAP excreted form cow at subclinical stage. Based on the knowledge, we developed an improved detection method of MAP in feces and the method was applied the field in Korea.

An improved detection method based on PCR-targeted IS900 and ISMAP02 was established by some modifications. Detection efficiency was enhanced by compared with previous methods. This method was applied to cattle fecws collected in national wide regardless of diarrhea. Until now, 46 samples of 563 samples were positive in both IS900 real-time PCR and ISMAP02 nested PCR. 7 herds were infected with MAP from total of 20 herds investigated. More investigations on the detection of MAP in feces of cattle are going on. In addition, isolation of MAP is carrying out from MAP-positive feces. Results from this investigation suggest that the method can detect cows which are secreting MAP into feces without any clinical signs. Although isolation and identification of MAP is still remaining as a gold standard, genetic detection of MAP by PCR should be considered as one of the early diagnostic methods. The consideration will help to control MAP infection because removal of fecal shedder is the most important factor in the strategy. This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (PJ008970)” Rural Development Administration, Republic of Korea.

Keywords:

detection, feces, Korea

Abstract P-02.38

DETECTION OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS IN BOVINE MILK SAMPLES FROM POSITIVE HERDS FOR PARATUBERCULOSIS

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Abstract text:

Paratuberculosis is a chronic granulomatous disease caused by Mycobacterium avium subsp. paratuberculosis (MAP) that causes a chronic and profuse diarrhea, refractory to treatment with antibiotics, and is responsible for major economic losses. The objective of this study was to detect the presence of MAP DNA in samples of cow's milk using the Polymerase Chain Reaction (PCR) and qPCR (Real Time) and conduct a study of agreement between the tests used. 121 samples of bovine milk coming from six positive herds for MAP by serological test (ELISA) were collected. These samples were taken to the Bacteriosis Laboratory of the Federal Rural University of Pernambuco. DNA extractions of the samples were performed using commercial kit. Of the 121 milk samples analyzed it was possible to detect MAP DNA in 20 (16.52%) samples using conventional PCR and in 35 (28.92%) using the qPCR. The DNA of the agent was detected in all of the six herds studied, ranging from 10.00% to 23.81% in the conventional PCR, and from 10.00 % to 42.11% in qPCR. The correlation between qPCR and PCR was moderate (Kappa = 0.52, $\chi 2$ = 36.34, $p<0.000$). The sensitivity and specificity of qPCR compared PCR was 82.2% and 85.0%, respectively. It is concluded that MAP was detected and that qPCR is a sensitive and rapid technique for detection of the agent in milk samples. Sanitary control measures should be undertaken, because this agent is responsible for major economic losses to livestock.

Keywords:

Molecular Biology, bovine, paratuberculosis.

Abstract P-02.39
PARATUBERCULOSIS ELISAS: IMPROVEMENTS ON THE HORIZON?

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[1]*IDVET ~ Grabels ~ France*

Abstract text:
ELISA serology has been used for many years, alone or in combination with other techniques, for Paratuberculosis diagnosis. It allows for the efficient identification of high shedders leading to a reduction in disease prevalence, although it is often difficult to obtain total disease eradication. In certified herds, serology aids in disease prevention.

The ELISA method, however, is known to lack in sensitivity, and despite numerous studies, the identification of new antigens (petides, recombinant proteins) to improve test sensitivity has been unsuccessful; the absorbed iELISA remains the method of choice.

Other problems are associated with the use of the ELISA method for Paratbuerculosis including:

1. Absence of an international reference standard to calibrate analytical sensitivity;
2. Discordant results obtained in infected herds between manufacturers, or between batches from the same manufacturer;
3. Specificity problems caused by the temporary or prolonged appearance of atypical reactions in certified disease-free herds;
4. Difficulty in identifying truly disease-free herds to determine test specificity.

IDvet has extensively examined each of these issues.

This study will present case studies of herds to illustrate the aforementioned points, as well as the solutions developed by IDvet to provide kits with a wider spectrum of detection (improved sensitivity), while maintaining high specificity and stable analytical sensitivity.

Keywords:
ELISA, paratuberculosis, evaluation

Abstract P-02.40
VALIDATION STUDY FOR PARATUBERCULOSIS ON GOAT MILK WITH PARACHEK® 2

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Abstract text:
Purpose: The purpose of the study was to find answers to the following two questions: (1) How many goats have to be infected before the antibodies show up in the bulk milk tank and (2) How does the paratuberculosis antibody-level in a bulk milk tank relate to the proportion of goats that is infected.

Methods: Three commercially available tests (PARACHEK® 2 from Prionics AG and two competitor tests) and goat milk from farms that don't vaccinate against paratuberculosis and from farms who do vaccinate were compared. Answers to the two questions mentioned above are important in order to setup a scheme to find the infected animals with the lowest number of tests to run. Samples from all individual goats were collected in order to be able to prepare "bulk milk" by recombining positive and negative samples. From the recombination an OD versus percentage infected animals can be calculated.

Results: The study is ongoing and results will be presented at the meeting in Parma.

Conclusion: The study should give valuable insights with regard to the sensitivity of commercially available Johne's Disease ELISAs for detecting antibodies in tank milk samples and should allow a risk based approach for the monitoring of Johne's Disease in goat milk.

Keywords:
Paratuberculosis on Goat Milk, Antibodies in the bulk milk, Monitoring of Johne's Disease in goat milk

Abstract P-02.41

COMPARATIVE ANALYSIS OF MILK AND SERUM ELISAS FOR JOHNE'S DISEASE IN IRISH CATTLE HERDS

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Abstract text:

Purpose: Herd screening is one of the main objectives of the Dairy Herd Pilot Johne's Disease Control Programme for Ireland which commenced at the end of 2013. This involves serology testing of blood serum or milk samples by ELISA to identify infected animals and herds and to provide knowledge and professional supports to control and reduce the disease over time and ultimately achieve a high confidence of disease freedom (bio-containment). In this study, over 1000 samples from routine submissions throughout Ireland were analysed by three commercial milk and serum ELISAs test kits.

Methods:

Three commercially available diagnostic ELISA kits for Johne's disease were compared: PARACHEK®2 (Prionics AG, Switzerland), Paratuberculosis Screening Ab Test (IDEXX, USA) and the ID Screen Paratuberculosis Indirect ELISA (IDvet, France).

Results:

There is generally a high agreement between the three ELISA kits. Agreement was highest for the comparison of the Prionics PARACHEK®2 and the ID Screen ELISAs with a kappa value of 0.736 followed by the Prionics PARACHEK®2 versus IDEXX Paratuberculosis Screening Ab test with an observed kappa value of 0.677. The lowest agreement with a kappa value of 0.626 was determined for the comparison of the ID Screen ELISA versus the IDEXX Paratuberculosis Screening Ab test.

Conclusion:

This comparative study shows that the all commercially available ELISA kits for Johne's disease show similar performance and that differences in the diagnostic sensitivity and diagnostic specificity are statistically not significant.

Keywords:

Parachek 2, ELISA, Johnes Disease

Abstract P-02.42

EVALUATION OF DIFFERENT METHODS TO DETECT MYCOBACTERIUM AVIUM SPP. PARATUBERCULOSIS (MAP) IN BOOT SWABS AND LIQUID MANURE SAMPLES

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Abstract text:
Boot swabs of fecal matter from cow traffic areas (BS) and liquid manure samples from manure storage sites (LM) are utilized for environmental sampling (ES) to identify MAP-positive dairy herds (MAP+H). Fecal culture (FC), the gold standard for MAP detection, is time consuming and prone to microbial overgrowth. Several direct fecal qPCR protocols are available as time saving alternative to FC. The present study aimed at evaluating three different qPCR protocols in comparison to FC for the detection of MAP in environmental samples.

Parallel BS and LM were taken once from 58 historically MAP+H and 19 certified MAP-negative herds (MAP-H) with a median number of 250 cows. Median within-herd prevalence of all MAPH estimated by individual FC was 2,4%. Cows from MAP-H had been monitored for three years by annual individual FC without any positive results.

Each sample was drawn in triplicate and one replicate each was tested in lab A, B, and C simultaneously by fecal culture (3 x HEYM) and either one of two different direct commercial qPCR methods (A, B) or in-house qPCR following 4-week cultural enrichment (C).

FC of BS and LM from MAP-H were negative while positive qPCR results were observed in two herds. MAP was culturally detected in BS from 41 and LM from 42 MAP+H. BS were positive in 37, 34 and 34 herds and LM in 38, 33, and 29 herds using PCR A, B or C, respectively. Six MAP+H could not be detected with any method. PCR A, B and C showed a moderate to good accordance to FC (κ: 0.808, 0.757 and 0,713 for BS and 0.514, 0.794 and 0.704 for LM). Correlation between quantitative qPCR and FC results was also noted ($P < 0.001$). Agreement was very good for FC results of BS and LM (κ: 0.840) and moderate to good for qPCR results of BS and LM (κ: 0.543 [A], 0.671 [B] and 0.784 [C]).

In conclusion, despite slightly lower sensitivity, qPCR A and B are valuable alternatives to FC of environmental samples. Single positive PCR results in MAP-H point to minor specificity problems.

Keywords:
boot swab, liquid manure sample, within-herd-prevalence

Abstract P-02.43

BACTOTYPE MAP REAL TIME PCR - COMBINING OPTIMIZED SAMPLE EXTRACTION WITH SENSITIVE DETECTION

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Abstract text:
Purpose of this study was to increase sensitivity of detection of Mycobacterium avium paratuberculosis (MAP) by combining a sensitive amplification and detection method with an optimized protocol for extraction of MAP from fecal samples.

Culture from fecal samples is in general regarded the gold standard of MAP detection in ruminants. But culture is labor intensive and takes up to several weeks. Therefore direct fecal PCR is becoming more widely used which allows a test result within one day.

But there are challenges extracting MAP DNA from fecal samples as MAP clusters in the sample, the thick cell wall of MAP and inhibitors.

QIAGENs approach is combining 3 strategies to optimize the extraction of MAP from fecal samples:

1. Lysis buffer
2. Mechanical disruption using bead beating instruments (e.g. Tissue LyserII) and Pathogen Lysis Tubes.
3. Heat treatment of the sample

A special MAP extraction protocol based on modifications of QIAGENs QIAamp® cador Pathogen Mini Kit or QIAamp DNA Stool Mini Kit will be presented.

bactotype MAP PCR Kit for amplification and detection of MAP DNA is a Duplex real-time PCR with a ready-to-use master mix and a heterologous control as extraction and amplification control integrated as separate component.

8 µl sample are added to 17 µl Master mix for a total of 25µl. The total run time is about 1.40 hours (Rotorgene).

The PCR kit is TagMan based and can be used on all common cyclers.

Analytical sensitivity assessed with Rotorgene Q by a titration series of MAP in vitro DNA performed in triplicates of ten-fold dilutions was proven up to 5 copies/well.

Data comparing different sample pretreatment procedures, different extractions kits and different amplification PCR kits on fecal samples with known status e.g. the 2012 Johne's Disease Individual Fecal Proficiency Panel will be presented.

Keywords:
MAP, PCR, sample extraction

Abstract P-02.44
DEVELOPMENT OF IS900 AND F57 FAST REAL-TIME PCR ASSAYS FOR THE DETECTION OF MYCOBACTERIUM PARATUBERCULOSIS

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Abstract text:
The present study aimed to develop Fast Real-Time PCR (RTPCR) assays to amplify the multicopy element IS900 for qualitative purposes and the single copy element F57 for the quantitative detection of Mycobacterium avium subsp. paratuberculosis (Map).

All the experiments were performed with a Map DNA extracted from the ATCC 19698 strain.

Fast-RT PCR conditions for IS900 and F57 were optimized and compared to the standard-mode protocol.

For each target, FastRTPCR produced overlapping, sometimes better, results respect to standard mode.

To evaluate LOD (Limit of detection) and LOQ (Limit of quantification), IS900 and F57 gene portions were amplified and cloned in pCR-Blunt vector. Obtained clones were checked by restriction and sequence analysis. IS900 and F57 clones were two-fold serially diluited (4000 copies to 31,25 copies) in TE supplemented with salmon sperm DNA (ssDNA). Different amounts of ssDNA in RT-PCR reaction were tested and the final amount of 200 ng gave the best results. Each target was tested in 10 replicates. In this case LOD is the lowest amount of DNA which can be reliably detected in 100% of replicates. LOD was 125 copies (31,25 copies/µl) for IS900 and 62,5 copies (16 copies/µl) for F57.

LOQ corresponds to the diluition of analyte in a sample for which RSD (Relative Standard Deviation) value is 25 or less. LOQ for F57 was 125 copies (31,25 copies/µl).

Robustness was evaluated by analyzing the same samples with two different Real Time PCR platforms obtaining comparable results. Diagnostic specificity and sensitivity were evaluated testing Map ATCC strain 19698, M. avium avium (D4ER strain), M.bovis (AN5 strain), M. bovis BCG and field strains (Map, M. caprae, M. microti and M.intracellularis). No mycobacterial DNAs other than Map were found positive by either assays.

The results show that IS900 and F57 Fast RT-PCR assays are rapid, sensitive, specific detection methods for Map and could be applied to the qualitative/quantitative diagnosis of paratuberculosis.

Keywords:
FastRTPCR, IS900, F57

Abstract P-02.45

DETECTION OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS INFECTION IN DOMESTIC RUMINANTS OF EASTERN PART OF KARNATAKA AND ADJOINING AREA OF TAMILNADU

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Abstract text:

Johne's disease or paratuberculosis is contagious, chronic granulomatous enteritis of ruminants caused by Mycobacterium avium subspecies paratuberculosis (MAP), the host range sheep, goat, cattle, buffalo, deer and wild bison and some non ruminant species. The objective of present study was the detection of this disease in domestic ruminants of eastern karnataka region. The area for sampling covers Kolar district of Karnataka and adjacent part of Krishnagiri district of Tamilnadu. For this study serum samples were collected from 79 animals (19 sheep, 30 goat and 30 cattle). Out of 79, 62 animals were also subjected for faecal sampling for investigation of acid fast organism. The prevalence was estimated by Indigenous ELISA kit (using Purified protoplasmic antigen of MAP S5 Indian Bison Type strain) and microscopic examination of faecal smears after Zeihl-Neelsen staining. The overall prevalence was 48.1% and 66.1% by Indigenous ELISA and microscopy of fecal smear respectively. Out of 62 animals tested by both tests, 22 animals were positive and 15 animals were negative by both tests. ELISA positive 6 animals were negative by microscopy and ELISA negative 19 animals were positive by microscopy. Further, species, sex and age wise sero-prevalence was calculated; 57.9% sheep, 60.0% goat and 30.0% cattle were positive; sex wise 34.2% male and 61.0% female were positive while age wise 30.2% animals of age below one year and 84.6% animals of age 1 year and above were positive. The higher prevalence emphasizes the necessity of initiation of control program to control the disease in this area.

Keywords:

Johne's disease, Mycobacterium avium subspecies paratuberculosis, ELISA

Abstract P-02.46

SECRETORY PROTEINS PROFILE OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS WITH DIFFERENT GROWTH PATTERNS

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Abstract text:

Culture filtrates (CF) secreted proteins profile of native 'Indian Bison Type' strain of Mycobacterium avium subspecies paratuberculosis was studied at different growth phases. Two loopful of MAP colonies on HEY medium were transferred to Middlebrook 7H9 medium supplemented with 10% ADC, PANTA and mycobactin J. Turbidity of cultures were measured every three days for 4 weeks by McFarland standards. Morphologically, thin floating sheets of mycobacterial cells were observed in 7H9-ADC medium. CF proteins were harvested from supernatant at different growth phases (Early to mid logarithmic) of liquid cultures at 1, 2, 3 and 4 weeks. Purification of CF proteins was done by BioLogic LP chromatography system (Biorad) using Bio-ScaleTM Macroprep(R) High Q column (strong anion exchanger). Analysis of purified fractions upto 4th week showed six narrow purified protein peaks (by LP Data View v1.03) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that the greater part of CF proteins had low molecular masses (<70 kDa) as 19, 30, 32, 35, 54 and 65 kda at 1st to 4th weeks of MAP growth.

Collectively, these results indicated that serological tests for paratuberculosis may be improved by using CF proteins instead of whole cell sonicated crude protoplasmic extract (PPA) and cocktail of CF secreted proteins could be the potential targets for developing diagnostics with improved sensitivity and high specificity in early or subclinical stages of disease. Furthermore, the immunogenicity of culture filtrates proteins secreted during different phases of growth (1st to 12th weeks) would be studied in the goat model to identify immuno-dominant proteins inducing strong antibody responses including above six for developing ELISA. We anticipate that the indigenously developed assays will serve as backbone of the future control programs in the country.

Keywords:

Mycobacterium avium subspecies paratuberculosis, Secretory proteins, SDS-PAGE

Abstract P-02.47

COMPARISON OF 3 FECAL CULTURE, 2 FECAL PCR, 2 SERUM ELISA, AND MILK ELISA FOR DIAGNOSIS OF PARATUBERCULOSIS IN US DAIRY CATTLE

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Abstract text:
The objective of this study was to conduct a comparison of multiple diagnostic tests for Johne's disease in US dairy cattle and to create a repository of well-characterized fecal, serum, and milk samples as a resource for future studies. Samples were collected from 499 cows in herds known to have infected animals, and 418 cows in dairy herds in a test-negative certification program. Fecal, serum, and milk samples were collected from cows in the second lactation or older.

Diagnostic tests applied to the samples included fecal culture on solid Herrold's Egg Yolk Medium (HEYM), 2 different liquid culture media systems (TREK Diagnostics; BACTEC MGIT, BD Diagnostic Systems), fecal PCR using two different platforms (Tetracore, Taqman), two serum ELISA (IDEXX, Prionics), and milk ELISA (IDEXX). Bayesian models for test evaluation when a "gold standard" test is not available were employed in estimation of diagnostic sensitivity of each test.

For organism based tests, the specificities were 81.2% for MIGT, 97.1% for TaqMan PCR, 98.1% for TREK, 100% for HEYM and 100% for Tetracore. Bayesian median sensitivity estimates were 48.5% for HEYM, 63.8% for TREK, 76.5% for TaqMan PCR, 78.5% for Tetracore PCR.

For antibody detection tests, all had specificity > 99.3%. The Bayesian median sensitivity estimates were 15.3% for IDEXX serum ELISA, 17.1 % for Prionics ELISA, and 46.8% for milk ELISA.

The results of this study provide a basis for selection of optimal diagnostic test protocols according to herd characteristics and management objectives. Repository samples are available for future study.

Keywords:
Diagnositics, ELISA, FECAL

Abstract P-02.48
DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN RAW MILK BY DIRECT QPCR ASSAY

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Abstract text:
Mycobacterium avium subsp. paratuberculosis (Map) infection is highly prevalent in Northern Italy where milk production is intensively developed. Control programs are hardly feasible, require long time and are limited on a voluntary basis. On the other hand the growing importance of guaranteeing dairy products safety poses the need for implementing quality assurance programs. Map-specific molecular diagnostic tools on bulk milk might represent a fast and reliable approach to determine the level of Map contamination.

In this study, we implemented a protocol for Map DNA extraction from raw milk based on sample centrifugation, pooling of cream and pellet, lysis in hot SDS buffer, bead-beating, proteinase K digestion and DNA purification through silica columns. This procedure was coupled to a previously published Map IS900 qPCR modified for quantitative assessment using recombinant plasmidic standards.

Analytical sensitivity was determined on raw milk samples spiked with 10-fold dilutions of both Map cultures and faeces from a naturally infected cow. The analysis revealed a LOD of 1 Map/ml (Ct<40) and quantitation was consistent above 10 Map/ml of milk. The assessment of the diagnostic sensitivity is ongoing on bulk milk samples under a regional milk quality assurance program.

Keywords:
milk, test evaluation, quantitative PCR

Abstract P-02.49

A LIQUID MEDIUM (M7H9C) FOR ROUTINE CULTURE OF MYCOBACTERIUM AVIUM SUBSP PARATUBERCULOSIS TO REPLACE BACTEC 12

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Cultivation of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) remains a definitive diagnostic test for Johne's disease. MAP is an obligate parasite therefore if it is detected there must be – or must recently have been – an infected animal. Despite a long history of endeavour, there are still many problems with culture and knowledge gaps remain: absolute values for diagnostic sensitivity and analytical sensitivity are lacking, contamination sometimes occurs but causes are unclear, results are not available for months, international agreement is lacking on the most suitable protocols, questions exist over the cultivability of some strains, and dormancy of MAP may occur in cultures. In Australia, culture is an important part of the National Johne's Disease Program. Once based mainly on Bactec radiometric culture, and in the absence of a validated commercial alternative capable of supporting the growth of the common strains of MAP in Australia (i.e. both S and C), animal health laboratories in Australia now use a locally produced, non-radiometric liquid medium and protocol. The new medium is "set and forget" for its 12 week incubation after an initial contamination check. Growth of MAP is confirmed by PCR examination and/or subculture of every broth. There is no growth indicator, but semi-automated processing of the broth for PCR examination is possible. The validation data for M7H9C have been published recently. Based on culture of 671 faecal samples and 113 tissue samples from both sheep and cattle from a range of farms and a range of disease stages, the performance of M7H9C and Bactec were not significantly different. M7H9C replaces Bactec medium in existing protocols with no changes required to sample preparation, decontamination or inoculation. The medium is not radioactive.

Reference:

Whittington R, Whittington A, Waldron A, Begg D, de Silva K, Purdie A, Plain K (2013). Journal of Clinical Microbiology 51, 3993-4000.

Keywords:
culture, media, accuracy

Abstract P-02.50

EVALUATION METHODS FOR THE DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN MILK SAMPLES FROM LOW PREVALENCE HERD.

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Abstract text:

Objective

Detection of cattle's infection with Mycobacterium avium subsp. paratuberculosis (MAP) based on milk samples examination and it's suitability for the diagnosis of Johne's disease (JD).

Animals

18 cows from low prevalence (< 2, 5%) herd – 5 seropositive, 1 doubtful and 11 seronegative by ELISA anti-MAP antibodies test. 4 cows were feces cultured positive.

Methods

Feces and milk samples were collected from selected animals 3 times: 1 and 6 months after first collection. Feces samples were tested by culture. Milk samples were tested by culture; direct isolation of DNA and the presence of an insertion sequence IS-900 (QIAamp DNA Mini Kit, Qiagen); presence of anti-MAP antibodies by ELISA test (Svanovir Para-TB Ab ELISA Kit, Svanova). The statistical analyzes were conducted using PQStat software.

Results

The obtained results allowed calculating the sensitivity and specificity of the tests, and positive and negative predictive value. The highest specificity demonstrated the detection of antibodies by ELISA, and the lowest direct isolation of DNA. The sensitivity of the ELISA test was the lowest, while the direct isolation of DNA from milk samples - the largest. The differences between the negative prediction values are much smaller than the positive predictive values difference between used three methods.

Conclusions

Examination of milk samples for the presence of MAP does not detect all infected cows. The highest sensitivity in detecting MAP shows direct isolation of DNA. The highest specificity shows anti-MAP ELISA

Keywords:

Mycobacterium avium subsp. paratuberculosis, milk, Johne's disease

Abstract P-02.51

RELATIONSHIP BETWEEN PRESENCE OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS ANTIBODY IN COLOSTRUM AND CALVES' SERUM.

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Abstract text:

Objective

Identify associations between the presence of Mycobacterium avium subsp. Paratuberculosis (MAP) antibodies in colostrum of the dam and the presence of MAP antibodies in calves' serum.

Material

60 samples of colostrum from infected and not infected dam and serum samples from their calves.

Methods

Colostrum was taken directly after calf's birth. Calves' serum samples were taken 3 times: 3 days, 6 months and 12 months after parturition. All samples (colostrum and serum) have been tested for antibodies against Mycobacterium avium subsp. paratuberculosis by ELISA test (Svanovir Para-TB Ab ELISA Kit, Svanova).

Results

MAP antibodies were detected in 5 samples of colostrum; 1 sample was defined as doubtful. In the examination of 3 days old calves' serum samples we found 3 samples ELISA positive (all from calves' from colostrum positive dam) and 4 samples ELISA doubtful (2 from colostrum positive and 2 from colostrum negative dam). In the age of 6 months - 4 positive (1 from colostrum positive and 3 from colostrum negative dam) and 3 doubtful (1 from colostrum positive and 3 from colostrum negative dam). In the age of 12 months - 4 positive (1 from colostrum positive and 3 from colostrum negative dam) and 4 doubtful (1 from colostrum positive and 3 from colostrum negative dam). All calves with positive serum samples in the age of 3 months were serum negative 6 and 12 months old.

Conclusion

The presence of anti-MAP antibodies in the colostrum is correlated with presence of anti-MAP antibodies in serum of new born calves. Most of anti-MAP seropositive serum samples from older individuals came from calves born to anti-MAP seronegative colostrum dam. Presence of anti-MAP antibodies in colostrum may affect the MAP infection in calves.

Keywords:

colostrum, ELISA, calves

O-03 MAP Control Programs

Abstract O-03.1: INVITED SPEAKER
BOTTLENECKS IN THE PREVENTION AND CONTROL OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS INFECTION

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Many Johne's disease (JD) control programs have been developed worldwide, due to the economic losses caused by this disease, in addition to a potential association between Mycobacterium avium subspecies paratuberculosis (MAP) and Crohn's disease. In the absence of neither a cure, nor even an effective vaccine, control of JD is currently based mainly on herd management to mitigate in utero and calf infection and to restrict transmission within or between herds. In this abstract, we identify the most pressing gaps in knowledge hampering JD control programs and we discuss research needs.

Prevalence. Prevalence estimates are required for modeling or simulating the spread of an infection and for designing surveillance, control or eradication programs. However, the use of various sampling strategies and case definitions make it difficult or impossible to directly compare prevalence studies and prevalence estimates of MAP infection. Therefore, a supranational standardized study should be done, comparable to the Neospora caninum seroprevalence study of cattle in 4 countries (Bartels et al., 2006).

Pathogen. Some of the variation in disease manifestation and progression might be due to the presence of multiple MAP genotypes (Stevenson, 2010). Dominant MAP strains exist in specific geographic areas suggesting that these strains are more successful in spreading among herds, although secondary strains are concurrently capable of persisting. A high prevalence MAP within a specific herd could be due to greater exposure to the organism or to greater ease of transmission of a particular strain under similar exposure scenarios. Whole genome based genotyping will identify which strains associate with high prevalence and efficient transmission on all geographical scales.

Tests. Ideal tests should identify an infectious animal or predict when an infected animal will become infectious. To develop and validate these tests, we need to better identify and define cases of infection in infected but asymptomatic animals versus those that are not infected. Therefore, populations of both categories in multiple species are key to evaluating diagnostic tests. However, large variability in infectious dose, age at infection, genetic makeup, routes of infection and MAP genotypes, along with interactions among these factors, make it difficult to determine true infection status and stage in each tested animal. Consequently, results from decades of test evaluations are of questionable quality (Nielsen and Toft, 2008).

Transmission. Although the most common prevention measure is to limit the exposure of calves to feces from infective adults, the potential risk of calf-to-calf transmission has been largely overlooked. Furthermore, with detection of MAP in dust samples, and intestinal infection occurring after exposure to MAP-containing aerosols and intra-tracheal infection, another

previously ignored route of transmission may have been identified (Eisenberg et al., 2012).

Studies implicating colostrum in MAP transmission generally used milk (not colostrum) containing very high numbers of bacteria. Some of this research is somewhat artificial, as spiked milk is used as a substitute for naturally infected milk and hot water baths instead of commercial pasteurizers. Therefore, whether on-farm heat treatment effectively reduces the number of viable MAP bacteria in colostrum has not been appropriately tested.

Most studies characterizing the rate of intra-uterine infection have examined fetuses of MAP-infected cows at various stages of the pregnancy, resulting in an underestimation of the proportion of calves born with a MAP infection. Furthermore, whether or when in utero-infected calves will start shedding MAP and whether the progression of the infection and the responses to the organism differ from those of calves infected orally in their first week of life have not been determined.

Across ruminant species transmission should also be considered, as many ruminants species are susceptible to MAP, including a number of common wildlife species. However, other than documented infection in a wide range of free-ranging and captive ruminants, to include deer and elk, the role of wildlife in MAP transmission in domestic agriculture species has not yet been proven or quantified.

Host susceptibility. In an infection trial, calves were susceptible to MAP infection until at least 12 months of age and shed MAP soon after infection. In most MAP-infected herds, a significant proportion of calves shed MAP in the feces resulting in a high proportion of MAP-contaminated pens (Mortier et al., 2013 and submitted; Wolf et al., submitted). Because group housing of calves is common, MAP control strategies based on best calf hygiene management practices need to be re-evaluated. It is noteworthy that this will not only help to control MAP, but also other fecal-orally transmitted infections, e.g. Salmonella, Escherichia coli, rota/corona viruses and Cryptosporidium.

Heritability. Susceptibility to MAP infection is multigenic or polygenic. The limited congruence among studies available is due to varied definitions of case and control animals, in light of the multiple stages of the disease, phenotypic data recorded (tissue culture, fecal culture, blood and milk ELISA), and the diagnostic outcomes in animals at the same stage of infection. Attempts to locate loci associated with resistance to paratuberculosis have proven to be more challenging than finding loci associated with susceptibility. Potential genetic influences on the immunological responses of the host can complicate detection of infected animals in experiments which use serological responses. Now that multiple SNPs have been associated with susceptibility to MAP infection, a series of follow-up experiments are needed. Next, experimental infection trials with specific genotypes should determine effects on infection dynamics.

Vaccination. Current vaccines against MAP can cause severe reactions at the injection site and interfere with tests to identify animals infected with other mycobacterial species. Furthermore, although they may reduce the clinical impact of infection, including sometimes a reduction in shedding, they do not prevent infection. Therefore, there is clearly a need for improved vaccine which goes hand in hand with improved diagnostic tests for JD. The successful vaccine strain will have to be sufficiently homologous in antigen composition with the majority of field strains.

Uptake. That nearly all JD programs worldwide are voluntary, their success therefore depends on enrolment and long-term retention of farms in these programs. To ensure participation and success, there needs to be sufficient awareness of the issue. Although there is evidence that participating farms are larger and better managed than non-participants (Kelton et al., 2012), it is not clear what motivates farmers to participate. It is important that potentially MAP-free and low-prevalence herds participate; however, to decrease herd- and animal-level prevalence, it is essential that high-prevalence farms also participate. Farmers differ in priorities and motivations; therefore, the likelihood of successful adoption of any management strategy or technology will vary among farms (Jansen et al., 2011). This is a relatively new avenue for research, but it has important implications for our approaches to motivating individual farmers to adopt optimal disease control practices.

References are available upon request.

Abstract O-03.2

BOVINE PARATUBERCULOSIS IN THE DAIRY CATTLE POPULATION: TESTING AN INNOVATIVE MODEL OF BLENDED LEARNING

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Abstract text:

In order to reduce the impact of Bovine Paratuberculosis in the dairy cattle population, a new training approach was tested by the Health Awareness and Communication Department of Istituto Zooprofilattico Sperimentale delle Venezie. The aim of the project was to increase the awareness of Italian veterinarians in relation to disease control and communication skills. The research process was divided into different phases: (1) assessment of veterinarians' knowledge and perception of cattle health and management with a special focus on paratuberculosis; (2) development of a training pathway for veterinarians; (3) assessment of veterinarians' knowledge after the training course. The target was veterinarians working in the Veneto region (North Eastern Italy), the 3rd milk producing region in Italy. In the first phase, a survey was designed to assess veterinarians' perception of the impact of the disease in cattle farms, their awareness of the health status of the farms and their willingness to participate in a training program. In the second phase a model of blended learning, referred to as flipped classroom, was adopted. The delivery of content was online through video lessons, extra reading material and discussion forums. In this way, classroom work focused on expert-led discussion of case studies and the provision of more detailed information on demand. Based on this model, a course addressed to 85 veterinarians was organized in: plenary-session; online video lessons on key content (Pathogenesis and epidemiology of paratuberculosis, Management of infected farms, Interpretation of diagnostic tests); discussion with the experts and the other participants, in the classroom. The assessment tests suggested that the adopted approach was highly effective: the participants positively assessed certain features of the method, as flexibility in individual study scheduling and the opportunity to discuss issues directly with experts and other professionals facing the same professional problems.

Keywords:

innovative training approach, blended learning, disease control

Abstract O-03.3
THE USE OF WEB BASED ECONOMIC HEALTH EVALUATOR TO INCREASE FARMER ENGAGEMENT WITH JOHNE'S CONTROL

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Introduction

The economic impact of Johne's Disease (JD) is traditionally divided between direct losses(milk loss, culling) and indirect losses and associations(e.g. udder health). The milk losses due to the subclinical and clinical infection have been estimated to be in excess of 4000kg per lifetime of affected individuals (Villarino 2005). Further unpublished work by National Milk Records (UK) illustrates the association between prevalence and average lifetime yields (total milk produced divided by total number of days alive). Herds with prevalence's greater than 6% typically produce 10-15% less milk per lifetime than lower prevalence herds (Fig 1).

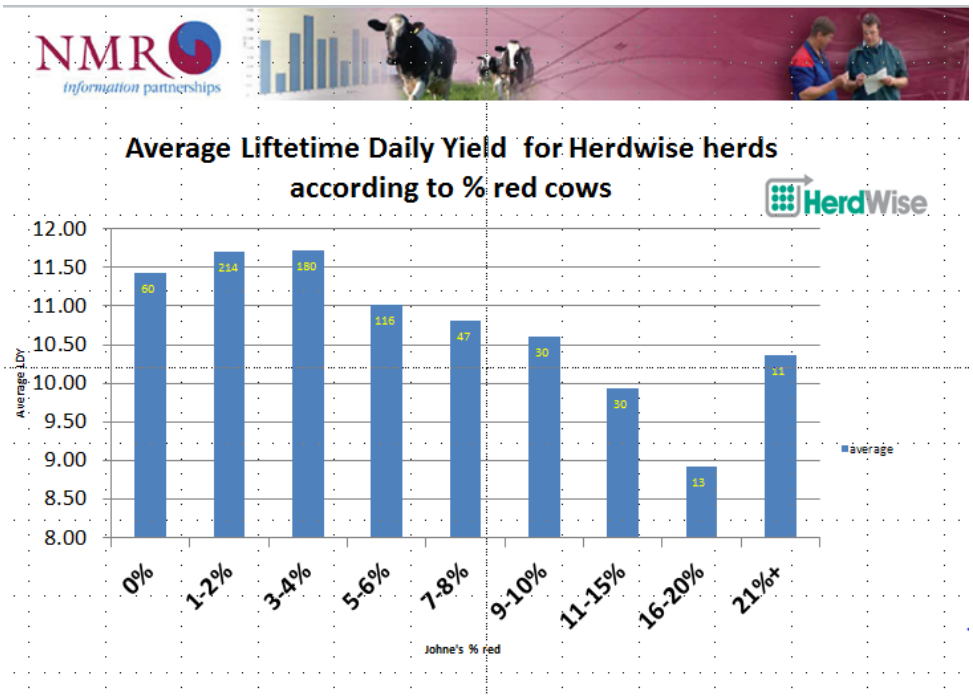

Fig 1. Average Lifetime daily yields compared to % repeat test Johne's positive cows (671 Herds, UK Herdwise Johne's program)

Premature culling of affected animals reduces the overall economic performance of the herd with the risk in some herds that animals are culled before the rearing costs have been recouped. This is further exacerbated in heavily infected herds with increased risk of culling in the first 100 days after calving (emergency culling) with significantly higher economic losses.

Typically Johne's disease costs are expressed as an average cost across all herds, for example,£27/ cow (Stott 2005). The prevalence within herds is defined by the risk factors for spread of the disease and if the risks are high the within herd prevalence can rise rapidly to over 10-15% with substantively greater economic losses than this average cost would suggest.

If Johne's disease is unchecked and the herd incidence rises further the disease may create extremely significant consequential losses due to retention of poorly fertile, lame or diseased cows

due to high forced removals of infected animals due to JD. These consequential losses are seldom included in the economic analysis of the herd thus underestimating the true impact of the disease.

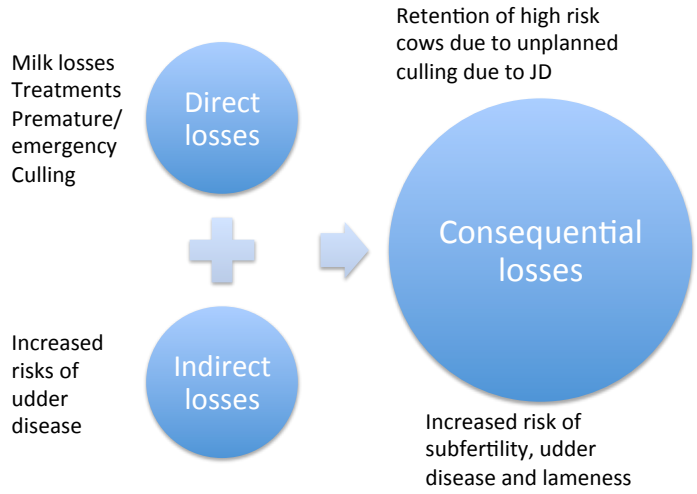

Fig 2. An illustration of the relationship between direct, indirect and consequential losses for Johne's disease in a dairy herd.

To fully engage farmers with Johne's control programme a full economic health evaluation can be used to simply illustrate the profit opportunities that can be achieved through effective control. If the audit is performed using agreed targets and costs, which are specific to the farm this will produce a credible figure that the farmer is more likely to comprehend. This then forms the foundations of a robust discussion on the most cost effective control option for the farm.

Methods

In 2007 a Health Evaluator was developed within a web based Health Planning tool (www.myhealthyherd.com) allowing both the vet and farmer to enter their own targets and costs for all the key health areas (fertility, udder health, lameness, sick cows/ metabolic, culling and youngstock). The program was populated with default incidence and cost targets for 29 health values and this allows for farm specific overall losses for health areas to be calculated. The economic benefit between the target for the herd and the actual performance is expressed as a "profit opportunity" which can be achieved by improving performance in that particular area. Within the program the culling reasons and costs are defined and split into losses due to no value culls/ deaths (£3,499), emergency culls (£2,182) and culls sold live at the end of lactation (£1,238). Analysis of 833 UK dairy herds (30,820 culls) revealed that 23% were culled due to sickness, death or casualty (Orpin 2010). In high JD incidence herds, without appropriate controls, the emergency and no value culling increases significantly. This substantively alters the overall economic impact of Johne's disease where a control plan is not instituted. Indeed in many herds the introduction of a testing program to identify high-risk cows can facilitate more justifiable economic removal for these cows on a planned and lower cost basis. The subsequent savings made by improved heifer placement and high risk cow removal can more than cover the investments in control compared to taking no positive action.

The direct, indirect and consequential losses within the program and are expressed in a £ per cow, per herd or pence per litre basis.

In the authors experience practical on farm health evaluation typically reveal that in herds with high prevalence's of Johne's disease the economic impact can be in excess of 1-3 pence per litre due to JD with these losses continuing (at a lower level) for several years before the disease is fully controlled. Johne's disease therefore in affected herds becomes significantly more costly than endemic infections due to IBR and BVD where the impact of the disease can be more readily controlled by vaccination and the disease process is more self-limiting as immunity develops.

Conclusions

The use of a farm specific Health Evaluator that illustrates direct, indirect and consequential losses using simple inputs with credible outputs is a useful method of motivating farmers to commit to long term JD control programs.

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Keywords:

Johne's, Economics, Control

Abstract O-03.4

*i*RAMP: AN ITALIAN RISK ASSESSMENT AND MANAGEMENT PLANNING TOOL + JUST-IN-TIME LEARNING WITHIN ONE PRACTICAL APPLICATION.

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Abstract text:

In 2012 Italy decided to institute a control program for paratuberculosis. As with any control program, education for veterinarians and producers is key to the program's success, so the Italian program created online modules for veterinarians who in turn educate producers. Eleven years before, the US made a similar decision to develop an education program for paratuberculosis. A variety of courses were developed, including a veterinary certificate program, virtual farm visits and practice simulations for veterinarians (JD Consult), modules for producers (dairy, beef, sheep, goats, cervids) and milk processors. With primary funding from the US Department of Agriculture, through JDIP (Johne's Disease Integrated Program) and the National Milk Producers Federation, the latest addition was JD-RAP (jdrap.org), a simulation for producers that embeds education within a risk assessment process where users enter their own data resulting in customized results and specific recommendations.

Creating quality education can be an expensive endeavor. Through an Italian-US collaboration we created new tools and modules for Italian veterinarians and producers in a cost-effective way by reusing and repurposing parts of existing, proven US modules. Funded by a US Fulbright award and the Italian Ministry of Health (Research Project 2012/001, "Creating an informative integrated system for Paratuberculosis"), the main focus of our project is an electronic version of a risk assessment for tablets, both iPad and Android, for veterinarians to use on the farm to facilitate paratuberculosis management and record maintenance. We mined and revamped elements from JD-RAP and other US modules and embedded mini-lectures, explanations, and examples, accessed through help buttons at strategic points, for just-in-time learning and review of key concepts.

Accessed through **http://iramp.izsler.it/**, *i*RAMP is a convenient online risk assessment tool that provides education and review at strategic points. Using the application the veterinarian collects background and risk assessment data. The program then makes calculations, analyzes the risks, and generates a prioritized list of recommendations for veterinarians and producers to choose from to jointly create a management plan and a testing scheme. The data is automatically uploaded to the national database where it can be retrieved and reviewed at any time, especially for comparison during subsequent risk assessments. This process establishes producer accountability and fosters responsibility.

The design and programming of the risk assessment tool lends itself easily to customization for other countries to adopt, translating to the local language, changing pictures to reflect local conditions and environment, and editing text for local laws and customs. Recycling existing content is very economical and efficient in terms of cost, effort, and time to create a similar application.

We are also creating an Italian version of JD-RAP that we have named Paratubercolosi: Analisi del Rischio per l'Allevatore, or PARA, and simple modules for dairy and beef producers, editing for Italy-specific rules, regulations, and management practices, to add to the educational arsenal for the diagnosis, control, and management of paratuberculosis in Italy.

Keywords:

online, education, modules, risk assessment

Abstract O-03.5
iCULL – A HERD-SPECIFIC TOOL FOR FINANCIAL EVALUATION OF THE IMPACT OF PARATUBERCULOSIS

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Abstract text:
iCull (intelligent Culling) is a newly started Danish project. The objective is to develop an economic, herd-specific model, which should be implemented in a smartphone app / computer software and serve as a tool for individual farmers to aid decision making on individual cows.
The main focus will be on infections with Mycobacterium avium subsp. paratuberculosis (MAP), which can have a significant impact on the farmer´s economy. The iCull model can be used to evaluate the effect of control scenarios, such as breaking transmission routes by removing calves from their dam after birth or through pasteurization of colostrum, as well as for financial optimization of the culling approach in their herd.

The iCull model estimates the retention pay-off (RPO) of each individual cow, while existing models address management decisions on herd-level. The iCull model can be used to assess actions deemed to lower the MAP infection pressure for a specific herd, and will aid the farmer´s decision making in ad hoc decisions about culling animals. For example, it can be used to test the optimal age for culling cows taking into account the milk yield and MAP infection status of the specific cow based on diagnostic test results. The model can also be used to evaluate if it is financially worthwhile to try to eradicate MAP from a farm or if it will be more beneficial to control the infection, or do nothing. Lastly, the iCull model can be useful for evaluating the financial impact of keeping cows with consistently low ELISA results in spite of a single positive value and culling cows with repeated positive results. It might be more ideal to use different test interpretations in different herds, e.g. adjusting the test cut-off or using more than just the latest ELISA test.

Keywords:
Economic, ELISA, Simulation model

Abstract O-03.6

QUANTIFYING THE COST OF REMOVING FECAL SHEDDERS IN A VOLUNTARY JOHNE'S DISEASE CONTROL PROGRAM

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Abstract text:

A voluntary Johne's Disease (JD) control program for dairy cattle herds was launched in Ontario, Canada in 2010 (www.johnes.ca). The program included an on-farm risk assessment (RA), funded milk or serum based testing of cows in the herd and terminal removal of high test positive animals. Milk testing was done with the Prionics Parachek Antibody ELISA. Cows were classified as test positive (TP) if they had a SP value >0.1, and as high TP (HTP) if they had a SP value >1.0 (high risk of fecal shedding of MAP). HTP cows had to be permanently removed from the herd without entering another dairy herd or the human food chain. Producers removing HTP cows within 90 days of testing were paid $500 CDN per cow. Of 243 HTP cows identified in 171 herds, 163 (67%) were permanently removed. A reason cited by farmers for not removing HTP cows was their belief that these cows were more valuable than the compensation being paid.

The module Cow Value of Dairy COMP 305 calculates the net present value of each cow (CWVAL), comparing her to the value of an average heifer for that farm. If the Cowval of a HTP cow was less than $500, the program compensation payment covered the cost of replacing that cow. If the Cowval was greater than $500 then the value of the cow exceeded the program compensation. Cowval was calculated for 86 HTP, 232 TP and 11,957 test negative cows in 80 herds. HTP cows had a mean CWVAL of $700, compared to $830 for TP and $1,200 for negative cows. One-third of the HTP cows had a CWVAL below $500, so the compensation more than covered the replacement cost. Dairy producers who permanently removed HTP cows lost $200 per cow on the transaction but gained non-monetary value for 'doing the right thing'.

Keywords:

Cost, Removal, Fecal Shedders

Abstract O-03.7
ECONOMICS OF PARTICIPATION IN A MANAGEMENT-BASED JOHNE'S DISEASE CONTROL PROGRAM

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Abstract text:
Introduction: The Alberta Johne's Disease Initiative (AJDI) is a control program which aims to reduce transmission of MAP through implementation of best-management practices. The objective was to estimate the economic benefit of participation in the program. Methods: A decision tree was constructed in TreeAge Pro and populated using data estimated in the AJDI and from published sources. Key assumptions were that farms participating in the project would successfully change their management, thereby decreasing disease prevalence but increasing management costs, whereas non-participating farms would maintain constant management. Data on prevalence, baseline management, and management changes as well as cost estimates were entered as distributions to enable probabilistic sensitivity analysis. Results: Farms participating in the AJDI had a net benefit of CDN$74 per cow over the course of 10 years. However, if all project costs were covered by participating farmers, the net benefit was CDN$27. In addition, modelling additional benefits through prevalence decrease of other fecal orally transmitted diseases resulted in higher calf revenues, but increased calf management costs for participating farms (net benefit of CDN$19). Based on one-way sensitivity analysis, participation would not be cost-effective if cows in early stages of MAP infection would not have decreased production or if MAP prevalence would not increase on farms with poor management. Conclusion: Although the primary impetus for Johne's disease control programs is the zoonotic potential of MAP, participation in a management-based Johne's disease prevention and control program such as the AJDI was cost-effective for the average dairy farm.

Keywords:
paratuberculosis, economics, control program

Abstract O-03.8

ARE CULLING DECISIONS BASED ON MICROBIOLOGICAL AND SEROLOGICAL TEST RESULTS FOR JOHNE'S DISEASE IN COMMERCIAL BEEF COW-CALF HERDS ECONOMICALLY JUSTIFIABLE?

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Introduction

Economic losses from Johne's disease (JD) in beef herds primarily occur due to lower weaning weight of calves (Bhattarai et al., 2013a), premature culling of affected animals, reduced body weight of culled animals and loss of potential markets (Roussel, 2011).The majority of US beef producers and other stakeholders are less aware about the losses associated with JD (Benjamin et al., 2010; Bhattarai et al., 2013b). Studies evaluating the economic aspect of JD control strategies in beef cow-calf herds are limited. The objective of the present study was to compare the costs of JD control using the scenarios of no-intervention, and currently available tests in an infected beef cow-calf herd using a stochastic decision tree model and to estimate benefits over multiple years as MAP prevalence is reduced through the yearly low-risk replacements with and without test-based culling.

Methods

The economic model was constructed using inputs from multiple sources including a survey of beef cow-calf producers (Bhattarai et al., 2013b; Bhattarai et al., 2014) and published reports (Greer et al., 1980; Apple, 1999; Collins et al., 2006; Kudahl et al., 2008; Bhattarai et al., 2013a; USDA, 2013b, a). A decision tree model was constructed within MS-Excel (Microsoft Corporation, Redmond, WA, version 2010) using Precision Tree (Palisade Corporation, Ithaca, NY, version 5.7). The first node of the constructed tree was the producer's decision of whether or not to test all adult breeding cows in the herd. The testing decision was structured to branch further into selection of test type: ELISA, bacterial culture of feces (BCF), or ELISA screening with BCF confirmation (EBCF). Probabilities for each branch were calculated based on sensitivities, specificities and prevalence. MAP associated losses attributed to lower production along with the cost of test, treatment, culling, and replacements were also incorporated. The economically prudent decision was the option with the highest return (or lowest cost) evaluated for prevalence levels between 0 to 100%.

Another independent spreadsheet model was developed to estimate yearly prevalence changes in a 100-cow herd at 10% true prevalence after implementation of the control program with all test-positive cows being culled and replaced with cows from low-risk herds. Total yearly costs and cumulative costs over the control program were estimated based on test costs and production losses attributable to MAP infection. Costs based on the scenarios of no-testing, testing with ELISA, testing with BCF, and testing with EBCF annually were calculated until the true herd prevalence was reduced to 0.5% or lower.

Summary of new and unpublished data

Average cow-level returns in the cow-calf herd decreased with increasing MAP prevalence. The return associated with the decision to cull test positives and replace with heifers from low-risk herds was lower compared to the return from a non-testing herd at all levels of prevalence. Evaluation of multi-year testing revealed that the use of BCF provided the fastest reduction in herd prevalence and lower cumulative cost over years followed by ELISA, EBCF, and no test, respectively.

A herd with 10% prevalence, purchasing low-risk replacements, and conducting a test and cull program based on BCF achieved a prevalence of less than 0.5% in four years while it took six years to reach the same prevalence with an ELISA-based control program. Testing and culling based on EBCF results took eight years to reach that threshold. The progress of non-testing herd selling all calves and purchasing all replacements from low-risk herds also reached the less than 0.5% prevalence threshold only after 11 years of continuous effort by the time when the native stock was completely replaced by low-risk replacements (Figure 1).

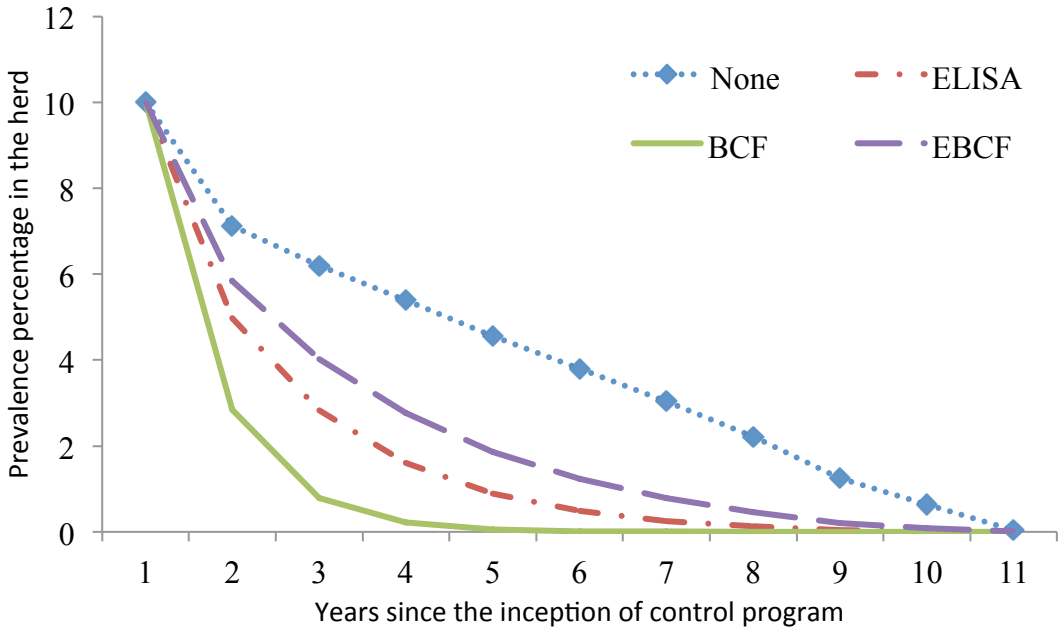

Figure 1: Year-wise prevalence after introduction of low risk replacements after test based culling in a beef cow-calf herd with 10% initial prevalence of *Mycobacterium avium* subspecies *paratuberculosis.* Estimated based on the following options: no-test (dotted line), ELISA (solid line), bacterial culture of feces (long dash), and ELISA screening followed by bacterial culture of feces confirmation (short dash).

Conclusions

A single microbiological and serological test based culling was not economically justifiable for a typical cow-calf herd at any MAP prevalence. However, when the priority is to reduce the infection, testing the herd annually and obtaining all replacements from low-risk herds is effective. BCF testing results the most rapid reduction in prevalence and lower overall cumulative costs attributable to MAP.

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Keywords:
beef, control, economics

Abstract O-03.9

A NATIONAL JOHNES DISEASE SERUM SURVEY AND ITS USE TO EVALUATE TARGETED SUB-SAMPLING IN IRELAND

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Abstract text:

The primary objective of this study was to estimate the herd-level and within-herd prevalence of MAP infection in Irish cattle during 2009. In addition, we evaluated the effectiveness of targeted sub-sampling to detect MAP-infected herds at a single screening, noting that this method has been suggested as a cost-effective alternative to testing of all adults. MAP ELISA was performed on samples, submitted during autumn 2009 under the national brucellosis surveillance programme, from all female animals and bulls over 12 months in 1647 randomly selected herds. For each of sixteen sub sampling strategies, we calculated the herd detection fraction, defined as the number of herds test positive to the targeted sub-sampling strategy divided by number of herds test-positive to the full adult screen. The overall true herd prevalence was 20.8%, higher in dairy (25.0%) than beef (14.6%) herds, and the true animal-level prevalence was 1.7% (95% CI 1.4% - 1.9%), each based on a test specificity (Sp) of 99.8% and a test sensitivity (Se) of 27.8%. The true within-herd prevalence varied from 0.9% to 30% in dairy herds and 1.5% to 55% in beef herds. The number of herds that would be incorrectly assigned a negative status if targeted sub-sampling was carried out varied from 5.0% if all animals over three years were tested to 81.9% when ten animals in dairy herds in the 5-8 year old age group were sampled at random. The MAP ELISA prevalence detected among herds in this survey is lower than has been reported in many other European countries using similar test strategies. We caution against the use of sub-sampling strategies in regions where within-herd prevalence is low, as this would result in a large number of infected herds being undetected.

Keywords:

targeted sub-sampling, Mycobacterium avium subspecies paratuberculosis, herd ELISA prevalence

Abstract O-03.10
SLAUGHTERHOUSE SAMPLING AS PART OF JOHNE'S DISEASE CONTROL IN SCOTTISH CATTLE

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Abstract text:
PARABAN is a Scotland-wide initiative to develop and deliver farm specific 'best practice' for the control of MAP in cattle through Knowledge Exchange. Project partners include nine 'Champion Farms' which each have a tailored monitoring and control programme devised with input from the farmer, his/her vet and PARABAN advisors, taking into account the history of the disease on farm, physical facilities available and farmer objectives. Culling decisions based on live animal test results have been incorporated into each farm-specific plan to complement the programme already in place. Post-mortem samples have been collected from all adult animals culled from PARABAN herds at slaughter or as fallen stock.

With the help of slaughterhouses throughout Scotland and the north of England, a length of terminal ileum and a draining lymph node were collected from each animal, irrespective of whether a positive or negative result had been obtained from serum ELISA or faecal PCR in life. These were examined histologically for evidence of MAP infection using H&E and Ziehl-Nielsen stains.

Between October 2011 and February 2014 352 animals were sampled from eight of the nine farms and the results were collated with those from live animal tests. The presence of MAP has been confirmed by histopathology on six of the eight PARABAN farms despite low or very low prevalence of ELISA positive animals. There is imperfect agreement with serum ELISA results, varying by farm; which indicates that despite extensive, and on some farms prolonged, efforts to control the disease MAP is still a problem to a greater or lesser degree. As part of the PARABAN project this work is supporting decision making on farm within the MAP control plan, influencing interpretation of the live animal test results relative to an expected decrease in MAP prevalence.

Keywords:
slaughterhouse sampling, preclinical animals, knowledge exchange

Abstract O-03.11

CHANGES IN MAP SHEDDING IN AUSTRALIAN SHEEP FLOCKS CONTINUOUSLY VACCINATED WITH GUDAIR FOR 10 YEARS

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Abstract text:

Previous research has demonstrated that Gudair vaccination of sheep flocks infected with ovine Johne's disease (JD) can reduce mortalities and shedding by 90% and consequently vaccination has evolved as a key strategy for controlling the disease in Australia. While the original research was conducted in heavily infected flocks many lower prevalence flocks also commenced vaccination. It was suggested that vaccination may be even more effective in lower prevalence flocks and modelling indicated that prevalence would fall rapidly after commencing a vaccination program depending on initial prevalence. This paper reports on changes in the prevalence of shedding over a decade in 12 infected flocks of variable prevalence after they commenced vaccinating 1-4 month old lambs in 2002.

Study flocks had variable disease prevalence and up to 350 sheep from each of the 3, 4, 5 and 6 year old cohorts had faecal samples collected every 2 years for 5 samplings. At the first sampling all age groups were unvaccinated while at the second sampling the 3 & 4 year olds were vaccinated and the 5 & 6 year-olds were not. At subsequent samplings all ages were vaccinated. Pools of 25 or 50 sheep were BACTEC cultured to detect MAP and the prevalence of shedding estimated.

The prevalence of shedding generally reduced over time as the proportion of vaccinated sheep in the flock increased. However, faecal shedding was present in 7 of the 10 flocks still in the study at the last sampling a decade after vaccination had commenced. The persistence of shedding for an extended period following the start of vaccination presents a risk for spread and recrudescence of ovine Johne's disease in sheep flocks.

Keywords:

vaccination, MAP shedding, sheep

Abstract O-03.12

EFFECT OF AGE AT VACCINATION ON MAP SHEDDING: AN EIGHT-YEAR FOLLOW-UP

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Abstract text:

The field trial on paratuberculosis vaccination in the Basque Country started in 2005 has been highly satisfactory for both farmers and regional animal health authorities and currently includes 21 vaccinated (VH) and 5 control herds on a Test & Cull (TCH) strategy. Here, we report the differences on fecal shedding and on tuberculosis Cervical Comparative Skin Test (CCST) diagnostic test related to age at vaccination. A commercial inactivated vaccine (SilirumTM, CZ Veterinaria, Spain) was applied to all animals present in the VH at the time of joining the trial, and then to all calves intended for replacement within their first 3 month of life (YV). At first herd visit and then yearly, faecal samples from animals older than 24 months were taken and tested by rtPCR. Simultaneously, the CCST was carried out according to European legislation. Thus far, 10842 CCST readings and 8149 faecal PCR results have been recorded. Starting with a fecal PCR prevalence of 10.61% and 13.14% in TCH and VH, respectively, two last years rate of shedding in animals vaccinated when older than 6 months (AV) was 5.07% (n=335) and 0.00% (n=128), respectively (p=0.0049) and 4.76% (n=63) and 0.37% (n=270) in YV (p=0.0225). No significant differences in shedding according to age at vaccination were observed (p=1). Age did not significantly affected the shedding (p=1), nor the overall frequency of positive reactions in the CCST (0.20% YV vs. 0.47% in AV) (p=0.1971). In conclusion, vaccination at both ages was similarly effective, and no clear reduction in CCST was obtained from early vaccination.

Keywords:

vaccine, cattle, shedding

Abstract O-03.13
ELIMINATION OF MAP FROM COWS WITH JOHNE'S DISEASE BY DAILY TREATMENT WITH DIETZIA SSP. C79793-74

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Abstract text:
The objectives were to determine whether Dietzia ssp. C79793-74, shown by Richards to inhibit growth of MAP under in vitro culture conditions, has therapeutic value for paratuberculosis. Animals were obtained from several local herds with evidence of disease based on positive serology and/or fecal shedding. Sixty-eight cows with initial evidence of Stage II or III paratuberculosis and two with an initial Stage IV disease were evaluated longitudinally. Animals were either treated daily with variable, disease-dependent doses of Dietzia (n = 48) or left untreated (n = 22). Clinical aspects of disease (diarrhea, emaciated, cachectic, appetite) and paratuberculosis parameters (serology ELISA/AGID and fecal culture assays done by Allied Monitor) were monitored over the lifetime of each animal until they either recovered or required euthanasia. The results indicated that daily treatment with Dietzia: (a) caused a longitudinal decline in ELISA values; (b) prolonged survival, the length of which was associated with initial ELISA values; and (c) reduced both ELISA and fecal shedding values to levels indicative that MAP was systemically eliminated. Unfortunately, this treatment modality is not cost-effective for producers. Therefore, assessment of whether Dietzia would prevent development of parameters indicative of paratuberculosis in calves naturally exposed in utero, at birthing and/or as neonates (colostrum) was undertaken. The results indicated that (a) 18 of 24 calves, untreated or treated with non-viable Dietzia, developed positive parameters as adults, (b) a daily, 60-day treatment with viable Dietzia prevented development of parameters in 10 of 10 animals. Thus, it is concluded that this cost-effective treatment, in combination with good management practices, has the potential to eradicate MAP from animals/herds. Such a consequence should significantly reduce human exposure to MAP, which in turn, could have relevance for the controversial role of MAP in multiple human diseases.

Keywords:
Dietzia probiotic, MAP elimination, cures

Abstract O-03.14: PERSPECTIVE
CONTROLLING INFECTION BY MYCOBACTERIUM AVIUM PARATUBERCULOSIS IN DAIRY COW POPULATIONS, MYTH OR REALITY? LESSONS LEARNT FROM EXPERIENCE AND REASONS FOR HOPE

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Control programmes based on understanding the epidemiology of infection by Mycobacterium avium paratuberculosis (Map) have been developed for more than 20 years. Their evaluation is quite complex: intervention studies have to last several years, because of the delay between improved prevention and reduction of observable rate of new infections (mainly due to the long latency and incubation periods). Moreover, when the infection persists in a herd, it is difficult to disentangle effects of incomplete compliance, insufficient effectiveness of preventive measures, and repeated introduction of the pathogen in a herd via purchase of latently infected animals. Still, learning from on-going efforts in the control of Map infection is an essential step to improve control programmes and to evaluate potential added value of existing and foreseen control measures.

1. Results of control programmes in commercial conditions

After several years of implementation, in many countries, programmes aim at reducing the prevalence of infection and at keeping it at a low level (and therefore at reducing its adverse consequences on health and performances) rather than at eradicating the pathogen which seems very difficult to achieve with Map.

There are a few programmes aiming at certification of freedom of herds, but in areas where Map infection is endemic they seem to have little to moderate success: often, only a small proportion of farmers enrol, probably because costs are high compared to benefits they can expect. Moreover, farmers and vets have to face reoccurrence of positive animals in herds previously certified free. This can be either associated with purchase of infected animals, or because some latently infected animals have remained undetected and the exposure in the herd hasn't been totally prevented.

In infected herds, results of intervention studies over several years are available for programmes based on eliminating infected animals (test-and-cull measures) and reducing risk of exposure of replacement calves. Different approaches to detect animals and to assess and reduce risks are proposed, but they all rely on similar principles and are progressively adapted as knowledge on Map transmission and tools to detect infection are developed. In many cases, the observed reduction in the within-herd prevalence is mainly due to culling of the positive animals, although a few studies also show a reduced incidence of infection in the cohorts of animals born after implementation of the programme. Nevertheless, efforts again can be jeopardized by introduction of infected animals. This is a specific difficulty in Map control: (i) sensitivity of diagnostic tests is very poor in younger animals, i.e. the ones that are traded between farms for replacement, (ii) test-and-cull measures often induce or increase the need to purchase animals in order to maintain the herd milk production and no or few animals from 'safe' sources are available on the market. Whatever the studies, within 6 to 10 years, prevalence of infection decreases on average, but Map is generally not eliminated from infected herds in that time lapse.

2. New insights to evaluate control programmes

In addition to intervention studies, modelling studies have been developed to predict long-term results of control programmes, simulating the population dynamics of a herd and the transmission

and effects of Map infection in the herd. These models can focus on the epidemiological effectiveness of a control programme, evaluated by reduction in prevalence and incidence or probability of and time to clearing a herd from Map (the absence of reintroduction being easily simulated) or assess its efficiency considering expected costs and benefits over a long-term horizon (e.g. by comparing net present value of different options). The first transmission models were developed in the early 90's and since then, new models have incorporated new knowledge on progression across infection stages, transmission routes, and quantification of the force of infection and of the effects of risk factors to define model parameters. Interestingly, models can account for many different situations and potential interactions. Then it is possible to rank different options for control and to determine what herd conditions can influence such rankings (the best option can indeed vary between herds). Moreover, in modelling studies, it is possible to simulate control options which are still incompletely known or assessed. For example, progress which can be expected from use of a vaccine has been simulated using different assumptions on the effects of the vaccine (e.g. reduced sensitivity to infection, reduced or delayed shedding of Map, etc.). Such approaches enable to determine the minimum effects a measure must have in order to provide effective results at a population level. Overall, modelling studies provide increased evidence of possible results of control programmes in a variety of situations, integrating available knowledge and data.

Another field of research has recently developed to better understand farmers' decisions and motivations. Indeed, compliance is often reported as being partial in farms enrolled, while uptake of voluntary Map control programmes can remain low. Means should be found to achieve good compliance for measures which are expected to provide the highest return in a given herd.

3. Perspectives from improved use of existing methods or from new control methods

Many control measures exist but they should be further assessed to evaluate their results in cow populations. First, better quantifying the effectiveness of preventive measures in reducing the likelihood of infection of young stock is still needed. Second, farmers and vets expect results on efficiency and not only effectiveness. Such assessments should account for farmers' decision-making as it is likely that Map control programmes will remain voluntary and full compliance will never be achieved. At a population level, possible effect of incentives on decisions and finally on prevalence of Map infection could be of interest.

Better understanding of the host response to Map and its determinants could provide new methods for control. Genomic studies aim at enhancing the ability of cattle to cope with exposure to Map via genetic selection. Other possible control actions, such as use of probiotics to reduce infection of exposed calves, are proposed. Their effectiveness in commercial settings should be evaluated before being considered as options to include in control programmes. As for existing measures, it is very likely that at best they might reduce the probability of being infected or becoming infectious when exposed. The expected rate of reduction should be quantified.

In conclusion, simple control programmes (based one only a few key measures) are not effective enough for Map control. Rather, programmes combining several control measures are more likely to be effective at a population level. Because they are more complex and difficult to implement, a special attention should be paid on understanding farmers' motivations, to obtain a good compliance. Besides, costs of the programmes can still be problematic in many circumstances and a better evaluation of cost-effectiveness is needed. Research to improve existing tools and to develop new methods is active. Combining results of experiments, data from field intervention studies and modelling studies to evaluate the long-term effectiveness and efficiency of control options should bring evidence of effective pathways to achieve a sustainable control of Map infection in dairy cow populations.

P-03 Control Programs

Abstract P-03.1

IMPACT OF CHANGING MANAGEMENT PRACTICES ON THE INCIDENCE OF FECAL EXCRETION (FE) OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS (MAP) IN DAIRY HERDS ENROLLED IN THE QUÉBEC VOLUNTARY PREVENTION AND CONTROL PROGRAM (QVPCP)

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Abstract text:
It is generally accepted that implementing changes to reduce the risk of transmission at the farm level should reduce the incidence of MAP. Our objective was to assess the impact of changing management practices on the incidence rate of MAP FE in animals born before and after the enrolment in the QVPCP. Twenty-one MAP positive dairy herds were enrolled in a 5 years longitudinal study. After an initial risk analysis, recommended changes (RC) were provided to all dairymen. Herds were visited once a year to take environmental and individual fecal samples. They were cultured in modified Middlebrook 7H9 liquid medium with Mycobactin J by the BACTEC™ MGIT™ 960 system. The prevalence and incidence rate of FE were determined at the individual and herd level. Herd prevalence varied from 0 to 20%. Herd incidence rate varied from 0 to 7,7 cases per 100 cow-year. Incidence rate of FE in the cohort born before the enrolment was 0,7 cases per 100 cow-year compared to 0,2 in the cohort born after (after 3 years of the study completed). The average age of the 2 cohorts were different (3,6 years versus 5,8 years). RC were implemented about 50% of the time. This percentage was however variable from one farm to the other and amongst recommendations. The most common RC were those involving the neonatal period in particular the calving pen hygiene. Changes regarding the buying behavior appear to be the most difficult to implement. Our results suggest a positive impact of enrolment in the QVPCP even if all RC are not fully implemented.

Keywords:
MAP fecal excretion, Incidence, Voluntary program

Abstract P-03.2
THE NATIONAL OVINE JOHNE'S DISEASE MANAGEMENT PLAN, 20 YEARS ON

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Abstract text:
OJD was first diagnosed in NSW in 1980 and because little was known about the disease the state department of agriculture adopted a regulatory approach in an attempt to limit disease spread while at the same time undertaking surveillance to try to determine the extent to which the disease had spread. The program was initially supported by producers but, as more became affected by the impact of quarantine on the ability to move stock, and the disease was identified in other states, producers sought a more sustainable and less punitive approach. A national evaluation program was initiated in 1998 to determine a longterm approach to the disease.

The initial approach extended until 2004, at which time control and management was determined to be the most effective and sustainable approach. Since then the program has progressed through several iterations, focussing on extensive research and development projects, surveillance and disease monitoring and promoting the benefits of disease risk mitigation activities.

The program has been most recently reviewed in 2012 and, with the benefit of additional projects in the field of social research, has identified the importance of an industry driven approach supported by extension services and livestock agents. The current program provides an agreed national framework that promotes disease risk mitigation through on-farm and regional biosecurity, as well as providing access to vaccination and extension advice for disease containment and reduction in endemic areas.

The current National OJD Management Plan places a strong emphasis on communication activities and producers taking responsibility for protecting their own business interests. Producer peak councils are also working to actively manage a number of other sheep endemic diseases as part of a broad-based approach aimed at improving sheep flock productivity.

Keywords:
ovine Johne's disease, control program, biosecurity

Abstract P-03.3
THE AUSTRALIAN NATIONAL JOHNE'S DISEASE CONTROL PROGRAM - SUSTAINING THE APPROACH

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Abstract text:
Australian governments and livestock industries have partnered in the control and management of Johne's disease since 1996. During this time activities undertaken as part of the National Johne's Disease Control Program (NJDCP) have been regularly reviewed and modified to reflect changing national policies on disease control and risk mitigation.

Like other animal health programs internationally, the NJDCP has been subject to resource constraints, in the form of reduced government regulation and funding. The advisory committee has had to adapt and modify the program to continue to deliver effective disease control and market assurance.

These objectives have been achieved by seeking synergies with related animal health activities such as on-farm biosecurity practices, or by adopting constructs used in other animal health programs such as 'compartmentalisation'. Increasingly producers, individually and collectively are accepting responsibility for Johne's disease control on farm. The ongoing commitment of producers to the management of endemic disease will ensure the ovine Johne's disease (OJD) program remains sustainable into the future.

Specific examples include the expansion of abattoir monitoring to include diseases other than OJD and to provide producers with an endemic disease animal health status report.

The NJDCP now also recognises regional biosecurity plans as a valid method of delivering a level of animal health assurance for domestic trading purposes. This construct has been expanded and refined on the promotion of farm biosecurity practices.

Smaller livestock industries have fewer resources available to expend on specialised disease control programs. They have sought to modify and apply the experiences of other industry sectors in a multi-disease approach to risk assessment and the development of mitigations.

Keywords:
control program, Australia, biosecurity

Abstract P-03.4
PARATUBERCULOSIS CONTROL IN GERMANY – CONCLUSIONS FROM FIVE YEARS VOLUNTARY CONTROL IN THURINGIA AND SAXONY

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Abstract text:
Efficacy of paratuberculosis control is disbelieved due to high efforts and low chances to eradicate the disease. Usually a complex of control measures including hygiene improvement, testing, culling and trade control is recommended which might overburden the farmers. Therefore, this study aimed to demonstrate the potential of incidence reduction in a control period of five years and focused on factors influencing the effect of control measures.

A cohort of 25 herds enrolled in the Thuringian and the Saxon voluntary regional control program was monitored for reduction of cumulative incidence (CI) of MAP shedders (new cases per herd and year). Initial within-herd prevalence was >5% for each study herd. On farm-status of hygiene was recorded in a structured questionnaire by experienced veterinarians. Mean survival days of MAP shedders after notification of the test results were used to reflect farmer's compliance concerning culling.

During the control period of five years mean cumulative incidence (CI) of MAP shedders (new cases per herd and year) decreased from 11.2% (minimum: 6.2%, maximum 46.9%) to 4.7% (minimum: 1.4%, maximum 25.4%). Separate calving pens for MAP shedders (spearman rank correlation coefficient rs = 0.40, p = 0.034) and staff hygiene (rs = 0.42, p = 0.028) significantly correlated to CI reduction. To a higher extend, survival days of MAP shedders in the herd correlated (rs = -0.544, p = 0.005) to CI reduction.

From our data we conclude, that (1) annual fecal culture based herd screenings are well accepted to identify of MAP shedders (2) five years of control are a realistic period of time to halve paratuberculosis incidence (3) compliance to cull MAP shedders influences CI reduction and (4) few hygienic measures are of outstanding importance to reduce the incidence of MAP shedders.

Keywords:
control measures, incidence reduction, compliance

Abstract P-03.5

M. BOVIS INFECTION DETECTION AFTER PARATUBERCULOSIS VACCINATION IN CATTLE

Garrido J.M.*[1], Minguijon E.[1], Urkitza A.[2], Geijo M.V.[1], A. Sevilla I.[1], Elguezabal N.[1], Arrazuria R.[1], Vazquez P.[1], Vordermeier M.[3], Whelan P.[3], Juste R.A.[1]

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Abstract text:
In spite of its proven efficacy, paratuberculosis (PTB) vaccine use in cattle has been limited by interference in tuberculosis control. In order to know whether or not M. bovis infection can be detected after PTB vaccination, 2-3 month-old calves in a feedlot were tested with standard IFN- γ release assay (IGRA) and vaccinated with 1ml of an inactivated PTB vaccine (T0). After 2 new tests 1 (T1) and 3 (T2) months later, 10 vaccinated and 5 non-vaccinated calves were moved to three boxes in level 3 biocontainment facilities. One month later, 5 vaccinated and 5 non-vaccinated animals were infected with 105 cfu of M. bovis in 2 ml of PBS by the intratracheal route resulting in the following groups: PTB vaccinated-M. bovis infected (VI), only PTB vaccinated (VNI) and only M. bovis infected (INV). All animals were submitted to IGRA then (T3I) and 2 (T4) and 4 (T5) weeks and 2 (T6) and 3 (T7) months later. Then calves were necropsied and sampled for pathological and microbiological studies. In addition to the standard IGRA with avian and bovine PPD, ESAT-6/CFP-10 and Rv3615c recombinant antigens were used in all the testings. An intradermal test with all antigens was carried out only at T7. The best results in the skin test were observed with the recombinant antigens. VI and VNI groups showed a predominant avian response until M. bovis infection. Specific bovine reactions were first observed two weeks afterwards and were predominant in all the infected individuals. However, at the manufacturer cut-off, vaccinated calves were slightly less frequently detected than non-vaccinated. The cocktail ESAT-6/CFP10 was more sensitive than RV3615C which instead was slightly more specific.

Keywords:
map vaccine, m bovis, differentiation

Abstract P-03.6
DETECTION, CONTROL AND INVESTIGATION OF JOHNE'S DISEASE INCIDENT IN QUEENSLAND

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Abstract text:
A large-scale response to detection of Johne's disease (paratuberculosis) in the beef cattle industry of sub-tropical Queensland is described. Infection risks on more than 280 extensively grazed properties were assessed and managed.

The detection and response in this case are notable in many key and novel areas, including confirmation of spread and establishment of paratuberculosis in extensively grazed livestock in a sub-tropical environment, the large scale and resources committed to the response, the first detection of 'bison' strain in Australia, the use of gene sequencing to identify relationships with other known isolates, the use of national electronic records to trace cattle movements, implementation of direct faecal PCR testing, assisting individual producers to meet the impact of regulatory controls for the benefit of the wider industry, technical and logistical management of animal health risks in remote and hostile environments and the adverse impact on access to sensitive markets.

The innovative tools and techniques used to resolve the technical, logistical and political challenges are discussed.

Keywords:
Queensland, Extensive beef grazing, Sub-tropical

Abstract P-03.7

ESTIMATE AGREEMENT BETWEEN ELISA AND CULTURE RESULTS FOR MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS IN SERA AND FECAL SAMPLES.

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Abstract text:
Diagnosis of paratuberculosis (Mycobacterium avium subspecies paratuberculosis - MAP) can be a challenge primarily in latent stages of the infection. In fact, the definite diagnosis of MAP is very difficult to perform, however the enzyme-linked immunosorbent assay (ELISA) is largely used. The aim of the study was to evaluate the predictive value of serological tests, in agreement with culture results, for the diagnosis of paratuberculosis.

Blood and fecal samples were collected from 300 lactating dairy cows in two known infected Friesian dairy herds. The serological tests are performed with two commercial kits: one for screening (PourquierÒElisa Paratuberculosis Antibody Screening-Montpellier-France) and one for confirmation (PourquierÒElisa Paratuberculosis Antibody Verification-Montpellier-France), according to the protocol provided by the manufacturer.

Culture results were classify as: negative, high shedder, moderate shedder, low shedder, while Elisa results were expressed in terms of the relationship between optical density value S/P (sample to positive values) in order to quantify the degree of positivity. To evaluate the agreement between the two tests the Intraclass Correlation coefficient (ICC) was calculated using GLM mixed model and the index Kappa was also estimated.

Data showed fair agreement between Elisa and culture (ICC=0.46). Culture negative samples showed S/P average of 0.91 (CI95%: 0.58-1.23), low shedder showed a S/P average of 1.54 (CI95%: 1.24-1.84), moderate shedder showed a S/P average of 1.82 (CI95%: 1.29-2.50) and high shedder showed a S/P average of 2.34 (CI95%: 1.72-2.96). Kappa 0.66 (CI95%: 0.42-0.80).

Data showed fair agreement between Elisa and culture with these premises, serological tests, expressed as S / P, can be considered a valuable predictive tool for the diagnosis of paratuberculosis when combined with excretion as infected head became ELISA 4 months prior then culture.

Keywords:
Serological tests, S/P, valuable predictive

Abstract P-03.8
ASSOCIATION OF MAP SPECIFIC ELISA-RESPONSES AND PRODUCTIVE PARAMETERS IN 367 DANISH DAIRY FARMS

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Introduction

The impact of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection on productive parameters such as milk yield and reproductive features is central in the culling decision on individual cows. This study aimed to compare the test-day milk yield and lactation specific conception probability in 94,064 Holstein cows in 314 dairy herds from the Danish control programme on paratuberculosis (Nielsen, 2007).

Methods

Herds participating in the Danish programme are tested 4 times per year using a commercial MAP specific antibody ELISA (Nielsen et al., 2013), which has been used since 15 October 2008. All data used are from this date and forward. A sample-to-positive ratio >0.2 was considered positive.
The milk yield is recorded either six or eleven times per year depending on the farm. For each of the 314 Holstein herds, these samples were pooled to fit Wilmink lactation curves for parity 1, 2, and 3+ per herd (Wilmink, 1987), leading to a total of 942 lactation curves. These curves represent the expected energy corrected milk yield of the average cow on each farm as a function of days in milk. For each milk yield point, the value relative to the average cow on the farm was then calculated. This relative energy corrected milk yield (rECM) indicated whether a cow at a certain test date had milked as the average cow on the farm (rECM=1) or for example 5% more (rECM=1.05), or 5% less (rECM=0.95). We then tested whether rECM depended on the MAP ELISA score. We also assessed if cows with no positive ELISA test at the test date but with a future positive test would differ in rECM from cows that never tested positive. This was tested using bootstrapping with 10,000 replicates under the null hypothesis that there is no difference in means between groups.

The conception probability was tested for the first three insemination attempts between the first and second lactation period. The cows were divided into two groups: one with animals that had no ELISA positive tests during their lifespan; and one for cows with a positive test result later than the fourth insemination attempt. Thus, no cows testing positive before the four insemination attempts are included. This procedure was used to avoid that a positive test would have caused a farmer to cull the cow, which could potentially change apparent conception probability. The conception probability was then tested in a generalized linear model using the binomial family with a logit link function, with farm and positive/negative ELISA test as fixed effects. Subsequently the test was also carried out using only cows that achieved a 2nd parity.

All data handling, analysis, and plotting were done using the free open source statistical software R (r-project.org). All data were extracted from the Danish Cattle Database (Knowledge Centre for Agriculture, Aarhus, Denmark, vfl.dk) for the use in the 'iCull' project (icull.dk).

Results

Test-day milk yield was negatively correlated with increasing milk ELISA value (Figure 1). The figure shows that an increase in MAP S/P ratio is correlated with a decrease in milk production relative to the average cow on each farm. At a MAP S/P-value of 1, the rECM was 0.94, while it was 0.78 at the MAP ELISA value of 5. Differences in conception probabilities are illustrated in Figure 2.

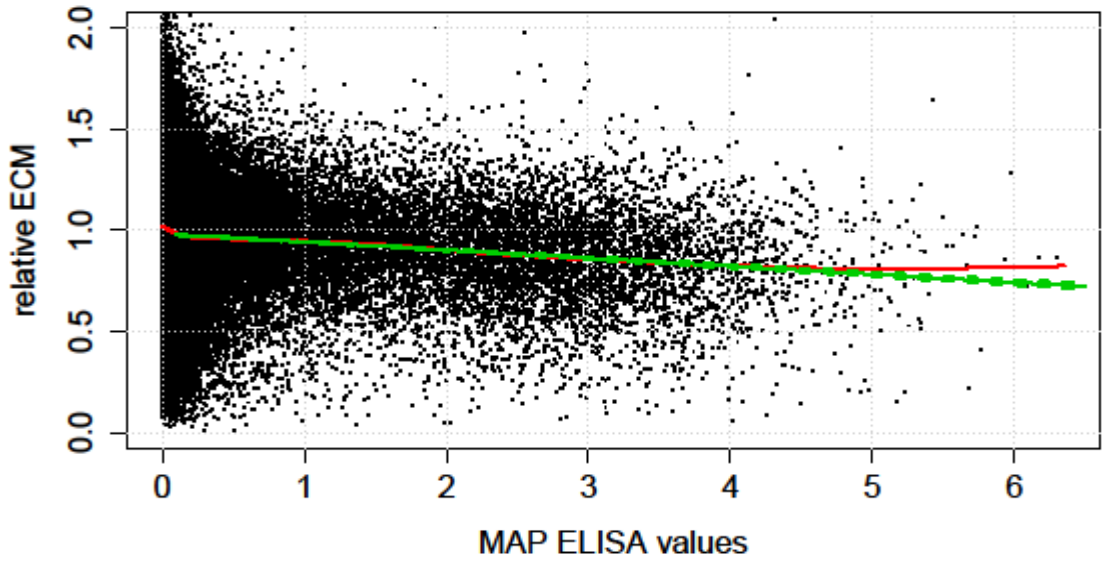

Figure 1: The figure shows the relative energy corrected milk yield (rECM), which is the milk yield relative to the average cow on each farm. The rECM is plotted as a function of the MAP S/P values measured on the same day as the rECM, based on a parity-stratified Wilmink-function. The **red line** is a local polynomial fit. The **green line** is a linear fit in the ELISA range [0.1;6.2], with the dashed green lines representing the 95% confidence interval of the linear fit.

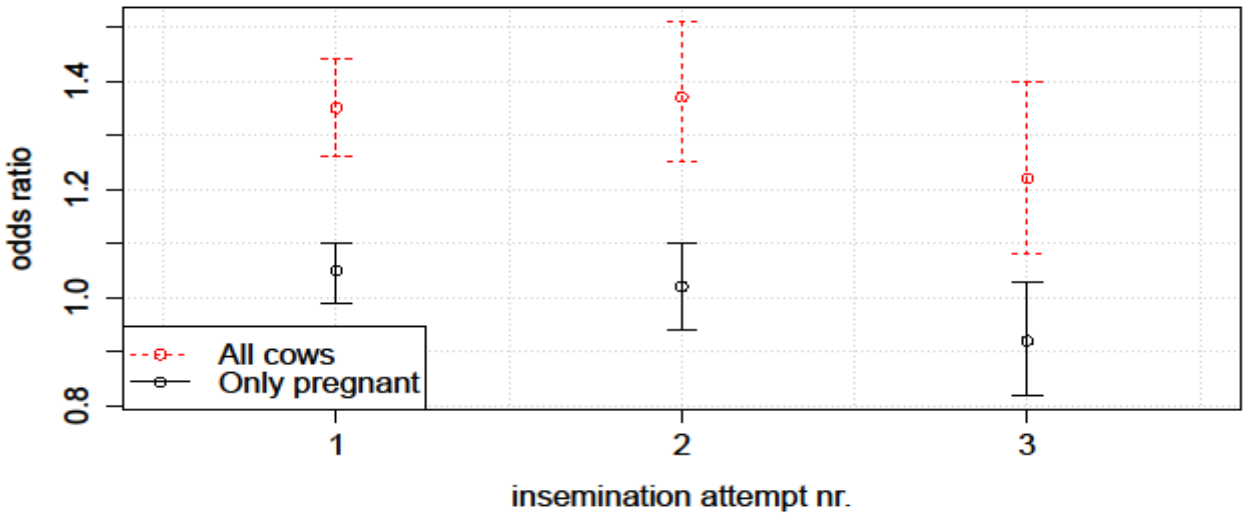

Figure 2: The odds ratio of the conception probability following inseminations 1 to 3 between first and second calving of animals that later tested MAP ELISA positive versus animals that never tested positive. The **red circles** with dashed lines represent the test with all cows, and the **black circles** represent the test with only the cows that achieved proceeding to the following lactation (parity 2), and thereby actually achieved becoming pregnant. Error bars represent the 95% confidence interval of the estimate. The test using all cows suggest that cows that test MAP positive later in life have a ~30% higher chance of a successful insemination. However, when using only cows that do become pregnant, then there is no significant difference between negative and later positive cows.

Milk production of cows prior to the positive MAP ELISA test was tested compared to cows never testing positive. Here the milk production was averaged over the lactation period and the result was that first parity cows that later tested positive produced 2.2% (95% CI: 1.7-2.6%) more milk than cows never testing positive. For the second parity cows, the numbers were 2.7% (95% CI: 1.9-3.3%), and third parity cows produced 3.3% (95% CI: 2.2-4.5) more.

Discussion

The data presented in figure 2 are biased in different ways. When including cows that do not become pregnant (red circle), we include cows that will not live for much longer as they will be culled when milk yield decreases. For this reason the discovery of a MAP infection is less likely as they do not live for long. Therefore, they will count as non-positive cows, and this group will get more cows that never become pregnant, and hence a reduced conception probability compared to later MAP positive cows. When only including cows that eventually become pregnant (black circles) then the excluded cows may have a different distributions of MAP positive animals than the included group, which may draw in either positive or negative direction. So the general problem is that we do not know whether animals are MAP positive, or rather would deliver a MAP positive sample in the future had they survived. However, basing the model design on cows that were already positive would subject the data to a potential decision bias of farmers: that they may be more likely to keep well-functioning MAP positive animals.

That future MAP positive cows produce more milk before testing positive, may be due to high-producing cows being more likely to lose control of the infection, as evidenced by occurrence of antibodies. Another potential reason is that stronger animals may be better to handle the infection. So that they produce more milk is possibly not an effect of the MAP, but because weaker animals succumb earlier to the infection, and the remaining are higher producing animals.

Conclusion

MAP infection prior to being ELISA positive does not appear to negatively influence conception, and may have a positive effect on milk yield for individual cows compared to cows at the same farm. However, positive ELISA values are correlated with a reduction in milk yield, with higher S/P values associated to greater reduction in milk yield.

Data originating from a database should be analysed with caution, because bias may enter in many forms such as bias resulting from farmers' decisions to cull infected but not positive cows, ELISA positive but non-affected (on milk yield or conception) cows etc.

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Keywords:

reproduction, milk yield, economic control

Abstract P-03.9

COMPARISON OF PARTICIPANTS AND NON-PARTICIPANTS IN A VOLUNTARY JOHNE'S DISEASE CONTROL PROGRAM IN ONTARIO, CANADA

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Abstract text:
A voluntary Johne's Disease control program for dairy cattle herds was launched in Ontario, Canada in 2010 (www.johnes.ca). The program included an on-farm risk assessment, a single funded milk or serum test of each cow in the herd and permanent removal of high test positive animals. Of 4,158 herds in the province, 2,153 completed all three parts (participants). Production and management data collected by the Dairy Farmers of Ontario and CanWest DHI were used to compare participants to non-participants to understand how best to increase future program participation. Additionally, using a unique ELISA test, a bulk tank sample from each herd in the province was tested once. The results of the ELISA test were used to determine if the prevalence of positive bulk tank tests differed between the 2 groups.

In the participant herds, 1% of cows tested positive and 26% of herds had at least one test positive cow. Just under 0.1% of all tested cows had high positive test results and were targeted for permanent removal. Compared to non-participant herds, participants were significantly larger (79 cows versus 74), produced more milk per cow ($6,666 milk value versus $6,378), produced higher quality milk (225,000 versus 246,000 bulk tank average somatic cell count) and had better management indicators (lower age at first calving and higher pregnancy rate). The prevalence of positive bulk tank tests was 5% higher in the non-participant group.

As the non-participants were more likely to be JD bulk tank test positive than the participants, there is a need to develop effective strategies to increase participation in the program by the dairy producers that have not yet participated.

Keywords:
Voluntary, Control, Participation

Abstract P-03.10
FINANCIAL IMPACT AT SLAUGHTER OF OVINE JOHNE'S DISEASE (OJD) IN A MERINO FLOCK IN AUSTRALIA

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Abstract text:
Aim: To estimate the financial loss at slaughter due to sub-clinical OJD in an infected merino flock in Tasmania.

Method: 278 mixed age merino ewes and wethers were presented for slaughter in November 2011. They were drafted into 2 groups based on skin quality: Group A - 217 sheep, good skins, Group B - 61 sheep, poor quality skins. Prices quoted are in Australian dollars determined at the time of slaughter.

Results: OJD was confirmed by histopathology on 3 representative ileum samples. The total potential value of each sheep to the producer was calculated at $88-55 based on an average carcase value of $66-00 (22kg carcase at $3-00/kg) and a skin value of $22-55. This yielded a total potential value of $24,617 for the 278 sheep. Thickening of terminal ileum attributable to OJD was seen in (22%) in Group A and 61 (100%) of sheep in Group B. Losses in Group B attributable to reduced carcase weight, meat value/kg, bone out percentage (49% versus 64%)and skin value ($11-10 v's $22-55) resulted in an estimated loss for Group B of $2127 (39% loss) and $1269 (7% loss) in Group A – a total loss of $3396 (14%).

The processor lost $568-70 (44% of potential value) from condemned runners in addition to the reduced bone-out percentage and the decreased efficiency of slaughtering lower weight/meat value carcases.

Conclusion: Lower condition score/carcase weight and poor skin quality are well recognised problems attributable to OJD. Vaccination and improved management are the primary tools for control and prevention of OJD. The cost to vaccinate these 278 sheep as lambs at $2-20 per head would have been $611-00 plus labour costs yielding a benefit cost ratio to the producer of >5:1.

In summary, sub-clinical OJD can incur major losses at slaughter both to the producer and the processor.

Keywords:
sub-clinical OJD, financial loss, slaughter

Abstract P-03.11
ESTIMATED ECONOMIC LOSSES DUE TO OVINE PARATUBERCULOSIS IN NEW ZEALAND

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Abstract text:
Ovine Johne's disease (OJD) is the clinical manifestation of infection of sheep with Mycobacterium avium subspecies paratuberculosis (MAP). Production loss occurs through deaths, premature culls of clinical animals, reduced carcass weight at slaughter, reduced fertility and lower growth rates. About 78% sheep flocks are infected with MAP whereas only few flocks experience a significant incidence of clinical disease, hence it has commonly not been regarded as a major production limiting factor. The objective of this work was to assess the economic loss attributable to ovine paratuberculosis in commercial lamb producers in New Zealand.

Firstly, we developed a mathematical model simulating the dynamics of OJD in a sheep flock, based on available data about the patho-physiology of MAP infection in sheep and considering the typical New Zealand sheep farming system with seasonal management of lambing, culling and replacement. Secondly, published reports about the production impact of OJD were used to inform the model about economic consequences of OJD. This model was then used to estimate the total economic loss associated with different levels of on-farm clinical incidence. Finally, we combined the results of simulation with demographic data of the sheep population in New Zealand and a range of assumptions concerning the distribution of clinical incidence of OJD in New Zealand sheep farms. Assumptions were based on nationwide farm-prevalence field studies and field estimates of the extent of clinical cases of OJD on properties infected by MAP. Results will be presented and discussed at the conference.

We conclude that modelling infection dynamics is useful for estimating economic impact and could become useful for simulating benefit-cost outcomes of interventions for disease control.

Keywords:
production effects, economics, modeling

Abstract P-03.12

APPLYING SOCIAL SCIENCE TO THE DEVELOPMENT OF HACCP-BASED TOOLS FOR THE CONTROL OF PARATUBERCULOSIS

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Abstract text:

Hazard Analysis and Critical Control Points (HACCP) is a preventive risk management approach that has been used throughout all stages of processing in EU food chains since 2004. The use of HACCP at the level of primary food production and specifically as a strategy to assist in disease management on farms is relatively limited but has been applied, for example, in mastitis control in Irish dairy herds (Beekhius-Gibbon et al., 2011). In the literature thus far, a crucial aspect of monitoring and optimising the application of the HACCP approach at farm-level has been the incorporation of sociological considerations relating to farmer behaviour (Beekhius-Gibbon et al., 2011). Sociological studies of farmers' attitudes and knowledge are systematically used to enhance the effectiveness of agricultural extension and stand to be incorporated to the design and approach of a wider range of veterinary disease control programmes. This paper, building on previous research, draws from an extended suite of qualitative research methodologies and sociological theories to generate a comprehensive framework for the design of HACCP-based tools for the control of paratuberculosis at farm-level. Guided by Participatory Learning and Action (PLA) approaches, which facilitate multi-disciplinary and inter-professional collaboration, we set out a methodology for synergistic co-design among scientists, veterinarians and farmers of HACCP-based tools for direct application on farms. Drawing from a vast sociological literature on farmer behaviour, we identify key themes that represent critical considerations for the successful implementation of HACCP-based paratuberculosis control programmes at farm-level. The proposition of our paper, endorsed by the corresponding literature on agricultural innovations, is that through pursuing a participatory co-design approach informed by sociological theories on behaviour, the HACCP-based tools will be more implementable by veterinarians and more interpretable and adoptable by farmers.

Keywords:

HACCP, social science, behaviour

Abstract P-03.13

EVALUATION OF DIAGNOSTIC TESTS TO CLASSIFY CATTLE, IN CONTROL PLANS, ACCORDING TO THEIR LEVELS OF EXCRETION OF MYCOBACTERIUM AVIUM PARATUBERCULOSIS.

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Abstract text:

The purpose of this study is to evaluate several tests for identification heavy shedders of Mycobacterium avium subspecies paratuberculosis (MAP) on 2692 cattle from 21 Normandy's dairy herds. Semi-quantitative results qPCR kit (ADIAGENE) on individual fecal sample (Ct value) and ELISA test (IDEXX) on serum (%E/P value) are compared to quantitative fecal culture considered as a Gold Standard (with positive + < 100 colony-forming unit (CFU) per gram, positive ++ between 100 and 500 CFU, positive +++ > 500 CFU). The sensitivity and specificity with optimal threshold of ELISA (résults +++) and PCR (results +++) are then calculated to detect heavy shedders (cows shedding more than 500 CFU). A positive correlation can be observed between the level of seropositivity (%E/P), the semi-quantitative PCR result (Ct value) and the culture. Serological ELISA test (%E/P > 125 % with Se = 0.89, Sp = 0.91 and area under the ROC curve: AUC = 0.89) and qPCR (Ct <31 with Se = 0.89, Sp = 0.96, AUC =0.96) detects with a relative confidence the heavy shedders in coproculture (>500 CFU). Furthermore, the prevalence of high Ct value was associated with prevalence of positive PCR inside a herd (Spearman correlation coefficient = 0.882, p-value < 0.0001).

Samples from 4 sampling sites per herd have also been analyzed in PCR and the results (Ct) was compared to herd heavy shedders serological (ELISA +++) prevalence. A correlation (Spearman correlation coefficient = -0.597, p-value 0.017) was observed. So, PCR on environmental select samples could be used as an indicator of heavy shedders prevalence in control's program.

In conclusion, ELISA and PCR can be used to detect the MAP heavy shedders with a high efficacy. The good correlations between the prevalence of results with low Ct value, overall livestock MAP PCR prevalence and PCR results obtained from environmental samples suggests the interest of reforming the heavy shedders in priority.

Keywords:

paratuberculosis, diagnostic, heavy shedders

Abstract P-03.14
ACTIVITIES PRECEDING A DECLINE IN THE PARATUBERCULOSIS TEST PREVALENCE

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Abstract text:
A voluntary control programme on Mycobacterium avium subsp. paratuberculosis (MAP) was initiated in Denmark in 2006 and has since 2007 included 25-28% of the dairy herds and 35-40% of the dairy cows. The programme was complemented with activities aimed to reduce the MAP infection prevalence. A challenge in evaluation of activities in a national programme it is essentially a sample size of one without a control group. Therefore, the apparent effect of activities on programme level can only be descriptive. Our objective was to describe the decline in the test-prevalence along with the activities preceding this decline.

The cohort of herds enrolled in 2006-2007 had an average estimated within-herd test-prevalence of 10% at start. By January 2014 this had declined to 2%. The test-prevalence in the cohort of herds enrolled in 2008-2010 started at approximately 6% and by January 2014 was reduced to about 3%. In addition to these data, a “sampling error” in August 2011 resulted in the availability of a sample of 99 herds that were not enrolled in the programme. They had a median test-prevalence of 5.5% compared to 3.1% in 268 herds from the 2006-2007 cohort tested in the same month. This sample and associated estimates were used to validate that a test prevalence reduction was due to the programme

The decline in test-prevalence has been statistically associated to herd-level factors such as low level of livestock purchases and culling of cows deemed to be infectious, but not really to other management factors. On national level, the first initiatives included training of herd health advisors, information campaigns including various sources of information material and farmer’s meetings. In the last approximately 5 years, the programme has run routinely with few specific extraordinary activities, except for a “continuous” flow of diagnostic test information four times annually in each herd. This information can be used for detection and management of infectious animals and for prevalence monitoring.

Keywords:
test prevalence reduction, control programme, cattle

Abstract P-03.15

A NATIONAL CONTROL PROGRAMME FOR JOHNE'S DISEASE IN NEW ZEALAND FARMED DEER, FROM 2007 TO THE PRESENT

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Abstract text:
Deer farming in New Zealand is a relatively new agricultural industry with the first farms established in the 1970s. Its growth has remained driven by the export of venison. Today the industry's approximate size is 3000 farmers managing one million deer and processing 3-400,000 annually for export.

The first confirmed case of Johne's disease (JD) in a New Zealand farmed deer was in 1986. Described as a rare occurrence in the early 1990s, it became an increasingly serious issue throughout the late 1990s and the early 2000s. Mortality rates in groups of young animals in excess of 10% were reported.

In response, the deer industry established Johne's Management Limited (JML) in 2007. JML is a small biosecurity company owned by the group of deer processing companies.

It has five main functions. Firstly, it is responsible for the administration and monitoring of a national slaughterhouse surveillance database, also established in 2007, to record lesions suspicious of JD in deer. Secondly, it coordinates a nationwide network of veterinarians with specialist training in the management of Johne's disease in deer, the Johne's Consultant Network. Thirdly, it communicates directly with deer farmers to link affected properties with network veterinarians. Fourth, it works with farmers and veterinarians to develop tailor-made on-farm Risk Management Plans for JD. Fifth, it supports but does not fund research utilising the slaughterhouse data which now exceeds three million individual animal level records.

The database structure, function, and validation are described in an associated paper.

The emergence of JD in the deer industry represents a rare opportunity to study this disease in a new and naive farming system. This paper will discuss industry and research data, highlighting interesting observations over eight years of monitoring and control efforts.

Keywords:
deer, control, New Zealand

Abstract P-03.16

VALIDATING A NATIONAL SLAUGHTERHOUSE SURVEILLANCE DATABASE FOR JOHNE'S DISEASE AGAINST ON-FARM DISEASE SEVERITY IN FARMED DEER IN NEW ZEALAND

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Abstract text:
Deer farming in New Zealand was first established in the 1970s, it's subsequent growth driven by the export of venison. Today there are approximately 3000 farms, one million deer, and 400,000 carcasses processed annually.

Johne's disease (JD) in New Zealand farmed deer was first confirmed in 1986 and emerged as a major risk to the Deer Industry.

Deer mesenteric lymph nodes (MLN) with macroscopically visible abnormalities and/or a circumference of >55mm have >95% likelihood of MAP infection. They are termed JD-suspect lymph nodes (JDSLN). Grossly normal MLN have a 45% likelihood of MAP infection. JDSLN are recorded by meat inspectors during routine carcass inspection with sensitivity and specificity measured at 13.3% and 99.9%.

In 2007 a national slaughterhouse surveillance database for JDSLN was established. It now holds 3.1 million animal-level records (>99% of deer processed) including age, sex, carcass weight and farm of origin, plus the location of JDSLN. Data are analysed quarterly to report spatial and temporal trends in the incidence of JDSLN to the industry and to identify and prioritise farms to assist with JD control. At a prevalence range of 0.2% (low season) and 1.0% (high season), the accuracy of predicting MAP infection through JDSLN scoring was 73-94%.

The relationship between JDSLN incidence and on-farm JD severity is a key uncertainty. To validate this, a phone survey was made of 150 farmers, representing 28% of deer processed in the 2012/13 production season. Farmer perception of mortality rate due to JD in that production season was recorded in addition to stock numbers, farm size, and demographic data. Economic impact of the disease on-farm was estimated using a standardised spreadsheet. Data on farmer perception of JD was also collected for analysis using 1000minds© methodology.

Results of validating JDSLN incidence against on-farm mortality rate and economic impact will be presented and discussed in this paper.

Keywords:
deer, New Zealand, control

Abstract P-03.17

COMPARING RISK FACTORS FOR ELISA PREVALENCE AND RECOMMENDATIONS FOR JOHNE'S DISEASE PREVENTION BETWEEN ORGANIC AND CONVENTIONAL DAIRY FARMS IN ONTARIO, CANADA

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Abstract text:
The aim of this study was to investigate differences between organic (ORG) and conventional (CON) dairy farms in terms of risk factors for Johne's disease (JD) milk ELISA test positive prevalence and recommendations for JD prevention.

Risk Assessment and Management Plan (RAMP) scores, recommendations, and herd ELISA results were available for analysis from the Ontario JD Program (www.johnes.ca) database. Data from 2103 herds including 42 ORG herds were used for descriptive statistics and multivariable mixed effect logistic regression analysis.

Overall, herd-level and within-herd prevalence did not differ between ORG and CON herds. However, if at least one positive cow was present, within-herd prevalence was higher in ORG herds. The overall RAMP score was not different between ORG and CON herds. ORG herds had higher RAMP section scores (indicating higher risk for JD transmission) in the areas of calving management and pre-weaning calf management and lower scores in the area of biosecurity. Generally, there was a positive association between RAMP section scores and the odds of receiving a recommendation for management change in that area. However, after accounting for the section score, ORG farms were less likely to receive recommendations in the calving and pre-weaned calf area.

The results indicate that between-herd transmission risk is not different, but within-herd transmission could be higher in affected ORG compared to CON herds. ORG farms conceivably have a different risk profile for JD transmission; however, recommendations given to the producers might not necessarily reflect those differences.

Keywords:
risk assessment, management recommendation, organic farming

Abstract P-03.18
PARTICIPATION IN THE ALBERTA JOHNE'S DISEASE INITIATIVE IS ASSOCIATED WITH HERD SIZE, BUT NOT WITH MAP HERD PREVALENCE OR ENROLLMENT IN A DHI PROGRAM

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Abstract text:

Introduction: At time of study initiation, approximately 60% of 591 Alberta dairy farmers participated in the voluntary Alberta Johne's Disease Initiative (AJDI), a management-based control program for Johne's disease. The objective of this study was to assess herd prevalence of participants and non-participants and investigate whether involvement with the AJDI is associated with *MAP* prevalence, herd size or enrollment in a dairy herd improvement (DHI) program.

Methods: Six environmental samples each were analysed from 239 farms enrolled in the AJDI and from 93 farms not enrolled in the AJDI. Samples were collected from September 2012 until December 2013 and processed using a standardized 3-day decontamination protocol that was followed by 48 days of culture using a TREK ESP culture protocol. All culture products were then analysed with conventional IS900 PCR. Number of lactating cows and use of milk recording programs were self-reported by the farmers. Herd information was collected at a farm visit using a standardized questionnaire.

Results: The apparent herd prevalence was 54% (95% CI: 47-60%) for participating farms and 51% (95% CI: 40-61%) for non-participating farms (p= 0.55). Participating farms had a geometric mean of 88 lactating cows (95% CI: 81-96) and non-participating farms had a geometric mean of 102 lactating cows (95% CI: 96-108 (p<0.01)). Of the participating farms 82% (95% CI: 77-87%) was enrolled in a DHI program compared to 76% (CI: 69-82%) of the non-participating farms (p=0.11). Conclusions: Participation in the AJDI is associated with herd size, but not with MAP herd prevalence or the use of DHI milk recording.

Keywords:
paratuberculosis, participation, prevalence

Abstract P-03.19

EXTENSION COMMUNICATION PREFERENCES OF ALBERTA DAIRY PRODUCERS

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Abstract text:

Introduction: The Alberta Johne's Disease Initiative (AJDI) is a management-based control program launched in 2010 with the aim to make producers more aware of Johne's disease and reduce disease transmission. Despite ongoing efforts to enhance farmers' participation, approximately 40% of all Alberta dairy farmers were not involved in the AJDI at time of study initiation. Aim of the study was to investigate extension communication preferences of these "harder to reach" producers.

Methods: Surveys including questions about communication preferences were conducted with Alberta dairy farmers that did not participate in the AJDI. All 250 non-participating farmers were contacted and 164 (66%) producers agreed to be interviewed in person. The interview was conducted using a standardized questionnaire with 38 questions that was first evaluated on 10 farms.

Results: On a scale from 1 to 5, dairy farmers regarded the veterinarian as most important source for information with a preference score of 3.6. Various other sources, such as newsletters, farm media, fellow producers or direct communication by mail and phone received scores ranging from 2.9 to 3.4. Seminars and workshops, as well as communication by email were rated least important with scores ≤ 2.6. With increasing age, years as producer, education and decreasing years until retirement, especially seminars were seen as less attractive. However, travel distance or time was not associated with producers' willingness to attend seminars.

Conclusions: Veterinarians play an important role as trusted advisers and are potent mediators between research and producers. Additionally, different communication strategies should be applied to target specific producer groups based on their preferences. Media, such as newsletters or radio, has great potential to reach a wide spectrum of producers. Seminars need to be attractive, particularly to older and more experienced farmers, in order to reach them effectively.

Keywords:
Communication, Extension, Control Programs

Abstract P-03.20
THE ONTARIO FOCUS FARM APPROACH TO JOHNE'S DISEASE CONTROL

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Abstract text:
This study evaluated the impact of a participatory-based, experiential learning program, Ontario Focus Farms (FF), which aimed to accelerate the adoption of on-farm management practices for Johne's disease (JD) control in the Ontario (ON) dairy industry. The FF approach is a learner-centered process, which utilizes an adult learning framework to facilitate behavioural change. Producers engage in 4 full-day sessions, where small groups discuss their on-farm problems and learn using many techniques (e.g. farm tours, risk assessments). Pre-post questionnaires collected data on 70 FF and 62 control respondents' perceptions, knowledge, attitudes, and behaviours; pre-post risk assessments were used to assess respondents' on-farm risk of JD transmission. Between 2010 and 2013, over 200 producers, across 8 regions of ON, participated in FF. Respondents held strong positive attitudes towards JD control and felt a moderate amount of social pressure from various sources to make on-farm changes. However, they questioned their ability to effectively control JD on the farm. Both groups exhibited a moderate level of knowledge on JD, on an objective knowledge assessment prior to the intervention period, with a median score of 75.9% (22/29). FF respondents significantly improved their score at the post-intervention, with a median of 82.8% (24/29); control respondents did not significantly change. The proportion of FF participants who reported making at least one on-farm change (81%) was significantly higher than that of control respondents (38%). Overall, FF respondents significantly lowered their risk score in 4 out 5 risk areas and had an average reduction of 13 points in their overall risk score between pre and post measurements; control respondents did not significantly change. The FF process was effective at influencing the adoption of on-farm management practices for JD control. Future JD control programs should consider the implementation of learner-centered extension processes, such as FF, to influence producer behaviour.

Keywords:
Control program evaluation, Behaviour change, Extension and communication

Abstract P-03.21
ONTARIO DAIRY PRODUCER PERCEPTIONS AND ATTITUDES TOWARDS JOHNE'S DISEASE CONTROL

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Abstract text:
This study investigated Ontario (ON) dairy producer, and veterinarian, perceptions and attitudes regarding Johne's disease (JD) control, and used this information to inform a novel online communication approach for producer education. In 2012, 8 focus groups were conducted with ON dairy producers, and 2 with regional veterinarians. Each group was comprised of 4-8 participants, a moderator, and a note-taker. Discussions were audio recorded and professionally transcribed. Thematic analysis was used to analyze the data; each transcript was read line-by-line and coded (i.e. labeled based on meaning). Codes from all transcripts were then analyzed for similarities and grouped into themes. With this information, a series of 'whiteboard scribing' videos were developed. They relay a simple message through a narrated script, which is cued to a series of time-lapse whiteboard drawings that match the speed of the narration. An important theme was 'barriers to the adoption of JD control measures', where producers and veterinarians discussed how physical barriers (e.g. time, money, infrastructure) and intrinsic barriers (e.g. priority, habits, motivation) inhibit on-farm adoption. Respondents highlighted that many producers do not view JD control as a priority, mainly because they don't perceive it as an on-farm problem. Producers also suggested that many on-farm recommendations are impractical and/or ineffective. They further suggested that lack of motivation is a barrier, and both extrinsic motivation (e.g. incentives, penalties, extension) and intrinsic motivation (e.g. pride and responsibility) is necessary for widespread change. Three videos were created to address these perceptions, and communicate the importance of, and methods for, JD control; the first of which is titled 'Johne's Disease in Canadian Dairy Herds: What it means for farmers' (bit.ly/HJhnjv). JD control programs can benefit from a better understanding of the specific perceptions and attitudes held by producers and veterinarians with respect to JD control.

Keywords:
Barriers to Johne's control, Producer perceptions and attitudes, Social epidemiology and extension

Abstract P-03.22
ASSESSING THE IMPACT OF SEED-STOCK PRODUCERS ON THE SPREAD OF BOVINE JOHNE'S DISEASE IN THE AUSTRALIAN BEEF INDUSTRY

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Abstract text:
Bovine Johne's disease (BJD) in Australia mainly occurs in dairy cattle herds in the south-eastern States. Infection in beef cattle herds is generally at a very low prevalence. Traditionally, contact with the dairy industry by grazing land previously grazed by adult dairy cattle or through introduction of dairy-cross animals has been considered the major risk for the beef industry. However, a number of recent incidents have identified that beef seedstock producers pose an additional potential risk for commercial producers that has not been well documented previously in Australia.

Since 2004 a financial and non-financial assistance (FNF) package has operated to assist affected beef producers. Further analysis of FNF data has been undertaken to assess the potential impact of seedstock producers on the spread of BJD in the Australian beef industry.

Of 176 infected herds identified, 36 (20%) were recorded as seedstock producers. This is a substantial over-representation compared to the expected percentage of seedstock producers in the beef industry as a whole.

Until recent years, recording of specific trace-forward animal movements has been incomplete, because the significance was not well understood and because producers who choose not to register with the FNF are not included in the data. As a result, 7 of 36 seedstock herds have tracings recorded. Numbers of tracings per herd range from 2 to 40 (3 herds >=20). Although the proportion of traces resulting in confirmed new infections is low (<5%), seedstock herds often sell many breeding animals each year to multiple purchasers, leading to opportunities for significant spread of disease. Commercial producers should now consider the level of assurance provided by seedstock producers as part of their business risk management strategy.

Keywords:
seedstock, disease risk, beef cattle

Abstract P-03.23

STRATEGIC OPTIONS FOR THE CONTROL OF PARATUBERCULOSIS IN DAIRY HERDS IN SOUTH WEST ENGLAND

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Abstract text:

Introduction

The paper will describe a regional herd health initiative in the South West of England which engaged 322 dairy herds in a Johnes Disease management program.

Method

Herds were classified into risk categories, and their Johnes status defined by targeted screens. Trained veterinarians gave informed advice and helped farmers select a control strategy that suited their current risks and status, and their aspirations and available resources. The control strategy options offered comprised seven different approaches to Johnes prevention and control, including husbandry and management changes to reduce transmission, identification of infectious animals by blood or milk antibody testing to identify high risk animals and prevent them transmitting infection to susceptible animals, a traditional test and cull program, vaccination, and the use of terminal sires to breed all indigenous breeding cows to beef sires whilst replacing breeding cows over time with cows of low MAP prevalence. The choice of control strategy was based upon the current estimated prevalence of MAP within the herd and the predicted future prevalence with current risks and husbandry practices. Available resources and future aspirations for the disease status of the herd were also taken into account.

Results

258 herds that were found to be significantly infected with MAP, 50% opted to use quarterly milk testing and a risk based control strategy to control the disease, 20% opted to improve farm management and use single annual testing to identify and manage high risk cows, and 18% chose to improve farm management for all cows without any testing. 8% opted for a traditional test and immediate cull policy. Only 2.3% decided to vaccinate, and a few farms used terminal sires and the purchase of replacements to manage the disease.

Conclusions

Providing a choice of control strategies is likely to increase engagement with MAP control. The choice of control strategy requires informed advice and background understanding of needs and aspirations.

Keywords:

Strategic Johnes control, Johnes control programs, Options for control of MAP

Abstract P-03.24

STATUS AND POTENTIAL OF 'INDIGENOUS VACCINE' IN THE THERAPEUTIC MANAGEMENT AND CONTROL OF JOHNE'S DISEASE IN THE ENDEMICALLY INFECTED POPULATION OF GOATS, SHEEP, CATTLE AND BUFFALOES IN INDIA

Singh S.V.*[1], Singh P.K.[2], Singh A.V.[3], Sohal J.S.[4], Gupta S.[1], Kumar N.[1], Bhatia A.K.[5], Srivastava A.K.[5], Singh S.N.[6], Bhattacharya T.[6], Chandel B.S.[7], Dadawala A.[7], Singh B.[6], Swain N.[8], Kumar A.[9], Singh A.V.[1], Bhushan S.[1], Chaubey K.K.[1], Rawat K.D.[1], Swarup D.[1], Sharma D.[1], Singh N.P.[1], Agarwal N.D.[1]

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Abstract text:

Study reports extensive trials of 'Indigenous Vaccine' developed using novel 'Indian Bison type' biotype of goat origin for the control of 'Johne's disease' in endemically infected population of domestic livestock in the country. Test and cull policy miserably failed to control JD in goats at CIRG, Mathura and there is ban on 'cow slaughter' in India, therefore vaccination is only alternative for management of bovine JD. A total of 4773 animals (2629 goats, 719 sheep, 1163 cattle and 262 buffaloes) from 15 livestock farms and 17 farmer's herds from Western, Northern, Central and Southern regions were vaccinated in last 8 years (2005-2013). Animals in 34 trials (21 in goats and sheep, 13 in cattle and buffaloes) were naturally infected and endemic for JD and were usually on sub-optimal nutrition. Therapeutic potential of 'Indigenous vaccine' in the management of clinical JD was evaluated on the basis of improvements in physical traits (gain in body weights, improvement in skin lustre, reduction diarrhea, etc), production parameters (growth rate, reproductive performance, total milk yield), mortality, shedding of MAP in feces (microscopy), sero-conversion (ELISA titre), and feed conversion efficiency. Following vaccination, substantial improvements were recorded in production parameters in each of the farms, where vaccine was used. Sero-monitoring of immune response by 'indigenous ELISA kit' showed enhanced 'herd immunity' for minimum 12 months period. Shedding of MAP in feces showed reduction for minimum of 7 months period depending on the nutritive status of animals. Only few of 34 trials could run for successive generations. 'Indigenous vaccine' developed for the control of JD in goats using 'Indian Bison Type' strain of MAP has shown promise to treat, manage and control MAP infection in endemically infected population of sheep, cattle and buffaloes in different geographical regions of the country.

Keywords:

Mycobacterium avium subspecies paratuberculosis, 'Indigenous vaccine', Indian Bison Type

Abstract P-03.25

COMMERCIALIZATION OF FIRST INDIAN VACCINE FOR THE CONTROL OF CLINICAL JOHNE'S DISEASE IN DOMESTIC LIVESTOCK POPULATION

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Abstract text:

India has dual distinction of highest in milk production (127.3 million tonnes) and livestock population (555.23 millions) [sheep (74.5m), goats (157.0m) cattle (219.8m) and buffaloes (112.9m)], but per animal productivity is very low (1/6 in Asian countries). Culling of cows is banned in India due to strong religious ethics, so, vaccination is better way to control Johne's disease in endemically infected livestock population of the country. First Indian vaccine (BioJD-Gel/BioJD-Oil) has been developed using well characterized strain 'S 5' 'Indian Bison Type' strain of MAP obtained from Central Institute for Research on Goats, Mathura under Public Private Partnership Project. Indigenous strain has been characterized in different stages since it isolation in 1998 from terminally sick Jamunapari goat and recently by whole genome sequencing. The strain has been adopted in Middlebrook 7H9 broth containing growth supplements (OADC and Mycobactin J) and different batches of antigen have been produced in 5-10 litre bottles and 100 litre vessel. Antigen was inactivated by heat and adjuvanted with Aluminium hydroxide Gel (BioJD-Gel) or mineral oil (BioJD-Oil). Vaccine is formulated on the basis of per ml dry weight of inactivated culture. Purity, safety and potency of the vaccine comply with OIE manual (2008). Both formulations were tested in sheep, goat and cattle for safety and efficacy. Monitoring of the vaccinated animals over a period of 1 year showed restriction in faecal shedding of MAP and antibody titres peaked around 65-90 dpv. Titres started decreasing 9-12 months post vaccination. Indigenous vaccine was effective in controlling clinical JD and protecting young animals. Test licence from DCGI, New Delhi has been obtained and large scale field trials are underway in sheep, goat, cattle and buffaloes in different agro climatic regions of the country. After getting commercial license, National Control programs against JD will be initiated first time in South Asian countries.

Keywords:

Johne's disease, Indigenous vaccine, Indian Bison Type

Abstract P-03.26
THE IRISH JOHNE'S DISEASE CONTROL PROGRAMME FOR DAIRY HERDS

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Abstract text:
This describes the details of a voluntary pilot Johne's Control Programme launched within the Republic of Ireland in 2013. Two organisations, Animal Health Ireland (AHI) and Animal Health and Welfare Northern Ireland (AHWNI), have been established within the island of Ireland to progress the control of some of the most significant endemic non-regulated infectious diseases. Operating in the two jurisdictions they work under a formal cooperation agreement sharing resources and technical knowledge. A technical working group was formed to develop the framework for a Johne's Disease control programme (JDCP). Their conclusions have been presented to implementation groups in each of the jurisdictions which comprise representatives from across the agri-food industry. Within the Republic of Ireland this group agreed to launch a voluntary pilot control programme for dairy herds in September 2013. Within Northern Ireland it is anticipated that a control programme will be launched using the same technical framework during the second quarter of 2014.

The overall objectives for the AHI JDCP are;
1. Bio-exclusion
To identify apparently infection free herds, allowing them to increase their confidence over time of being free of infection and to protect them from the risk of introduction of infection.
2. Bio-containment
To provide herds identified as infected with the knowledge and professional support to allow them to reduce the prevalence of the disease over time and where possible to achieve a high confidence of disease freedom.
3. Market reassurance
To underpin the quality of Irish dairy and beef produce in the international marketplace.

Keywords:
Ireland, Johne's, Control

Abstract P-03.27
ESTIMATION OF ECONOMIC LOSSES ASSOCIATED WITH JOHNE'S DISEASE IN DAIRY HERDS OF NORTHERN ITALY

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Abstract text:
The aim of this study was to estimate the economic losses related to Mycobacterium avium subsp. paratuberculosis (MAP) infection in dairy herds of the Emilia-Romagna Region, Northern Italy.

Results of a monitoring plan carried out on bulk milk samples in 2011-2012 were used to recruit case (n=40) and control dairy herds (n=46). Herds were tested using a commercial ELISA every four months, for a total of three tests. Cases were selected among herds positive to at least one bulk milk test. In these herds, serological testing was carried out on heads older than 36 months using the same ELISA to confirm MAP infection and estimate prevalence. In 175 herds where all the bulk milk tests were negative, we serologically tested a sample of 30 cows older than 36 months, and collected six fecal environmental samples for PCR and cultural tests. If all these tests were negative, the herd was considered as control. Each case was matched with at least one control according to the geographic location and herd size.

For the selected herds, we collected data on cows older than 24 months reared during the two years preceding the individual serological testing. Data such as ear-tag, sex, breed, type of movement, date of birth, date of death/culling were extracted from the farm register of the Cattle National Database. A total of 19,215 cows, mostly Friesian, were considered. For cases we found a shorter life of culled cows (62.3 vs 64.5 months; $p<0.01$), a higher culling rate (31.7% vs 27.3%; $p=0.02$), and a higher mortality rate (3.6% vs 2.6%; $p=0.07$). We used a spreadsheet to quantify costs due to the observed differences. For case herds we estimated a loss of more than 200€/cow/year: 60% of costs were related to reduced milk production due to the shorter life of cows.

This study, the first on economic impact of Paratuberculosis in Italy, suggests that MAP is associated with herd-level economic losses, supporting the implementation of control programs to recover producers profitability.

Keywords:
Bovine Paratuberculosis, Economics, dairy cattle

Abstract P-03.28

AWARENESS OF FARMERS ABOUT CHRONIC BACTERIAL DISEASES WITH PARTICULAR REFERENCE TO JOHNE'S DISEASE IN DAIRY ANIMALS IN NORTHERN INDIA

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Abstract text:

India is basically an agriculture based economy with huge populations of livestock (500 million). Recent studies suggested that tuberculosis (TB), Johne's disease (JD) and brucellosis have spread to livestock of villages (unorganised sector), thus further complicating their control. Participation of farmers and their awareness on these diseases is important in success of any control programme. In this study, we evaluated the farmer's awareness to develop extension strategies for control of these diseases. A total of 180 livestock owning families were selected randomly from 9 villages from Western Uttar Pradesh, India. Quantitative and qualitative data were collected personally through a structured and pre-tested interview schedule. Socio-economic profile, education of the farmers, extension services available, etc, were documented. Their awareness about TB, JD and brucellosis was recorded by asking questions directly on the basis of specific clinical symptoms. 70% farmers were maintaining 1-3 animals; cows or buffaloes or both, 25% had 4-6 animals and about 5% had >10 animals. Bovine TB was known to 18.9% (34) farmers by symptoms and about 6% by disease only when they contacted veterinarians. Fourteen farmers (7.8%) were somewhat aware of the symptoms of brucellosis. Surprisingly, JD was known to <3% of farmers after the contact with the veterinarian based on the symptoms like prolonged diarrhoea, severe weight loss and reduction in the milk yield in their animals. 14.4% farmers were aware of transmission of infection amongst animals and also to humans. Thus, farmers had very poor knowledge of these diseases especially JD and reasons for these were also documented. Education status and exposure of farmers to institutional trainings/camps had direct impact on the knowledge level of farmers. Based on the baseline data generated, training strategies specifically targeting JD are being developed for the farmers so as to get their participation in control programme.

Keywords:

Control programme, Farmers, Johne's disease

Abstract P-03.29
CHANGE MANAGEMENT FOR OVINE PARATUBERCULOSIS CONTROL IN AUSTRALIA

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Abstract text:
Ovine paratuberculosis (Johne's disease; OJD) is a serious disease of Australian sheep, estimated from abattoir surveillance as having infected approximately 5% of flocks and continuing to spread, despite a series of national OJD control programs. Since the introduction of Gudair™ vaccination in 2002, the catastrophic losses that occurred prior to using vaccine are now uncommon, with significant declines in within flock prevalence where vaccination use has exceeded five years. To assist understanding of the responses by producers and other stakeholders to improved knowledge of OJD control, relevant change management factors were examined including: drivers and motivation for change; resistance to change; knowledge management; farming systems dimensions; and industry leadership. It was concluded that extension programs addressing disease risk factors including the introduction of a risk-based trading system, have been relevant to improved attitudes to disease risk management and on-farm biosecurity by producers, although noteable differences were observed between those where OJD has become endemic and those where the disease has not yet established, explaining many of the difficulties experienced by supporters of the national programs. However improvements in OJD disease control practices were considered largely attributable to the introduction of vaccination. Persistent use of OJD vaccination to continue disease suppression when clinical cases are undetectable and continually improving biosecurity, remains a challenge for animal health authorities. Despite concerns of vaccine efficacy and safety plus the need for and cost of OJD vaccination, the trend appears that OJD control is being achieved in Australia. Persistent vaccination is having a profound impact on the health of Australian sheep, although the effectiveness of prolonged use of vaccine in suppressing OJD needs further recognition.

Keywords:
Change management, Paratuberculosis Control, Vaccine

O-04 Host Response and Immunology

Abstract O-04.1: INVITED SPEAKER
ROLE OF THE MAP ANTIGEN AND/OR ADJUVANT IN PATHOGENESIS OF HUMAN DISEASES

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Introduction:
All bacteria have special relationships with the host. Most bacteria are harmless and some even are beneficial. In contrast, harmful bacteria invade animal and human bodies, causing infectious diseases and other problems. To control the infection or prevent the disease, we need to know what happened in every step of the infection. The host response is generated differently in different steps and different times of the initial invasion when the host has only natural immunity but then generates acquired immunity. In paratuberculosis, we do not understand all steps of the infection well, despite exhaustive efforts. Before our study on experimental induction of Crohn's disease like mice colitis, Mycobacterium avium subsp. paratuberculosis (Map) antigen has been treated mainly as a tool for diagnosing paratuberculosis and vaccine development. There are many antigen candidates for diagnosis of cellular and humoral immune response, but a very specific definitive antigen has not yet been discovered. For Map antigen, we need to consider the fact that they were not created to induce a host immune reaction but should have their own biological purpose to achieve successful invasion and infection and to facilitate persistent infection and shedding from the host. For example, we do not understand how Map acquires iron for their metabolism and growth in the host macrophages. The life of Map depends on every antigen function that can switch or control the host system. Every event caused by Map in the host body seems to be done with very kind help of host cells, especially macrophages and the immune system. We have demonstrated the passive invasion and shedding mechanism of Map that can control host macrophages. These manipulations of host cell must be done by their antigen function.

Brief introduction of our experiment
We recently discovered that Map-derived lipophilic antigen can cause Crohn's disease (CD) like colitis in mice, however the detailed pathogenesis is still being investigated. The mouse experiment suggested that the Map antigen component can affect human health as well. The experiment method was similar to those in the 2,4,6-trinitrobenzene sulfonic acid (TNBS) induced colitis model. Therefore, we considered that the colitis lesions were caused by specific or nonspecific mycobacterium antigen, or as an adjuvant or hapten function. It's interesting to note that the number of reports offering clues to the mystery of incurable human diseases in relation to Map is increasing. This direction of immunological and molecular study is very beneficial for developing knowledge and techniques to control and eradicate ruminant paratuberculosis as well.

Role of common antigenic determinants
Mycobacteria have been considered candidates for inducing autoimmune reaction including host tissue damage. Heat-Shock Proteins (HSPs), human HSP 60, and M. leprae HSP 65, for example, are popular candidates that may be responsible for tissue destruction in leprosy and other diseases. Mycobacterial heat-shock protein hsp-65 has been proposed to lead to production of

autoantibodies against human lactoferrin and considered to contribute pathogenesis of autoimmune disease. However, some papers have pointed out the contamination of anti mycobacterial HSP65 in antiserum induced with complete Freund's adjuvant (CFA) including mycobacterial HSP65. Recent works on the role of Map epitopes homologous to the T-cell receptor gamma-chain in the pathogenesis of multiple sclerosis (MS) is a new approach using the modern molecular immunological technique developed by Seci's group in Italy. They have shown a similar mechanism in type 1 diabetes as well. The role of the molecular mimicry mechanism in autoimmune pathogenesis seems to be opening new doors.

Role of the Map antigen as an adjuvant

A form of Mycobacteria antigen, CFA, has been used as a famous representative of immune adjuvant. This is a mixture of oil and mycobacterium cell wall antigen. This component induces nonspecific up-regulation to Th1 immunity against antigen administered together with CFA, in addition to anti mycobacterial immunity. Oral administration of Mycobacterium phlei prior to live Newcastle disease F strain vaccination leads to an enhanced cell-mediated Immune response against the vaccine. The use of immunological adjuvants has been repeatedly shown to be essential for improving the immunogenicity in FMDV vaccines. Generally, oral administration of antigen has been considered to lead to oral tolerance, but there are new approaches free from traditional ones. Oral Mycobacterium bovis BCG vaccine and an inactivated M. bovis preparation for wild boar seems to effectively control bovine tuberculosis in wild animal species. These reports suggest orally administered mycobacterial antigen induces immunomodulation. Immunomodulation could be caused by Map-antigen contaminated foods worldwide.

Risk by exposure of Map antigen by the oral route

The effect of Map antigen-complex that can contaminate dairy products and meat from Ptb-infected cattle on human health has not been discussed well. However, paratuberculosis is spreading like an epidemic without sufficient control by each country. As many previous studies have indicated, the detection of Map-specific DNA IS900 by PCR from human intestines, blood, and feces or by in situ hybridization in intestines is evidence of continuous exposure to the Map antigen complex.

Need of research on the role of Map antigen-complex

Map cells are composed of many different antigen epitopes with various functions for their metabolism, to survive in an environment and control infectious host functions. Some map components may enhance Th1 type and/or Th2 type and some reduce the host functions. Map has an antigen that strongly induces IL-10 and seems to be their important weapon for the host, but the entire role of the antigen in all steps of in bovine paratuberculosis. We don't know the role of the antigen that induces IL-10 is responsible in human diseases. Recently, the role of pathogenic Th17 cells in autoimmunity has attracted much attention. There are many antigen-presenting cells (APCs) in laminapropria mucosa and other layers of the intestine. APCs produce TGF-beta, IL-6, IL-1, and IL-23 and affect T-cells (Th17, Tc17, and NKT-17), cells in natural immunity (LTi-like cells), and non-immune cells (intestinal epithelial cells, Paneth cells).

Conclusion

We IAP members know that mycobacterial antigens are among the strongest stimulants to APC and about the serious spreading of disease and contamination of food worldwide. We should not ignore the issue.

Abstract O-04.2

CD16HIGHCD14LOW CELLS: A NOVEL MYELOID CELL POPULATION IN PERIPHERAL BLOOD IS ELEVATED IN CATTLE WITH CLINICAL AND SUBCLINICAL JOHNE'S DISEASE

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Abstract text:
Myeloid cells are a heterogeneous population including monocytes, macrophages and dendritic cells (DC), subpopulations of which can be identified by the differential expression of CD16 and CD14. Using these two markers we were able to identify and functionally characterise a novel myeloid cell subset which is CD16highCD14low in bovine peripheral blood. We have now investigated the dynamics of this population during Mycobacterium avium paratuberculosis (MAP) infection in vivo.

This novel cell population has a pro-inflammatory profile, secreting relatively high levels of IL-12 and IL-1β after LPS stimulation in comparison to other blood monocyte populations. In addition, the CD16highCD14low population expresses higher levels of MHC-II and co-stimulatory molecules, is more phagocytic and able to induce stronger allogeneic responses when compared to CD14+ monocytes. These findings suggest this subset may be similar to the human blood DC subset referred to as Slan DCs.

Human studies have shown an increase in the percentage of Slan DCs in patients with inflammatory disorders including Crohn's disease. Since the bovine CD16highCD14low cells resemble Slan DCs, we hypothesised that the bovine cells may be involved in the inflammation seen in JD. Assessment of myeloid cells in cattle with clinical JD and, importantly, cattle with sub-clinical MAP infection, revealed an increased proportion of the CD16highCD14low cells in comparison to healthy animals or animals with other inflammatory diseases. These results were also confirmed in calves experimentally infected with MAP when compared to uninfected controls. We hypothesise that the CD16highCD14low cells identified in bovine PBMC are a specialised subset of blood DCs that are associated with MAP infection and disease progression and could provide insight into the pathogenesis of JD.

Keywords:
myeloid cells, pro-inflammatory, bovine PBMC

Abstract O-04.3
SELECTION FOR HERITABLE RESISTANCE IS AN OPTION TO CONTROL JOHNE'S DISEASE IN RUMINANTS!

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Abstract text:
While different breeds of cattle, deer and sheep display heritable resistance (R) or susceptibility (S) to Johne's disease (JD), little progress has been made to identify genetic markers that contribute to either R or S traits, in livestock.

Our 10 year study of an elite deer herd (>2,500 animals), including 7 disparate breeds of European red deer (Cervus elaphus), in a herd that had been chronically infected with M. ptb for more than 5 years. Sires with a confirmed Resistant (R) or Susceptible (S) phenotype were used in an AI programme with crossbred females, and the phenotype of the progeny was confirmed immunologically, microbiologically and histopathologically, following experimental infection with virulent M. ptb. In a two year breeding programme, 14/18 progeny (78%) from R sires were confirmed as R and 15/18 S progeny (84%) expressed the S phenotype. Detailed RNA-seq and transcriptome analysis of R and S animals was used to determine the relative expression levels of immune genes, measured by qPCR. Multiple genes involved in functional pathways of innate and adaptive immunity have been evaluated using a 'systems biology' approach to target overall cell function.

Animals with a S genotype expressed significantly higher levels of iNOS, IL-1α, TNF-α and IL-23p19 genes, associated with pro-inflammatory reactions and genes related to chemotaxis (CXCL10, CSF3, and CCL8) and type 1 interferons (RSAD2, IFIT1, IFIT2, ISG12, ISG15, USP18, and HERC6). Higher levels of IL-2, IL-4, IL-12, GATA3, Foxp3 and increased levels of Apoptosis was seen in R animals.

Animals with S phenotypes express dysfunctional innate immunity and appear to be incapable of clearing mycobacterial pathogens. By contrast, R animals express markers of adaptive immunity, that combined with adequate levels of innate immunity, promote protection.

Keywords:
Heritable, Resistance, Susceptibility

Abstract O-04.4

USE OF NEW JOHNIN IN GAMMA-IFN TEST TO DETECT MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP) INFECTED CATTLE: PRELIMINARY DATA

Mazzone P.*[1], **Corneli S.**[1], **Vitale N.**[2], **Di Paolo A.**[3], **Biagetti M.**[1], **Maresca C.**[1], **Ricchi M.**[4], **Mangili P.**[1], **Papa P.**[1], **Pezzotti G.**[1], **Raeber A.J.**[5], **Arrigoni N.**[4], **Cagiola M.**[1]

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Abstract text:

Traditional diagnosis of Paratuberculosis (PTB) is based on serology and fecal culture. Gamma Interferon (γ-IFN) and Skin Test (ST), already used for ante-mortem diagnosis of bovine Tuberculosis (bTB) could be also useful for PTB diagnosis. γ-IFN test detects cytokine production of T lymphocytes after stimulation with purified protein derivatives (PPDs), from M. bovis (PPDB) and from M. avium (PPDA). In order to achieve an early detection of subjects exposed to MAP infection, 3 new PPDs extracted from culture of MAP (PPDJ a, b, c) were produced, assessed in the γ-IFN test, compared to classic PPDs. In the ongoing study, 68 cattle from bTB officially free herds and with previous PTB clinical cases were tested by IDVet ELISA, PCR and culture. PPDJs were used in γ-IFN test (BOVIGAM ELISA) at dilutions 1:5 and 1:10. Samples have been considered positive when Optical Density (OD) value was, at least, twice OD of Nihil. Relative specificity (Sp) of PPDB and PPDJ in bTB diagnosis was calculated using ST as Gold Standard (GS). Relative sensitivity (Se) of PPDJ in PTB diagnosis was calculated using, as GS, the positivity in at least one of classical tests and PCR. Out of 55 animals PTB positive, 46 reacted to both PPDA and PPDJ, 4 only to PPDJ. For bTB diagnosis, the comparative use of PPDA and PPDJ against PPDB in 68 bTB negative cows yielded a 100% Sp. For PTB diagnosis, 1:5 dilution of PPDJ a, b and c showed respectively 83.6%, 76.4% and 74.5% Se. Relatively low Se in PTB diagnosis is influenced by tests used as GS, which are able to detect only advanced stages of disease. In fact 4 out of 9 subjects, previously classified as PTB negative, but positive to PPDJ, became positive 8 months later to serological and culture tests. Our preliminary results highlight the ability of our γ-IFN to avoid false positivity for bTB. For PTB diagnosis we plan to include more subjects with a suitable follow up. Projects RCIZSUM 11/2008 and RCIZSUM 04/2011funded by Italian Ministry of Health

Keywords:

Mycobacterium avium subsp. paratuberculosis, Johnin, Gamma Interferon

Abstract O-04.5

MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS REACTIVE CD4+ T CELLS CULTURED FROM BLOOD OF GOATS WITH NATURALLY ACQUIRED PARATUBERCULOSIS

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Abstract text:
The aim of this study was to establish a protocol for generation of MAP-specific T cell lines from the peripheral blood of goats, to investigate the properties of such T cells, and to use them for future evaluation of vaccine candidate antigens.

T cell lines were made from Norwegian dairy goats naturally infected with MAP. CD4+ T cells were positively selected by using a mouse anti-caprine CD4 mAb followed by incubation with Dynabeads pan mouse IgG. The sorted CD4+ cells were stimulated and cultivated in the presence of PPDj or E. coli sonicate, recombinant ovine IL-2 and recombinant human IL-15. Restimulation was performed with PHA, IL-2 and IL-15. At the end of the cultivation cycle the T cells were harvested and tested for recall responses in a proliferative T cell assay. The phenotype and activation status of T cell lines was assessed by staining of surface markers and intracellular cytokines for flow cytometric analysis.

Phenotyping of the T cell lines showed a mean percentage of 73 % CD4+ T cells, 26 % CD8+ T cells and only 4 % γδ TCR+ T cells. The proliferative response of the T cell lines were in average 92 % for PPDj, and -3 % for E. coli sonicate. Intracellular staining for IFN-γ in T cell lines stimulated with PPDj showed a 6 fold increase in IFN-γ production in CD 4+ T cells compared to controls. For CD8+ T cells the increase in IFN-γ production was 2 fold, while there was no increase in the IFN-γ production in γδ TCR+ T cells. We were not able to detect any intracellular IL-10 in any of the T cell subtypes after PPDj stimulation.

These results indicate that our CD4+ T cell lines are specific to MAP, and that we have established a protocol for generation of MAP-specific T cell lines in goats. Our T cell lines appear to be of a classical Th1 nature as they produce mainly IFN-γ after antigen stimulation. These T cell lines will be a valuable tool for identifying potential antigens for use in vaccines, and for studying T-cell responses to MAP.

Keywords:
CD4+ T cells, MAP specific responses, Th1 phenotype

Abstract O-04.6

UNDERSTANDING VACCINE FAILURE IN RELATION TO IMMUNE PARAMETERS IN MAP INFECTION

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Abstract text:

Vaccination is one of the strategies used to control the spread of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection. Gudair® is a widely-used vaccine in sheep and goats and is the only vaccine approved for use in sheep in Australia and New Zealand. This vaccine reduces mortality due to MAP infection by up to 90% but some sheep remain infectious despite vaccination. In this study our aim was to assess differences in immune parameters between vaccinated MAP-exposed sheep in which the vaccine was effective compared to those in which it failed to protect against disease. Using an experimental infection model in sheep we assessed immune parameters such as MAP-specific IFNγ, IL-10 and lymphocyte proliferative responses and serum antibody levels. At the end of the trial, 72% of non-vaccinated sheep and 24% of vaccinated sheep were infected, as defined by the detection of viable MAP in intestinal tissues when the trial was terminated at 49 weeks post exposure. There were significant differences in the proliferation of $CD4^+$, B and γδ T cells in vaccinated sheep in which the vaccine failed to protect against infection compared to the non-infected vaccinated sheep. There were no significant differences in the IFNγ response or serum antibody levels between the vaccinated infected and vaccinated non-infected sheep. These results emphasise the importance of lymphocyte subsets in protecting against MAP infection, especially in vaccinated sheep, and that immune parameters other than the commonly used IFNγ and antibody tests are required when assessing vaccine efficacy.

Keywords:

Immune response, Vaccine, Sheep

Abstract O-04.7

EVALUATION OF DIFFERENT VACCINATION STRATEGIES ON MYCOBACTERIUM AVIUM SUBSP PARATUBERCULOSIS INFECTION IN RABBITS

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Abstract text:

Map infection occurs early on in life and because of this vaccination of newborn animals is encouraged. However, little is known about the difference of being vaccinated prior to contact with Map or after contact with Map. The goal of the study was to evaluate the efficacy of vaccination with a killed vaccine (Silirum®) before and after challenge with Map in a rabbit infection model. NZW rabbits were divided in three groups: healthy non-exposed control (CNI, n=4), infected control challenged with Map (CI, n=5), vaccinated and challenged one month after with Map (VSR-I, n=5) and challenged with Map and vaccinated two months later (I-VSR, n=4). Humoral response was assessed by immunoblot and PPA-3 ELISA adapted for use in rabbits. Tissue culture and tissue F57 qPCR and histopathology were used to define final disease status. Vaccinated animals showed a boost in humoral response as seen by immunoblot and ELISA, whereas infected animals barely presented anti-Map antibodies. VSR-I animals showed higher ELISA rates compared to infected animals which were significantly different in most samplings. Both vaccination strategies decreased the number of tissues containing Map. However, bacterial load was decreased in the VSR-I group and elevated in the I-VSR group. As for histological findings, CI animals presented greater lesion extension in sacculus rotundus and mesenteric lymph node whereas VSR-I presented greater lesion extension in vermiform appendix. Vaccination induced a strong humoral response and decreased Map load and colonization in tissues in Map infected rabbits.

Keywords:

vaccine, animal model, rabbit

Abstract O-04.8

POST-EXPOSURE VACCINATION WITH MULTI-STAGE VACCINE SIGNIFICANTLY REDUCES MAP LEVEL IN TISSUES WITHOUT INTERFERENCE IN DIAGNOSTICS

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Abstract text:

A new (FET11) vaccine against paratuberculosis based on recombinant antigens from acute and latent stages of Map infection was developed to be used without interference with diagnostic tests for bovine TB and Johne's disease.

Calves were orally inoculated with 2x10E10 live Map in their third week of life and randomly assigned to four groups of seven calves each. One group was left unvaccinated, while other calves were post-exposure vaccinated with either a whole-cell vaccine at 16 weeks, or FET11 vaccine at 3 and 7, or 16 and 20 weeks of age, respectively. Antibody responses were measured by ID Screen® ELISA and individual vaccine protein ELISAs along with FACS and IFN-γ responses to PPDj and to individual vaccine proteins. At termination 8 or 12 months of age, Map burden in a number of gut tissues was determined by quantitative IS900 PCR and histopathology.

FET11 vaccination of calves at 16/20 weeks returned a 1.1 log10 reduction of Map burden compared to non-vaccinated animals ($p<0.05$). Non-significant reduction was observed in early FET11 and whole-cell vaccinated calves. Antibody and CMI responses corroborate observed vaccine efficacy: Six of the seven (85%) non-vaccinated calves seroconverted in ID Screen® ELISA indicating the progression of infection, while only three of 14 (20%) FET11 vaccinated calves seroconverted. PPDj induced IFN-γ responses also increased over time, but FET11 vaccinated calves had significantly reduced responses in PPDj IFN-γ assay from 40 to 52 weeks compared to non-vaccinated calves. All 14 FET11 vaccinated calves were negative in both single and comparative tuberculin testing. All whole-cell vaccinated calves seroconverted after vaccination, and four and one animal tested positive in the single and comparative tuberculin skin test, respectively. These results indicate the FET11 vaccine can be used to accelerate eradication of paratuberculosis while surveillance or test-and-manage control programs for TB and JD remain in place.

Keywords:

Vaccine, post-exposure, multi-stage antigens

Abstract O-04.9
EVALUATION OF NOVEL ORAL VACCINE CANDIDATES AND VALIDATION OF A CAPRINE MODEL OF JOHNE'S DISEASE

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Abstract text:
Johne's disease (JD) caused by Mycobacterium avium subspecies paratuberculosis (MAP) is a major threat to the dairy industry and possibly some cases of Crohn's disease in humans. A MAP vaccine that reduced of clinical disease and/or reduced fecal shedding would aid in the control of JD. The objectives of this study were 1) to evaluate the efficacy of 5 attenuated strains of MAP as vaccine candidates compared to a commercial control vaccine using the protocol proposed by the Johne's Disease Integrated Program (JDIP) Animal Model Standardization Committee (AMSC), and 2) to validate the AMSC Johne's disease goat challenge model. Eighty goat kids were vaccinated orally twice at 8 and 10 weeks of age with an experimental vaccine or once subcutaneously at 8 weeks with Silirum® (Zoetis), or a sham control oral vaccine at 8 and 10 weeks. Kids were challenged orally with a total of approximately 1.44 X 109 CFU divided in 2 consecutive daily doses using MAP ATCC-700535 (K10-like bovine isolate). All kids were necropsied at 13 months post challenge. Results indicated that the AMSC goat challenge model is a highly efficient and valid model for JD challenge studies. None of the experimental or control vaccines evaluated prevented MAP infection or eliminated fecal shedding, although the 329 vaccine lowered the incidence of infection, fecal shedding, tissue colonization and reduced lesion scores, but less than the control vaccine. Based on our results the relative performance ranking of the experimental live-attenuated vaccines evaluated, the 329 vaccine was the best performer, followed by the 318 vaccine, then 316 vaccine, 315 vaccine and finally the 319 vaccine was the worst performer. The subcutaneously injected control vaccine outperformed the orally-delivered mutant vaccine candidates. Two vaccines (329 and 318) do reduce presence of JD gross and microscopic lesions, slow progression of disease, and one vaccine (329) reduced fecal shedding and tissue colonization.

Hines, II M., Turnquist S., Ilha M.R.S., Rajeev S., Jones A.L., Whittington L., Bannantine J.P., Barletta R.G., Gröhn Y., Katani R., Talaat A.M., Li L., and Kapur V. Evaluation of Novel Oral Vaccine Candidates and Validation of a Caprine Model of Johne's Disease. *Frontiers in Cellular and Infection Microbiology.* March 4, 2014. vol 4:1-14. doi: 10.3389/fcimb.2014.00026.

Keywords:
Mycobacterium avium subsp paratuberculosis, vaccine efficacy, mutant vaccines

Abstract O-04.10

AD5 PRIME – MVA BOOST HAV VACCINE PROTECTS CALVES AGAINST MAP CHALLENGE

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Abstract text:

Introduction

Previous MAP vaccines have been shown to have some efficacy in decreasing clinical disease, but struggle to reduce excretion or herd prevalence and interfere with tuberculin testing. This study investigates the efficacy and immunological reactivity of a non-replicative prime boost viral delivery vaccine using four MAP specific epitopes to protect against subsequent MAP challenge in calves.

Methods

An Ad5 prime – Modified Vaccinia Ankara (MVA) boost delivery regimen of a multi-epitope MAP specific polypeptide (HAV vaccine) was administered to 8 week old calves and subsequently challenged orally with MAP. Calves were followed for 9 months and compared immunologically and microbiologically against sham vaccinated MAP challenged calves.

Results

HAV vaccine stimulated distinct immune responses which resulted in significant protection (>2 log reduction) against MAP challenge in the gut of HAV vaccinated calves, one calf being negative in all gut wall samples, combined with a complete lack of detectable faecal shedding of MAP in all vaccinated animals. HAV vaccination did not interfere with tuberculin testing and a vaccine specific peptide assay was able to differentiate vaccinated from infected unvaccinated animals (DIVA) over the whole 11 month study period. Vaccination showed no adverse effects and HAV vaccine was not excreted. Correlates of protection included early stimulation of Th-17 related reactivity, early and sustained stimulation of MAP specific IFN-γ, decreased IL-10 and IL-1β responses and retention of PMBC killing capacity on MAP challenge.

Conclusion

These results suggest that HAV vaccine could represent an effective tool for MAP infection control in cattle.

Keywords:

HAV vaccine, Protection, DIVA

Abstract O-04.11: PERSPECTIVE
Current thoughts and future directions on host response to MAP

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Johne's disease (JD) or paratuberculosis, caused by Mycobacterium avium subsp. paratuberculosis (MAP), affects many ruminants and, after several years of infection, a chronic wasting disease develops which may ultimately result in death. JD occurs in domestic and wild animals worldwide. Prevalence of JD in cattle in Australia, New Zealand, Europe and the U.S. in some studies was estimated to range from 10 to 60%. USDA reported that 68.1% of dairy herds in the U.S. are contaminated with MAP, and the most recent report estimated that actual herd-level prevalence of MAP was higher than 90%. Due to the reduced milk production and premature culling of JD-affected cattle, JD causes an estimated annual loss of $220 million to the US agricultural economy. In addition to the significant economic loss, MAP has also been suspected as an etiological agent of Crohn's disease in humans. The heavy economic burden on the agricultural industry and potential public health concern urged scientific community to develop effective control measures for JD.

Test-&-cull strategy in conjunction with risk management for JD is currently recommended for JD control in dairy herds. However, current diagnostic tests suffer low sensitivity and mathematical modeling studies predicted that this approach (test-&-cull) would require long-term efforts to reduce JD prevalence in dairy herds. The other JD control measure is based on vaccination of young animals. In the U. S., only one vaccine, called Mycopar, is currently approved to be used for JD control. Mycopar was shown to be only partially effective – reduces fecal shedding and clinical symptoms but does not prevent new infections. A recent mathematical modeling study predicted that such an imperfect vaccine has a limited impact on reducing JD prevalence in dairy herds. Thus, development of new vaccines with an increased efficacy is an urgent need for JD control, especially in countries with high JD prevalence. One of the major reasons for the continuing struggle with JD vaccine development is that there are many unknown factors in JD pathogenesis and in particular, in immune responses that mediate protection against the disease. For example, the host immune responses to MAP that lead to persistence and sudden exacerbation of the infection, are poorly understood.

MAP infections typically occur through ingestion of manure, milk, or colostrum contaminated with the bacterium. Also, vertical (in utero) transmission of MAP was reported to occur in 20-60% of fetuses from clinically affected dams. A recent meta-analysis study indicated that the fecal-oral transmission of MAP to calves is the most important transmission route of MAP in dairy herds. Young animals are the most susceptible to MAP infection but recent reports indicated that heifers and adult cattle can also be infected. After a long incubation time of 2-5 years, MAP-infected cattle start shedding the bacterium in their feces, colostrums, and milk, which spread the disease to other susceptible animals in the herd.

After ingestion, MAP organisms are taken up by M cells (and by enterocyte to a lesser extent), pass through the cells by transcytosis, and are then engulfed by submucosal macrophages residing at the basolateral side of the intestinal epithelial cells. The uptake of MAP by a M cell and a macrophage was suggested to occur through a5b1 integrin (via fibronectin) and complement receptor 3, respectively. Also, opsonization of MAP with serum or fibronectin was found to facilitate macrophage uptake of the bacteria. Macrophages infected with MAP were shown to be capable of killing the intracellular bacteria when activated by interferon (IFN)-g. However, the killing is not always successful and MAP can persist in macrophages by inhibiting phagosome

maturation. A recent study indicated that MAP infection suppresses apoptosis of primary bovine macrophages, which may further allow the intracellular MAP to live in the host cells. MAP infection also modifies cytokine productions from macrophages. Cytokines released from macrophages upon MAP infection include tumor necrosis factor (TNF)-a, interleukin (IL)-1, IL-6, IL-8, IL-10 and IL-12.

In subclinical JD, infected animals present with granulomas in intestinal tissues, intermitted shedding of MAP in feces, and low (or undetectable) level of anti-MAP serum antibody but do not show any clinical symptoms. TNF-a produced by macrophages induces recruitment of immune cells by up-regulating expression of chemokines (CC and CXC) and promotes formation of granuloma. Increased expression of the cytokine along with IL-1b and IL-6 was observed in intestinal tissues infected with MAP. In early stages of MAP infection, Th1 cytokines, such as IFN-g, IL-2,and TNF-a, were expressed in infected animals. IFN-g (and IL-12) drives precursor Th (Th0) cells into Th1 cells and suppresses Th2 differentiation. Also, IFN-g is known to antagonize functions of IL-4. Thus, in subclinical JD, T-cell populations are skewed toward Th1 immune responses while Th2 responses are being suppressed. Further, IFN-g plays a key role in controlling bacterial infection by promoting macrophage activation to kill intracellular bacteria, T cell differentiation, and MHC expression.

In clinical JD, infected animals shed a significant number of MAP in feces while producing a high level of anti-MAP serum antibodies, indicating that antibodies are not effective enough to control MAP infection. Hostetter et al. demonstrated that antibodies enhances phagocytosis of MAP and that intracellular proliferation of MAP was limited when the bacteria had been ingested by activated macrophages via Fcg receptors, suggesting that the antibody has an ability to reduce the number of MAP at the site of infection. Production of IFN-g and IL-2 was reduced in cows with clinical JD whereas expression of a Th2 cytokine (IL-4) was elevated. Th2 cytokine, IL-4, suppresses macrophage activation caused by IFN-g and also inhibit autophagy-mediated killing of intracellular mycobacteria. IL-4 also opposes Th1 differentiation by inhibiting expression of IL-12 receptor b chain. Another Th2 derived cytokine, IL10, was shown to inhibit proliferation and activation of lymphocytes. Thus, Th2 cytokines inhibit Th1 effector functions and differentiation. A recent study indicated that MAP inhibits IFN-g induced signaling, which may in turn enhance Th2 effector functions because IFN-g is shown to antagonize IL-4 functions. These findings suggest that the decline in IFN-g level (and thus Th1 response) in clinical JD may be a driving force of the Th1-Th2 switch observed in JD. However, recent experimental infection studies showed that IFN-g level stayed high in late stages of MAP infections and that timing of Th1-Th2 switch varies in individual animals, posing a question on the hypothesis.

Since Th1 responses are shown to be protective, vaccine that is capable of preventing the Th1-Th2 shift may be effective in controlling MAP infections. Indeed, Beggs et al. showed that animals with predominant Th1 response over Th2 response had low grade MAP-infected lesions. A similar finding was noted in experimental infection of calves where animals with strong and persistent Th1 response showed no (or negligible) Th2 response, fecal shedding of MAP, clinical symptoms, and gross lesions at slaughter.

Details of the above mentioned current knowledge in JD immunology will be summarized. Also, our efforts toward better understanding of JD immunology through mathematical modeling approaches will be presented with some perspectives suggested by the modeling projects.

P-04 Host Response and Immunology

Abstract P-04.1

SHORT TERM MYCOBACTERIUM AVIUM SUBSP PARATUBERCULOSIS INFECTION MODELS IN RABBITS

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Abstract text:

In order to develop a short paratuberculosis (PTB) infection model we hypothesized that increasing protein levels in the diet of rabbits, a species that has previously shown variable susceptibility rates, would induce a digestive stress that could enhance MAP infection. Fifteen New Zealand white rabbits were divided in three diet groups: regular (R, n=5), high protein (HP, n=5) and high fibre (HF, n=5). Groups HF and HP underwent diet stress during Map strain K10 challenge and received regular diet thereafter. Diet group R did not receive stress diets and was fed regular diet food during the entire experiment. Body weight was recorded, faecal culture and F57 qPCR were used to monitor disease in samplings that took place twice a month. Post-mortem tissue culture and F57 qPCR along with histopathology were used to evaluate final disease status. Minimal weight loss was recorded and significant differences were observed towards the end of the experiment, when diet group R showed lowest weight. Faecal shedding was confirmed in all groups being diet R the one that accounted for a higher number of shedders and highest bacterial load. Histopathology revealed PTB- compatible lesions affecting sacculus rotundus and vermiform appendix of all groups, although diet group R animals presented a larger lesion affected area. This was most evident in sacculus rotundus where significant differences were observed when diet R was compared to diet HF ($p=0.003$) and diet HP ($p=0.023$). Tissue qPCR showed high bacterial loads in sacculus rotundus and vermiform appendix of diet R and HF groups. Microbiological and pathological quantitative assessment showed that PTB infection was established in all groups, but that diet group R animals were the most affected.

Keywords:

rabbit, animal model, shedding

Abstract P-04.2
PRODUCTION OF MUCOSAL ANTIBODY IN RESPONSE TO PARATUBERCULOSIS INFECTION

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Abstract text:
Many studies have examined the serum antibody response to MAP infection but at this time no published studies have been reported on the mucosal antibody response. This study examined longitudinal mucosal IgG and IgA responses from experimentally inoculated sheep. ELISAs were used to measure the MAP specific IgG and IgA levels in sheep faecal samples collected regularly up to 23 months post inoculation (PI). Corresponding serum IgG responses and MAP culture of the faeces were also examined. The animals were divided into 3 groups: “un-inoculated controls” (10 sheep), “clinicals” (8 sheep)- inoculated animals that developed clinical disease and “survivors” (11 sheep)-inoculated animals that did not show histological lesions at the end of the trial. Serum IgG responses gradually increased in the surviving and inoculated animals, peaking at 12 and 16 months PI. A significant increase in the MAP specific faecal IgG and IgA was measured at 16 and 17 months PI in the survivors while the un-inoculated controls and clinicals remained at background levels. The sampling at 16 months PI was 1 week after the removal of 3 sheep developing clinical disease, a further 3 clinically affected sheep were removed several weeks before the 17 month PI sampling. By 19 months PI there was no difference in the groups and their faecal Ig responses. All the clinical sheep displayed typical disease including high to moderate MAP faecal shedding prior to necropsy. The data indicate that surviving animals are producing MAP specific IgA and IgG in the intestinal mucosa, which is released into the faeces. It also leads to the hypothesis that the surviving animals are producing faecal IgG and IgA in direct response to MAP contamination from the clinical animals, as the antibody levels were high immediately after removal of the clinical animals and returned to background in the following months.

Keywords:
Paratuberculosis, Mucosal, antibody

Abstract P-04.3

EVALUATION OF PROTECTIVE EFFICACY OF SAPONIN AND FREUND'S INCOMPLETE ADJUVANTED PARATUBERCULOSIS KILLED VACCINE IN MURINE MODEL EMPLOYING AGAR GEL IMMUNODIFFUSION TEST

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Abstract text:

Johnes disease is primarily an enteric infection of ruminants and other wild type species causing substantial economic losses in many countries. At present, there is no cure for Johnes disease and the only way to control the disease to reduce the economic loss is to vaccinate the animals. Though various types of vaccines have been developed against Johne's diseases, killed vaccine remains the most practically applicable vaccine in field condition. Recently, studies conducted using killed vaccine employing saponin adjuvants have been found to have less faecal shedding and systematic side effects.

Therefore, the present study was planned to compare the protective efficacy of Map killed vaccine(ATCC 19698) using two different adjuvants (Saponin Freunds Incomplete adjuvant) and also to evaluate diagnostic efficacy of AGID in predicting the clinical and subclinical disease.

The present study was conducted in murine model for a period of 11 months employing 80 numbers of healthy mice of about 2 weeks old of either sex.The mice were grouped into 4 groups (Gr I-mice vaccinated with killed Map adjuvanted with saponin, GrII- for FIC, Gr III- Saponin control and GrIV- FIC control). A total of 76 serum samples, 52 faecal samples and 20 tissue samples were collected at different intervals during the study period. Ziehl Neelsen method and AGID test were employed for analysis of samples. Acid fast staining revealed 37 (71%) and 5(20%) samples positive for faecal and tissue samples respectively. In AGID test, 24(31.6%) samples revealed positive result. The tests results obtained revealed that killed Map vaccine adjuvanted with saponin adjuvant have higher protective efficacy in terms of reduced systemic side effects as well as faecal shedding as compared to killed Map vaccine adjuvanted with FIC. The present study also reveals that AGID test is a highly specific test in diagnosis of paratuberculosis in subclinically infected animals.

Keywords:

Paratuberculosis killed vaccine, Saponin and Freund's Incomplete adjuvant, Agar Gel Immunodiffusion test

Abstract P-04.4
INCREASED BLOOD-CIRCULATING INTERFERON GAMMA, INTERLEUKIN 17, AND OSTEOPONTIN LEVELS IN BOVINE PARATUBERCULOSIS

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Abstract text:
Paratuberculosis infected cattle initially develop an effective cell-mediated immune response which however wanes as the disease progresses. Blood remains one of best sources for analyzing mediators related to chronic diseases. The objective of this study was to evaluate the cow-level association between blood cytokines, the influence of serum on immune cell proliferation, and Mycobacterium avium ssp. paratuberculosis (MAP) naturally infected dairy cows. Positive animals (n = 41) from 19 herds were selected on the basis of two positive fecal culture results and further divided in two groups: single-positive, which were serum ELISA-negative cows (n = 32) and double-positive, which were cows with positive results for both mycobacterial culture and serum ELISA (n = 9). Negative animals (n = 39) were selected from paratuberculosis-negative herds in which at least 80% of the animals had been diagnosed as negative by fecal culture and ELISA without any positive results in the herd during the 2 year study. Analysis of the plasma levels of IL-4, IL-10, IL-17, IFN-γ, and osteopontin cytokines were performed and revealed distinct patterns. The ELISA-positive cows with MAP shedding had similar plasma concentrations of IL-4 and IL-10 but elevated levels of IFN-γ, IL-17, and osteopontin, suggesting an inflammatory disease in these subclinical cows. In vitro MAP infection of bovine macrophages confirmed the establishment of a T-helper type-17 immune response. To determine the systemic influence of serum on immune cell functions, lymphoproliferation assays were also performed. The presence of serum from shedding cows showed 15% less proliferation. These results suggest that infected cows have a lower systemic capacity to maintain a protective immune response and that, as the disease progresses, an emerging T-helper type-17 immune response is established.

Keywords:
interleukin 17, osteopontin, cell proliferation

Abstract P-04.5
GENETIC MARKERS FOR RESILIENCE AND SUSCEPTIBILITY TO JOHNE'S DISEASE IN RED DEER

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Abstract text:
Johne's disease in deer has been studied for many years and while polarised outcomes following infection have been described little is known about the particular immune pathways which may be involved with protective immunity or disease related immunopathology. There is evidence, however, that host genetics may contribute significantly to resilience or susceptibility to Johne's disease and that particular subsets of genes exhibiting either increased or decreased expression may be associated with a resilient or susceptible phenotype.

The purpose of this study was to identify genes of the innate and adaptive immune response that contribute to the resilient or susceptible phenotype in red deer which may be of use as candidate biomarkers. Progeny animals were bred from sires that had a resilient or susceptible phenotype confirmed by post-challenge slaughter of previous progeny and pathology from infected tissues. Animals were challenged orally with Mycobacterium avium subspecies paratuberculosis (MAP). Animals were bled routinely and blood monocyte derived macrophages and whole peripheral blood mononuclear cells were obtained by in vitro cultures. Cells were stimulated with MAP to induce transcription of immune associated genes. Recovered RNA was used in qPCR assays to quantify the expression of candidate genes known to be differentially expressed in the immune response following stimulation.

Preliminary results suggest that in susceptible animals there is a trend towards increased expression of targets associated with the innate immune system (such as IL-1a and IL-12 p35) whereas resilient animals exhibit increased expression of genes associated with the adaptive immune system (such as IFN-y and IL-2). We propose that a susceptible phenotype may manifest due to an inappropriate innate immune responses whereas a resilient phenotype may be conferred by the adaptive immune response.

Keywords:
Immunology, Genetics, Biomarkers

Abstract P-04.6
EXPERIMENTAL INFECTION OF NEONATAL PIGS WITH MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP)

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Littermates from each of 4 sows were study groups. Pigs were orally challenged on days 12, 14, 16 and 18 of life. Group A pigs received homogenized tissue from a clinical case of bovine paratuberculosis suspended in 4.0 mL UHT milk (5.5 x 10^4 MAP/mL). Group B pigs received cultured MAP suspended in 4.0 mL UHT milk (5.5 x 10^7 MAP/mL). Two pigs in groups A and B were not challenged but served as contact controls. Group C pigs received cultured *M. avium* subsp. *avium* (MAA) suspended in 4.0 mL UHT milk (2.8 x 10^7 MAA/mL). Group D, controls, received only UHT milk. All pigs were observed daily and weighed weekly. MAP serology and MAP fecal cultures were done at 10 days of age (pre-challenge) day 21 (3 days post-challenge) and every 3 weeks to the end of the trial. Pigs were necropsied at 21 weeks.

All pigs remained clinically normal. Fecal shedding of MAP was detected in most MAP-challenged pigs one week post-challenge and then again weeks 8-15 post-challenge, after which the pigs were fecal culture-negative for MAP. Serum antibody to MAP was detected only in Group B pigs and was first detected 6 weeks post-challenge. Mesenteric lymphadenitis was seen grossly in most MAP-challenged pigs (groups A & B). MAP was recovered from the ileum and mesenteric and ileocecal lymph nodes of group B pigs, including one of two contact controls. MAA was recovered from these same tissues from group C pigs, but less frequently. Only MAP-challenged pigs had histologic lesions and these were primarily in mesenteric, ileal and ileocecal lymph nodes. In these tissues the main finding was abundant type III (necrotic/caseous centers with fibrous capsule) and coalescing type IV (necrotic/caseous and calcified centers with fibrous capsule) granulomas. No intestinal lesions were induced by MAP up to the end of the trial when the pigs were 150 days old.

The findings show that pigs are susceptible to infection by MAP and that MAP is more virulent for pigs than is MAA. Granulomas seen in lymph nodes were more typical of tuberculosis than Johne's disease. This study highlights that different animal species respond to MAP infection differently. A limitation of the study may have been its short duration. It would have been interesting to observe if the MAP infections progressed or resolved with time.

Keywords:
Swine, Model, Pathology

Abstract P-04.7

IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN SLC11A1 AND CARD15 GENES AND THEIR ASSOCIATION WITH INFECTION BY MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS

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Abstract text:
Using the approach of the candidate gene, a case-control study on Friesian cattle, naturally infected by Mycobacterium avium subspecies paratuberculosis (MAP), was carried out.

Solute carrier family 11 member 1 (SLC11A1) and Caspase recruitment domain 15 (CARD15) genes were analysed. Cases and controls were defined considering blood serum ELISA, faecal culture, and PCR tests using a Bayesian probability weighting function. Twelve polymorphisms were reported in CARD15 and two were statistically associated ($p<0.05$) with susceptibility to MAP infection. Presence of polymorphism c.2886-14 A>G, at intron 10 was found in infected animals 2-fold higher than in healthy ones (OR=2.35; CI 95%: 1.08-5.10; X2=4.87 p=0.03); it was formerly reported associated to health and productive traits in Canadian Holstein. Analysis based on TFSearch software showed, when mutation was present, the alteration of the site for GATA3 transcription factor, involved in phagocytosis and apoptosis. Interestingly GATA3 and Interleukin 5 expression was reported altered in MAP-infected cattle. We confirmed the role of mutation 1908C>T, at 3'UTR, (OR=2.04; CI 95%: 1.03-4.99; X2=4.45 p=0.03), previously associated with susceptibility; this could be attributed to changes in a regulatory motif comprising the mutation site.

Seven polymorphisms were reported in SLC11A1; one associated with susceptibility, found in the promoter region, c.-90 C>A (OR=2.04; CI 95%: 1.03-4.04; X2=6.02 p=0.01); and the second, already described, c.1157-91 A>T, at intron 11, related to susceptibility when the wild-type allele A is present (OR=3.46; CI 95%: 1.37-8.74; X2=6.86 p=0.01).

The association with infection, of polymorphisms c.-90 C>A and c.2886-14 A>G is described here for the first time.

These data could be considered for future application in programs to control paratuberculosis in cattle, in which highly susceptible animal could be negatively selected according to the genotype.

Keywords:
paratuberculosis, CARD15, SLC11A1

Abstract P-04.8

INTERACTIONS BETWEEN M. PARATUBERCULOSIS AND BOVINE MACROPHAGES: CHALLENGES AND OPPORTUNITIES FOR VACCINE DEVELOPMENT

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Abstract text:

Though our understanding of how M. paratuberculosis (MAP) survives in host macrophages has improved significantly over the past few years, there is still much we need to uncover. While it is clear that MAP actively prepares the infected macrophage for its own survival, precisely how this happens and it consequence to the overall immune response is less well documented. MAP infected macrophages produce significant amounts of IL-1α and the anti-apoptotic protein TRAF 1. MAP-infected macrophages also fail to fully activate caspases 3/7, 8, and 9 in response to external stimuli and are therefore highly resistant to apoptosis. For caspases 3/7 and 8, a reduction in activation is complemented by a loss in gene expression. This presumably prevents efferocytosis of MAP containing apoptotic blebs by other antigen presenting cells and thus proper antigen presentation to T cells. In addition, MAP infected macrophages show defects in activation by CD40ligand, a major T cell co-stimulatory factor. Gene expression analyses by our group and others demonstrate that infection by MAP induces massive changes in abundance of numerous host mRNAs as early as 2 and 6 hours post-infection. By 24 hours post-infection, many of these gene expression changes have normalized. MAP infected macrophages also produce large amounts of IL-10, an immune suppressive cytokine in cattle, suggesting that MAP infected macrophages adopt an alternatively activated, or M2 phenotype. Of interest, macrophages that have been pre-polarized into M1 macrophages by exposure to IFNY become resistant to persistent infection by MAP. Many of the features of MAP infected macrophages inform the design and analysis of modified live vaccines against MAP. In this presentation, these opportunities and some challenges will be discussed.

Keywords:

macrophage, apoptosis, vaccine

Abstract P-04.9
EVALUATION OF THE EXPRESSION OF IFN-G IN THE DIFFERENT TYPES OF LESION ASSOCIATED WITH BOVINE PARATUBERCULOSIS

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Abstract text:
Animals infected with Mycobacterium avium subsp paratuberculosis (MAP) show a variety of lesions: focal or multifocal forms, composed of granulomas with no bacteria located exclusively in the intestinal lymphoid tissue o adjacent lamina propria, associated with subclinical stages; diffuse granulomatous enteritis, with large numbers of epithelioid cells harbouring large numbers of MAP (multibacillary) or lymphocytes with scattered small granulomas with none or few MAP (paucibacillary). The immune response of the host plays an important role in the development of these lesions. The purpose of the present work is to evaluate the local expression of IFN-γ using immunohistochemical methods in tissue samples of MAP-infected cows showing different types of lesions.

Only lymphocytes, both in the intestine and lymph nodes, were positively labelled. In the control, non-infected animals, they were scattered throughout the lamina propria and occasionally in the lymphoid tissue. In focal and multifocal lesions, lymphocytes expressing IFN-γ were more frequent but only in close relation to granulomas. They were surrounding the granuloma and also infiltrating among the macrophages. Similar findings were observed in the paucibacillary lesions where, despite the high amount of lymphocytes in the lesion, positive labeling was only found in lymphocytes closely related to the granulomas. In contrast, in the multibacillary lesions, few or none IFN-γ positive lymphocytes were seen in relation to the granulomas.

These findings would suggest a strong relation between the activation of macrophages in focal or paucibacillary lesions and the presence of IFN-γ secreting lymphocytes closely related to the granulomas, in contrast to multibacillary lesions, characterized by the absence of IFN-γ secreting lymphocytes. The markedly focal distribution of the immunolabelled cells should be bearing in mind when carrying out studies on cytokine expression in intestinal samples.

Keywords:
Granulomas, Interferon-gamma, Immunohistochemistry

Abstract P-04.10
IMMUNOPHENOTYPIC ANALYSIS OF MACROPHAGES SUBSETS WITHIN GRANULOMATOUS LESIONS IN BOVINE PARATUBERCULOSIS

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Abstract text:
Macrophages, the main cell population within the granulomatous inflammatory infiltrate characteristic of paratuberculosis, can be further differentiated in two subsets, i.e.M1 or M2, depending on the influence of molecules secreted by T lymphocytes. The aim of this study is to characterize the immunophenotype of macrophages in the different types of lesions observed in experimentally and naturally infected cows. Intestinal tissue samples embedded in paraffin were immunohistochemically labelled using different primary antibodies against iNOS (M1), CD163 and TGF-β (M2), Mac387 (calprotectin, indicator of maturation), CD68 (lysosomal membranes) and lysozyme. Number of stained cells and staining intensity were assessed in relation to the type of lesion.

Lysozyme and CD68 expression was found in macrophages with no evident differences among the type of lesions. Macrophages forming focal granulomas restricted to the lymphoid tissue, seen in subclinically infected animals and with no bacilli, were positively labelled for iNOS and few of them to Mac387 as well, while negative for TGF- β or CD163. Similar results were observed in paucibacillary diffuse granulomatous lesions. However, the macrophages present the multibacillary lesions, also characterized by a granulomatous infiltrate, were positively labelled for CD163, TGF- β and Mac387, while negative for iNOS.

The frequent expression of Mac387 in the macrophages forming multibacillary lesions suggests that, in this type lesion, there is an inflammatory active but ineffective response, with most of the macrophages polarized towards a M2 phenotype, characterized by CD163 and TGF- β expression. In contrast, granulomas in the focal and paucibacillary lesions would have a latent character, with macrophages not recently recruited (as denoted by the low Mac387 expression) but effective in controlling bacterial multiplication, polarized towards a M1 phenotype (iNOS +).

Keywords:
Macrophages, Immunophenotyping, calprotectin

Abstract P-04.11
LEUKOCYTE SUBSET CHANGES IN GOATS WITH JOHNE'S DISEASE

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Abstract

Johne's disease is a chronic disease in domestic and wild ruminants divided into four stages: (1) silent stage, (2) subclinical stage, (3) clinical stage, (4) advanced clinical stage. While there are many studies on the immune responses in the clinical and advanced clinical stages there is a large knowledge gap on the first two stages. Recent studies analyzed only the cytokine and humoral immune responses, but not leukocyte subset population changes. Here we report our findings on the leukocyte subset population changes during the first two years of infection in the goat model. Peripheral blood was collected at weeks 1, 3, 5, and 8 as well as every four weeks thereafter from the infected goats and the ten negative control goats of same breeds and age. While we did not see any significant changes in the overall granulocyte, lymphocyte, or monocyte population in the first year, we detected an increase of the granulocytes and monocytes towards the end of the second year. We identified CD4+ T cell increases for the infected goats during the first year, which were normalized at the end of two years, while the CD8+ T cells showed the opposite trend. While the gamma delta T cells during year one demonstrated differences between infected and uninfected goats with the infected having lower numbers of gamma delta T cells, this T cell subset was normal throughout the end of year two. We also analyzed the two antigen presenting cell markers MHCII and CD1. We noticed a short increase of both markers at the end of year one, but could not detect the same differences during year two.

In summary, at the end of year two there is a trend for an increase of granulocytes and monocytes in the peripheral blood of infected goats while the T cell subpopulation differences switch from CD4+ T cells and gamma delta T cells to CD8+ T cells.

Introduction

Johne's disease (JD) is an inflammation of the intestine in domestic and wild ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Most chronic mycobacterial infectious diseases have a late onset and Johne's disease is no exception with an average of two years but up to five years. However in contrast to other mycobacterial diseases, the time of infection with the pathogen can be placed right after birth within the first few weeks and months of life. Because of the delayed onset of clinical symptoms the disease itself is divided into four different stages or phases with specific characteristics. The first two stages do not have any clinical symptoms or signs that are usually characteristic for Johne's disease. The very first stage is called the silent stage because there are no clinical or laboratory diagnostic indications for the infection. One of the characteristics of Johne's disease is the fecal shedding of the pathogen and the earliest time it could be detected is during the subclinical stage. This shedding is usually temporary and sporadic. Diagnostics tests are seldom indicative for the disease and fecal culturing might be the only indication for Johne's disease. During the next two stages, clinical stage and advanced clinical stage, diagnostic tests are more often positive and animals show symptoms and signs for Johne's disease with weight loss and chronic diarrhea as the key characteristics. Even during these stages serological diagnostics are not always positive. Shedding usually occurs more often but not in all animals.

The only effective control measure currently applied by most dairy farmers in the US is culling infected animals and/or instituting good herd management practices. While there are strong

efforts to develop excellent vaccines, no successful efforts have been seen on the development of new diagnostic approaches that would help in identifying infected animals during the first two stages. Most diagnostic tests were evaluated at only one time point and without any knowledge about the stage of the disease at testing time. Long-term studies on Johne's disease were performed in the past, however, with limited numbers of animals and thus without significant statistical evidence.

There are two key questions associated with the first two stages: Why is there a late onset of the clinical characteristics, and what could be done to improve detection of infected animals. Long-term studies with experimentally infected animals might provide more insights into the immune responses during the early stages. In addition, following peripheral blood leukocyte population changes might provide indication on what might happen at the local level in the intestine. Here we present our analyses on peripheral blood leukocyte populations during the silent stage of Johne's disease in experimentally infected goats.

Materials and Methods

Animals: All research and housing procedures were approved by Colorado State University IACUC and the approval number is #11-3120A. Twenty goat kids aged two to five days old were purchased from CCI/Juniper Valley Products (Canon City, Colorado), a Johne's disease-free operation for decades, and transferred to the final location at CSU Foothills Campus. All goats were housed together in a closed barn until the age of seven weeks at which half of the goat kids were orally inoculated with MAP. The pre-infection location and the location for the negative control group are JD-free, while the location for the infected group was used prior the study for goats with JD. The goat kids were individually fully milk fed for the first two months with warmed whole cow milk (three times a day). Before inoculation of goats within the infected group, goats were separated into different locations. Milk feeding was reduced to twice a day for the next six weeks followed by once a day for another four weeks. During the weaning alfalfa hay was introduced to supplement the goats' nutrition needs. At 12 weeks post infection all goats received only alfalfa hay as food supply.

Goat infection and preparation of the inoculum: Goats were inoculated with MAP strain K-10. We used the second passage from the original sample received from Dr. Kapur. The pathogen was grown on Middlebrook 7H11 supplemented with 10% OADC and 2 µg/ml mycobactin J. Cells were harvested and aliquots of 100 mg wet cell pellet in PBS (pH 7.2) were made for inoculation. 100 mg of wet cells equals roughly 10^9 cfu the required dose per inoculum. Cells were suspended in 20 ml warm whole cow milk and transferred to a 20 ml sterile syringe. The individual nipple was place on top the syringe. Ten goats were inoculated three times with a suspension of 10^9 cfu at three consecutive days. The inoculation was performed when the goat kids were 7 weeks old.

Flow cytometry: Samples for flow cytometry were analyzed via flow cytometer (FACSCantoll, BD, USA) equipped with BD FACSDiva software. A minimum of 10,000 events were collected per sample. Profiles were analyzed with FlowJo software (TreeStar, Ashland, OR, USA). The following panels were used: CD4/CD8, CD4/WC1, CD1/CD14, and MHC class II/CD14. Thereafter, the fluorescences of the positive cell signals were compared to their corresponding isotype-matched controls. The percentage of positive cells and intensity of fluorescence was recorded as percentage of positive cells and mean fluorescence channel (MFC), respectively. Specific cell populations ($CD14^+$ granulocytes or $CD14^+$ monocytes) were back gated to show the presence and amount of such population within the selected cell types.

Statistical analyses: Statistical analysis was done using SAS 9.3. A separate repeated measures analysis was done for each cell type using Proc Mixed. The within-subjects factor is time and the between-subjects factor is treatment group. A time*treatment interaction term was also included

in the model. The arh(1) covariance structure was used, allowing for unequal variances at the different time points. Comparisons of means between treatment groups at each time point were considered. A Benjamini-Hochberg adjustment was applied to account for multiple testing across time points separately for each cell type. Statistical significance was defined as p-value ≤ 0.05.

Results

The overall leukocyte counts in the peripheral blood were only significantly elevated during the first weeks in the infected goats – but still normal. In the following weeks while still elevated in the infected goats, the overall counts of peripheral leukocytes were not significantly different from those of the uninfected goats.

While no differences were seen in the lymphocyte, monocyte, and granulocyte amounts during the first year, the second year is characterized by increased granulocytes and monocytes in the infected goats. Interestingly, the CD14+ granulocytes of the infected goats were elevated in the first year and did not exhibit differences in their amount in the second year from those of the uninfected goats.

The lymphocyte subpopulations in the infected goats exhibit different profiles for year 1 and year 2. In year 1, the CD4+ T cells and the WC1+ γδ T cells had different amounts in the two groups (with lower numbers in the infected goats for both subsets), whereas year 2 is characterized by reduced amounts of CD8+ T cells and no differences between the two groups for CD4+ and WC1+ γδ T cells.

Conclusions

In summary it can be concluded that while it could be assumed that year 1 represents the silent phase and year 2 probably the subclinical phase, both years have their specific characteristics. While in the silent stage only minor changes are detected, the year 2 (or subclinical stage) is characterized by more significant changes.

Keywords:
Goat Model, Silent and Subclinical Stages, Leukocyte subsets

Abstract P-04.12

FACING INTERPRETATION DIFFICULTIES OF POSITIVE SEROLOGY IN PRESUPPOSED PARATUBERCULOSIS-FREE HERDS: LONGITUDINAL STUDY AND RESULT COMPARISON OF COMMERCIAL ELISAS

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Abstract text:

Serology is widely used to monitor paratuberculosis, and assays are considered to have a high specificity. However, in paratuberculosis-free herds, unexpected positive reactions are sometimes detected, raising thus interpretation and herd management difficulties.

In three well-controlled herds (annual serological survey for at least 5 years) with no history nor clinical signs for paratuberculosis, few cattle were serology-positive since 2010. To further investigate the frequency and persistence of the positive reactions, sera were assessed with two commercial assays (IDEXX, and ID.Vet). Herd status was also investigated by other assays: fecal PCR (Life technologies, and Biomérieux), IFNg assays using PPDj and several Map antigens (IDVET), and culture on pooled feces (n=10, Trek Para JEM, Thermo Fisher). Furthermore, individual and herd-level responses against Map were assessed again ten months later.

ELISA results did not correlate between commercial assays indicating that an antibody response could not be constantly detected and was largely assay-dependent. Furthermore, none of the positive results can be confirmed using other assays such as direct PCR on feces or IFNg assay. After ten months, all animals were sampled again. Animals that show ELISA discrepant positive results ten months earlier were negative with both assays and fecal PCR, indicating that the frequency of non-specific responses may be high and assay-dependent in some herds. At this occasion, one animal was found positive with both ELISAs but was fecal PCR-negative.

In herds with a presupposed Map-free status, new seropositive cases should be considered with caution and need confirmation using comparison amongst serological assays or alternative methods.

Keywords:
serology, specificity, diagnostic

Abstract P-04.13

INTERACTION OF BOVINE INTESTINAL SUBEPITHELIAL MYOFIBROBLASTS WITH DIFFERENT STRAINS OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS

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Abstract text:
Intestinal subepithelial myofibroblasts (ISEMFs) are mesenchymal cells located beneath the mucosal epithelium of the small and large intestine. Beside their role in wound healing and fibrosis, a growing body of research shows that ISEMFs can act as nonprofessional antigen presenting cells (APCs) and affect the recruitment, retention and activation of immune cells.

Paratuberculosis, caused by Mycobacterium avium subsp. paratuberculosis (Map) is a chronic enteritis that affects many domestic ruminants and other wild animals worldwide. The disease causes intestinal lesions consisting of epithelioid cell granulomas of various sizes with differing numbers of acid-fast bacilli (AFB). However, it is not clear how the surrounding stromal cell population interacts with MAP and affect development and maintenance of the infection.

In this work, we analyzed ileal sections from paratuberculosis affected-cows and observed the presence of alpha-SMA+, vimentin+ ISEMFs inside and surrounding AFB-positive lesions. In order to study how MAP interacts with ISEMFs, primary intestinal myofibroblasts from healthy cows were infected with genetically diverse MAP strains and the expression of inflammatory cytokines and tissue remodelling factors (e.g. MMPs, TIMPs) was evaluated showing strain dependent differential activation. In addition, MAP-induced effects were compared with Mycobacterium avium subsp. avium and LPS-induced responses.

Keywords:
myofibroblast, fibrosis, inflammation

Abstract P-04.14

CYTOKINE GENE EXPRESSION AND DETECTION PROBABILITY OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS IN ORGANS OF EXPERIMENTALLY INFECTED MICE WITHOUT HISTOLOGICAL LESIONS

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Abstract text:

Mycobacterium avium subspecies paratuberculosis (MAP) can infect ruminants and remain subclinical for long periods within herds. Identification of organs more susceptible to infection and evaluation of cytokine expression at the site of infection in subclinical phase is important for pathophysiology of the disease. It was evaluated the detection probability of MAP-specific DNA by IS900 nested-PCR and expression of cytokines by quantitative real time RT-PCR in subclinical organs of C57BL/6 infected mice. Briefly, 20 female were inoculated intraperitoneally with 250 µL of inoculum containing MAP66115-98 strain at a concentration of 7.5 x 10^7CFU/mL and eight with PBS. At 30, 60, 90 and 120 days post-inoculation (p.i.), five challenged mice and two controls were euthanized. Immediately, the mice were necropsied and liver, spleen, terminal ileum, colon and Peyer's patches were collected aseptically for histopathological and molecular analyzes. No clinical manifestation or histological lesions were found, but even so, the presence of MAP should not be discounted. The spleen showed more positives, 27.8% (17/61), followed by colon 24.6% (15/61), liver 19.7% (12/61), terminal ileum 18.0% (11/61) and Peyer's patches 9.8% (6/61). The spleen was also the main organ to detect the presence of MAP in a systemic subclinical infection after 60 days (29.4%). Relative risk showed that spleen had 1.54 times more risk to be positive compared to ileum and 2.00 times more risk than Peyer's patches ($p<0.05\%$). Sixty days p.i. was the period showed significantly higher expressions of cytokines compared to the other days. During this period, the spleen and liver were responsible for the significant expression of pro-inflammatory TNF-α and IFN-γ. In the ileum, besides TNF-α, IL-4 was verified, indicating that anti-inflammatory cytokines can be expressed even in subclinical stages of infection. Financial Support: CNPq, CAPES, FAPEMIG.

Keywords:

paratuberculosis, mice, experimental model

Abstract P-04.15

TRU-SEQ DEEP SEQUENCING REVEALS MAJOR DIFFERENCES IN THE ILEOCECAL LYMPH NODE TRANSCRIPTOME OF PAUCIBACILLARY AND MULTIBACILLARY PARATUBERCULOSIS INFECTED SHEEP.

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Abstract text:

Mycobacterium avium subspecies paratuberculosis (MAP) the causative organism of Johne's disease or paratuberculosis, an infectious chronic enteric disease of ruminants. In sheep, paratuberculosis presents as two distinct pathological forms; paucibacillary or multibacillary disease. Paucibacillary disease is associated with an inflammatory Th1/Th17 response and multibacillary disease with a macrophage/Th2 response; and we hypothesise that these different responses influence the pathogenesis of MAP infection. The focal site of infection is the terminal ileum of the small intestine and the immune response to pathogens in this region develop in the ileocecal lymph node. To understand the relationship between the developing immune response and pathology, we have performed Illumina Tru-seq transcriptome analysis of ileocecal lymph nodes from sheep with paucibacillary and multibacillary paratuberculosis as well as uninfected controls. The 100-cycle multiplex paired-end reads (average >50 million reads per sample) were aligned to the sheep genome v3.1. Reads aligning to exons, genes and splice junctions were counted, permitting quantitative differential gene and transcript analysis between the two disease forms and also paratuberculosis-negative sheep. These data advance our knowledge of the complex molecular and immunological events involved in the different outcomes of MAP infection in sheep.

Keywords:

Sheep, Paratuberculosis, Transcriptome

Abstract P-04.16

IN-VITRO ASSESSMENT OF THE FUNCTIONAL ROLE OF THE 3' UTR OF THE SLC11A1 GENE OF GOATS (CAPRA HIRCUS), IN CONNECTION TO EXPOSURE TO MYCOBACTERIUM AVIUM SUBSPECIES PARATUBER-CULOSIS (MAP)

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Abstract text:
The purpose of this study was the in-vitro assessment of the functional role of the 3' UTR (untranslated region) of the SLC11A1 gene of goats (Capra hircus), in connection to MyAP exposure.

Primary cultures of monocyte-derived macrophages were established from blood of 54 goats selected based on the following polymorphic sites (regions A and B) consisted of guanine-thymine repeats (GTn) of the 3′ UTR of the SLC11A1 gene: A(GTn)15/16 - B(GTn)8/8, A(GTn)15/16 - B(GTn)7/7, and A(GTn)15/16 - B(GTn)7/8. Stimulation was performed using MyAP, bovine purified protein derivative, interferon gamma, and lipopolysaccharides in various combinations. Real time polymerase chain reaction and western blot analysis was performed before, and 1, 3, and 24 hours after stimulation. Gene activity variation was correlated to that of two interleukins, one pro (IL-1a), and the other anti-inflammatory (IL-10). The investigation was repeated with plasmids reconstructed by the insertion of regions A and B. Finally, in silico analysis was performed to predict post-transcriptional active motifs.

The results recorded indicate that the A region alone especially containing the polymorphism GT16, and even more so in combination with the GT8 polymorphism of the B region, is a potent SLC11A1 up-regulator. In transcriptional and translational level the homozygous GT7 polymorphism (B7/7) was found to be strongly associated with up-regulation of the SLC11A1 and IL-1α genes. In-silico analysis predicted five transcriptional regulatory elements and 85 miRNAs within the targeted genomic region.

This study provides for the first time strong evidence at translational, transcriptional and post-transcriptional level that the specific polymorphisms of 3′UTR of the SLC11A1 gene are functional.

Keywords:
SLC11A1, Monocytes, goat

Abstract P-04.17
HIGH PREVALENCE OF LATENT PARATUBERCULOSIS AND GENETIC RISK OF PATENT FORMS

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Abstract text:

Latency of *Mycobacterium tuberculosis* infection is the most prevalent form of human tuberculosis, accounting to more than 90% of all infections. The existence of this form, however, remains controversial for both *Mycobacterium bovis* and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infections in cattle. Recent microbiological and immunological observation on immunopathological forms of paratuberculosis have shown that delimited *focal* granulomatous lesions are also the most frequent form of presentation of natural MAP infections in adult Friesian cattle (39%; n=986), and have been proposed to be defined as *latent* forms. These forms showed a low frequency of humoral immune responses (3%), a moderate rate of MAP detection in intestine and lymph nodes (30%) and a relatively low MAP viability rate (below 50%) compared with the classical *patent* forms (*multifocal* and *diffuse intermediate*, lymphoplasmacytic and *histiocytic*) which were 70% positive in ELISA, 91% positive in tissue PCR and had a viability rate of 90%. Although these data suggest that these *latent* forms are not an immediately cause MAP spread or clinical damage, but rather represent some degree of resilience, they might become critical in the overall spread of paratuberculosis as they are unlikely to be identified by standard paratuberculosis tests, but have a potential for re-activation. Further support for the pathogenetic meaning of these forms is provided by the association of four specific combinations of twenty-four single-nucleotide polymorphisms (SNPs) in six immunity-related genes with different risk levels of *patent* (versus *latent* and *apparently free*) PTB forms (40%, 10%, 0%, 0%, n=502).

Keywords:

latency, tuberculosis, paratuberculosis

Abstract P-04.18

EXPRESSION OF IMMUNOMODULATORY CYTOKINES BY PBMC IN THE COURSE OF AN EXPERIMENTAL MAP INFECTION OF GOATS

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Abstract text:
Host responses deciding whether an animal will overcome MAP infection or will succumb to disease are still not fully understood. Immunoregulatory processes during early MAP infection were studied by analyzing release or gene expression of different cytokines by PBMC at different time points in the course of an experimental MAP infection of goats. Cytokine responses were related to the bacterial organ burden at necropsy (BOBn).

Thuringian goats (n=26) were inoculated with a bovine MAP isolate (JII-1961) ten times beginning at the tenth day after birth, 16 age matched goats served as controls. PBMC were isolated from blood samples taken at intervals of four weeks and stimulated for 24h with jPPD. IFN-γ and IL-10 were quantified by an in-house ELISA. Gene expression of IL-12, IL-18 and TNF-α was examined by real-time RT-PCR. All results were adjusted to differences during sample preparation by comparison to the house-keeping gene GAPDH.

In contrast to control animals, the jPPD-induced IFN-γ response of MAP-infected goats started to increase at 7 weeks post infection (wpi) and reached peak levels between 15 and 22 wpi. Variable responses were measured until necropsy at 51-54 wpi. Animals with moderate BOBn were characterized by very early (11-14 wpi) and high IFN-γ peak responses compared to those with high BOBn. JPPD-induced IL-10 production of MAP infected goats was elevated between 7 and 18 wpi independent from the BOBn. Expression of IL-12 was increased in PBMC of all MAP infected animals while IL-18 and TNF- α were differentially expressed in animals with moderate vs high BOBn. JPPD-stimulation induced a further increase of the IL-12 and TNF-α gene expression, IL-12 expression being especially high between 27 and 38 wpi in animals with moderate BOBn.

In conclusion, the efficacy of the host response to MAP is related to temporal and quantitative differences in the expression of immunomodulatory cytokines.

Keywords:
cytokine gene expression, experimental MAP infection, goats

Abstract P-04.19

DIAGNOSIS AND TREATMENT OF INFECTION BY MYCOBACTERIUM AVIUM PARATUBERCULOSIS (HUMAN PARATUBERCULOSIS) IN A COHORT OF FAMILY MEMBERS WITH SEVERAL DISEASES OF UNKNOWN ETIOLOGY (INCLUDING CROHN'S DISEASE)

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Abstract text:

A cohort of family members with diseases including Crohn's disease, asthma, complex regional pain syndrome, type 1 diabetes mellitus, and lymphangiomatosis and/or evidence of infection by Mycobacterium avium paratuberculosis is described in this series of case reports. The organism was cultured from the blood of those members affected by the first four diseases and there was accompanying elevated antibody to the organism in these cases. The patient affected by the fifth disease has a markedly elevated Map antibody titer. The two patients affected by the first three diseases have been treated with a combination of antibiotics and ultraviolet blood irradiation therapy with resolution of the disease symptomatology and inability to culture the organism and an absence or diminution of antibodies directed against Map in post treatment blood samples. Genetic testing for mutations commonly seen in mycobacterial susceptibility was also performed on the family members. This series of case reports in patients with infections by Mycobacterium avium paratuberculosis provides supportive evidence of a pathogenic role of Mycobacterium avium paratuberculosis in the human host.

Keywords:

Mycobacterium avium paratuberculosis , human, pathogenicity

Abstract P-04.20
GENOME WIDE ASSOCIATION STUDY FOR MAP INFECTION IN GERMAN HOLSTEIN COWS

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Abstract text:
The classical control programs for paratuberculosis in cattle are based on management arrangements and culling of infected animals. A low to moderate heritability for Mycobacterium avium ssp. paratuberculosis (MAP) infection susceptibility is described and documents a genetic background of the disease, especially on the basis of fecal culture in dairy cattle. Therefore, breeding is an additional tool to inhibit the distribution of MAP on a sustainable basis. A genome-wide association (GWA) study was performed in German Holsteins cows within a case-control study, based on fecal culture test method for MAP. All animals were routinely tested within a voluntary program in Thuringia, Germany. Additional all animals of the case-control study were tested by serum ELISA. Because within the herds the cows were annually tested for MAP, we could exclude possible stratification factors and used the following criteria for the control group: same farm, same sire and same age (or older). The Illumina Bovine SNP50BeadChip was used for genotyping. The first analysis showed no significant associations between the SNP's and the fecal test results. This is different to results from other GWA studies based on different test methods for MAP infection (milk or serum ELISA). One reason could be the difference in sensitivity of the diagnostic tests used for the association studies. In addition the different diagnostic tests represent different reactions of the host and therefore different chromosomal regions and genes may be involved. Therefore a standardized phenotypic characterization of the animals is a prerequisite to compare GWA studies as basis for estimating breeding values.

Keywords:
fecal culture, genome-wide association study, German Holstein

Abstract P-04.21

THE DOWN-REGULATION OF FERROPORTIN1 MRNA IN MURINE J774 MACROPHAGES TREATED WITH MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS

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Abstract text:
Mycobacterium avium subspecies paratuberculosis (MAP) has high iron requirements and its in vitro growth is mycobactin-dependent. Ferroportin is a unique iron exporter in macrophages which is down regulated directly by the binding of Hepcidin, the key regulator of iron metabolism in mammals. To analyze the effect of MAP on the iron metabolism of the host organism, we examined the level of ferroportin1 (FPN1) mRNA expression by real-time PCR in mouse macrophage cell line (J774) treated with MAP or MAP antigen. We found that J774 cells treated with 50-200mmicrograms/ml of whole cell lysate of MAP (ATCC 19698) decreased by 25% in relative FPN1 mRNA expression at 6hr after the treatment. Infection with live MAP did not have a significant effect on relative FPN1 mRNA levels at 6hr.When we up regulated expression of FPN1 mRNA with iron-overload treatment of 400mmicromolar ferric nitrilotriacetate, the presence of live MAP (MOI 1:20) resulted in a reduction of relative FPN1 mRNA by >70% at 6hr. These data demonstrate the inhibitory-effect of MAP in FPN1 mRNA expression, and suggest a mechanism for MAP by which the host iron metabolism accommodates the pathogen by increasing macrophage intracellular iron levels. Possible implications for disease pathology in ruminants will also be discussed.

Keywords:
iron, ferroportin1, macrophages

Abstract P-04.22

DEVELOPMENT OF LESIONS DURING THE FIRST YEAR AFTER EXPERIMENTAL INFECTION OF GOATS WITH MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP)

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Abstract text:

Progress of infection and development of lesions during the long clinically inapparent phase of paratuberculosis are not completely resolved yet. The objective of this study was to investigate tissue lesions and presence of Mycobacterium avium subsp. paratuberculosis (MAP) during the first year after experimental infection of goats. Goats were necropsied at 3 (n=7), 6 (n=6), 9 (n=6) and 12 (n=7) month after oral inoculation (mpi) with MAP. Sham inoculated goats (n=3, at 3, 6, 9 and 12 mpi) served as controls. At necropsy, macroscopic lesions were documented and samples collected from the intestinal tract, lymph nodes and other representative organs. Histological lesions were assessed in hemalaun and eosin stained paraffin sections. Presence of MAP was examined by immunohistochemistry and culture. Characteristic granulomatous lesions were detected in gut-associated lymphoid tissue (GALT), intestinal lymph nodes and/or proximal and mid jejunum of all kids that had received MAP, but in none of the sham-inoculated goats. Thickened Peyer's patches in the jejunum (JPPs) were seen in 3/7 kids as early as 3 mpi and were often associated with ulcerations and circumscribed serositis. GALT and intestinal lymph nodes were affected initially. Beginning at 6 mpi, lesions were increasingly found in intestine without GALT. Segmental granulomatous infiltrates in small arteries of the intestinal wall may have contributed to the spreading into mucosa. The morphology of granulomatous lesions was rather uniform in kids at 3 mpi, whereas inter-individual differences varying from mild focal paucibacillary to severe diffuse multibacillary infiltrates were most marked at 12 mpi. These differences are most likely indicators for the progression of the infection later on.

Keywords:

pathology, experimental MAP infection, goats

Abstract P-04.23

ZAP-70, CTLA-4 AND PROXIMAL T CELL RECEPTOR SIGNALING IN COWS INFECTED WITH MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS

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Abstract text:

Paratuberculosis is a chronic intestinal disease of ruminant animals caused by Mycobacterium avium subsp. paratuberculosis (MAP). A hallmark of paratuberculosis is a transition from a cell-mediated Th1 type response to a humoral Th2 response with the progression of disease from a subclinical to clinical state. The objective of this study was to investigate the expression of two crucial molecules in T cell function, ZAP-70 (zeta-chain associated protein of 70 kDa) and CTLA-4 (cytotoxic T-lymphocyte antigen-4), in cows naturally infected with MAP. Peripheral blood mononuclear cells (PBMCs) isolated from control non-infected cows (n=5), and cows in clinical (n=6) and subclinical stages of paratuberculosis (n=6) were cultured alone (medium only), with concanavalin A, and a whole cell sonicate of MAP for 24, 72 and 144 hours to measure the dynamic changes of ZAP-70 and CTLA-4 expression on CD4, CD8, and gamma delta T cells. Flow cytometry was also performed to measure ZAP-70 phosphorylation to examine proximal T cell receptor signaling in animals of different disease status. It was found that the surface expression of CTLA-4 is dramatically increased in animals in subclinical stage of infection while levels of ZAP-70 are significantly decreased in CD4+ T cells of both subclinical and clinical animals, indicating a change in T cell phenotype with disease state. Interestingly, proximal T cell receptor signaling was not altered in infected animals. This study demonstrates changes in crucial signaling molecules in animals infected with MAP elucidating T cell alterations that are associated with disease progression.

Keywords:

T cell, Immune response, T cell receptor signaling

Abstract P-04.24
DIVERSE EXPERIENCES ARISING FROM A MAP INFECTION MODEL IN RED DEER

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Introduction: Johne's disease emerged as a significant problem on many deer farms in New Zealand over 15 years ago. In sheep and cattle Johne's disease is sporadic, it occurs most commonly in 2-4 year old animals and tends to become slowly progressive. In deer it causes clinical disease in young red deer aged 9-27 months, it can have an attack rate of up to 20% and it progresses quite rapidly in young animals.

In order to study the disease, an experimental oral challenge model was developed, which has allowed us to investigate the pathogenesis of disease, immune responses, host genetic factors and test vaccines. This paper describes the oral challenge model, how it has been used and summarises findings over the last 10 years.

Methods: Key factors in the red deer oral challenge model;
· Age (3-6 months old)
· Strain of MAP (bovine)
· Source of MAP— recovered from enlarged mesenteric lymph nodes (LN) of young deer with severe multibacillary (MB) lesions confirmed by histopathology
· Oral dose (daily for 4 days with 4 x 10^8 - 4 x 10^9 cfu)
· Monitoring:
  · Animals are closely monitored by weekly weighing
  · Clinical assessment
  · 2-4 weekly blood sampling for Paralisa^R testing
  · 2-4 weekly faecal qPCR for enumeration of MAP
· Clinically affected animals are promptly euthanized when they start to lose weight
· Samples of the mesenteric lymph nodes were taken under general anaesthetic 8-13 weeks post challenge for histopathology, culture and/or qPCR in some studies
· Histopathology of mesenteric LN and intestines to assess lesion severity score (LSS 1-13) and MB/PB (paucibacillary); LSS 1-4 mild, LSS 5-8 moderate, LSS 9-13 severe.
  (Clark et al., 2010, 2011)

Results: This challenge model resulted in a range of disease outcomes that were typical of those seen on deer farms, ranging from mild subclinical infection to clinical disease. Usually the first signs of clinical disease, such as nil weight gain and diarrhoea, occurred 15-35 weeks post challenge and the animals were euthanized within 2-3 weeks due to weight loss and loss of muscle mass over the loins. Subclinically affected animals usually had some degree of enlargement of the mesenteric lymph nodes and these had small foci or caseous lesions, as well as histopathological lesions of varying severity.

The model has been used to study:
· Dose response of MAP (Mackintosh et al., 2007)
· Bovine vs ovine strain MAP (Mackintosh et al., 2007)
· Age susceptibility (Mackintosh et al., 2010)

· Vaccine efficacy (Mackintosh et al., 2008)
· Longitudinal pathogenesis study (Mackintosh et al., 2012)
· Immunological and pathological responses of red deer resistant or susceptible genotypes (Mackintosh et al., 2011)
· Refinement of diagnostic tests, including the Paralisa^{R} and IFN-gamma and faecal qPCR
· Development and refinement of a panel of tests to predict heritable phenotypes for resistance and susceptibility to paratuberculosis in the absence of MAP challenge

Table: Summary of details from 7 studies showing the number of red deer in each group, their age and genotype (unselected or of known or suspected resistant R or susceptible S genotype), the oral dose and strain of MAP, number of clinical cases, when the remaining animals were killed, proportion of animals identified as having MAP infection and the average Lesion Severity Score (LSS)

Study	No. deer	Age	Genotype	Oral dose	Strain MAP	Clinical Johne's	Week killed	MAP +ve	Mean LSS
Dose response and	16	4 mo	unselected	10^9	Bovine	5	11	16/16	4.8
bovine vs ovine	16	4 mo	unselected	10^7	Bovine	0	16	16/16	2.9
MAP	16	4 mo	unselected	10^3	Bovine	0	16	8/16	0.9
	16	4 mo	unselected	10^7	Ovine	0	16	11/16	0.9
Age susceptibility	27	3 mo	unselected	4 x 10^9	Bovine	10	17	26/27	4.9
	20	15 mo	unselected	4 x 10^9	Bovine	0	20	19/20	3.5
	20	adult	unselected	4 x 10^9	Bovine	0	20	18/20	1.1
Vaccine efficacy - Control	30	4 mo	unselected	4 x 10^8	Bovine	0	30	29/30	3.7
Vacc 1	30	4 mo	unselected	4 x 10^8	Bovine	0	30	25/30	2.0
Vacc 2	30	4 mo	unselected	4 x 10^8	Bovine	0	30	27/30	3.6
Pathogenesis study	5	4 mo	Sire A	4 x 10^9	Bovine	0	50	5/5	7.6
of R/S deer	9	4 mo	Sire B	4 x 10^9	Bovine	0	50	9/9	9.3
Immunological study	9	4 mo	Sire R	4 x 10^9	Bovine	1	49	8/9	5.9
of R/S deer	9	4 mo	Sire S	4 x 10^9	Bovine	2	49	9/9	11.7
2012/13 R/S study	3	4 mo	Sire R	4 x 10^9	Bovine	0	43	3/3	8.6
	6	4 mo	Sire M (R?)	4 x 10^9	Bovine	0	43	6/6	8.2
	5	4 mo	Sire S	4 x 10^9	Bovine	3	43	5/5	12.6
	4	4 mo	Sire 8 (S?)	4 x 10^9	Bovine	4	43	4/4	13.6
2013/14 R/S study	5	4 mo	Sire R	4 x 10^8	Bovine	0	37	1/5	3.4
	5	4 mo	Sire Br (R?)	4 x 10^8	Bovine	0	37	0/5	1.6
	5	4 mo	Sire S	4 x 10^8	Bovine	0	37	5/5	8.2
	5	4 mo	Sire Bo (S?)	4 x 10^8	Bovine	0	37	4/5	8.8

Conclusions: If used within recommended parameters, this model reliably produces a range of infection/disease outcomes typical of naturally acquired paratuberculosis in red deer, which has accelerated research into this disease and led to the development of a number of key tests and findings.

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Keywords:
red deer, challenge model, reliable outcomes

Abstract P-04.25
CLONING AND EXPRESSION OF THE FUR PROTEIN OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS, FOR USE AS A VACCINE

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Abstract text:

Introduction
The Fur protein of Mycobacterium avium subsp. paratuberculosis (MAP), which is known to cause paratuberculosis is located in a pathogenicity island 38 Kb. Hypothetically its function is recognized as having sequence similarities to other bacteria such as, regulating the expression of proteins involved in the metabolism and storage of iron. Therefore, it is important for the development of vaccines against MAP.

Objective
Clone and express of the Fur protein MAP in E. coli, for use as a vaccine.

Materials and Methods
Designing primers for amplification of the fur gene MAP ATCC strain K10 was conducted and cloning performed of the plasmid TOPO pCR 2.1, pRSET-A by Invitrogen and subsequent transformation into DH5 α E. coli TOP 10 F.

Results
Cloning in the pCR 2.1 TOPO plasmid was performed and of the 20 clones conducted six were checked by PCR. These were found to be positive for the fur gene, and the resulting plasmid band was obtained from 420 bp accounted fur by digestion with enzymes SacI and Hind III. Cloning in the expression vector pRSET-A, 28 clones were characterized by PCR where clone 10 was selected. The expression was performed by IPTG with a band of approximately 15 kDa obtained in the polyacrylamide gel, which is specific for Fur.

Conclusions
Paratuberculosis is a chronic disease in ruminants and related to Crohn's disease in humans. To date there is no treatment for this disease so; the necessity to develop vaccines, together with the management practice of newborn animals and sanitation herds would help to control this chronic disease.

Keywords:
MAP, protein, vaccine

Abstract P-04.26
VACCINATION WITH MAP SPECIFIC PEPTIDES REDUCES MAP BURDEN IN TISSUES OF INFECTED GOATS

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Abstract text:
As an alternative to protein-based vaccines, we investigated the effect of post-exposure vaccination with Map specific peptides in a goat model aiming at developing a Map vaccine that will neither interfere with diagnosis of paratuberculosis nor bovine tuberculosis.

Peptides were initially selected by two strategies 1) in silico selection of unique Map peptides (compared to other Mycobacteria) and with predicted binding to 5 known bovine MHC class II molecules or 2) hydrophobic peptides unique to Map from selected proteins. Based on immunogenicity studies in goats a subselection of 23 MAP peptides (20 µg each) was formulated in a cocktail with Montanide ISA 61 VG adjuvant in a ratio of 1:1½.

A post-exposure vaccination study was performed with 10 goats orally inoculated with 4x10E9 live Map in their third week of life and randomly assigned to two groups of five goats each. One group was left unvaccinated, while the other was vaccinated at 14 and 18 weeks post Map inoculation with the peptide cocktail. At termination 32 weeks post Map inoculation, Map burden in 15 gut tissues and lymph nodes was determined by quantitative IS900 PCR.

Of the 75 tissue samples from the 5 unvaccinated goats only 5 samples were IS900 qPCR negative. In contrast, only 9 samples in total from the 5 peptide-vaccinated goats were IS900 positive with a highly significant ($p<0.0001$) difference in Map numbers between the groups. All immunized goats responded with strong IFN-γ responses to the peptide pool from 2 weeks post 1st immunization and throughout the study while unvaccinated goats were unresponsive to the peptides at all times. IFN-γ responses to PPDj were low in all goats at all times, except for one peptide vaccinated goat that responded from 26 weeks post inoculation and onwards. A single goat in the unvaccinated control group seroconverted in ID Screen® ELISA at last sampling prior to euthanasia.

These results indicate that a subunit vaccine against Map can induce a protective immune response against paratuberculosis in goats.

Keywords:
Vaccine, peptide, goat

Abstract P-04.27

LONGITUDINAL EVALUATION OF DIAGNOSTICS IN YOUNG CALVES INFECTED WITH MAP, BOTH IN THE CLINICAL AND SUBCLINICAL STAGE OF JOHNE'S DISEASE

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Commercially available diagnostic tests for MAP seem to lack sensitivity and performance of these tests strongly depends on the stage of Johne's disease (JD) at the time of testing. In an experimental infection trial, 2 steers developed clinical JD and provided the opportunity to identify the onset of positivity for routinely used diagnostic tests.

Both calves were part of a group (n=5) inoculated at 2 wk of age with 5 x 10^9 CFU of MAP on 2 consecutive days. Whole blood, serum and feces were collected weekly during the first month after inoculation and thereafter monthly until necropsy at 16 or 17 months of age. Gross lesions and histology were assessed at necropsy and samples collected for tissue culture. Before clinical signs became apparent, these 2 calves were consistently MAP fecal culture-positive starting 2-3 weeks after inoculation, whereas antibody ELISA was positive as of 4-5 months after inoculation. Two of the asymptomatic calves in this group shed MAP intermittently and the third calf was shedding persistently towards the end of the trial; 1 was ELISA-negative, 1 had a transient response, and 1 was ELISA-positive as of 10 months after inoculation. In contrast, asymptomatic and clinical calves had a similar IFN-γ response with an increase in IFN-γ at 2-3 months after inoculation. At necropsy, all 3 asymptomatic calves were less severely affected compared to clinical calves, based on gross lesions, histology and tissue culture. In conclusion, these 2 steers exhibited clinical JD at a very young age. As of 15 months (shedding) and 1 year (ELISA) before the onset of clinical symptoms, these calves consistently tested positive, in contrast to those that were asymptomatic.

Keywords:
clinical JD, experimental infection, diagnostics

Abstract P-04.28

IMMUNE RESPONSE IN CALVES EXPERIMENTALLY INFECTED WITH DIFFERENT GENOTYPES OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS

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Abstract text:

The aim of this work was to evaluate immune response induced after experimental infection of calves with two different bovine genotypes (by VNTR) of Map from Argentina. Holstein calves (6-8 weeks old) were infected using two oral doses (246 mg of total wet weight) of genotypes A (MA, n=3) or C (MC, n=2) and mock infected (MI, n=2). Specific IgG, IgG1 and IgG2 against Map whole bacteria (wMap), protoplasmic antigen (PPA) were evaluated by ELISA. Avian purified protein derivative (PPDa), bovine purified protein derivative (PPDb) specific proliferation, IFN-γ production and CD4, CD8, WC1 and CD25+ cell subpopulations were determined and intradermal comparative test (ICT) was measured at day 160. We detected significant increases of wMap-specific IgG and IgG1 in sera from both infected groups from day 80. MC showed higher levels of Igs than MA. Remarkably, wMap-specific IgG2 was the first isotype detected from day 50. Meanwhile, PPA-specific IgG and IgG1 were low during the experiment, PPA-specific IgG2 were positive in MC from day 80. Antibody levels in MI remained under cut off values. From day 30 Map, PPDa and PPDb stimulation index (SI) were greater in infected groups than in MI ($p<0.05$). The highest SI was detected in MC at days 90 and 120, showing significant differences with MA group at day 120. IFNγ production was variable, detecting an increase of PPDa specific in MC at day 90. Peripheral CD8+ cells decreased in MC group at day 60 and WC1CD25 expression was increased in both infected groups at day 90 ($p=0.01$). In addition, the ICT showed higher reactions in MA than MC. Our results demonstrate that humoral and cellular immune response are early induced in Map experimental infection. Data also suggest that differences in early immune responses in experimentally infected calves could be related with the specificity, isotypes and genotypes of Map evaluated.

Keywords:

immune response, calves challenge, genotypes

Abstract P-04.29
CYTOKINE GENE EXPRESSION PROFILE IN THREE PATHOLOGICAL FORMS OF PARATUBERCULOSIS IN BULLOCKS

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Abstract text:
In the present study the profile of certain cytokines were analysed in the infected tissues in three different forms of bovine paratuberculosis. The ileum and mesenteric lymph node (MLN) tissue samples were collected from 11 paratuberculosis infected adult bullocks which were identified as paratuberculosis infected by histopathology, IS900 gene PCR, qPCR, ZN (Ziehl Neelsen) staining and IPT. The mRNA gene expression of cytokines viz. IL-1α, IL-10, TGF-β, IFN-γ and iNOS were measured by RT-qPCR from ileum and MLN. Histologically these infected bullocks were divided into 3 pathological forms viz. type 1, type 2 and type 3 based on the nature and severity of lesions, type of cellular infiltration and number of bacteria in the lesions. Type 1 bullocks were characterized by diffuse lymphocytic infiltration in small intestine and focal granuloma in MLN. Type 2 bullocks showed focal granulomas in small intestine as well as in MLN. Both type 1 and type 2 bullocks showed scarce AFB (acid-fast bacilli) in small intestine and MLN. Type 3 bullocks showed diffuse epithelioid cell infiltration, multifocal granuloma, giant cells and abundant AFB in the small intestine and MLN. The cytokine studies revealed significant increase in the expression level of IL-1α and TGF-β in type 1, type 2 and type 3 bullocks as compared to non-infected bullocks, which indicated their important role in inflammatory process and progression of paratuberculosis infection in cattle. Increased expression of TGF-β and IL-10 was observed in type 3 bullocks as compared to control, type 1 and type 2 bullocks indicated their roles in the clinical or multi-bacillary form of the disease. The expression level of iNOS was significantly higher in ileum of type 2 bullocks compared to uninfected control bullocks. This enhanced iNOS expression could be associated with focal granulomatous lesions observed in type 2 bullocks.

Keywords:
Bullocks, Cytokine, Paratuberculosis

Abstract P-04.30

PATHOLOGY OF CLASSICAL AND NON-CLASSICAL FORMS OF JOHNE'S DISEASE IN BULLOCKS NATURALLY INFECTED WITH MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS (MAP) BISON TYPE

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Abstract text:

The pathology and efficacy of different diagnostic tests in detection of paratuberculosis infection in bullocks was investigated. Small intestine and mesenteric lymph node (MLN) tissue samples were collected from 404 bullocks, of which 35 (8.66%) were found to be positive for paratuberculosis by histopathology, immunoperoxidase test (IPT), Ziehl Nielsen (ZN) stain, bacterial culture, IS900 PCR and qPCR. These bullocks were classified into classical (2) and non-classical (33) forms on the basis of histopathological findings and mycobacterial loads. Microscopically, two types of lesions were observed in the non-classical form; the diffuse lymphocytic (15) and the focal (18). The diffuse lymphocytic lesions were characterized by infiltration of mononuclear cells without granuloma formation in the small intestine, however, MLNs of these bullocks showed granuloma. The focal type showed focal granulomas along with lymphocyte and macrophage infiltration in the small intestine as well as in the MLN. The classical form showed diffuse epithelioid cell infiltration, multifocal granulomas, giant cell formation and abundant acid-fast bacilli in the small intestine and the MLN. Bullocks with classical lesions were positive in the bacterial culture, whereas all the bullocks with non-classical form were found to be negative. The IS900 PCR, qPCR, IPT and ZN detected both (100%) bullocks of classical form and 19 (57.58%), 23 (69.70%), 11 (33.33%) and 6 (18.18%) bullocks with non-classical form, respectively. The findings of the present study indicates that paratuberculosis due to bison type is prevalent in bullocks in India causing mostly the non-classical form of the disease. The qPCR was found to have greater sensitivity in detection of non-classical form in comparison to conventional PCR, culture, IPT and ZN staining.

Keywords:

Bullocks, Paratuberculosis , Pathology

Abstract P-04.31

CYTOKINE RECEPTOR POLYMORPHISMS ASSOCIATED WITH T-CELL DIFFERENTIATION IN SHEEP PARATUBERCULOSIS

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Abstract text:

Paratuberculosis in sheep presents as two distinct disease forms; paucibacillary (or tuberculoid) and multibacillary (or lepromatous) disease. The immunopathological responses associated with these two disease forms have been characterized as an inflammatory Th1/Th17 and a macrophage/Th2 response respectively. Differential polarization of the immune response determines pathology; animals that develop high antibody levels generate little IFNγ, characteristic of a Th2 response, and cannot control bacterial replication. This project aims to identify the molecular basis of T-cell polarization in the two pathological forms of sheep paratuberculosis. The cytokines IL-23A and IL-25 are key to the development of Th17 and Th2 responses by the interaction with their complex receptors; IL23R/IL12RB1 and IL17RB/IL17RA respectively. In other species these receptor genes are polymorphic and linked to various inflammatory diseases of the gut. We have previously identified genetic and/or transcript variants for both these cytokines; and this project is focussed on identifying gene/transcript variants in the cytokine receptors. Sequencing the transcripts and genes of the cytokine receptors has identified transcript variants present in both IL12RB1 and IL17RB genes. RT-qPCR assays have been developed for the individual variants of each cytokine receptor and used to quantify their expression levels in the ileocecal lymph node isolated from paucibacillary and multibacillary sheep to try and link individual transcript variation to disease phenotype.

Keywords:

Immunology, Cytokine, Polymorphisms

Abstract P-04.32

EFFECT OF VACCINATION AGAINST PARATUBERCULOSIS ON FECAL SHEDDING AND PRODUCTIVITY OF HEAVILY INFECTED CATTLE HERDS

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Introduction

Since its first occurrence in 1961, paratuberculosis has become a common disease of ruminants in Slovenia. During the next three decades, no cases of paratuberculosis were reported, but since 1993, when the disease was found in a sheep flock, several outbreaks in cattle, goats and sheep have been documented. With the exception of a period between 1995 and 2001, systematic screening of the disease in cattle has never been performed in Slovenia. The last seroprevalence study in randomly selected cattle herds was conducted in 2008, revealing a fairly low true prevalence at the herd level (Kušar et al., 2011). However, later studies indicated that presumably a lot more dairy cattle herds are infected with *Mycobacterium avium* subsp. *paratuberculosis* (Map) (Starič et al., 2011). In addition, up to 89% of the animals within a herd were found to be infected with Map (Logar et al., 2012) which leaves limited options to the farmers interested in controlling the disease – either to cull the animals or to implement very restrictive control measures. The objective of this paper is to present the findings related to vaccination of two heavily Map-infected cattle herds. In both herds, an almost 50% prevalence of Map infection was established by faecal culture of all cattle older than two years.

Methods

Milk and faecal samples of cattle of different age groups were investigated by culture and qPCR (Logar et al., 2012) for the presence of Map before and after vaccination.

On farm A, a total of 91 milk and 141 faecal samples were collected in December 2010. Vaccination with 1 mL Gudair® vaccine (CZ Veterinaria, Spain), administered subcutaneously, was carried out in December 2011. Post-vaccination sampling was performed in May 2012, including 48 milk and 103 faecal samples.

On farm B, 96 milk and 138 faecal samples were collected in July 2011. Vaccination followed in January 2012 and post-vaccination sampling of 38 milk and 120 faecal samples in January 2013. Average milk yield in standard lactation on both farms was recorded. No specific paratuberculosis control measures were implemented on the farms.

Summary of new and unpublished data

Vaccination was not equally effective on both farms. On farm A, infection was decreased on a herd level as there was a reduction of Map in all tested samples. Meanwhile, on farm B vaccination obviously reduced the shedding of Map in faeces and milk but the infection on the herd level was not controlled as the number of positive faecal samples increased. It should be noted that the management of the herds differs between the farms which might have influenced the results of the study. Farm A is a family-run holding, exclusively depending on the income from the farm while farm B is managed by hired workers and represents only an additional source of finances for the owner. However, in both herds the average milk yield increased for about 600 kg per cow and, most importantly, the shedding of Map in milk decreased after vaccination. Detailed results of investigations are presented in Table 1.

Table 1: Cultivation and PCR results of samples taken before and after vaccination in both cattle herds

sample	farm A		farm B	
	before	after	before	after
faeces culture	19.1%	13.6%	30.4%	35.0%
faeces-qPCR	93.6%	35.9%	83.3%	97.5%
milk-qPCR	41.76%	2.08%	27.08%	7.89%
N Map/g faeces[1]	257	126	624	369
milk yield/cow[2]	6194.2 kg	6837.3 kg	5142.7 kg	5735.5 kg

[1] average number of Map in one gram of faeces, [2] average milk yield per cow per year

Conclusion

In the infected herds, control measures should be proposed on individual basis, taking into account the infection rate and type of cattle breeding. Vaccination apparently reduces losses and Map shedding in milk; it should therefore be considered for controlling the disease, but only as a last option. As shown in previous studies, vaccination alone is not sufficient for paratuberculosis control. Efficient control should necessarily include biosecurity measures, in addition to culling of the shedders.

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Keywords:

vaccination, map shedding, production

Abstract P-04.33
ASSOCIATIONS OF CANDIDATE GENES INVOLVED IN SUSCEPTIBILITY TO MAP INFECTION IN HOLSTEIN COWS

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Abstract text:
The understanding of the genes associated with MAP infection is fundamental to evaluate them in to breeding schemes and control the disease. The aim of this study was to investigate the genetic variability within a pool of 8 genes and achieve a study of association with MAP infection in a population of Holstein cattle classified as MAP-positive and MAP-negative using both ELISA and faecal cultural results.

A case-control study was designed by using a total of 324 German Holstein cows from 15 different farms tested for paratuberculosis using faecal culture. In addition the plasma was tested for MAP antibody by ELISA. Buffy coat was used for isolation of DNA. The genes were investigated for genetic variability by PCR and sequencing. Twenty SNPs were genotyped and finally 9 SNPs were included in the association study.

The SNP rs43390642 in the WNT2 promoter region was significantly associated with MAP infection using both faecal and ELISA diagnostic test. The linkage disequilibrium with the SNP rs134692583 in DLD might suggest a combined mechanism of action of these neighbour genes into MAP infection. Significant association was also found between the faecal culture status of the animals and allele variants of the SNP AY518738S04:g.521G>A in NOD2. The other SNPs within the genes: LAMB1, PRDM1, SOCS5, PTGER4 and IL10 showed no associations.

This study clarifies the involvement of the investigated loci in MAP infection. The identification of SNPs associated with MAP infection is the first step to set up marker assisted selection programmes to improve the health status by breeding.

Keywords:
Assosiation study, MAP infection, Holstein cattle

Abstract P-04.34

VIRULENCE ATTENUATION OF AN INOCULUM PREPARED FROM GUT MUCOSA AFTER BACTERIAL CULTURE IN EXPERIMENTAL OVINE PARATUBERCULOSIS

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Abstract text:
The inoculum used in experimental infections with Mycobacterium avium subsp paratuberculosis (MAP) is one of the factors that can influence notably the outcome of the challenge. The aim of this study is to compare the results of an experimental challenge in lambs using either an inoculum prepared from gut mucosal tissue or cultured bacteria obtained from the same intestinal homogenate.

A total of 12 one-month-old lambs were divided into two groups and infected with a total amount of 1x108 mycobacteria per lamb of a suspension obtained from the ileal mucosa of a diseased sheep (group M) or the low passage isolate (group C) obtained after the culture of the mucosal homogenate employed in group M. A third group of 6 animals was kept as uninfected control. The peripheral immune response was evaluated and pathological and nested PCR analysis were carried out in three lambs from each group culled at 120 days post infection (dpi) and in the remaining animals euthanized at 220 dpi.

All lambs from group M showed specific peripheral cellular (evaluated by IFN-γ production) and humoral (indirect ELISA) immune responses from day 90 and 120 respectively, whereas only some lambs from group C showed positive IFN-γ response at 90 dpi and no antibody response was detected in this group. Lesions were observed in all lambs from group M: at 120 dpi animals have multifocal lesions, with granulomas widespread to several areas of the intestinal mucosa and at 220 dpi, one lamb had a focal form and the remaining multifocal lesions. However, only focal lesions, with no acid fast bacteria, were seen in the Peyer's patches of three lambs from group C, two killed at 120 and one at 220 dpi. MAP DNA was detected by nested PCR in tissues from all lambs from both groups.

The MAP isolate cultured from an intestinal homogenate clearly had significantly lower virulence than the inoculum directly prepared from the intestinal homogenate.

Keywords:
Virulence, Culture, Homogenate

Abstract P-04.35

CONSISTENT GENE EXPRESSION PANELS IDENTIFIED AT EARLY TIME POINTS THAT PREDICT CLINICAL OUTCOMES OF JD IN SHEEP

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Abstract text:

A study was undertaken to identify gene expression changes in Merino sheep experimentally exposed to MAP in comparison to control animals and to correlate these changes with clinical outcomes (paucibacillary, multibacillary, no clinical disease). The objective was to identify consistently regulated gene panels associated with the specific clinical outcomes. Affymetrix GeneChip Genome Array technology is a tool for probing the expression of thousands of genes in a single experiment and works by exploiting the ability of messenger RNA to bind specifically to the DNA template from which it originated. A comparison of hybridisation between samples derived from control and exposed animals provides an overview of gene expression patterns in response to infection. Twenty MAP-exposed and ten unexposed control sheep were sampled at four early time points post inoculation (2, 4, 6 and 9 months) and just prior to emergence of clinical signs (12 months); RNA was isolated from the white blood cell fraction of the blood and the resulting samples were processed to gene expression analysis. Clinical outcome was verified by histopathology, faecal culture, HT-J faecal PCR analysis and serum ELISA. The clinical outcome was correlated with the gene expression changes and this resulted in the identification of panel of genes. These genes had consistent differential expression through the pre-clinical phase of the infection in comparison to control sheep and were associated with specific clinical outcomes. qRT-PCR verification of 12 selected genes verified these GeneChip findings. This study is the first to successfully identify specific gene panels that are indicative of pathological outcome long before the animals progress to clinical disease.

Keywords:

Gene expression, Disease pathogenesis, Sheep

Abstract P-04.36
EVALUATION OF MAP-SPECIFIC PEPTIDES FOLLOWING VACCINATION OF GOATS

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Abstract text:
Our aim is to develop a subunit MAP vaccine not interfering with the diagnosis of paratuberculosis or bovine tuberculosis. This study's objective was to evaluate MAP-specific peptides defined by in silico analysis.

Peptides were picked by 1) comparing MAP genomes to that of other mycobacterium species or 2) selected based on "experience". Peptides predicted to bind bovine MHC II by in silico analysis were included in further studies, resulting in two panels 1) genome-based and 2) selected. Initially, two groups of 15 healthy goats were vaccinated with one of the two panels (50 µg/peptide in CAF01 adjuvant/CAF04 for boosting). Four MAP-infected goats were also vaccinated. In a second vaccination trail, groups of 8 healthy goat kids were vaccinated with genome-based peptides, selected peptides or selected peptides linked together in a recombinant protein (20 µg/peptide or 50 µg protein in Montanide ISA61 adjuvant). IFN-γ responses were measured by ELISA and ELISPOT upon stimulation with peptide pools or individual peptides. T cell lines were made by cultivating CD4+ cells in the presence of antigen, feeder cells plus cytokines, and used to evaluate responses to peptide pools and individual peptides.

IFN-γ responses in healthy goats after the first vaccination were low, but testing of T cell lines from MAP-infected goats identified peptides inducing strong proliferative responses. Peptides for a second vaccination were selected by combining results from this study with a parallel cattle study. In the second trial, goats in the genome-based and the selected peptide group had solid IFN-γ responses while goats in the protein group had modest responses. Only a moderate boosting effect was seen in the second trail. The genome-based pool induced the strongest CD4+ T cell line responses and had the highest number of immunogenic peptides.

This study shows 1) that detection of immunogenic antigens using in silico predictions and T cell lines work, and 2) the identified MAP-specific peptides show potential for use in a subunit vaccine.

Keywords:
Peptide vaccine, T cell lines, in silico analysis

Abstract P-04.37

IMMUNOLOGICAL ANALYSIS OF 35 NEW ANTIGENS OF M. PARATUBERCULOSIS IN MICE INFECTED WITH M. PARATUBERCULOSIS, M. AVIUM, M. TUBERCULOSIS.

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Abstract text:
The goal to this study is to evaluate the immunogenicity and the specificity of 35 novel M. paratuberculosis proteins in BALB/c and C57BL/6 mice experimentally infected with M. avium subsp.paratuberculosis (Map), M.avium subsp. avium, and M. bovis.

23 of these 35 candidates were identified in standard Map biofilm cultures (surface pellicle) on Sauton medium by proteomics and immunoproteomics (Leroy et al, 2007). 6/35 were identified in latency models based on Sauton cultures submitted to different stress conditions (hypoxia, acidic pH, nutrient starvation or non-toxic NO). Finally, 6/35 were identified using an in silico analysis of Map genome. The 35 proteins were produced as recombinant histidine-tagged proteins in E. coli and purified by affinity chromatography on immobilized nickel-chelate (Ni-NTA) columns. Mice were infected intravenously with M. bovis AN5 or with luminescent Map or M. avium ATCC 15769 strains obtained by transformation with pSMT1 plasmid. At day 1 and 2, 5, 8, 12 and 20 weeks after infection, mice were killed and organs were individually analysed for bacterial replication by luminometry and by/or standard plating. Spleen cell IFN-γ production following in vitro stimulation with the 35 proteins and serum antibody levels were measured by ELISA.

Of the three isolates, M. avium subsp. avium was the most virulent strain as reflected by highest bacterial numbers in spleen, lungs and liver. M. bovis and Map showed a preferential replication in lungs and liver, respectively. Strong antibody responses were only detected in M. avium infected mice.

All proteins were immunogenic and recognized at least once during experimental infection. However, although some proteins were recognized more strongly in Map/M.avium than in M. bovis infected mice, no real species-specific antigens could be identified.

Keywords:
MAP antigens, mice, IFN-g

Abstract P-04.38

ASSOCIATION BETWEEN SINGLE NUCLEOTIDE POLYMORPHISMS AND SUSCEPTIBILITY TO MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS INFECTION IN NATIVE INDIAN CATTLE POPULATION

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Abstract text:

Pathogenesis of Johne's disease caused by Mycobacterium avium subspecies paratuberculosis is complex and has not been completely understood yet. It is evident from some recent studies, that genetic variations in the host genes may also play important role in the development of JD. Study analyzed nine known SNPs belong to four candidate genes (SLC11A1, TLR2, NOD2 and IFNG) in case-control cattle population. On the basis of clinical sign and positivity in diagnostic tests, 94 animals were broadly categorized into 'Case' (n=47) and 'Control' (n=47) animals or groups. Results of univariate logistic regression analysis revealed that there was no significant effect of 'breed' on disease (JD) occurrence. On the basis of frequency and distribution of genotypes (as revealed by PCR-RFLP of targeted SNPs) in case-control population, only TLR2 showed a significant effect ($p<0.05$) on occurrence of JD. Distributions of AA, AC and CC genotypes in 'Case' groups were 0.66, 0.04 and 0.3 respectively whereas 0.57, 0.21 and 0.21 were found in 'Control' population. Estimated Odd ratios for this locus (TLR2) revealed the relative resistance of homozygotes (AA), while heterozygotes were more abundant in 'Case' than 'control'. Rest of the three genes (SLC11A1, NOD2 and IFNG) failed to distinguish between infected and healthy animals. Although the observations seem biologically plausible however in view of certain limitations of this study, further population and functional studies are needed to confirm these relationships and to clarify the underlying mechanisms.

Keywords:

Indian cattle, Mycobacterium avium subspecies paratuberculosis, SNPs

Abstract P-04.39

TISSUE REDUCTION OF MAP NUMBERS AFTER POST-EXPOSURE VACCINATION WITH SINGLE LATENCY ANTIGEN IS IMPROVED BY COMBINATION WITH ACUTE-STAGE ANTIGENS IN GOATS

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Abstract text:
A new (FET11) multi-stage vaccine against paratuberculosis has been shown to reduce Map numbers in cattle after post-exposure vaccination. Here we investigated the effect of vaccination with latency antigen alone, or two different constructs of the multi-stage vaccine in a goat model.

Goats were orally inoculated with 4x10E9 live Map in their third week of life and randomly assigned to four groups of five goats each. One group was left unvaccinated, while other goats were post-exposure vaccinated at 14 and 18 weeks post Map inoculation with either a single FadE5 protein (100 µg), FET11 multi-stage vaccine with FadE5 (60 µg) and ESAT-fusion protein (40 µg), or FET13 with FadE5 and ESAT proteins combined in a single fusion protein (100 µg), respectively. All proteins were delivered in CAF09 adjuvant (DDA/MMG/Poly I:C). Antibody responses were measured by ID Screen® ELISA and individual vaccine protein ELISAs along with IFN-γ release assay with PPDj and vaccine proteins. At termination 32 weeks post Map inoculation, Map burden in 15 gut tissues and lymph nodes was determined by quantitative IS900 PCR.

FadE5 vaccination induced a significant protective effect in goats with consistently reduced Map numbers compared to unvaccinated control goats. FET11 and FET13 vaccination, however, provided even stronger protection with absent or very low Map numbers in tissues ($P<0.0001$). No goats seroconverted in ID Screen® ELISA, except for a single goat in the unvaccinated control group at last sampling prior to euthanasia. PPDj responses were low in all goats at all times.

These results indicate a combination of acute and latent stage antigens in appropriate adjuvant formulation induces a protective immune response against paratuberculosis and corroborate previous findings of the FET11 vaccine in calves.

Keywords:
Vaccine, goats, multi-stage antigens

Abstract P-04.40
INVASION CAPACITY AND CYTOKINE INDUCTION IN EPITHELIAL CELLS BY MAP STRAINS OF DIFFERENT GENOTYPES

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Abstract text:
Mycobacterium avium subsp. paratuberculosis (MAP), causing chronic enteritis in cattle and other ruminants, has a distinct intestinal organ tropism compared to other mycobacteria. After oral ingestion, intestinal epithelial cells are the first to interact with MAP. Only little data are available about the interaction of MAP with intestinal epithelial cells of ruminants, an important component of the pathogen-host communication system.

The aim of our study was to investigate the ability of five different MAP-strains to invade and modulate cytokine responses of the bovine intestinal epithelial cell line FKD-R 971. Reference strain DSM 44135, bovine field isolate JII-1961, red deer isolate JII-0821 (all major Type II) and sheep field isolate JIII-86 (Type III) were compared to laboratory adapted type strain ATCC 19698. FKD-R cells were inoculated with MAP at a MOI of 100 and cultivated for 4-72 h. Viable internalized bacteria were quantified as colony counts. Response of FKD-R 971 cells to infection was characterized by analysing pro-inflammatory cytokines expression (IL-1 beta, IL-8, MCP-1, GM-CSF, IL-18) via RT-PCR.

All MAP strains were able to invade the intestinal epithelial cell line, but invasion efficacy differed. ATCC 19698, DSM 44135 and JII-1961, all of bovine origin, exhibited a higher invasion rate than the red deer isolate, also of genotype II and the sheep isolate (Type III). After infection, cytokine expression of FKD-R 971 cells was variably modulated by the different strains. Infection with the bovine isolates resulted in a significant up-regulation of IL-1 beta, IL-8, MCP-1 and GM-CSF within 8 hpi. In contrast, FKD-R 971 cells infected with the sheep and red deer isolate, genotype III and II respectively, showed a reduced pro-inflammatory response compare to bovine isolates. The results led us to assume that differences were more dependent on the host animal species of origin than the strain type.

Keywords:
Intestinal epithelial cells, Host-pathogen interaction, Strain diversity

Abstract P-04.41

GAMMA-INTERFERON RELEASE FROM PBMC OF GOATS EXPERIMENTALLY INFECTED WITH MYCOBACTERIUM AVIUM SSP. PARATUBERCULOSIS (MAP)

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Abstract text:
T cell activation, detectable by antigen-induced gamma-interferon (IFN-γ) release from peripheral blood mononuclear cells (PBMC), is an important feature of paratuberculosis. In adult goats, it was mainly attributed to CD4+ T cells. In this study IFN-γ-production in goat kids was traced back to T lymphocyte subsets in order to better characterize T cell activation in the early pathogenesis of MAP infection.

Thuringian goats (n=21) were orally inoculated with bovine MAP isolate JII-1961 ten times beginning at the 10th – 21st day after birth (MAP+). Ten age matched goats served as controls. PBMC were isolated from every goat in four-weekly intervals up to 48 weeks post infection (wpi). Cells were stimulated with Johnin purified protein derivative (jPPD) for 15 h, re-stimulated with PMA/ionomycin in the presence of brefeldin A for further 3 h, immuno-labeled for surface markers (CD4, CD8, γδ-TcR) and intracellular IFN-γ, and analysed by flow-cytometry. Cells without or with PMA/inonomycin treatment only served as controls.

PBMC of control goats consisted of more CD4+ T cells (about 30%) than CD8+ T cells (about 15%). From 11 wpi onward proportion of CD4+ T cells was even higher in MAP+ goats. The portion of γδ T cells increased until 4-5 month p.p. to 30% but increase was less prominent in MAP+ goats. This shift in T cell subsets in MAP+ goats went along with a significant increase in the proportions of CD4+ and CD8+ T cells producing IFN-γ upon jPPD+PMA/ionomycin re-stimulation starting 11-14 wpi and reaching peak levels at 30 wpi and 26 wpi, respectively. Even PMA/ionomycin re-stimulation alone induced marked IFN-γ production in T cell subsets of MAP+ goats. Preceding stimulation with jPPD further increased the proportion of CD8+IFN-γ+ cells - but not CD4+IFN-γ+ cells - in these animals.

After experimental MAP infection of goats early antigen-specific IFN-γ-production in CD8+ T cells is accompanied by a considerable but non-specific activation of CD4+ and CD8+ T cells.

Keywords:
IFN-gamma-production, T cell subsets, goats

O-05 Genotyping and Map Diversity

Abstract O-05.1: INVITED SPEAKER
Protection Against Johne's Disease in the Era of Genomics

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Johne's disease (JD) is a contagious, chronic, and potentially fatal infection caused by MAP which affects the small intestine of ruminants [1]. All ruminants are susceptible to Johne's disease, specifically dairy cattle. Cattle infected with MAP can suffer from chronic diarrhea, weight loss, low milk yield and low (but persistent) mortality, causing significant economic losses in the dairy industry, with estimated annual losses of $200 to $500 million in the USA alone. JD is a worldwide health problem for dairy herds that carries a heavy economic burden for producing safe food. The prevalence of the disease on the herd level ranges from as low as 3-4% of the herds in regions with low incidence (such as England), to levels as high as 90% in some areas within the USA [2]. Worldwide prevalence of the disease is estimated at >15% in Canada, >20% in Australia and >50% of herds in Europe. The disease spreads primarily through shedding MAP by infected animals in feces or milk. The incubation period of JD in cows is usually 2-4 years, with intermittent MAP shedding. Finding an early diagnostic test and effective vaccine to help control JD in livestock is of paramount importance to provide a safe food supply and alleviate the economic burden of JD.

Currently, no feasible antibiotic regimen or efficient control strategy is approved to combat paratuberculosis. This is complicated by the lack of reliable diagnostic tools to detect early stage infection, even though molecular protocols have been introduced to facilitate JD detection. For cattle, there is only one vaccine (Mycopar®, Boehringer Ingelheim) approved for limited use in the USA and a few other countries. This vaccine causes significant granuloma formation at the site of inoculation [3], which persists throughout the animal's life, increasing the possibility for tissue condemnation at the slaughterhouse (Fig. 1). Despite the ability of this vaccine to induce cell mediated immunity in animals [4], shedding of MAP from vaccinated animals continue to cause a problem for transmitting the disease to naïve animals. In a long-term study of the effect of killed vaccine on dairy herds to reduce the transmission of the disease, no significant difference in prevalence was found between vaccinated and non-vaccinated herds [5]. More efforts are needed to better understand the pathogenesis of JD and to plan an effective control strategy. An efficient vaccine against JD could be the cornerstone for such control strategy.

Fig. 1 Granuloma in the brisket area of 5 years old cow following immunization with Mycopar®.

Genomics is the solution. Fortunately, the genome sequence of MAP K-10 was completed [6] which jump-started our efforts to screen for virulence factors and regulatory elements encoded in this genome. Sequence analysis of MAP genome indicated the presence of many regulatory proteins (~150 genes) including 14 two-component regulatory systems (2CR), and 19 σ factor genes [6]. Our sequencing of 10 more MAP genomes [7] confirmed the conserved nature of most of the GGR including the 4 of the 2 CR systems conserved among all mycobacteria (Fig. 2). This myriad of regulatory proteins is required for cell adaptation to survive under wide spectrum of microenvironments. For example, the 2CR have been associated with virulence in M. tuberculosis as well as other intracellular pathogens. In MAP, limited studies were done to examine the importance of the regulatory proteins belonging to the 2CR system and transcriptional regulators,

in general. Among the 19 σ factors, a total of 13 were orthologous of- their counterparts in M. tuberculosis, including 2 copies of sigF. Interestingly, 6 additional σ factor genes (termed ECF1-6) with no similarity to M. tuberculosis genes were identified [6]. Such disparity in the σ factor genes between these two pathogens is another example of the disparity in their pathogenesis and virulence mechanisms. Previously, a total of 7 σ factor transcripts were identified when MAP were exposed to stress conditions including sigJ which was activated during Caco2 cell infections [8].

Global Gene Regulators as vaccine candidates. The use of Next-generation sequencers enabled us to sequence all transcripts in a given RNA sample, hence named RNA sequence (RNA-Seq) profiling. This naming reflects the ability of the high throughput sequencers (e.g. HighSeq2000, Roche 454) to be used for in-depth sequencing of millions of transcripts in a single run. Because of the global nature of transcriptional regulation under control of σ factors, we pursued the characterization of the sigH transcriptome using RNA-Seq profiling utilizing an Illumina-based sequencer operated by the University of Wisconsin Biotechnology Center (UWBC). Our stress experiments showed that the ΔsigH mutant was hypersensitive to elevated temperature and diamide exposure, each resulting in impaired growth. Accordingly, we hypothesize that sigH may play an important role in directing transcriptional control under unfavorable environmental conditions. To test this hypothesis, both wild-type MAP K10 and ΔsigH mutant transcriptomes were profiled before and after diamide exposure [9]. Interestingly, when the transcriptome of wild-type strain was compared to the ΔsigH mutant, approximately 15% of the MAP genes (~307 induced and ~344 repressed) were found to be differentially regulated at 3 h post exposure to diamide stress. Many induced genes were involved in detoxification and maintaining cellular redox homeostasis (e.g. trxB2, adhE) during oxidative stress as detailed before.

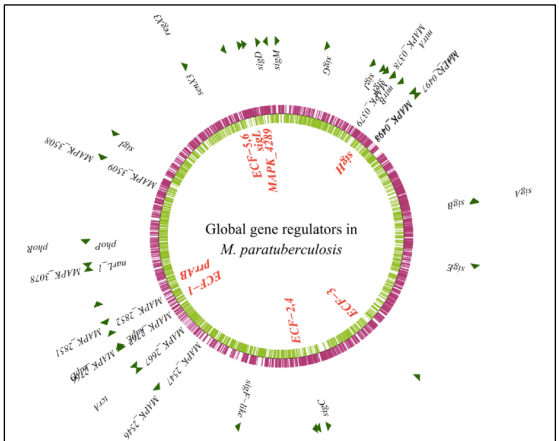

Fig. 2. A circular gnomic map shows location (green arrows) of selected regulatory elements (2CR and σ factors) in *M. ap* K10 genome. Inside of the circle shows list of candidate genes (red) targeted in our study.

Because they control a large number of genes, we hypothesize that GGR mutants will be attenuated but able generate enough immune responses to serve as live attenuated vaccines that could control JD. To examine the vaccine potential of ΔsigH and ΔsigL mutants as live-attenuated vaccines using the C57BL/6 mouse model of paratuberculosis. Mock-immunized mice with (Phosphate Buffered Saline, PBS) showed the highest colonization levels of MAP in their organs following challenge with MAP K10. On the other hand, mice vaccinated with the attenuated mutants (ΔsigH and ΔsigL) showed a significant reduction in bacterial colony levels in the intestine at 6 weeks post challenge (WPC). Lesion scores in liver and spleen were also reduced in immunized animals compared to mock-immunized mice. Overall, both vaccine candidates were effective in inducing protection against virulent MAP K10 strain compared to ΔsigH vaccination. In conclusion, we developed postulates that focus on MAP GGR. We plan to focus on GGR to decipher their role in MAP pathobiology.

In conclusion, we started our analysis using genomic information and were able to identify potential vaccine candidates that could control Johne's disease. We are working on a diagnostic test that could help in the differentiating infected from vaccinated animals (DIVA). In addition, we were able to define the MAP transcriptomes that shed more light on the pathogenesis of this important disease.

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Abstract O-05.2

EXPLORATION OF PHYLOGENETIC RELATIONSHIPS BETWEEN STRAINS OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS USING GENOME SEQUENCING

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Abstract text:

The differentiation of isolates is essential for an in depth understanding of the epidemiology and pathogenesis of Mycobacterium avium subspecies paratuberculosis (MAP). Different strain groups have been identified using various molecular typing techniques, but some confusion exists as to the phylogenetic relationships between these strain groups. Genome sequencing was undertaken to investigate the genetic diversity and phylogenetic relationships between 134 MAP isolates of different provenance, representing 17 countries and 9 host species. The study confirmed the existence of two major lineages corresponding to the previously described S-type and C-type strains. The Type I and III strains identified by pulsed-field gel electrophoresis and Gyr SNP analysis comprised two distinct phylogenetic clusters within the S-type lineage. 'Bison type' strains did not comprise a distinct phylogenetic cluster and were scattered among different clusters within the C-type lineage. The majority of isolates in the panel were typed by pulsed-field electrophoresis and Mycobacterial Interspersed Repetitive Unit-Variable Nucleotide Tandem Repeat (MIRU-VNTR) analyses. The MIRU-VNTR data based on eight loci was mapped to the phylogenetic tree and showed poor concordance with the SNP-based phylogeny. This suggests that while MIRU-VNTR typing can distinguish between some strain types, it detects diversity within a limited region of the genome and should not be the typing technique of choice for tracing the source of infections or transmission studies. Genome sequencing is clearly the most effective technique for strain differentiation but is expensive and impractical in many circumstances. However, as more isolates are sequenced, a SNP-based approach may offer a suitable alternative.

Keywords:

Phylogenomics, MAP strain types, Genome sequencing

Abstract O-05.3

PHYLOGENOMIC ANALYSES ELUCIDATE MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS DIVERSITY AT A GLOBAL, NATIONAL AND PROVINCIAL SCALE

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Abstract text:

Introduction: A thorough understanding of MAP genomic diversity provides key insight into the transmission dynamics and potentially into genotype specific fitness characteristics that may influence the spread and progression of MAP infection. In this study the phylogeny of Canadian MAP isolates from dairy herds was determined at the provincial, national and global level to dissect the relationship of these overlapping populations.

Methods: Whole genome sequencing of 89 MAP isolates from Johne's disease control initiatives in British Columbia, Alberta, Saskatchewan, Ontario, Quebec and the Atlantic provinces was performed, in which 250bp paired-end reads were mapped to the K10 reference genome. Bases were called using a combination of samtools and bcftools and reads were filtered to identify high quality variant sites. A maximum likelihood phylogenetic tree was constructed based on concatenated variant sites. Additional MAP sequences (n=26) from a global collection (n=137) were selected based on their phylogenetic position and analyzed alongside the Canadian isolates.

Results: Nearly 2,700 SNPs were identified among the Canadian isolates, compared to more than 7,000 including the global sequences. Phylogenetic analysis revealed a dominant clade which includes 5 of the global isolates and more than 80% of the Canadian isolates. The next dominant clade is composed of strictly Canadian isolates from multiple provinces and is genetically distinct from the nearest global isolates. In Alberta alone, 3 distinct clades were identified compared to 8 in the entire Canadian collection. Additionally, an 8 loci MIRU-VNTR analysis indicated poor concordance with the observed phylogeny based on SNPs.

Significance: Our approach provides a detailed and comprehensive analysis of MAP diversity and establishes a framework for future work to identify the interaction of genotype, phenotype and transmission in the context of different geographical scales.

Keywords:

whole genome sequencing, phylogenetics, diversity

Abstract O-05.4
EXPLORING A VIRULENCE HYPOTHESIS PART I: RELATIONSHIP BETWEEN MAP GENOYPES AND JOHNE'S DISEASE PROGRESSION IN NZ SHEEP

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Abstract text:
Molecular typing of isolates of Mycobacterium avium subspecies paratuberculosis (MAP) has provided insights into Johne's disease (JD) epidemiology in NZ. A previous subtyping study by VNTR/SSR indicated that different species often harboured different MAP subtypes, but when co-grazed they frequently carried identical subtypes. Cattle subtypes (C) were found in dairy cattle with one predominant subtype accounting for 70% of the isolates. A different C subtype was isolated from 76% of deer, and the common cattle subtype and sheep subtypes (S) from some deer. In contrast, 86% of sheep and 68% of beef cattle that were grazed on the same farm carried the same S subtype suggesting transmission between species. It is hypothesized that the virulence of MAP is associated with molecular subtypes in different ruminant hosts. If this hypothesis is true, it could potentially be exploited in control schemes. As a test of this hypothesis we are correlating MAP subtypes of isolates from NZ sheep with Johne's disease progression on the basis of the extent of histologically scored lesion severity.

MAP isolates are being cultured and subtyped by VNTR and SSR methods (7 DNA loci) from 98 below average body condition ewes from 18 commercial farms of merino and other breeds and correlated with histopathology scores. Sheep had an array of different levels of pathology, 34 were type 1 (mild/non-specific), 4 were type 2 (moderate), 15 were type 3a (clinical), 32 were type 3b (severe clinical), and 13 were type 3c (severe/non-specific). Chisquare and proportional similarity index analysis will be employed to evaluate associations between histological progression sheep breed and MAP subtype. Results will be presented and discussed at the conference.

Keywords:
MAP virulence, VNTR/SSR typing, Histological lesion scores, sheep

Abstract O-05.5

EXPLORING A VIRULENCE HYPOTHESIS PART II: RELATIONSHIP BETWEEN MAP SUBTYPES AND JOHNE'S DISEASE SEVERITY IN NEW ZEALAND RED DEER

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Abstract text:
Molecular typing of isolates of Mycobacterium avium subspecies paratuberculosis (MAP) has provided insights into Johne's disease epidemiology in New Zealand (NZ). Recent VNTR/SSR subtyping analyses indicated that different ruminants often harbour different MAP subtypes, but that when co-grazed they frequently carry the same subtypes. Dairy cattle harbour a variety of C subtypes with one predominant subtype (CDA) accounting for 70% of the isolates, whereas most deer (76%) harbour a different C subtype (CDE). In contrast, 86% of sheep and 68% of beef cattle that are co-grazed carry the same S subtype. It is hypothesized that the virulence of MAP subtypes varies between ruminant hosts. If true, this could be exploited in JD control schemes. To test this hypothesis we are assessing the relationship between MAP subtypes and the severity of infection in NZ deer.

MAP subtypes that were isolated from the lymph nodes of 93 NZ red deer were correlated with the number of acid fast organisms (AFOs) detected in these tissues. A variety of MAP subtypes were isolated from tissues from which no AFOs were detected (57 samples) including CDA and CDE subtypes as well as less common cattle and sheep subtypes. The remainder of the MAP isolates, 29 CDE, 6 CDA and 1 sample with multiple types, were from lymph nodes with detectable AFOs, with load level ranging from 1 AFO/section to >60 AFO detected in multiple macrophages. The results support the conclusion that C subtypes are more virulent in deer than S subtypes, but the sample size was too small for firm conclusions to be drawn. In addition, correlation of MAP subtypes isolated from faeces from 102 2 year old hinds seropositive for JD, with MAP shedding as measured by qPCR, will be presented.

Keywords:
virulence, deer, subtype

Abstract O-05.6
NOVEL SNP-BASED ANALYSIS FOR THE DIFFERENTIATION OF ISOLATES OF MYCOBACTERIUM AVIUM SUBSP PARATUBERCULOSIS

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Abstract text:
In the past decade a variety of molecular typing techniques have been investigated to discriminate between different strain types and isolates of Mycobacterium avium subsp paratuberculosis (MAP) but the recent advances and availability of new generation sequencing has extended possibilities. The aim of this study was to investigate the utility of single nucleotide polymorphisms (SNPs) for accurately distinguishing phylogenetic groups within MAP. SNP analysis was performed using genome sequence data from one hundred and thirty three separate isolates of MAP from different host species and distinct geographic regions around the world plus the available genome sequence of MAP K10. From the phylogenetic cluster analysis more than twenty five thousand SNPs were found between all the strains and phylogenetic relatedness is not reflected by Mycobacterial Interspersed Repetitive Unit- Variable Number Tandem Repeat analysis. We calculated a minimum of 93 SNPs was necessary to distinguish among all the strains and we focused on defining the minimal panel of SNPs required to distinguish between major groups of isolates, based on the principal branches of the phylogenetic tree. Primers were designed for the amplification of fragments containing each identified SNP which were confirmed by sequencing. SNPs recognised by restriction enzymes were selected for the first branches of the phylogenetic tree to permit identification of the SNP by restriction analysis as an alternative to sequencing of the amplicon. We have developed a novel work flow based on sequential analysis of sixteen SNPs that allows us to distinguish between fourteen phylogenetic clusters of MAP strains by standard PCR methods. This SNP analysis is a rapid, user-friendly typing technique that can be performed in any laboratory around the world.

Keywords:
SNP analysis, phylogenomics, new generation sequencing

Abstract O-05.7: *Richard S. Merkal Award winner*
IN-DEPTH SURVEY OF MAP GENOTYPES IN GERMANY

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Abstract text:
Paratuberculosis is a chronical disease with long incubation period. Sources of infection often remain undisclosed. The objective was to elucidate the utility of multi-target genotyping for appraisal of MAP diversity and identification of transmission routes. About 350 bovine isolates were sourced from 315 cattle in 147 herds in 12 Federal States. Additionally, 46 isolates originating from sheep, goat, donkey and deer - wild living, held in captivity in private ownership or in zoos - and a human isolate were included. Multi-target genotyping using IS900-RFLP (BstEII, PstI), MIRU-VNTR (8 loci)-, and SSR (3 loci)-analysis was applied. An unprecedented detailed overview of MAP diversity at a national scale is presented. Sixty two combined genotypes were detected for bovine isolates, 22 genotypes for isolates of the other hosts. Eight of the latter were only found in sheep, deer, donkey or human. Most isolates belonged to the main type-II-group, only 8 to the type-III-group, i.e., 7 sheep and one cattle isolate. Four predominant, five common, some of rare and 39 unique genotypes including new profiles were detected. In Federal States with long history of paratuberculosis the highest number of unique genotypes was discovered. The common genotypes showed a different frequency among the Federal States pointing to traditional trade habits. For example, the distribution and frequency of specific genotypes reflect spread of MAP by animal movement from Lower Saxony to the New Federal States in the nineties. Introduction of MAP from Hesse to a Thuringian herd could be confirmed. The diversity of MAP within individual herds varied and was correlated with the frequency of animal purchase. Within a National Park with known shared habitats of cattle and wild-living red deer results imply that specific strains had been transmitted between and within these species. Furthermore, both species maintained a reservoir for specific genotypes not found in other areas of Germany. Multi-target genotyping may help to unveil MAP transmission routes.

Keywords:
multitarget genotyping, national scale, different hosts

Abstract O-05.8

MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS ISOLATES INDUCE IN VITRO GRANULOMA FORMATION AND SHOW SUCCESSFUL SURVIVAL PHENOTYPE, COMMON ANTI-INFLAMMATORY AND ANTI-APOPTOTIC RESPONSES WITHIN OVINE MACROPHAGES REGARDLESS OF GENOTYPE OR HOST OF ORIGIN

Abendaño N.*[1], **Tyukalova L.**[1], **Barandika J.F.**[1], **Sevilla I.A.**[1], **Balseiro A.**[[2], **Garrido J.M.**[1], **Juste R.A.**[1], **Alonso-Hearn M.**[1]

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Abstract text:

The analysis of the initial macrophage responses, including bacterial growth within macrophages, represents a powerful tool to characterize the virulence of clinical isolates of *Mycobacterium avium* susbp. *paratuberculosis* (Map). The present study represents the first assessment of the intracellular behaviour in ovine monocyte-derived macrophages (MDMs) of Map isolates representing distinct genotypes (C, S and B) and isolated from cattle, sheep, goat, fallow deer, deer, and wild boar. Intracellular growth and survival of the selected isolates in ovine MDMs was assessed by quantification of CFUs in the initial inoculum and inside of the host cells at 2 h p.i. (day 0) and 7 d p. i. using an automatic liquid culture system (Bactec MGIT 960). Small increases in the estimated $\log_{10}$ CFUs from days 0 to 7 were observed for all the clinical isolates. After 7 d of infection, variations in the estimated $\log_{10}$ CFUs between all the isolates were not statistically significant. In addition, ovine MDMs exhibited enhanced anti-inflammatory, antiapoptotic and antidestructive responses when infected with two ovine isolates of distinct genotypes (C and S) or with two C-type isolates from distinct hosts (cattle and sheep); which correlated with the successful survival of these isolates within ovine MDMs. A second objective was to study, based on an *in vitro* granuloma model, latter stages of the infection by investigating the capacity of two *Map* isolates from cattle and sheep with distinct genotypes (C and S) to trigger formation of micro-granulomas. Upon 10 days p.i., both isolates of *Map* were able to induce the formation of *in vitro* granulomas with a well-defined edge and comparable to the granulomas observed in clinical specimens with respect to the cellular components involved.

Keywords:

Map diversity, Map-Host interaction, ovine macrophages

Astract O-05.9: PERSPECTIVE

MAP TYPING – THE TRANSITION FROM YATMS (YET ANOTHER TYPING METHOD) TO GENOMES

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Abstract text:
Genetic diversity of Mycobacterium avium subsp. paratuberculosis (MAP) has progressively evolved with the advent of higher resolution technologies ranging from anonymous biallelic interrogations to whole genome sequencing. Despite this advancement, most all studies have used isolates from existing local collections to define and calculate the genetic diversities and have ignored a primary component of evaluating molecular diversity – use of well-characterized isolate collections with multiple methodologies to objectively identify the value of each method. This imposes the possibility of an ecological fallacy when comparing genotypes or unoptimized genotyping methods across the globe. Multiple tools have been evaluated on MAP – PFGE, IS900-RFLP, VNTRs, ML-SSRs and now whole genome sequence based SNP analyses. Indeed, in the current ICP, we have papers presented on MAP isolates from multiple hosts representing at least 10 countries – all of which have applied one or the other genotype discrimination method id in cross-sectional collection of isolates from their respective geographic locations. What is missing? And, do we need another typing method or can we apply the currently available genomic tools in well-designed longitudinal studies to understand the dispersal of MAP in multiple hosts? Are we missing key information on the extent of diversity by culturing this organism in the laboratory – a likely reason for the apparent lack of overall diversity identified to date?

Defining genetic diversity in the post-genomics era has become a major emphasis in the molecular biology and epidemiology of Johne's disease research. These new high- resolution genome-wide SNP based methods can now be used to define extent of diversity of strains and their dispersal on farms. Combinations of multiple methods or intelligent use of combinations of genome-wide SNPs can also provide discrimination sufficient for dependable strain tracking. Current technology is also capable of allowing for direct de novo SNP typing of fecal samples (without need for cultures) eliminating the cultivation bias that may constrain or select for only those genotypes capable of growing in-vitro. An overall optimization globally harmonized methods on identical sets of isolates to define diversity within geographic locations followed by local longitudinal studies are required to effectively utilize these methods to understand MAP dispersal (transmission) within and between farms, geographic localities and hosts.

P-05 Genotyping and Map Diversity

Abstract P-05.1

A WEB TOOL FOR ANALYSIS THE GENOTYPING OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS STRAINS AND OTHER MAC MEMBERS

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Abstract text:
Background: Genotyping applied to strains of Mycobacterium avium subsp. paratuberculosis (MAP) becomes an indispensable tool for epidemiological surveillance of this significant veterinary pathogen. For MAP, multi-locus variable number tandem repeat analysis (MLVA) targeting mycobacterial interspersed repetitive units (MIRUs) and other variable number variable-number tandem repeats (VNTRs) was established using 8 markers. Within the past 5 years this standard, portable, reproducible and discriminatory typing method has been frequently use alone or in combinations with other molecular method. As such, a number of genotypes (termed INVM) of strains of diverse origins have been identified in our laboratory. However, no web tools are available for knowing the already existing INMV profiles and storing newly defined genotypes.

Objective: To develop a web application called “Mac INMV database”. This freely accessible service allows users to compare the genotyping data of their strains analysed by MLVA with those of existing reference strains.

Results: a freely accessible database was created with genotyping data of strains of MAP and of other M. avium complex (MAC) members. This database gathers all INMV profiles obtained in the laboratory and those published in the literature. The database information include incrementing species and subspecies status, INMV profiles with corresponding alleles/repeat numbers at each locus, and combined numerical genotypes. The user can consult and query all the existing INMV profiles in order to see if profiles identified in his lab already exist. If profiles are not known, the user can request new attribution numbers online. All information can be exported under EXCEL or PDF format. An extensive documentation regarding the genotyping method, protocols, primer sequences, marker information, is available to the users.

Keywords:
Genotyping, Mirus VNTR, Database

Abstract P-05.2
CANDIDATE GENES FOR PARATUBERCULOSIS RESISTANCE IN SHEEP BY GENOMIC SCAN

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Abstract text:
Not only does Paratuberculosis cause severe economical loss in livestock, but furthermore attracts attention because of possible relationship with Crohn' s disease in human subjects. It is difficult to control by common methods including vaccines and very few reports are available on natural resistant Genotypes. In this study, a genome-wide scan using the Illumina Ovine SNP50K Beadchip was performed in the attempt to identify genomic regions associated with disease resistance in sheep. The ovine genomic regions encoding putative candidate genes were searched on OARv3.1 Genome Assembly for the presence of genes that play a potential role in the immune responses. Diagnosis of paratubercolosis, performed with the ELISA test on serum of 759 adult sheep, from a single flock, allowed to select fifty positive and fifty negative individuals; the negative results were further confirmed with PCR. The sheep of the two selected groups were genotyped on the Illumina Ovine SNP50K Beadchip. For each marker, two parameters were evaluated: the Fst value of differentiation between the two groups, and the Fisher's exact test of significance of differences of allele frequency between the two groups. Thirteen markers showed Fst ≥ 0.07 and P (Chi2) ≤ 0.0007; 11 of them were located within, or close to, described genes. Three of these genes, CD109, PCP4 and ITFG2 are referred in literature to play a role in either disease resistance or cell-mediated immune response.

Keywords:
genes, paratuberculosis, snp50k

Abstract P-05.3

INSIGHTS IN THE GENOME OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS BY NEXT GENERATION SEQUENCING APPROACHES

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Abstract text:
Mycobacterium avium subsp. paratuberculosis (MAP) is the causative agent of paratuberculosis in farmed and wild animals. In the present study the genomic variability of field strains of MAP isolated from different hosts and from several regions, in Italy was analyzed by whole genome sequence comparison using next generation sequencing approaches. The preliminary results on 15 strains are presented.

All the MAP strains were isolated from single animals from cattle herds located in 6 provinces in the north of Italy, in collaboration with National Reference Centre for Paratuberculosis (Piacenza, Italy). Nine samples were paired end sequenced at 150bp end and six samples at 75bp per end on the Illumina Myseq. The K-10 (NC_002944.2) strain was used as the reference, and bioinformatic analyses were performed using the BWA-mem, FreeBayes, GATK, and SNPEff software.

The reads were mapped to the K-10 reference sequence with 99.96% reads finding a match, with a mean coverage of 138.26 X. In total 844 variants were identified, of which 698 SNP, 23 MNP, 45 INS, and 78 DEL. Each strain has 25.90% private SNP on average. The variants identified are 1.40% missense, 1.14% nonsense, and 37.46% silent. About 43% of variants were located in coding regions, while 25% were in the -60 upstream regions.

Fewer variants were identified than expected. This could be functional, due to the position of the variants, which mainly fall in coding and regulatory sequences. In the future, phenotypic information linked to MAP strains and their hosts may help to disentangle the genetic variability linked to virulence and MAP population substructure.

Keywords:
Mycobacterium avium subsp. paratuberculosis, Next Generation Sequencing, Genetic Variability

Abstract P-05.4
GENETIC DIVERSITY OF MAP ISOLATES FROM A PARATUBERCULOSIS CONTROL PROGRAM

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Abstract text:

Introduction

A compulsory paratuberculosis (PTB) control programme by government regulation started in Austria in 2006. For a better understanding of transmission routes and PTB epidemiology in Austria MAP isolates of different origin were subjected to molecular characterization.

Material and methods

218 MAP isolates from cattle, 2 isolates from sheep, 19 isolates from goat and 6 isolates from red deer were analysed by IS900 RFLP with two restriction enzymes (BstEII, PstI) and MIRU-VNTR at 8 genomic loci (MIRU292,X3; VNTR25,47,3,7,10,32). These field strains included MAP strains isolated from 92 different cattle farms and various breeds. MAP strains from 4 typical local Austrian cattle breeds and 8 different imported cattle breeds were tested.

Results

The combination of RFLP and MIRU-VNTR analysis allowed a differentiation of 20 MAP types classified Austria (AT) 1 to AT 20. 19 different MAP types were detected in cattle, 4 different types in red deer, 2 different types in sheep and 1 type in goat.

Discussion

This is the first study carried out in Austria providing data about MAP strain types on a national scale.

It could be shown, that AT 2 is the most frequent MAP type in Austria and was detected in 48% of all tested cattle herds. Additionally some MAP types showed association with certain cattle breeds and herds. In comparison to the isolates from red deer and sheep which were also found in cattle, the MAP isolates from goat turned out goat specific. 8 new RFLP patterns and 1 new INMV profiles were determined within the 218 analysed field strains. 11 herds were infected with more than one MAP type. Our examinations have shown that single animals can even be infected with different MAP strains. Molecular characterization of MAP strains isolated within a PTB control program provided valuable information about routes of transmission between herds and the epidemiology of PTB in Austria.

Keywords:

Map diversitiy, epidemiology, control program

Abstract P-05.5

FIRST ISOLATION AND GENOTYPING OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN ITALIAN MEDITERRANEAN BUFFALO (BUBALUS BUBALIS) IN ITALY

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Abstract text:
The current study aimed at reporting the first isolation and typing of Mycobacterium avium susp. paratuberculosis (MAP) in the Italian Mediteranean Buffalo, in Italy.

In the international scientific literature, few data are available about MAP in buffalo. In Italy, Mycobacterium avium subsp. paratuberculosis in buffalo was isolated in 1999 even if at that time it was not genotyped. In this study, we tested two adult subjects breeded in two different farms. The first animal showed clinical signs (weight loss, diarrhea) but ELISA tests resulted repeatedly negative. The culture tests on Herrold's Egg Yolk Medium (HEYM) yielded a positive result for mesenteric lymph node, ileum, ileocecal valve and stool samples. Kinyoun stain confirmed the presence of acid-fast bacilli. The second animal was asymptomatic but showed positive results in ELISA. Culture and microscopic tests on stool samples resulted positive and both animals were high shedders. These results were confirmed by IS900 commercial end-point PCR. The isolates were sent to the National Reference Centre for Paratuberculosis (IZSLER- Piacenza, Italy) to be confirmed by F57-PCR and typed by IS1311 REA-PCR and by PCR-DMC. The isolates were confirmed as MAP and identified as type C (II or cow type).

Keywords:
Mycobacterium avium subsp. paratuberculosis, buffalo, typing

Abstract P-05.6

LIPID PROFILING OF VARIOUS STRAINS OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS FROM STOCKS MADE IN THE EARLY 1990S AND 20 YEARS LATER

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Abstract

Mycobacterium avium subsp. *paratuberculosis* (MAP) is one of five subspecies of *M. avium*. MAP itself has two distinct types: the C-type and the S-type. Mycobacteria are known for the high lipid content of their cell walls, and one of the key lipids in MAP is the antigenic lipopeptide Para-LP-01. The main question about the use of Para-LP-01 as an antigen in diagnostic tests has been whether or not it is in all strains and isolates.

Here we report our findings on the lipid profiles of various MAP isolates. We analyzed 12 newly obtained strains comprised of six animal isolates (including two S-type strains and four C-type strains) and six isolates from humans with Crohn's disease (UCF strains), and 12 strains that were stored as frozen stocks since the early 1990s.

Purified whole-cell lipids were analyzed by HPLC/MS in the positive and negative modes. We observed three major HPLC profiles in the positive mode, and five different HPLC profiles in the negative mode. All 12 of the isolates from the 1990s exhibited the same HPLC profiles in both the positive and negative modes. Four of the newly obtained strains (K-10, 527, S-type strain 467, UCF-4) shared a unique HPLC profile, while the remaining eight newly obtained isolates shared an HPLC profile that is different from the other profiles.

All 24 MAP isolates contained Para-LP-01 but with varying intensities. Among the newly isolated strains, UCF-4, K-10 and C-type strain 517 demonstrated a Para-LP-01 MS intensity of 10^6 while the others (including all the human isolates) had low intensities between 10^2 and 10^3. All strains from the early 1990s showed MS intensities in the mid-range of $5x10^4$ to $7x10^5$. In conclusion, all MAP isolates contained Para-LP-01, regardless of origin. There was no apparent correlation between the MS intensity of Para-LP-01 and the lipid profile.

Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP) is one of five different subspecies of *M. avium*. MAP itself has at least two distinct types: the C-type (cattle type) and the S-type (sheep type). Few other types are in discussion, such as types isolated from buffalo (primarily in India), goat, and human. There are also different forms of Johne's disease associated with the two major types: Johne's disease in cattle is multibacillary with heavy shedding and chronic diarrhea, while the disease in sheep can be both multibacillary or paucibacillary combined with shedding but no diarrhea. It is not clear yet if the differences in the clinical appearance of the disease is due to the different types or due to the ruminant species.

Sequencing the genome of the two types revealed several interesting insights into the evolutionary relationship between the subspecies of M. avium and the types of MAP. One interesting feature was the presence and absence of the gene cluster responsible for the biosynthesis of the glycopeptidolipids. These highly immunogenic surface molecules encode for

the 28 different serotypes of *M. avium* subsp. *avium* and subsp. *hominissuis*. Since MAP does not exhibit GPLs it was not a surprise to see the gene cluster truncated and partially deleted from the C-type of MAP. However, this gene cluster is fully intact in the S-type, but the bacteria do not synthesize GPLs. While there are clear differences between the types, a valid hypothesis is that there are also differences between strains of the same type.

Another observation for Johne's disease is the strong variation of immune responses throughout the course of the disease and between animals. This is mostly seen in the diagnostics in which some animals shed the bacteria and have a positive serology, while others have either a positive fecal culture or a positive serology. Some animals are even negative for both. The hypothesis for this observation is that these differences are due to differences within the individual immune systems of each animal.

A combined hypothesis addresses both observation might try to explain the variation in immune responses through differences in the appearance of antigens in each strain and in differences in the immune system of each individual host.
To evaluate one part of the hypothesis we analyzed the lipidome of various strains from MAP representing the C-type and the S-type and demonstrate the clustering of the lipid profiles for the different types.

Materials and Methods

MAP strains: Twenty-four strains were included in this study: twelve recently obtained strains comprised of six isolates from animals (four cow isolates [C-types] and two sheep isolates [S-types]) (cow isolates: K-10, 517, 7879, 7964; sheep isolates: 467, 7556) as well as six isolates from humans (kindly provided by Dr. Saleh Nasser [University of Central Florida]) (UCF-1, UCF-3, UCF-4, UCF-5, UCF-7, UCF-8). Strains 7879, 7964, and 7556 were kindly provided by Dr. Sreevatsan (University of Minnesota). Twelve strains were obtained from an old collection of frozen stocks made in the early 1990s (BB410, K-10 [1993], 8-5, NJDA Bambi, K-39 [1993], MBC 3, 05817 #82, 35516 #23, ILADL, 69, NJDA 1704, Holland).

Growth conditions: All strains were plated directly from frozen stocks to Middlebrook 7H11 agar plates supplemented with 10% OADC and mycobactin J (2 µg/ml). Plates were incubated at 37°C for 8 weeks.

Lipid isolation: Cells were harvested from plates into Middlebrook 7H9 media, centrifuged and the cell pellets were lyophilized. Lipids were extracted from lyophilized cells with choloroform/methanol (2:1) for three hours at 55°C. The crude extract was transferred to a new tube and dried down under nitrogen. Crude extract was purified by Folch wash.

Lipid analyses through Liquid chromatography/Mass spectrometry: Analyses were performed on 40 ng total lipids as described recently by Sartain et al. (2011) on an Agilent 1200 HPLC combined with an Agilent 6220 time-of-flight (TOF) mass spectrometer with an Agilent ESI/atmospheric-pressure chemical ionization (APCI) multimode source.

Results

Strains of MAP grown on Middlebrook 7H11 plates had different growth appearances. While strain obtained recently appeared as rough, dry and beige/white, strains obtained in the early 1990s grew roughish with a tough of being smooth, as well as grayish and small. Although the strains

from the early 1990s were roughish they did not appear dry as the recently obtained strains. There was no difference in grown appearance between the C-type and the S-type.

There were at least three major HPLC pattern for the various strains: (1) representing small numbers of only new strains (K-10, 517, UCF-4, and 467); (2) representing the remaining new strains from UCF and U of Minnesota (UCF-1, UCF-3, UCF-5, UCF-7, and UCF-8 from University of Central Florida; 7556, 7879, and 7964 from the University of Minnesota); and (3) all strains obtained in the 1990s. The various HPLC pattern were independent of the ion analyzed (positive versus negative).

The major differences in the total lipid profiles in mass spectrometry were seen for the amount of Para-LP-01 within the various profiles. While all strains obtained in the early 1990s (except for strain K-10 obtained in 1993, which had an intensity of $4x10^4$) had similar amounts (intensities of 3 x 10^5 to 8 x 10^5), the strains recently obtained varied strongly. The strains K-10 and 517 had the highest amounts of 10^6, while the remaining strains (including the two S-type isolates) had very small amounts ranging from 2 x 10^2 to 1 x 10^4.

Conclusions

- HPLC pattern vary among recently obtained strains of MAP, which differ from chromatograms of strains obtained in the 1990s
- While the total lipid mass spectrometry pattern are very similar among strains the amount of the major cell surface immunogenic lipid Para-LP-01 varies significantly.
- Variations in the HPLC pattern and the amount of Para-LP-01 might contribute to different immune responses observed in cattle with Johne's disease.

References

Sartain MJ, Dick DL, Rithner CD, Crick DC, Belisle JT. 2011. Lipidomic analyses of Mycobacterium tuberculosis based on accurate mass measurements and the novel "Mtb LipidDB". J Lipid Res. **52**:861-72.

Keywords:
Para-LP-01, Lipidomics, Mass spectrometry

Abstract P-05.7

UNIQUE GENOMIC PROFILES OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS ISOLATED FROM SMALL RUMINANTS OF QUÉBEC, CANADA

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Abstract text:

Johne's Disease, is an economically important disease impacting sheep & goat industries. However, information on prevalence and epidemiology of disease in small ruminants in Canada is scarce. Strain typing is a valuable tool for epidemiology investigations. Present study was undertaken to investigate the genetic diversity of Mycobacterium avium subspecies paratuberculosis (MAP) in small ruminants of Québec, Canada. A total of 30 isolates were subjected to primary typing using IS1311 PCR-REA. Sheep isolates (20) originated from 5 different farms and were grown from intestinal/ lymph node tissues on LJ/ MB7H10 slants. Goat isolates (10) originated from 8 different farms and were first grown in MGIT and then passaged on MB7H10. Further typing was performed using VNTR analysis at 13 loci. Primary typing revealed that 16/20 isolates from sheep belonged to "sheep type" or "S type". On the other hand, 8/10 isolates from goats belonged to "cattle type" or "C type". VNTR typing identified 9 different genotypes (G-5 to G-13) for sheep isolates and 4 different genotypes (G-1 to G-4) for goat isolates. VNTR profiles of 'C type' isolates were common profiles often reported for cattle isolates from different countries. Interestingly, with the exception of one sheep isolate, VNTR profiles of all "S type" isolates from both sheep and goat were unique and had never been reported before. In addition, VNTR profiles of "S type" isolates from sheep were different from goat "S type" isolates. This is the first report of MAP genetic diversity in the small ruminant populations of Québec; overall results suggest significant diversity among strains.

Keywords:

Biotyping, VNTR analysis, Small Ruminants

Abstract P-05.8

THE GENOME OF A MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS ISOLATE RECOVERED FROM A CROHN'S DISEASE PATIENT REVEALS CLOSE GENETIC ASSOCIATION WITH ISOLATES RECOVERED FROM CATTLE

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Abstract text:

Several studies have identified Mycobacterium avium subspecies paratuberculosis (MAP) in human patients with Crohn's disease (CD). Preliminary investigations have also suggested that MAP isolates from humans bear considerable genetic similarity to MAP from cattle, but not from sheep. In order to characterize genetic differences between MAP isolates recovered from humans and those associated with bovine Johne's disease (JD), we sequenced to completion the genome of an isolate of MAP documented to have been recovered from a patient with CD. Massively parallel sequencing approaches were used to generate a total of 88.5 million base pairs of high quality sequence from a randomly sheared MAP genomic DNA library. These sequences were subsequently assembled into contiguous fragments and an iterative primer walking approach was applied to close gaps and areas of low quality coverage re-sequenced in order to obtain the complete circular genome. Comparative analysis with the MAP K10 and other cattle isolates revealed near identity in genome organization. The analysis showed that the genome of strain K10, the only other MAP isolate whose complete genome sequence have been characterized thus far, is ~3.0kb larger as a result of several sequence insertions, including one additional copy of the insertion sequence element, IS900. The analysis revealed over 200 single nucleotide polymorphisms (SNPs), with a majority of these occurring in protein coding regions. Genome-wide comparisons of SNPs in multiple MAP isolates recovered from humans with CD and cattle with JD strongly suggest that MAP isolates from either human or cattle are indistinguishable based on genotype.

Keywords:

Crohn's disease (CD), genome, genetic diversity

Abstract P-05.9

MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS DIAGNOSIS AND GENO-TYPING IN KOREAN BLACK GOAT (CAPRA HIRCUS AEGAGRUS)

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Introduction

Black goat is a type of indigenous breed of Korea, and its population is estimated at 266,000 animals in about 21,000 herds; it is a majority of small ruminant herd of the country. Korean black goats are mainly raised for meat and healthy food supplement. Currently, very little information of MAP infection in Korean black goat is available. This study reports the results of investigation of MAP infection in Korean black goat by using ELISA, fecal culture and molecular assays.

Methods

The serum and fecal samples of each 491 Korean back goats (≥1 year of age) from 100 goat herds were simultaneously collected. A commercially available ELISA kit (Institute Pourquirer, Montpelier, France, ELISA paratuberculosis antibody screening) was used to detect MAP antibodies in the serum. Fecal samples were cultured on Herrold's Egg-Yolk medium (HEYM) and confirmed by PCR. The PCR amplification of IS1311 sequence was performed by using primers M56 (5′-GCG TGA GGC TCT GTG GTG AA-3′) and M119 (5′- ATG ACG ACC GCT TGG GAG AC-3′) and 608-bp PCR product was digested using *Hinf*I enzyme (Enzynomics^{TM}, Korea).

Results

Among the 491 goats from 100 herds screened, 4 (0.8%) and 3 (0.6%) goats were defined positive for MAP by ELISA and fecal culture, respectively (Table 1). Both ELISA and fecal culture tests revealed that 6 goats (1.2%) in 6 different herds (6%) were positive for MAP.. All the 3 isolates were positive for IS900 PCR that confirmed as MAP. Two isolates were "cattle type" which resulted in fragments of 323, 285, 218, and 67 bp and one isolate was "bison type" which resulted in fragments of 323 and 218 and 67 bp (Fig. 1).

Table 1: ELISA and fecal culture test results for *Mycobacterium avium* subsp. *paratuberculosis* in 491 Korean black goats.

Geographical areas	Number of animals	Number of herds	ELISA-positive (animals/(%))	Fecal culture-positive (animals/(%))
Chungbuk	81	20	1 (1.2)	0
Chungnam	33	9	1 (3.0)	1 (3.0)
Gyongnam	150	34	2 (1.3)	0
Gyongbuk	81	22	0	0
Jeonbuk	51	5	0	0
Jeonnam	95	10	0	2 (2.1)
Total	491	100	4 (0.8)	3 (0.6)

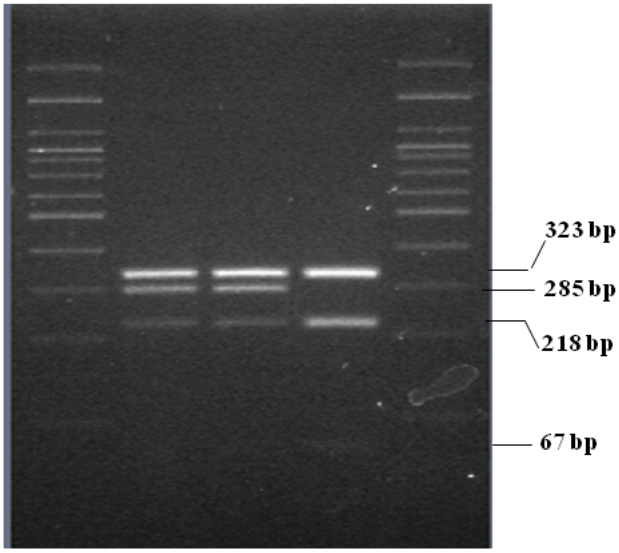

Figure 1. Agarose gel electrophoresis of IS1311 PCR products digested with *Hinf*I. Lanes 1, 2: “cattle type”; lane 3: “bison type”; M: molecular marker (100 bp; Bioneer).

Conclusion

In conclusion, the results obtained in this study showed serological, microbiological, and molecular evidence of MAP infection in Korean black goat with both “cattle type” and “bison type” genotypes. Therefore, there is a need to implement a national MAP control programme in order to reduce the incidence and limit transmission among animal species.

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Keywords:

Mycobacterium avium subspecies paratuberculosis , Korean black goat, diagnosis

Abstract P-05.10

VIABLE MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN RETAIL ARTISANAL COALHO CHEESE FROM NORTHEASTERN BRAZIL

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Abstract text:
The artisanal Coalho cheese is the most consumed and produced dairy product in Northeastern region in Brazil, it has great cultural importance and represents the main source of income for many smallholder. Considering that Mycobacterium avium subsp. paratuberculosis (MAP) has already been detected from many types of cheese in different countries, the aim of this study was report the detection of MAP in raw retail Coalho cheese in Brazil by molecular test and microbiological culture. During November 2011 to January 2012 were randomly collected 30 samples of artisanal Coalho cheese in formal and informal trade in Parnaíba city, Piauí State. A total of 30 g of each sample was placed in sterile bags and 125 mL of 1% NaCl preheated were added into the bags and mixed at stomacher blender for 2 minutes at 260rpm. For culture, 30 mL of the resultant suspension were decontaminated with 10 mL of 0,75% Hexadecyl pyridinium chloride for 5h and 250uL aliquot of the suspension was inoculated onto two Herrold's egg yolk medium (HEYM) with and without Micobactin J. Another 30 mL of the result suspension were used for DNA extraction. The primers BN1 and BN2 that amplifies 626 bp fragment, based on the insertion sequence IS900 were used. MAP-specific DNA was detected by conventional PCR in three of Coalho cheese samples and one of these samples verified the growth in culture. They were submitted to sequencing and were compared with sequences deposited in GenBankand revealed 99% similarity with strain MAP UFV-JJ insertion sequence IS900. The results of our study confirmed the presence of MAP-specific DNA and viable cells in artisanal Coalho cheese from Piauí State, Northeast Brazil and evidence that MAP might remain viable in retail cheeses. This study has important implications since MAP can be acoadjuvant agent in Crohn's disease, with a potential risk for susceptible peopleby ingesting dairy products contaminated with viable MAP. This is the first report of viable MAP in cheese in Brazil.

Keywords:
viable, cheese, detection

Abstract P-05.11

GENOTYPING AND SUB-TYPING OF ISOLATES OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS USING MOLECULAR EPIDEMIOLOGICAL TOOLS TO STUDY THE STRAIN VARIATION BETWEEN ISOLATES OF NATIVE AND FOREIGN ORIGIN

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Abstract text:

Paratuberculosis is endemic in domestic and wild ruminants in India. However, information about the genotype diversity and interspecies transmission of Mycobacterium avium subspecies paratuberculosis (MAP) isolates is limited in the country. In present study, 41 MAP isolates, 3 MAP genomic DNA and 36 MAP positive fecal DNA samples from different livestock species (cattle, buffaloes, goat, sheep and bison) and geographical regions were included to investigate the genotype diversity and strain variation among MAP isolates. Of the 41 MAP isolates, 25, 3, 2 and 11 were from India, Canada, Spain and USA, respectively. Three MAP genomic DNA were Portugal origin. DNA was extracted from all MAP isolates and sample DNA (n=80) were analyzed for the presence of IS00 sequence and further subjected to genotype differentiation using IS1311 PCR-REA and IS1311 Locus 2 specific PCR-REA methods. All DNA samples were positive for the presence of IS900 sequence. IS1311 PCR-REA showed that all DNA samples of Indian origin were 'Bison Type'. Of the non-Indian DNA samples (n=19); 2, 15and 2 belonged to 'Bison type', 'Cattle type' and 'Sheep type', respectively. In IS1311 L2 PCR-REA test restriction profile of 'Indian Bison type' was different from non-Indian DNA samples. Present study indicated that 'Indian Bison Type' genotype of MAP is predominant genotype shared by different species in India and IS1311 L2 PCR-REA method is rapid, easy to perform method for the differentiation of 'Bison type' MAP isolates of Indian origin from other non-Indian MAP isolates.

Keywords:

IS1311PCR- REA, Indian Bison Type , Mycobacterium avium subspecies paratuberculosis

Abstract P-05.12

GENOME SEQUENCING OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS STRAIN S-5 OF INDIAN ORIGIN: A NEW BIOTYPE, ('INDIAN BISON TYPE') ISOLATED FROM A GOAT WITH CLINICAL JOHNE'S DISEASE

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Abstract text:

Novel strain 'S5' of Mycobacterium avium subspecies paratuberculosis was recovered from a goat with advance clinical Johne's disease. Strain was bio-typed as 'Indian Bison Type' and so far only reported from India. This new bio-type was later reported from other domestic and wild ruminants, other animals, primates and human beings. It was important to understand the pathogenic mechanisms employed by this biotype and to understand genomic structure and its homo/ heterogeneity with other biotypes. Whole genome of 'S5' was sequenced by Illumina GA IIX and Ion Torrent technologies produced a total of 112,487,226 paired-end reads of length 101 nucleotides (nt) and a total of 1,151,448 reads of length 5 to 202 nt respectively. NGS QC toolkit v2.2.1 was used to filter the raw sequencing data. Reference-assisted genome assembly of filtered data was performed with MAP strain K10 (GenBank accession no. NC_002944.2) using Velvet v1.2.08. There was a total of 178 contigs of size 4,798,157 nt, with an N50 contig length of 58,516 nt with GC content of 69.25%; the largest contig assembled measured 199.4 kb and was produced as the draft genome, annotated by RNAmmer 1.2 and the Prokaryotic Genome Annotation Pipeline (PGAAP) of the National Center for Biotechnology Information (NCBI). A total of 4,288 protein-coding sequences (CDSs), 3 rRNAs, and 46 tRNAs were predicted. Phylogenomics comparison of strain S5 have been done with Mycobacterium avium 104, MAP K-10, MAP4, MAP S397, MAP S5 and M. tuberculosis H37Rv; on the basis of 31 phylogenetic marker sequences (i.e. amino acid sequences) extracted from each compared strain. Concatenated neighbor-joining tree (1000 bootstrap) of all these 31 markers shows that strain MAP4 is the closest neighbor of strain S5. Nucleotide based whole genome comparison of strain S5 by BLAST and Mummer both, showing 99.91% similarity with strain MAP4, which is a human isolate from USA.

Keywords:

Whole Genome sequencing, Mycobacterium avium subspecies paratuberculosis, Indian Bison Type

Abstract P-05.13

UNIQUE AND DIVERSE GENOMIC PROFILES OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS BELONGING TO 'INDIAN BISON TYPE' BIOTYPE

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Abstract text:

Mycobacterium avium subspecies paratuberculosis (MAP) is a multi-species pathogen and different biotypes of this organism exist. Four principal biotypes have been identified for MAP; Cattle type (C type), Sheep type (S type), Bison type (B type) and Indian Bison type (IB type). Though disease does not have geographical restrictions but unlike other biotypes IB type has only been reported so far from India. Our previous studies have shown that IB type is recent to evolve and IB type has the ability to infect multiple species including domestic (goats, sheep, cattle, buffaloes) and wild (deer, blue bulls, bison) ruminants, free ranging animals (rabbits), primates and human beings. Present study was undertaken to understand whether genotype diversity exists within IB types or it's a homogenous group of strains with ability to infect multiple species. IB type MAP isolates isolated from different domestic livestock species (ovine, caprine, bovine and buffaloes were subjected to genomic analysis of VNTR and SSR loci. Results indicated that IB type isolates from different species possess four different kinds of MIRU-VNTR profiles. Comparison of this data with standard table of INMV I, similar profile for the reported strains could not be found in literature. In SSR analysis we could get 3 different profiles. Since this data is not complete so could not be compared but still reflected the high diversity of Indian MAP strain. Repetition of this exercise may lead to identification of more diversity. This is the first evidence of genomic diversity within IB type MAP isolates. This study provides further evidence that IB type MAP has evolved to adapt and infect different species. In conclusion, we may draw inference that MAP strains have to do genomic adjustments to adapt and infect different species.

Keywords:

Mycobacterium avium subspecies paratuberculosis, VNTR profiles, Indian Bison Type

Abstract P-05.14

NOVEL MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP) GENOTYPE REPORTED IN BOVINES IN SOUTHERN CHILE

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INTRODUCTION

Mycobacterium avium subsp. *paratuberculosis* (MAP) is a ubiquous, obligate intracellular bacterial pathogen, and the causative agent of paratuberculosis or Johne's disease (JD) (Manning and Collins, 2001). MAP is transmitted principally via the faecal-oral route, causing a chronic intestinal infection mainly in domestic and wild ruminants (Lombard, 2011). In addition, this agent has been associated with Crohn's disease in humans (Lee et al., 2011). As in most countries with a developed dairy industry, MAP infection is widely distributed among Chilean cattle herds, especially in southern Chile, which concentrates the largest cattle population. The estimated infection rate in southern Chile at both individual and herd level is high (Kruze et al., 2013), and this might suggest different epidemiological scenarios in relation to the infection pressure, finding herds with high, medium and low MAP infection rates. However, a low heterogeneity or variability in MAP strains between bovine isolates has been described (Motiwala et al., 2003). Therefore, the aim of the present study was to explore MAP genotypes present in some cattle herds of Southern Chile.

MATERIAL AND METHODS

The study was carried out in 11 cattle herds from the Los Ríos Region. To determine herd infection level, individual fecal and/or tissue samples were collected and cultured in the BACTEC-MGIT 960 automated system, and souspicious tubes confirmed by IS*900* real time PCR. All confirmed positive samples were sub-cultured on solid Herrold's Egg Yolk Medium with mycobactin J in order to obtain pure MAP colonies. Finally, from those HEYM cultures showing typical MAP colonies, a randomly selected subsample of colonies from different herds (n=20) were molecularly characterized by MIRU-VNTR using 5 polymorphic loci (TR 292, X3, 25, 47 and 3) as described by Thibault et al. (2007). Primers were designed to target flanking regions of the MIRU-VNTRs and the conditions of the PCR amplification were carried out as previously described (Thibault et al., 2007). Briefly, PCR mixture for each MIRU-VNTR locus per sample comprised 5 µl of template DNA, 12.3 µl of nuclease-free water, 2.5 µl of buffer, 0.5 µl of magnesium chloride (1.5 mM), 1 µl of dimethyl sulfoxide (Sigma-Aldrich Corp. St. Louis, Missouri, 63118, USA) (except for TR X3), 1 µl (1µM) (each) of primers, 1 µl (0.2 µM) of DNTP mix (dATP, dCTP, dGTP, and dTTP) and 0.2 µl of Taq polymerase (5U/ µl) (Invitrogen Ltd., Paisley, UK) in a final volume of 25 µl. The reactions were carried out in a Swift MaxPro thermocycler (ESCO, Portland, Oregon, USA) under the following conditions: after one cycle of 5 minutes at 94ºC the thermal profile of the PCR was 30 cycles of denaturation for 30 seconds at 94ºC, annealing for 30 seconds at the appropriate temperature for each locus, and extension for 30 seconds at 72ºC. The annealing temperature was 58ºC in the case of loci 1, 2 and 3, 64ºC for locus 4, and 60ºC in the case of locus 5. To detect differences in repeat numbers, the PCR products were analyzed by electrophoresis using 1.5% agarose gels. Repeat numbers (alleles) were determined according to amplified fragment sizes that were estimated using the image analysis software of Quantum Capt ST4 v 15.18 (Vilber Lourmat) for fragment size calculation.

RESULTS AND DISCUSSION

Three cows belonging to two different herds showed a different MIRU genotype with 31332 pattern repetitions rather than 42332 and 32332 found in the other herds. The latter isolated MAP strains are in agreement with the highly conserved strains found around the world in various domestically managed ruminant species (Motiwala et al., 2003), and it has already been reported locally (Salgado et al., 2011; Salgado et al., 2013). Besides, these two identified genotypes are the most prevalent in many countries (Thibault et al., 2007). However, the genotype 31332 has been reported to belong to a MAP vaccine strain obtained from the Veterinary Laboratories Agency Weybridge (Thibault et al., 2007), without any previous information on local occurrence.

CONCLUSION

We described the finding of a novel MAP genotype in cattle from southern Chile not described previously. This finding should be further investigated in order to reach a better knowledge about its potential influence on MAP infection, epidemiology, and transmission.

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Keywords:
MAP, MIRU-VNTR, southern Chile

O-06 Epidemiology

Abstract O-06.1: INVITED SPEAKER
DO WE HAVE THE RIGHT TOOLS FOR ASSESSING HERD INFECTION?

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The precise quantification of herd infection status and the proper interpretation is not straightforwardly assessable, thus at times, misclassification causing invalid outcomes could occur without knowing. Epidemiological tools have been continuously developed over years to deal with the challenges caused by uncertainty and/or variability related to performance of diagnostic tests, sampling and testing methods, budget constraint, etc. Subject being discussed in this talk include effects of variables influencing classification of dairy herd infection status for Mycobacterium avium subsp. paratuberculosis (MAP); use of culture of pooled fecal samples for detection of MAP in large dairy herds; use of Bayesian approach for performance estimation of imperfect diagnostic tests without gold standard and for development of a logistic regression model predicting the probability of herd having a high seroprevalence on the basis of various herd characteristics and management practices; epidemiological and financial impacts of targeted sampling of subpopulation (of cows) versus random sampling in relation to detection of an MAP-infected herd.

Categorization of MAP- infection status into infected versus non-infected herds often requires testing many cattle at high cost, and most of the time, categorization is not feasible when tests are imperfect, within-herd prevalence is very low (eg, < 2%), and/or additional evidence for classification of herd status is not available. Increasing the number of cattle tested in each herd to increase herd sensitivity often affects herd specificity if the herd is not infected and the testing method used is < 100% specific. Most available testing methods for MAP are highly specific, but all have specificities < 100%, which could result in misclassification of non-infected herds, depending on the number of cattle tested. Here, interrelationships among variables influencing classification of MAP-herd infection status were evaluated by use of simulated data for various herd size, true within-herd prevalence, and sampling and testing methods. We determined how these variables affecting the number of test-positive cattle when a subset of cattle in a herd and an entire herd were tested for various simulated scenarios, and evaluated the confidence level of detecting infected herds by estimating the probability of finding ≥ 1 infected animal in herds with varying true prevalences for various testing methods and sample sizes. Consequently, these estimates were used to construct figures (or tables) that could be used by veterinarians or herd owners to classify (with 95% confidence) herd infection status on the basis of the maximum number of test-positive cattle or culture positive fecal pools detected.

Use of a Bayesian modeling approach offers a few advantages. It is a flexible tool for accounting for hierarchical levels and allows incorporating previous information providing an appropriate setting for complex models and missing data problem. The approach yields results in a form of probability distribution that is interpretable intuitively. When MAP prevalence is extremely low, testing of pooled samples could provide data similar to that for testing of samples from individual cattle but at a substantially lower cost, providing that the sensitivity of culture for individual and pooled samples is comparable. Here, the Bayesian approach was used to estimate true prevalence of MAP-infected cows (animal-level prevalence) on the basis of results for culture of individual fecal samples and for culture of pooled fecal samples, and herd-level sensitivity for culture of pooled fecal samples. The method was based on the principle that prior knowledge about test

characteristics and infection prevalence (which can be acquired from experts or published experiments) can be incorporated into the model and used to update inferences for these variables. Results showed that the use of pooled fecal samples from 10 cows was a cost-effective tool for herd screening and may provide a good estimate of the percentage of MAP-infected cows in dairy herds with a low prevalence of MAP.

A Bayesian logistic regression model was developed for predicting the probability of a herd having a high seroprevalence on the basis of various herd characteristics and management practices. The covariates included herd size, breed of cow, percentage of first lactation cows, and type of housing, disease history including previous detection of clinical signs consistent with paratuberculosis (diarrhea and persistent weight loss), purchase policy for replacement heifers; management practices for colostrum, milk, and manure handling, and location and management of the calving area, etc. The informative Bayesian approach improved the model estimates by incorporating prior information on herd management factors. The seroprevalence of a herd infected with MAP could be reduced by improvements in herd management practices.

Targeted sampling is a useful tool when the primary objective is disease detection, and the prior knowledge of disease clustering by factors such as age or clinical signs exist and are available. Targeted sampling may aid substantially in the detection of MAP infection at the herd level because in prior studies in cattle, associations were detected between age, stage of lactation, and milk production in infected cows and infection with MAP. Here, the epidemiologic and financial impacts of targeted sampling of subpopulations versus random sampling were investigated by using Monte Carlo simulation method. The probability of detecting an MAP-infected herd was estimated for various different sampling scenarios, and a marginal cost-effectiveness analysis was used for determination of the cost increase relative to increase in the detection probability. Sampling cows in relation to the stage of lactation, lactation number, and milk production significantly increased the detection probability of herds infected with MAP, and costs of testing were substantially reduced by targeted sampling groups of cows.

Abstract O-06.2: *Richard S. Merkal Award winner*
DETECTION OF ANTIBODY RESPONSES SOON AFTER INOCULATION IN DAIRY CALVES INFECTED WITH TWO DOSES OF MAP AT FIVE AGES

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Abstract text:
Serological testing for Mycobacterium avium subspecies paratuberculosis (MAP) infection in the early stages of Johne's disease is generally not recommended. In previous studies, however, an antibody response has been detected shortly after inoculation using specific antigens. In this study, the presence of antibody responses in experimentally infected calves was evaluated with a commercial ELISA. Fifty-six calves from MAP-negative dams were randomly allocated to 10 MAP challenge groups (5 calves per group) and a negative control group (6 calves). Calves were inoculated orally on 2 consecutive days at 2 wk or 3, 6, 9 or 12 mo of age. Within each age group, 5 calves received either a high or low dose (5 x 10exp9 or 5 x 10exp7 CFU, respectively). A humoral immune response was detected in 42% of the inoculated calves and was present in all age and dose groups except for the 6-mo low-dose group. Antibody response profiles differed among individual calves, for example persistent responses were observed but also peak and bimodal peak responses. Three calves inoculated at 12 mo were ELISA-positive within 4.5 mo after inoculation, whereas calves inoculated at a younger age generally took longer to become ELISA-positive. Furthermore, calves inoculated with a high dose of MAP more often became ELISA-positive when inoculated at 2 wk or 3 mo of age than calves inoculated with a low dose. In conclusion, a dose-dependent humoral immune response was detected by ELISA in a large proportion of calves soon after inoculation. Susceptibility of calves up to 1 y of age and transient responses should be taken into account in control programs.

Keywords:
serology, age-dependent, dose-dependent

Abstract O-06.3

VALIDATION OF A SERUM AND MILK ELISA FOR ANTIBODIES AGAINST MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS ACROSS LACTATIONIN GREEK DAIRY GOATS.

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Abstract text:
In this study we validated a commercial (IDEXX Pourquier, Montpellier, France) serum- and milk-ELISA for the detection of Mycobacterium avium subsp. Paratuberculosis(MAP) antibodies, across lactation, in Greek dairy goats. A total of 1268 paired milk/colostrum and blood samples were collected from lactating goat that were sampled at four consecutive times starting from kidding and covering the early, mid and late lactation stage. Bayesian mixture models for continuous correlated responses were used to derive the distribution of the serum- and milk-ELISA response for the healthy and the MAP-infected individuals at each lactation stage. In all lactation stages, serum and milk-ELISA were of average and similar overall discriminatory ability as measured by the area under the curve. For each test, the lowest overlap between the distribution of the healthy and the MAP infected does was at late lactation. Both tests had comparable sensitivities and specificities at the recommended cut-offs, across lactation. Lowering the cut-offs led to an increase in the sensitivities without serious loss in the specificities. Milk-ELISA can be as accurate as the serum-ELISA especially at the late lactation stage and could be preferred to the latter, during the implementation of MAP control programs because milk sampling is a non-invasive, rapid and easy process.

Keywords:
milk ELISA, dairy goat , validation

Abstract O-06.4
FINANCIAL IMPACT OF OVINE JOHNE'S DISEASE ON THE PROCESSING SECTOR IN AUSTRALIA

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Introduction: Johne's disease causes considerable economic loss to the livestock industries (Webster and Hall, 2000; Bush et al., 2008); however, the financial impact of Ovine Johne's Disease (OJD) in sheep at slaughter has not been previously investigated. Monitoring under the National Sheep Health Monitoring Program (NSHMP) for OJD at an abattoir in Tasmania in 2011 confirmed an increasing prevalence of infected flocks, with many consignments having >10% lesions. The aims of this pilot study were: 1) to estimate financial loss to producers and processors attributable to OJD by monitoring in a Tasmanian abattoir; 2) to investigate potential associations between OJD carcase lesions with carcase quality and economic cost of the disease; and, 3) Investigate producer attitudes towards abattoir disease feedback and OJD management practices.

Methods: Consignments of sheep (lamb, hogget & mutton) were monitored at slaughter at a Tasmanian abattoir for the presence of OJD (evidenced by thickened terminal ileum) or OJD vaccination abscesses (attributed to poor technique) during two periods (total 5 months) in 2012/13. Other conditions (such as *Cysticercus ovis*, sarcocystis, arthritis and grass seeds) were also monitored. Individual carcase weights (corrected for all conditions), amount of meat trimmed for each condition (including condemns), fat score and slaughter interval (in seconds) were also recorded. Consignment details included average skin value, price per kg to producer, breed (Merino, crossbred, other), property identification code (PIC), average chain speed (carcases/hr) and extra staff numbers/hours required to trim affected carcases. OJD was confirmed by histopathology on terminal ileum/ileocaecal valve of up to three animals showing intestinal thickening per consignment. In addition, a retrospective study was undertaken on mutton consignments (2 years or older) slaughtered at the same abattoir in 2011/2012 and monitored for OJD, vaccination abscesses and other conditions under the NSHMP. Individual carcase weight, fat score and condemn details and PICs were provided. Skin values and the number of animals affected with each condition were recorded on a consignment basis. Statistical analyses were conducted using GenStat software (©2000-2012 VSN International Ltd, Hemel Hempstead, UK). Linear and logistic regression analyses were conducted to investigate the association of the presence of OJD and OJD vaccination lesions with a series of animal and carcase characteristics
A general framework was developed for the economic analysis of direct and indirect costs at slaughter. To investigate producers' attitudes towards abattoir disease feedback, specifically on OJD, a sample of 20 producers with consignments positive for OJD or vaccination abscesses was selected for phone interview.

Results: *Study data:* Data from 358 consignments (203 lamb, 17 hogget & 138 mutton) and 31,858 individual carcases were collected from the abattoir. Sheep breed was available for 231 consignments (107 Merino, 114 crossbred and 10 mixed). Most lamb consignments were crossbred (95.3%) and most mutton were Merino (81.8%). Six mutton consignments (all Merino) were OJD positive, with a median apparent within consignment prevalence of 4.6% (5-95%, 3.5% - 16.1%). Forty-seven consignments had OJD vaccination lesions [2 hoggets (11.8% of consignments), 6 lambs (3.0%), 40 mutton (28.3%)]. Presence of OJD vaccination lesions was significantly ($p<0.001$) associated with breed, with a higher proportion of Merino consignments

(33.6%) with vaccination lesions than mixed (10.0%) and crossbreed (2.6%) consignments. However, these results should be treated with caution as most crossbreds are not vaccinated and are consigned to slaughter as lambs. In addition, there was a significant random effect of property of origin on the presence of OJD vaccination lesions, suggesting clustering within properties.
No trim loss was associated with the presence of OJD other than affected intestines/runners lost during processing. The mean carcase weight, carcase value, fat class, slaughtering time and skin price per consignment were not associated with the presence of OJD or OJD vaccination lesions. In contrast, the individual carcase weight was associated with the presence of OJD vaccination lesions ($p = 0.008$). As expected the individual carcase weight was also associated with the amount of trim due to vaccination lesions, with every kg increase in trim estimated to yield a decrease of 0.65kg in carcase weight. The mean proportion of total consignment weight trimmed due to OJD vaccination lesions was 0.18% (0 – 0.73%) – an estimated 0.59kg of trim. The random effect of property of origin on individual carcase weight was also significant. Of all the conditions monitored, Sarcocystosis yielded the highest estimated mean trim per affected consignment (1.87kg) and per carcase (5.6kg).

The average skin price was not associated with the presence of OJD or the presence of vaccination lesions; however, further investigations are required to better understand the variables that impact on skin values. The intestines from OJD affected mutton carcases lost during processing were valued at $4.50 (at 20th February 2013), which represents a significant proportion (eg 15%) of the average mutton carcase value (approximately $30 in the current study).

Retrospective Data: All 6 PICs identified as OJD positive in the current study had been previously monitored between 2007 and 2012, with 4 PICs identified as positive previously (16 consignments). Among properties with OJD vaccination lesions (n=27), 81.5% were previously monitored and 40.9% were found to be OJD positive. The 353 consignments included from the NSHMP monitoring during 2011/12 comprised 336 mutton, 9 lamb & 8 hogget. Breed information was available for 233 of the mutton consignments (77.3%, Merino; 22.7%, Crossbred).. There were 63 (18.7%) mutton consignments positive for OJD. The presence of OJD in the consignment was not associated with breed ($p = 0.192$) , with 15% of Merino and 22.6% of Crossbred consignments being laboratory confirmed as positive for OJD. The average carcase weight was significantly associated with the presence of OJD in the consignment, with an estimated 1.01kg lower among OJD affected consignments than unaffected consignments. The average carcase value based on the price per kg at 20th February 2013, was also significantly associated with the presence of OJD in the consignment ($p=0.007$). Median average carcase value was $28.80 ($16.90-$40.50) for positive consignments compared with $32.30 ($18.20- $40.70) for negative consignments.

***Economic Framework*:** The low prevalence of OJD found in consignments sent to the abattoir during the study period might have been due to changes in farmer behaviour. Mutton production is a dynamic system responding to changes in the commercial environment with OJD operating as one factor of that system. The linkages between different players in the mutton supply system and the influences of OJD could be explored using a value chain approach. The value chain is largely controlled by demand for the product, which depends on feedback from the market, including the abattoir. Abattoirs are commercial operations (as are farms) and therefore the interactions (through price and other signals) provided by abattoirs to farmers would be expected to change the behaviour of the farmers in relation to the animals they present for slaughter. Understanding the interactions between activities to control and manage OJD (including selection of animals to be culled) forms an important part of the mutton supply system and impacts the potential costs of OJD during the slaughter process. A framework for the economic analysis was developed based on

direct and indirect costs at slaughter. The direct costs are due to meat, viscera and skin loss and the waste disposal costs; while, the indirect costs are due to increased labour costs.

Producer attitudes towards abattoir disease feedback: Most producers (95%) participating in the case study believed the disease feedback received from the abattoir was useful and prompted changes in their attitudes towards these conditions and management practices. Some of these changes included reviewing the OJD vaccination protocols and techniques, contacting their veterinarian to discuss ways of reducing the problems identified and starting vaccinating for OJD. Costs due to reduced payment from the abattoir were the most important motivator for management change.

Discussion: Interpretation of the presented results for OJD is difficult due to the small number of OJD positive consignments identified. Possible explanations include diversion to other abattoirs due to better prices or desire to avoid monitoring, retention of sheep for an extra year due to losses from OJD impacting flock size, increased availability of grazing land due to opening up of forest areas in Tasmania or reduction in regional OJD prevalence. Nonetheless the project was successful in developing procedures to better define the economic impact of OJD vaccination lesions (and a range of other conditions) on an individual animal and consignment basis. Results from the retrospective data contrast with results from the current study and suggest that there is a potential economic impact of OJD on the processing sector and on returns to producers.
Prior to the current study, the financial loss to producers and processors in Tasmania due to OJD in mutton sheep at slaughter was estimated to be substantial. Smaller carcases take the same time to process as larger ones but result in lower throughput of meat per unit of time and hence less efficient use of capital resources. Abattoir management would be likely to pass these costs onto the individual producer through sheep fitting a lower weight grid price. Alternatively they could spread the cost over all producers by a general reduction in prices paid. The initiative and interest of the participating abattoir in the current study, indicates the magnitude of the financial concern for the industry – both producers and processors - particularly in circumstances where OJD prevalence had been escalating and there were concerns that OJD was not being effectively controlled. Feedback to producers on the presence of conditions in combination with processing/financial information should act as a catalyst for altered management as well as provide an ongoing assessment of sub-clinical disease prevalence in the flock as a result of management changes or continued within flock spread.

Acknowledgements: This pilot study has been funded by Meat and Livestock Australia and is a collaborative effort between government, university and the private sector. Thanks are extended to Chris Cocker, inspectors, management and support staff at Tasmanian Quality Meats for provision of monitoring data. The NSHMP, which generously provided retrospective data, is funded by Sheepmeat Council of Australia and WoolProducers Australia through the National OJD Program managed by Animal Health Australia (Lorna Citer, Manager Endemic Diseases). The assistance of sheep producers is greatly appreciated.

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Keywords:
Ovine Johne's Disease, Financial impact, Abattoir feedback

Abstract O-06.5
DETECTION LIMIT OF BOOT SWAB AND LIQUID MANURE SAMPLING FOR HERD LEVEL SCREENING

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Abstract text:
Boot swabs (BS) taken from areas with high cow traffic were shown to be a simple, reliable and economic sampling tool to identify MAP-positive herds (MAP+H). The aim of this study was to determine the minimal within-herd prevalence (WHP) which can be identified by separate or combined analysis of BS and liquid manure (LM) samples with defined probability.

Corresponding BS and LM were taken once from 58 MAP+H and 19 certified MAP-negative herds (MAP-H) with a median WHP of 2.4% estimated by individual fecal culture (FC) of 22,940 (883 MAP+, 22,057 MAP-) cows. The cows of all MAP-H had been monitored for three years by annual individual FC without any positive results. Samples were tested directly by FC and qPCR after appropriate preparation. Estimates of WHP limits which allow detection of a MAP+H at 50% and 90% probability using BS, LM or a combination of both were calculated with an asymptotic logistic regression model.

Using FC, 37 BS and 36 LM samples from MAP+H were tested MAP+, whereas PCR was positive for 37 BS and 38 LM samples, respectively. Samples from 19 MAP-H were negative in FC and PCR except one sample each that showed a positive PCR result. When tested with both FC and qPCR, WHP thresholds for classification of MAP+H as positive at 50% and 90% probability were estimated to be 2.4% and 5.8% for BS, and 1.8% and 5.2% for LM, respectively. Estimated WHP thresholds decreased to 1.3% and 3.3% by combined testing of BS and LM. When only qPCR was applied, the 90% probability threshold value increased to 10.7%, 7.7% and 5.9% for BS, LM and combined BS and LM samples, respectively.

Assuming that herds with WHP of >5% account for the highest risk of MAP shedding into the environment and the food chain, we conclude, that a single BS and LM sampling identifies those MAP+H with adequate probability and specificity. This may advance implementation of control measures and the entry into a paratuberculosis control program. Testing with PCR only saves time but nearly doubles the estimated detection threshold.

Keywords:
herd level screening, boot swabs, within-herd prevalence

Abstract O-06.6
TEST CHARACTERISTICS AND LIKELIHOOD RATIO INTERPRETATIONS FOR TWO COMMERCIAL MILK ELISAS

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Introduction:
To apply milk ELISA diagnostics as part of herd testing programs, it is crucial to understand the test characteristics. The majority of milk ELISA evaluations have utilized in-house kits, and test characteristic estimates may not be transferable to commercial systems. Additionally, important diagnostic information may be lost by focusing exclusively on dichotomous outcomes. The objective of this study was to estimate sensitivity, specificity, and categorical likelihood ratios for two commercial milk ELISAs.

Materials and Methods:
Milk and fecal samples were collected from 1829 cows in 15 MAP-infected herds and 1889 cows in 17 non-infected herds. The two commercial milk ELISAs used were milk ELISA A (Prionics AG, Switzerland) and milk ELISA B (IDEXX, USA). Both pseudogold and latent class methods were used for test characteristic estimation.

Results:
Similar results were obtained for each of the statistical methods utilized. The sensitivity of the ELISAs ranged from 28.4% to 34.6%, and the specificity was between 99.2% and 99.7%. Likelihood ratio analysis demonstrated a strong relationship between the quantitative ELISA result and the likelihood for a cow to be shedding MAP in its feces. For ELISA A, cows in the 0.1 to < 0.3 category (just above threshold) and cows ≥ 0.5 were 19 and 196 times more likely to be shedding MAP in their feces, respectively. For ELISA B, cows with ELISA values ≥ 0.4 but < 0.7 (just above threshold) and cows ≥ 1.0 were 34 and 465 times more likely to be shedding MAP, respectively. Post-test probabilities for a cow to be MAP-infectious, given the prevalence of infection within her herd (the pretest probability), further extended the practical application of the categorical likelihood ratio data.

Conclusion:
These results establish the test characteristics of commercial milk ELISAs for practical applications. In addition, the results indicate that quantitative interpretation can further extend the utility of laboratory milk ELISA values for application within herd testing programs.

Keywords:
latent class analysis, milk ELISA, likelihood ratio

Abstract O-06.7
EVALUATION OF SEVEN PARATUBERCULOSIS DIAGNOSTIC TESTS IN THE DAIRY GOAT AND DAIRY SHEEP INDUSTRIES OF ONTARIO, CANADA

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Abstract text:
A cross-sectional study of the Ontario dairy sheep and dairy goat populations was performed between October 2010 and August 2011. The objective was to estimate the test sensitivity and specificity of seven commercially available paratuberculosis tests: faecal culture, faecal polymerase chain reaction (PCR), two serum enzyme-linked immunosorbent assays (ELISAs), agar gel immunodiffusion (AGID) and two milk ELISAs. Twenty-nine dairy goat herds were selected using stratified random sampling based on herd size, and 21 dairy sheep flocks were conveniently selected. Twenty lactating animals over the age of two years were randomly selected from each farm resulting in 580 samples from lactating does and 397 samples from lactating ewes. Each animal was sampled for faeces, blood, and milk.

Statistical analysis was performed using frequentist methods and also using latent class analysis (LCA) with Bayesian methodology. The apparent animal-level prevalence estimate using faecal culture as the reference standard was 18.3% (goats) and 7.6% (sheep), while the estimates derived from the 7-test 1-population model estimated a prevalence of 19.0% in goats and 9.4% in sheep. Faecal culture had the highest sensitivity in the goat population (81.1%), while in the sheep population, faecal culture demonstrated a sensitivity of 49.5% and faecal PCR a sensitivity of 42.4%. Faecal culture in both populations had an estimated specificity of 98.1% in goats and 97.4% in sheep while the specificity for PCR in both species was <90.0%. Both serum ELISAs in sheep and goats demonstrated higher sensitivities than the serum AGID.

Keywords:
Canada, diagnostic tests, dairy small ruminants

Abstract O-06.8

PRESENCE OF MAP DNA AND/OR MAP SPECIFIC ANTIBODIES IN COLOSTRUM FED TO NEONATAL CALVES RAISED IN AN ENDEMIC ENVIRONMENT IS NOT A DOMINANT RISK FACTOR FOR BECOMING A MAP SHEDDERS

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Abstract text:
Mycobacterium avium subsp. paratuberculosis (MAP) is known to be shed in feces, colostrum and milk by infected dairy cattle at calving. The uptake of MAP by neonatal calves is regarded the most important moment of transmission. Therefore, minimal contact between calves and adult cattle and restrictions in feeding colostrum are advised. Although a dominant role for the infectious status of the dam in transmission is accepted actual dam-calf data is scarce. This study aimed at quantification of the association between MAP status of dams and subsequent MAP shedding of their heifer calves at approximately 2 years of age.

A cohort of 117 dam-heifer calf pairs were enrolled on eight MAP positive commercial dairy farms in the Netherlands. To identify MAP exposure at birth colostrum and fecal samples of dams were collected at parturition. Blood and fecal samples of heifer calves were taken at approximately one and two years of age. Colostrum and fecal samples were analyzed for the presence of MAP using an IS900 real-time PCR. Colostrum and blood samples were tested by Pourquier ELISA. Data were analyzed by multinomial logistic regression.

After 1 year 24 heifers showed fecal shedding of MAP; one year later this number increased by 11 calves. Logistic regression revealed that the baseline probability of shedding of MAP by heifers born in a MAP contaminated environment at early stages of infection was 0.3. However, no association between colostrum quality or fecal shedding of the dam at parturition and fecal shedding of the heifer could be established.

We conclude that dam infection status at time of parturition is not likely to be the predominant risk factor for the occurrence of MAP shedding in offspring up to the age of two years when raised in a contaminated environment.

Keywords:
colostrum, transmission, calves

Abstract O-06.9

QUANTITATIVE SURVEY ON BULK TANK MILK CONTAMINATION BY MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP) IN EMILIA-ROMAGNA REGION

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Abstract text:
Milk can be contaminated by Mycobacterium avium subsp. paratuberculosis (MAP) through both direct excretion in the milk and fecal contamination during milking of soiled udders. Many extensive surveys have been carried out on bulk milk, but very few quantitative data are available; for this reason it is very difficult to estimate the exposure of consumers to MAP through the consumption of contaminated milk.

A quali-quantitative survey on bulk tank milk (BTM) was carried out during the period March-September 2013, testing almost all bovine dairy herds in the Emilia-Romagna Region (3052 samples).

MAP detection in BTM was performed by a peptide-magnetic separation (PMS) protocol, followed by IS900-qPCR. Briefly, 50 ml of milk were centrifuged (15' at 2500g) and the pellet was suspended in 1 ml of PBS. PMS capture of MAP was done by addition of an equal volume of magnetic beads coated with peptides aMp3 and aMptD. After capture, DNA extraction was performed by Chelex resin. Quantitative PCR was done targeting IS900 and absolute quantification of MAP cells was performed calibrating the assay with an IS900-plasmid standard solution [LOD: 1.3-1.6x100 cells/ml (10 replicates), corresponding to 23-67 CFU/ml].
Overall, 167 (5.47%) samples were positive by IS900 qPCR. Considering the efficiency of the detection system, only 12 out of 167 (7.19%) were estimated to contain more than 100 MAP cells/ml, while 83 (49.70%) ranged between 10 and 100 cells/ml and 72 (43.11%) less than 10 cells/ml.

Although MAP contamination of bulk milk in dairy herds of Emilia Romagna Region is limited, this survey suggests that the exposure cannot be considered negligible.
Supported by Ministry of Health, RF-2009-1545765.

Keywords:
Milk, Human exposure, qPCR-quantification

Abstract O-06.10

MOLECULAR EPIDEMIOLOGY OF PARATUBERCULOSIS: USING STRAIN TYPING DATA TO INFORM THE TRANSMISSION BETWEEN FARMS VIA LIVESTOCK MOVEMENTS

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Abstract text:
The purchase of infected stock is thought to be the primary factor of introduction of Mycobacterium avium subsp. paratuberculosis (MAP) onto properties primarily free, although no documented evidence of farm-to-farm transmission of MAP exists so far. This has important implication for the control of paratuberculosis (PTB) in areas and farms free of infection. The objective of this work was to analyze molecular data combined with animal movement data to demonstrate the possible transmission of MAP between farms.

The data for this study were provided by Landcorp Farming Limited (LC), which owns 119 farms throughout New Zealand (dairy, beef, sheep and deer). Longitudinal movement data of livestock between LC properties were available from 2006 to 2010. All farms were screened for evidence of MAP infection in a subset of 20 animals per farm and species in 2010. The strains isolated from fecal samples on each farm were typed using 5 loci by VNTR, and 1 locum by SSR methods. We combined social network analysis with molecular data to analyze the probability for 2 in-contact farms to share the same strain type, as a function of their relative position in the contact network. A significant association ($p<0.01$) was found between the contact structure of the LC farms and the distribution of strains: the closer two farms were connected via livestock movements the higher the probability of sharing the same strain. Physical distance between farms was not associated with strain similarity.

Results support the role of livestock movements in the transmission of PTB between farms, contributing to the maintenance of relatively high farm-level prevalence (sheep 48%, deer 63%, beef 43%, dairy 74%). The findings suggest that farm biosecurity could be used to control PTB even in populations where MAP is endemic.

Keywords:
molecular epidemiology, farm-to-farm transmission, livestock movements

Abstract O-06.11: PERSPECTIVE
MAP epidemiology: current trends and perspectives

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Abstract text:
Epidemiology of Mycobacterium avium subsp. paratuberculosis (MAP) infection is challenging in many ways. MAP infection remains latent for a long period and there are not highly accurate and cheap diagnostics for the early ante mortem detection of MAP. The course of infection differs between species and environmental conditions, management practices and genetic factors play a critical role on whether initial entrance and persistence of the microorganism will lead to clinical manifestations, be restrained during the productive life of infected animals or even be cleared out. Hence, valid studies are needed to map the situation on MAP. Thankfully, quality standards for the conduct of epidemiological studies and the reporting of results have been recently published and are already applied, though not thoroughly yet.

One challenge ahead is the constantly increasing limitations in the available financial and human resources. Data collection must be cost effective while, at the same time, it should not compromise the quality of the data collected. These fall within the context of a risk based approach in disease surveillance and prevention. On the other hand, the application of advanced bioinformatics, like the use of automated systems for data recording and diagnosis, will offer an intuitive way for the gathering of high quality information at a fraction of the cost. Furthermore, modern analytical tools, which are not yet popular, will provide solutions in the analysis of data beyond the current capabilities but within the present and future demands. For instance, neural networks can be used to differentiate between factors that are simply correlated with MAP infection and factors that have direct dependence and are true risk factors, a possibility not available in regression modelling. However, the proper use of these or other advanced tools, in the future, cannot substitute for the sincere interest of all parties involved in the study of MAP epidemiology. Thus I would like to finish by acknowledging the importance of social sciences in understanding the farmers' perceptions, attitudes and motives. This is and will always be crucial for the study of MAP epidemiology and the subsequent implementation of control interventions.

P-06 Epidemiology

Abstract P-06.1

FLOCK-LEVEL FACTORS ASSOCIATED WITH THE RISK OF MYCO-BACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP) INFECTION IN GREEK DAIRY GOAT FLOCKS.

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Abstract text:
A cross-sectional study was conducted from May to September 2012 in Greek dairy goat flocks in order to identify flock-level risk factors for Mycobacterium aviumsubsp.paratuberculosis (MAP) infection. A total of 1599 milk samples were collected from the does of 58 randomly selected goat flocks. The samples were skimmed and tested with a commercial milk ELISA (IdexxPourquier, Montpellier, France). Results were interpreted at a cut-off that maximized the sensitivity (Se) without serious loss in the specificity (Sp) of the test. A questionnaire was administered through a face-to-face interview with the farmers in order to collect data on farm management practices and productivity indices. A Bayesian multivariable logistic regression model, which adjusted for the imperfect Se and Sp of the milk-ELISA, was used to identify potential risk factors for MAP-infection. In all analyses flock was included as a random-effect term. Use of common water troughs, communal grazing, water from surface water (streams, shallow wells) and kids' spending more than 10 hours per day with their does were associated with higher odds of MAP infection. Contrarily, the alternating use of different anti-parasitic compounds was associated with lower odds of MAP infection. These results provide insight to managerial practices that should be targeted for intervention during the implementation of MAP control efforts in Greek dairy flocks.

Keywords:
dairy goat flock, risk factor, milk ELISA

Abstract P-06.2
SCREENING FOR MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN SOUTHERN ITALIAN DAIRY HERDS BY BULK MILK ELISA AND IN LINE MILK FILTER PCR

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Abstract text:
The knowledge of Mycobacterium avium subsp. paratuberculosis (MAP) infection status of each herd is a key factor for the control of the disease, for informed decisions of risk managers and for creation of positive conditions for the conscious commerce of animals and their products. Few data are available about paratuberculosis prevalence in Southern Italian dairy herds; therefore an investigation to detect MAP infected herds by repetitive screening tests was performed. This screening (S-MAP) was based on analysis of Bulk Tank Milk (BTM) samples by a commercial ELISA test (ID VET, France) and of In Line Milk Filter (IMLF) samples by IS900-qPCR.

BTM and ILMF were collected twice from 569 dairy herds in 3 Italian Regions. Additionally, a total of 12312 individual milk samples were collected and analysed by ELISA, 9509 from 102 herds which resulted positive to the initial screening (S-MAP positive) and 2803 from 24 herds which resulted negative to the initial screening (S-MAP negative). The S-MAP positive herds in these regions ranged between 18.8% and 23.9%; no significant differences were shown between regions in the prevalence of S-MAP positive herds. The within-herd Apparent Prevalence (AP) ranged between 0.00% and 22.73% and no significant differences were shown between Regions. The within-herd AP appears to be comparable to that reported in other Italian Regions.

A highly significant correlation was shown between positivity to S-MAP and within-herd AP. In fact, S-MAP detected a minimum of 56.3% of low prevalence herds (within-herd AP < 2.00%), up to a maximum of 100% of high prevalence herds (within-herd AP > 8.00%). Overall, the S-MAP detected 85.6% of positive herds. Although it cannot be used for MAP-free herd certification, S-MAP could be a useful tool to prioritise appropriate control measures in the context of widespread control plans aimed at reducing the prevalence of infection in dairy herds and milk contamination in dairy production.

Keywords:
Paratuberculosis, screening sampling plan, dairy herds

Abstract P-06.3

PREVALENCE OF PARATUBERCULOSIS IN THE DAIRY GOAT AND DAIRY SHEEP INDUSTRIES OF ONTARIO, CANADA

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Abstract text:
The objective of this study was to determine herd-level and within farm prevalence of paratuberculosis in each of the dairy goat and dairy sheep industries in Ontario and identify potential management practices that may contribute to farm prevalence.

This cross-sectional study took place between September 2010 and August 2011. A random sample of 29 dairy goat herds and a convenience sample of 21 dairy sheep flocks were visited once, whereupon 20 animals (lactating and 2 years of age or older) per farm were randomly selected and sampled for blood and faeces. During this visit a questionnaire was administered to producers inquiring about current management and biosecurity practices.

Faecal samples were analyzed using bacterial culture (BD BACTEC™ MGIT™ 960) and polymerase chain reaction (Tetracore®), and the sera samples were tested with the Prionics® Parachek™ serum ELISA.

Data was evaluated using both frequentist (faecal culture as gold standard) and latent class/Bayesian analysis. Using Bayesian analysis, in dairy goats, true herd-level prevalence was estimated to be 83.0% (95% PI: 62.6-98.1) and in dairy sheep was estimated to be 66.8% (95% PI: 41.6-91.4).

Analysis of the questionnaire data indicated deficiencies in biosecurity and youngstock management. These data indicate a need for a paratuberculosis control program in Ontario based on education, and the implementation of strategies to reduce on-farm transmission between animals.

Keywords:
prevalence, Canada, dairy small ruminants

Abstract P-06.4
SPREAD AND CONTROL OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP) IN A METAPOPULATION OF CATTLE HERDS

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Abstract text:
The spread of Map between herds is mainly due to animal movements, which form a complex network linking farms geographically close or distant. Moreover, the diversity of farming systems in a region (dairy vs beef, variable herd size and structure) and of contacts among farms (frequency, types of animals purchased) can also influence Map spread and control at the scale of the metapopulation of herds.

Here we study Map spread between cattle herds to evaluate, at a regional scale, control strategies based on the management of animal movements between herds depending on their epidemiological status.

Regional infection dynamics are represented by coupling intra and inter herds dynamics. For each herd, a stochastic compartmental model in discrete time describes realistic population dynamics and Map spread accounting for all the transmission routes and detailed infection progression. Intra-herds dynamics are coupled within a metapopulation model through animal movements. We use animal trade data from the French cattle identification database (2005–2009). A subset of the dairy farms network in the Finistère department in Northwestern France is considered based on the type and size of the herds.

Various tests at purchase are implemented, defined by the test sensitivity (Se) and specificity, the animal age and status at purchase and the delay between the test and the removal of detected animals (no delay if testing occurred before animal introduction until a delay of a few weeks).

The initial number of infected herds, their prevalence, and the network structure highly influence the speed of Map spread and the endemic state reached 20 to 50 years after the first disease occurrence. Testing purchased animals with Se greater than 0.75 achieves to stop Map spread but not to reduce the regional prevalence. Therefore, a combination of strategies should now be evaluated to also target a decrease in the within-herd contamination level. In parallel, an extension to beef herds is under way.

Keywords:
control strategies, animal movements, modeling of pathogen spread

Abstract P-06.5
PROVIDING ABATTOIR MONITORING FEEDBACK AS A CATALYST FOR PRODUCER BEHAVIOURAL CHANGE

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Abstract text:
Abattoir inspection of the viscera of sheep has been used as a tool to identify ovine Johne's disease (OJD) infected flocks for a number of years. The information has been used to calculate regional flock prevalence, for the early identification of infection in regions where the disease prevalence was low and to determine the effectiveness of on-farm disease control programs.

For a number of years this information has been returned to producers in the form of animal health status reports. Initially the focus was on disease identification, but more recently producers whose flocks have returned a negative detection result have been encouraged to implement biosecurity plans and to minimise the risk of introducing disease.

The provision of animal health status reports has been supported by a range of extension activities, including field days, vaccination technique workshops and the opportunity to visually inspect carcasses to see the impact of OJD first hand.

The National OJD Management Plan has facilitated the development of extension partnerships to ensure producers have access to contemporary advice on Johne's disease control and management.

Most recently the loss of income to producers from the inclusion of sub-clinically affected sheep in mobs presented for slaughter has been quantified in abattoirs. This information has been made available to producers in the form of a fact sheet and face to face workshops.

Results from a survey have indicated that producers are receptive to this information and have modified purchasing and disease management approaches.

Keywords:
abattoir monitoring, vaccination, financial impact

Abstract P-06.6

CASE STUDY OF A LARGE BEEF HERD IN SOUTHERN AUSTRALIA MANAGING ENDEMIC JOHNE'S DISEASE WITH VACCINATION AND HERD RISK STRATIFICATION

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Abstract text:

In 2012 a large beef herd in the southeast of SA was diagnosed as infected with Johne's Disease and placed under quarantine. All adult cattle (over 2 years of age) on the property were ELISA tested and positive cases further tested with faecal cultures or autopsy, in accordance with National Guidelines.

Forty one (41/ 1382) cattle were identified as ELISA positive and fifteen (15) of these were faecal culture positive. Other ELISA positive cattle were culled to slaughter prior to confirmation of status. Of the 15 infected cattle 11 of these were confirmed with S strain, and 4 with C strain.

S Strain is increasingly reported in cattle with clinical disease in areas where ovine Johne's disease is endemic and S strain was confirmed on this property.

The Cattle Council of Australia provides funding through the Financial and Non-financial assistance (FNF) package to assist producers with infected herds return to unrestricted trading. An FNF BJD Counsellor worked with the owners and government veterinarian to develop a disease management plan

Based on test results, all confirmed and reactor cattle were removed from the property and a number of strategies were put in place to manage rather than eradicate the disease in the short term including:

- Herd stratification according to disease prevalence and risk.
- Grouping by risk and managed according to status.
- Whole herd vaccination with Silirum®, including adults and female calves and this will continue in the future.
- Land management to reduce contamination
- Monitoring of high risk stock for clinical signs and culling.

Vaccination of cattle in Australia against Johne's Disease is rare, expensive and only undertaken under permit .The owner reports improved health within the herd following the management changes.

Keywords:

Vaccination in Australia, large Australian Beef herd, Risk stratification

Abstract P-06.7

SEROLOGICAL SURVEY OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN DAIRY CATTLE IN ESPÍRITO SANTO STATE, BRAZIL

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Abstract text:

Giving the lack of studys and the relevance of MAP in the dairy industry we verified the occurrence of antibodies to Mycobacterium avium subsp. paratuberculosis (MAP) in dairy cattle from Espírito Santo state, in Brazil. This country is one of the world's biggest milk producers, and most part of that milk is produced in the southwest area, which Espirito Santos's state belongs. For the diagnostic of Paratuberculosis the fecal culture is considered golden-standart method. However as MAP is a fastidious bacteria and has a slow grow, the culture means an expensive and long-lasting method, leading to the practice of the other methods, as the imunoenzimatic assay (ELISA). This test is practical and low cost, allowing the exam of large number of samples, also presenting an important tool in international control programs. A total of 1,450 serum samples from dairy cattle (males and females) been taken of all regions of the state and were analyzed for antibodies anti-MAP, using indirect ELISA. One hundred sixty-five (11.4%) samples were positive for anti-MAP, 33 (2.3%) were considered suspicious, and 1,252 (86.3%) were negative. In all regions, seropositive animals were found, indicating that the agent is spread by the State, posing a threat to the local dairy farming and neighboring states as Minas Gerais, the biggest milk producer in Brazil. as well it represents a risk to public health, since MAP can be involved with Crohn's disease in humans trough the ingestion of raw and maybe, pasteurized milk. This result presents the first MAP survey in dairy cattle of Espírito Santo. Seen the socioeconomic and public health importance of this bacteria, we hope that this study alarm the sanitary authorities and contribute to prevention and control programs of this disease, in Espirito Santo's state or even in Brazil.

Keywords:

milk, ELISA, antibody

Abstract P-06.8

SYSTEMATIC REVIEW OF THE PREVALENCE OF PARATUBERCULOSIS IN CATTLE, SHEEP, AND GOATS IN LATIN-AMERICA AND THE CARIBBEAN

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INTRODUCTION

The prevalence of an infection at the herd and animal level is often a key issue when decision or policy makers determine whether the infection should be considered important or not, and which measures to apply (Nielsen and Toft, 2009). The herd or flock prevalence in regions and countries is loosely associated with their history of animal importation, level of industrialization and degree of economic concentration in animal agriculture (Manning and Collins, 2010). Information on paratuberculosis for policy makers, academics, producers and consumers in Latin American and Caribbean countries is mostly based on information produced outside these countries (Europe, USA, Canada and Australia). The aim of the present study was to systematically review the prevalence of paratuberculosis among cattle, sheep, and goats in Latin America and the Caribbean.

METHODS

The present study was carried out following the procedures for Systematic Reviews in Health Care (frequency and rate) suggested by Glasziou et al. (2001). The process of identification of relevant studies included the search for existing systematic reviews, published primary studies, and unpublished primary studies. For this purpose, a systematic approach was undertaken. The searching criteria were defined by all authors. Firstly, the question for the systematic review was defined as how frequent or prevalent is paratuberculosis in farmed animals (cattle, sheep, and goats) in Latin America and the Caribbean? The search terms used to find relevant studies in databases were (diagnosis OR frequency OR prevalence OR occurrence) AND (paratuberculosis OR Johne's) AND (bovine OR cattle OR sheep OR ovine OR goat OR caprine). The initial search for existing publications reporting systematic reviews and primary studies was carried out by searching the available databases by January 2014. No language restriction was imposed. Only studies as from 1990 to January 2014 were included. Studies conducted on other farmed animal species or wild animals were excluded. The following databases were searched for published primary studies: Scopus, PubMed, Redalyc, and the Virtual Health Library. The proceedings of the 3rd, 4th, 5th, 6th, 7th, 8th, 9th, 10th and 11th International Colloquia on Paratuberculosis (ICP) were manually searched for existing systematic reviews and published primary studies. Studies from these proceedings were included in the systematic review if they had not appeared in peer reviewed journals, which were preferred for eventual inclusion. Database search was done using searching criteria by (J.A. F-S). The reference lists of relevant papers were searched for additional systematic reviews and published primary studies not found through database searching ('snowballing' procedure). The snowballing procedure was continued until no further study was found. Unpublished but cited primary studies were searched using the same procedure. Study was defined as a research aiming the determination of the frequency of paratuberculosis in a population of a specific animal species tested with one test. In case of publications including both animal level and herd level estimates, each one was counted as one study. Reports where two tests were used on the same population also counted as two studies.

For the final selection of studies an initial screening for basic eligibility and a detailed appraisal of quality was done. Both processes were always done by the three authors independently. The

decision for final inclusion of a relevant study in the initial screening and in the final selection was always obtained through consensus. The initial screen for basic eligibility was carried out using titles and abstracts of relevant studies found through search in databases, through search in the ICP proceedings, and through 'snowballing' procedure matching the general inclusion criteria on population, study factor, and outcome. After the initial screening, the detailed appraisal of quality was done. Potentially eligible studies were checked in full-text versions for three principal issues: minimization of selection bias of animals or herds / flocks, adequate assertion of final outcomes, and minimization of measurement or misclassification bias.

After final selection of studies, data on country and region, study period, population, inclusion criteria, selection, diagnostic test, sensitivity and specificity used (if available) for adjusting prevalence estimation, and results in terms of proportion, were extracted. Studies without crude numbers on frequency or proportion were excluded, unless the information provided allowed its calculation.

Results of the studies were analyzed according to animal species at individual and at herd / flock level, as well as according to diagnostic test used to reduce the influence of different test measurements. For the analysis of the data and the graphical representation of results, the software Microsoft Excel was used following the procedures described by Neyeloff et al. (2012). The analysis included the calculation of the outcome (effect size, es) and the standard error (SE), the computation of variance (Var), the individual study weights (w) and each weighted effect size (w*es). In addition, the analysis included the calculation of the Q test measures heterogeneity among studies and the calculation of the I^2 quantity. Finally, the analysis included the calculation of the appropriate effect summary (es) model (fixed effects or random effects model) according to the results obtained in the Q and I^2 tests (Neyeloff et al. 2012).

RESULTS

The 24 publications finally selected reported 52 studies: 38 (73.1%) from cattle (21 at animal level and 17 at herd level), 6 (11.5%) from sheep (all of them at animal level), and 8 (15.4%) from goats (6 at animal level and 2 at flock level). Thirty-three (63.5%) were animal level studies, while 19 (36.5%) were herd / flock level studies. No studies on prevalence in sheep at flock level that fulfilled the inclusion criteria of the detailed appraisal were found. In general, the studies were carried out in Brazil 25% (13/52), Mexico 21.2% (11/52), Chile 21.2% (11/52), Argentina 13.5% (7/52), Venezuela 7.7% (4/52), Puerto Rico 3.8% (2/52), Grenada and Carriacou 3.8% (2/52), and Costa Rica 3.8% (2/52). Only half of the studies reported information on study period, these studies were carried out in the period from 1990 to 2011. None of these selected studies provided complete and sufficient information on the issues for the quality appraisal. All studies were cross-sectional studies. Case definition was absent from all studies finally included. Diagnostic tests used in the selected studies to determine whether an animal or a herd / flock were considered a case were very diverse. ELISA (67.3%, 35/52) was the diagnostic test most commonly used , followed by fecal culture (individual or pooled, 7.7%, 4/52), skin test (7.7%, 4/52), culture of environmental samples (5.8%, 3/52), AGID (3.8%, 2/52), fecal PCR (3.8%, 2/52), individual milk culture (1.9%, 1/52), and bulk-tank milk PCR (1.9%, 1/52).

Prevalence in cattle at animal level: prevalence studies in cattle at animal level in Latin American and Caribbean countries using a random effect model revealed an overall prevalence of 16.9% (95% IC: 13.2-20.5). According to the type of diagnostic test used, the studies that used the ELISA test and the skin test (random effect model) revealed a prevalence of 19.5% (95% IC: 15.4-23.5) and 4.3% (95% IC: 2.5-6.1), respectively. Heterogeneity of all studies (I^2=80.1%) and of the ELISA-based studies (I^2=81%) was high. On the contrary, heterogeneity of results of the skin test-based studies (I^2=0%) was low. Prevalence in cattle at herd level: prevalence studies in cattle at herd

level in Latin American and Caribbean countries using a random effect model revealed an overall prevalence of 75.8% (95% IC: 50.1-101.5). According to the type of diagnostic test used, the studies that used the ELISA and other type of tests (environmental culture, bulk-tank milk qPCR, pooled fecal culture and skin test) using a random effect model revealed a prevalence of 74.0% (95% IC: 47.3-100.6) and 37.3% (95% IC: 25.3-49.4), respectively. Heterogeneity of all studies (I^2=0%), ELISA-based studies (I^2=0%) and environmental culture, bulk-tank milk qPCR, pooled fecal culture, and skin test studies (I^2=0%) was low. Prevalence in sheep at animal level: prevalence studies in sheep at animal level in Latin American and Caribbean countries using a random effect model revealed a prevalence of 16% (95% IC: 7.9-24.1). According to the type of diagnostic test used, the studies that used serological tests (ELISA and AGID) and direct methods (fecal culture and fecal PCR) using a random effect model revealed a prevalence of 17.9% (95% IC: 2.5-33.3) and 14.7% (95% IC: 4.3-25.1), respectively. Heterogeneity of all studies (I^2=60%) and of the serological tests studies (I^2=62.5%) was moderate to high, while heterogeneity in studies that used direct methods (I^2=44.9%) was moderate. Prevalence in goats at animal level: prevalence studies in goats at animal level in Latin American and Caribbean countries using ELISA for diagnosis and a random effect model for analysis revealed an overall prevalence of 4.3% (95% IC: 1.9-6.8). Heterogeneity of all studies (I^2=79%) was high. Prevalence in goats at flock level: prevalence studies in goats at herd level in Latin American and Caribbean countries using a fixed effect model revealed a prevalence of 3.7% (95% IC: 0.1-7.4). Heterogeneity of all studies (I^2=0%) was low.

CONCLUSION

In general, results of prevalence reported by the studies included in the systematic review were insufficient to accurately answer the question on how frequent or prevalent is paratuberculosis in farmed animals (cattle, sheep, and goats) in Latin America and the Caribbean. Several flaws in the studies design limit the quality of evidence on frequency of paratuberculosis in Latin American and Caribbean countries. Nevertheless, overall apparent prevalence for paratuberculosis in cattle in Latin America and the Caribbean appear to be around 17% and 76% at animal and herd level, respectively. Overall apparent prevalence for paratuberculosis in sheep is around 16% at animal level, whereas prevalence at flock level could not be determined. Overall apparent prevalence for paratuberculosis in goats at animal level is around 4.3%. Overall apparent prevalence for paratuberculosis in goats at flock level is around 3.7%, but the low number of studies included in the review as well as their homogeneity makes this low result unlikely to reflect the prevalence in Latin America and the Caribbean.

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Keywords:

Prevalence, Latin-America and the Caribbean, Farmed animals

Abstract P-06.9
MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN WILD RUMINANTS OF ITALIAN CENTRAL ALPS

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Abstract text:
Investigation on Mycobacterium avium subsp. paratuberculosis (MAP) diffusion in wildlife encounters many difficulties: collection of suitable samples, limits of serological tests (validated only for domestic ruminants) and difficult growth of some MAP strains. We examined samples of free ranging wild ruminants (red deer, roe-deer, chamois and mouflon) from Italian Central Alps. During 2012-2013 hunting season, we collected 760 samples for direct diagnosis (290 tissues and 470 feces) and 1656 sera. Nine animals showed gross pathology: in 7 young hinds/stags and one adult stag we observed loss of body conditions, rough coat, enteritis and lymph nodes extremely enlarged; in a roe-deer enlarged lymph nodes with micro-calcifications. Tissues and feces were examined by IS900-qPCR and the positive samples were cultured, both in solid (HEYM) and liquid media (VersatrekTM instrument). Sixty-nine samples (9%) resulted positive to PCR and 10 strains of MAP, all from deer, were isolated. All animals with gross lesions resulted positive to PCR and to histopathology. All deers positive to PCR came from an area of Stelvio National Park where MAP is endemic in this species and deer population density is high (winter density: 8.5 animals per 100 ha). The positive roe deers, chamoises and mouflons came from Bergamo Alps. For serology, we used a commercial ELISA kit (IDVET) and AGID. Less than 1% of samples resulted positive for AGID and ELISA. On 37 selected sera (all from PCR positive animals), we tested different ELISA kits in parallel. Out of these 37 sera, 5 were positive (13.5%) for all the kits. The majority of positive sera (60%) came from animals with macroscopic lesions, suggesting that it is necessary to improve serological sensitivity.

In conclusion, PCR resulted the most sensitive method for the surveillance of MAP infection in wild ruminants; on the contrary, serological and cultural methods didn't provide sufficient sensitivity.

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Keywords:
Wild ruminants, MAP, Paratuberculosis

Abstract P-06.10
ASSESSING THE PREVALENCE OF PARATUBERCULOSIS: A COHORT STUDY IN TWO FRIESIAN FARMS IN NORTH-WESTERN ITALY.

Romano A.[1], Vitale N.[1], Chiavacci L.[1], Rossi F.[1], Zoppi S.[1], Bergagna S.[1], *Gennero M.S.[1], Varello K.[1], Richelmi G.[1], Acutis P.L.[1], Boin C.[1], Arrigoni N.[2], Goria M.[1]**

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Abstract text:
A prospective longitudinal study in 2 Friesian dairy herds in North-western Italy was carried out in order to improve knowledge about incidence of seroconversion and fecal shedding, complemented with study on genetic resistance and susceptibility to Paratuberculosis (PTB). Three hundred cows, previously positive at PTB serological test, were selected and split into 3 different age categories (from 1.5 to 4.5 years). Each animal was tested every 4 months for antibodies in serum (ELISA test), for MAP isolation and detection respectively by culture and PCR on stool; PCR and culture were as well applied to monitor environmental contamination. Slaughtered cattle were also submitted to post mortem tests, collecting stool and target organs (lymph nodes, rectum, cecum, small intestine and ileo-cecal valve) for culture, PCR and histo-pathological examinations. A total of 1789 feces (80 of which from environmental samples), 2345 sera and 249 organ samples were tested during our study. In vita tests have been used to classify animals as "PTB positive" or "PTB negative", using a Bayesian algorithm that assesses sensitivity and specificity of combined tests serially to estimate the prevalence. This classification leads to build the base for genetic study on PTB resistance. The overall prevalence in Farm 1 ranged from 55% (CI95%: 33-78%) by PCR to 21% (CI95%: 14-30%) by culture, while in Farm 2 it ranged from 34% (CI95%: 24-44%) by PCR to 17% (CI95%: 10-25%) by culture. The estimated prevalence calculated on the basis of post mortem test on slaughtered cattle (n=49) was 57.3% (CI95%: 51-64%). Different infecting pressures were observed in the 2 herds and the highest prevalence occurred with the presence of high-shedders. These data highlight how in both farms, even with different management and exposure conditions, environmental contamination is maintained despite progressive removal of PTB positive animals.

Keywords:
Cohort Study, PTB Prevalence, Friesian farm

Abstract P-06.11
JOHNE'S DISEASE: ISOLATION OF M. AVIUM SUBSP PARATUBERCULOSIS (MAP) FROM A DAIRY HERD IN PERNAMBUCO, NORTHEASTERN-BRAZIL.

Gomes M.J.P.*[1], Costa N.D.A.[2], Mendonça C.L.[2], Pires Jr J.B.[2], Dantas A.C.[2], Afonso J.A.B.[2], Souza A.L.D.[3], Lopes B.C.[3], Gomes V.T.M.[3]

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INTRODUCTION

Bovine paratuberculosis or Johne's disease is a chronic enteropathy caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map), leading to malabsorption, cachexia and death. It is not a new disease in Brazil. After the first cases detected in 1915, there were other reports in the states of Rio de Janeiro, Santa Catarina, and Rio Grande do Sul between the 1950s and 1980s, by pathologists who regarded it as an exotic or sporadic disease associated with the import of infected animals. Epidemiological studies were carried out to estimate the prevalence of Map infection in herds from the states of Mato Grosso do Sul, São Paulo, Rio Grande do Sul, Rio de Janeiro, Pará, Pernambuco and Minas Gerais (Gomes, 2002; Gomes *et al*. 2002; Gomes *et al*. 2005; Gomes *et al*. 2007; Carvalho *et al*. 2009; Mota *et al.* 2010). Most serological surveys revealed the presence of infection in virtually all of the tested herds. However, most of these prevalence rates have been overestimated, since *M. bovis* or environmental mycobacteria in countries affected by bovine tuberculosis can interfere with the serological tests used for the diagnosis of bovine paratuberculosis, mainly with ELISA.

The aim of this case report was to provide a clinical and pathoanatomical description of Johne's disease, based on Map isolation and on the estimated prevalence of infection in a dairy herd in the state of Pernambuco.

METHODS

The first cases of the disease were observed in September 2005. The owner reported the disease among adult cows only, in both lactating and dry cows, which was characterized by intermittent chronic watery diarrhea, initially olive green and then blackish in color, often passed profusely, causing progressive emaciation, partial or total reduction in milk production and enlargement of superficial lymph nodes. The disease progressed within weeks or months, always leading to death. The herd also had a previous history of tuberculosis.

Four cows were autopsied in Pernambuco (PE). Fragments of lymph nodes, small intestine, large intestine, and of several other organs, were collected; the samples were fixed in 10% formaldehyde buffer and processed using conventional methods for histopathological analysis. Sections from the intestines and from mesenteric lymph nodes were stained by the Ziehl-Neelsen (ZN) method. Stool, tissue, and serum samples from PE herds were sent to the Laboratory of Bacteriology of the School of Veterinary Medicine of Universidade Federal do Rio Grande do Sul for detection of antibodies against Map.

Eight stool samples and terminal ileum samples (1 to 2 g) from the PE herd were treated and processed according to the modified classic Cornell technique, as described in Gomes (2002). The samples were inoculated into four tubes containing Herrold's egg yolk medium (HEYM), two with mycobactin and the other two without it. The cultured samples were kept in an oven at 37ºC and

were checked fortnightly for 12 months. The following criteria were used for Map identification: time of microbial growth, morphology, and mycobactin dependence (Gomes, 2002).

The isolated colonies were submitted to polymerase chain reaction (PCR) for amplification of insertion sequence IS*900* specific to the colony with suspected Map and for confirmation of the species, as described in Carvalho *et al*. (2012).

A total of 328 serum samples were collected from the PE herd, including all animals in that herd. Commercially available indirect ELISA (Allied Monitor, Fayette, MI, USA) with protoplasmic antigen (PPA) was used for the identification of antibodies against Map. The immunoassay was carried out according to the protocol supplied by the antigen manufacturer (Allied Monitor, Fayette, MI, USA) with minimal modifications (Gomes, 2002)

SUMMARY OF NEW AND UNPUBLISHED DATA

The clinical findings of chronic diarrhea in adult cows associated with weakness and reduction in milk and beef production have been frequently described in bovine paratuberculosis. The herd had a previous history of bovine tuberculosis.

The four autopsied animals showed fair to poor nutritional status; the serosa of small intestine had a cerebroid appearance, with thick and corrugated membranes and irregular reddish areas, with some foci of whitish dots on the corrugated surface. The mesenteric and ileocecal lymph nodes were enlarged.

The lesions observed in the animal tissues was characterized by pronounced, diffuse, granulomatous inflammatory infiltrate in the small intestine, from the duodenum to the large intestine, and in the mesenteric lymph nodes. The infiltrate was constituted chiefly of macrophages, lymphocytes, plasma cells, eosinophils, epithelioid cells, and several Langhans giant cells.

Mycobactin-supplemented HEYM cultures yielded colonies identified as Map according to their phenotypic and ZN staining properties. Map was isolated from the tissue/stool samples of six Girolanda cows (60%) with Johne's disease among 10 inoculated samples from a single dairy herd in the state of Pernambuco.

Amplified fragments (626 bp) of stools/tissues samples from six animals were observed using primers based on the insertion sequence IS*900* in dairy cows with clinical/subclinical signs of bovine paratuberculosis.

Absorbed ELISA using the PPA antigen detected 84 positive animals (25.3%) and 88 (26.5 %) suspect samples among 332 animals tested. The relatively low prevalence was probably due to the use of ELISA on all herd animals, associated with the presence of other mycobacterial infections, especially bovine tuberculosis.

CONCLUSION

Map was isolated and identified by ZN staining, culture, and PCR reaction in dairy cows with clinical/subclinical signs of paratuberculosis in Pernambuco, and antibodies against the causative agent were found in a large proportion of clinical/subclinically infected cattle.

The prevalence rate obtained by ELISA was high compared with international data. This suggests that further studies are necessary so that control programs can be implemented for dairy herds on

a nationwide basis. The estimated infection rate is high and worrying since we have a previous history of tuberculosis and there is no program for protecting our cattle herd from paratuberculosis and from milk-borne diseases.

ACKNOWLEDGMENTS
The authors express their thanks to Allied Monitor, especially to Dr. Chris Murdock, for donating the PPA antigen, Absorben (*M. phlei*) and positive and negative controls utilized in the serum ELISA tests.

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Keywords:
JD, Map isolation, Map infection

Abstract P-06.12
SURVIVAL OF HIGH TEST POSITIVE COWS IN HERDS PARTICIPATING IN A VOLUNTARY JOHNE'S DISEASE CONTROL PROGRAM

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Abstract text:
A voluntary Johne's Disease (JD) control program for dairy cattle herds was launched in Ontario, Canada in 2010 (www.johnes.ca). The program included an on-farm risk assessment (RA), funded milk or serum based testing of cows in the herd and terminal removal of high test positive animals. Milk testing was done with the Prionics Parachek Antibody ELISA. Cows were classified as test positive (TP) if they had a SP value >0.1, and as high TP (HTP) if they had a SP value >1.0 (high risk of fecal shedding of MAP). Of 4,158 herds in the province, 2,153 completed all three parts of the program between 2010 and 2013, and were paid for testing (program-paid). An additional 161 herds completed a herd test but either did not remove their HTP cows or did not complete a risk assessment, so were not paid (program-unpaid). An additional 1,711 herd tests were completed during the same 4 year period outside of the program (non-program). The objective of this study was to evaluate the impact of paying $500 CDN for permanent terminal removal of HTP cows within 90 days of testing.

Milk ELISA test results from 153,784 cows were matched to their DHI record to access parity, days in milk, production, end of lactation and subsequent calving date data. Logistic regression and survival analysis was used to compare the removal risk of test negative (TN), TP and HTP cows in each of the three herd groups. In non-program and program-unpaid herds, the TP cows were 3 times as likely, while HTP cows were 6 times as likely to be culled in the current lactation as TN herd mates. In program-paid herds HTP cows were 45 times as likely to be culled in the current lactation. Compensating dairy producers $500 per HTP cow removed altered their behaviour and resulted in the removal of two-thirds of the HTP cows identified as part of the voluntary program.

Keywords:
Survival, Test Positive, Compensation

Abstract P-06.13

REPEATED MILK ELISA TESTING FOR DETECTION OF MAP-INFECTED DAIRY CATTLE

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Abstract text:

Repeated milk ELISA testing may increase detection of MAP-infected cows. A potential gain in sensitivity, and concomitant loss in specificity, from repeat testing must be assessed before implementation of within-herd control programs. Study objectives were to investigate sensitivity and specificity of initial and repeated ELISA combinations, relative to fecal culture, and to evaluate factors influencing the probability of an initial ELISA-negative, MAP-infected cow to be positive on a repeated test. Milk and fecal samples were collected from 3,145 cows in 34 herds. Repeated ELISA tests were interpreted in parallel. For a 6-mo test interval, sensitivity of the milk ELISA increased from 22.0% for the initial test to 32.6% for combined initial and repeated tests. Specificity was 99.6%, and 99.2% for initial and combined tests, respectively. For a 12-mo interval, sensitivity was 25.6% for the initial test, and increased to 45.3% for combined tests. Specificity was 99.6%, and 98.9% for initial and combined tests, respectively. In MAP-infected cows, the magnitude of an initial negative ELISA was predictive for a repeated ELISA to be positive (OR=1.18 (95% CI: 1.08-1.28); *P*-value ≤ 0.001). Initially ELISA-negative cows tended to be more likely ELISA-positive with a 12-mo test interval than a 6-mo interval (OR=2.88 (95% CI: 0.84-9.90)). Repeated testing improves detection of MAP-infected cows, with minimal loss of specificity, and reduces risk of misclassification based on a single ELISA result. Retesting at 12-mo, with special focus on cows with negative ELISA results close to the cutoff, may be a practical option in herd testing programs.

Keywords:

milk ELISA, repeated testing, within-herd control programs

Abstract P-06.14
META-ANALYSIS OF PARATUBERCULOSIS PROGRESSION IN EXPERIMENTAL SHEEP STUDIES INFORMING MATHEMATICAL MODELING

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Abstract text:
The patho-physiology of the progression of ovine paratuberculosis is complex and still poorly understood. The objective of this work was to use data from published reports about the pathogenesis of ovine paratuberculosis to develop a state-transition model reflecting within-host progression of paratuberculosis in sheep.

Several experimental and natural infection studies with Mycobacterium avium subspecies paratuberculosis (MAP) were subjected to systematic review and meta-analysis. The review generated parameters of infection dynamics and pathogenesis for developing a mathematical state-transition model of paratuberculosis in sheep. We identified different possible pathways following infection with MAP in sheep and the relative frequency and duration for these infection routes, i.e. the proportion of animals entering different pathways, the possible duration of different stages of infection and the effect of covariates such as age, dose, and strains of MAP on the fate of sheep infected with MAP. A state-transition model was developed for paratuberculosis in sheep in agreement with the results of the meta-analysis and parameterized based on the reviewed data.

This study provided a robust framework for mathematical modeling of ovine paratuberculosis.

Keywords:
Meta-analysis, patho-physiology, modeling

Abstract P-06.15

PRELIMINARY RESULTS: SEROPREVALENCE OF PARATUBERCOLOSIS IN DAIRY HERDS REARED IN CASERTA, SOUTHERN ITALY AREA

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Introduction

Mycobacterium avium subspecies paratuberculosis (MAP) infection causes substantial economic losses and serious problems affecting the world's ruminant industry (Chiodini et al. 2012). Many countries have implemented control programs to prevent transmission among and within herds. In Italy, there is not mandatory national plan, only in some northern Italian area there is regional control activity. Efficient programs need knowledge of paratubercolosis seroprevalence, also to estimate the risk.

The seroprevalence of MAP is still unknown in many regions of the world especially in Italy regions. Some authors have shown that MAP infection affects over 70% of the dairy farms located in the Lombardy and Veneto regions (Pozzato et al. 2011). Other studies investigate on ovine paratubercolosis seroprevalence in two provinces of Marche region (central of Italy) (Attili et al. 2011). Therefore, only fragmentary and incomplete information on paratuberculosis prevalence in Italy is available.

The aim of the current study is to contribute to add another piece of this puzzle. So we investigate on seroprevalence of Mycobacterium avium subspecies paratubercolosis in dairy herds located in Caserta area, in Southern Italy. Furthermore, because of limited information about paratubercolosis seroprevalence in buffaloes, our study also point to investigate on this species.

Methods

A total of 1888 serum samples were collected from 48 herds. In tested herds, 30 raising only buffaloes and 18 raising both bovines and buffaloes, mixed farming. All animals tested were older than three years. All serum samples were tested using the ID Screen® Paratubercolosis Indirect Confirmation test, according to manufacturer's description (ID-Vet, France). This is an indirect ELISA test for detecting MAP specific IgG. To remove some cross-reacting antibodies, there is a pre-absorption step with *M*. *phlei* in order to reach more specificity.

Resulting optical density (OD) value were transformed to serum- positive percentage (S/P) according a formula cited by manufacturer. The sample was considered positive if S/P is ≥ 70%, negative if S/P is ≤ 60%, doubtful if S/P is > 60% and <70%, as recommended by manufacturer.

Results

Of 48 tested herds, a total of 13 were found to have at least one seropositive animal to Mycobacterium avium subspecies paratuberculosis, resulting a prevalence of 27%. Among positive herds, 12 positive ones raise only buffaloes and the other one breeds bovines and buffaloes in the same environment.

Conclusion

Bovine is a different species from buffalo and this suggests a different attitude to contract certain diseases. Our survey finds that buffalo and bovine seem to have similar attitude regarding to this pathogen. In fact, the only positive mixed farming shows the same percentage of positivity in both bovines and buffaloes.

Our data show 27% herds positivity and this percentage is less than Northern Italian area. However, it suggests to implement a good prevention and monitoring plan to reduce the spread of this pathogen.

Exhaustive study of paratubercolosis seroprevalence will be of some help to facilitate the design of prevention and control programs.

Nowadays, there are limited information on prevalence of paratuberculosis in Southern Italy herds. Our findings give a preliminary indication on seroprevalence of MAP in Caserta province. Further investigations, such as conventional culturing or molecular methods, should be performed to understand the real spread of this pathogen in Southern Italy.

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Keywords:

Paratubercolosis, seroprevalence, dairy herds

Abstract P-06.16
PARATUBERCULOSIS SEROPREVALENCE IN DAIRY CATTLE IN TWO REGIONS OF CENTRAL ITALY: UMBRIA AND MARCHE

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Abstract text:
Mycobacterium avium subsp. paratuberculosis (MAP) is the etiologic agent of Paratuberculosis (PTB). In Europe the prevalence of infected herds varies from 7% to 55% and in northern Italian regions published data report herd-level apparent prevalence (h-AP) ranging between 48% and 65%. Dairy cattle herds from two bordering central Italian regions (Umbria and Marche) were the target populations of investigation; they were evaluated together, being similar in consistence and breeding type. All sera of cattle older than 24 months were collected and analyzed as part of monitoring programmes in 2009–2011. Moreover, the farmers were requested to fill a questionnaire, aimed at evaluate risk factors for MAP spreading in herds. MAP specific antibodies in individual serum samples were tested by IDVet ELISA kit. In total, we tested 10524 subjects, coming from 178 herds out of 395 dairy farms present in the two regions (45%); 94 herds were positive with 52.8% h-AP (LC 95% 47.6%-58%). Positive animals were 492, with 4.7% individual-level AP (LC 95% 4.3%-5.1%). We found significant correlation between housing type and presence of infection in herds: in particular free-stall housing is more closely related to infection than tie-stall housing. Other significative risk factors are related to water supply (water trough vs bowl), management (replacement ≥ 20%) and previous PTB cases in last 5 years. The heterogeneity of the various studies (analytical technique, age, productive attitude of tested subjects) makes difficult to compare results obtained in whole Italy.

However, Umbria and Marche PTB h-AP can be considered similar to that calculated in other Italian regions. The most significant associations between risk factors and the presence of infection in the herd are the type of housing and watering. The high level of h-AP suggests in the future the application of guidelines for PTB control and certification, recently approved at a National level.

Research project RCIZSUM 072008 funded by Italian Ministry of Health

Keywords:
Paratuberculosis, Seroprevalence, Italian dairy cattle

Abstract P-06.17

SEROPREVALENCE OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS (JOHNE'S DISEASE) AND RISK FACTORS ASSOCIATED WITH SEROPOSITIVITY IN CATTLE HERDS OF OMAN

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Abstract text:

A cross-sectional country wide study of paratuberculosis (PTB) in the Omani cattle population was conducted during 2009-2011. For this purpose, 2209 cattle sera from 584 geographically marked randomly selected holdings were tested through commercial indirect ELISA (LSIVetTM Ruminant Paratuberculosis Advanced Serum ELISA Kit, Life Technologies, France). Data was analyzed and prevalence along with 95% confidence interval (CI) was calculated. Chi-square test was used to compare the differences in prevalence ($p<0.05$). Odds ratio and 95% confidence interval (CI) was calculated to measure the association between risk factors and disease. In total 66 (11.3%, CI: 8.8-14.2) herds were positive (with at least one positive cattle) for PTB and herd level prevalence ranged from 7.7% to 60% ($\chi2=28.42$, $p<0.01$). The individual prevalence ranged from 2.3% to 20% in different governorates ($\chi2=38.2$, $p<0.01$). The true prevalence at herd and individual level was calculated as 21% (CI: 19.3-22.7) and 4.5% (CI: 3.9-4.9) respectively. Significantly higher ($p=0.038$) percentage of females (3.6%, CI: 2.8-4.6) were positive for MAP as compared to males (1.6%, CI: 0.7-3.3). Prevalence of MAP was higher in local breeds (4.1%, CI: 3.1-5.4) followed by crossbreed (2.2%, CI: 1.3-3.5) and imported (1.3%, CI: 0.3-3.8) cattle ($p=0.02$). Cattle above 5 years of age were found more seropositive (3.8%, CI: 0.3-3.8) as compared to those below 5 years of age (3.2%, CI: 2.3-4.2). Different risk factors associated with higher prevalence were; cattle herds with history of chronic diarrhea (OR: 2.28, CI: 1.29-4.02), dairy settings (OR: 5.36, CI: 0.88-32.71), large (>30 cattle) herd size (OR: 1.34, CI: 0.73-2.45) and no or very little access to pasture (OR: 1.53, CI: 0.91-2.55). Present study is a first document on seroprevalence of PTB in the cattle of Oman and a detailed molecular epidemiological and risk factor study is required to devise a comprehensive control program.

Keywords:

Paratuberculosis, Cattle, Oman

Abstract P-06.18
SEROLOGICAL PREVALENCE OF PARATUBERCOLOSIS IN DAIRY CATTLE HERDS IN NORTHERN ITALY

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Abstract text:
A control program involving all dairy herds and cows older than 36 month was applied in Trento province during 2012 and 2013. The aim of the study is to describe the epidemiological pattern of this infection and to identify potential risk factors useful to improve the program.

Overall 20544 cows >36 months old from 1181 herds and 20406 cows from 1176 herds were sampled in 2012 and in 2013 respectively. An Elisa test (ID-VET) was applied on blood samples; results were interpreted by the following scheme: S/P≤0.6: negative, S/P≥0.7: positive, any other value: doubtful.

The results of 2012 campaign showed that 506 (2.5%) of samples and 212 herds (18%) were positive. Among these latter ones, 138 had a single positive cow, 30 had 2 positive cows, while 46 had 3 or more positive cows. In 2013, the number of positive cows decreased to 346 (1.7%) and the herds to 157 (13.4%). The control program did not include compulsory culling of positive animals. Therefore, in 2013, 234 positive cows were sampled again: 116 of these cows (49.6%) were negative, 7 doubtful, while 114 confirmed as serologically positive; however, when herds were considered, 102 herds out of the 212 positive ones in 2012 were negative and only 106 confirmed to host positive cows, while 4 were not sampled. However, among the 969 negative herds, only 820 confirmed their status, while 49 had at least one positive cow.

These results showed that the dynamic of serological positive results is peculiar with a significant decrease of sero-positive animals in 2013, even in absence of compulsory culling. The detection of new positive herds in 2013 suggests the importance to better define the criteria to classify a herd as infected.

In addition, the present program was also conducted with the aim to gain insight into the relationship between the sero-prevalence at a herd level and several factors that could influence the epidemiological pattern of this infection such as: herd size, animal movement, replacement rate, culling of infected animals, herd hygiene.

Keywords:
paratubercolosis, dairy herd, Italy

Abstract P-06.19
MAP SHEDDING PATTERNS IN DAIRY CALVES

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Abstract text:
In an experiment conducted at the University of Calgary, a high proportion of experimentally infected calves shed MAP in their feces. Because the relevance of this finding for the field was unclear, the aims of this study were to determine the proportions of: 1) MAP-shedding calves on MAP-infected farms; and 2) MAP-contaminated calf housing facilities. Fecal samples of all young stock (newborn to 24 mo, n= 2460) environmental fecal samples of all group-housing pens (n=104), and dust samples of all barn airspaces (n=29) were collected on 17 known MAP-infected dairy farms in Alberta, Canada. All individual fecal samples were processed using both IS900 and F57 rtPCR. Resulting positives in any of the two PCR methods, as well as all environmental and dust samples, were processed using the TREK ESP liquid culture system.

Results: 286 (14%) of the 2112 cattle on 14 farms were positive in at least one PCR method. Culture results from 11 farms are currently available; 23 (20%) of 113 rtPCR-positive samples, were also fecal culture-positive. The overall proportion of MAP-culture positive calves was 1.8%, with positive calves on 9 of 11 farms. Furthermore, 13% of 77 environmental samples from 5 farms were culture-positive. However, no dust samples were culture-positive. Conclusion: on most MAP-infected dairy farms, young stock shed MAP in their feces, resulting in a high proportion of pens that were MAP contaminated. This study used a serial testing scheme with direct PCR and subsequent culture of positive samples; therefore, the proportion of positive calves should be interpreted as conservative.

Keywords:
paratuberculosis, shedding, calves

Abstract P-06.20

ISOLATION AND MOLECULAR CHARACTERIZATION OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS FROM RUMINANT AND NONDOMESTIC-NONRUMINANT ANIMALS DURING AN OUTBREAK IN A DAIRY FARM IN EGYPT

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Abstract text:

Mycobacterium avium subspecies paratuberculosis (MAP) is a global pathogen that causes serious economic problems in the veterinary field and possesses a climbing public health concern. The disease was first reported in Egypt in 2004 and from that time it was repeatedly reported in different localities across the country. The current study investigates an outbreak of JD in a mixed breeding cattle – camel farm in Sinai Peninsula. Fecal samples were collected from 24 apparently healthy dairy cattle and from 15 one humped Arabian camel in which 3 suffered from chronic diarrhoea. Moreover, intestinal tissue samples were provided from 7 cats and 2 rats that were caught from the same farm and were euthanized before necropsy. Samples were examined using traditional culture and IS900 PCR techniques together with the application of BstEII-IS900 RFLP assay for typing of obtained isolates. Eight cows were identified by culture (33.3%) and14 cows by PCR (58.3%). All diseased camels (n=3) yielded MAP in their feces by both culture and PCR, whereas 2 of the apparently healthy ones (16.6%) were identified only by PCR. Furthermore, of 7 examined cats 3 were positive with culture and PCR while none of the examined rats was positive. The segregation of Map isolates from 3 hosts in a single farm reflects similarities and differences in the prevalence and diversity of obtained types. All fast growers recovered from cattle (n=8) and cat (n=3) clustered in the C18 and C1 types respectively. Slow growers from camel (n=3) clustered in a separate new type related to sheep strains and was named CS type. The obtained results denote an outbreak of JD in such a farm and highlight the potential role of non-ruminant and non-domestic animals in the epidemiology of MAP under our local conditions in Egypt, a subject which needs further investigation and might have a public health importance being cat a common member of many families

Keywords:

MAP, Camels and noruminants, Egypt

Abstract P-06.21
OCCURRENCE OF SHEEP STRAIN JOHNE'S DISEASE IN THE AUSTRALIAN BEEF INDUSTRY

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Abstract text:
The objectives of Australia's National BJD Strategic Plan are to: 1) Minimise contamination of farms and farm products by M paratuberculosis; 2) Protect non-infected herds while minimising disruption to trade and 3) Minimise the social, economic and trade impact of BJD at herd, regional and national levels.

Historically, the largest threat to the beef industry has been from the infected dairy industry, particularly in areas where the industries co-exist. The beef industry overall has a very low prevalence of Johne's disease, although prevalence is higher in dairying areas than elsewhere. Current risk management measures for the beef industry are targeted at minimising risk from the dairy industry. These measures are also supported by a program of financial and non-financial assistance to affected producers, overseen by Cattle Council of Australia.

However, in recent years there have been increasing numbers of cases of sheep (s) strain M. paratuberculosis in beef herds. This paper provides an analysis of data collected on beef herds in south-eastern Australia, with a view to evaluating the occurrence of sheep-strain infection as a source of risk to the beef industry.

Of 176 cases of paratuberculosis in beef herds between 2003 and 2013, 20 were due to s strain only, 4 due to a mixed infection of s and cattle (c) strains, 9 as c strain only and 143 were untyped. Overall, 14% of cases were due to s strain (or mixed infections). When summarised by geographic region, there were 0% s strain infections in the major dairying areas of Gippsland and the NSW coastal area compared to about 50% in the mixed farming areas of central and southern NSW and south-western Victoria. South Australia had 2 cases (9%), of which at least one was introduced from western Victoria, north-east Victoria also had 2 (9%) and Tasmania had 3 (30%).

Keywords:
sheep strain, beef cattle, epidemiology

Abstract P-06.22
TECHNICAL VALIDATION OF THE AUSTRALIAN JOHNE'S DISEASE MARKET ASSURANCE PROGRAM FOR SHEEP

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Abstract text:
The Australian Johne's disease Market Assurance Program for sheep (SheepMAP) is a voluntary program designed to provide a pool of low-risk animals for purchase by flock owners wishing to avoid the introduction of paratuberculosis into their flock. The SheepMAP includes elements of biosecurity to prevent introduction of infection and testing to demonstrate low risk of infection within the flock. Testing is designed to demonstrate 98% confidence that paratuberculosis is not present at a prevalence of 2% or greater. Flocks can progress their status through the SheepMAP, providing greater levels of assurance at each level, from Monitored Negative 1 (MN1) to Monitored Negative 3 (MN3).

The SheepMAP is now operating in a very different disease environment than when it was introduced in 1997 and is currently subject to a technical review. The ultimate objective of developing a simpler yet robust SheepMAP is to continue to deliver a high level of assurance to flock owners, sheep industries and prospective purchasers of sheep.

To assess the technical validity of the SheepMAP, an epidemiological model was developed to simulate the sampling process in candidate flocks. The model allowed for varying underlying flock-prevalence in the region of origin of sampled flocks, as well as for several different testing strategies (pooled faecal culture, alone or in combination with abattoir screening and on-farm veterinary surveillance of thin sheep). The model demonstrated that flocks at the highest level of assurance (MN3) presented a risk that was less than that for unscreened sheep from an area with a prevalence of <0.5% of infected flocks. Flocks at lower levels in the program that were from low or medium prevalence areas also provided a very high level of assurance. Overall, the modelling demonstrated that the program is meeting its objectives for level of assurance provided.

Keywords:
modelling, market assurance, testing

Abstract P-06.23

A DECADE (2002-2013) OF MOLECULAR EPIDEMIOLOGY (BIOTYPE PROFILES) AND INTER-SPECIES SHARING OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS IN DOMESTIC AND WILD RUMINANTS (BLUE BULLS, DEER, BISON), OTHER ANIMALS, PRIMATES AND HUMAN BEINGS IN INDIA

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Abstract text:

A decade (2002-2013) of study monitor biotype diversity and interspecies sharing of MAP using IS1311 PCR-REA, in highly diverse biotic [domestic livestock, wild ruminants (blue bulls, deer, bison), other animals, primates and human population] environment of the country. In 2002, first time Dr. R.J. Whittington (Australia) revealed bio-type of MAP strains infecting goats and sheep population as 'Bison Type' and again by Dr. R.A. Juste (Spain) in 2004. Native strains have since been characterized as 'Indian Bison Type', a new biotype. Domestic livestock (Goat, sheep, cattle & buffaloes): In first 5 years (2004-08), of 70 representatives IS900 positive DNA, from domestic livestock of different geographical regions, 91.4% were 'Indian Bison Type' and rest were 'Cattle type'. In next 5 years (2009-13), of 255 representative DNA, 96.4% were Indian Bison Type and rest were 'Cattle type'. Decade of biotyping revealed 'Indian Bison type' as major biotype (95.3%) infecting diverse domestic livestock population. Goats, sheep and buffaloes were exclusively infected with 'Indian Bison type'. Wild ruminants: Blue bulls: In 2005-07, of 8 representatives DNA, all were positive for 'Indian Bison Type'. Bison: Screening of 8 representatives DNA all were 'Indian Bison Type'. Rabbits: Of 15 representatives DNA (2009-11) all were 'Indian Bison Type'. Primates: On bio-typing of 2 DNA, both were 'Indian Bison Type' (2010). Human beings: In 10 years (2004-13) period, screening of 62 representatives IS900 positive DNA, from human population from different geographical regions, 82.2% were biotyped as, 'Indian Bison Type'. Dominant presence of 'Indian bison type' bio-type in domestic livestock species, wild animals (blue bulls, bison and rabbits), primates and human population in India, indicated pathogenicity of 'Indian Bison Type', infecting multiple species.

Keywords:

Mycobacterium avium subspecies paratuberculosis, 'Indian Bison Type', Cattle type

Abstract P-06.24

STATUS AND DYNAMICS OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS BIOLOAD IN THE BIOTIC (DOMESTIC LIVESTOCK SPECIES, WILD RUMINANTS, OTHER ANIMALS, PRIMATES AND HUMAN BEINGS) ENVIRONMENT OF COUNTRY: A FACT SHEET OF 28 YEARS

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Abstract text:

Study reports status and dynamics of MAP bioload in domestic livestock population of the country. In farmer's animals: Of 23,622 livestock head screened in 28 years (1985-2013), bioload of MAP was 23.6%. Analysis of 28 years data showed that bio-load of MAP was static (11.4, 13.1 and 11.1%) in 1985-1990, 1991-1995, 1996-2000 time periods, respectively. However, from 2001 to 2013, there was rapid rise in bio-load (24.2, 28.9 and 38.8% in 2001- 2005, 2006-2010 and 2011-2013, respectively) at five yearly intervals. Species-wise, bioload of MAP was highest (40.2%) in cattle followed by sheep (32.7%), buffaloes (29.4%) and goats (20.1%). Bioload of MAP was significantly lower in 1985-2000 ($p<0.001$) and 2000-2010 ($p<0.03$), as compared to 2010-2013. Farm animals: Of 8053 goats and 1527 sheep died at CIRG farms between 1988 and 2013, 8.8 and 3.0% deaths were due to JD, respectively. On the basis of JD and suspected JD, 11.3 and 32.9% goats and 2.0 and 45.8% sheep, respectively were culled in 2011-13. Screening of 396 tissues also showed that bioload was high in farm animals. 'Indian Bison Type' was the dominant biotype, irrespective of domestic livestock species and geographical region sampled. High bioload has also been reported in wild animals (48.6%), rabbits (25.3%), primates (40.0%) and in human beings (34.0%). These findings indicated that MAP is the major challenge for the bio-security of human and animal health. Bioload of MAP has shown increasing trend in our domestic livestock of country, there is urgent need to control the spread of MAP infection in biotic environment of the country. In order to reduce threat to human life it is essential to control bioload of MAP in animals. This is the first major study reporting bioload of MAP outside developed countries. A similar situation may exist in major part of the world where JD screening is still a distant dream.

Keywords:

MAP infection, Prevalence, Johne's disease

Abstract P-06.25

ECONOMIC LOSSES DUE TO THE BREAKOUT OF CLINICAL JOHNE'S DISEASE IN HIGH YIELDING HOLSTEIN FRIESIAN COWS LOCATED IN A DAIRY FARM AT ALWAR DISTRICT OF RAJASTHAN, INDIA

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Abstract text:

On the basis of clinical symptom [loss in body condition, drop in milk production (30 to 2 litres/day), forced culling and untimely deaths] and necropsy findings, local veterinarian suspected breakout of JD in a dairy farm consisting of high yielding Holstein Friesian cows in the Alwar district of Rajasthan. JD outbreak was confirmed by screening of 30.0% cows by fecal microscopy (68.5%), indigenous ELISA kit (92.3% in serum and 60.8% in milk) and 35.7% in IS900 blood PCR. All cows above 4 months of age were vaccinated against MAP using 'Indigenous vaccine' and economic losses before and after JD vaccination were analyzed. As compared to healthy cows infected cows suffered from significant reduction in milk yield ($p<0.05$). Of the 20 cows, that completed 8 months of milking, 10 showed significant ($p<0.05$) reduction, while other 10 cows exhibited reduction but was not significant ($p>0.05$). Total milk production (407 litres/day) was reduced by 183 litres/day after JD outbreak and was 224 litres/day. After vaccination, there was increasing trend (>2.1 litres/day) in milk yield and at 2 months post vaccination it improved by 2-8 litres/day (increase of 49 litres/day). Losses due to culling, mortality and reduced productivity (infertility, stunted growth etc.) were also analyzed and growing heifers showed weakness, stunting and onset of heat was delayed. Due to delayed breeding and reduced fertility (repeat breeding and no conception), there was loss of Rs 163800.0 in 180 days. Losses caused by untimely deaths (weakness and JD) and early culling (weakness and emaciation) were Rs 105000.0 and Rs. 167000.0, respectively. High yielding H/F cows were susceptible to MAP therefore suffered from JD breakout. 'Indigenous vaccine' using native strain 'S 5' of goat origin helped in 'therapeutic management' of clinical JD in high yielding H/F cows and helped in improving physical condition and milk yield of cows and restore productivity of cows.

Keywords:

Economic losses, Clinical Johne's disease, Indigenous Vaccine

Abstract P-06.26

BULK-TANK MILK ELISA FOR ESTIMATING THE PREVALENCE OF PARATUBERCULOSIS IN DAIRY HERDS OF A NORTHERN ITALIAN REGION

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Abstract text:

The aim of this study was to estimate the herd prevalence of paratuberculosis in dairy herds of the Emilia-Romagna Region, Northern Italy.

Between 2011 and 2012, a monitoring plan was carried out in 2859 dairy herds, 80% of all regional dairy herds. We tested bulk tank milk every four months, for a total of three samplings per herd, using a commercial ELISA kit (ID VET, France). On the whole, bulk milk tested positive at least once in 440 herds (15.4%). Adjusting for test sensitivity (Se=0.35) and specificity (Sp=0.995), the estimated real herd prevalence was 43.2% (95%CI: 39.4%-47.2%). Apparent herd prevalence increased according to herd size, reaching 50% among herds larger than 500 cattle. Conversely, we found no association between sampling season (four-month periods) and probability to have a positive result.

An additional sampling was performed in 175 herds, randomly selected from those always negative to ELISA bulk milk test: in each herd we collected 30 serum samples from cows older than 36 months for ELISA, and six environmental faecal samples for PCR and culture. Out of these 175 herds, 79 (45.1%, 95%CI: 37.6%-53.0%) were MAP infected (at least one positive serum and/or environmental sample). Five herds tested positive for environmental samples only. In 60 out of these 79 positive herds within-herd seroprevalence was lower than 5%. Also for this additional sampling, as for ELISA bulk milk results, herd-level prevalence increased according to herd size; in fact 8 out of the 10 investigated herds over 500 cattle were infected.

In the Emilia-Romagna region, the estimated MAP herd prevalence was about 45%, but it is likely to be higher among large-size herds. As found in other researches, repeated bulk milk ELISA testing seems a useful tool to screen herds with high prevalence.

Keywords:

Bulk tank milk, Prevalence, Monitoring

Abstract P-06.27

SEROPREVALENCE OF BOVINE PARATUBERCULOSIS IN ORGANIZED AND UNORGANIZED FARMS IN INDIA BY AN INDIGENOUS AND A COMMERCIAL ELISA

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Abstract text:

The objective of this study was to estimate the sero-prevalence of bovine paratuberculosis from different parts of India using a highly specificcommercial ELISA kit (InstitutPourquier, France) and indigenously developed ELISA. Bovine serum samples (n= 531) were collected from adults and calves of either sex from slaughterhouse (269) and different organised farms (262) of Nagpur, Akola, Bareilly, Durg and Palampur region of India.The serum samples collected were subjected to commercially available ELISA kit (Pourquier, France) and indigenous ELISA and the results were compared.Out of 531 sera tested, 43 (8.09%) were positive and 488 were negative by Pourquier ELISA. The seroprevalence of paratuberculosis was 13.5% in cattle of the organised farms and 4.83% in unorganised farms.The indigenous ELISA detected 36 (6.78%) positives and 495 negatives. Of these, 6 sera tested positive by the indigenous ELISA but negative by Pourquier ELISA, were considered “false positive”. While 13 sera tested positive by Pourquier ELISA, but negative by indigenous ELISA, were considered “false negative”.A total of 32 (6.02%) sera were found positive by both the tests and were considered as “true positive” while 480 sera, negative in both the tests were considered as “true negative”. The sensitivity and specificity of indigenous ELISA were found to be 77.1% and 98.76%, respectively, with accuracy of 96.42%. The predictive value for the positive test was 84.21% and predictive value for negative test was 97.36%. On the basis of Kappa value (0.72) calculated in present study to know agreement between the two tests, the indigenous ELISA used in this study was found to be in good agreement with commercial Pourquier ELISAand has a potential to be used in screening for MAP infection in cattle

Keywords:

Bovine, ELISA, Paratuberculosis

Abstract P-06.28
CASES OF PARATUBERCULOSIS DETECTED IN CATTLE DURING THE YEARS OF 2003-2011 IN ESTONIA

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Abstract text:
Estonia is currently considered to be free of bovine tuberculosis (M. bovis) – last sporadic cases of bovine tuberculosis were registered in 1986 and a case of tuberculosis in a pig in 1970. At the same time several non-tuberculous mycobacterial infections (primarily M. avium complex – MAC, but probably also M. avium subsp. paratuberculosis - MAP) are widely spread in Estonia. The problem is becoming particularly severe because of dramatic increase in HIV-infected people in Estonia that is accompanied by grown number of disease cases caused by MAC. The main source for human infections with non-tuberculous mycobacteria, are food-producing ruminants and derived products. Due to the unstudied situation, there exists a potential threat of contamination of raw milk with MAC and M. avium subsp. paratuberculosis (MAP) – showing the increased importance of ensuring safety and quality of dairy products in human epidemiology.

Information about the distribution of bovine paratuberculosis in Estonia is rather scarce. There was only one serology study on randomly selected cattle herds in 2002 that revealed ca 2% of seropositive animals. No further pathogen detection on those seropositive herds was carried out that time. However, some random examinations of 108 cattle samples regarding to MAP were performed over the period of 2003-2011. By PCR analysis with IS900 fragment specific primers, M. avium subsp. paratuberculosis from 7 cows (3 milk- and 4 feces samples) and from 1 sample of cow faeces from Latvia was detected.

Nevertheless, because of the lack of a proper survey, this data will not reflect an actual epidemiological situation in the country and further more profound studies are needed.

Keywords:
cattle paratuberculosis, Estonia, IS900

O-07 Public Health and MAP in the Environment

Abstract O-07.1: INVITED SPEAKER
CROHN'S DISEASE; AN ABERRANT IMMUNE RESPONSE TO INTESTINAL BACTERIA OR AN INFECTIOUS DISEASE?

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Crohn's disease (CD) is an intestinal disorder characterized by granulomatous inflammation. The etiology is still unknown, but it is generally believed that an inappropriate inflammatory response to commensal bacteria is involved. Over the last decade it has become clear that risk of developing CD is associated with polymorphisms in several genes involved in innate immune response, autophagy and inflammation. A recent meta-analysis of genome-wide association studies (GWAS), including more than 75000 patients and controls, demonstrated extensive sharing of susceptibility genes between CD and ulcerative colitis (UC). It is, however, notable that genes related to innate recognition of bacteria like NOD2, ATG16L1 and IRGM are all risk factors for CD but not UC. This indicates that some of the inflammatory pathways are likely shared between the two conditions, while the importance of immune handling of bacteria differentiates CD pathophysiology from UC.

The hypothesis that intestinal bacteria play an important role in the development of CD is supported by mouse models of colitis where disease is associated with microbiota exposure and observations in humans where antibiotic treatment and diverting the faecal stream away from sites of disease can reduce activity of IBD. As to which intestinal bacteria are involved in the pathogenesis of CD, much uncertainty remains. Characterisation of the microbiota in affected individuals and control subjects has demonstrated a relative increase in numbers of Proteobacteria and Actinobacteria and a decrease in Firmicutes in IBD patients. Enterobacteriaceae seem to be increased particularly in CD while less dysbiosis were often observed in UC. But importantly, there is no general bacterial signature for IBD patients, and it is not clear whether the observed bacterial dysbiosis is the cause or the result of the disease.

It is thus increasing amount of evidence supporting the understanding that CD is related to the ability of the immune system to handle bacteria. However, some important questions remain. Does the genetic predisposition in certain individuals influence the host response to particular bacteria or is it a more general effect to a wide range of gut bacteria? Can the disease be triggered by different bacteria depending on the host's genotype? Is CD a dysregulated/excessive immune response to bacteria or is it in fact an immune deficiency? Indications for the latter came from the above mentioned meta-analyses were it was noted that IBD loci were also markedly enriched (4.9-fold, $p < 10{-}4$) in genes involved in primary immune deficiencies, which are characterized by a dysfunctional immune system resulting in severe infections. The immune deficiency theory implies that otherwise harmless or opportunistic bacteria may cause a chronic infection in susceptible individuals that the host is unable to clear. The result is CD, where the immune system mounted in an attempt to clear the infection, causes more damage than the infection itself. Hence the use of immunosuppressive drugs as the standard treatment. Mycobacterium avium subsp. paratuberculosis (MAP) fits well into this scenario, and polymorphism in several genes are shared between CD patients and susceptibility to mycobacterial infections. Several groups have documented the presence of MAP or related mycobacteria in CD patients. Together with all the genetic susceptible data emerging over the last decade, it is very hard to reject the hypothesis of mycobacteria being involved in the development of CD in at least a sub-cohort of patients.

Abstract O-07.2

PREDICTORS FOR POSITIVE ENVIRONMENTAL SAMPLES COLLECTED IN VARIOUS AREAS ON DAIRY FARMS

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Abstract text:

In Western Canada, both the Alberta (AB) and Saskatchewan (SK) Johne's disease control programs use 6 environmental samples from 3 sampling locations for detection of MAP positive dairy farms. To estimate sensitivity among locations, we tested 761 sets of samples from AB dairy farms (average herd size 150) and 151 sets from SK (total of 513 dairy farms). Sample locations were manure storage areas from adult cows, lactating-cow pens, and dry, sick or calving pens. Each sample consisted of 4 subsamples. To maximise information gained from these samples, each subsample was also a mixture of manure from as many mature cows as possible, thereby avoiding individual-cow samples. Clear instructions were given for sample collection, labeling and shipment. Results: 60% of sample sets were culture negative, whereas the other 40% varied from 1 to 6 positive environmental samples. There were positive tests from approximately 20% of all locations within lactating dairy cow pens, whereas for manure storage areas, positive tests ranged from 14% for manure piles to 28% for lagoons. Dry cow areas were more variable, with positive samples from14% of bedding packs to 30% of alleyways (with similar variability for sick-cow and calving-pen samples). Within duplicates of one sample location, agreement was highest for lactating-cow pens. Based on multilevel logistic regression adjusted for clustering by herds, manure lagoon samples had higher odds, whereas manure pile samples and samples taken from bedding packs had lower odds of testing positive compared to lactating-cow alleyway samples. Herd size was a confounding factor for positive outcomes. Conclusions: Environmental samples should be collected from alleyways instead of bedding packs and from lagoons instead of manure piles to increase the chance of getting positive samples from infected herds. Presumably, having manure from more cows in these samples increased the accuracy of the method.

Keywords:

paratuberculosis, environmental samples, diagnostics

Abstract O-07.3

PERSISTENCE OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS IN DAIRY CATTLE SLURRY AFTER CHEMICAL TREATMENTS.

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INTRODUCTION: The application of animal slurry to the soil is a common agricultural management practice as fertilizer due to its contribution of nutrients and organic matter. Slurry consists of a mixture of cattle feces and urine with large amounts of water from rain and/or cleaning water, bedding of cattle housing, and feed wastes (Pain and Menzi 2003). An important disadvantage of the use of slurry as fertilizer could be its high content of pathogens that can put both animals and public health at risks. Livestock slurry can carry a variety of bacterial, viral and protozoan pathogens (Mawdsley *et al* 1995, Pell 1997, Hooda *et al* 2000, Hahesy *et al* 2002, Grewal *et al* 2006) resulting in a potential contaminant of surface and ground water. Indirect transmission of an infectious agent may occur through contamination of water, pasture, feed or food fertilized with insufficiently treated manure (Sahlström *et al* 2006). Among fecal-oral transmitted pathogens there is scarced published information on the persistence of members of the *Mycobacterium* genus, such us *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in slurry (Hahesy *et al* 2002, Grewal *et al* 2006). The aim of the present study was to evaluate the effect of chemical treatments on MAP survival in dairy slurry.

MATERIAL AND METHODS: Slurry, naturally contaminated with MAP, was collected from an experimental dairy herd and aliquots were treated with one of the following chemicals: 3% CaO; 0.5% NaOH; 0.087% H_2SO_4; 0.11% H_2SO_4; 0.14% H_2SO_4, 1% $KMnO_4$ and 2.5% $KMnO_4$. Treated slurry samples were stored at room temperature in glass flasks and evaluated at 24, 48 and 72 h, and 7, 15, 30 and 60 days for viable MAP using the BACTEC MGIT 960 liquid culture system. A positive signal in the liquid culture system was confirmed for MAP presence by real-time IS*900* PCR. MAP cell numbers (genome equivalent) was estimated on the uncultured original slurry based on the molecular weight of the genome of MAP strain K-10. Strain type discrimination by MIRU-VNTR pattern analysis was performed on MAP isolates from control and treated slurry samples. To evaluate the effect of chemical treatments on MAP survival, ANOVA for repeated samples followed by test Tukey multiple comparisons was initially proposed. Since results did not show homocedasticity and were not normally distributed, the Friedman ANOVA test was used, followed by the multiple comparative Dunn test. Student's *t*-test for paired samples was used to determine differences of MAP load between treatments and between one single treatment and control. All analysis was performed using the Statistix 8.0 program.

RESULTS AND DISCUSSION: Treatments based on lime increased slurry pH from 7 to 12 pH which resulted in a reduced survival of MAP, although it took 72 h to produce a significant reduction or elimination of MAP in the slurry. Highly significant differences ($p = 0.0001$) were found among treatments regarding MAP viability. The Dunn test indicated that the statistical difference between treatments is based on 2.5% $KMnO_4$ and CaO treatments versus control. Treatment with 2.5% $KMnO_4$ completely eliminated viable MAP right after treatment. At 24 and 48 h, CaO 3 % diminished significantly the viable organism ($P < 0.05$), however at 72 h complete elimination was recorded. Acid treatments did not affect MAP survival. MAP from control and treated slurry samples preliminarily had the same MIRU-VNTR patterns.

CONCLUSION: Under experimental conditions, this study suggests practical methods to control of MAP in slurry. Both lime and $KMnO_4$, potent oxidizing agents, were effective and affordable in the concentrations used. This information could be incorporated into management recommendations for slurry management on dairy farms. This information should be considered for future management plans for infection control in susceptible animal populations and could be considered as a best management practice to reduce pathogens for slurry management on dairy farms. Further studies are necessary to evaluate the optimum concentration and the potential secondary effects of these treatments on slurry composition and characterization, as well as its advantages and disadvantages from a biological, environmental, economical and practical point of view.

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Keywords:
MAP, Slurry, Persistence

Abstract O-07.4

SURVEY ON THE PRESENCE OF MAP IN GROUND BEEF FROM AN INDUSTRIAL MEAT PLANT

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Abstract text:
Paratuberculosis, at advanced stages of the disease, is characterized by a systemic dissemination of Mycobacterium avium subsp. paratuberculosis (MAP) in tissues and organs. Moreover, MAP has been associated with Crohn's Disease and other human pathologies. Dairy and beef cattle infected with Paratuberculosis, are routinely sent to slaughter and the consumption of their meat could be a possible route of human exposure to MAP. MAP has been demonstrated in muscles and lymph nodes of clinical and asymptomatic cows. However, few studies on the presence of MAP in ground beef are available.

We carried out a survey on the ground beef produced in an industrial meat processing plant. During the period November 2013 - February 2014, around 120 samples, each representing a single batch of ground meat, were collected and analyzed by both qPCR and a liquid culture method.

Three ml of sterile saline solution were added to 3 g of meat; after homogenization, the liquid phase was collected, centrifuged and DNA was extracted from the pellet with a commercial kit (BioSprint® 96 One-For-All Vet, QIAGEN); qPCR was then performed targeting IS900.

The liquid culture was performed from the same homogenization step described above, followed by decontamination with hexadecylpyridinium chloride 0.75%. The pellet was suspended in 1 ml of PBS, inoculated in para-JEM® Broth and placed into the Versa-TREK® instrument (Thermo Scientific). Positive samples were confirmed by F57-PCR.

The limit of detection (LOD) of both methods was around 6.3 x 100 MAP cells/g (corresponding to 1.1 x 100 CFU/g) for two MAP strains tested (ATCC 19698 and a field strain).

No samples were positive by direct IS900 qPCR, while one sample resulted positive to culture (F57-PCR confirmed).

Our preliminary data suggest that presence of live MAP in raw minced meat, although possible, is rare, namely less than 1%. Moreover, the level of exposure for humans could be considered even lower following cooking of meat.

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Keywords:
MAP, Human exposure, Ground beef

Abstract O-07.5
THE SUBMUCOSAL MICROBIOME IN CROHN'S DISEASE

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Abstract text:
In recent years there has been a great deal of effort devoted to the detection of M. paratuberculosis in patients with Crohn's disease using IS900 PCR as a biomarker for specific detection of the organism. To define the ecological niche occupied by M. paratuberculosis in patients with Crohn's disease, we sought to define the total microbial communities (microbiome) inhabiting the diseased submucosal and mucosal intestinal tissues and in blood. Methods were used to physically and chemically separate mucosal and submucosal tissues from resected intestinal specimens and mucosal and submucosal DNA isolated from diseased, disease-margin, and normal tissues from the same patient and controls. DNA was subjected to deep 16s metagenomic sequencing by 454 pyrosequencing to a depth of 0.0001% of the total bacterial population. The Q25 sequence data derived from the sequencing process was processed using a proprietary analysis pipeline and operational taxonomic units (OTU's) were taxonomically classified against a curated database, clustering at 3% divergence (97% similarity). A total of 105 resected tissues were examined representing 35 patients.

There were clear differences between Crohn's disease and controls even at the Kingdom level, with Bacteria representing 99% of detected organisms in Crohn's disease while in controls Bacteria represented only 60% followed by Metazoa (40%). Fungi and Viridiplantae were poorly represented. Even in blood, there was 3X more bacteria as compared to controls. In total, over 330 bacteria genera representing 695 distinct bacterial species were identified within submucosal and/or mucosal tissues. Although many species were common between mucosa and submucosa, each section tended to have distinct bacteria and different percentages suggesting both the presence of a submucosal microbiome and differences in the ability of certain genera/species to penetrate the mucosal barrier. OTU's corresponding to M. paratuberculosis were not detected in any intestinal or blood sample even in cases where IS900 was detected by PCR.

Keywords:
Crohn's disease, microbiome, molecular biology

Abstract O-07.6

BEHAVIOR OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP) DURING MANUFACTURING AND AGING OF ITALIAN HARD CHEESES

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Abstract text:
The aim of this study was to investigate the survival of MAP during the manufacturing and aging of Italian PDO cheeses (Parmigiano Reggiano and Grana Padano). These cheeses are produced with partially skimmed (through a natural creaming process) raw cow milk. The natural whey starter is added immediately before the curd heating at 53-56 °C for 30-70 min; then the cheese is ripened for 9-24 months. The study was conducted in two stages: a first initial evaluation of MAP behavior during the overnight creaming of the milk and a secondary challenge test during cheese manufacturing and ripening. The natural creaming process was reproduced in laboratory, using raw bovine milk, spiked with a MAP reference strain (ATCC 19698, Log 5-6 CFU/ml), maintained for 12 h at 18 and 27 °C. Samples of skimmed milk and cream-layer were collected for MAP enumeration. Cheese was manufactured in an experimental cheese factory located in Mantua (Lombardy, Italy), according to traditional procedures. Semi-skimmed raw milk was experimentally contaminated (final estimated concentration Log 5-6 MAP CFU/ml of milk) and then poured into two traditional copper vats. The first vat was inoculated with ATCC 19698, while the second one, with three different MAP field isolates. MAP survival was monitored from the beginning of cheese manufacturing, continuing throughout the ripening period. Samples of milk, cream, homogenized curd and cheese were cultured, without decontamination step, on HEYM-VAN, Middlebrook 7H9 (VersaTrekTM system) and HEYM, the last two supplemented with MIGIT PANTA, penicillin and nisin. Currently, only data related to the laboratory-scale creaming process are available. We observed a decrement of MAP load (Log 1) after natural creaming process at both temperature conditions, suggesting natural creaming process is effective in reducing 90% of MAP contamination of milk. Data on MAP survival during the manufacturing process will be further available.

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Keywords:
PDO Italian cheeses, Survival, MAP

Abstract O-07.7

TRENDS IN BIO-LOAD OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS INFECTION IN HUMAN POPULATION WITH SPECIAL REFERENCE TO INDIVIDUALS SUFFERING WITH CHRONIC AILMENTS FROM NORTH INDIA IN LAST DECADE (2004 TO 2014)

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Abstract text:

Bio-presence of MAP was first reported in 2008 in 5 patients of Crohn's disease by stool culture, IS900 PCR (blood and stool) and indigenous ELISA kit (80.0% in each test). Of 8 suspected cases of IBD (animal handlers), 75.0 and 62.2% were positive for MAP infection in ELISA and culture, Whereas, of 71 apparently healthy persons, 38.0% were positive in ELISA, respectively. In 2011, screening of animals and non-animal keepers, showed 2.1 to 36.0% were positive for MAP infection by ELISA, microscopy and PCR (stool and blood). From 2011, a large scale screening of bio-load of MAP in human population of Mathura district was initiated, wherein of 23,196 serum, 3093 blood and 101 stool samples were collected from different pathology laboratories, 34.0, 8.4 and 5.9% were positive in ELISA, blood PCR and microscopy, respectively. Bio-load of MAP was higher in patients suspected with ion imbalance (51.2%) followed by abdominal (42.5%) and liver disorders (41.8%), tuberculosis (41.1%), typhoid (40.7%), anemia (37.4%), inflammatory illness (36.5%), thyroid (29.5%) and diabetes (28.0%). Recently in 2014, screening of confirmed cases of diabetes type I from Chatarpur region of Madhya Pradesh (35.7%) were positive in ELISA. and IS900 PCR IS1311 PCR_REA bio-typing showed 'Indian Bison Type' was the most prevalent (83.8%), followed by 'cattle biotype (16.1%) in the above studies on human samples. In 2013, a follow up of 25 patients suffering from various kinds of chronic ailments were screened. Of these, 68.0, 44.0 and 28.0% were positive in microscopy, ELISA and PCR, respectively. Of these patients, majority had clinical symptoms indistinguishable to IBD and two cases on anti-MAP therapy showed substantial recovery from symptoms. Study indicated large scale exposure of human populations with MAP in North India and provides evidence for association between MAP especially in cases of IBD.

Keywords:

IBD, Mycobacterium avium subspecies paratuberculosis, IS900 PCR

Abstract O-07.8
DETECTION OF VIABLE MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP) IN INFANT FORMULA

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Abstract text:
Infant formula (PIF) donations were solicited from colleagues; 68 samples from 19 donors were received March through July, 2013. Samples were from 18 countries and represented 40 brands; 66 milk-based and 2 soy-based formulations. PIF products were reconstituted based on manufacturer's instructions; typically ~7.5 gm/50mL sterile water. On each PIF product peptide-mediated separation (PMS) followed by a phage amplification assay (PMS-phage assay) was performed to assess the number of viable MAP as described (Foddai et al., 2010). Only when plaques were verified to contain MAP DNA by IS*900* PCR were PIF samples considered MAP-positive.

By PMS-phage assay, 30 of 68 (44.1%) PIF samples tested positive for viable MAP. Among MAP-positive samples, counts ranged from 4 to 678 MAP PFU/50mL. Both soy-based PIF products were found positive for MAP by PMS-phage assay with counts of 66 and 175 MAP PFU/50mL.

Of the 18 countries from which PIF products were donated, only 5 had no MAP-positive products by PMS-phage assay. A total of 16 products were purchased from USA retailers and of these 9 (56%) were MAP-positive by PMS-phage assay. Two of 3 products originating from Sweden were MAP-positive by PMS-phage assay (88.3 ± 4 MAP PFU/50mL). MAP-positive products were found among all major brands of PIF tested.

These provocative findings require further validation. Such studies are in progress. If confirmed, they highlight the persistence of MAP during the manufacturing of powdered milk products, the possibility of cross-contamination of soy-based products during manufacturing, and explain exposure of human infants in many countries to MAP.

Keywords:
infant formula, epidemiology, crohn's disease

Abstract O-07.9: PERSPECTIVE
MAP disease in humans: An inconvenient truth or trivial dalliance?

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Abstract text:
One of the most interesting and challenging questions in MAP research addresses the implication that MAP may be a zoonotic agent. Several human diseases have been linked to this pathogen but it is particularly relevant to the hypothesis that MAP infection triggers Crohn's disease (CD) just as it does Johne's Disease (JD). There is an ongoing alarming increase in the prevalence of Crohn's disease worldwide particularly in paediatric and Asian populations. This is an important emerging issue in public health and if linked conclusively to MAP could represent an inconvenient truth that would have profound consequences for food providers and health strategies around the world. Although still unproven, it remains incumbent on us as experts in this field to assess and plan for such an eventuality with the provision and application of knowledge on all aspects of MAP pathogenesis and disease control.

Current concepts suggest CD does not develop as a result of auto-immunity but from a unique combination of genetic predispositions that importantly always require chronic exposure to environmentally acquired triggers. These drive or enhance immunological dysregulations, eventually releasing a characteristic cocktail of destructive inflammatory mediators which induce phases of gastro-intestinal hyper-responsiveness. Importantly, this is not an acute infectious disease but one that presents with episodes of acute symptoms that are a result of chronic inflammation driven indirectly and possibly distantly from the triggering agent. Presentations can manifest in several biotypes and locations including early and late onset, possibly as a result of the multiple combinations of genetic predisposition, age and triggering agent exposure. As there is no accepted cause we also have no idea of infective trigger dose, nor whether characteristic relapses are a result of re-crudescence from dormancy, re-infection with the triggering agent or even loss of tolerance.

So is MAP the culprit? Is it a lone perpetrator? Or just one of a range of triggers? Contrarily, could it be that previous inconsistencies in MAP isolation from human tissue and the lack of exclusivity in MAP detection from patient samples with and without disease is an indicator that MAP only represents a bystander infection and theories of MAP as a causative agent should be banished to conjecture and tales of coincidence?

The answers to these questions, like in all good detective stories, lies in careful investigation of the manner and motive for the crime; in this case dissecting the critical mechanisms that are required to initiate and maintain CD and demonstrating how candidate triggers may achieve this. Unfortunately, ethics (and health and safety) will no longer let us re-enact the crime! …but if we are to get a lasting conviction we must aim to show that the suspect had the means and opportunity to deliver, then importantly place them at the scene.

This perspective will discuss the latest evidence in the field for MAP as a causal agent, including new evidence that MAP is widely prevalent in the environment, compounded by low priority treatment of animal waste and persistent in a range of niches, some of which have the capacity to allow MAP proliferation. Further studies prove presence of viable MAP in a variety of both fresh and preserved common food products including meat and dairy, confirming that human exposure

to MAP is beyond doubt a continuous event. Important new evidence suggests viable MAP are also present in food stuffs including dairy products consumed within regions with a low incidence of MAP related disease. If confirmed this could break a long held dogma that MAP is not a credible candidate for triggering because it is not always present in high CD prevalence areas. The current trend for Asian populations to take on Western diets, as seen by recent doubling of demand for milk and dairy products, may in some small way play into this scenario. The reality is that long term exposure of humans to MAP, in the western world at least, is currently inevitable and substantial over long periods, making MAP a triggering candidate with ample opportunity.

As for method, new studies have shown that both CD and JD disease processes develop through significantly similar specific immunological reactivities and share an ever increasing list of host susceptibility gene loci associated with enhancing these pathways. Work in early onset CD correlates particular genetic phenotypes specifically with MAP presence in the gut, whilst interesting studies on late onset CD have linked differing disease biotypes to defined dysbiosis of normal gut flora that associate in a mutually exclusive manner with either MAP or other invasive, persistent bacterial species of as yet undetermined identity.

Definitive proof of causality in this day and age will only come from showing a pathogen specific therapy can prevent progression of the disease. With no cure for CD on the immediate horizon, we must remain speculative. The evidence however is still strongly in favour of an invasive, long term persistent intracellular organism that retains a capacity for dormancy, is prevalent in the environment, remains viable in food and can cause selective dysregulation of innate and adaptive immune processes in a manner highly reminiscent to that of MAP in JD. Finally, suggestions that MAP infection may additionally present specific cellular mimic peptides influencing autoimmune disease linked processes may be a complexity too far, but as we well know, MAP holds its secrets close.

Abstract O-07.10

SYNOPSIS: THE PARMA 12 ICP: A SUMMATION OF THE IAP SILVER JUBILEE (1989-2014) COLLOQUIUM

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P-07 Public Health and MAP in the Environment

Abstract P-07.1

POTENTIAL CURATIVE TREATMENT FOR IBD---MODELED AFTER PROPHYLAXIS OF JOHNE'S DISEASE

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Abstract text:
Outside the probiotic, Dietzia ssp. C79793-74, there are no preventive/curative therapies for Johne's disease. Interestingly, the causative agent, MAP, is at the center of controversy as to its role (cause, perpetuate, bystander) in different human diseases. Conventional therapies, including biologics, are in general directed at curtailing processes that are intricate parts of inflammation, with the goal to induce and maintain remission. Most therapies for IBD have side effects of varying severity, lose therapeutic value, are not directed to reduce/eliminate MAP, and most importantly, do not result in long lasting remissions or in cures. It is envisaged that both amelioration of diarrhea and attainment of long-term remissions are attainable since both were attained in Dietzia-treated cattle. Achievement will depend upon curtailment of (a) inflammation of the intestine, presumed to be due to an immune response to an etiologic agent (MAP in cattle) and (b) eradication of the etiologic agent. Interestingly, the value of present-day treatments aimed at reducing inflammation in light of findings that steroids actually resulted in an increase in MAP in blood and intestinal tissues should be questioned? Supportive evidence for this question is that treatment with dexamethasone along with Dietzia, while reducing diarrhea and MAP-specific antibody titers, resulted in an increase in fecal shed MAP and no curtailment of Johne's disease. Therefore, reduction and elimination of MAP in both humans and cattle appear to require some aspect of immune activity (functional phagocytes, B-cells, T-cells). And yet immune functions alone are also insufficient; Dietzia is required. Thus, Dietzia must have unique attributes that are essential to control GI symptoms and to eliminate MAP, irrespective of its role. Since Dietzia appears to be far superior in effectiveness and safety to all other treatments for human GI diseases, this report will present arguments for the value of Dietzia and how/why it should be evaluated.

Keywords:
DIETZIA PROBIOTIC, IBD, NEW TREATMENT

Abstract P-07.2
M. PARATUBERCULOSIS AND PARKINSON'S DISEASE – IS THIS A TRIGGER

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Introduction: Genome wide analysis and genetic linkage studies have suggested an association between *Mycobacterium avium* ss. *paratuberculosis* (MAP) and several inflammatory diseases. Polymorphisms of the CARD15 and SLC11a1 genes have been central to these investigations [1]; genes that harbor defects imparting susceptibility to both mycobacterial infection and autoimmune/inflammatory diseases have provided a "genetic trail" that has linked MAP to Crohn's disease [2-4] sarcoidosis [5], Blau syndrome [6], autoimmune diabetes [7-9], autoimmune thyroiditis [10-12] and multiple sclerosis [13,14]. Similarly, a parallel trail is emerging that provides a link between MAP and Parkinson's disease (PD). Polymorphisms of the LRRK2 and PARK genes are associated with PD and polymorphisms of the same genes are associated with susceptibility to mycobacterial infection: PD genes are "surprisingly" involved with leprosy (M. Leprae) and Crohn's disease (putatively, MAP) [15-18].

***Mycobacterium avium* ss. *paratuberculosis* (MAP)** is the cause of an enteric inflammatory disease mostly studied in ruminant animals called Johne's disease. MAP is the putative cause of the very similar human enteric inflammatory disease, Crohn's disease[2-4] MAP is present in pasteurized milk [19,20], infant formula made from pasteurized milk [21], surface water [22,23], soil [24], cow manure "lagoons" that leach into surface water, cow manure in both solid and liquid forms that is applied as fertilizer to agricultural land [23,24], and municipal tap water [25,26], providing multiple routes of transmission to humans.

Parkinson's disease (PD) is a common, progressive degenerative disease of the nervous system that manifests itself clinically as motor symptoms, including slowness of movement, tremor, rigidity and difficulties with balance and walking,. These symptoms occur after the pathology already has reached an advanced stage [27-29]. A prerequisite for the postmortem diagnosis of both the presymptomatic and symptomatic phases of the pathological process underlying PD is evidence of specific inclusion bodies called Lewy bodies [30-33]. In PD, only a few specific types of nerve cells are prone to develop the lesions. The major component of Lewy bodies is an aggregated form of the normally presynaptic protein – synuclein [34] Lewy bodies target the dopamine producing cells of the brain particularly the substantia nigra [35].

Anatomic staging of PD progression suggests that an unidentified neurotropic pathogen in the intestinal lumen triggers abnormal synuclein aggregation that initiates a "prion-like" process in the enteric nervous system (ENS) eventually achieving access to the central nervous system (CNS) via the vagus nerve. [34-39] This protein aggregation, manifest as Lewy bodies, spreads to the brain in "prion-like" fashion targeting and destroying dopamine producing cells resulting in the classic motor and non-motor symptoms of PD [39,40].

Contemporary thought on Parkinson's Disease: [Refs. for bullet points: 35-40]

- Proposed enteric pathogen invades the enteric nervous system (ENS).
- Parkinson pathology (Lewy body) progresses from ENS to the vagus nerve (cranial nerve X).
- Neuroinvasion of CNS ensues via retrograde propagation in the vagus nerve.
- In CNS, Lewy body pathology first presents in the dorsal motor nucleus of the vagus nerve.
- Lewy body pathology "spreads" in "prion-like fashion" resulting in targeted destruction of the substantia nigra in the midbrain.
- The pathologic process targets dopamine-producing cells.

Infectious Trigger of Parkinson's Disease: There are known infectious and toxic triggers of PD. Of the infectious triggers, *Nocardia asteroides* is the most studied. As a member of Actinobacteria this bacterium is phylogenetically related to MAP. The finding of immunosupressed patients with *Nocardia* neuroinfection presenting with acute symptoms of PD and demonstrating *Nocardia* in the substantia nigra has prompted exhaustive study of this bacterium and PD.

- Animal model of PD triggered by Nocardia – Nocardia attacks substantia nigra - affecting dopamine producing cells
- Sheroplasts (cell wall deficient) bacteria in substantia nigra of PD patients
- These speroplasts shown to not be Nocardia in PD patients
- MAP well known to form spheroplasts [Refs. for bullet points: 42-47]

Discussion: How can genes associated with neurodegeneration play a role in the host defense against bacterial infections: in addition to autophagy, parkin and LRRK2 also promote xenophagy, another autophagic pathway implicated in the removal of intracellular pathogens [48,49].This article proposes these same genetic polymorphisms associated with PD allow persistent infection of MAP and that MAP is the "unidentified enteric pathogen" that triggers synuclein aggregation. The cell wall deficient forms that are found in the substantia nigra of PD patients – found to not be *Nocardia* may be MAP. It is postulated that the loss of function leading to protein aggregation (Lewy bodies) is due to "consumptive exhaustion" of the processes that both maintain cellular protein homeostasis and effect removal of intracellular pathogens.

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Keywords:
Parkinson's, MAP, Autophagy

Abstract P-07.3
EVALUATION OF ENVIRONMENTAL SAMPLING FOR HERD-LEVEL SCREENING IN BEEF COW-CALF HERDS

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Abstract text:
Testing environmental samples (ES) for Mycobacterium avium spp. paratuberculosis (MAP) by fecal culture (FC) or PCR is established for herd-level screening in dairy herds. Usually three to six composite fecal samples from high cow traffic areas and the manure storage area are sampled and tested. For beef cow-calf herds, evidence for this approach is weak. This epidemiological field trial aimed to evaluate ES in high (HP) and low (LP) prevalent and certified paratuberculosis unsuspicious (CU) beef herds.

According to within-herd prevalence (WHP) in earlier years, six HP herds (WHP ³5%) and six LP (0% < WHP < 4.9%) herds were included into the study. In the six CU herds, the cows had been monitored for three years by annual individual FC without any positive result. In HP and LP herds, WHP was estimated by individual FC of each cow (n= 2095) resulting in a median WHP of 7.6% (min. 4.6%; max. 43.1%) and 3.6% (min. 2.7%; max. 3.8%) in the HP and LP herds, respectively. A mean number of six ES were taken from areas of high cow traffic (feed alley, drinking system, running alleyway) as well as from the holding pen in the barn or open air and the calving pen at the end of the winter holding period.

Out of 109 ES tested by fecal culture 19 samples showed a positive result, 17 of them originated from HP and two from LP herds. All samples from CU herds were MAP-negative. For HP and LP herds, percentage of positive ES and positive IFC samples correlated significantly (spearman rank correlation coefficient rs=0.747; p<0.01). In all HP herds at least one ES was found to be positive, whereas in LP herds positive ES originated only from one herd.

We conclude, that ES allows to detect beef herds with WHP >5% as MAP-positive. Relative to WHP, testing of ES from beef herds is as reliable as from dairy herds when sampling is performed at the end of the winter season. ES sampling in beef herds is an easy and cheap approach to identify those herds that account for the highest amount of MAP-shedding into the environment.

Keywords:
herd level screening, environmental samples, beef herds

Abstract P-07.4

OCCURRENCE OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS IN CONSECUTIVE INDIVIDUAL AND BULK TANK MILK FROM A DAIRY HERD WITH A LOW PREVALENCE OF JOHNE'S DISEASE

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Abstract text:
The aim of the study was to investigate the risk for the entry of MAP in the food chain via milk from dairy farms with subclinical Johne's disease (JD).

During 23 months, single and bulk tank milk samples from a MAP-positive dairy herd were collected. Milk, fecal, and blood samples were taken from all cows older than 1.5 years of age at the beginning and the end of the trial. Additionally, 63 cows (33 assumed MAP infected and 30 assumed MAP not infected) were sampled monthly. Raw and pasteurized bulk tank milk samples also were collected on a monthly basis. The milk samples were tested for MAP by real-time quantitative PCR (qPCR), and the fecal by qPCR or solid culture. Serum and milk samples were tested for specific antibodies by ELISA.

Based on the results of all cows older than 1.5 years, the prevalence of fecal positive animals in the herd was around 5%. No cases of clinical JD were observed during the study. The results of the ELISA showed high variation, with 2.1 to 5.1% positive milk samples and 14.9 to 18.8% ELISA-positive blood samples. Monthly milk sampling revealed low levels of MAP shedding into the individual milk samples of both MAP-infected and not infected cows. Only 13 of the sampled cows shed the bacterium into milk at any time during the study period. MAP was not detected by qPCR in any raw or pasteurized bulk tank milk sample throughout the study. A significant positive association could be found between MAP shedding into milk and feces.

From the results of the present study, it can be concluded that MAP is only present in a small number of cows with subclinical JD and for a limited period of time only, leading to dilution of the bacterium below the detection level of qPCR within the bulk tank milk. These findings indicate that dairy herds subclinically infected with JD pose a minor source for human MAP consumption with milk and milk products.

Keywords:
Johne´s disease, Milk, Shedding pattern

Abstract P-07.5

MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS EPITOPE HOMOLOGOUS TO EBNA1-400-413 AND HUMAN MYELIN BASIC PROTEIN-85-98 IS RECOGNIZED IN MULTIPLE SCLEROSIS PATIENTS

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Abstract text:

Mycobacterium avium subsp. paratuberculosis (MAP) was recently associated with Multiple Sclerosis (MS), a chronic neurological disease of young adults. MS etiology is thought to be triggered by environmental factors operating on a permissive genetic background. We have previously investigated the binding activity of 9-mers peptides, belonging to the MS related protein MAP_2694, against MS sera and we have demonstrated that two epitopes are immunodominant antibody targets within MAP_2694.

In this study, BLASTp analyses showed that MAP121-132 epitope, which derives from MAP_0106c, presents highly sequence homology to EBNA1400-413, the most important MS associated Epstein Barr Virus peptide. Noteworthy, this viral peptide shares conformational homology with human Myelin Basic Protein (MBP)85–98 epitope.

We asked whether antibodies targeting MAP121-132 epitope could cross-recognize self and no-self peptides. Antibodies against MAP121-132 were found in 12 out of 48 (25%) MS patients and only in 1 out of 34 (3%) HCs (AUC=0.70, p=0.0016), whereas 23 out of 48 (48%) MS patients and 2 out of 34 (6%) HCs were positive for MBP85–98 (AUC=0.84, p<0.0001). A competitive inhibition assay confirmed that MAP121-132 efficiently blocked MBP85-98-coated binding, proving that antibodies anti-MAP121-132 targeting the same conformational MBP85-98 epitope are cross-reactive.

Moreover, to test whether MAP121-132 is able to up/down regulate the cytokine response, we detected cytokine secretion of PBMCs isolated from MS patients and HCs after stimulation for 48 hours with MAP121-132. The ELISA cytokine assay revealed that PBMCs in MS patients were capable of secreting Th1 and Th17 related cytokines such as IL-6, IL-10, IL-17A and TGFbeta1.

In conclusion, MAP121-132 epitope seems to induce both an humoral and a T-cell mediated immune response. These results supports the theory that MAP might trigger autoimmunity via molecular mimicry mechanism.

Keywords:

MAP_0106c, molecular mimicry, Multiple Sclerosis

Abstract P-07.6

RECOGNITION OF ZINC TRANSPORTER 8 AND MAP3865C HOMOLOGOUS EPITOPES BY NEW-ONSET TYPE 1 DIABETES CHILDREN FROM CONTINENTAL ITALY

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Abstract text:

Type 1 diabetes (T1D) is an autoimmune disease characterized by a T cell-mediated destruction of insulin-secreting pancreatic β-cells. T1D results from the interaction of multiple gene variants and environmental factors, albeit the environmental factors remain poorly defined. Several autoantigens are known, the latest is Znt8. There are several pieces of evidence indicating that Mycobacterium avium subspecies paratuberculosis (MAP) infection is linked to type 1 diabetes (T1D) in Sardinian patients. Indeed, anti-MAP and anti-ZnT8 antibodies (Abs) were present in both T1D adults and newly diagnosed T1D children from Sardinia. What is more, an association between MAP and T1D was recently observed in an Italian cohort of pediatric T1D individuals, characterized by a different genetic background. We set up this study to investigate further the prevalence of anti-MAP Abs outside Sardinia. Our major objective was to assess the prevalence of anti-MAP/ZnT8 Abs in another pediatric population from continental Italy, looking at several marker of MAP presence in new-onset T1D subjects. Newly diagnosed children, compared to age matched healthy controls (HCs) were tested by indirect enzyme-linked immunosorbent assay (ELISA) for the presence of Abs towards the immunodominant MAP3865c/ZnT8 homologues epitopes, the recently identified C-terminal MAP3865c281-287 epitope and MAP specific protein MptD. Abs against MAP and ZnT8 epitopes were more prevalent in the sera of new-onset T1D children compared to HCs. These findings support the view that MAP3865c/ZnT8 cross-reactivity is involved in the pathogenesis of T1D and addition of Abs against these peptides to the panel of existing T1D biomarkers should be considered. It is important now to investigate the timing of MAP infection during prospective follow-up in at-risk children to elucidate whether Ab-titers against these MAP/ZnT8 epitopes are present before T1D onset and if so, if they wane after diagnosis.

Keywords:

MAP3865c, new-onset type 1 diabetes, Children

Abstract P-07.7
EVALUATION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (MAP) SURVIVAL IN TWO BIOGAS PLANTS IN ITALY

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Abstract text:
New legislation on renewable energies allows the use of animal manure in biogas plants. The final product of anaerobic digestion process (AD), namely digestate (DG), could be used as crop fertilizer. Although positively contributing to energy and environmental problems, this use raises health-related concerns regarding bacteria potentially resistant to AD treatment. In particular, MAP, the causative agents of Paratuberculosis (PTB), is a very resistant agent, widespread in cattle manure of affected herds.

We here report results relative to the survival of MAP in two different Italian biogas plants, both operating in mesophilic conditions (38-40°C).

The first plant was characterized by a double-stage Digester, supplied with manure from 29 bovine herds, 7 of which (24.1%) resulted infected by serological or culture tests. Samples were collected at Pre-tank (PT), Primary Digester (D1) and Secondary Digester (D2). Out of 230 samples submitted to culture, 27 were positive: 12/18 (55,5%) in PT, 14/70 (20.0%) in D1, 1/70 in D2 (1.4%), 0/36 in solid and 0/36 in liquid DG. MAP load showed a significant decrease from PT to D1 and D2; moreover it has never been detected in final DG. This might be due both to AD inactivation and to dilution effect.

The second plant was characterized by a single-stage Digester, supplied with manure from a single herd, affected by PTB (apparent prevalence: serology 14.3%; culture 11.6%). Out of 112 samples analyzed, 65 were positive to culture: 36/48 (75.0%) in PT, 26/48 (54.2%) in Digester, 1/13 in solid DG and 2/3 in liquid DG. The higher survival rate of viable MAP reported in this second study might suggest that this single-stage system is more permissive than the double-stage plant. We conclude that the use of DG as a fertilizer can increase the risk of MAP spreading on agricultural soil. To reduce this risk, we suggest, especially for single-stage digesters, the use of manure from PTB negative herds.

Research project RCIZUM 072010 funded by Italian Ministry of Health

Keywords:
MAP, Biogas plant, Anaerobic digestion

Abstract P-07.8
INVESTIGATING THE USE OF FREE-LIVING ENVIRONMENTAL AMOEBA AS A TOOL FOR DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS

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Abstract text:
Free-living amoebae are single-celled protozoans ubiquitously in terrestrial and aquatic environments. They voraciously feed on bacteria. Many bacterial pathogens have been identified which resist the phagocytic killing mechanisms utilized by amoeba to survive and often multiply, protected, within the amoebal host. Amoeba are able to form highly resistant cysts during adverse conditions which may also help to further protect internalized bacteria and facilitate the persistence, transmission and dispersal of pathogens. Many mycobacterial species, including Mycobacterium avium subsp. paratuberculsosis (MAP) have been identified as amoeba-resistant bacteria. However, much of our knowledge comes from studies utilizing highly lab-adapted strains of amoeba. Little is known about the interactions between Mycobacterium spp. and resident environmental amoeba. As a result, the role that free-living amoeba play in their persistence, transmission and dispersal is unknown. This is attributable to the inherent difficulties commonly encountered during the primary isolation of amoeba. In this study we developed methodologies for the improved isolation of free-living amoeba from dairy farm operations and fisheries. A 77% successful isolation rate was achieved using the developed methodologies. Environmental amoeba isolates were used for co-culture with 12 pathogenic and environmental Mycobacterium spp. Individual free-living amoebae, but not the lab-adapted Acathamoeba polyphaga were found to differ in their abilities to clear the Mycobacterium spp. tested. MAP but not M. a hominissuis survived repeated cycles of citation and trophozoite growth. An amoeba-based pathogen detection system has potential to be used as a surveillance tool for environmental pathogens.

Keywords:
Free-living amoeba, MAP bacteria, Amoeba cultures

Abstract P-07.9

THE MYCOBACTERIAL DISEASES OF ANIMALS (MDA) MULTISTATE INITIATIVE - A COOPERATIVE EFFORT ADDRESSING ANIMAL DISEASES

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Abstract text:
Johne's Disease Integrated Program (JDIP) efforts are well known and documented. Primary funding was through USDA grants that allowed leveraging of additional public and private resources to expand the effort. The grants have come to an end so a plan for the future was needed.

JDIP addressed many knowledge gaps, but much work remains, so a range of options for the consortium were considered. Primary objectives were to maintain the networking, collaboration and basic infrastructure developed through JDIP, allowing participants to identify, obtain and share resources needed to address Johne's and other mycobacterial diseases. A proposal was developed and later, approved by USDA's National Institute for Food and Agriculture (NIFA) to begin operation as Multistate Initiative - NE1201, "Mycobacterial Diseases of Animals (MDA)".

The multi-state initiative (MI) is focused on two mycobacterial disease complexes - paratuberculosis (Johne's disease; JD) and the tuberculosis complex of diseases (TBc; i.e bovine tuberculosis). The initiative includes five objectives:

1: Increase understanding of the epidemiology and transmission of MDA, including predictive modeling;
2: Develop and implement new generations of diagnostic tests for JD and TBc;
3: Improving our understanding of the biology and pathogenesis of MDA, as well as the host response to infection;
4: Develop programs to evaluate and develop new generations of vaccines for JD and TBc; and,

Outreach: Develop and deliver JD and TBc education and outreach material in electronic and print form for use by producers and other stakeholders. Use trade media, producer organizations and other outlets to aid in dissemination of information.

Projects within each objective, with cross-cutting contributions, are designed to address major animal, human, and societal issues surrounding detection and control of mycobacterial infection, including how these organisms move and spread within cattle, small ruminant and wildlife populations.

Keywords:
JDIP, multi-state initiative, knowledge gaps

Abstract P-07.10
CATALYZING INNOVATION AND SCIENTIFIC DISCOVERIES IN MYCOBACTERIAL DISEASES OF ANIMALS

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Abstract text:
Mycobacterial diseases such as Johne's disease (JD) and bovine tuberculosis (bTB) are extremely costly to the livestock industry in North America and throughout the world. Public and private research has worked to address these diseases, but much remains to be done. For example, the prevalence of JD remains high and new cases of bTB continue to be found. Around the world, bTB remains a problem, resulting in significant human disease. Concerns over human health links for both diseases remain, with the link between some human Crohn's disease cases and M. paratuberculosis becoming more evident, and new research suggesting a link between M. paratuberculosis and other autoimmune disorders in humans. While the impact and threat associated with these diseases remains high, federal and state funding for research, education, outreach and coordination of efforts has continued to shrink. Thus, there are growing concerns that grant funding shortfalls will hamper basic and translational research and education efforts to prevent and control JD and other mycobacterial diseases in animals. To help catalyze continued innovation, and to assist producer groups, researchers, regulators, and funding agencies by promoting scientific research, education and extension activities in developing and implementing science based solutions for the prevention and control of mycobacterial diseases, we have recently developed a new structure, the American Association of Mycobacterial Diseases (AAMD). The AAMD is organized as a not- for-profit corporation focused on promoting translational research, providing resources, and enabling networking amongst key stakeholders so as to assist producers, educators and researchers efforts to address major issues relating to mycobacterial diseases of animals. The AAMD also provides a novel platform to facilitate the continued use of resources developed through JDIP, and helps disseminate information and develop tools to address JD and other major mycobacterial diseases in the future.

Keywords:
Mycobacterial diseases, American Association of Mycobacterial Diseases (AAMD), education

Abstract P-07.11
EVALUATION OF AN ALTERNATIVE ENVIRONMENTAL SAMPLING METHOD FOR DETECTION OF MAP

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Abstract text:
Introduction: Based on 3 years of experience with environmental sampling in a management-based Johne's disease control program in Alberta, collection of 6 environmental samples can be challenging (e.g. not enough cows in sick cow pens, lagoons are frozen in winter, etc.). However, sampling socks as used for Salmonella spp. testing in poultry are potentially a cheap and simple alternative for conventional environmental samples and only require sample collection in lactating-cow pens. The objective of the present study was to compare the accuracy of sampling socks adapted for use in MAP testing and conventional environmental samples. Methods: 6 environmental samples and 2 sock samples were collected during the same visit on 90 AB dairy farms. Sock samples were composed of 12 x 12 cm swipes of commercial absorbent material attached to the bottom of single-use plastic boot covers. Sampling strategy included walking up and down alleyways once with the socks attached. Environmental samples were collected in lactating cow pens, sick cow pens and manure storage areas. All samples were sent to the University of Calgary where they were processed using a TREK ESP liquid culture protocol, with subsequent PCR confirmation. Results: 53 farms (58%) had positive environmental samples and 28 farms (31%) had positive sock samples. Whereas 15 of 16 farms with 5 or 6 positive environmental samples had at least 1 positive sock sample, only 1 of 11 farms with 1 positive environmental sample had a positive sock sample. Furthermore, 30% of environmental samples collected in lactating-cow pens and 24% of sock samples tested positive. Conclusions: The sock method is an alternative for MAP testing as it is cheap, simple and readily standardized. However, assuming nearly perfect specificity for both methods, on low-MAP prevalence farms, the sock method had lower accuracy compared to conventional environmental samples.

Keywords:
paratuberculosis, diagnostics, environmental samples

Abstract P-07.12
HISTOLOGICAL AND IMMUNOLOGICAL FEATURES OF AN INTRAVENOUS AND INTRAPERITONEAL CHALLENGE MODEL IN MICE INFECTED WITH MAP

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Abstract text:
The aim to this study was to compare the intravenous and the intraperitoneal infection route of Map on immunogenicity, bacterial burden and histopathological lesions in three susceptible mouse strains.

BALB/c, C57BL/6 and mutant C57BL/6OlaHsd-Lystbg/bg (B6bg/bg) beige mice were infected either intraperitoneally with 108 CFU or intravenously with 106 CFU of luminescent M. paratuberculosis S23. The IP dose and the MAP strain used in these experiments were based on the recommendations by Hines II et al., 2007. At day 1 and 2, 5, 10, 15, weeks after infection, the mice were killed and spleen, liver and mesenteric lymph node were individually analysed for bacterial burden using luminometry. At 5 and 15 weeks post-infection, histopathological lesions in spleen, liver and mesenteric lymph node and immune analysis (IFN-γ production) with splenocytes and mesenteric cell in response to in vitro stimulation with Map culture filtrate, PPD, Ag85A and Ag85B from MAP and with 14 MAP proteins were evaluated.

All three mouse strains showed the susceptible phenotype following intravenous and intraperitoneal infection, although bacterial replication in spleen, liver and MLN was slightly higher (but more variable) by IP than IV route. Early local immune responses were stronger in animals infected by the IP route. Histopathological examination 5 weeks post-infection revealed extra medullar haematopoiesis (EMH) both in the spleen and liver, with strongest EMH in liver of C57BL/6 and beige mice infected by the intravenous route. This EMH was still present after 15 weeks, but no prominent histopathological differences could be detected neither between the 3 mouse strains, nor between the two infection routes.

IV and IP route are two efficient ways of infection in susceptible mouse strains, but local, mesenteric immune responses are strongest following the IP route.

Keywords:
intravenous, intraperitoneal, mice

Abstract P-07.13

BIO-PRESENCE OF MYCOBACTERIUM AVIUM SUBSPECIES PARATUBERCULOSIS IN MILK AND MILK PRODUCTS IN INDIA AND ITS' PUBLIC HEALTH SIGNIFICANCE

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Abstract text:

India is the leading milk producing country in the world and milk is the staple diet of every Indian. In recent years, trend of using milk and milk products made from pasteurized milk has increased, which is a visible deviation from centuries old practice of using milk after boiling. Johne's disease caused by Mycobacterium avium subspecies paratuberculosis is endemic in Indian domestic and wild ruminant population. But country is yet to realize widespread bio-presence of live MAP bacilli both in biotic and abiotic environment and in pasteurized milk and milk products, therefore pose serious food safety concern and threat to public health as it escapes standard pasteurization conditions. After the first Indian report of live MAP from milk of infected goat in 2004, bio-presence of MAP in milk has been recorded as 7.6-69.8% in goats and 23.0-84.4% in cattle population using 'Indigenous milk-ELISA' and milk-PCR, respectively in 2007. Further 69.8 and 96.1% milk samples were positive for MAP in goats and cattle using milk-culture, respectively. Bio-presence of MAP in un-pasteurized and pasteurized milk samples was 43.7 and 72.2% using m-culture, and 6.2 and 38.8% using m-PCR, respectively in 2010. Whereas, bio-presence of MAP in milk products made from pasteurized milk was 55.5 and 22.2% using m-culture and m-PCR, respectively. Lacto-prevalence of MAP in raw milk has been reported as 88.4% using m-ELISA. Recently in 2013, MAP has also been reported from paneer made from milk of goatherds endemic for JD and 16.6% (12.5% in fat and 4.2% in sediment fractions) were positive in m-microscopy. It is therefore very important to estimate National bio-presence of MAP in milk and milk products made from pasteurized milk. For the safety of human health from contaminated milk and milk products made from pasteurized milk, it is essential to control infection of MAP in the endemically infected domestic livestock population of the country.

Keywords:

Mycobacterium avium subspecies paratuberculosis, Public health, Milk & milk products

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