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ICP 2024 INDIA
International Association for Paratuberculosis

# 16th INTERNATIONAL COLLOQUIUM ON PARATUBERCULOSIS

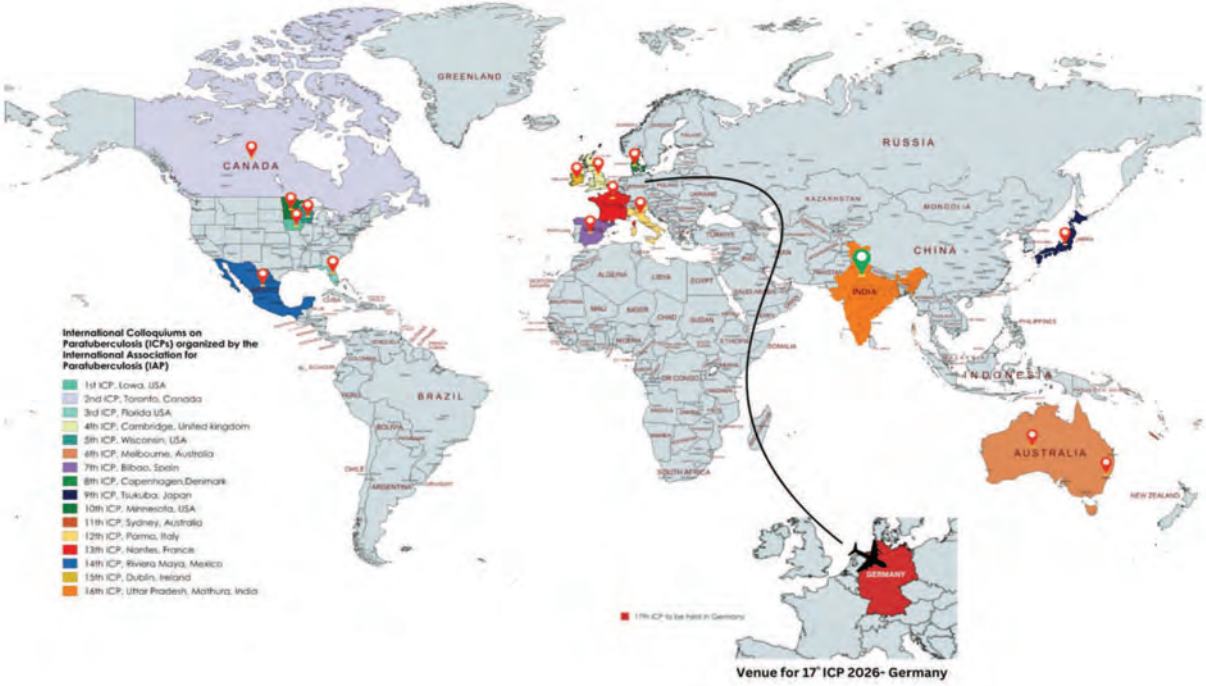
**October 21-25, 2024**

Organized by:

Department of Biotechnology
GLA University, Mathura,
Uttar Pradesh-281406-INDIA

www.gla.ac.in/icpindia

# Profile of Colloquia 1st to 16th held around the World




Venue for 17th ICP 2026- Germany

16th International Colloquium on Paratuberculosis
2024 INDIA

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# PROGRAMME

**DAY 1, Monday , 21 October**

| | |
|---|---|
| 09:30 onwards | Registration |
| 10:00 -12:30 | Opening Ceremony and Hi-tea |
| 12:30 -14:00 | Lunch |
| 14:00 -15:30 | IAP Board Meeting |
| 15:30-17:30 | Visit to Chandrodaya Temple, Gausala, Akshaya Patra |
| 18:30 - 19:30 | Cultural Program |
| 19:30 onwards | Welcome Dinner |

**DAY 2, Tuesday , 22 October**

Session 1: Epidemiology, MAP Diversity and Economic Burden

| | |
|---|---|
| 08:30 - 10:00 | Invited Talks |
| 10:00 -11:30 | Oral Presentations |
| 11:30 -11:45 | Coffee Break |

Session 2: Immunology, Immuno-genetics and Host response

| | |
|---|---|
| 11:45-13:15 | Invited Talks |
| 13:15-14:30 | Lunch and Poster Viewing |
| 14:30-16:00 | Oral Presentaions |

Session 3: Diagnostics and Omics

| | |
|---|---|
| 16:00 -17:30 | Invited Talks |
| 17:30-17:45 | Coffee Break |
| 17:45 -19:00 | Oral Presentations |

**DAY 3, Wednesday , 23 October**

Session 4: Public Health, MAP in Food, Environment and Wildlife

| | |
|---|---|
| 08:30 -10:00 | Invited Talks |
| 10:00 -11:30 | Oral Presentations |
| 11:30 -11:45 | Coffee Break |

Session 5: Management and Control Programs

| | |
|---|---|
| 11:45-13:15 | Invited Talks |
| 13:15-14:30 | Lunch and Poster Viewing |
| 14:30-16:00 | Oral Presentaions |

Session 6: Other Mycobacterial Infections of Animals and Human Beings

| | |
|---|---|
| 16:00 -17:30 | Invited Talks |
| 17:30-17:45 | Coffee Break |
| 17:45 -18:45 | Oral Presentations |

**DAY 4, Thursday , 24 October**

| | |
|---|---|
| 07:00 -08:00 | Hi-Tea cum Breakfast |
| 08:00 -13:00 | Departure and Visit to Tajmahal, Agra |
| 13:00 -14:00 | Grab and go lunch |
| 14:00 -16:00 | Visit to Sadar Baazar, Agra |
| 16:00 - 18:00 | Return Back |
| 19:30 onwards | Gala Dinner |

**DAY 5, Friday , 25 October**

Session 7: Alternative Therapeutics for Mycobacterial Infections

| | |
|---|---|
| 08:30 - 10:00 | Invited Talks |
| 10:00 -11:00 | Oral Presentations |
| 11:00 -11:15 | Coffee Break |
| 11:15-11:45 | Oral Presentations (Merkal Awardees) |
| 11:45 - 13:30 | Awards and Valedictory |
| 13.30-14:30 | Lunch and Poster Viewing |
| 14.30-15:00 | General IAP Body Meeting |
| 15:00-15:30 | Invitation to the 17th ICP in Germany |

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Dr. Manthena Navbharatha

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Dr. Saurabh Gupta
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Dr. Himanshu Gupta
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Mr. Harshit Bansal

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Chief Guest
**Prof. N. K. Ganguly**
FMR Director General,
Indian Council of Medical Research

Special Guest
**Padma Shree Dr. M. L. Madan,**
FMR DDG (AS), ICAR New Delhi
VC DUVASU, Mathura & PDAU, Akola.

Special Guest
**Dr V M Katoch**
FMR Director General, ICMR, New Delhi
FMR Director, NJIL_OMD, Agra

Guest of Honour
**Dr Nagendra Sharma**
FMR Director, ICAR_CIRG & NDRI, Karnal, &
FMR Vice Chancellor – SKUAST, Jammu

Special Guest
**Dr. William Selvamurthy**
President in Amity University,
FMR Chief Controller R&D, DRDO

Guest of Honour
**FMR Lt Gen PR Venkatesh**
Director General RVC
PVSM, SM (retd.), Indian Army

Guest of Honour
**Dr Nem Singh**
FMR Director, ICAR_IVRI Bareilly

Guest of Honour
**Dr M. C. Sharma**
FMR Director, ICAR_CIRG & IVRI &
VC Deemed to be University, Bareilly

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**Dr Lal Krishna**
FMR AH Commissioner (DADF),
& ADG (AH), ICAR, New Delhi

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**Prof. (Dr.) A. K. Srivastava**
Vice Chancellor, DUVASU, Mathura

Guest of Honour
**Dr K.M.L Pathak**
FMR DDG, ICAR (AS), FMR Director
ICAR_NRC Camel, &
FMR- VC_DUVASU, Mathura

Guest of Honour
**Dr B N tripathi**
VC, SKUAST-Jammu &
DDG (AS), ICAR, New Delhi

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**Dr. Mohammad Aslam**
FMR Senior Advisor, DBT &
Director, BIRAC, New Delhi

Guest of Honour
**Dr. Praveen Malik**
FMR Animal Husbandry Commissioner (GOI),
CEO, ICAR_Agro-Innovate, India, ltd.

Guest of Honour
**Dr A. K. Rawat**
Co- Chairman, TRPVB &
FMR Senior Advisor, DBT, New Delhi

# EMINENT SPEAKERS

**Dr. David Kelton**
Ontario Veterinary College,
University of Guelph, Guelph,
Ontario, Canada

**Dr. Herman Barkema**
Faculty of Veterinary Medicine,
University of Calgary,
Calgary, Alberta, Canada

**Dr. Matteo Ricchi**
National Reference Centre for
Paratuberculosis,
Gariga, Italy

**Dr. Vivek Kapur**
College of Agricultural Sciences,
Pennsylvania State University,
PA, USA

**Dr. Adel Talaat**
School of Veterinary Medicine,
Univeristy of Wisconsin,
Madison, USA

**Dr. Jerome DeBuck**
Faculty of Veterinary Medicine,
University of Calgary,
Calgary, Canada

**Dr. Tim Bull**
Professor of Cell and Molecular
Biology
St George's, University of London, UK

**Dr. Vineet Ahuja**
Professor, Department of
GastroEnterology
AIIMS, New Delhi

**Dr. Shoor Vir Singh**
Prof. & Head,
Dept. of Biotechnology,
GLA Univ. Mathura, India

**Dr. K. Gururaj**
Senior Scientist, ICAR- CIRG,
Mathura, India

**Dr. Sayeed Ahmad**
Professor & Head
Faculty of Pharmacy
Jamia Hamdard University,
New Delhi, India

**Dr. Amit Awasthi**
Professor, THSTI,
Faridabad, India

**Mr. Sarwar Azam**
Scientist-D DBT-NIAB
Hyderabad, India

| Session 1: Epidemiology, MAP Diversity and Economic | |
|---|---|
| **Invited Talk** | **The Epidemiology of Paratuberculosis: New Answers to Old Questions…and More Questions**<br>David Kelton |
| **Invited Talk** | **Infection, persistence and pathogenicity of Mycolicibacterium avium subspecies paratuberculosisin humans: A perspective**<br>Tim Bull |
| **Oral Presentation** | |
| **OI.01** | **Signature of Selection in *Mycobacterium avium* subsp. *paratuberculosis* Reveal Candidate Genes for Host Preferences**<br>Lamontanara Antonella, Orrù Luigi, Garbarino Chiara, Filippi Anita, Russo Simone, Ricchi Matteo |
| **OI.02** | **Prevalence of *Mycobacterium avium* subsp. *paratuberculosis* Infection in Dairy Calves in Southern Chile**<br>JM Hernández, H Barkema, P Steuer, C Tejeda, F Ulloa,M Salgado |
| **OI.03** | **Paratuberculosis in Bull Dams' Herds: Insight into Slovenian Situation**<br>Joze Staric, Urska Zajc, Tanja Knific, Jožica Ježek, Rok Marzel, Marija Klopčič, Matjaž Ocepek |
| **OI.04** | ***Mycobacterium avium* subsp. *paratuberculosis* (MAP) Infection in Mithun in North Eastern States of India: A Pilot Study**<br>Tapan Kumar Dutta, Parimal Roychoudhury, Prabhati Yadav, Ankush Dhillon,Shoor Vir Singh, Saurabh Gupta |
| **OI.05** | **Ecological Risk Mapping for the Distribution of *Mycobacterium avium subspecies paratuberculosis* Across Uganda and Sudan**<br>Julius Boniface Okuni, Kamal Ali Eltom, Elsagad Eltyayeb, Abdallah M. Samy,JudahbSekitoleko, Sanna Idris Mohamed Idris Lonzy Ojok, Uwe Truyen, Ahmed Abd El Wahed |
| **Poster Presentation** | |
| **PI.01** | **Incidence of Paratuberculosis (*Mycobacterium avium* subsp. *paratuberculosis,* Map) in Target Organs of Buffaloes in Malwa Region of Madhya Pradesh**<br>Gaya Prasad Jatav, Vishambhar Dayal Sharma, Supriya Shukla, Nidhi Shrivastava, Rashmi Choudhary, A. K. Jayraw, Mukesh Shakya, Vivek Agrawal |
| **PI.02** | **Biotyping of *Mycobacterium avium* subspecies *paratuberculosis* infecting cattle and buffalo population in Eastern Madhya Pradesh**<br>S.D. Audarya, K.K. Chaubey, S. Gupta, B. Bharti, N. Pathak, D. Chhabra, S. Matoli,A.K. Mishra and S.V. Singh |
| **PI.03** | ***Mycobacterium avium* subsp. *paratuberculosis* (MAP) Influences the Multiplication of *Staphylococcus aureus* in Milk**<br>Pedro Ferreira de Sousa Júnior,David Germano Gonçalves Schwarz, Rivanni Jeniffer Souza Castro, Francisco Alyson Silva Oliveira,Maria Aparecida Scatamburlo Moreira,Sandra Maria Ferraz, Ricardo Antônio Pilegi Sfaciotte |
| **PI.04** | **The Importance of Communal Pastures for Paratuberculosis Transmission Between Dairy Herds in Slovenia**<br>Matjaž Ocepek, Urška Zajc, Jože Starič, Jožica Ježek, Maja Kavalič, Tina Pirš,Tanja Knific |
| **PI.05** | **Caprine Paratuberculosis: Cross-Sectional Study in Italy and Evaluation of an Indirect ELISA Test's Performance in Milk**<br>Attili Anna-Rita[1*], Desirè Ombrosi[2], Toso Lucia[2], Corradini Corrado Maria[3], GigliFrancesca[1], Galosi Livio[1], NguNgwa Victor[4], Cuteri Vincenzo[1] |

| | |
|---|---|
| **PI.06** | ***Mycobacterium avium* subsp. *paratuberculosis* (MAP) Infection in Yak (*Bos grunniens)*in North Eastern states of India: A Pilot Study**<br>Tapan Kumar Dutta, Parimal Roychoudhury, Prabhati Yadav, Ankush Dhillon, Shoor Vir Singh and Saurabh Gupta |
| **PI.07** | **Paratuberculosis in Sudan: A Nationwide Study Reveals High Prevalence and Phylogenetic Relationships of *Mycobacterium avium* subsp. *Paratuberculosis***<br>Sanaa M, Idris [1,2,8], Wisal A. Elmagzoub[1,3,8], Saeed E. Enour[4], Wadeiaa N. Younis[5,6], Enas M. Abdalla[1], Darein K. Mohamed [4], Abbas M. Ahmed[6], Yousuf Abdelwahab[6], Julius B **Okuni[7], Lonzy Ojok [7], Uwe Truyen[8], Ahmed Abd El Wahed[8], ElSagad Eltayeb [9], Ahmed A. Gameel [2], Kamal H. Eltom[1*]** |
| **PI.08** | **Molecular Strain Typing of *Mycobacterium avium* subspecies *paratuberculosis* Isolates Recovered from Different Hosts**<br>Samiksha Agrawal[1], Saurabh Gupta[1*], Shoor Vir Singh[1], Ankush Dhillon and Nishant Vasdev[1] |
| **PI.09** | **Detection of *Mycobacteriumavium* subspecies *paratuberculosis* antibodies in goats of the Malwa region of Madhya Pradesh in India by using an Indigenous indirect enzyme-linked immunosorbent assay**<br>S.D. Audarya, M. Singh, R. Sharda, D. Chhabra, K.K. Chaubey, S. Gupta, R. Gangil, Kratika and S.V. Singh |
| **PI.10** | **Estimation of bio-load of *Mycobacterium avium* subspecies *paratuberculosis* in patients suffering with thyroid and arthritis disorders using multiple tests**<br>Sheetal Rajput, Saurabh Gupta, Samiksha Agrawal and Shoor Vir Singh |

| Session 2: Immunology, Immuno-genetics and Host Response | |
|---|---|
| **Invited Talk** | **Intelligent Vaccine Design: What we learned so far from the immunopathogenesis of Johne's disease?**<br>Adel M. Talaat |
| **Invited Talk** | **"Role of micronutrients in the regulation of gut inflammation"**<br>Rajdeep Dalal |
| **Oral Presentation** | |
| **OII.01** | **Changes in the Bacterial Gut Microbiome of Goats with Paratuberculosis (Paratb)**<br>P. Möbius, E. M. Liebler-Tenorio, H. Köhler, C. Pickrodt, T. M. Fuchs |
| **OII.02** | **Does MAP Infection Disturb Tissue Homeostasis of Eicosanoids?**<br>Heike Köhler, Anna Krauß, Anja Zigan, Michael Rothe, Volkmar Liebscher, Elisabeth M. Liebler-Tenorio |
| **OII.03** | **Cytokine Expression in Subjects with *Mycobacterium avium* ssp. *paratuberculosis* Positive Blood Cultures and a Meta-Analysis of Cytokine Expression in Crohn's Disease**<br>Todd Kuenstner, Qiang Xu , Tim J Bull, Antonio C G Foddai, Irene R Grant, Saleh A Naser, Raghava Potula, Peilin Zhang, Ira Shafran, Serhat Emre Akhanli, Svetlana Khaiboullina, Russell Kruzelock |
| **OII.04** | **Evaluation of *Mycobacterium avium* subsp. *paratuberculosis* Δ*Map_1152* Mutant as a Live Attenuated Vaccine in Holstein Calves**<br>M A Colombatti Olivieri[1,2*], M Hanafy[3], D K Zinniel[3], J P Bannantine[1], and R G Barletta[3] |
| **OII.05** | **Commercial Paratuberculosis Vaccine Does Not Interfere with Bovine Tuberculosis Diagnostic Tests and Lends Protection Against Lung Bacterial Load in Experimental *M. Bovis* Infections**<br>R A Juste, M G Magri-Danree, I A Sevilla, J M Garrido, E Minguijon, L de Brun, A Suanes, M Altuna, M Luzardo, V Grolero, C Gortazar, J de la Fuente, L Dominguez, P Vazquez, M V Geijo, E Molina, M Serran |
| **OII.06** | **Localized Immune Responses Induced by BacA Oral Vaccine Against *Mycobacterium avium subsp. paratuberculosis* in Calves**<br>Razieh Eshraghisaman<br>i, Antonio Facciuolo, Jeroen De Buck |
| **OII.07** | **Development of a Bovine Three-Dimensional (3D) *In Vitro* Intestinal Mucosa Model: Isolation, Culture and Characterization of Primary Bovine Intestinal Epithelial, Stromal and Immune Cells**<br>Alejandra Isabel Navarro Leon, Rosa Casais, Stefan Przyborski, Marta Muñoz |
| **OII.08** | **Infliximab (Remicade®) Increases the Acidification of *Mycobacterium paratuberculosis*- Containing Phagosomes in Macrophages**<br>Horacio Bach[1*], Cesar Monjaras-Avila[1], Ana Lorenzo-Leal[1] |
| **OII.09** | **Pathophysiologic Characteristics of CRISPRi-Generated Mutants of *Mycobacterium avium* subsp. *paratuberculosis***<br>Jun Ho Lee[1], Su Min Kyung[1], Eun-Seo Lee[1], Xi-Rui Xiang[1], Han Sang Yoo[1*] |

| Poster Presentation | |
|---|---|
| PII.01 | **Cytokeratin Expression and Distribution Pattern of Epithelioid Macrophages in Granulomatous Lesions of Animals with Different Types of Paratuberculosis-Associated Histological Lesions: Cytokeratin as a Biomarker of Resilience**<br>Natalia Iglesias[1], Alejandra Isabel Navarro León[1], Marta Muñoz[1], Cristina Blanco-Vázquez[1], Tania Iglesias[2], María Canive[3], Gerard Badia-Bringué[3], Marta Alonso-Hearn[3], Ana Balseiro[4], and Rosa Casais[1] |
| PII.02 | **Effects on the Induction or Inhibition of Autophagy in Intracellular Survival of *Mycobacterium avium* subsp. *paratuberculosis* in Bovine Macrophages**<br>M A Colombatti Olivieri[1,2*], J P Bannantine[1] |
| PII.03 | **Characterization of *Mycobacterium avium* subsp. *paratuberculosis* Δ*lprG-p55* Mutant in Bovine Macrophage and BALB-C Mice**<br>Damián Moyano, María Alejandra Colombatti Olivieri, Elena Scarel, Alonso María Natalia |
| PII.04 | **Early Growth Response Factor 4 (EGR4) Expression in Gut Tissues and Regional Lymph Nodes of Cattle with Different Types of Paratuberculosis-Associated Lesions**<br>Alejandra Isabel Navarro León, Marta Alonso-Hearn, Marta Muñoz, Natalia Iglesias, Gerard Badía-Bringué, Tania Iglesias, Ana Balseiro, Rosa Casais |
| PII.05 | **Apoptosis of Bovine Mammary Gland Epithelial Cells (MAC-T) Infected by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and Co-Infected with Bacteria that Cause Bovine Mastitis**<br>Junnia Luísa Pena, Arthur William de Lima Brasil, Leandro Licursi de Oliveira, David Germano Gonçalves Schwarz, Fernando Alberto Paolicchi,Maria Aparecida Scatamburlo Moreira |
| PII.06 | **Comparison of Immunopathological Mechanisms in the Early Stage of *Mycobacterium avium* subsp. *paratuberculosis* Infection Through the Murine Model by Different Administration Route**<br>Jun Ho Lee[1], Su Min Kyung[1], Eun-Seo Lee[1], Xi-Rui Xiang[1], Han Sang Yoo[1*] |
| PII.07 | **Cloning, Expression, Purification, and Immunological Testing of a New *Mycobacterium avium* subsp. *paratuberculosis* Antigen Encoded by the *Map2191* (MAP_RS11140) Gene**<br>Zahra Hemati[1*], Abdollah Derakhshandeh[2], ShoorVir Singh[3], Kundan Kumar Chaubey[4] |

| Session 3: Diagnostics and Omics | |
|---|---|
| **Invited Talk** | **Omics in epidemiology/diagnostic of MAP**<br>Matteo Richi |
| **Invited Talk** | **Omics: Unrevelling the Scretes of Tuberculosis**<br>Amit Kumar Pandey |
| **Oral Presentation** | |
| **OIII.01** | **Development and Validation of a Genomics Informed Real-time PCR Assay for the Detection and Strain Typing of *Mycobacterium avium* subsp. *paratuberculosis.***<br>R Hodgeman, Y Liu, S Rochfort, B Rodoni |
| **OIII.02** | **Differences in Serum Metabolic Parameters and Milk Production in *Mycobacterium avium* subsp. *paratuberculosis* Infected Goats with Different Shedding Levels and Non-Suspect Goats**<br>C. Pickrodt, H. Köhler, T. Gärtner, U. Moog, E. Gernand, K. Donat |
| **OIII.03** | **Bayesian Accuracy Estimates of Commercial Antibody-ELISA and qPCR to Detect *Mycobacterium avium* subsp. *paratuberculosis* Infected Cows in Herds with Historical MAP Infection Status**<br>W L N Nguekap, B Nathalie, S Dufour, O Séverine, J P Roy, G Fecteau, Dave Kelton, J C Arango-Sabogal |
| **OIII.04** | **Human Antibodies Against *Mycobacterium avium* ssp. *paratuberculosis* for the Selection of Patients with Crohn's Disease for Anti-Mycobacterial Therapy**<br>J Todd Kuenstner*, Jean-Michel Galarneau, Horacio Bach, Peilin Zhang, Raghava Potula |
| **OIII.05** | ***Mycobacterium avium* subsp. *paratuberculosis*: composition of the bacterial microbiota in blood samples from patients in the groups Crohn's disease and patients with ulcerative colitis**<br>Richard Costa Polveiro, David Germano Gonçalves Schwarz; Isis de Freitas Espeschit Braga, Maria Aparecida Scatamburlo Moreira1. |
| **OIII.06** | **Stimulation of Bovine Ileal Organoids by Bovine RANK-L and the Uptake of *Mycobacterium avium* subspecies *paratuberculosis* into Intestinal Epithelial Cells**<br>Rachel Richardson, Ambre Chapuis, David Smith, Jo Moore, Craig Watkins |
| **OIII.07** | **A microRNA-based Johne's disease diagnostic predictive system: preliminary results**<br>Fabio Albanese, Paul Capewell, Arianne Lowe, Spiridoula Athanasiadou, David Wilson, Eve Hanks, Robert Coultous, Michael Hutchings and Javier Palarea-Albaladejo |
| **OIII.08** | **Identification of Novel Bovine Biomarkers Associated with Paratuberculosis Tolerance**<br>Alejandra Isabel Navarro Leon, Susana Belen Bravo Lopez, Marta Alonso-Hearn, Natalia Iglesias, Gerard Badía-Bringué, Ana Balseiro, Rosa Casais |
| **Poster Presentation** | |
| **PIII.01** | **Fatty Acids that Distinguish C-type and S-type Strains of *Mycobacterium avium* subspecies *paratuberculosis***<br>M A Colombatti Olivieri, S C Duffy, M A Behr, N P J Price, J P Bannantine, F Biet |

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| **PIII.02** | **microRNA Biomarkers for Improved Detection of Infectious Diseases**<br>Ryan Farr, Carlos Miranda Rodrigues, Annaleise Wilson, Christina Rootes, Jenny Su, Christopher Cowled, Nagendra Singanallur, Marina Alexander, Cameron Stewart* |
| **PIII.03** | **Digital PCR (dPCR) to Quantify the Load of *Mycobacterium avium* supsp. *paratuberculosis* (MAP) Present in Feces as a "Tool" to Define Priorities of Interventions in an Infected Cattle Herd**<br>Garbarino Chiara[1*], Filippi Anita[1], Ventura Giordano[2], Boldini Massimo[2], Fabio Ostanello[3], Alice Giuliani[4], Russo Simone[1], Ricchi Matteo[1] |
| **PIII.04** | **Detection of Mycobacterium avium subsp. paratuberculosis in fecal samples by culture in dairy farms in Panama**<br>Dayan Palacio, Roselin Chérigo, Daniela Candanedo, Sara Miranda, Priya Patel1, Anel Pérez, Elvin Cano, Mitchelle Morán, Dilcia Sambrano, Venancio Polanco, Edith Maldonado, Gilberto Chávez-Gris, Fermín Acosta, Richard Whittington, Amador Goodridge |
| **PIII.05** | **miRNA in stool as Paratuberculosis prognostic biomarkers in beef cattle: extraction methods comparison**<br>Torricelli M., Sebastiani C., Fratto A., Madeo L., Petrucci L., Ciullo M., Biagetti M., Ricchi M., Garbarino CA., Mazzone P. |
| **PIII.06** | **Electrochemical Biosensors for *Mycobacterium avium* subsp. *paratuberculosis*: Diagnostic Hurdles and Emerging Solutions**<br>Ankush Dhillon, Shoor Vir Singh, Jagdip Singh Sohal |
| **PIII.07** | **Duplex PCR for Zoonotic Threats: Validation and Detection of *Mycobacterium paratuberculosis* and *Mycobacterium bovis***<br>Saurabh Gupta, Swadha Pandey, Samiksha Agarwal, Shivani Mahor, Tamanash Mondal, Ankush Dhillon, Anukool Vaishnav, Jagdip Singh Sohal and Shoor Vir Singh |
| **PIII.08** | **The State and Diagnosis of Animal Johne's Disease**<br>Zahra Hemati[1,*] Zohre Hemati[2], Reza Ameri[1], Shoor Vir Singh[3], Azadeh Salimi[2] |

| Session 4: Public Health, MAP in Food, Environment and Wildlife | |
|---|---|
| **Invited Talk** | ***Mycobacterium avium* subspecies *paratuberculosis* – an important food borne pathogen of high public health significance with special reference to India**<br>Shoor Vir Singh |
| **Invited Talk** | **"Public Health, MAP in Food, Environment and Wildlife"**<br>Shubham Prasad |
| **Oral Presentation** | |
| **OIV.01:** | **Network inference of gut microbial communities in a multiple sclerosis cohort with Mycobacterium avium subspecies paratuberculosis infection**<br>Hajra Ashraf, Umer Zeeshan Ijaz, Elena Simula and Leonardo A. Sechi |
| **OIV.02** | **Empowering Learning Through Student-Created Study Materials: Enhancing University Students' Understanding of *Mycobacterium avium* subsp. *paratuberculosis* Infections**<br>Jože Starič |
| **Poster Presentation** | |
| **PIV.01** | **Surveillance of Paratuberculosis in Alpine Red Deer (*Cervus Elaphus*) in Northern Italy**<br>C Garbarino, M Nava, A Bianchi, L Corlatti, L Gugiatti, L Pedrotti, M Ricchi, I Bertoletti, C Luzzago, C Filippi |
| **PIV.02** | **The Effect of Environmental Conditions, Nematodes, and Gut Microbiome Species- Composition, on the Transmission of *Mycobacterium avium* subspecies *paratuberculosis*(MAP) Infections in Sheep (*Ovis aries*)**<br>Charlotte Winspear, Dave Bartley, Lisa Avery, Andrew Free, Craig Watkins, Eulyn Pagaling |
| **PIV.03** | **Screening of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in a Highly Threatened Species of *Camelus bactrianus* (Double Humped Camel) in the Temperate Nubra Valley of the Himalayas**<br>Shoor Vir Singh[1*], Prabhati Yadav[1], Ankush Dhillon[1], Saurabh Gupta[1], Mohd Altaf Bhat[2*], Zahid Amin Kashoo[2], Shaheen Farooq[2], Abdul Qayoom Mir[2], R K Sawal[3], A Sahoo[3], Rakesh Ranjan[3*] |
| **PIV.04** | **In Vitro Study of Interactions between *Mycobacterium avium paratuberculosis* and HERV-W derived-peptides with Human Pancreatic Islets: Implications for Type 1 Diabetes Pathogenesis.**<br>*Marta Noli, April Joy Vergara, Alishba Fayyaz, Elena Rita Simula, Flemming Pociot, Reza Yarani, Leonardo Antonio Sechi* |
| **PIV.05** | **Molecular Epidemiology of *Mycobacterium avium* subspecies *paratuberculosis* isolated from Captive Wild Animals**<br>Swarup Sadashiv Lingayat, Tawheed Ahmad Shafi, Meera Pundlikrao Sakhare, Abdul Mujeeb Syed, Prashant Ramchandra Suryawanshi and Shoor Vir Singh |
| **PIV.06** | ***Mycobacterium avium* subspecies *paratuberculosis* biotyping by using milk samples from infected large ruminants of Madhya Pradesh**<br>S.D. Audarya, K.K. Chaubey, S. Gupta, B. Bharti, N. Pathak, D. Chhabra, S. Matoli, A.K. Mishra and S.V. Singh |

| Session 5: Management and Control Programs | |
|---|---|
| **Invited Talk** | **Improved control of Johne's disease in dairy cattle through advancements in diagnostics, testing and management of young stock**<br>Larissa Martins[1], Karin Orsel[1], Razieh Eshraghisamani[1], Jose Miguel Hernández-Agudelo[2-3], A. Caroline Pereira[1], Waseem Shaukat[1], Ad P. Koets[4], John P. Bannantine[5], Caroline Ritter[6], David F. Kelton[7], Richard J. Whittington[8], Maarten F. Weber[9], Antonio Facciuolo[10-11], Navneet K. Dhand[8], Karsten Donat[12], Susanne Eisenberg[13], Miguel A. Salgado[2], John P. Kastelic[1], Jeroen De Buck[1], and **Herman W. Barkema[1]** |
| **Oral Presentation** | |
| **OV.01** | **CONTROL OF JOHNE`S DISEASE: A CALL FOR SCIENTIFIC RENEWAL**<br>Benedictus,G, Hesselink,J.W. & Vellema,P. |
| **OV.02** | **The UK National Johne's Tracker Database 2010 to 2023.**<br>James Hanks, Pete G. Orpin, Nicholas M. Taylor[1], Emma N. Taylor |
| **OV.03** | **Phase 3 of the UK National Johne's Management Plan (NJMP)**<br>Pete G. Orpin, James Hanks, Nicholas M. Taylor, Emma N. Taylor |
| **OV.04** | **Successful control of paratuberculosis in a dairy herd with high initial prevalence – a case study**<br>K. Donat, E. Einax, D. Rath, A. Klassen |
| **OV.05** | **Immunoinformatics Approaches for Designing Paratuberculosis Antigen** Vaccine Candidates<br>Maryam Sadat Moezzi[1,*], Abdollah Derakhshandeh[1], Farhid Hemmatzadeh[2] |
| **OV.06** | **The management and control of Johnes disease in 12 commercial dairy herds using regular milk testing for antibodies to Mycobacterium avium subspecies paratuberculosis (MAP) and targeted risk management.**<br>R. J. Sibley |
| **OV.07** | **Effect of Manure Processing Method on Presence of *M. avium* subsp. *Paratuberculosis* in Recyc led Manure Solids Bedding on Mid west US Dairy Farms**<br>Felipe PeñaMosca; SandraGodden; ErinRoyster; Douglas Albrecht, ScottWells ; Brian A.Crooker[2]; NicoleAulik |
| **OV.08** | **A novel Vaccine Candidate for the Control of MAP Infection in Small Ruminants**<br>Abdollah Derakhshandeh and Shoor Vir Singh |
| **OV.09** | **Paratuberculosis case definition by the WOAH**<br>Juste RA, Whittington R, Thibault-Poisson VC, Daptardar M, Garrido JM, Sevilla I, Elguezabal N, Alonso M, Chng C, Torres G |
| **OV.10** | **Rise and decline of Johne's disease Market Assurance Programs (MAPs) in Australia**<br>Lawrence C Gavey, Lorna Citer and Rob Barwell |
| **PV.01** | **Using InterHerd+ to record Johne's Disease data in a multi-herd database.**<br>James Hanks, Nicholas M. Taylor, Emma N. Taylor |
| **PV.02** | **The probability of freedom from the disease as a measure for assessing and managing the risk of MAP introduction into cattle herds**<br>Anne Klassen, Annika Wichert, Elisa Kasbohm, Karsten Donat |
| **PV.03** | **Age of first time shedding *Mycobacterium avium* subsp. *paratuberculosis* and seropositivity in young stock on MAP-infected Alberta dairy farms**<br>A. Caroline Pereira, Karin Orsel, Jeroen De Buck, Larissa Martins, Marit M. Biesheuvel, and Herman W. Barkema |

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| **PV.04** | **The control of clinical and subclinical Johnes Disease in a large commercial dairy herd by targeted risk management**<br>Richard Sibley |
| **PV.05** | **Developing a Successful Johne's Disease Control Program within a Veterinary Practice**<br>Pete G Orpin, Dick Sibley, James Hanks |
| **PV.06** | **Longitudinal Study on *Mycobacterium avium* subspecies *paratuberculosis* Infection in Goats**<br>Attili Anna-Rita, Toso Lucia, Desirè Ombrosi, Corradini Corrado Maria, Gigli Francesca, Galosi Livio, NguNgwa Victor, Cuteri Vincenzo |
| **PV.07** | **Paratuberculosis in *Camelus dromedarius* (single-humped camel) in India**<br>Shoor Vir Singh, Ankush Dhillon, Prabhati Yadav, Saurabh Gupta, Rakesh Ranjan, R K Sawal, Meet Pal, Kashi Nath and Amita Ranjan |
| **PV.08** | **Out: a comparative study of *Mycobacterium avium paratuberculosis* in Sheep (*Ovis aries*) across Scottish farmland.**<br>Charlotte Winspear, Lisa Avery, Dave Bartley, Andrew Free, Craig Watkins, and Eulyn Pagaling |
| **PV.09** | **Impact of COVID-19 pandemic on a livestock farmer and management of goats infected by *Mycobacteriumavium* subspecies *paratuberculosis***<br>S.D. Audarya, R. Sharda, R. Gangil, D. Chhabra, K.K. Chaubey , S. Gupta and S.V. Singh |
| **PV.10** | **Microscopical examination of fecal samples of cattle at Rewa in Madhya Pradesh detected mixed infection of *Mycobacterium avium* subspecies *paratuberculosis* and *Cryptosporidium* species**<br>S.D. Audarya, K.K. Chaubey, A.K. Pal, S. Sharma, Y. Chatur, G.K. Mishra and S.R. Upadhyay |

| Session 6: Other Mycobacterial Infections of Animals and Human Beings | |
|---|---|
| **Invited Talk** | **"Other Mycobacterial Infections of Animals and Human Beings"**<br>Ajay Vir Singh |
| **Oral Presentation** | |
| **OVI.01** | **Isolation and identification of IS*900*qPCR positive Mycolicibacterium species from human blood and ascites that are not MAP.**<br>Tim J Bull, Ken Haines, Bina Makwana, Metin Yalcin, Dan Forton, Adam Witney. |
| **OVI.02** | **Enhancing Paratuberculosis Diagnosis: Novel Universal Reference Material and Direct Digital PCR Method for Precision Livestock Management**<br>Debelhoir Valentin[1*], Berthet Amandine[1], Barbier Sandrine[2], Caquineau Laurent[2], Legall Ghislaine[2], Klubkova Valeriia[1], Sellal Eric[1] |
| **OVI.03** | **Mass spectrometric analysis of lipids from *Mycobacteriumavium* subsp. paratuberculosis**<br>Soundarya Udaiyar, Sajan George, Shoor Vir Singh, Sabareesh V1 |
| **OVI.04** | **Investigation of endemic *Mycobacterium bovis* infection in a small beef breeding herd using a comprehensive testing programme**<br>R. J. Sibley |
| **OVI 05** | **Chronic Diseases control under AMR strategy**<br>Ashwini Kumar Singh, Nihar N. Mohanty, Vikas K Gupta, Alok Kumar Yadav, Himanshu Sharma |
| **Poster Presentation** | |
| **PVI.01** | **Detection of *Mycobacterium avium* subsp. *paratuberculosis (MAP)* in slaughtered goats using histopathological and molecular approaches**<br>Fatemeh Namazi* and Elmiraz Khatamsaz |
| **PVI.02** | **Assessment the bio-load of *Mycobacterium paratuberculosis* and genotype frequencies of A1 and A2 β-casein in raw bovine milk samples from Braj region using molecular assays**<br>Deeksha Sharma, Samiksha Agarwal, Rasanpreet Kaur, Saurabh Gupta* and Shoor Vir Singh |
| **PVI.03** | **Co-Infection with *Mycobacterium tuberculosis* in Patients Positive for *Salmonella*: A Clinical study in Northeast India.**<br>Jayanta Deb[1], Saurabh Gupta[2], Tapan Majumdar[1] |

| Session 7: Alternative Therapeutics for Mycobacterial Infections | |
|---|---|
| **Invited Talk** | **Alternative therapies for mycobacterial diseases, with focus on prophylactic phage therapy for Johne's disease**<br>Jeroen De Buck |
| **Oral Presentation** | |
| **OVII.01** | **Tolerance of MAP strains to copper stress suggest more virulence?: a preliminary experimental study**<br>Salgado, M, Tejeda C, Steuer, P, Barkema, HW, Hernández, JM, Ulloa, F |
| **OVII.02** | **Deciphering the Potential of Synergistic Herbal Formulation from *Asparagus racemosus* and *Sapindus mukorossi* for Veterinary Vaccines**<br>Saurabh Gupta, Rasanpreet Kaur, Vidhi Mishra, Ravi Tiwari, Shoor Vir Singh, Jagdip Singh Sohal |
| **OVII.03** | **Evaluation of the Phytotherapeutic Potential of *Moringa oleifera* and *Chenopodium album* extracts Against *Mycobacterium avium* subsp. *paratuberculosis* Infections.**<br>Shoor Vir Singh, Vijay Kumar Dubey, Saurabh Gupta, Ravindra Kumar, Ankush Dhillon, Manthena Navabharatha, Deendayal, Harshit Bansal, Shivani Mahor |
| **Poster Presentation** | |
| **PVII.01** | **Inhibitory effects of common detergents on *Mycobacterium avium* subsp. *paratuberculosis***<br>Soundarya Udaiyar, Sabareesh V, Shoor Vir Singh, Sajan George |
| **PVII.02** | **A synthetic tunicamycin derivative evaluated for treatment of Johne's disease in Holstein cows**<br>Maria A. Colombatti Olivieri; Neil P. J. Price; Michael A. Jackson; John P. Bannantine |
| **PVII.03** | **Response of *Mycobacterium avium* subsp. *paratuberculosis* isolates to copper ion stress**<br>Salgado M[1], Steuer P[1], Barkema HW[2], Tejeda C[1], Hernández JM[1,3], Ulloa F[1,3] |

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**Invited Talk**

## The Epidemiology of Paratuberculosis: New Answers to Old Questions…and More Questions

**Professor David Kelton**

*Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada, N1G 2W1*

From an epidemiological perspective Johne's Disease control is based on understanding and manipulating 3 categories of risk factors. These risk factors are attributes of the host, the agent and the environment. While our understanding of each of these groups of risk factors is changing, if not necessarily improving, our efforts to control JD has been slow at best, and largely unsuccessful in many instances. This presentation will delve into what we know, and don't know, about characteristics of the host, the agent and the environment, including the roles of genetic selection, test application and interpretation, newborn calf management and dairy farm biosecurity in JD control.

**Invited Talk**

## Infection, persistence and pathogenicity of *Mycolicibacterium avium* subspecies *paratuberculosis* in humans: A perspective

**Professor Tim Bull**

*Institute of Infection and Immunity, St George's University of London, Cranmer Terrace, London*

The widespread presence of viable MAP in the environment, potable water and food such as milk indicates that human exposure to MAP is inevitable and most probably semi-continuous depending on lifestyle. MAP, like other pathogenic mycobacteria, can enter and passage through mucosal epithelia to infect human cells including macrophages and innate cell populations. Unlike other mycobacteria however, viable MAP remains persistent within white cell fractions from a significant proportion of humans. As with Mycobacterium tuberculosis (already latent within one third of the world), initial intracellular establishment can drive MAP into a viable non-culturable (VNC) phenotype. To facilitate chronic persistence, intracellular MAP has capacity to interfere with the normal host processing of the invading organism and dysregulate host immune reactivities in both cellular and humoral pathways. MAP colonisation of humans however fails to develop from this latent phase into acute destructive immunological manifestations and high transmissible bacterial loads in (respiratory) mucosal tissue as seen with MTB reactivation (Tuberculosis). Neither does it evoke, as in animals, major shifts in immunological reactivity and high transmissible bacterial loads in (gut) mucosal tissue (Johne's Disease). In most humans therefore MAP infection is tolerated and quiescent. The organism is contained as a VNC at low loads but fails to realise immunological reactivity that could result in clinical consequences. Thus, MAP encounters although regular are never overwhelming, are tolerated and are likely cleared from time to time through normal host cell turnover. The presence of host genetic traits inferring aberrant intracellular antigen processing or irregular immunological reactivities can potentially break host tolerance. Traits already identified in animals, favour chronic MAP persistence and increase the propensity for MAP infected cells to trigger dysregulated immune disease states. GWAS studies in humans with chronic inflammatory conditions, link mutations in similar host genes, or their epigenetic ncRNA controllers, found in animals implicating MAP involvement in human disease. However as with all chronic immune-related conditions the network of response is complex. No one trait is universally found in IBD patients. Traits subgroup to either a raised susceptibility to disease initiation, specific tissue site involvement or severity (including possible resistance) reflecting perhaps the wide spectrum of disease manifestations and prognoses that can result from a long-term underlying condition. This discourse examines the latest attempts to determine if the presence of a ubiquitous pathogen like MAP in patients with disease is causative, contributory, consequential or only a function of local variance in natural exposure/carriage. It will outline the unique and potentially immunomodulatory characteristics of the VNC MAP phenotype developed once established in human cells and discuss how these are hindering effective study. Lastly it will describe outcomes of interventions with mycobacterial targeting antibiotics and a trial of an anti-MAP therapeutic vaccine in Crohn's Disease patients that may be finally revealing the true subversive nature of paucimicrobial intracellular persistence of MAP in susceptible humans.

# OI.01: Signature of Selection in *Mycobacterium avium* subsp. *paratuberculosis* Reveal Candidate Genes for Host Preferences

**Lamontanara Antonella[1], Orrù Luigi[1], Garbarino Chiara[2], Filippi Anita[2], Russo Simone[2], Ricchi Matteo[2*]**

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[2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Territorial unit of Piacenza, National reference centre for paratuberculosis and WOAH Reference Laboratory for Paratuberculosis, Podenzano PC, Italy*

*Corresponding Author

**Abstract**

The *Mycobacterium avium* subsp. *paratuberculosis* (MAP) can be further typed in two major clades, one including Type I and Type III sub-lineages and circulating mainly in sheep and another one, defined as Type II, which is mainly isolated from cattle and other animals. The mechanisms of host invasion and colonization by MAP have been partially characterized and little is known about the genomics differences between these two groups that may explain their mechanisms of host preference. In this study, the genomes of 21 MAP isolated from goat were sequenced by WGS approach and analyzed together with the genome sequences of 474 MAP obtained from NCBI database. Orthologs genes were identified using Orthofinder, while MAP types were determined characterizing the gyrA and gyrB mutations. To identify the genes that may have relevance to understand the differences between the Type I and Type II and their interaction with the different hosts, we measured the evolutionary pressures on protein-coding genes known to have a role in host colonization. To this aim, the Ka/Ks ratio of the virulence genes between lineages were calculated to detect evidence of positive selection that could have led to pathoadaptive mutations affecting host preference. Using this approach, we identified several virulence genes showing signatures of adaptive evolution between lineages. The identified genes may provide information toward understanding the differences in MAP host preference.

## OI.02: Prevalence of *Mycobacterium avium* subsp. *paratuberculosis* Infection in Dairy Calves in Southern Chile

**J M Hernández[1,2], H Barkema[3], P Steuer[1], C Tejeda[1], F Ulloa[1,2], M Salgado[1*]**

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[3]*Department of Production Animal Health, University of Calgary. Calgary, Canada*

*Corresponding Author

**Abstract**

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection poses a significant economic burden on the dairy industry, and its potential prevalence in southern Chile raises concerns due to the region's substantial contribution to national milk production.

The need to identify all sources and transmission routes of MAP infection for effective control programs has become increasingly evident in recent years. This includes understanding the role of calves as potential sources and transmitters of infection to others within shared facilities. The lack of knowledge regarding MAP transmission in calves hinders the development of efficient control strategies.

This study aimed to determine the prevalence of MAP infection in dairy calves in southern Chile. A multistage, cross-sectional study design was employed. Thirty-nine dairy herds from Los Ríos and Los Lagos regions (representing 82% of national milk production) were conveniently selected based on a list provided by the Chilean Dairy Consortium. Herds were categorized by annual milk production volume (small, medium, large). Subsequently, 10% of calves within the age criteria were randomly selected from each herd. Sample size was calculated considering a 5% margin of error, 95% confidence level, and an expected prevalence of 50%. Between July and September 2023, a single visit was conducted to each farm, where 5-10 grams of fecal samples were collected directly from the rectum of each calf using disposable sleeves. Phagomagnetic separation (PhMS) followed by qPCR targeting the IS900 insertion sequence was utilized to detect MAP.

Analysis of fecal samples revealed that 6.14% (35/570) of the calves tested positive for MAP infection. Furthermore, 41% (16/39) of the dairy farms had at least one positive calf.

These findings highlight the presence of MAP infection in dairy calves in southern Chile, and emphasize the need for further research to understand transmission dynamics and develop effective control strategies.

## OI.03: Paratuberculosis in Bull Dams' Herds: Insight into Slovenian Situation

**Jože Starič[1*], Urška Zajc[1], Tanja Knific[1], Jožica Ježek[1], Rok Marzel[1], Marija Klopčič[2], Matjaž Ocepek[1]**

[1]*University of Ljubljana, Veterinary faculty,*

[2]*University of Ljubljana, Biotechnical faculty, Department of animal science*

*Corresponding Author

**Abstract**

Paratuberculosis caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is a chronic granulomatous enteritis that affects cattle worldwide and negatively impacts the sustainability of cattle production systems. The disease is endemic in Slovenia and there is no control program for it. Epidemiological studies have shown that paratuberculosis poses a considerable threat to bull dams' herds, mainly due to its insidious nature and long subclinical phase. Bulls can act as potential carriers and transmit the infection within and between herds. Accurate and timely diagnosis is crucial for effective control of the disease. However, MAP infection diagnosis is challenging as available diagnostic tests have limitations. The Slovenian situation regarding paratuberculosis in Holstein Friesian bull dams' herds is presented.The study involved 84 Holstein Friesian (HF) bull dams' herds (out of total 88 HF bull dams' herds in Slovenia) where the environmental fecal samples from two or three locations most used by the animals (near the drinking trough, at the end of the scraping area, in the waiting area of the milking parlour or the calving area) were collected. Samples (the size of a walnut) were taken manually with a rectal sleeve from the corners and edges where more mixed feces accumulated. Samples were analysed by PCR (gene F57). Six (7.14%) herds had at least one MAP-positive sample in two consecutive years. In five positive herds, we sampled the manure of all animals aged two years and older individually. The average prevalence of MAP-positive animals in individually sampled herds was 13.6%. The situation with MAP in Slovenian Holstein Friesian bull dams' herds seems relatively favourable. Preventive measures such as education of farmers, biosecurity protocols, herd testing, and culling infected animals play a crucial role in disease mitigation. Integrating advanced technologies such as whole genome sequencing and mathematical modeling can improve our understanding of paratuberculosis dynamics in bull dams' herds and optimize control strategies.

# OI.04: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) Infection in Mithun in North Eastern States of India: A Pilot Study

**Tapan Kumar Dutta[1*], Parimal Roychoudhury[1], Prabhati Yadav[2], Ankush Dhillon[2], Shoor Vir Singh[2], Saurabh Gupta[2]**

[1]*Department of Veterinary Microbiology, CVSc & AH, Central Agricultural University, Selesih, Aizawl, Mizoram*

[2]*Department of Biotechnology, GLA University, Mathura, UP*

*Corresponding Author

**Abstract**

Mithun (*Bos frontalis*) is a semi-domestic unique bovine species in Southeast Asia including India, Bangladesh, Myanmar, and Bhutan. In India, Mithun is available in the North eastern states including Arunachal Pradesh, Manipur, Mizoram and Nagaland. Mithun used to extend their habitat from low to high altitude areas and prefers a cool climate with temperatures ranging from 20°C to 30°C. Along with the source of milk, meat, and skin mithun is also a symbolic representative of peace and communal harmony in this region. The icon animal used to suffer from various infectious diseases, which poses a great threat to the population. Among them, Johne's disease (JD) is one of the major concerns. In the present study, a total of 18 fecal samples were collected mithun of Lachung, Sikkim. During collection, all the animals were found to apparently healthy without any significant clinical manifestation. All the samples were processed for detection of mycobacteria by microscopy, polymerase chain reaction and isolation and identification of the organism. A total of 44.4%, 16.6%, 33.3%, and 38.8% of the samples were found be positive for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) by acid-fast staining, *IS900* PCR assay, Taqman probe qPCR assay, and culture study, respectively. The affected animals might be acting as asymptomatic carrier and may pose a serious threat for the other animals in the herd as well as the entire population of the region. This is the first pilot study of MAP infection in mithun in Sikkim and large-scale screening of the mithun population in this region is essential to protect the species.

## OI.05: Ecological Risk Mapping for the Distribution of *Mycobacterium avium subspecies paratuberculosis* Across Uganda and Sudan

**Julius Boniface Okuni[1*], Kamal Ali Eltom[2], Elsagad Eltyayeb[3], Abdallah M. Samy[4,5], Judahb Sekitoleko[1,6], Sanna Idris Mohamed Idris[2,7] Lonzy Ojok[1,8], Uwe Truyen[9], Ahmed Abd El Wahed[9]**

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**Abstract**

The aim of this study is to provide a scientific basis for prioritization of future areas of surveillance of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in Uganda and Sudan. MAP infection in cattle has recently gained attention in two counties in the last decade since the publications of initial survey reports on the occurrence of MAP in livestock in both countries. However, the surveys have not covered the entire geographical areas of the two countries. We are constructing ecological niche models based on maximum entropy algorithm using Kuenm package in R program, based on data from the recent studies in Uganda and Sudan and the most recent epidemiological knowledge on MAP to show hotspots and areas with different likelihoods for MAP occurrence in livestock in both countries. Occurrence of MAP at different areas are modeled against environmental and social economic factors to create the risk map. The study presents and describes risk maps for the occurrence of MAP infection in cattle and other livestock in Uganda and Sudan.

These maps will not only guide the surveillance but the practicing veterinarians, farmers and policy makers on issues of MAP diagnosis, prevention and control.

# PI.01: Incidence of Paratuberculosis (*Mycobacterium avium* subsp. *paratuberculosis,* Map) in Target Organs of Buffaloes in Malwa Region of Madhya Pradesh

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## Abstract

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) causes chronic persistent diarrhoea in ruminants by infecting the small and large intestine, and is known as Johne's disease (JD). In this study, 100 (35 females and 65 males), non-productive buffaloes were slaughtered at Cantonment Board slaughterhouse, Mhow. Tissues collected from target organs were for screened for MAP infection. Age of buffaloes was between 1 - 12 years. Incidence of JD was 41.0% (41/100), based on faecal smear examination and 37% (37/100) on gross lesions and impression smear examination of target organs; intestines and mesenteric lymph nodes (MLN). Intestinal wall was thickened (variable degree), with congestion in various regions. Intestinal mucosa was also thickened in mucosal folds in the form of transverse corrugations, congesion and haemorrhage in various regions of the intestine. Mucosa at ileocaecal junction was also thickened, congested, edematous, and had transverse corrugations. MLN were swollen, enlarged and congested. Histopathologically, formation of initial granuloma, giant cells were seen in target organs. Serological screening by 'indigenous ELISA kit', revealed 60.9% (25/41) positive buffaloes. Molecular examination of 41 tissues (intestine or MLN), by *IS*900 PCR revealed, 19 (46.3%) tissues positive for MAP infection.

# PI.02: Biotyping of *Mycobacterium avium* subspecies *paratuberculosis* infecting cattle and buffalo population in Eastern Madhya Pradesh

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## ABSTRACT

Paratuberculosis or Johne's disease in large ruminants is caused by *Mycobacterium avium* subspecies *paratuberculosis*. Disease in large ruminants is characterized by chronic diarrhea, weakness and emaciation. Apart from the production losses, the presence of disease in animals and its unresponsiveness to common drugs significantly impact the economic condition of livestock owners. There are few reports on disease prevalence in the large ruminant population of Eastern Madhya Pradesh. However, the disease has been reported from the other parts of this central state of India. Fecal samples from the cattle and buffalo population of this area were investigated for the presence of *Mycobacterium avium* subspecies *paratuberculosis*(MAP). Microscopic examinations revealed the presence of acid-fast bacilli indicating possible involvement of MAP bacilli. Infection in the cattle and buffaloes was confirmed at molecular level by specific amplification of 413 bp product in the IS*900*PCR. MAP specific amplification of 608 bp in the IS*1311* product followed by restriction endonuclease analysis revealed the infection of 'Indian Bison Type', biotype.

# PI.03: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) Influences the Multiplication of *Staphylococcus aureus* in Milk

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**Abstract**

*Staphylococcus aureus* and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) can infect the mammary gland with distinct inflammatory potential in cows. Understanding these co-infective processes allows for the improvement of bovine mastitis prevention and control protocols. The objective of the present study was to evaluate whether the presence of MAP in bovine milk would be capable of altering the multiplication of *S. aureus* in vitro. A standard strain of MAP K-10 at $10^6$ CFU/mL and an isolate of *S. aureus* at 1.5 x $10^8$ CFU/mL were used. *S. aureus* and MAP cultures were centrifuged at 12,000 x g for 5 min to obtain the pellet. After discarding the supernatant, the *S. aureus* and MAP pellets were resuspended in 15 mL and 10 mL of pasteurized whole bovine milk (Piracanjuba®), respectively. Then, isolates were combined in tubes: i) milk + *S. aureus*; ii) milk + *S. aureus* + MAP; and iii) bacteria-free milk. Incubation was carried out at 37°C for 2h, 4h, 6h, 8h, 10h, 12h, and 24h, and plate counts for *S. aureus* were performed. It was verified that after 12 h of incubation, there was an increase in the concentration of colonies in the solution with MAP (4.7 x $10^7$ CFU/mL) compared to the solution without MAP (3.1 x $10^7$ CFU/mL). Similarly, it was found that at 24h of incubation, 5.1 x $10^8$ CFU/mL was found in the solution with MAP and 3.3 x $10^8$ CFU/mL in the solution without MAP. These findings demonstrate that MAP can interfere with the multiplication of *S. aureus* in milk, increasing the multiplication rate. In practical terms, the preliminary findings could indicate a greater speed of multiplication of mastitis agents when MAP is eliminated by the mammary gland.

# PI.04: The Importance of Communal Pastures for Paratuberculosis Transmission Between Dairy Herds in Slovenia

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## Abstract

The aim of this study was to investigate the role of communal pastures in the transmission of paratuberculosis caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in dairy herds in Slovenia, where the disease is endemic. Movement restriction is a common control measure, as animal movements contribute significantly to MAP transmission between herds. Slovenia's cattle movement network exhibits distinctive seasonal pattern due to grazing, making communal pastures potential transmission routes between herds, although this has not yet been thoroughly investigated.

Cattle movement data from 2011 to 2021 were analysed together with herd information to identify communal pastures. Environmental samples (faeces and water) were taken from the selected pastures in 2022 and 2023 to detect MAP infections. The network analysis of cattle movements over thirteen years was focussed on dairy herds engaged in communal grazing.

Each year, about 1,100 herds (out of approximately 37,000) grazed on about 170 communal pastures, with an average of eight herds and 45 animals per pasture. Only 26 pastures had more than 100 animals, but animals from the same herd generally did not constitute a large proportion of their herd. The analysis of the network's islands consisting of communal pastures and connected herds revealed that certain herds with frequent trade played an important role in connecting other herds, similar to the pastures themselves. The importance of specific premises (pastures and farms) for the network connectedness varied greatly over time.

Suspected MAP cases were detected during sampling in the second year, limiting further research into the role of pastures in the MAP spread. Nevertheless, the study suggests that pastures may be less crucial for MAP transmission than previously assumed.

## PI.05: Caprine Paratuberculosis: Cross-Sectional Study in Italy and Evaluation of an Indirect ELISA Test's Performance in Milk

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**Abstract**

A cross-sectional epidemiological study was carried out on goats reared in Macerata Province (Marche Region, Italy) in order to: -assess the apparent and true *Mycobacterium avium* subsp. *paratuberculosis* (MAP) seroprevalence at flock- and animal-level; -evaluate the performance and the applicability of PARAS (ID.vet, France) on goat milk and bulk tank milk (BTM), as screening tool for flock health status; - evaluate the efficacy of the whole IDVet system for MAP analysis in faeces. In May 2022, a simple randomized sampling at farm-level, and a screening at animal-level (n=217) were conducted in 5 flocks ($CI_{99\%}$), representative of goat flock population reared in Macerata Province (Italy), stratifying the animals according to age (young: ≤6 months; adult:6-24 months; elderly: ≥24 months) and sex. Indirect ELISAs and qPCR IS900 were performed on blood, milk and bulk tank milk (BTM) sera, and faecal samples. MAP prevalence at farm-level was 80% (4/5) while the goat apparent- and true-blood seroprevalences at animal-level were 8.29% (18/217) and 14.02%, respectively. No significant differences were observed for seroprevalence and antibody level in relation to age (elderly:9.8%, S/P%=19.73±37.04; adult:8%, S/P%=13.71±23.91; lambs:2.5%, S/P%=11.73±32.15; $p$=0.9). Male goats (16.67%, 3/18) resulted seropositive but not shedders. Milk seropositivity of 22.22% (26/117) was observed and ELISA test on BTM confirmed the 80% of infected flocks. In females, significant higher seroprevalence was recorded in milk *vs*. blood (22.22%-7.54%, $p$=0.0001), both in adult (25%-6%, $p$=0.0192) and elder goats (21.50%-11.02%, $p$=0.0206). The strong and weak/passive-intermittent faecal shedders were 2.80% and 6.54%, respectively. All strongly shedders were elder female and seropositive, both in blood and milk. Considering the bovine (30%) and sheep (>21.38%) cuts off, the ELISA ID Screen® Paratuberculosis Indirect Screening test (ID.vet, France) on goat milk showed the following performance: Sensitivity=92.3%, Specificity=86.5%, Positive and Negative predictive values of 46.2% and 98.9%, respectively; Accuracy=87.2% and a moderate agreement (Cohen kappa=0.55).

# PI.06: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) Infection in Yak (*Bos grunniens)* in North Eastern states of India: A Pilot Study

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## Abstract

Yak (*Bos grunniens)* is a semi-domesticated unique bovine species located in North-eastern (NE) states of India and Ladakh, which is the home of more than 95% yak population of India. Yaks provide several resources including milk, meat, leather, hair, and excrement, which are very important source of livelihood for the poor and marginal farmers in this region. Yak thrives well in a fodder scared cold desert with harsh cold, high altitude, extreme hypoxic conditions. But the existence of such unique animal species is also under threat due to various infectious diseases, of which paratuberculosis (Johne's disease) is one of the major diseases of concern. JD is a chronic enteritis infection, which appear mainly in domestic and wild ruminants. *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease. MAP causes early culling, reduced growth rate, progressive weight loss, and reduced production. However, no systematic detailed study on paratuberculosis infection in Yak has been attempted in India so far. In the present study, a total of 16 fecal samples were collected from the yaks of Arunachal Pradesh and Sikkim states of India. During collection, all the animals were found to apparently healthy without any significant clinical manifestation. All the samples were processed for detection of mycobacteria by microscopy, polymerase chain reaction and isolation and identification of the organism. A total of 31.2% (5/16), 37.5% (6/16), 25.0% (4/16), and 37.5% (6/16) specimens were found positive for paratuberculosis infection in acid-fast staining, *IS900* PCR, Taqman probe PCR, and culture study, respectively. The affected animals might be acting as asymptomatic carrier and may pose a serious threat for the other animals in the herd as well as the entire population of the region. This is the first pilot study of MAP infection in yak in Sikkim and large-scale screening of the yak population in this region is essential to protect the species.

# PI.07:Paratuberculosis in Sudan: A Nationwide Study Reveals High Prevalence and Phylogenetic Relationships of *Mycobacterium avium* subsp. *Paratuberculosis*

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## Abstract

Although paratuberculosis (PTB) was diagnosed in Sudan in 1964, itwas neglected and only a few reports on it have been made since then.The present study aimed at investigating the current situation of the disease in Sudanand to identify the phylogenetic relationships betweenSudanese isolates of *Mycobacterium avium* subsp. *paratuberculosis* (MAP)and other strains worldwide. A total of 270 faecal samples from wild animals were collected from the Dinder Reserve Park; serum and faecal sampleswere collected from 2,168 domestic animals in 291 herds (172 cattle, 111small ruminants and 8 camel herds) distributed over eight states representing the main five regions (Western, Southern, Eastern, Northern and Central) of the country. A Recombinase PolymeraseAmplification (RPA) and an Enzyme- Linked Immunosorbent Assay (ELISA) tests were used to detect MAP DNA in faeces and MAP antibodies in serum, respectively. The overall prevalence of MAP DNA in wild animals was 33.7%.The true prevalence at animal and herd levels, respectively,in cattle were 18.6 and 42.2%,andin small ruminants was 10.7 and 41.8%. Thetrue animaland herd seroprevalence was8.0% and 55.8% in cattle and 1.7% and 8.5% in small ruminants, respectively. Although the trueprevalence of MAP DNA in camel was 16.3% and 75.0% at animal and herd levels, respectively, no anti-MAP antibodies were detected in camelsera. Phylogenetic analysis of IS1311gene sequence revealed close relatedness of MAP camels, cattle, sheep and goat Sudaneseisolates to each other and to worldwide S type (I/III) strains, thus indicating that one type of MAP is circulating in the Sudan.In view of animal resources as one of the main pillars of the country's national economy and essential for the livelihood of people, high prevalence of PTB at herd level necessitates adoption of control and prevention of the disease in the country.

# PI.08: Molecular Strain Typing of *Mycobacterium avium* subspecies *paratuberculosis* Isolates Recovered from Different Hosts

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**Abstract**

Short sequence repeats, known as SSRs, have recently gained widespread acceptance as markers for typing and species distinction in many microorganisms and are used for epidemiological studies. The *Mycobacterium avium* subsp. *paratuberculosis* (MAP) genome contains SSRs that can be used for a simple and reliable high-resolution typing technique to identify new native MAP isolates. In the present study, 56 faecal samples (Cattle, Buffaloes, Goats, and Sheep) were collected from different herds/flocks of the Mathura region and identified MAP positive by F57 PCR and IS900 PCR. Molecular strain typing of MAP-positive isolates was done by IS1311 PCR_ restriction enzyme analysis (REA), IS1311 PCR Locus 2_REA, and SSR typing to comprehend the genetic diversity of *Mycobacterium avium* subspecies *paratuberculosis* from different hosts. Out of 56 samples, 13 animals (Cattle-03, Buffaloes-02, Goat-05, and Sheep-03) were positive for MAP. Indian bison type was found predominant and was the exclusive genotype recovered from goats, sheep, buffaloes, and cattle. SSR typing revealed that all MAP Indian bison-type isolates had profile 7g4ggt concerning G and GGT repeat SSR loci. Our findings showed low diversity in MAP genotypes and different species and breeds of the Mathura region shared the 'Indian Bison type'. Moreover, the study is continued with a large sample size to relate genetic differences between MAP isolates, influenced by geographic and host factors, which could aid in tracing new paratuberculosis isolates and inform future epidemiological investigations.

**Keywords:** Short sequence repeats (SSRs), *Mycobacterium avium* subsp. *Paratuberculosis*, F57 PCR, IS900 PCR, Indian Bison Type

# PI.09: Detection of *Mycobacteriumavium* subspecies *paratuberculosis* antibodies in goats of the Malwa region of Madhya Pradesh in India by using an Indigenous indirect enzyme-linked immunosorbent assay

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## ABSTRACT

Johne's disease (JD)/ paratuberculosis (pTB) is a chronic intestinal infection caused by *Mycobacteriumavium* subspecies *paratuberculosis* (MAP). This is a fatal disease is characterized by cachexia, and in some species diarrhea, after a long pre-clinical phase. pTB causes economic losses due to direct and indirect complications. MAP infection has been reported in domestic livestock, human beings and wildlife. Different diagnostic tests/examinations have been employed to diagnose MAP infection in domestic animals: direct microscopic identification and isolation of MAP from fecal samples, serological and molecular biological tests and flow cytometry. The prevalence of MAP was second highest in goats after cattle in the domestic livestock population of the country. Hence in the present study, a serological test (indigenously developed indirect enzyme-linked immunosorbent assay) was employed to detect MAP antibodies in goat serum samples. More than 70% of goats (125 positives out of 173 tested) tested positive for MAP antibodies. A vaccine against pTB was developed and produced in India which works for both prophylactic and therapeutic purposes. Increasing MAP infection in the goats and other livestock populations suggests a national-level vaccination program for goats and other domestic livestock populations.

# PI.10: Estimation of bio-load of *Mycobacterium avium* subspecies *paratuberculosis* in patients suffering with thyroid and arthritis disorders using multiple tests

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## Abstract:

Aim of the current study was to estimate the bio-load of MAP from the confirmed cases of thyroid and arthritis clinical samples (blood and serum) which were collected from the community healthcare center Bhangel campus in the Noida NCR region. 111 blood samples (76 thyroid and 35 arthritis) were collected and screened by IS*900* blood PCR, indigenous ELISA, and Taqman probe qPCR. IS*1311* PCR_REA. From 111 serum samples analysed by indigenous ELISA, 19 (17.1%) were positive for MAP antibodies (Thyroid -18.4% and arthritis-14.2%). 111 blood samples, 10.8 and 11.7% were confirmed as positive for MAP by IS900 PCR and Taqman probe qPCR, respectively. Percent bio-incidence of MAP in thyroid patients was 9.2 to 14.2% and in arthritis was 10.5 to 14.2% by IS900 PCR and Taqman probe qPCR, respectively. Substantial agreement between the ELISA and PCRs was found and in IS*1311* PCR_REA molecular strain typing 'Indian Bison Type' was found most prevalent biotype. The study indicated moderate accessibility of the human population to MAP in Noida NCR region and presence 'Indian Bison Type' biotype suggests a possible zoonosis transmission route of MAP from livestock products to humans. In order to validate these findings in broader cohorts, large sample size of thyroid and arthritis patients is required.

**Invited Talk**

# Intelligent Vaccine Design: What we learned so far from the immunopathogenesis of Johne's disease?

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Johne's Disease (JD) is a chronic devastating condition caused by the infection with *Mycobacterium avium ss. paratuberculosis (M. ap),* that affect many ruminants with a significant impact on the productivity and health of dairy herds. *Our hypothesis is that betterunderstanding of the disease's mechanisms could inform the design of more immunogenic and highly protective vaccine candidates against JD*. We call this endeavor an intelligent vaccine design.Recent studies have shown that live-attenuated vaccines generated by targeting specific virulence factors (e.g.*lipN*gene, auxotrophs)may provide superior protection against JD compared to the traditional, inactivated vaccine. These findings suggest that the use of genetically modified strains could enhance vaccine efficacy by promoting a more effective immune response. Moreover, the role of epitope mapping and immune-informatics in vaccine design has gained prominence in designing better vaccines against multiple infectious diseases. By identifying B- and T-cell epitopes from *M. ap* proteins, researchers can create multi-epitope vaccines that target multiple components of the immune response. This approach not only increases the potential for robust immune responses, but also allows for the tailoring of vaccine components to enhance safety and efficacy. For example, subunit vaccines and nanovaccines utilizing specific antigens from *M. ap* were able to stimulate a robust immunity without the risk associated with using live-attenuated vaccines. Overall, the integration of computational tools in the vaccine discovery facilitates the identification of promising vaccine candidates and optimizes their design. In addition, the application of artificial intelligence (AI) tools in vaccine design could help in the analysis of vast datasets to predict immune responses and identify optimal vaccine candidates, streamlining the development process. In this presentation, we will discuss fundamental immunopathogenesis steps of JD and how we could use innovative approaches for vaccine design targeting JD.

## OII.01: Changes in the Bacterial Gut Microbiome of Goats with Paratuberculosis (Paratb)

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**Abstract**

The aim of this study was to reveal potential changes in the composition of intestinal bacterial communities in goats infected with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and diseased with ParaTB compared to goats free of MAP without ParaTB. In addition, bacterial factors that may be involved in the pathogenesis of ParaTB should be identified.

Goats of the breed "Thüringer Wald Ziege" from dairy herds in Thuringia with and without ParaTB were sampled. Health status of autopsied goats was checked by macroscopy, histology and microbiology. In contrast to goats without ParaTB, characteristic intestinal lesions and MAP were detected in goats with ParaTB. The microbiome compositions in the contents and mucosa of different intestinal compartments were estimated through 16S rRNA amplicon sequencing.

As is known for diseases that cause dysbiosis in the gut microbiome, α-diversity was reduced in large intestine and β-diversity was different in the small and large intestine of goats with ParaTB compared to goats free of MAP infection ($p \leq 0.05$). Differential analysis revealed various changes in the relative abundance of different taxa in the microbiome of gut content in goats with ParaTB: at the phylum level, there was an increase in *Firmicutes* and a decrease in *Bacteroidota* and *Proteobacteria* ($p \leq 0.01$) in the small intestine; an increase in *Actinobacteria* ($p \leq 0.05$) and a decrease in *Desulfobacterota* ($p \leq 0.01$) in the large intestine and various changes at the family level. The mucosa-associated microbiome of the jejunum and colon of goats with multibacillary ParaTB showed an increase in *Actinobacteria* (*Mycobacteriaceae*) and a decrease in *Proteobacteria* (*Mitochondria, Rhodobacteraceae*) compared to MAP-free goats ($p \leq 0.05$).

In goats with ParaTB, changes in the gut microbiome signature were found, which should be confirmed by further studies. Next, we will apply PICRUSt2 to predict microbial community functions and metabolites that may play a role in microbiome-host interactions and progression of ParaTB.

## OII.02: Does MAP Infection Disturb Tissue Homeostasis of Eicosanoids?

**Heike Köhler[1*], Anna Krauß[2], Anja Zigan[1], Michael Rothe[3], Volkmar Liebscher[2], Elisabeth M. Liebler-Tenorio[1]**

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**Abstract**

Eicosanoids, derivatives of cell membrane-derived 20-carbon polyunsaturated fatty acids, have multiple, sometimes pleiotropic effects in inflammation. In search for alternative biomarkers of paratuberculosis, alterations in the local eicosanoid profiles were examined in goats experimentally orally infected with *Mycobacterium avium* subspecies *paratuberculosis* (MAP), with *Mycobacterium avium* subspecies *hominissuis* (MAH) and mock-infected controls. MAP-infected goats remained clinically inconspicuous until necropsied three (n=5), six (n=5) and 12 (n=10) months after inoculation (m.p.i.). Six MAH-infected goats developed severe depression, fever and diarrhea, and died or had to be euthanized within 11 weeks after inoculation (w.p.i.). Seven animals with milder symptoms survived and were necropsied 12 m.p.i., as were the control goats (n=7). At necropsy, infection was culturally confirmed in all MAP- or MAH-inoculated goats. MAP infection resulted in granulomatous inflammation predominantly in Peyer´s patches (PP) and granulomas in regional lymph nodes (LN). MAH infection caused extensive ulceration of jejunal and ileal PP (JPP, IPP), caseous granulomas in many organs and disseminated intravascular coagulopathy at 11 w.p.i. and granulomas in IPP and regional LN at 12 m.p.i. JPP, IPP, jejunal, ileocolic and mediastinal LN were sampled for eicosanoid analysis. More than 50 different eicosanoids were quantified by HPLC-MS/MS. Principal component analysis revealed that, in all tissues examined, the eicosanoid profiles of the severely affected MAH-infected animals could be clearly discriminated from all other groups. This was due to a decrease of the anti-inflammatory metabolites $PGE_3$ and $TXB_3$ and the resolvin precursor 18-HEPE. In the IPP, the pro-inflammatory metabolite 20-HETE was increased. In contrast, the eicosanoid profiles of all MAP-infected goats and the MAH-infected goats at 12 m.p.i. overlapped largely in all tissues, and did not vary significantly from the controls. It appears that tissue homeostasis of eicosanoids is not significantly dysregulated by chronic MAP-induced inflammation. Thus, potential MAP-specific eicosanoid biomarkers could not be identified.

## OII.03: Cytokine Expression in Subjects with *Mycobacterium avium* ssp. *paratuberculosis* Positive Blood Cultures and a Meta-Analysis of Cytokine Expression in Crohn's Disease

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### Abstract

The objectives of this study were to 1) Culture *Mycobacterium avium* ssp. *paratuberculosis* (MAP) from blood, 2) assess infection persistence, 3) determine Crohn's disease (CD) cytokine expression, 4) compare CD cytokine expression to tuberculosis, and 5) perform a meta-analysis of cytokine expression in CD.

The Temple University/Abilene Christian University (TU/ACU) study had a prospective case control design with 201 subjects including 61 CD patients and 140 non-CD controls. The culture methods included MGIT, TiKa and Pozzato broths, and were deemed MAP positive, if IS900 PCR positive. A phage amplification assay was also performed to detect MAP. Cytokine analysis of the TU/ACU samples was performed using Simple Plex cytokine reagents on the Ella ELISA system. Statistical analyses were done after log transformation using the R software package. The meta-analysis combined three studies.

Most subjects had MAP positive blood cultures by one or more methods in 3 laboratories. In our cytokine study comparing CD to non-CD controls, IL-17, IFNg and TNFa were significantly increased in CD, but IL-2, IL-5, IL-10 and GM- CSF were not increased. In the meta- analysis, IL-6, IL-8 and IL-12 were significantly increased in the CD patients.

Most subjects in our sample had MAP infection and 8 of 9 subjects remained MAP positive one year later indicating persistent infection. While not identical, cytokine expression patterns in MAP culture positive CD patients in the TU/ACU study showed similarities (increased IL-17, IFNg and TNFa) to patterns of patients with Tuberculosis in other studies, indicating the possibilities of similar mechanisms of pathogen infection and potential strategies for treatment.

# OII.04: Evaluation of *Mycobacterium avium* subsp. *paratuberculosis* Δ*Map_1152* Mutant as a Live Attenuated Vaccine in Holstein Calves

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**Abstract**

Development of an effective vaccine against Johne's Disease is essential to prevent disease or reduce transmission. In this study, we infected bovine monocyte-derived macrophages (bMDM) to determine bacterial survival, and we evaluated the immune response and pathogenicity in a calf challenge experiment. For the in-vitro assays, bMDM were obtained from healthy cows and infected for 48h with wild-type (WT) strain K-10 and Δ*Map_1152* (DMAP52-unm) to evaluate survival. For the calf trial, eleven male Holstein calves were used in two experimental groups of 5 animals infected at 2 to 3 weeks of age with three doses of 2 x $10^{11}$ CFU/mL of K-10 and DMAP52-nm, and an uninfected animal was used as a control. At pre-infection, two weeks, and at 1, 2, 3, 6, and 8.5 months post-infection, the immune response was evaluated by antibody production, lymphocyte/monocyte populations, and skin test.

The attenuation of virulence for the DMAP52-unm mutant strain in bMDMs was confirmed. For the calf trial, some calves succumbed to a rotavirus outbreak and pneumonia, leaving the mutant and the WT strain groups with only 3-4 calves. The infectious dose used in the WT group was significantly lower than that used in the DMAP52_unm group. Although the results between the two groups are not comparable, we confirmed infection in the WT group. We did not detect a significant humoral or cellular immune response in the DMAP52_unm group within 8.5 months of infection, and DMAP52_unm was undetectable in the tissues by PCR. These results suggest that the mutant is attenuated. Further studies with more animals and for a more extended period are required to determine the potential vaccinal use of this strain.

# OII.05: Commercial Paratuberculosis Vaccine Does Not Interfere with Bovine Tuberculosis Diagnostic Tests and Lends Protection Against Lung Bacterial Load in Experimental *M. Bovis* Infections

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**Abstract**

Reluctance to use vaccination against paratuberculosis in cattle is due to its supposed interference with diagnostic in bovine tuberculosis (bTB) control programs. We have run a series of experiments in different settings comparing homologous and heterologous vaccines diagnostic interference and protection against TB. To do this, we orally or parenterally vaccinated over 100 newborn calves with the commercial paratuberculosis vaccine (Silirum), BCG, and a heat killed *M. bovis* (HIMB) and then followed them for 6 months. Additionally, we ran 4 experiments with the same vaccines on smaller groups of calves that were later challenged with *M. bovis* and post-mortem examined at 75 days post infection or more.

We observed limited reactivity both to homologous *M. bovis* vaccines and to Silirum in the bTB tests. Only the high dose HIMB parenteral vaccine caused increased IFN responses, while the cervical comparative test easily differentiated paratuberculosis vaccinated calves.

Both BCG and HIMB vaccines yielded over 90% reduction relative to controls in the bacterial counts in lung. Silirum still yielded an 81% protection. Including latent lymphoid tissue infection, vaccination yielded about 50% bacterial burden reduction. This, in agreement with recent work showing that reducing excretion and increasing resistance to infection decreases prevalence as both effects add up to diminish R coefficient, indicates that either vaccine could be useful for bTB control. Extrapolating from previous commercial herds results showing *Mycobacterium avium* subsp. *paratuberculosis* shedding eradication by vaccination, we predict that it should be possible to eradicate *M. bovis* from infected herds in less than 8 years without killing animals and at a much lower cost than current test and cull strategies in less affluent regions or where sociocultural factors make it impossible a test and cull strategy.

# OII.06: Localized Immune Responses Induced by BacA Oral Vaccine Against *Mycobacterium avium subsp.paratuberculosis* in Calves

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## Abstract

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) primarily invades the small intestine of ruminants by targeting the Peyer's patches (PP) located in the ileum and jejunum. Despite continuous efforts to develop an effective vaccine against MAP infection, a significant knowledge gap exists concerning the impact of live-attenuated vaccines on mucosal immunity. Previous investigations have indicated that the BacA oral vaccine provides localized protection against MAP within the small intestine of vaccinated calves. This protection was associated with a high occurrence of peripheral blood immune cells exhibiting pro-inflammatory and memory characteristics, along with antigen-specific stimulation of pro-inflammatory gene expression.

The main aims of this study were to evaluate the localized immune responses induced by the oral BacA vaccine within the continuous PP of the ileum and discrete PP of the jejunum and to elucidate the differences in protection conferred by the oral vaccine between these sites. To achieve this, mucosal immune cells were profiled, and gene expression upon restimulation and RNA-seq transcriptome analyses were performed.

Oral immunization with BacA enhanced the frequency of CD4+IFNγ+, CD4+TNFα+, and the ratio of T effector memory cells to T central memory cells in the ileum and jejunum of the group vaccinated with BacA and subsequently challenged with wild-type MAP. In contrast, the infection control group, where animals were solely challenged with wild-type MAP, showed no such enhancements. The immune cells isolated from the ileum of the vaccinated-challenged group exhibited significant upregulation of IFNγ, IP-10, TNFα, IL-2, IL-15, and IL-17 following restimulation, compared to the uninfected control group. However, only minimal differences were observed in the jejunum under similar conditions. The RNA-seq data also indicated a more pronounced host response in the ileum of vaccinated, vaccinated-challenged, and infection control groups compared to the jejunum. Gene ontology analyses of differentially expressed transcripts implicated genes associated with epigenetic reprogramming in the ileum and jejunum of the vaccinated group and increased phagocytic and apoptotic activities in the vaccinated- challenged group.

In conclusion, the variation in the effectiveness of the BacA oral vaccine appeared to be more associated with differences in antigen-specific gene expression between the ileum and jejunum, with the ileum showing a more vigorous host response.

# OII.07: Development of a Bovine Three-Dimensional (3D) *In Vitro* Intestinal Mucosa Model: Isolation, Culture and Characterization of Primary Bovine Intestinal Epithelial, Stromal and Immune Cells

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**Abstract**

The development of new effective strategies to control paratuberculosis (PTB) requires a comprehensive study of the interactions between *Mycobacterium avium* subsp. paratuberculosis (Map) and its host.

In recent times, mucosal epithelial 3D scaffold-based cell *in vitro* models have revolutionized our understanding of the host-pathogen interactions as they faithfully resemble different aspects of the *in vivo* microenvironment. However, no 3D scaffold- based in *vitro* model accurately reflecting the physiology of the small intestinal mucosa of ruminant host and its interactions with intestinal pathogens is available.

The present study aimed to generate an innovative, reproducible, reliable, and functional 3D scaffold-based model of the bovine intestinal mucosa containing epithelial, stromal fibroblasts and immune cells. This model will mimic the multicellular and multilayer architecture of the intestinal mucosa, support long-term culture, and offer an alternative to animal experimentation to study the host-pathogen interactions.

We have optimized the isolation and culture of intestinal primary epithelial, macrophages and stromal cells from distal jejunum (DJE) samples of Holstein-Friesian cows (<3 years old) from North-Spain local abattoirs. The separation of the cell populations has been achieved using their different plating time characteristics. Subsequently, cell-type characterization of goblet cells, epithelial, macrophages and stromal cells was performed by immunofluorescence staining using rabbit anti-mucin, rabbit anti-pan cytokeratin, mouse anti-Iba1 and anti-vimentin primary antibodies, respectively. Finally, stromal cells were seeded and cultured in commercially available scaffolds (Alvetex™). After two weeks, stromal cells showed the capacity to produce their own extracellular matrix.

These preliminary results established a stable subepithelial compartment that gives the foundation for seeding the epithelial lining and perform future physiological studies.

# OII.08: Infliximab (Remicade®) Increases the Acidification of *Mycobacterium paratuberculosis*-Containing Phagosomes in Macrophages

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**Abstract**

The association between *Mycobacterium avium* ssp. *paratuberculosis* (MAP) and Crohn's disease (CD) although debatable, is supported by several studies which have reported the detection or isolation of MAP from human tissues including serum, body fluids, and high levels of TNF-a was found secreted by the gut mucosa in MAP-associated CD patients. Infliximab is a monoclonal antibody that specifically inhibits TNF-a and is used as a current therapy for CD.

Previously, we have reported that MAP can infect, reside, and multiply intracellularly in human macrophages (THP-1), suggesting that the pathogen can subvert the host's immune response to avoid its demise even at the earliest stage of the infection. A previous study from our lab showed that THP-1 cultured under the presence of infliximab reduced the survival of MAP *in vitro*.

To determine the mechanism by which infliximab reduces MAP's growth, a proteomic study was developed using iTRAQ that included the following groups: infected THP-1 with live MAP, infected THP-1 with killed MAP, and uninfected THP-1 (negative control). These groups were assessed by iTRAQ and the results indicated that the V-type proton ATPase subunit S1-like protein (V-proton pump) was upregulated in infected macrophages cultured with infliximab. Further analysis by qPCR showed that the V-proton pump transcript was significantly increased in the presence of infliximab, but not in the group exposed to killed MAP or untreated cells.

To determine the significance of this upregulation, the endogenous pH of phagosomes from infected macrophages was measured. Results indicated that the presence of infliximab reduces and maintains an acidic pH of ~4.0 in the phagosome, suggesting that this pH may reduce MAP survival.

# OII.09: Pathophysiologic Characteristics of CRISPRi-Generated Mutants of *Mycobacterium avium* subsp. *paratuberculosis*

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## Abstract

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the etiologic and causative agent of Johne's disease, a chronic debilitating disease in ruminants. To control this disease, it is crucial to understand intracellular survival of MAP. However, the precise mechanism of survival is not fully understood. Therefore, we tried to reveal the mechanism by characterization of phenotypes of mutants, generated using the CRISPRi system on intracellular survival related genes. Four genes (PknG, Icl, MAP1981c, and Mdh), known as related to mycobacterial virulence, were mutated with CRISPRi system and inhibition of gene expression in mutants was confirmed by DEG analysis by RNA-seq. The optimal gene expression inhibition concentration was confirmed at the mutants ($P$<0.05) after the culture for 7 days using 30 μg/ml of anhydrotetracyline (ATc). Growth patterns of each mutant were determined by measuring $OD_{600}$ and CFU counting. Growth of Mdh gene mutant was decreased by the inhibition of the gene expression. The survival of those mutants was determined under stress conditions (nutrient starvation, oxidative and acidic stress). Also, colony morphology, cell aggregation and envelope alteration were observed. The survival of MAP mutants on Icl and MAP1981c genes was gradually decreased in nutrient starvation and oxidative stress, respectively and both in acidic stress when inhibited the gene expression with ATc. Morphological changes of colony were observed with PknG and MAP1981c gene mutants in the nutrient starvation and oxidative stress conditions, respectively. Similar morphological changes in the colony and aggregation of the Icl gene mutant were also observed under nutrient starvation and oxidative stress conditions. Morphological changes of the above mutants were irregular forms on the margin of MAP colony. Our study indicates these phenotypic and physiological characteristics might give important insights to reveal the involvement of those genes in MAP pathogenesis in the stages of infection and intracellular survival.

# PII.01: Cytokeratin Expression and Distribution Pattern of Epithelioid Macrophages in Granulomatous Lesions of Animals with Different Types of Paratuberculosis-Associated Histological Lesions: Cytokeratin as a Biomarker of Resilience

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## Abstract

A recent genome-wide association study identified 92 genetic variants in Holstein Friesian cattle with paratuberculosis (PTB)-associated multifocal lesions. Pathway analysis with the identified candidate genes revealed a significant enrichment of the keratinization (KRT) pathway in those animals.

The aim of the present study was to confirm that enrichment and its biological significance investigating the number and distribution pattern of CK-expressing cells in granulomas of distal jejunum (DJE) and jejunal lymph nodes (JELN) of animals with different PTB-associated lesions (focal, multifocal and diffuse) and in control animals without lesions was determined by quantitative double-immunohistochemical analysis using Iba1 (ionized calcium-binding adapter molecule-1) and CK as specific markers of macrophages and epithelial cells, respectively. Epithelioid macrophages (EMs) are activated macrophages that resemble epithelial cells present in the granulomas.

Animals with multifocal lesions showed the highest numbers of double-Iba1/CK positive cells (EMs) showing significant differences with focal, diffuse and control animals in JELN and higher numbers of single-CK expressing cells in JELN and DJE. Two distribution patterns of the EMs in the granulomas were observed. In focal and multifocal animals EMs were forming a barrier surrounding the granuloma while in animals with diffuse lesions EMs were throughout all the extension of the granuloma. As expected, focal and multifocal animals (most subclinical) had significantly lower Map levels than clinical animals with diffuse lesions.

In summary, our results confirm the enrichment of the KRT pathway and underline the importance of the distribution pattern of the EMs in the granuloma. Multifocal animals might be resilient to the disease as they control the shift from subclinical to the clinical through formation of ordered granulomas where EMs have a relevant role preventing Map dissemination and maintaining tissue integrity. Since CK expression was enriched in cattle with multifocal lesions, it could be considered as a potential biomarker of PTB resilience.

# PII.02: Effects on the Induction or Inhibition of Autophagy in Intracellular Survival of *Mycobacterium avium* subsp. *paratuberculosis* in Bovine Macrophages

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**Abstract**

*M. avium* subsp. *paratuberculosis* (Map) is a facultative intracellular parasite that replicates inside macrophages. Autophagy is an essential cellular mechanism that plays multiple roles, including as a defense mechanism to control intracellular pathogens. Previous studies have shown that induction of autophagy limits mycobacterial survival, but little is known about its role in MAP infection.

The aim of the present study was to evaluate autophagosomal co-localization and intracellular survival of Map in bovine monocyte-derived macrophages (bMDM) after induction or inhibition of autophagy. Monocytes were purified from bovine peripheral blood and cultured for one week to obtain differentiated macrophages. Macrophages were then infected with three different strains of Map (MOI 20) for 2 hours followed by treatment with DMSO (5uL/mL) as a negative control, rapamycin (25uM), chloroquine (CQ, 40uM) or 3-methyladenine (3-MA, 5mM) for 2 hours. In addition, untreated wells (RPMI only) were left as a negative control for CQ. At 0 and 1 days post-infection, confocal microscopy and CFU counts were performed. Treatment with 25uM of rapamycin for 2h was effective in inducing autophagy as Map showed an increase in autophagosome colocalization, but only reduced the intracellular survival of 285 Map strain. On the other hand, treatment with CQ increased the viability of Map but was statistically significant only for the Kay strain. Treatment with 3-MA did not alter the survival of Map. Future experiments will use higher drug concentrations and longer incubation times to further test Map survival.

# PII.03: Characterization of *Mycobacterium avium* subsp. *paratuberculosis* Δ*lprG-p55* Mutant in Bovine Macrophage and BALB-C Mice

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**Abstract**

Currently, more effective detection methods and vaccines are required to improve the prevention and control of Paratuberculosis. To do this, it is essential to know the genes or factors associated with the virulence or pathogenesis of *Mycobacterium avium* subsp *paratuberculosis* (Map) in the host. In this work, we mutated the *lprG-p55* operon, potentially associated with virulence in Map, and its survival and cytokine production in bovine monocyte-derived macrophages (bMDM) was evaluated. Furthermore, its protection against Map infection in BALB-c mice was also addressed.

For the in-vitro assays, bMDM were obtained from healthy cows, then infected with Map WT or Map*ΔlprG-p55,* and Map survival was evaluated after 1, 2, 4, 6, and 8 days post-infection. We observed that Map*ΔlprG-p55* had lower survival than Map WT. Then, we evaluated the expression levels of cytokines measured by qPCR after two hours of Map infection. We found similar expression levels of IL-10, IL-1β, and TNF-α and different expression levels of IL-12, IL-6, IL-8, and MHC-II when compared Map WT vs Map*ΔlprG-p5* infected bMDM.

Finally, we aimed to evaluate Map*ΔlprG-p55* as a live attenuated vaccine candidate in mice. To do this, BALB-c mice were vaccinated with two doses of Map*ΔlprG-p55* or PBS and then challenged with a Map virulent strain, Map1347. Animals were sacrificed at 3-, 20- and 60-days post-challenge and colony forming units (CFU) were evaluated from mice spleen homogenate. We observed a significant reduction in CFU in animals vaccinated with the mutant strain in comparison with PBS vaccinated group. Moreover, Map*ΔlprG-p55* induced IgG production in vaccinated animals. Overall, the obtained results demonstrate that Map*ΔlprG-p55* is attenuated and immunogenic. Vaccination with this mutant strain in mice reduced the CFU count of the challenge Map strain. Further studies are required to determine the potential use of this mutant strain as a vaccine.

# PII.04: Early Growth Response Factor 4 (EGR4) Expression in Gut Tissues and Regional Lymph Nodes of Cattle with Different Types of Paratuberculosis-Associated Lesions

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## Abstract

Selective breeding of animals with natural resilience to *Mycobacterium avium* subsp. *paratuberculosis* (Map) infection is a well-recognized strategy for paratuberculosis (PTB) control. Using summary-data based Mendelian randomization (SMR), a novel cis- expression quantitative loci (cis-eQTL) was found associated with the upregulation of the early growth response factor 4 (EGR4) expression and the presence of multifocal lesions (p=0.002).

The aim of this study was to evaluate the expression of EGR4 in gut tissues of animals with PTB-associated lesions or without lesions to confirm the SMR results. The number of EGR4-expresssing cells per $\mu m^2$ was analyzed in ileocecal valve (ICV), ileocecal lymph nodes (ICVLN), distal jejunum (DJE) and jejunal lymph nodes (JELN) of animals with focal (n=7), multifocal (n=13) and diffuse lesions (n=14) and in controls without lesions (n=6) by immunohistochemistry using a rabbit polyclonal anti-EGR4 antibody. Ten randomly selected fields (400X) per individual and tissue section were examined for each group. Quantification and statistical analysis were performed using QuPath version 0.4.3 and R Statistical Software version 4.1.3, respectively.

The number of positive EGR4-immunolabelled cells per $\mu m^2$ was significantly higher in gut tissues of animals with multifocal lesions than in the rest of the groups (multifocal *vs* control $p<0.001$, multifocal *vs* focal $p=0.002$ and multifocal *vs* diffuse $p<0.001$) which confirmed the SMR results. Morphologically EGR-4 expression was mostly detected in goblet cells, enterocytes and argentophilic cells of DJE and ICV and in lymphocytes of JELN and ICVLN.

EGR4 is a transcriptional factor that modulates the nuclear factor kappa β (NF-kβ)- induced proinflammatory response which upregulation could serve as a brake of T-cell activation preventing excessive immune responses and tissue damage promoting resilience in animals with multifocal lesions in gut tissues.

The use of this validated cis-eQTL in marker-assisted breeding programs could contribute to selection of resilient animals and consequently to reduce PTB economic losses.

# PII.05: Apoptosis of Bovine Mammary Gland Epithelial Cells (MAC-T) Infected by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and Co-Infected with Bacteria that Cause Bovine Mastitis

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**Abstract**

In the mammary gland, MAP infects epithelial cells without causing acute inflammation. However, these previously infected cells may be capable of associating with other classic mastitis bacteria, altering infective and cell injury parameters. The objective of this study was to determine the degree of apoptotic impairment of MAC-T cells infected by MAP and co-infected by bacteria that cause bovine mastitis through the quantification of caspase-9. MAC-T cells were infected by MAP isolates (standard strain K10, isolate from Argentina and isolate from Brazil) and co-infected separately by *S. aureus*, *S. agalactiae*, and *E. coli* at 4 h, 12 h, and 24 h, in two biological replicates. These cells were lysed and subjected to an enzyme-linked immunosorbent assay to quantify caspase-9. The results were submitted to Friedman's ANOVA test with 5% significance. There was a significant increase in caspase-9 of 7.37 ng/mL in MAP isolates from Argentina when co-infected with *S. aureus* within 24 h. In the remaining isolates, caspase-9 was quantified independently of the infectious process, however, with statistically non-significant results. The presence of caspase-9 only in the isolate from Argentina indicates that the apoptosis of mammary epithelial cells by MAP is influenced by the type of isolate, its origin, and also by the association with certain bacteria that cause mastitis. Taken together, these results demonstrate that the intensity of production losses due to MAP in dairy herds co-infected with bovine mastitis bacteria can be heterogeneous between regions and influenced by infection pressure.

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# PII.06: Comparison of Immunopathological Mechanisms in the Early Stage of *Mycobacterium avium* subsp. *paratuberculosis* Infection Through the Murine Model by Different Administration Route

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**Abstract**

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) is a pathogen responsible for Johne's disease (JD). JD causes chronic granulomatous enteropathy in ruminants and has threatened the livestock industry and public health. However, the pathogenesis and diagnosis of the disease are still not totally understood. In this study, an *in vivo* murine model according to oral (OA) and intraperitoneal (IP) administration was compared to identify suitable models in the early stages of MAP infection. Upon MAP infection, spleen and liver size and weight were increased in the IP groups compared to the OA groups. Analysis was performed using cytokine and splenocyte to observe various immunological characteristics according to different groups of infection. Higher amounts of TNF-α, IL-10, and IFN-γ were produced at the early stage of IP-infected mice while production of IL-17 was produced at different times and in infected groups. This event may represent the immunological transition from Th1 to Th17 during the overtime of MAP infection. Gene expression was analyzed in mesenteric lymph nodes (MLN) and spleen of 6 weeks post-infection mice with significant histological and immunological characteristics according to OA and IP routes. The expression of genes related to immune response and lipid metabolism was analyzed by canonical pathway using the ingenuity pathway analysis (IPA). Reduced glucose use of host cells due to increased secretion of pro-inflammatory cytokine ($P<0.05$), and cholesterol release in host cells via cholesterol efflux pump to disturb the energy source of MAP proposed an immunological mechanism according to the host-pathogen interaction. These results indicate the immunopathological and metabolic responses in the early stage of MAP infection through the development of murine models. This work is supported by BK21 FOUR and Research Center and Research Institute for Veterinary Science, Seoul National University, Seoul, Republic of Korea.

## PII.07: Cloning, Expression, Purification, and Immunological Testing of a New *Mycobacterium avium* subsp. *paratuberculosis* Antigen Encoded by the *Map2191* (MAP_RS11140) Gene

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**Abstract**

Despite investigations in John's disease (JD) diagnosis, there is no optimal primary antigen candidate for immunization or immunodiagnosis of *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The detection of a highly immunogenic antigen can improve early diagnosis, vaccine development, disease prevention, and control. We want to design a MAP immunogenic antigen candidate to create humoral and cellular immunity for host protection.

Map2191's N-terminal region had potential T- and B-cell epitopes predicted in silico. This study successfully amplified, sub-cloned, and produced a distinct Mce-truncated protein encoded by a specific section of the Map2191 gene in *E. coli* for the first time. The recombinant protein was purified using a batch technique, employing a ni-NTA gel matrix. Its purity was confirmed through western blot analysis, using anti-His tag monoclonal antibodies. The reactivity and immunogenicity of this protein against MAP infection were assessed using ELISA. The Mce-truncated protein was compared to commercial ELISA using the same sera for sensitivity and specificity. We then investigated Mce-truncated protein as a JD subunit vaccine in experimentally challenged goats. Six healthy goat kids received this protein, and two were controls. Live MAP bacilli were twice given orally to all goat kids. MAP culture was performed on all necropsied tissues to confirm JD.

The results demonstrated that recombinant Map-truncated protein can detect JD with high sensitivity and specificity by ELISA method. Only pooled vaccinated goat sera responded with this protein in western-blot. In vaccinated goats, humoral immune response to Mce protein increased considerably. Vaccinated goats gained greater weight than controls and did not shed MAP or had necropsy tissue histopatological lesions or colonization. The innovative Mce protein-based vaccine protected goats from live MAP bacilli. The study data is expected to contribute to the development of novel ways for preventing JD and designing veterinary and medicinal vaccines.

## OIII.01: Development and Validation of a Genomics Informed Real-time PCR Assay for the Detection and Strain Typing of *Mycobacterium avium* subsp. *paratuberculosis.*

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**Abstract**

Specific and sensitive detection of Map is highly important for on farm management and control of Johne's disease. The currently available PCR's lack sensitivity and specificity and strain typing assays are time consuming, labour intensive and lack discriminatory power. In this study specific genomic targets that were identified through pan-genome analysis of Map were analysed for their sensitivity and specificity and ability to detect Map directly from faeces.

A probe-based qPCR using a Map specific novel target was identified and found to be more specific and sensitive than current JD PCR assays with a detection limit of 0.0002 fg/µl as compared to 0.02 fg/µl for the IS900 PCR. This novel assay was 100% reproducible and repeatable and was able to detect Map directly from faeces. In addition, a Type S strain specific probe-based qPCR was developed and was found to be more sensitive than the IS1311 PCR and REA with a sensitivity of 40fg as compared to 4.7 x $10^6$ fg/µl. The combination of these two assays can differentiate between Type C and Type S Map.

This study developed and validated two genomics informed qPCR assays, and found both assays to be highly specific and sensitive for the detection of Map from culture and directly from faeces. These assays provide an alternative approach to current testing methods that are rapid, more cost effective and are more informative for the diagnosis of Map in sheep and cattle. Both assays outperformed the currently available PCR assays.

# OIII.02: Differences in Serum Metabolic Parameters and Milk Production in *Mycobacterium avium* subsp. *paratuberculosis* Infected Goats with Different Shedding Levels and Non-Suspect Goats

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**Abstract**

Infection with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) causes chronic inflammation of the gut mucosa in ruminants and decreased nutrient absorption eventually leading to weight loss. The impact hereof on metabolic and milk production parameters in goats was examined in a case-control-study.

In a commercial 400 head organic dairy goat herd vaccinated against paratuberculosis, 75 clinically inconspicuous goats were classified as infected with MAP based on results of repeated fecal culture, and 71 goats with no MAP detection were matched as control according to age. Blood and fecal samples were collected from the individual animals at five sampling times, each three months apart. Blood serum was analyzed by spectrophotometric methods using an automated analyzer for concentrations of albumin, total protein, beta-hydroxybutyrate, non-esterified fatty acids, cholesterol, bilirubin, creatinine, urea, calcium, iron, magnesium, and phosphorus. Additionally, the serum activities of alkaline phosphatase, aspartate aminotransferase, creatine kinase, gamma- glutamyltransferase, and glutamate dehydrogenase were measured. Milk yield, protein, fat, lactose, urea, and somatic cell count were obtained from monthly milk recording. Linear mixed models using generalized estimating equations were applied to statistically evaluate disparities in three different group comparisons. Group definitions were based on a) MAP-infection status (non-suspect or infected), b) current fecal culture result (MAP-shedders or non-shedders) and c) combination of both characteristics (non-suspect, infected non-shedders, infected light-shedders, infected heavy- shedders).

High MAP shedding was associated with the largest deviations, particularly concerning serum concentrations of calcium, iron, total protein, and albumin. Furthermore, a milk yield reduction of 23.9% compared to non-suspect goats was observed. Albumin, iron, glutamate dehydrogenase, and milk yield differed between currently MAP-shedding goats and non-shedders.

These results confirm that metabolism and milk production can be affected even in the subclinical stage of paratuberculosis. However, significant changes are most likely to be detected in goats in advanced stages of MAP infection.

# OIII.03: Bayesian Accuracy Estimates of Commercial Antibody-ELISA and qPCR to Detect *Mycobacterium avium* subsp. *paratuberculosis* Infected Cows in Herds with Historical MAP Infection Status

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**Abstract**

Applying biosecurity measures and test-and-cull strategies should reduce the prevalence and incidence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infections. Understanding diagnostic accuracy of available tests is paramount to make adequate culling decisions in MAP-positive herds. Our objective was to estimate the diagnostic accuracy of serum-ELISA and fecal-qPCR to identify MAP infected cows in herds with historical MAP infectious status using a Bayesian latent class model (BLCM).

We analyzed a database of serum-ELISA and fecal-qPCR test results from a companion project. Cows originated from MAP-positive dairy herds in Québec (1157 cows; 14 herds) and Ontario (1718 cows; 8 herds). ELISA was performed on serum samples using the IDEXX Paratuberculosis Kit. Two independent DNA extractions were performed on each fecal sample using the Zymo Research Fecal DNA Kit and a qPCR assay (VetMAX™-Gold MAP Detection Kit) was performed on each DNA extraction (qPCR-1 and qPCR-2). Samples were declared positive by ELISA if the sample to positive ratio was ≥55% and by qPCR if the cycle threshold was <38. A three-tests (ELISA, qPCR-1, qPCR-2) hierarchical BLCM, allowing for conditional dependency between the two qPCR assays, was fitted to estimate the sensitivity, specificity, positive and negative predictive values (PPV and NPV), and 95% Bayesian credible intervals (95% BCI).

For serum ELISA, qPCR-1 and qPCR-2, the median sensitivity (95% BCI) was 30.5% (25.6-35.9), 78.4% (68.7-86.8) and 71.4% (62.4-79.4), respectively. The median specificity (95% BCI) was 97.8% (97.3-98.3), 97.3% (96.3-98.2) and 96.8% (95.7-97.7), respectively. The median PPV (95% BCI) was 63.2% (48.4-76.6), 78% (64.3-87.8), 73% (58.6-84.5), respectively. The median NPV (95% BCI) was 92% (86.3-95.3), 97.4% (95-98.8), 96.5% (93.3-98.2), respectively. The median within-herd MAP prevalence (95% BCI) of participants herds was 10.9% (6.6-18.1).

In this prevalence context and using these thresholds, fecal qPCR was more sensitive than serum ELISA to identify MAP infected cows.

# OIII.04: Human Antibodies Against *Mycobacterium avium* ssp. *paratuberculosis* for the Selection of Patients with Crohn's Disease for Anti-Mycobacterial Therapy

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**Abstract**

Increasing evidence links a worldwide bacterial infection of cattle and other animal species by *Mycobacterium avium* ssp. *paratuberculosis* (MAP) to Crohn's disease (CD). A large, FDA phase 2/3 controlled clinical trial of combination antimycobacterial antibiotic therapy for CD has been completed, and the report describing the trial is pending publication. The identification of MAP infection in CD patients will become increasingly important. The objective of this study is to develop MAP tests that can accurately predict which CD patients have a MAPinfection.

A Prospective, case-control laboratory test study of 199 subjects (61 CD patients and 138 non-CD controls). MAP antibodies, including Hsp65, PknG, PtpA, CL1, and MAPIDEXX, were measured under blind conditions in the plasma of the 199 subjects. Compared to any MAP antibody, combinations of antibodies showed improved CD diagnostic performance. For the Hsp65 antibody, the sensitivity (SEN), specificity (SPE), positive predictive value (PPV), negative predictive value (NPV), correct classification(CC), and area under the curve (AUC) were 59.02%, 58.70%, 38.71%, 76.42%, 59.3% and 0.606, respectively. For the best combination of MAP antibodies (Hsp65 and PknG), the SEN, SPE, PPV, NPV, CC, and AUC were 59.02%, 60.87%, 40.00%, 77.06%, 60.30%, and 0.631, respectively. Further improvement of the CD diagnostic performance was achieved by combining IFN-□, IL-8, and IL-17 cytokines with MAP antibodies, yielding SEN, SPE, PPV, NPV, CC, and AUC of 62.3%, 62.32%, 42.22%, 78.9%, 62.31% and 0.708, respectively.

Hence, the combinations of MAP antibodies and cytokines yield better CD diagnostic predictive performance than any single MAP antibody.

## OIII.05: *Mycobacterium avium* subsp. *paratuberculosis*: composition of the bacterial microbiota in blood samples from patients in the groups Crohn's disease and patients with ulcerative colitis

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**Abstract**

*Mycobacterium avium* subspecies *paratuberculosis* (MAP), the etiologic agent of paratuberculosis in ruminants, is also isolated from humans with Crohn's disease (CD). However, the involvement of MAP in the pathogenesis of CD and other intestinal diseases is unclear. The present study aimed to analyze the microbiota in samples of human blood and its relationship with the identification of MAP: 24 samples from CD, 8 samples from ulcerative colitis (UC), and 8 from control samples (H). For the detection and identification of MAP in blood samples, the IS*900* and F57 genes were investigated by real-time PCR, and CD and UC samples were positive for MAP. The V3/V4 hypervariable regions of the 16S rRNA gene were amplified for the Illumina MiSeq platform. Bioinformatic analyses followed pipelines executed in the R program using "DADA2," version 1.20.0, and the package Phyloseq, version 1.46.0. The main phylum found were *Firmicutes* (92%: all groups), *Actinobacteria* (CD: 6.2%; H: 6.5%; UC: 6.2%), *Proteobacteria* (1%: all groups), and others such as *Bacteroidota*, *Bacillota,* and *Actinomycetota*. The *Mycobacteriaceae* family was identified in greater abundance in CD, followed by the UC and H groups. *Mycobacterium* sp. was only identified in CD. *Mycolicibacterium* sp. was identified in a greater proportion in CD, followed by H and UC. On the other hand, *Mycolicibacter* sp. was identified in a greater proportion in UC, followed by CD and H. Therefore, although MAP has been more frequently detected in the blood of patients with CD, it appears that MAP may be present in other intestinal inflammations and even in mucous membranes. Still, the greater presence of the genus *Mycobacteria* sp. in the blood of patients with CD indicates greater susceptibility to the maintenance of this genus in this inflammatory process when compared to UC.

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# OIII.06: Stimulation of Bovine Ileal Organoids by Bovine RANK-L and the Uptake of *Mycobacterium avium* subspecies *paratuberculosis* into Intestinal Epithelial Cells

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**Abstract**

It is known that *Mycobacterium avium* subspecies *paratuberculosis* (*MAP)* infects the GI tract via M-cells within the Peyer's patches of the ileum. Additionally, it has previously been shown that treatment with RANK-L induces the differentiation of M-cells. This study aims to investigate the route of invasion of *MAP* by establishing an *in vitro* bovine ileal organoid model to characterize the molecular mechanisms of *MAP* infection, and to identify putative factors involved in *MAP* invasion.

Bovine ileal organoids were cultured as previously described by Hamilton *et al.* (2018). Briefly, after 9 days of differentiation and RANK-L stimulation, organoid monolayers were challenged with MAP for 6h and 24h, organoids were then lysed for RNA extraction or fixed and stained for immunofluorescent imaging.

It was observed that, after treatment with RANK-L, *MAP* uptake into epithelial cells was increased compared to controls, suggesting that RANK-L stimulation influences gut epithelial cell differentiation and increased transcytosis capability of the monolayer cultures. Additionally, RNA sequencing identified putative factors involved in epithelial attachment and invasion of *MAP*.

We successfully developed a organoid monolayer system and demonstrated the uptake of *MAP* in the presence and absence of RANK-L. Through the employment of transcriptomic analysis of this infection model, we identified changes to the cellular composition of the gut and identified factors involved in epithelial attachment and invasion of *MAP*. Therefore, this model provides an opportunity to better understand the molecular mechanisms of *MAP* entry into the intestinal epithelium.

# OIII.07: A microRNA-based Johne's disease diagnostic predictive system: preliminary results

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## Abstract

Johne's disease, caused by *Mycobacterium avium subsp. paratuberculosis* (MAP), is a highly contagious chronic enteritis impacting welfare and productivity in cattle. Prevention and control of Johne's disease is difficult as MAP is environmentally resilient and vaccine efficacy is variable and controversial. Despite detection being a central component of control, diagnosis can be challenging as clinical signs are insidious and non-specific. A promising approach to early Johne's disease diagnostics is to harness the information contained in the expression profiles of small non-coding RNA molecules involved in gene regulation (microRNAs), which are altered during mycobacterial infection.

This preliminary study measured the expression level of 24 microRNAs affected by mycobacterial infection in sera from MAP-positive ($n$=66) and MAP-negative ($n$=65) cattle samples. A curated collection of 15 predictive models were thoroughly tuned, trained, and benchmarked to determine an optimal classifier for the diagnosis of Johne's disease using miRNA profiles. Random Forest was the best-performing model, which provided 72% accuracy, 78% AUC, 73% sensitivity and 71% specificity on average to detect MAP-infected versus healthy cattle. Although control samples were collected from farms nominally MAP-free, low sensitivity in current diagnostics means animals may be misclassified.

This preliminary study indicates that miRNA profiling in combination with advanced predictive modelling, accurately discriminated between healthy and MAP-infected animals. Efforts are currently ongoing to expand and validate the model with more sample data, which will further improve accuracy and expand the approach to characterise Johne's disease stage, enhance early-stage diagnosis and distinguish between alternative mycobacterial pathogens through miRNA diagnostics methods.

# OIII.08: Identification of Novel Bovine Biomarkers Associated with Paratuberculosis Tolerance

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**Abstract**

Proteomics is a promising tool for the identification of biomarkers of tolerance to PTB. Tolerance is defined as a host defense mechanism genetically determined that reduces the negative impact of infection on host fitness without affecting the pathogen burden. Tolerance also decreases host susceptibility to tissue damage and other fitness costs caused by the pathogens or the immune response against them.

The present study aimed to identify bovine protein biomarkers associated with two phenotypes of tolerance to PTB: phenotype 1) animals with multifocal lesions but without clinical signs and phenotype 2) animals with *Mycobacterium avium* subsp *paratuberculosis* (Map) load but without lesions in gut tissues. Sera and ileocecal valve (ICV) samples collected from naturally infected cattle with phenotypes 1 and 2 and controls were analyzed using high-throughput and label-free quantitative proteomics (SWATH-MS) to identify proteins differentially expressed (DE) between groups. Three comparisons were performed: phenotype 1 (n=5) *versus (vs*.) control 1 (animals without lesions (n=3)), phenotype 2 (n=11) *vs.* control 2 (animals with focal lesions negative by PCR and bacteriological culture of tissues (n=17)) and phenotype 2 *vs*. phenotype 1. Proteomic results were validated by Scaffold Software version 5.3.3. Proteomic profiles revealed both common and unique protein expression in response to infection. Ninety-eight, 52 and 103 DE serum proteins and 92, 211 and 350 DE ICV samples were identified in the phenotype 1 *vs* control 1, phenotype 2 *vs* control 2, and phenotype 2 *vs.* phenotype 1 comparisons, respectively. These DE proteins (p-value≤ 0.05) were further analyzed with FunRich Software and STRING to generate functional protein association networks for the discovery of the underlying mechanisms and key biological processes relevant to the development of tolerance to PTB.

Those biomarkers will be very useful for the detection of cattle able to tolerate Map without compromising health and milk production.

## PIII.01: Fatty Acids that Distinguish C-type and S-type Strains of *Mycobacterium avium* subspecies *paratuberculosis*

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**Abstract**

Branch-chain fatty acids are a predominant cell-wall component among species belonging to the *Mycobacterium* genus. One of these is tuberculostearic acid (TBSA), which is a long-chain middle branched fatty acid used as a diagnostic marker for *M. tuberculosis* but also has industrial application as a bio-lubricant. In this study, we demonstrate that TBSA production can be inhibited either by addition of pivalic acid to mycobacterial growth cultures or by a *bfaA*-gene knockout encoding an FAD-binding oxidoreductase. *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) growth and TBSA production was inhibited in 0.5 mg/ml pivalic acid supplemented cultures, but higher concentrations were needed to have a similar effect in other mycobacteria including *Mycobacterium avium* subspecies *hominissuis*. While *Map* C-type strains will produce TBSA in the absence of pivalic acid, the S-type *Map* strains do not produce TBSA in any condition. A SAM-dependent methyltransferase, encoded by *bfaB*, and FAD-binding oxidoreductase are both required in the two-step biosynthesis of TBSA; however, S-type strains contain a SNP in the *bfaA* gene, inactivating the oxidoreductase enzyme. This results in the production of an intermediate, termed 10-methylene stearate, which is detected only in S-type strains by gas chromatography/mass spectrometry. Fatty acid methyl ester analysis of a C-type *Map* *bfaA* knockout revealed the loss of TBSA production, but the intermediate was present in this mutant similar to the S-type strains. Collectively, these results demonstrate the subtle biochemical differences between two primary genetic lineages of *Map* and other mycobacteria as well as explain the resulting phenotype at the genetic level. Control of TBSA production is important for industrial purposes as well as understanding the biology of mycobacteria. Finally, this work sheds further light on the mechanism used by mycobacteria to produce tuberculostearic acid.

## PIII.02: microRNA Biomarkers for Improved Detection of Infectious Diseases

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**Abstract**

The diagnosis of infectious diseases is sometimes hampered by lengthy incubation periods, unreliable pathogen shedding or poor antibody responses. For example, the incubation period for paratuberculosis (Johne's disease (JD)), a chronic enteritis of ruminants caused by *Mycobacterium avium* susp *paratuberculosis* (MAP), can extend for several years. This causes poor test sensitivity for histopathology, culture, PCR and serology, particularly during early disease stages. Similarly, early detection of *Mycoplasma bovis* (*M. bovis*) is not always achievable through serology or molecular diagnostics.

Having recognised these limitations, our team has built a biomarker discovery platform to improve diagnostic test sensitivity for select infectious diseases. Our approach involves the measurement of host-encoded microRNAs (miRNAs), which have emerged as key regulators in both innate and adaptive immune responses to infection. Our platform employs artificial intelligence and machine learning (AI/ML) to identify specific miRNA signatures associated with disease status. The current test format involves PCR assays coupled to software that translates PCR data into a test result (i.e. probability of infection). We recently identified differential miRNA expression patterns in COVID-19 patients[1], showing the potential of miRNAs as predictors for infection severity and patient stratifications.

Our team has received support from the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) and the New Zealand Ministry of Primary Industries (MPI) to develop miRNA-based diagnostics for JD and *M. bovis*, respectively. Both activities include milestones for test validation according to World Organisation for Animal Health (WOAH) guidelines. Results to date suggest miRNAs detect these diseases with >95% sensitivity and specificity, with early indications suggesting successful detection of asymptomatic cases.

# PIII.03: Digital PCR (dPCR) to Quantify the Load of *Mycobacterium avium* supsp. *paratuberculosis* (MAP) Present in Feces as a "Tool" to Define Priorities of Interventions in an Infected Cattle Herd

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**Abstract**

Both sub-clinically and clinically paratuberculosis infected animals can spread MAP into the environment through feces and the ingestion of food and water contaminated by infected feces is the major route of transmission of the disease. Since MAP has a very high environmental resistance, despite is unable to multiply outside hosts, the level of environmental biocontamination relies on both the number of shedding animals in the farm and the MAP load excreted by every single animal.

We report here our experience in a Northern Italy dairy herd (about 900 milking cows) with paratuberculosis infection, where Digital PCR aimed at detecting MAP from bovine faeces was used in order to define priorities of interventions. The farm risk analysis pointed out the following critical points: purchased animals with no sufficient health garantees, young animals bred very close to adults paddocks; poor environmental hygenic conditions.

Sera and feces from all 1019 animals ≥24 months old were collected. Apparent sero-prevalence resulted 7.3% while fecal prevalence, determined by qPCR, was 6.5%. Samples showing a qPCR results ≤ 30 Cq underwent to F57 digital PCR (dPCR), whith the scope of quantifying the load of MAPs eliminated by each single cow. Overall, just five animals resulted shedding a very high load of MAP (Super shedders, defined as ≥ $10^7$ MAP genome copies/g of feces) and were considered the first to be segregated, followed by a group of six High shedders (shedding from $10^5$ to $10^7$ copies/g of feces). Moreover, 7 out of 11 environmental samples resulted positive, but dPCR was not carried out because resulted > or = 30 Cq .

The use of the dPCR allowed the direct quantification of the MAP cells load present in the feces in a quicker way than cultural assay, providing useful information for the development of an effective health management plan.

# Detection of *Mycobacterium avium* subsp. *paratuberculosis* in fecal samples by culture in dairy farms in Panama

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**Abstract**

*Mycobacterium avium* subsp. *paratuberculosis* (MAP), poses economic challenges to dairy farms worldwide therefore, its detection and control strategy is crucial. We aim to evaluate the performance of liquid and solid culture media for isolating MAP from infected cows and describe livestock management practices using standardized questionnaires. We collected stool samples from 14 dairy farms (Chiriquí, Panama), and analyzed them using acid-fast bacilli staining, direct PCR, liquid, and solid culture, and end-point PCR targeting IS900, F57, ISMAv2, and Locus 255. The bacteriological culture was evaluated with liquid media M7H9C and A7H9J and solid media HEYM-PS, 7H11-M, and 7H9-OP. Results indicated that liquid medium M7H9C yielded higher bacterial clumps than A7H9J at the sixth week of incubation (1.3 vs. 0.2 clumps/field; $p < 0.05$). Solid medium 7H9-OP demonstrated higher bacterial growth than HEYM-PS and 7H11-M from the third week of incubation. Evaluation of 443 stool samples using M7H9C liquid medium revealed 38 animals with acid-fast bacilli (9.0 clumps/field average). End-point PCR analysis of the IS900 gen detected MAP DNA in 26% (10/38) culture with bacterial growth; both F57/Locus 255 showed a 3% detection rate, ISMav2 did not detect. Solid medium 7H9-OP identified 5 animals with acid-fast bacilli (1 CFU each); end-point PCR detected MAP DNA in 60% (3/5). Management practices on culture-positive farms showed that 71% (5/7) used animal purchase as a replacement method, and 60% (3/5) knew their health status. No farms applied quarantine for new animal introduction. Additionally, 71% (5/7) of the farms housed calves with their mothers after calving, and 29% (2/7) used manure to fertilize pastures. Our findings highlight the effectiveness of liquid and solid cultures in isolating MAP and the relationship of culture-positive farms with poor management practices. We recommend culture to aid control measures in the dairy industry and track the spread of paratuberculosis in herds.

## PIII.05: miRNA in stool as Paratuberculosis prognostic biomarkers in beef cattle: extraction methods comparison

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**Abstract**

Circulating microRNAs (miRNAs) are small non-coding RNAs involved in gene expression regulation and protein translation. They are detectable in different matrices and used as prognostic biomarkers for different human diseases and more recently, for animal ones. So far, molecular contribution of miRNA from animal faeces has been poorly investigated. Thus, this abstract reports about the comparison of miRNA extraction methods from cattle faeces in order to investigate the diagnostic/prognostic role of some faecal miRNA targets in bovine Paratuberculosis (PTB). Faeces were taken from a herd monitored for PTB where cattle were categorized into three groups "negative", "affected", "infected" on the basis of ELISA, IFN-γ assay, and faecal MAP qPCR outcomes. RNAs were extracted comparing 3 extraction kits: RNeasy Power Fecal; Power Microbiome; miRNeasy. All protocols were slightly modified to fit the experimental purpose. Trials were then performed using TaqMan_MicroRNA Reverse Transcription Kit, with RT stem-loop specific primer and with specific TaqMan_MicroRNA Assays for bta-miR-658, hsa-miR-501-5p, bta-miR-92a, chosen from the unique concerning study of Shaughnessy et al. 2020. In order to choose the more performing extraction method, qualitative/quantitative criteria were assessed. Considering the concentration of extracted miRNAs and total RNAs, as quantitative parameter, and the absorbance ratio values, as qualitative index, the best protocol resulted RNeasy Power Fecal (Table 1). The selection of the best faecal miRNA extraction method is pivotal in order to down-stream evaluate, among the groups of interest, the differential expression of potential miRNAs PTB prognostic biomarkers.

Table 1. Outcomes of qualitative and quantitative parameters derived from miRNA extraction methods comparison

| | | QUBIT (ThermoFisher) microRNA Assay | QUBIT (ThermoFisher) RNA BR Assay | Biophotometer (Eppendorf) | | |
|---|---|---|---|---|---|---|
| Extraction mehtods/kits | Sample | miRNA (ng/µl) | RNA (ng/µl) | RNA (ng/µl) | 260/280 ratio | 260/230 ratio |
| **RNEasy Power Fecal (QIAGEN)** | 1N | 68 | 236 | 252 | 1.85 | 1.50 |
| | 2N | 60 | 235 | 250 | 1.80 | 1.45 |
| | 3P | 44 | 102 | 105 | 1.85 | 1.45 |
| | 4P | 58 | 259 | 120 | 1.80 | 1.50 |
| **Power Microbiome (QIAGEN)** | 1N | 4 | 17 | 17 | 1.40 | 0.40 |
| | 2N | 3 | 16 | 25 | 1.30 | 0.50 |
| | 3P | 5 | 18 | 28 | 1.40 | 0.42 |
| | 4P | 3 | 11 | 23 | 1.35 | 0.75 |
| **miRNeasy (QIAGEN)** | 1N | 29 | 92 | 163 | 1.40 | 0.70 |
| | 2N | 72 | 103 | 300 | 1.30 | 0.81 |
| | 3P | 61 | 95 | 265 | 1.35 | 0.75 |
| | 4P | 66 | 96 | 267 | 1.30 | 0.81 |

N: PTB negative bovine

P: PTB positive or affected bovine

*rounded values

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## PIII.06: Electrochemical Biosensors for *Mycobacterium avium* subsp. *paratuberculosis*: Diagnostic Hurdles and Emerging Solutions

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**Abstract**

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD), a chronic enteritis in animals. The infection is also associated with Crohn's disease in humans, raising public health concerns. The clinical manifesto of the disease is chronic intermittent diarrhea, gradual weight loss despite a good appetite, reduced productivity, and infertility. A large number of animals shed bacilli without showing clinical symptoms (sub-clinically infected) thus making the whole herd vulnerable to infection. Therefore, early and accurate diagnosis is indispensable, especially in a country where domestic livestock are known for low productivity. Current laboratory tests require specialized equipment and training; tests like ELISA and PCR are lab-intensive. Existing biosensors require isolated DNA and many laboratory resources, making them time-consuming. In contrast, the lateral flow assay (LFA) developed for detecting MAP-specific antibodies showed variable diagnostic sensitivity (84.2%) and specificity (83.3%), when compared to the indigenous ELISA.

Developing a rapid, inexpensive sensor-based point-of-care diagnostic assay will help in the JD control program in third-world countries. To address these challenges, this study introduces a novel electrochemical biosensor (ECB) that targets MAP-specific secretory proteins (1693c, 2168c, 2677c, 4308c, and 3547c) immobilized on a nanoparticle-enhanced electrode. The ECB is designed to improve the detection sensitivity and specificity, providing more accurate and rapid diagnostic outcomes. Early trials expect to indicate that the ECB surpasses traditional methods in both detection limits and accuracy, offering a promising solution for point-of-care diagnostics in the control of Johne's disease, especially in resource-limited settings. This innovation could play a pivotal role in veterinary diagnostics by facilitating more efficient disease management and control programs.

**Keywords:** Paratuberculosis, *Mycobacterium avium* subsp. *paratuberculosis,* Johne's disease, Crohn's disease, electrochemical biosensors

## PIII.07: Duplex PCR for Zoonotic Threats: Validation and Detection of *Mycobacterium paratuberculosis* and *Mycobacterium bovis*

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**Abstract**

Being considered among the major zoonotic concerns, we developed the Duplex PCR method for identifying *Mycobacterium bovis* and *M. paratuberculosis* targeting different genes including RD9, 16s rRNA, IS1081, IS900, and F57. Duplex PCR yielded three different patterns showing amplification either for IS900 (P90B/P91B) with IS1081, IS900 (150C/921) with IS1081 and F57 with IS1081. We have developed a diagnostic assay to identify *M. bovis* that improves upon previously published methods and can reliably identify *M. bovis* from bacterial culture or infected tissue. This assay can also differentiate between strains of *M. bovis*, which have been suggested to be associated with different rates of adverse events. The assay has a limit of detection of 1 pg *M. bovis* lysate DNA and was shown to detect *M. bovis* in both pure cultures and experimentally infected fecal samples. For internal validation, we spiked the pure milk samples with each mycobacteria for different CFU/gram concentrations $10^6$, $10^4$, $10^2$, and 10. The duplex PCR assay was tested on the isolated DNA from all the spiked milk samples and LOD for the assay was suggested best upto $10^2$ CFU/gram. The optimized Duplex PCR assay is simply implementable in laboratories with minimal resources and has an appropriate sensitivity. The high frequency of diseases with zoonotic origins necessitates the quick application of control measures.

## PIII.08: The State and Diagnosis of Animal Johne's Disease

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*Mycobacterium avium* subspecies *paratuberculosis* (MAP) causes irreversible Johne's disease (JD) in domestic animals, wild ruminants, and primates. MAP bacilli replicate in the intestinal mucosa and are expelled in feces, milk, semen, and vaginal secretions of infected animals. Infected moms' colostrum and milk, semen, and postpartum environment can pass on infection. Infants are highly vulnerable to illness and typically acquire infections throughout their early life through oral intake of contaminated food, water, or milk from their infected moms. Nonspecific symptoms include weight loss, weakness, and emaciation. Since these symptoms occur in older animals due to the extended incubation time, environmental stress, nutrition, concurrent diseases, production conditions, and overpopulation can affect them. Clinical diagnosis is easy, but preclinical diagnosis is difficult. Infected animals without symptoms release MAP bacteria in their milk and dung, contaminating the environment and silently infecting additional herd members. The ‘silent transmission' phenomenon lasts long before it is eliminated from animal groupings. Breast milk from MAP-infected mothers, pasteurized milk, and baby formula contain live MAP.MAP is found in several cattle species and regions. Information on this topic is scarce, especially in underdeveloped nations. The lack of locally developed tests and kits is the main cause. Many classical and molecular assays have been developed and used. For key antigen detection, microscopy, culture, indirect FAT, IS900 PCR, IS1311 PCR_RE, Taqman probe test, and Sybrgreen RT_PCR are used. LAT, indirect, and dot ELISA are antibody detection standards. Requirements and goals determine screening and confirmation test selection. Multiple tests are advised for persistent infections.The classic 'Test and cull' approach proved ineffective in controlling the transmission of disease among animal herds and flocks. JD (MAP bio-load) in domestic livestock must be managed and eliminated immediately through broad and diverse study. Since it is incurable, this is essential to prevent human infection.

**Keywords:** Paratuberculosis, Domestic livestock, Bio-load, Milk and milk products.

**Invited Talk**

# *Mycobacterium avium* subspecies *paratuberculosis* – an important food borne pathogen of high public health significance with special reference to India

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***Abstract***

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) is an important pathogen transmitted through food and associated with serious public health consequence. The causative agent of Johne's disease (paratuberculosis), a chronic granulomatous enteritis in ruminants, translates to substantial economic losses for the livestock industry. Not only in veterinary terms, but MAP also draws increasing association with Crohn's disease in humans, thus raising the concern of its zoonotic potential. Another reason that has made this pathogen more important in food safety is its capacity to withstand pasteurization and remain viable in the food product, both dairy and meat products.

India houses one of the highest consumption rates of dairy products in the world, which makes it a danger zone for MAP. The pathogen has been reported in most studies to have attained broad prevalence in domestic livestock, especially cattle, buffalo, goats, and sheep. This organism has been detected from raw milk, pasteurized milk, and milk products, which suggest possible routes of transmission to humans. Poor farm hygiene, unregulated milk processing, and lack of systematic screening worsen the threats of MAP contamination. Unfortunately, there is also no robust compulsory surveillance program against this and, coupled with the complexity of MAP infection diagnostics, makes it underreported and misconceived in animals and humans.

Immediate magnitudes for establishing strong diagnostic tools, effective food safety regulations, and control measures are awaiting their mitigation for impact. Continued efforts toward improvement in surveillance programs and increasing awareness about the zoonotic potential of MAP are important steps to ensure the health of both livestock and humans in India.

## OIV.01: Network inference of gut microbial communities in a multiple sclerosis cohort with Mycobacterium avium subspecies paratuberculosis infection

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### Abstract

In gut ecosystems, there is a complex interplay of biotic and abiotic interactions that decide the overall fitness of an individual. Divulging the microbe-microbe and microbe-host interactions may lead to better strategies in disease management, as microbes rarely act in isolation. Network inference for microbial communities is often a challenging task limited by both analytical assumptions as well as experimental approaches. Even after the network topologies are obtained, identification of important nodes within the context of underlying disease aetiology remains a convoluted task. In this study, we present a network perspective on complex interactions in gut microbial profiles of individuals who have multiple sclerosis (MS) with and without *Mycobacterium avium subspecies paratuberculosis* (MAP) infection. Our exposé is guided by recent advancements in network-wide statistical measures that identify the keystone nodes. We have utilised several centrality measures, including a recently published metric, Integrated View of Influence (IVI), that is robust against biases. The ecological networks were generated on microbial abundance data (n=69 samples) utilising 16S rRNA amplification. Using SPIEC-EASI, a sparse inverse covariance estimation approach, we have obtained networks separately for MAP+, MAP- and healthy controls (as a baseline). Using IVI metric, we identified top 20 keystone genera and regressed them against covariates of interest using a generalised linear latent variable model (GLLVM). Our analyses suggest *Eisenbergiella* to be of pivotal importance in MS irrespective of MAP infection. For MAP+ cohort, *Pyarmidobacter,* and *Peptoclostridium* were predominately the most influential genera, also hinting at an infection model similar to those observed in Inflammatory Bowel Diseases (IBDs). In MAP- cohort, on the other hand, *Coprostanoligenes group* was the most influential genera that reduces cholesterol and supports the intestinal barrier. The identification of keystone species, their co-occurrences, and associations with the exposome (meta data) advances our understanding of biological interactions through which MAP infection shapes the microbiome in MS individuals, suggesting the link to the inflammatory process of IBDs. The associations presented in this study may lead to development of improved diagnostics and effective vaccines for the management of the disease.

# OIV.02: Empowering Learning Through Student-Created Study Materials: Enhancing University Students' Understanding of *Mycobacterium avium* subsp. *paratuberculosis* Infections

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## Abstract

This paper presents the significance of university students creating their study materials as a component of their academic assignments on ruminant medicine, including *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infections, which are subsequently evaluated. By engaging in the process of writing their notes, summaries, and study guides, students can achieve a deeper comprehension of this complex subject matter. This method encourages active learning, critical thinking, and the ability to synthesize information. It enhances retention and recall of studied information, as rephrasing and organizing information aids memory consolidation. Evaluating students-created study materials not only provides educators with insights into students' understanding and learning processes but also motivates students to take a more active role in their educational journey. By examining educational research and my own experience, it can be concluded that incorporating student-generated study materials into the curriculum is an effective approach for promoting academic success and cultivating lifelong learning skills, particularly in understanding the intricate details of MAP infections. It resulted in slightly better grades for the class. The downside of this approach is additional work for teachers, as they need to evaluate the quality and accuracy of the student-created study materials. Further research and an extended evaluation period are necessary to determine if this approach provides sufficient benefits to be integrated as a permanent component of the study course.

## PIV.01: Surveillance of Paratuberculosis in Alpine Red Deer (*Cervus Elaphus*) in Northern Italy

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**Abstract**

In Alpine habitats, the risk of cross-transmission of pathogen between domestic and wild ruminants on summer pastures is of particular concern. In Italy, the Stelvio National Park has played a key role in health surveillance for paratuberculosis, as the first cases of this disease in wildlife were found in red deer in the early 1990s. Subsequently, the prevalence of paratuberculosis in red deer was estimated and recently showed a decreasing trend from 20.2% (2011-2015) to 5.9% (2018 and early months of 2022).
The aim of this study was to verify the decreasing trend in the prevalence of *Mycobacterium* avium subsp. paratuberculosis (MAP) in red deer, in the context of the European Animal Legislation which extended surveillance to both domestic and wild ruminants. This study is part of a larger research project conducted in the Lombardy region (Park and neighbour area) to define the role of red deer in the epidemiology of paratuberculosis.
In this area (9,600 ha), the red deer population counts approximately 2,300 animals. Taking advantage of hunting, culling activities and passive surveillance during the autumn of 2022 and 2023, individual animals were examined for signs of disease or lesions, and faecal samples were collected and then analysed by IS900-qPCR for MAP detection.
Four out of 372 individuals examined tested positive for MAP, giving a prevalence of 1.1% (95% CI 0.4 – 2.7). These data, together with the absence of clinical signs of paratuberculosis and gross lesions in the carcasses examined, support the presence of animals in the early stages of the disease and with subclinical infections.
Moreover, the low prevalence trend in the recent years and the low environmental MAP load in red deer faeces, already observed in a previous study, suggest this species plays a marginal role in the maintenance of MAP in this Alpine area.

# PIV.02: The Effect of Environmental Conditions, Nematodes, and Gut Microbiome Species- Composition, on the Transmission of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) Infections in Sheep (*Ovis aries*)

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## Abstract

Johne's Disease, caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), affects the intestine of ruminants. It is also known to live within the environment, including in soil and water, for extensive periods of time (years), presenting a challenge to farmers when considering management strategies. Despite efforts to combat Johne's, we have a limited understanding of how environmental factors affect MAP transmission within a farm setting. Given the longevity of MAP persistence within soil and water, a better understanding of MAP's environmental preferences, along with an understanding of MAP infection within livestock is crucial to disease control/management. This study aims to provide a comprehensive overview of MAP distribution on-farm via environmental and animal sampling.

A range of samples were collected from five independent farms across Scotland over a one-year period. The sampling included rectal-fecal (x700 in total) which was used for DNA extraction, gut microbiome analysis, and fecal egg count/Nemabiome analysis. In addition, environmental samples including water (x40), soil (x300), and sock (x120) samples were also collected. All samples were then processed and quantified via an optimized multiplex qPCR assay, targeting IS900 and F57 to identify and quantify the presence of MAP within these samples.

The qPCR assay was validated using known positive and negative samples, alongside real-world samples. The validated assay was used to determine the presence and absence of MAP across all collected samples. The outcome of which, alongside nematode co-infection and microbiome data, will be used to inform a generalized linear mixed model to predict factors of disease.

This study aimed to better understand the contribution of both environmental and animal factors in the spread of Johne's, with the overall goal of providing a more comprehensive understanding of MAP longevity and persistence. This understanding can then be utilized to develop and implement better control strategies in a real-world setting.

# PIV.03: Screening of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in a Highly Threatened Species of *Camelus bactrianus* (Double Humped Camel) in the Temperate Nubra Valley of the Himalayas

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**Abstract**

Johne's disease is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a chronic granulomatous enteritis of domestic and wild ruminants. The study aimed to estimate the bio-load of MAP infection in the highly threatened species of *Camelus bactrianus* (Double Humped Camel or DH camel) found in the Nubra valley located in the 'temperate region' of the Himalayas (Ladakh). Of the total camel population in the world, there are 94.0% single-humped camel and only 6.0% are DH camels limited to the Asian continent. Of these, 304 DH camels (150 males and 154 females) are left in Nubra Valley, where temperature ranges from -40$^0$C to 35.0$^0$C. In our earlier studies, we have reported the incidence of MAP in the wild animals (Mithun, hog deer, blue bulls, bison) living in the sub-tropical regions of the country. Our other studies also reported a high bio-load of MAP (32.7, 20.1, 23.8 and 39.3% in sheep, goats, buffaloes, and cattle, respectively) in domestic livestock. Randomly 70 fecal samples were collected from the herd of animals that did not exhibit clinical symptoms of MAP. On screening of samples against MAP infection, 21.4, 5.7, 32.8, and 20.0% were positive for MAP infection by microscopy, *IS900* PCR, Taqman probe qPCR, and fecal culture, respectively. On bio-typing using IS1311_PCR_RE, MAP isolates belonged to, 'Indian Bison Type'. It showed the wide distribution of 'Indian Bison Type, cutting across, species, wild and domestic animals, and geographical region. Along with MAP infection, 22.9% of animals were also positive for Cryptosporidium, which causes immune suppression, leading to the progression of MAP infection in the body. These asymptomatic animals were a source of infection to other DH camels and other domestic and wild ruminants. Since they share grazing land with domestic and wild animals. This is the first report on the incidence of MAP infection in the Double Humped camel population located in the Nubra Valley.

## PIV.04: In Vitro Study of Interactions between *Mycobacterium avium paratuberculosis* and HERV-W derived-peptides with Human Pancreatic Islets: Implications for Type 1 Diabetes Pathogenesis.

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**Introduction:** Type 1 diabetes is a chronic autoimmune disease resulting from the destruction of pancreatic β-cells. Previous studies have shown that individuals with T1D, particularly at the onset of the disease, exhibit elevated antibody titers against peptides derived from Mycobacterium avium paratuberculosis (MAP) and Human endogenous retrovirus-W (HERV-W). However, the role of MAP and HERV-W in the pathogenesis of T1D remains to be elucidated. Human pancreatic islet was used to analyze the potential involvement of these two factors in beta-cell destruction. This in vitro model allows us to analyze potential interactions with beta-cells and also with different cell populations within the Human pancreatic islet. Our study aims to evaluate and compare protein transcripts by analyzing the whole secretome, collected post-treatment for 24h. Although, this would facilitate identifying and quantifying the proteins present, thereby providing valuable insights into the proteomic responses of the islets to the treatments. **Materials and methods:** Two highly immunogenic peptides (MAP 3865C125-133 and HERV-Wenv93-108) were chosen and tested on healthy human islets from a post-mortem donor. Human islets were seeded with a density of 120-130 islets per well in 12-well plates and were stimulated for 24 hours with 100.000 ng/mL of MAP and HERV-W derived peptides. The same plate also included a negative control of islets maintained in culture without conditioning and a positive control provided by cytokine cocktails comprising IL-1β and IFNγ. The supernatant, potentially containing proteins and the EVs secreted by the islets in response to the treatments, was carefully collected and preserved for subsequent proteomic analysis. **Results:** After a 24-hour conditioning period, an apparent decrease in viability of the treated islets is observed. MAP and HERV-W-treated islets show surprisingly similar cell viability to that observed with cytokines. This suggests these peptides probably exert effects comparable to cytokines on islet viability. We also documented the morphological characteristics of islets, where it is possible to appreciate the effects of the treatments that subject the islets to stress or disaggregation and death.

## PIV.05: Molecular Epidemiology of *Mycobacterium avium* subspecies *paratuberculosis* isolated from Captive Wild Animals

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**ABSTRACT**

The study was undertaken to understand the molecular epidemiological of *Mycobacterium avium* subsp. *paratuberculosis* in captive animals of Siddharth Garden Aurangabad Municipal Corporation Zoo, Maharashtra, India. A total 126 individual / pooled faecal samples (45 Carnivores, 24 Herbivores, 3 Omnivores, 21 Birds and 33 Reptiles) were examined by direct microscopy out of which 29 were found positive as acid fast bacilli. Samples positive under microscopy were further processed for confirmation by Real Time-PCR targeting IS*900* gene that detected 14 samples correctly, comprising of 2 Omnivores, 5 Carnivores and 7 Herbivores. Overall prevalence of MAP infection was 11.11% (66.66% Omnivores, 29.16% herbivores, 11.11% carnivores, 0% Birds and 0% Reptiles). Molecular typing and genetic diversity of 14 positive MAP isolates using IS1311 PCR restriction endonuclease analysis revealed all belonged to 'Indian Bison type', biotype. This study first time revealed presence of MAP infection in tigers from India and highlights, the silent interspecies transmission of 'Indian Bison type' biotype from herbivores to omnivores and carnivores. The findings of the present study highlight the need for implementing effective management practices that will be helpful to minimize spread and reduce burden of this chronic life-threatening disease in captive animal species.

**Keywords:** Captive Wild Animals, Molecular Epidemiology, *Mycobacterium avium* subsp. *Paratuberculosis,* Genotyping

# PIV.06: *Mycobacterium avium* subspecies *paratuberculosis* biotyping by using milk samples from infected large ruminants of Madhya Pradesh

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## ABSTRACT

Paratuberculosis or Johne's disease in large ruminants is caused by *Mycobacterium avium* subspecies *paratuberculosis*. Disease in large ruminants is characterized by chronic diarrhea, weakness and emaciation. Apart from the production losses, the presence of disease in animals and its unresponsiveness to common drugs significantly impact the economic condition of livestock owners. There are few reports on disease prevalencein the large ruminant population of Eastern Madhya Pradesh based on milk sample screening. However, the disease has been reported from the other parts of this central state of India. Milk samples fromthe large ruminant population of this area were investigated for the presence of *Mycobacterium avium* subspecies *paratuberculosis*(MAP). Microscopic examinations revealed the presence of acid-fast bacilli indicating possible involvement ofMAP bacilli. Infectionin large ruminants was confirmed at the molecular level by specific amplification of 413 bp product in the IS*900*PCR. MAP-specific amplification of 608 bp in the IS*1311* product followed by restriction endonuclease analysis revealed the infection of 'Indian Bison Type', biotype. The presence of MAP infection in milk samples is of public health importance. Hence, further studies are warranted on the livestock population of this area as well as the exposed human population.

# Improved control of Johne's disease in dairy cattle through advancements in diagnostics, testing and management of young stock

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## ABSTRACT

Johne's disease (JD; paratuberculosis) control programs have been regionally implemented across the globe, but few have successfully eradicated the pathogen (*Mycobacterium avium* subsp. *paratuberculosis* (MAP)) causing this disease. The limited success may partly be attributed to excluding young stock (calves and replacement heifers or bulls) from testing strategies aimed at identifying MAP-infected cattle. Young stock can shed MAP in feces and can have detectable MAP-specific antibodies in blood, as confirmed in experimentally and naturally infected cattle. Furthermore, MAP transmission causes new infections in young stock. Calves and heifers are often included in JD management strategies on dairy farms but excluded from conventional diagnostic tests due to a presumed lag between infection and detection of MAP shedding and/or MAP-specific serum antibodies. We summarize evidence of MAP shedding early in the course of infection and discuss promising diagnostics, testing and management strategies to support inclusion of young stock in JD control programs. Improvements in fecal Polymerase Chain Reaction, interferon-gamma release assay (IGRA), and enzyme-linked immunosorbent assay (ELISA) enable earlier detection of MAP and specific early immune responses. Studies on IGRA and ELISA have focused on evaluation of new antigens and optimal age of testing. There are new diagnostics, including phage-based tests to detect viable MAP, and gene expression patterns and metabolomics to detect MAP-infected young stock. In addition, refinements in testing and management of calves and heifers may enable reductions in MAP prevalence. We provide recommendations for dairy farmers, researchers, veterinarians, and other stakeholders that may improve JD control programs with an objective to control and potentially eradicate JD. Additionally, we have identified the most pressing gaps in knowledge that currently hamper inclusion of young stock in JD prevention and control programs. In summary, transmission among young stock may cause new MAP infections, and appropriate use of new diagnostic tests, testing and management strategies for young stock may improve the efficacy of JD control programs.

## OV.05: CONTROL OF JOHNE`S DISEASE: A CALL FOR SCIENTIFIC RENEWAL

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**Abstract**

In the absence of a definitive solution, this is a call to explore a new approach to combating Johne's disease (JD). The proposed renewal is based on the recognition of the multifactorial nature of infections with *Mycobacterium avium* subsp. *paratuberculosis* (MAP).

Various efforts have been made to control JD. Extensive research has resulted in a shift from the individual animal to an integrated approach, including biosecurity and business and risk management, but a fundamental and sustainable approach is still lacking.

To be able to reduce the incidence and impact of JD, we must approach a dairy farm as an integrated system with complex relationships between external and internal factors. Genetic susceptibility is an example of the latter. External factors for instance related to competition, overstocking, housing facilities or ambiguity in the ranking order due to a frequent change in herd composition, can cause variation between individual circadian patterns within a herd. It has been shown that this can lead to a lower resilience, defined as the ability of animals to be minimally affected by challenges that can cause disease and, if affected, to recover quickly.

A combination of disease resistance, tolerance and robustness form disease resilience in animals when faced with MAP. Housing and management factors that better meet the natural behavioural and physiological needs of cows, can be considered as important factors to enhance the irresilience for MAP.

The conclusion of this review is a call for scientific renewal to identify and quantify the management practices which can be used as indicators of resilience to MAP.

# OV.02: The UK National Johne's Tracker Database 2010 to 2023.

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**Abstract**

The National Johne's Disease (JD) Tracker Database, which was established in Autumn 2023, is an anonymised InterHerd+ database containing milk recording and IDEXX milk ELISA data for ~2000 herds which test on a quarterly basis. This study describes the trends in 'JD parameters' from 2010 to 2023 and current distribution of JD amongst herds. Within this database, the JD Tracker tool calculates a series of 'JD parameters' including indicators of new infections, chronic infections and the management of chronically infected cattle.

From 2010 to 2023, the median milk ELISA average test value (ATV) decreased by 25% from 7.6 to 5.7. The median within-herd prevalence (based on a cut-off of 30) reduced by 60% from 4.9% to 2.0%. The median proportion of milk ELISA tests >60 decreased by 75% from 2.5% to 0.6% and median proportion of milk ELISA tests >100 decreased by 86% from 1.3% to 0.2%. The median proportion of J4 (newly infected) cows improved by 61%, from 2.7% to 1.0%. The median proportion of J5 (chronically infected) cows improved by 42% from 2.6% and 1.5%. The median proportion of priority cull cows (1 test >100 or 2 consecutives tests >60) improved by 70% from 1.9% to 0.6%. The relative risk of J5 cows being served or culled improved up to 2022, but data for 2023 cannot be calculated until summer 2024.

Milk ELISA positive cows are not equally distributed amongst the herds. When herds are ranked by ATV, quartile 4 herds (the 'worst' 25% of herds) contain 53.9% of the Johne's positive tests, which is equivalent to 8.9 times more JD positive cows than the 'best' 25% of herds. All JD Tracker parameters within the National JD Tracker Database have improved but the burden of JD is disproportionately greater in herds with higher ATVs.

# OV.03: Phase 3 of the UK National Johne's Management Plan (NJMP)

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## Abstract

In Great Britain, Phase 1 (2015 to 2017) of the National Johne's Management Plan (NJMP) focused on education and engagement. Phase 2 (2018 to present), requires British Cattle Veterinary Association Johne's Certified Veterinary Advisors and farmers to conduct an annual risk assessment, examine the herd Johne's status and agree a management plan. In 2019, participation in the NJMP or an equivalent scheme became mandatory for dairy farms within the Red Tractor Assurance scheme, so management plans are now completed by >11,000 UK dairy farms. Following the development of the National Johne's Tracker Database in Autumn 2023, the industry group Action Group Johne's are developing Phase 3 of the NJMP.

The National Johne's Tracker Database is an anonymised database containing milk recording and milk ELISA data for ~2,000 herds which test on a quarterly basis. Since the launch of the NJMP in 2015, the median within-herd prevalence has decreased by 64% from 5.5% to 2.0% and median proportions of newly infected cows and chronically infected cows have dropped by 60%. However, milk ELISA positive cows are not equally distributed amongst the herds. When ranking herds according to their milk ELISA average test value (ATV), the 'worst' 25% of herds contain 53.9% of the Johne's positive cows, which is equivalent to 8.9 times more JD positive cows than the 'best' 25% of herds.

To effectively control Johne's at a national level, herds at all levels of prevalence need to be engaged. In response, Action Group Johnes are introducing an 'aspirational goal' for UK farmers as part of Phase 3 of the NJMP. With support from milk processors and retailers, farmers will be asked to aim for a specified herd-level milk ELISA ATV. The value of the 'aspirational goal' is under consideration by Action Group Johne's and will be announced in Autumn 2024.

# OV.04: Successful control of paratuberculosis in a dairy herd with high initial prevalence – a case study

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## Abstract

Due to the high tenacity of MAP, the limited sensitivity of diagnostic tests and the long incubation period, successful elimination of MAP from a cattle herd is doubted, particularly in case of high initial MAP shedder prevalence. This longitudinal case study provides an in-detail report of the process towards a zero MAP shedder prevalence in a closed 450 head commercial dairy herd. According to the regulations of the regional voluntary paratuberculosis control program, the herd was certified as non-suspect of having paratuberculosis in 2022. In the study herd, annual individual fecal culture, and MAP-antibody ELISA were applied in parallel on all cows annually. Half of the testing costs were subsidized by the program fund. For each annual sampling, the kappa coefficients for test agreement, and the survival rates of MAP positive and MAP negative cows were calculated. Applying a multivariable linear regression model revealed significantly lower fat corrected 305 days milk yield for MAP positive cows. True prevalence of MAP shedders reduced from 24.2 % in 2012 to 0.4 % in 2019, and in 2020-2022 no MAP shedder was identified suggesting successful elimination of the infectious agent. Test agreement was substantial in the initial year (kappa = 0.46) and low in the following years. Fecal culture showed positive results earlier than the ELISA. In the first years of control, survival of MAP shedders was longer than in the final stage. In conclusion, elimination of MAP from a dairy herd might be feasible within a decade. Changes in test agreement must be considered. Closing the herd, the timely removal of MAP excreta, hygiene in calf rearing and in colostrum supply are key. Voluntary control programs are effective to control paratuberculosis in closed herds if an adequate diagnostic, logistic and financial support is provided for farmers.

# OV.06: Immunoinformatics Approaches for Designing Paratuberculosis Antigen Vaccine Candidates

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## Abstract

Due to challenges in controlling infection and limitations of existing vaccines, paratuberculosis has become a worldwide disease among ruminants. Till date, there are no ideal antigen candidates to deal with *Mycobacterium avium subsp. paratuberculosis* as an etiological agent of John's disease. So, the purpose of the present study was to use immunoinformatics approaches for designing efficient and secure antigens with the capability of stimulating both arms of adaptive immunity. In the first step, the conserved and hydrophilic parts of MAP2191 and FAP-P proteins were determined, and the antigenicity and solubility properties of the candidate sequences were predicted. The sequences were evaluated in terms of various physicochemical properties. 3D structures of selected parts were predicted via homology modeling. Then, the sequences were screened to detect cytotoxic T-lymphocyte (CTL) and Helper T-lymphocyte (HTL) epitopes. Also, linear and discontinuous B-cell epitopes of ht-MAP2191 and ht-FAP-P were identified. Eventually, predicted protein constructs were evaluated for molecular docking simulations against TLR4. Initial assessments demonstrated that ht-MAP2191 and ht-FAP-P are immunogenic, highly water-soluble, non-toxic, and non-allergenic. A total of 25 and 18 CTL epitopes were generated for ht-MAP2191 and ht-FAP-P, respectively. Also, 36 and 14 epitope profiles were generated for MHC class II. It was found that ht-MAP2191 and ht-FAP-P possess potent linear and conformational B-cell epitopes. Besides, ht-MAP2191 and ht-FAP-P showed a strong binding affinity with the TLR4 in the molecular docking process. According to the results, this in silico analysis suggests that ht-MAP2191 and ht-FAP-P are potentially able to evoke cellular and humoral immune responses to combat paratuberculosis effectively. Still, in vitro and in vivo assays are needed to validate these outcomes.

## OV.06: The management and control of Johnes disease in 12 commercial dairy herds using regular milk testing for antibodies to Mycobacterium avium subspecies paratuberculosis (MAP) and targeted risk management.

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**Abstract**

12 commercial dairy herds of varying size (comprising between 85 milking cows and over 1000 milking cows) were engaged in a Johnes management and control programme to reduce the prevalence of Johnes disease in the herd, as part of the UK National Johnes Management Plan. Each of the farms used a quarterly testing and risk management programme for more than ten years as a strategic approach to control, with varied success.

The worst affected herds had test prevalences of over 25%, with a mean starting test prevalence of 12.0% for all the herds in the study. Two herds managed to achieve complete success by reducing their test prevalence to 0%, whilst most achieved their objective of reducing test prevalence to less that 1% over the ten year duration of the study. 3 herds failed to control Johnes disease, resulting in no reduction of test prevalence or an increase in test prevalence over the ten years of engagement with the control programme.

Success was best achieved by the rigorous implementation of risk management systems that reduce the risk of neonatal infections along with a structured and comprehensive culling programme to reduce the infective load within the herd. Failure was associated with incomplete risk management and the retention of infected cows which became potential supershedders and superspreaders.

This paper will demonstrate the successes and failure of Johnes management using regular elisa testing for MAP using milk samples, and the adoption of specific risk management systems to prevent new infections.

## OV.07: Effect of Manure Processing Method on Presence of *M.avium* subsp. *paratuberculosis* in Recycled Manure Solids Beddingon Midwest US Dairy Farms

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While many US dairies use green (GRN) recycled manure solids (RMS) bedding, some first process slurry through an anaerobic digester (DIG), and others use secondary (SEC) processing methods such as mechanical composters, hot air dryers, or infrared drying in an effort to lower mastitis pathogen counts in ready to use (RTU) solids. These processing methods may also reduce other pathogens in RTU solids. The objective of this study was to investigate the relationship between use of processing methods and *M. avium* subsp. *paratuberculosis* (MAP) in RTURMS. Twenty-seven dairies in Minnesota and Wisconsin were visited once in summer 2021 to collect slurry and bedding samples before and after each processing step within the system and samples were submitted to the Wisconsin Veterinary Diagnostic Lab for MAP detection (liquid culture with PCR confirmation). Farms were categorized into four system types: GRN (n=6), DIG only (n=9), SEC only (n=5), or DIG+SEC (n=7). Logistic regression was used to compare the odds for MAP positivity in initial versus final RTU RMS samples within each of the four system categories. For MAP, 80%,68%,60%, and58%of initial raw slurry samples, and 40%, 0%, 20% and 0% of finished RTU solids samples were MAP-positive, for GRN, DIG,SEC, or DIG+SEC systems, respectively. When evaluating individual processing steps within a system, the odds (95% CI) for MAP detection in a post- (vs pre-processed) sample varied in magnitude and significance depending on the step evaluated. Despite small sample sizes for some systems, results show that either DIG or SEC processing can result in a substantial numerical, if not statistical, reduction in risk for MAP detection. DIG alone cannot be counted upon to eliminate MAP, but no MAP was detected in RTU solids when a combination of DIG plus SEC processing was used.

## OV.08: A novel Vaccine Candidate for the Control of MAP Infection in Small Ruminants

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**Abstract**

Paratuberculosis is a crippling, chronic and incurable disease of goats and sheep worldwide leading to heavy losses (decreased milk production, progressive loss of weight, reduced fertility, rough hair coat, skin pliability is lost, feces loose / hard etc.). Vaccination is one of the most effective measures to control *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infections. For the effective control of Caprine Johne's disease (CJD), we evaluated the protective potential of the new subunit vaccine using truncated Mce protein in goat model. In silico epitope prediction, mapping the whole length of the MAP2191 protein, revealed potential T and B-cell epitopes in the C-terminal portion of the protein. Novel Mce-truncated protein encoded by the selected region of the MAP2191 gene was expressed, purified using Ni-NTA gel matrix, and confirmed by SDS PAGE and western blot. Six healthy goat kids were immunized with the Mce-truncated protein, while two were kept as controls. All kids were orally challenged twice with live MAP strain, and shedding of MAP was monitored in tissues to know status of MAP infection.

There was significant increase in the humoral immune response against Mce protein in vaccinated goats as compared to the control group. Vaccinated goats exhibited higher body weights, and none of them shed MAP or showed histopathological lesions or colonization of MAP in tissues. Study demonstrated significant immunity in goats, as they successfully faced the challenge with live MAP bacilli. Though vaccine exhibited high potential as vaccine candidate, however, large scale trials are will help to release the vaccine for CJD control programs in Iran and elsewhere.

## OV.09: Paratuberculosis case definition by the WOAH

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**Abstract**

The World Organization for Animal Health (WOAH) was founded just one century ago as the Office International del Epizooties (OIE) with the goal of facilitate animal trade by controlling animal infectious diseases. At that time, the causative agent of paratuberculosis, Mycobacterium avium subsp. paratuberculosis (MAP) had just been isolated in cattle and the disease had been recognized also in sheep. Since then, it has been reported from all the continents and countries where it has been investigated. Starting in the 1960s the knowledge of the disease has been growing and its economic impact has led to develop diagnostic and control measures in many countries as a recent review has shown. The WOAH has always recognized the disease and issued guidelines for diagnostic and control (Terrestrial code and Terrestrial manual), but until now had not adopted a case definition like in other diseases as a common base to standardize disease country reporting. Currently, the WOAH tools for paratuberculosis incidence monitoring show a globally stable but highly variable country outbreak dynamics that is at odds with the paratuberculosis research and control national efforts reflected in the veterinary scientific literature. WOAH records go back to 1996 and show that, even though paratuberculosis is repeatedly recognized in the literature to have a worldwide distribution, only 10 countries reported at least one outbreak in 20 or more years out of 28. Of these, only three countries Spain, Japan and Germany account for most of the world declared outbreaks (72%). In its February meeting, the WOAH Scientific Commission supported the Secretariat proposal to prioritize in this year the case definition for the two main ruminant slow infections still lacking it: paratuberculosis and small ruminant lentivirus infections. We hope that this will spread paratuberculosis awareness, improve reporting and help reduce its impact.

# OV.10: Rise and decline of Johne's disease Market Assurance Programs (MAPs) in Australia

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## Abstract

This paper collates data from Australia's Animal Health Surveillance Quarterly to chart farmer participation in SheepMAP and CattleMAP, and compares fluctuations against key developments, concerns, and impacts of risk and controls. The national approach to Johne's disease (JD) management from 1995 was initiated by industries strongly motivated to control disease and coincided with development of Animal Health Australia, an alliance of governments and industries. Cattle MAP was an early initiative of this collaboration, facilitating voluntary, market-driven risk mitigation through objectively identifying sources of low-risk dairy and beef breeder cattle. Cattle MAP gained immediate acceptance and uptake, encouraged by subsidised testing. Sheep MAP followed one year later, driven by concerns over mortality and productivity impacts of infection and variable regulatory approaches between jurisdictions. Goat MAP and Alpaca MAP followed. MAPs are based on sound epidemiology, incorporating biosecurity management and herd testing. They embraced technical innovations and flexibility to meet user needs, improve testing and reduce costs. Adopted primarily by pedigree herds and flocks, they demonstrate high health standards, and also reduce risks of infection spread between zones and at shows and sales. JD programs may impose adverse impacts, sometimes severe, on farms regarded as high-risk due to actual infection or by association with geographic location or industry sector risks. Stakeholders increasingly perceived these impacts as exceeding the benefits of standardised control and management, especially for cattle seedstock producers who saw little clinical impact and for sheep producers once vaccination became established as a cost-effective mitigation tool. Sheep MAP includes provision for vaccinated flocks, and continues to be valued by some sectors of the sheep industry although participation has halved from its peak. In contrast, cattle industries selected individual farmer risk management rather than maintaining a MAP. Australia leverages the learnings from the MAP narrative to build networks, capacity and capabilities in livestock biosecurity for the future.

# PV.02: The probability of freedom from the disease as a measure for assessing and managing the risk of MAP introduction into cattle herds

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## Abstract

The control of paratuberculosis at farm level is lengthy and, depending on the diagnostic method, also cost-intensive. The voluntary Thuringian Paratuberculosis Control Programme (TPCP) provides cattle farmers a pathway to eliminate the infectious agent *Mycobacterium avium* ssp. *paratuberculosis* (MAP) from the herd and certify the herd as non-suspect of having paratuberculosis if no MAP had been detected for at least three years. For this purpose, an annual faecal culture testing of all adult cattle was necessary. Since 2023, monitoring of certified herds relies on sufficiently high probability of freedom from paratuberculosis ($P_{Free}$) as determined by various diagnostic approaches including analysing pooled samples for MAP and MAP antibodies. An essential element of this monitoring concept is the control of animal traffic into the herd.

In 2022, 148 farms in Thuringia were enrolled in the TPCP. At the end of this year, 63 (42.6%) farms were certified as "non-suspect", and a further 11 farms were in the certification phase to achieve this status. Considering the limited sensitivity of faecal culture testing and the program rule to close the herd at least from the begin of the certification phase onward, were calculated a $P_{Free}$ of 99% that had to be maintained by annual testing and facilitating the animal's movement requirements, as the incautious purchase of animals is the main cause of MAP introduction. During the first year of applying this approach, we observed that (i) the higher the sensitivity or the more repetitions of the diagnostic test at herd level, the higher the $P_{free}$ and, in contrast, (ii) the higher the risk of introduction via purchase, the lower the $P_{free}$.

The preliminary results suggest that monitoring of cattle herd certified as non-suspect of having paratuberculosis by $P_{Free}$ provides a sufficient level of confidence and enables farmers and program managers apply different monitoring approaches.

## PV.01: Using InterHerd+ to record Johne's Disease data in a multi-herd database.

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**Abstract**

In the UK, in addition to its use for day-to-day herd management, the InterHerd+ program is extensively used by technical advisers to benchmark herd performance and focus on-farm discussions to areas in need of improvement and subsequent management changes. Data are automatically downloaded from milk recording organisations. Parameters include, but are not limited to; milk yield and composition, somatic cell count, fertility events (such as; services and pregnancy diagnosis), vaccinations and health-related events (such as; Johne's Disease test results and lameness). Data from multiple herds are stored in a single cloud-based InterHerd+ database. Data can be accessed and analysed at individual or multiple herd-level, for example; at veterinary practice or milk-pool level.

The UK National Johne's Tracker database is a single anonymised database of ~2,000 dairy herds which regularly milk record and conduct whole herd IDEXX milk ELISA testing on a quarterly basis. It is an invaluable research resource which has allowed the tracking of Johne's Disease parameters (including within-herd prevalence), generation of benchmarks and development of a new 'aspirational standard' for UK farmers. InterHerd+ is used in Africa and Asia to improve the traceability of livestock and record events across multiple livestock systems. Users access a central database via mobile devices with the level of access controlled by their user permissions. The existing Johne's Disease analysis tools in InterHerd+ could easily be adapted and extended to extensive smallholder livestock systems. Currently, the InterHerd+ Johne's Disease analysis tool uses IDEXX milk ELISA data. However, data from other diagnostic tests and clinical observations could also be inputted. This would provide smallholders with a unique opportunity to monitor Johne's Disease transmission and prevalence, as well as assess the effectiveness of control plans.

## PV.04: The control of clinical and subclinical Johnes Disease in a large commercial dairy herd by targeted risk management

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**Abstract**

A large commercial dairy herd, comprising 1000 Holstein Friesian intensively managed dairy cows producing an average milk yield of 11,500 litres per cow per year, embarked on a Johnes management plan in 2010. The programme was prompted by an increasing incidence of clinical Johne's disease and growing evidence of subclinical disease such as high culling rates for poor yield, performance, loss of condition etc.

A testing programme comprising MAP elisa antibody testing of milk from all lactating cows every four months enabled a focussed risk management plan to prevent new infections, particularly directed at preventing infection spreading from high risk cows to calves and youngstock. Management procedures included the segregation of test-positive cows prior to calving, the immediate removal of newborn calves from high risk cows, the use of pasteurised colostrum and milk for calf feeding, and general improvements in hygiene, particularly around the pre-calving cows and neonates.

A rigid programme of accelerated culling of infected cows with clear rules to quickly remove animals that were considered at high risk of shedding, and to stop breeding from cows that were likely to progress to become shedders using the milk test results, was also introduced.

The test prevalence fell from a peak of 14.5% in 2011, to 1% in 2023, with no clinical cases since 2020. The rate of progress was measured by the proportion of the herd testing positive, the overall herd average test value, and the proportion of the herd with persistently high test values.

This paper demonstrates that the management and control of clinical and subclinical Johnes Disease can be successful in large dairy herds, using regular milk testing and targeted management procedures to reduce the infective load and minimise new infections within the herd.

## PV.03: Age of first time shedding *Mycobacterium avium* subsp. *paratuberculosis* and seropositivity in young stock on MAP-infected Alberta dairy farms

A. Caroline Pereira, Karin Orsel, Jeroen De Buck, Larissa Martins, Marit M. Biesheuvel, and Herman W. Barkema

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**Abstract**

Understanding shedding dynamics of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in young stock is crucial for JD management. Calves under 12 months also contribute to MAP spread within dairy herds. However, conventional JD control programs often overlook calves in testing strategies, potentially perpetuating MAP infection within herds. The study aimed to determine the age at which young stock (< 12 months) start shedding MAP and become seropositive in field conditions. Eight Alberta dairy farms that had at least one MAP-positive environmental sample were followed for 14 months. Serum ELISA and fecal qPCR (ISMAP02 gene) were used. Of a total of 679 young stock, 12% tested positive for MAP by qPCR, with 4% exhibiting positive ELISA results. Additionally, a single herd demonstrated a significantly elevated prevalence of shedding, with rates reaching as high as 56% among young stock. Notably, animals positive for ELISA did not correlate with fecal qPCR positivity. In herds with higher MAP prevalence, more young stock tested positive for MAP. Shedding of MAP in young stock occurred as early as 4 months with qPCR, whereas the earliest a calf became ELISA-positive was 3 months of age. Additionally, the utilization of pooled fecal qPCR targeting the ISMAP02 gene offers a cost-effective screening method for JD. Unlike traditional fecal culture, which is costly, and time-consuming, pooled fecal qPCR provides an economical approach to detect MAP shedding in young stock. Early identification is crucial for timely removal and reduced transmission risk. As animals age, positive cases increase, emphasizing continuous monitoring. The study provides vital insights to enhance JD control, stressing targeted sampling in young stock.

# PV.06: Longitudinal Study on *Mycobacterium avium* subspecies *paratuberculosis* Infection in Goats

**Attili Anna-Rita[1*], Toso Lucia[2], Desirè Ombrosi[2], Corradini Corrado Maria[3], Gigli Francesca[1], Galosi Livio[1], NguNgwa Victor[4], Cuteri Vincenzo[1]**

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**Abstract**

A longitudinal epidemiological study was conducted from May 2022 to May 2023 in three Italian goat flocks that tested positive for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection in a previous cross-sectional study carried out in Macerata Province (Marche Region). The aims were to assess: -the dynamics of immune response in blood, milk and bulk tank milk (BTM); -the dynamic of faecal excretion by qPCR; -the incidence trends of MAP infection; -the effects of a one year '*test and cull*' and biosecurity measures application on strongly shedders goats as control program of caprine Paratuberculosis; -the efficacy of the whole IDVet system for MAP analysis in faeces and the use of TPC-MAP as decisional threshold for estimation of culling priority. Goat population was stratified by age (young: ≤6 months; adult:6-24 months; elderly: ≥24 months), and sex to be screened at $T_0$, after 6 ($T_6$) and 12 ($T_{12}$) months. Blood (n=200), milk (n=114), and bulk tank milk (BTM: n=9) samples were collected for indirect diagnosis (ELISA-IDScreen®-Paratuberculosis, ID.vet, France), while faecal samples (n=402) were submitted to qPCR IS900 investigation (IDGene®-Easy-Preparation, and IDGene™-Paratuberculosis-Duplex, ID.vet, France). Blood-seroprevalence decreased from 8.29% ($T_0$) to 0.99% ($T_6$) to 0% ($T_{12}$). At $T_0$, $T_6$ and $T_{12}$, milk-seroprevalences of 22.22%, 4.26% and 4.48% were observed. From $T_0$ to $T_{12}$, MAP incidence rates decresead to 0% and to 3.77% testing blood and milk, respectively. The BTM confirmed the blood seronegativity observed in all flocks at $T_6$, while at $T_{12}$ the individual milk seropositivity of 12% (3/25) in one flock was revealed by BTM seropositivity (S/P% 17.128), although all goats resulted not strong/active MAP shedders at $T_{12}$. The study highlights a milk "fluctuating" antibody trend in blood seronegative and non-shedder goats. The use of PCR with TPC as a threshold, proved to be a useful tool to prioritize culling among seropositive animals.

## PV.05: Developing a Successful Johne's Disease Control Program within a Veterinary Practice

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**Abstract**

Creating a robust infectious disease control program in aveterinary practice requires a robust structure to ensure the aims of the programme are delivered. This paper describes a commonly used system used to manage Johne's Disease within the UK.
The process can be divided into 10 steps.

1. Agree on a database which can be used to manage test results, risks and control plans. The format can be adaption of existing software or use of third party application (Myhealthyherd.com).
2. Arrange a meeting of the whole practice team and agree the communication plans and approaches that will be used with farmers.
3. Develop a "~Johne's Disease Champion" who will lead the project.
4. Arrange educational events to raise awareness and stimulate interest within the farmers. Share successes with JD control and facilitate peer to peer learning amongst the farmers.
5. Follow up individual farmers after the event to explore their beliefs, relative priorities and appetite to commit. Share information visually using PowerPoint slides/ Webinars.
6. Undertake structured risk assessments and initial surveillance to estimate prevalence (e.g. targeted milk ELISA samples from 30 high risk cows).
7. Discuss and agree the most appropriate strategy for control based on farmers aims, resources and assessment of disease prevalence using the JD Tracker tool.
8. Develop an agreed control plan and ensure all farm workers understand the importance and aims of the programme.
9. Explain that disease may get worse before it improves. Celebrate successes and deliver positive feedback.
10. Review the risk assessment and control plan annually and ensure compliance with the agreed plan.

    Developing a proactive JD control plan process delivers more effective engagement and control. A systematic process is eminently more effective than a reactive approach based on problem herds.

## PV.07: Paratuberculosis in *Camelus dromedarius* (single-humped camel) in India

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**Abstract**

Paratuberculosis infects both animals and humans and is a major concern globally. Survival of bacilli in extremely harsh environments. The dromedary camel (*Camelus dromedarius)* is an important livestock of the desert ecosystem of tropical Rajasthan and Gujarat states. Camels are primarily used for draught purposes. The farmer's families consume milk, milk by-products, leather, and other by-products to add to the rural income. Studies in dromedary camel are limited in India. Prevalence in Oman and Saudi Arabia has been reported, at 9.1% and 16.1%, respectively. In Eastern Saudi Arabia, it was 15.0 and 16.1%, respectively, in ELISA and *IS900* PCR. A study from Gujarat reported a 33.3% prevalence. In the present study, of 88 fecal samples from different rural tribal areas of districts (Bikaner, Jodhpur, Sirohi, Nagaur), of Rajasthan, 46.6, 15.9, 28.4, and 20.4% of camels were positive for MAP infection in acid-fast bacilli, *IS900* PCR, SYBER Green qPCR, and fecal culture, respectively. Bio typing of MAP DNA using IS1311 PCR_REA all belonged to 'Indian Bison Type'. Clinical symptoms (diarrhea/weakness) were absent in positive Dromedary camels (sub-clinical positive). Therefore, early detection of MAP is crucial for the control of JD in camel herds and to prevent human infection. Since camel owners share a close relationship.

## PV.08: Out: a comparative study of *Mycobacterium avium paratuberculosis* in Sheep (*Ovis aries*) across Scottish farmland.

Charlotte Winspear[1], Lisa Avery[2], Dave Bartley[1], Andrew Free[3], Craig Watkins[1], and Eulyn Pagaling[2]

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The causative agent of Johne's Disease, *Mycobacterium avium paratuberculosis* (MAP) is a persistent issue in livestock globally, with several attempts to combat it producing an unclear picture going forwards. Many studies in the UK and Ireland focus upon cattle, with sheep not as widely researched, despite a similar problem and known issues around the world.

However, emerging evidence about the environmental preferences of MAP, such as its known persistence in soil and water; alongside several identified wildlife vectors and potential co-infection with parasitic nematodes, prompted investigation into connections between these aforementioned factors and disease incidence/prevalence, to provide a larger picture of exactly what is happening at the farm-level.

The study involved environmental (soil, water, and sock) and faecal samples from across selected farms in Scotland (UK), which were tested for MAP via QPCR, as well as faecal egg counts, soil chemistry analysis, and gut microbiome analysis comparisons. This data will then be analysed and compared across several different categories, in the hopes of finding correlations and possibly even predicting factors for disease.

The aim of this study is to gain a greater understanding of MAP's behaviour at its root on-farm, and how many different factors may influence its persistence. The problem of Johne's Disease is a multi-faceted one, and by approaching the problem at a slightly more holistic angle, we may ultimately find solutions for farmers and livestock where it is needed most.

# PV.09: Impact of COVID-19 pandemic on a livestock farmer and management of goats infected by *Mycobacteriumavium* subspecies *paratuberculosis*

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## ABSTRACT

Johne's disease (JD)/ paratuberculosis (pTB), is a chronic intestinal infection of domestic ruminants caused by *Mycobacteriumavium* subspecies *paratuberculosis* (MAP). India has a total goat population of 148.88 million and Madhya Pradesh stands fifth in the country with 11.06 million goats. The increase in the goat population of the state indicates the profound interest of the livestock farming communities in goat farming. MAP infection has been reported in various animal populations in the country including small ruminants. A livestock farmer in the Indore district in Madhya Pradesh noticed extreme debilitation in his goats and reported this condition to the author in the college at Mhow in late 2019. Acid-fast staining revealed acid-fast bacilli in fecal smears. Further examinations confirmed the outbreak due to MAP infection. Hence, on instructions the livestock farmer visited the Central Institute of Research on Goats to procure a vaccine (therapeutic and prophylactic). Upon vaccination, in one of the visits to the farm, a positive change in the bodily conditions of the animals was noticed. But soon COVID-19 pandemic struck and the livestock farmer had to dismantle the farm due to difficulties related to the pandemic. The livestock farmer suffered psychologically and had to undergo treatment. In such a pandemic scenario, it was tough to keep the farm alive.

# PV.10: Microscopical examination of fecal samples of cattle at Rewa in Madhya Pradesh detected mixed infection of *Mycobacterium avium* subspecies *paratuberculosis* and *Cryptosporidium* species

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## ABSTRACT

Paratuberculosis (pTB) or Johne's disease (JD)is a contagious disease of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis*(MAP). The diseaseis characterized by chronic diarrhea, weakness and emaciation in ruminants. Apart from the production losses, the presence of the disease in animals and its unresponsiveness to the common drugs are also big challenges. These factors significantly impact the economic condition of livestock owners. Fecal samples from cattle (animals healthy and those with a known history of diarrhea) in this area were investigated for the presence of MAP. Microscopic examinations revealed the presence of acid-fast bacilli indicating possible involvement of MAP bacilli with the disease. Along with MAP, most cattle fecal samples detected positive for the presence of typical red-colored eggs of *Cryptosporidium* species. Further investigations are ongoing for molecular detection, isolation and typing of MAP from domestic livestock in this area. The main purpose of this study is to know about the pathogens causing diarrhea in cattle in this region to make a strategy to control the spread of the infections to other species.

**Invited Talk**

# Epidemiology of *Mycobacterium bovis* induced Zoonotic tuberculosis in India

**Dr. Ajay Vir Singh**

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## Abstract

Zoonotic tuberculosis (zTB), caused by *Mycobacterium bovis* and *Mycobacterium orygis*, is an emerging public health concern in India. While *M. bovis* primarily infects cattle and can transmit to humans through the consumption of unpasteurized dairy products and direct animal contact, *M. orygis* has been increasingly identified as a zoonotic pathogen with a broad host range, including domestic and wild animals. India, with its high burden of tuberculosis and a large cattle population, faces significant challenges in the surveillance and diagnosis of zoonotic tuberculosis. However, limited epidemiological data make it difficult to estimate the true prevalence of these zoonotic *Mycobacterium* species in human tuberculosis cases.

Despite advancements in molecular diagnostics, differentiation between *M. tuberculosis* and *M. bovis*/*M. orygis* in routine tuberculosis testing remains inadequate, potentially leading to underreporting of zTB. Studies suggest that *M. bovis* accounts for a small but significant proportion of extrapulmonary TB cases in India, particularly among populations with occupational exposure to livestock. The role of *M. orygis* in human TB remains less understood but is increasingly being recognized in South Asia. Strengthening diagnostic capacities, implementing stringent disease control measures in livestock, and enhancing public awareness regarding the risks associated with zoonotic transmission are essential for effective disease management.

This study highlights the need for systematic surveillance and research on *M. bovis* and *M. orygis* in India to better understand their epidemiology, transmission dynamics, and public health impact, ultimately aiding in the development of targeted intervention strategies.

**OVI.01: Isolation and identification of IS*900*qPCR positive Mycolicibacterium species from human blood and ascites that are not MAP.**

✓ **Tim J Bull**, Ken Haines, Bina Makwana, Metin Yalcin, Dan Forton, Adam Witney.

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**Study**
Evaluation of IS*900* mycobacteriophage plaque assay plus a novel growth resuscitation medium (TiKa14D) to determine mycobacterialprevalence in the blood/ascites of patients with liver disease.

**Methods**
Whole blood/ascites, from 60 patients with and without liver diseasewere split equally. Half screened for IS*900* usingmycobacteriophage plaque assay. Half decontaminated with TiKa-Kic, incubatedin TiKa14D resuscitation medium theninoculated onto TiKa14D solid media. Sub-cultures were re-tested for IS*900*by Taqman qPCR. Colonieswith strong IS*900* positive PCR were confirmed by MAP specific PCR targeting mptD (MAP3733c). Coloniesshowing weak IS*900* signals were further speciated using whole genome sequencing (MinION).

**Results**
IS*900*PCR positive mycobacteriophage plaques were detected in 23/60 WBC samples and 2/9ascites. 10 WBC with high IS*900* positive plaque counts, grew MAP. 3WBC and 2 ascites withhigh plaque counts but weak IS*900*plaque positive signals, grew smooth uneven edged white colonies containing long acid-fast bacilli, weakly IS*900* positive, visible on TiKa14D media within 5 days identified as *Mycolicibacterium aubagnense* (Maub) by WGS. Alignments to Maub reference genome showed gene deletion regions and absence of known plasmidssuggesting a novel variant. Maub isolate genomesincludedIS*110*-family transposable elements (which includes IS*900*).

**Conclusions**
TiKa14D resuscitation media promoted MAP isolation from blood samples of patients with liver disease that showedhigh counts of IS*900* PCR positive mycobacteriophage plaques. The presence of *Mycolicibacterium aubagnense* variants in some samples generated high plaque counts but onlylow IS*900*qPCR positive amplification.MAP studies should be wary of relying on single target molecular detection methods.

# OVI.02: Enhancing Paratuberculosis Diagnosis: Novel Universal Reference Material and Direct Digital PCR Method for Precision Livestock Management

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## Abstract

*Mycobacterium avium Paratuberculosis* (MAP) causes paratuberculosis, a chronic disease mainly affecting ruminants. Transmitted fecal-orally, it leads to chronic diarrhea and weight loss, impacting productivity and health. It is generally accepted that a bacterial quantity exceeding $10^4$ MAP/gram of feces indicates active infection; below this, passive shedding is presumed. This data is crucial for controlling and managing this disease through a regular herd surveillance and accurate diagnostic methods like quantitative PCR (qPCR). Currently, most existing qPCR assays target the IS900 gene, which does not allow precise quantification due to the variable number of gene sequences across different strains, potentially leading to diagnostic errors.

To enhance the paratuberculosis diagnosis, BioSellal collaborates with Labocéa (Quimper, France) to validate newly developed digital PCR (dPCR) and qPCR workflows using a quantified Reference Material (RM). It consists of spiked and lyophilized feces quantified by dPCR in copies of F57 per gram. These workflows leverage the MAP F57 monocopy gene and allow the analyzes on both fecal and environmental samples. The study includes the use of qPCR and dPCR kits, which are currently under development and will be validated following the specifications of French regulations NF U47-600 and the MIQE guidelines.

Development of the RM and two quantification methods is ongoing: a direct dPCR method quantifying IS900 and F57, and a relative quantification RM-based qPCR method with the same targets. Initial results show significant advantages in combining IS900 for sensitivity and F57 for specificity and accurate quantification. The RM, calibrated to $10^4$ MAP copies/gram, appears to be a reliable standard for harmonizing extraction and PCR methods. dPCR is proving particularly valuable in livestock environments, thanks to its enhanced inhibitors resistance and its precision, which together simplify the definition of herd status. Thus, BioSellal and Labocéa aim to offer an innovative approach to the management of paratuberculosis diagnosis.

## OVI.03: Mass spectrometric analysis of lipids from *Mycobacterium avium* subsp. *paratuberculosis*

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**Abstract**

Purpose of this study: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is responsible for the Johne's disease in cattle and goats and is also attributed as the cause of many autoimmune and chronic inflammatory disorders in humans. The aim of this study is, to determine the lipidome of MAP using mass spectrometry (MS). *We hypothesize that MAP and M. tuberculosis share common and unique lipid molecular features, which are responsible for its pathogenicity and zoonosis*.

Methods: Clinical isolates of MAP were obtained from dairy farms and grown in agar slants of Harrold's Egg Yolk Medium in the laboratory of Prof. Shoor Vir Singh, GLA University, Mathura, UP. An inoculum with MAP culture density determined as 5 McFarland (Optical Density 600 nm) was pelleted down by high-speed centrifugation and the lipid extraction was done using methanol and chloroform. The extracted lipids were investigated using liquid chromatography - electrospray ionization - MS (LC-ESI-MS; 6540 Q-ToF attached to 1290 Infinity LC, Agilent Technologies), in positive ion mode. A linear gradient elution was applied on a reverse phase (C18) column (Phenomenex), using acetonitrile and water. All data were processed by MassHunter Workstation Software.

Preliminary Results: Mass spectra corresponding to two different retention times in a LC-ESI-MS chromatogram were chosen for further analysis. The isotope peak *m/z* values suggested that a greater number of lipids were detected in singly protonated form. The *m/z* values in the range 400 - 1400 Daltons were considered for current analysis. Since, lipid databases are not yet available for MAP, two already available databases on *M. tuberculosis*: MtbLipidDB and MycoMass were used for interpretation of the observed *m/z* values. The search results showing delta-M < 0.3 Daltons (delta-M = |*m/z* (observed) – *m/z* (database)|), only were considered for analysis. Outputs from database searches showed glycopeptidolipids, mycobactins, phosphatidyl inositols, cardiolipins, phosphatidyl ethanolamines, etc.

**OVI.04: Investigation of endemic *Mycobacterium bovis* infection in a small beef breeding herd using a comprehensive testing programme**.

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**Abstract**

A small beef breeding herd created in 2016 comprised approximately 20 breeding females and two bulls. The herd was established in a biosecure environment where the entire grazing area was securely fenced to ensure no contact with other cattle or potentially diseased wildlife such as deer or badgers. All biosecurity risks were managed effectively to minimise the risk of bovine tuberculosis entering the herd. In 2019, evidence of Mycobacterial bovis infection was disclosed by a routine statutory testing programme for bovine tuberculosis using the single intradermal comparative cervical tuberculin *test* (*SICCT*). Statutory tests disclosed further reactors with confirmed positive cultures of M.bovis. Additional tests were then used, including Interferon gamma, Idexx elisa, and a novel phage test to identify infected animals that were not disclosed by the SICCT. The enhanced testing programme comprised a statutory SICCT every 60 days, with additional interferon gamma, Idexx elisa and actiphage tests on all cattle three times per year.

Over the three years of regular testing, 99 animals have entered the testing programme and 82 have tested positive to one or more of the tests for bovine tuberculosis. 42 animals have been positive to a statutory test that has required their compulsory slaughter. There is strong evidence that undisclosed infected animals retained within the herd are a reservoir of infection which transmits to their offspring vertically or pseudo-vertically as well as transmission by direct contact.

The origin of the tuberculosis now endemic in the herd is likely to have been through the introduction of an infected bull when the herd was established. The biosecurity systems obviated the risks of infection through wildlife, direct cattle contacts or environmental sources. The case study indicates that statutory testing programmes using SICCT leave undisclosed infected animals in the herd that can be a reservoir of infection for further spread.

## OVI 05: Chronic Diseases control under AMR strategy

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**Abstract**

Control of Chronic diseases rarely find place in the immunization programmes. But vaccination against these diseases is important as it limits the spreading and reducing the severity and strengthen immune response against other infectious disease. Diseases know no borders. As global trade and travel expands, chronic zoonotic diseases are increasingly posing concerns worldwide. Every day, new health challenges emerge at the human-animal-environment interface. To face these threats, collaboration, coordination, communication, and concerted action between different sectors are needed, using a multisectoral, One Health approach. One Health also includes other health threats; for example, food safety and antimicrobial resistance (AMR). Chronic diseases continue to be a threat to global health, causing millions of deaths and economic losses every year. To support countries to control these diseases, the Tripartite organizations (FAO, OIE and WHO) in 2019 launched a guide entitled 'Taking a Multisectoral, One Health Approach: A Tripartite Guide to Addressing Zoonotic Diseases in Countries'. The zoonotic chronic diseases do not only affect human health, but also animal health and welfarse, causing lowered productivity (milk or egg or meat quality and its safety), or death and consequently affecting farmers' livelihoods and countries' economies.

To control the Chronic zoonotic diseases, antibiotics are effective in treating bacterial infections associated with these conditions but prolonged use can raise concerns about antibiotic resistance. Antimicrobial resistance (AMR) is a global health emergency affecting humans and animals, diminishing the effectiveness of medication used to treat illness.AMR was directly responsible for 1.27 million global deaths in 2019 and contributed to 4.95 million deaths. A huge number of interdependent factors related to healthcare and agriculture govern the development of AMR through various drug-resistance mechanisms. The agri-food sector has attracted increased attention for imprudent antimicrobial use (AMU) and its contribution to AMR besides inappropriate use by Individuals and health practitioners.

Vaccination has played a crucial role in eradicating or nearly eliminating several diseases and reduces the AMR.The Smallpox has been completely eradicated worldwide. WHO declared small pox eradication from globe in 1980. Rinderpest disease was declared eradicated in 2011 and second disease to be eradicated after smallpox. Polio has been eradicated in most part of the world. However, this disease still exists in a few countries like Afghanistan and Pakistan. Vaccination has also significantly reduced the incidence of other diseases, such as Measles, Rubella and Diphtheria. Therapeutic vaccines are now days playing big role in treatment of cancer and other conditions like Chronic infection, Cancer, Hypertension etc.

There must be identification of the most prevalent and important bacterial infections in cattle, sheep and goats by WOAH that are commonly farmed and associated with high antibiotic use, and associated prevalent bacterial infections in those species. An assessment of antibiotic use in response to the syndromic indication or diagnosed disease. This may be categorised as high, medium or low in the context of considered use compared with the total use of antibiotics in that animal species.

Johne's disease (JD), also known as paratuberculosis, is a severe production-limiting disease with significant economic and welfare implications for the global cattle, sheep and goat production. Caused by infection with *Mycobacterium avium* subspecies *paratuberculosis* (MAP), JD manifests as chronic enteritis in infected cattle. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) causes Crohn's disease (CD) in humans also known as Inflammatory bowl disease (IBD). Therapeutic vaccines against IBD aim to activate the body's immune system to generate specific antibodies, thereby offering a potential avenue for treating IBD.

The assessment for availability of a vaccine(s) will be done, and if available, their effectiveness will be assessed. The potential for a new or improved vaccine to reduce the need for antibiotic treatment should be checked. The vaccine research could have a significant impact, particularly if it addressed the following four priority gaps: 1- Maternal antibody interference 2- Cross-protection or inclusion of relevant strains in vaccine formulations 3- Occurrence of immunological interference in multivalent vaccines 4- Innovative delivery systems to enable mass-vaccination. There is need to invest for new or improved vaccines in order to reduce antibiotic use in the animals.

# PVI.01: Detection of *Mycobacterium avium* subsp. *paratuberculosis (MAP)* in slaughtered goats using histopathological and molecular approaches

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**Abstract**

Paratuberculosis or John's disease (JD), caused by *Mycobacterium avium* subsp. paratuberculosis (MAP), is one of the major economically important diseases of small ruminants throughout the world. The present study was aimed to detect MAP from slaughtered goats of southern of Iran using IS900-PCR assay and H&E and Ziehl- Nielsen stains on 66 samples collected from the ileum and mesenteric lymph nodes of suspected carcasses to Johne's disease with necropsy lesions including intestinal mucosal thickening and enlarged lymph node. 25.75% of all samples were positive by PCR. Among 66 collected samples, nine samples were positive in both methods (13.63%). Eight samples were positive in PCR method while no lesion related to Johne's disease was observed in their histopathological sections. There was no sample with positive histopathological and negative PCR result. According to the present findings, although both histopathological and PCR methods are appropriate for detecting Johne's disease, PCR is more sensitive than H&E staining.

## PVI.02: Assessment the bio-load of *Mycobacterium paratuberculosis* and genotype frequencies of A1 and A2 β-casein in raw bovine milk samples from Braj region using molecular assays

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**Abstract**

Study investigated the genetic variants of beta-casein in native and crossbred cattle in the Braj Region of Mathura, Uttar Pradesh, focusing on the prevalence of A1 and A2 alleles associated with health impacts due to the bioactive peptide β-casomorphin-7 (BCM-7). To determine the genotypic frequency of beta-casein variants, DNA was extracted from 90 raw milk samples collected from local cattle. Using allele-specific PCR and restriction digestion, the genotypic frequencies of A1A1, A1A2, and A2A2 were found to be 0.17, 0.39, and 0.42, respectively. The higher prevalence of the A2 allele suggests a potential for safer milk production, as A2 does not produce BCM-7, linked to various health disorders. Additionally, the study assessed the prevalence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in raw bovine milk samples, revealing a significant presence (44.0% by microscopy, 29.0% by ELISA, and 11.0% by IS900 milk PCR), raising concerns about zoonotic risks associated with dairy consumption. These findings underscore the need for breeding strategies prioritizing A2 variants and addressing MAP contamination to enhance public health safety. This study examines the genetic variants of beta-casein in native and crossbred cattle from the Braj Region of Mathura, Uttar Pradesh, focusing on the prevalence of A1 and A2 alleles and their associated health implications. Cow milk, a vital nutritional component of the human diet, contains proteins like casein that play crucial roles in providing nutrients and immunological protection. The study highlights the biologically active peptide β-casomorphin-7 (BCM-7), released from beta-casein during digestion, which has been linked to various health issues, including diabetes and heart disease. The study discusses the health implications of consuming milk containing A1 beta-casein, which has been associated with adverse health outcomes, contrasting with potential benefits from A2 beta-casein. Given the intermittent shedding of MAP by subclinically infected animals, the study suggests that the actual prevalence may be higher than reported. The findings underscore the zoonotic risk posed by MAP, emphasizing the need for enhanced monitoring and control measures to ensure public health safety. The study advocates for breeding policies aimed at increasing the frequency of A2 alleles in cattle to reduce health risks associated with A1 beta-casein and to mitigate the presence of MAP in dairy products.

# PVI.03: Co-Infection with *Mycobacterium tuberculosis* in Patients Positive for *Salmonella*: A Clinical study in Northeast India.

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## Abstract

Co-infections with *Salmonella* and *Mycobacterium tuberculosis* (MTB) can be very difficult to diagnose and treat, particularly in areas where both bacteria are highly prevalent. A major concern to world health is still tuberculosis (TB), especially in poor nations. In addition, *Salmonella* infections—particularly those caused by *Salmonella enterica*—raise the burden of infectious illnesses. Since these infections typically affect immune-compromised persons, overlapping symptoms and diagnostic problems might complicate treatment results. Despite their significance, little is known about the frequency and consequences of co-infections with *Salmonella* and MTB in environments with inadequate resources. At a tertiary care hospital in northeast India, the purpose of this study is to evaluate the prevalence, clinical characteristics, and outcomes of patients who are co-infected with MTB and *Salmonella*.
This 12-month cross-sectional research involved 250 patients who tested positive for *Salmonella* and was carried out at a tertiary care hospital in Tripura. For the purpose of isolating *Salmonella*, blood samples were cultured aerobically using the BacT/ALERT microbial detection system. Confirmed isolates were then examined utilizing serotyping and biochemical testing. Individuals exhibiting respiratory symptoms or suspected tuberculosis underwent further testing for MTB using the Ziehl-Neelsen (ZN) staining, which detects acid-fast bacilli (AFB).
In 38 cases (15.2%) out of 250 individuals with positive *Salmonella* tests, MTB co-infection was discovered. Patients with co-infections, who were primarily male (63%) and had immunosuppressive diseases such as diabetes and HIV, showed signs of respiratory distress, weight loss, and persistent fever. In 92% of instances, the Ziehl-Neelsen (ZN) staining confirmed MTB. Patients with co-infections recovered more slowly and died at a rate of 8% as opposed to 3% for those with *Salmonella* alone.
Co-infection with MTB and *Salmonella* poses a serious health risk, especially to those with weakened immune systems. Better clinical outcomes depend on early diagnosis and integrated diagnostics, necessitating aggressive treatment plans and more study to enhance management practices in endemic areas.

**Invited Talk**

# Alternative therapies for mycobacterial diseases, with focus on prophylactic phage therapy for Johne's disease

**Jeroen De Buck**

*Faculty of Veterinary Medicine, University of Calgary*

Mycobacterial diseases, including tuberculosis, leprosy, and non-tuberculous mycobacterial (NTM) infections, are notoriously difficult to treat due to their persistence, slow growth, and inherent resistance to many antibiotics. Although the focus here is on Johne's disease (JD) caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), insights are drawn from therapies used or proposed for other mycobacterial diseases. This includes the use of antibiotics, probiotics, phytochemicals, immunomodulators, and bacteriophages as potential treatments or preventive measures.

Phage therapy, which harnesses bacteriophages to target and kill specific bacterial pathogens, offers particular promise in the prevention of JD. In our recent work, we isolated and characterized mycobacteriophages capable of lysing diverse MAP strains. An experimental infection trial in calves demonstrated that prophylactic phage administration successfully prevented MAP shedding and protected intestinal tissues from infection, providing a compelling case for the use of phages in breaking the transmission cycle of JD.

In addition to phages, alternative therapies such as probiotics like *Dietzia* spp., which have shown potential in modulating immune responses and preventing JD, and phytochemicals such as quercetin and resveratrol, known for their anti-mycobacterial properties, will be discussed. Immunomodulatory agents, including vitamin D and cathelicidin LL-37, are also being explored for their ability to enhance the host's immune response against mycobacterial infections.

By leveraging the lessons learned from therapies used in other mycobacterial diseases, this presentation aims to highlight the potential of these alternative strategies for combating JD, while emphasizing the need for continued research to fully realize their benefits for the dairy industry.

## OVII.01: Tolerance of MAP strains to copper stress suggest more virulence?:a preliminary experimental study

Salgado, M[1],Tejeda C[1], Steuer, P[1], Barkema, HW[2], Hernández, JM[1,3], Ulloa, F[1,3].

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**Abstract**

Copper (Cu) ionarewell established for their ability to inactivate bacteria. This includes *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the causative agent of Johne's disease (JD). However, MAP is notorious for its resilience to harsh conditions. Interestingly, the MAP genome harbors genes associated with Cutolerance, suggesting a potential survival mechanism. However, some studies suggest that bacteria tolerant to stressors can exhibit increased virulence. To investigate this, we examined the differential gene expression of three MAP field isolates and the reference strain ATCC 19698 upon exposure to Cuion stress.

We focused on genes related to Cutolerance (*csoR, ctpV, mmcO, mymT,*mctB), alongside two ROS (reactive oxygen species) antioxidant genes (*sodA, katG*), and virulence genes (*umaA1, papA2, kdpC, impA*) linked to host tissue colonization and other pathogenic activities. Gene expression levels were evaluated before and after Cutreatment using a SYBR green-based qRT-PCR protocol, allowing us to calculate the fold change.

All Cu homeostasis genes were upregulatedafterCutreatment. Notably, across all MAP isolates the *ctpV* gene displayed the highest expression. Similarly, expression of anti-ROS antioxidant genes increased, with *katG*havingthe most significant increase, followed by *sodA*. However, surprisingly MAP virulence gene expression decreased after Cutreatment.

These findings suggest a potential link between Cuexposure and virulence attenuation in MAP, possibly a strategy to establish persistent colonization within the host. Further research is crucial to fully elucidate the implications of these observations for understanding MAP infection biology.

# Deciphering the Potential of Synergistic Herbal Formulation from *Asparagus racemosus* and *Sapindus mukorossi* for Veterinary Vaccines

**Saurabh Gupta[1*], Rasanpreet Kaur[1], Vidhi Mishra[1], Ravi Tiwari[2], Shoor Vir Singh[1], Jagdip Singh Sohal[3]**

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## Abstract

Tri-terpenoids are a natural immunopotentiator and vaccine adjuvant, and plants are an important source of tri-terpenoids in particularly food additives, pharmaceuticals, cosmetics, traditional medicines development. The safety and effectiveness of secondary metabolites obtained from plants, as an adjuvant for vaccine formulations and medicines have been scientifically validated in this work. Secondary metabolites were detected by phytochemical examination of the root and pericarp extracts from *Asparagus racemosus* and *Sapindus mukorossi*, respectively, in both aqueous and hydro-alcoholic preparations. Synergistic formulation of hydro-alcoholic extract from both *Asparagus racemosus* and *Sapindus mukorossi* was prepared in 1:1 ratio for further animal studies. In the in-vivo studies, a dose of 50, 100, 200, 500 and 1000 mg/kg bw was administered for 28 days and no toxic effects, death, or abnormal signs, nor changes in body weight were observed in rats treated with these dosages. The histopathological findings of the vital organs in this study did not produce any changes in architecture of liver, kidney, lung, heart and spleen in comparison to normal group. Treatment with the synergistic formulation at 1000 mg/kg bw did not show much changes in serum creatinine and uric acid concentrations significantly compared to the normal control group, only indicating mild significant changes in the kidney. The result of 28-day oral toxicity concluded that after treatment with synergistic formulation did not produce any sign or symptoms of toxicity by oral route at single dose in wistar rats and above formulation can be categorized as potential candidate. Based on the these finding, above formulation can be an important natural ingredient used in animal vaccines like Johne's disease that helps to create a stronger immune response in the host.

# OVII.03: Evaluation of the Phytotherapeutic Potential of *Moringa oleifera* and *Chenopodium album* extracts Against *Mycobacterium avium* subsp. *paratuberculosis* Infections.

Shoor Vir Singh[1], Vijay Kumar Dubey[1*], Saurabh Gupta[1], Ravindra Kumar[2], Ankush Dhillon[1], Manthena Navabharatha[1], Deendayal[1], Harshit Bansal[1], Shivani Mahor1

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## Abstract

Without control measures, the prevalence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection has risen in dairy animals. Bacilli is resistant to conventional pasteurization, which has led to a significant rise in inflammatory bowel disorders (IBD), in the human population. There is no effective therapy in contemporary medicine, for the management of MAP infection both in animals and human beings. However, alternative therapeutic strategies hold promise in this scenario. Potential of the herbal therapies against MAP and related chronic incurable infections has not yet been fully explored. We evaluated anti-mycobacterial activity in the plant-based synergistic combined feed pellet derived from *Moringa oleifera* & *Chenopodium album* against MAP infection. Plant material was authenticated as the peer of Ayurveda Pharmacopoeia of India. Comprehensive qualitative and quantitative analyses of the plant material were performed utilizing advanced chromatographic and spectroscopic techniques. *In-vitro* pharmacological investigations revealed that hydroalcoholic and aqueous extracts were nontoxic and had no adverse effects on body cells. The synergistically combined feed pellet had shown potential for combating MAP pathogen, the cause of significant morbidity and mortality in goats. Findings open new possibilities for the development of effective therapeutic interventions against MAP infection and associated complications.

# PVII.01: Inhibitory effects of common detergents on *Mycobacterium avium* subsp. *paratuberculosis*

**Soundarya Udaiyar[1,2], Sabareesh V[1], Shoor Vir Singh[3], Sajan George[4]***

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## Abstract

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) is an environmental pathogen causing Johne's disease in cattle and perhaps, ulcerative colitis and colonic Crohn's disease in humans. Objective: This project explored therapeutic options against the drug resistance developed in MAP that leads to high risk of animal to human transmission. Rationale: We had observed that linear alkyl benzene sulfonates (LABS), an ethyl acetate extract prepared from garlic was inhibiting *M. tuberculosis* H37Ra. *So, we hypothesized that similar other detergent-like compounds might have inhibitory effects on the growth of MAP.* Methods: We engaged Resazurin Microtiter Assay (REMA) to explore the inhibitory effects of LABS on MAP. We also investigated the inhibitory effects of four common sulfobetaines, which are zwitter-ionic molecules with a positive charge along with a negatively charged sulfonate group. In fact, sulfobetaines contain long hydrocarbon chain without an aromatic group (isoniazid, having an aromatic ring served as the positive control). Additionally, we tested the inhibitory effects of sodium dodecyl sulfate (SDS), which also lacks an aromatic group on the growth of MAP. The detergent, SDS has a single negative charge and a long hydrocarbon chain, which help us to know, whether negatively charged molecules are effective against MAP or not. Results: We found that the 'negative charge' of the sulfonate group in LABS are essential for inhibiting MAP, in addition to its aromatic moiety and a long hydrocarbon chain. The use of sulfobetaines and SDS were helpful in elucidating the role of charged molecules on the growth of MAP. Conclusion: This study gives us an indication about the inhibitory effects of zwitter-ionic and charged molecules on the growth of MAP, an important pathogen in One health program with epidemic and pandemic potential.

## PVII.02: A synthetic tunicamycin derivative evaluated for treatment of Johne's disease in Holstein cows

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### Abstract

Tunicamycin (Tun) is an antibiotic synthesized by *Streptomyces* spp. that inhibits the initial steps of cell wall biosynthesis but also affects eukaryotic cells. TunR2 is a modified tunicamycin that showed reduced toxicity on eukaryotic cells while retaining its antibacterial properties, showing a MIC between 16-32 ug/mL for *Mycobacterium avium* subsp. *paratuberculosis* (Map) strains. In the present work, we evaluated the pharmacokinetics, safety, and efficacy of low doses of TunR2 to treat adult cows with clinical John's Disease (JD). Treatment was administered intravenously to three Holstein cows in advanced stages of JD, and a control animal inoculated with a drug vehicle only. The animals received three doses of TunR2 administered every 48 hours. The first dose was 100 mg, the second 50 mg, and the last 45 mg/cow. Blood, feces, urine, and milk samples were collected for clinical analysis, MAP quantification, and drug kinetics at three time points over 90 days. At the end of the study, animals were euthanized, and tissues were collected. The drug was detected in blood, milk, and feces, with a cumulative effect after the 2nd and 3rd doses and was no longer detected after 20 days. Regarding toxicity, renal and hepatic toxicity was nil or extremely low after the third dose (day 4), which could be due to the cumulative effect of TunR2. Overall, the treatment was ineffective, as demonstrated by the persistence of Map shedding in feces and milk and the detection of Map in various tissues. However, it should be noted that the dose used was below the MIC, the dose required to achieve it is probably above 500 mg/animal, and only three doses were administered. Further studies with increased dose and frequency of administration are needed to evaluate the potential use of TunR2 for the treatment of John's disease.

## PVII.03: Response of *Mycobacterium avium* subsp. *paratuberculosis* isolates to copper ion stress

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**Abstract**

*Mycobacterium avium* subsp. *paratuberculosis* (MAP), a persistent pathogen in ruminants causing Johne's disease (JD), is also a potential zoonotic threat. Copper ion have shown promise in combating MAP by damaging its genetic material and proteins. However, the response of field isolates, obtained from MAP-infected cows, may differ from laboratory-adapted reference strains. This study explores these differences in copper tolerance.

We compared the response of three clinical MAP isolates and the reference strain ATCC 19698 to copper ion treatment. Their viability, protein/lipid damage, and reactive oxygen species (ROS) production (all performed in triplicate) were evaluated. Additionally, whole-genome sequencing was conducted.

Copper treatment resulted in a reduction in MAP load for all isolates. Interestingly, one isolate of a clinical JD cow displayed a longer survival time compared to the reference strain. However, protein concentration, lipid peroxidation, and ROS production were statistically similar across all isolates. Whole-genome sequencing revealed genetic similarities between field isolates and the reference strain, but also identified variations in genes associated with specific virulence factors.

While copper treatment affected MAP isolates similarly in some aspects, the observed difference in copper tolerance between a clinical isolate and the reference strain highlights the need for further investigation. Understanding how MAP responds to stress during natural infections and harsh environments is crucial for improved disease management in livestock.