IOM

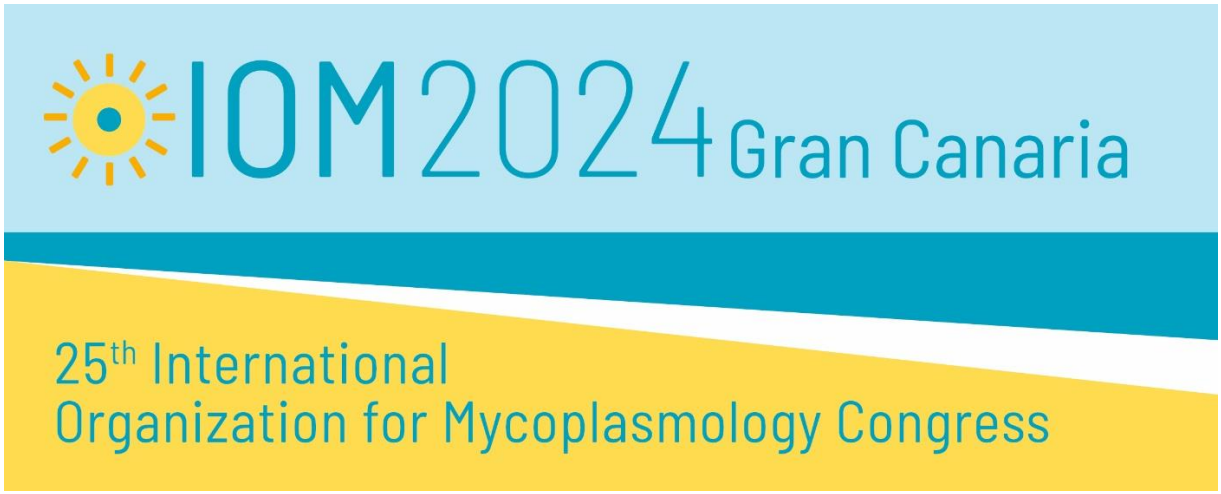
IOM 2024 Gran Canaria

25th International Organization for Mycoplasmology Congress

25th Congress of the International Organization for Mycoplasmology

July 7th–11th, 2024, Gran Canaria, Spain

Program and abstracts



25th Congress of the International Organization for Mycoplasmology

July 7th–11th, 2024, Gran Canaria, Spain

Program and Abstracts

Edited by Eric Baranowski, Christine Citti, Ana S. Ramírez and Rubén S. Rosales

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Welcome Message

from the Local Organizing Committee

In order to realign with the pre-pandemic tradition of hosting the IOM congress in even-numbered years, we were requested to organize the subsequent congress within a single year, rather than the customary two-year interval. Although we gladly accepted this decision, it added additional pressure to the process of organizing this congress in order to produce a congress with the standards that all members of the mollicutes scientific community deserve. After a year of hard work, it is with great pleasure, on behalf of the Local Organizing Committee, to welcome you to the 25th International Organization for Mycoplasmology (IOM) Congress, taking place from July 7th to 11th, 2024, in the beautiful and vibrant city of Las Palmas de Gran Canaria, Spain, representing the first time that this congress is being held in a Spanish city.

This IOM congress represents a unique opportunity for experts, researchers, and practitioners from around the globe to come together, share their latest findings, and discuss advances in the field of mycoplasmology. We are thrilled to host this esteemed event on our sunny island, known for its rich cultural heritage and stunning landscapes. Our amazing scientific program, designed by Christine Citti, Eric Baranowski, and the Scientific Program Committee, highlights the most recent innovations and developments in mycoplasma research. We have an exciting lineup of keynote speakers, including Dr. Héctor Argüello-Rodríguez, Dr. Benjamin Roche, and Prof. Saskia Hogenhout. We believe these speakers will inspire fruitful discussions and collaborations among participants, fostering the growth and progression of our field.

In addition to the academic and scientific activities, we have arranged several social events to provide opportunities for networking and to experience the unique charm of Las Palmas de Gran Canaria. From our welcoming reception to the gala dinner, we hope these events will enhance your experience and allow you to create lasting memories. The venue is located in the Hotel Cristina, situated at Las Canteras Beach, renowned for its stunning natural beauty and unique features such as soft golden sand, a natural barrier, crystal-clear waters, a picturesque promenade, and, on the other side of the beach, fantastic waves for surfing. We extend our heartfelt thanks to all the speakers, sponsors, and volunteers who have worked tirelessly to make this congress a success. Your contributions are invaluable, and your dedication to advancing the field of mycoplasmology is greatly appreciated.

Once again, welcome to Gran Canaria and the IOM Congress 2024. We are confident that this week will be filled with insightful exchanges, new friendships, and unforgettable moments.

Sincerely,

Ana S. Ramírez and Rubén S. Rosales

Chairs, Local Organizing Committee IOM Congress 2024

Local Organizing Committee Members

Chair of the Local Organizing Committee

Ana S. Ramírez (Universidad de Las Palmas de Gran Canaria)
Contact details: anasofia.ramirez@ulpgc.es

Co-chair of the Local Organizing Committee

Rubén S. Rosales (Universidad de Las Palmas de Gran Canaria)
Contact details: ruben.rosales@ulpgc.es

Members of the Local Organizing Committee

José B. Poveda (Universidad de Las Palmas de Gran Canaria, Spain)
Marisa A. Andrada (Universidad de Las Palmas de Gran Canaria, Spain)
Francisco Rodríguez (Universidad de Las Palmas de Gran Canaria, Spain)
Soraya Déniz (Universidad de Las Palmas de Gran Canaria, Spain)
Begoña Acosta (Universidad de Las Palmas de Gran Canaria, Spain)
Inmaculada del Rosario (Universidad de Las Palmas de Gran Canaria, Spain)
Ayose Castro (Universidad de Las Palmas de Gran Canaria, Spain)
Antonio Fernández (Universidad de Las Palmas de Gran Canaria, Spain)
Magnolia Conde-Felipe (Universidad de Las Palmas de Gran Canaria, Spain)
Sergi Torres Puig (Universität Bern, Switzerland)
Luis Serrano (Centre of Genomic Regulation, Barcelona, Spain)
Javier González de Miguel (Centre of Genomic Regulation, Barcelona, Spain)
María Lluch (Pulmobiotics, Barcelona, Spain)
Jaume Pinyol (Universidad Autónoma de Barcelona, Spain)
Marina Marcos (Universidad Autónoma de Barcelona, Spain)
Ignacio Fita (Consejo Superior de Investigaciones Científicas, Spain)
Jesús Martín Romero (Consejo Superior de Investigaciones Científicas, Spain)
Christian de la Fe (Universidad de Murcia, Spain)

Co-organizers

ULPGC Universidad de Las Palmas de Gran Canaria

Gobierno de Canarias
Consejería de Universidades, Ciencia e Innovación y Cultura
Agencia Canaria de Investigación, Innovación y Sociedad de la Información

Welcome Message from IOM Chair

Dear Colleagues and Friends,

I am thrilled to welcome all of you to the milestone XXV Congress of the International Organization for Mycoplasmology in Gran Canaria! September of 2024 marks fifty years since over three hundred of our predecessors met in Bordeaux and decided to establish the IOM as a formal entity. Preparing to write this message and develop opening remarks for the congress by reviewing photos, meeting proceedings, newsletters, and other archived materials, I find myself deeply humbled to hold the chair position at this particular moment. The IOM has always been an organization that fosters collaborations and professional network development, but in my experience it has been a venue where decades-long friendships have been formed as well. If this is your first congress, it is my most sincere hope that your experience mirrors my own.

The meeting we are about to enjoy is the result of a tremendous amount of work by a relatively small number of people, and these folks hold the unique distinction of having to conduct their work in half the amount of time usually allotted. The Local Organizing Committee chair Ana Ramírez, co-chair Rubén Rosales, and their team are responsible for everything around us, from the venue we are holding sessions in, to the refreshments we are offered, to assembling the very program book you are currently reading. It is an enormous job, and I could not be more appreciative of their hard work. The Awards Committee chair Inna Lysnyansky and her team reviewed and extensively discussed nomination packages for several qualified candidates, and their work will continue through the congress to evaluate nominated posters and select winners for the Louis Dienes and Harry Morton awards. I would like to take this moment to thank Inna and the committee for their work, and to congratulate the awardees. The Awards Committee's work contributes anchoring features to the congress program in the form of four award lectures, which have to mesh seamlessly into the work of the Scientific Program Committee, co-chaired by Christine Citti and Eric Baranowski. Christine, Eric, and their team were tasked with identifying keynote speakers who create excitement among IOM members and were available on fairly short notice. This alone is no easy task, and the committee then worked at breakneck pace to review abstracts, select submissions that would make engaging platform presentations, and organize cohesive symposia around award and keynote lectures that represent the wide breadth of topics studied by members of the IOM. This is a very difficult task, and I thank and commend the program committee for making it look so easy. Finally, I want to thank members of the IOM Board of Directors, who do the quiet work of making sure the organization runs smoothly. In particular I am grateful to our Secretary-General Pascal Sirand-Pugnet (who keeps me in line and on task) and to our Chair-Elect Amir Hadji Noormohammadi (who routinely serves as my sounding board); both extremely good-natured about my tendency toward chaotic nonsense (and terrible jokes).

I am very excited to spend this week meeting new colleagues and visiting with those current and past, seeing your latest scientific developments, and exploring the beautiful island of Gran Canaria. ¡Bienvenidos a la IOM 2024!

Meghan May

Chair, International Organization for Mycoplasmology

Supporting Organizations

Gold

ECO ANIMAL HEALTH

DISCOVER NATURE Poema del Mar AQUARIUM

Silver

BIOPROPERTIES The Vaccine Innovators

SBT

vetworks®

GranCanaria Convention Bureau

Bronze

ULPGC Universidad de Las Palmas de Gran Canaria | Consejo Social

ESGMAC ESCMID STUDY GROUP FOR MYCOPLASMA AND CHLAMYDIA INFECTIONS
European Society of Clinical Microbiology and Infectious Diseases

GD AHEAD IN ANIMAL HEALTH

Mycoplasma Experience

Others

veterinarios COLEGIO OFICIAL DE LAS PALMAS

veterinarios Ilustre Colegio Oficial de Veterinarios de de Santa Cruz de Tenerife

Biotein S.L.

ad DiagNost s.a.

ULPGC Universidad de Las Palmas de Gran Canaria | IUSA Instituto Universitario de Sanidad Animal y Seguridad Alimentaria

The Conference Center

Hotel Cristina by Tigotan

C/ Gomera, 6, Playa Las Canteras, 35008-
Las Palmas de Gran Canaria, España
Tlf. Tel. +34 928 268 050
www.dreamplacehotels.com

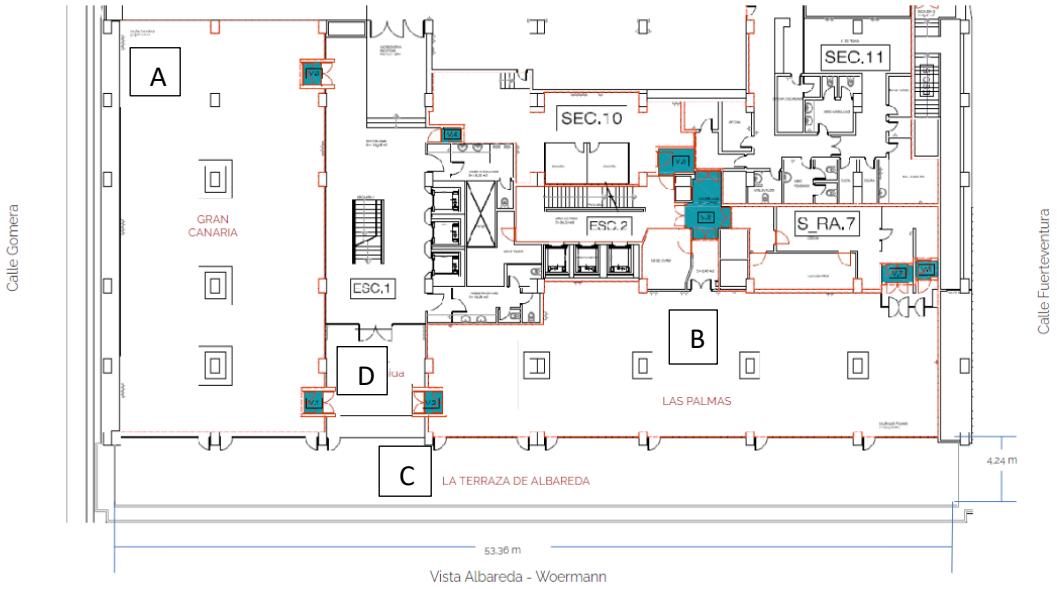
Level P

Oral sessions: Gran Canaria's room (A)

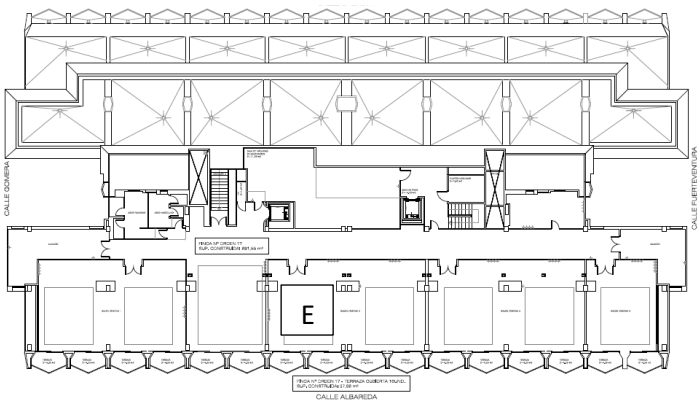
Poster sessions: Las Palmas' room (B)

Coffee breaks: Albareda Terrace (C)

Secretary Office (D)



Level 11th



Santa Catalina's room (E)

General information

Registration Desk

The IOM 2024 Registration Desk will be located in level P between Gran Canaria' and Las Palmas rooms. The desk will be open 17:00h-19:00h on Sunday, July 7, and 08:00h-18:00h on Monday and from 9:00h to 13:00h for the rest of the congress. All delegates and speakers will be provided with a name tag to be worn at all times within the Conference Hall. The name tag serves as entry to all sessions, exhibits, coffee break, meals, and social events. If you misplace your name tag, a replacement can be obtained at the Registration Desk.

Internet Access

Free Wifi is provided in oral and poster rooms as below.

Net: IOM

Keyword: IOW2024!

Mobile Phones

Please respect the delegates and speakers by ensuring phones are silenced during all conference sessions.

Platform presentation instructions

Presenters should bring their PowerPoint file on a pendrive to the session convenors in the Gran Canaria's room during the break before their session. The convenors will mount the presentations on the computer desktop immediately before the session starts, then remove the files from the desktop at the end of each session.

Poster presentation instructions

Posters should be mounted on Monday, in the stand with you assigned number at Las Palmas room. They will remain on display until Wednesday (9th July) afternoon or early Thursday (11th July). The posters that are not removed then, will be discharged.

Dietary requirements

Please notify the catering staff or IOM 2024 staff at the Reception Desk of any special dietary requirements.

Electricity and tap water

The voltage in Spain is 220 V/50 Hz and the electrical outlets are type C (with two roundly plugs). Tap water from Las Palmas de Gran Canaria is mostly desalinated water. And although it is safe to drink it, locals prefer bottle mineral water for drinking.

Local currency

The local currency is the Euro.

IOM Board of Directors

Chair: Meghan May (USA)

Chair-Elect: Amir Hadjinoormohammadi (Australia)

Past Chair: Steven Geary (USA)

Secretary-General: Pascal Sirand-Pugnet (France)

Treasurer: Maria Pieters (USA)

Membership Secretary: Sabine Pereyre (France)

IRPCM Chair: Chih-Horng Kuo (Taiwan)

Information Officer: Yonathan Arfi (France)

Board Members-At-Large: Emma Sweeney (Australia), Prescott Atkinson (USA), Ana Sofía Ramírez (Spain), Joerg Jores (Switzerland), Shigeyuki Kakizawa (Japan).

IOM Scientific Program Committee

Chairs of the Scientific Committee

Christine Citti (INRAE, France)
Eric Baranowski (INRAE, France)

Members of the Scientific Committee

Cécile Bébéar (University of Bordeaux, France)
Salvatore Catania (Istituto Zooprofilattico Sperimentale delle Venezie, Italy)
Aizhen Guo (Huazhong Agricultural University, China)
Miklós Gyuranecz (Institute for Veterinary Medical Research, Hungary)
Birgit Henrich (University of Düsseldorf, Germany)
Veronica Maria Jarocki (University of Technology Sydney, Australia)
Shigeyuki Kakizawa (National Institute of Advanced Industrial Science and Technology, Japan)
Calvin C. Ko (Iowa State University, USA)
Sathya Kulappu Arachchig (University of Melbourne, Australia)
Rubén Rosales (University of Las Palmas de Gran Canaria, Spain)
Sergi Torres Puig (University of Bern, Switzerland)
Li Xiao (University of Alabama, USA)
Amit Yadav (National Centre for Cell Science, India)

IOM Award Committee

Chair of the Award Committee:

Inna Lysnyansky (Kimron Veterinary Institute, Beit Dagan, Israel)

Members of the award committee:

Amir Hadjinoormohammadi (Melbourne University, Melbourne, Australia)
Tsuyoshi Kenri (National Institute of Infectious Diseases, Tokyo, Japan)
Roger Dumke (Institute of Medical Microbiology and Virology, Dresden, Germany)
Laure Beven (University of Bordeaux, Bordeaux, France)
Birgit Henrich (University of Düsseldorf, Düsseldorf, Germany)
Amit Yadav (National Centre for Cell Science, Pune, India)
Aizhen Guo (Huazhong Agricultural University, Wuhan, China)
Jose Perez-Casal (International Vaccine Centre, Saskatoon, Canada)
Sanjay Vashee (The J. Craig Venter Institute, Rockville, USA)
Wei Wei (United States Department of Agriculture, Beltsville, USA)

International Research Program on Comparative Mycoplasmology (IRPCM)

The International Research Programme on Comparative Mycoplasmology (IRPCM) is a permanent standing committee of the International Organization for Mycoplasmology (IOM). Its objective as defined in the Constitution is to advance and disseminate knowledge on all aspects of mycoplasmas, a term that refers to all members of the class Mollicutes.

Webpage: https://irpcm.ipmb.sinica.edu.tw/

IRPCM Board:

Chair: Chih-Horng Kuo (Taiwan)

Vice Chair: Marco Bottinelli (Italy)

Past Chair: Christine Citti (France)

Team Leaders

- Avian Mycoplasmas: Anneke Feberwee (Netherlands)
- Chemotherapy of Mycoplasma Infections: Florence Tardy (France)
- Clinical Aspects of Human Mycoplasmas: Tsuyoshi Kenri (Japan; 2023-2024); Patrick M. Meyer Sauteur (Switzerland; 2024-2026)
- Molecular Genetics and Cell Biology: Meghan May (USA; 2023-2024); Rohini Chopra-Dewasthaly (Austria; 2024-2026); Maureen Diaz (USA; 2024-2026)
- New and Emerging Species: Mary Brown (USA)
- Plant and Arthropod Mycoplasmas: Kenro Oshima (Japan)
- Ruminant Mycoplasmas: Nadeeka Wawegama (Australia)

Advisory Members:

- Alain Blanchard (France)
- Glenn Browning (Australia)
- Gail Gasparich (USA)
- Steven Geary (USA)
- Amir Noormohammadi (Australia)

Information Officer: Yonathan Arfi (France) (IOM Information Officer)

Treasurer: Maria Pieters (USA) (IOM Treasurer)

Representative of the IOM: Carole Lartigue-Prat (France)

Representative of the ICSP Subcommittee on the Taxonomy of Mollicutes: Vicki Chalker (UK; 2023-2024); Gail Gasparich (USA; 2024-2026)

International Committee on Systematic of Prokaryotes (ICSP)

Subcommittee on the Taxonomy of Mollicutes

The International Committee on Systematics of Prokaryotes (ICSP) is a standing committee of the Bacteriology and Applied Microbiology division of the International Union of Microbiological Societies (IUMS). The IOM is a member society of the IUMS. The ICSP Subcommittee on the Taxonomy of Mollicutes was established in 1966 to provide formal recommendations and general advice regarding identification, classification, and nomenclature of mycoplasmas according to the principles and rules of the International Code of Nomenclature of Prokaryotes. Although it is not directly affiliated with the IOM, the Subcommittee meets biennially in conjugation with the IOM Congress. Its minutes are published regularly in the International Journal of Systematic and Evolutionary Microbiology.

Members of the ICSP Subcommittee on the Taxonomy of Mollicutes

Past Chair: Victoria Chalker (UK)

New Chair: Gail Gasparich (USA)

Past Secretary: Meghan A. May (USA)

New secretary: Chih-Horng Kuo (Taiwan)

Mitchell F. Balish (USA)

Assunta Bertaccini (Italy)

Alain Blanchard (France)

Ludwig Hölzle (Germany)

Peter Kuhnert (Switzerland)

Lucia Manso-Silvan (France)

Ana Sofía Ramírez (Spain)

Joachim Spergser (Austria)

O. Brad Spiller (UK)

Emma Sweeney (Australia)

Dmitriy Volokhov (USA)

Wei Wei (USA)

IOM Emmy Klieneberger-Nobel Award

Monday 08 (9:30)

From Gene Cloning to Genome Engineering: A Mycoplasmology Journey

Professor Alain Blanchard
University of Bordeaux, France

The **Emmy Klieneberger-Nobel Award**, bestowed in recognition of outstanding contributions to mycoplasmology research, was awarded to **Prof. Alain Blanchard** (France) for his prominent and sustained scientific study of mycoplasmas. These efforts have significantly advanced our understanding of *Mollicutes*, particularly in terms of molecular biology, microbe-host interactions, genomics, and evolution. Prof. Blanchard's work has resulted in approximately 100 high-quality publications with significant scientific impact. Alain Blanchard has also made strong contributions to the mycoplasmology community by serving in various roles on the IOM board and its committees.

IOM Robert F. Whitcomb Award

Monday 08 (15:00)

How a mollicute induces developmental changes in its host and attracts insect vectors

Professor Saskia A. Hogenhout
John Innes Centre, UK

The **Robert F. Whitcomb Award**, recognizing outstanding research achievements in plant and insect mycoplasmology, was bestowed upon **Prof. Saskia A. Hogenhout** (UK) for her groundbreaking research in establishing the field of phytoplasma effector biology. This research has led to a deeper understanding of interactions among plants, insects, and microbes. With an outstanding track record, including over 100 publications of substantial scientific impact and several patents, Prof. Hogenhout has demonstrated her exceptional contributions to the field. Additionally, Prof. Hogenhout remains an active member of the IRPCM (Plant and Arthropod Mycoplasmas) group, along with other IOM committees.

IOM Peter Hannan Award

Monday 08 (17:40)

A 40-year struggle with Mycoplasma synoviae: an insidious pathogen hiding as a commensal

Dr. Chris Morrow
University of Melbourne, Australia

The **Peter Hannan Award**, recognizing outstanding research achievements in the field of applied medical or veterinary clinical mycoplasmology, was conferred upon **Dr. Chris Morrow** (Australia) for his significant contributions to research on avian mycoplasmas and the development of an effective live attenuated MS-H vaccine against *M. synoviae*. This vaccine is now registered in over 66 countries worldwide, which was a factor resulting in the massive decrease in antibiotic dependence of poultry industries. In addition, Dr. Morrow has been involved in the development of various PCR strain identification and differentiation assays for *M. synoviae*. Collectively, these advancements enable effective diagnosis and control of this pathogen. Dr. Morrow demonstrated a long-term commitment as the IRPCM team leader of the avian group.

IOM Derrick Edward Award

Tuesday 09 (11:40)

Mycoplasma pneumoniae: delayed re-emergence, new diagnostics, and evaluation of antibiotic treatment

Dr. Patrick Meyer Sauteur
University Children's Hospital Zurich, Switzerland

The **Derrick Edward Award**, given in recognition of outstanding in mycoplasmology research by young investigators entering the field, was awarded to **Dr. Patrick Meyer Sauteur** (Switzerland) for his important work on community-acquired *M. pneumoniae* infection. His work on surveillance of infection provides a unified geographical context for understanding *M. pneumoniae* epidemiology over time. Dr. Sauteur has a successful track record, with publications in high-impact journals, and demonstrates a strong ability to organize collaborations. He is a member of the IRPCM (Human Mycoplasmas) group.

IOM Louis Dienes Award

The Louis Dienes Award is made in memory of Louis Dienes. This award is given to recognize an outstanding poster in mycoplasmology by an author who is a postdoctoral fellow and who obtained a terminal degree no more than five years previously.

Nominees

O-08 **Elisabet Frutos Grilo**
Mycoplasma-Derived Vesicles: Novel Therapeutic Vehicle for Lung Disorders
Centre for Genomic Regulation, Spain

O-39 **Sathya Kulappu Arachchige**
Assessment of tracheal mucosal thickness is the most powerful and reproducible method for evaluation of the efficacy of vaccines against *Mycoplasma gallisepticum* in poultry
University of Melbourne, Australia

O-41 **Manoel Neres Santos Junior**
Microorganism-host interaction and the immunomodulatory mechanisms of synthetic genome *Mycoplasma mycoides* subsp. *capri* (Mmc) strains in caprine peripheral blood mononuclear cells culture
Federal University of Bahia, Brazil

O-61 **Federico Gabriel Mirkin**
The structural landscape of the secretome of phytoplasmas, master modulators of plant architecture
John Innes Centre, UK

P-38 **Yamile Ana**
Molecules secretion and exposure optimization in *Mycoplasma pneumoniae* chassis
Centre for Genomic Regulation, Spain

IOM Harry Morton Student Award

The Harry Morton Student Award is made to honor Harry Morton and is given in recognition of an outstanding poster presentation in mycoplasmology by a graduate student at a regular Congress of the IOM

Nominees

O-04 **Imdad Ullah**
Molecular detection and isolation of *Mycoplasma bovis* strains from tissue samples of *Bos granniens* (Yak)
University of Agriculture Peshawar, Pakistan

O-07 **Javier Gonzalez de Miguel**
Engineering Mycoplasma for the treatment of Idiopathic Pulmonary Fibrosis
Centre for Genomic Regulation, Spain

O-09 **Jitra-Marie Jittasevi**
Deciphering genome transplantation mechanisms as a step towards understanding basic principles of life
INRAE, University of Bordeaux, France

O-11 **John W. Sanford**
A Surface Post-Translational Modification System is Conserved in *Mycoplasma genitalium*, *Mycoplasma myco*ides, and JCVI-Syn3A
University of Alabama, USA

O-23 **Calvin Ko**
Development of a viability-qPCR detection system and in vitro growth evaluation of *Mycoplasma hyopneumoniae*
Iowa State University, USA

O-29 **Patrick Hogan**
Improved CRISPR-base editor tools for genome edition in *Mycoplasma bovis*: Application to surface proteins
INRAE, University of Bordeaux, France

O-30 **Marina Marcos Silva**
Heterologous expression of *Mycoplasma pneumoniae* major adhesins in the close related species *Mycoplasma genitalium*
Autonomous University of Barcelona, Spain

O-32 **Lars Vogelgsang**
From Sequence to Activity: Insights into the HgaI-RM-System of *Mycoplasma hominis*
University of Duesseldorf, Germany

O-34 **Beatriz Almeida Sampaio**
Assessment of immune response in goats using recombinant P40 and MAG_1560 as vaccine candidates for *Mycoplasma agalactiae*
Universidade Estadual de Santa Cruz, Brazil

O-35 **Hani Alnakhli**
Development of a Subunit Vaccine Against *Mycoplasma gallisepticum* Utilizing Diverse Surface Proteins
University of Liverpool, UK

O-42 **Sahar Zare**
Could the GAPDH-Enhanced MS-H Strain Redefine Vaccine Efficacy?
University of Melbourne, Australia

O-44 **Thatcha Yimthin**
Immune responses elicited by Janus-faced *Mycoplasma mycoides* - glycans make the difference
University of Bern, Switzerland

O-54 **Anna-Marie Ilic**
Power of Codon Usage Analysis for Phylogenetic Reconstruction of the *Mollicutes*
University of Hohenheim, Germany

O-66 **Morgan Hunte**
In vivo disease severity in a mouse model of *Mycoplasma pneumoniae* is influenced by the adherence state of bacteria during inoculum preparation
University of Connecticut, USA

O-69 **Elyse Levenda**
Interactions of *Mycoplasma penetrans* with urethral epithelial tissue culture cells
Miami University, USA

O-71 **Alexandra Burne**
The invertebrate *Galleria mellonella* confirms differences in virulence potential between *Mycoplasma alligatoris* and *Mycoplasma crocodyli*, two closely related Crocodilian pathogens
University of Florida, USA

P-21 **Congriev Kumar Kabiraj**
Evaluation of diagnostic methods for the detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infections in poultry
University of Liverpool, UK

P-27 **Raquel Toledo Perona**
Mycoplasma spp. presence in vagina, prepuce and raw milk microbiota of sheep: preliminary metagenomic study
Universidad CEU Cardenal Herrera, Spain

P-45 Kanishka Kamathewatta Walawwe
Protection of day-old chicks with the Vaxsafe MG304 live-attenuated *Mycoplasma gallisepticum* vaccine: a transcriptional profile
University of Melbourne, Australia

P-48 Juliet Masiga
Quality Control of CBPP Vaccines: Current Status and Challenges
International Livestock Research Institute, Kenya

P-53 Xiao-Hua Yan
Genome-scale analysis of the hominis group mycoplasmas and the taxonomic implications
Institute of Plant and Microbial Biology, Taiwan

P-54 Shen Chian Pei
Investigation into the putative effectors of poinsettia-associated *'Candidatus Phytoplasma pruni'*
Institute of Plant and Microbial Biology, Taiwan

P-60 Julien Berlureau
Efforts towards the purification of the putative F1-like X0 ATPase involved in the MIB-MIP system
INRAE, University of Bordeaux, France

P-65 Mina Yaa Serwaah Amoah
Macrophages join forces with neutrophils in *Mycoplasma bovis* mastitis
Koret Veterinary Institute, Israel

IOM Travel Award

Awardees

O-02 **Manon Vastel**
β(1-6)glucan homopolymer in mycoplasma capsules
ANSES, France

O-04 **Imdad Ullah**
Molecular detection and isolation of *Mycoplasma bovis* strains from tissue samples of *Bos granniens* (Yak)
University of Agriculture Peshawar, Pakistan

O-11 **John W. Sanford**
A Surface Post-Translational Modification System is Conserved in *Mycoplasma genitalium*, *Mycoplasma myco*ides, and JCVI-Syn3A
University of Alabama, USA

O-23 **Calvin Ko**
Development of a viability-qPCR detection system and in vitro growth evaluation of *Mycoplasma hyopneumoniae*
Iowa State University, USA

O-30 **Marina Marcos Silva**
Heterologous expression of *Mycoplasma pneumoniae* major adhesins in the close related species *Mycoplasma genitalium*
Autonomous University of Barcelona, Spain

O-34 **Beatriz Almeida Sampaio**
Assessment of immune response in goats using recombinant P40 and MAG_1560 as vaccine candidates for *Mycoplasma agalactiae*
Universidade Estadual de Santa Cruz, Brazil

O-39 **Sathya Kulappu Arachchige**
Assessment of tracheal mucosal thickness is the most powerful and reproducible method for evaluation of the efficacy of vaccines against *Mycoplasma gallisepticum* in poultry
University of Melbourne, Australia

O-41 **Manoel Neres Santos Junior**
Microorganism-host interaction and the immunomodulatory mechanisms of synthetic genome *Mycoplasma mycoides* subsp. *capri* (Mmc) strains in caprine peripheral blood mononuclear cells culture
Federal University of Bahia, Brazil

O-42 **Sahar Zare**
Could the GAPDH-Enhanced MS-H Strain Redefine Vaccine Efficacy?
University of Melbourne, Australia

O-44 **Thatcha Yimthin**
Immune responses elicited by Janus-faced *Mycoplasma mycoides* - glycans make the difference
University of Bern, Switzerland

O-49 **Salim CHIBANI**
Breaking down the complexity of Mycoplasma hominis
Institut Pasteur de Tunis, Tunisia

O-54 **Anna-Marie Ilic**
Power of Codon Usage Analysis for Phylogenetic Reconstruction of the *Mollicutes*
University of Hohenheim, Germany

O-61 **Federico Gabriel Mirkin**
The structural landscape of the secretome of phytoplasmas, master modulators of plant architecture
John Innes Centre, UK

O-62 **Hengning Wu**
Structural analysis and Molecular dynamics simulations of urease from Ureaplasma parvum
Osaka Women's and Children's Hospital, Osaka, Japan

O-64 **Doukun Lu**
Identification of Nucleomodulins of Mycoplasmopsis bovis by Magnetic bead enrichment and Proximity-Based Biotinylation Approaches
Huazhong Agricultural University, Wuhan, China

O-69 **Elyse Levenda**
Interactions of *Mycoplasma penetrans* with urethral epithelial tissue culture cells
Miami University, USA

O-71 **Alexandra Burne**
The invertebrate *Galleria mellonella* confirms differences in virulence potential between *Mycoplasma alligatoris* and *Mycoplasma crocodyli*, two closely related Crocodilian pathogens
University of Florida, USA

P-21 **Congriev Kumar Kabiraj**
Evaluation of diagnostic methods for the detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infections in poultry
University of Liverpool, UK

P-48 **Juliet Masiga**
Quality Control of CBPP Vaccines: Current Status and Challenges
International Livestock Research Institute, Kenya

P-54 **Shen Chian Pei**
Investigation into the putative effectors of poinsettia-associated *'Candidatus Phytoplasma pruni'*
Institute of Plant and Microbial Biology, Taiwan

Keynote Speaker 1

Monday 08 (15:00)

How a mollicute induces developmental changes in its host and attracts insect vectors

Prof. Saskia Hogenhout
John Innes Centre, UK

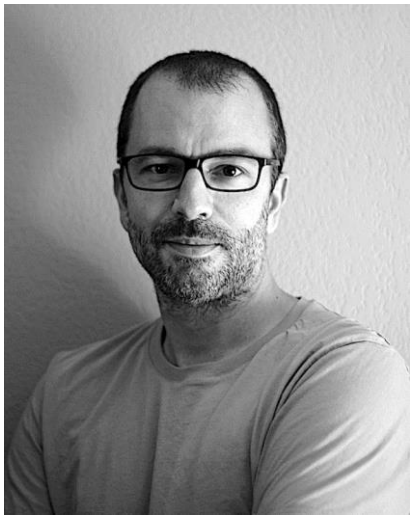
Professor Saskia Hogenhout is a Group Leader in the Department of Crop Genetics at the John Innes Centre and Honorary Professor at the School of Biological Sciences, University of East Anglia, Norwich, UK. Her research primarily delves into the intricate dynamics between plants and insects, particularly emphasizing the influence of microbes within these relationships. A significant focus of her work is the study of phytoplasmas—pathogens transmitted by insect vectors—which induce severe morphological changes in plants, such as witches' brooms and phyllody. Her team has made pioneering discoveries, revealing that these pathogens emit virulence proteins, or effectors, that specifically target and disrupt plant transcription factors crucial for developmental regulation. This interference promotes breakdown of these transcription factors, effectively short-circuiting plant pathways and circumventing the usual ubiquitination process, resulting in dramatic developmental defects. Furthermore, her research has highlighted that plants exhibiting symptoms of these diseases attract insect vectors more effectively, thereby facilitating the widespread dissemination of phytoplasmas. In addition, she has been instrumental in developing genomic tools and conducting functional analyses of effectors found in the saliva of various sap-feeding insects from the Hemiptera order, which are known carriers of plant pathogens. Her contributions span an array of fields including genomics, molecular genetics, entomology, plant pathology, virology, bacteriology, and recently, population biology and mechanistic modelling. Professor Hogenhout has authored over 100 articles in prestigious international journals, including Cell, PNAS, Molecular Biology and Evolution, PLoS Pathogens, among others.

Keynote Speaker 2

Tuesday 09 (9:00)

Understanding the interplay between host and pathogen diversities: opportunities for prevention of emerging infections?

Dr. Benjamin Roche
Research Institute for Development (IRD), France



Dr Benjamin Roche holds a PhD in Ecology and Evolutionary Biology. He is currently a Research Director at the French National Institute for sustainable Development (IRD), where he is the "One Health" scientific advisor of the CEO, as well as an associate professor at the National Autonomous University of Mexico (UNAM). He is one of the co-founders and co-coordinator of PREZODE, an international initiative aiming at preventing zoonotic diseases emergence. His research focuses on a cross-disciplinary approach between evolutionary ecology and public health. Most specifically, he is interested in how host and pathogen diversity can affect the transmission of infectious diseases. He has also studied the interactions between ecological and societal processes in pathogens propagation. Although rooted in fundamental research, his projects offer operational opportunities to improve public health strategies. Benjamin Roche has published more than 100 articles in international journals (The Lancet, Ecology Letters, PLoS Biology, PNAS, PLoS Pathogens, Trends in Parasitology, eLife, Philosophical Transactions of the Royal Society among others).

Keynote Speaker 3

Wednesday 10 (9:00)

Disclosing pig-pathogen interactions through meta-omics tools

Dr. Héctor Argüello-Rodríguez

University of León, Spain

Dr. Héctor Argüello-Rodríguez is a Doctor in Veterinary Medicine and holds a Ph.D. in Animal Health. After acting as Research Officer at the Food Safety Department at Teagasc (Dublin, Ireland) and at the Genetics Department of the University of Cordoba (Spain), he joined the University of Leon, Spain, as an Associate Professor. He is also a diplomate of the European College of Veterinary Microbiology. His research interests are focusing on host-pathogen-microbiota interactions in intestinal infectious diseases in pigs, on strategies for controlling antimicrobial and on the study of microbiome-resistome interactions in pig industry. Dr. Argüello is the co-author of a relevant number of articles (Anim. Microbiome, Gut Pathog, Vet. Res., Microbiome among others) and actively collaborates with EFSA, FAO and national research agencies.

Social Program

Welcome reception

Sunday July 7, 2024

Hotel Cristina by Tigotan

Swimming pool terrace at 19:00h

C/ Gomera, 6, Playa Las Canteras, 35008-Las Palmas de Gran Canaria, España

Tlf. Tel. +34 928 268 050

www.dreamplacehotels.com

Congress Gala Dinner

Wednesday July 10, 2024

Poema del Mar aquarium

Av. de Los Consignatarios, s/n, 35008, Las Palmas de Gran Canaria, España

https://www.poema-del-mar.com/

Post-congress tour

Friday July 12, 2024

Wonders of the south

The dunes of Maspalomas, the picturesque Puerto de Mogán and the Guanche caves

Admission information in https://iom2024.ulpgc.es/?page_id=110

For further information contact the secretary office

Sunday 07	Monday 08	Tuesday 09	Wednesday 10	Thursday 11
	9:00 Opening Ceremony	9:00 Keynote Lecture 2	9:00 Keynote Lecture 3	9:00 Seminar 4
	9:30 Emmy Klieneberger-Nobel Award Lecture	9:50 Symposium 2	9:50 Symposium 3	
	10:30 Coffee Break	10:30 Coffee Break	10:30 Coffee Break	10:30 Coffee Break
Taxonomy meeting 12:00-14:00 Room: Santa Catalina	11:00 Seminar 1	11:00 Symposium 2	11:00 Symposium 3	11:00 General Meeting & Closing Ceremony
		11:40 Derrick Edward Award Lecture	11:40 Short Talks 3	
		12:10 Short Talks 1		
	13:00 Lunch	13:00 Lunch	13:00 Lunch	13:00 Lunch
	14:00 IRPCM	14:00 IRPCM	14:00 IRPCM	
IRPCM meeting 15:00-17:00 Room: Santa Catalina	15:00 Keynote Lecture 1 & Robert F. Whitcomb Award	15:00 Seminar 2	15:00 Seminar 3	
	15:50 Symposium 1			
	16:30 Coffee Break	16:30 Coffee Break	16:30 Coffee Break	
	17:00 Symposium 1	17:00 Short Talks 2	17:00 Short Talks 4	
17:00 – 18:30 IOM Board meeting Room: Santa Catalina		17:30 – 19:00 Poster Session 1	17:30 – 19:00 Poster Session 2	
	17:40 Peter Hannan Award Lecture			
Welcome Reception Swimming pool terrace 19:00	IOM Board Dinner Meeting point at the hotel at 19:00	**Old Town Visit** Meeting point at the hotel at 19:00	**Gala Dinner** Meeting point at the hotel at 19:30	

IRPCM meetings

Rooms	Monday 08 14:00 - 15:00	Tuesday 09 14:00 - 15:00	Wednesday 10 14:00 - 15:00
Santa Catalina (11th level)	Clinical Human	-	Avian
Las Palmas	Chemotherapy of mycoplasma infections	Porcine	New and Emerging Species
Gran Canaria	Ruminant	Molecular Genetics and Cell Biology	Plant and Arthropod

Scientific Program Monday 08

09:00 – 09:30 **Opening Ceremony**

09:30 – 10:30 **Emmy Klieneberger-Nobel Award Lecture**
Chairs: Inna Lysnyansky

Alain Blanchard
From Gene Cloning to Genome Engineering:
a Mycoplasmology Journey

10:30 – 11:00 **Coffee Break**

11:00 – 13:00 **Seminar 1 - Virulence & Diagnostic**
Chairs: Aizhen Guo & Salvatore Catania

11:00 **Florence Tardy**
O-05 *Mycoplasma equirhinis*, a neglected player in equine respiratory disorders?

11:15 **Jennifer Guiraud**
O-18 *Mycoplasma pneumoniae* epidemic in France, 2023-2024

11:30 **Elyse Levenda**
O-69 Interactions of *Mycoplasma penetrans* with urethral epithelial tissue culture cells

11:45 **Anneke Feberwee**
O-67 Virulence of two recent Dutch *Mycoplasma synoviae* isolates in broilers and demonstration of *M. synoviae* in lesions by an *in-situ* hybridization test

12:00 **Alexandra Burne**
O-71 The invertebrate *Galleria mellonella* confirms differences in virulence potential between *Mycoplasma alligatoris* and *Mycoplasma crocodyli*, two closely related Crocodilian pathogens

12:15 **Calvin Ko**
O-23 Development of a viability-qPCR detection system and in vitro growth evaluation of *Mycoplasma hyopneumoniae*

12:30 **Nadeeka Wawegama**
O-01 Development of a model of *Mycoplasma bovis* mastitis in sheep

Scientific Program Monday 08

13:00 – 14:00 **Lunch**

14:00 – 15:00 **IRPCM**

15:00 – 16:30 **Symposium 1**
Virulence, pathogenesis and control
Chairs: Li Xiao & Glenn Browning

15:00 **Keynote Lecture and Robert F. Whitcomb Award**
Saskia Hogenhout
How a mollicute induces developmental changes in its host and attracts insect vectors

15:50 **Martina Seruga Music**
O-60 *'Candidatus Phytoplasma solani'* predicted effectors SAP11-like and SAP54-like are individually sufficient to alter phenotype of transformed Arabidopsis plants

16:10 **Wei Wei**
O-25 Artificial Intelligence-Based Diagnosis of Phytoplasma Diseases: A Case Study on Tomato Plants Infected by Potato Purple Top Phytoplasma

16:30 – 17:00 **Coffee Break**

17:00 **Gang Zhao**
O-38 Mycoplasma Nucleomodulin MbovP475 promotes inflammatory cytokines transcription by remodeling chromatin of macrophages

17:20 **Xiong Qiyan**
O-37 Efficacy evaluation in pigs of a combined inactivated vaccine against *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis*

17:40 – 18:10 **Peter Hannan Award Lecture**
Chairs: Li Xiao & Glenn Browning

Chris Morrow
A 40-year struggle with *Mycoplasma synoviae*:
an insidious pathogen hiding as a commensal

Scientific Program Tuesday 09

09:00 – 10:30 **Symposium 2**
Diversity, outbreaks and surveillance
Chairs: Cécile Bébéar & Alain Blanchard

09:00 **Keynote Lecture**
Benjamin Roche
Understanding the interplay between host and pathogen diversities: opportunities for prevention of emerging infections?

09:50 **Joachim Spergser**
O-56 Species diversity within family *Mycoplasmataceae* – a fresh perspective on the expansion of genera *Mycoplasma* and *Ureaplasma* with 140 novel species entities

10:10 **Michael Kube**
O-50 Genomic Insights into the sugar beet pathogen '*Candidatus Phytoplasma solani*' driving the outbreak in Germany

10:30 – 11:00 **Coffee Break**

11:00 **Maureen Diaz**
O-28 Harnessing clinical laboratory data sources for surveillance of *Mycoplasma pneumoniae* infections in the United States

11:20 **Mary Brown**
O-57 *Mycoplasma agassizii:* Coevolution with ancient land tortoises or introduction to wild populations?

11:40 – 12:10 **Derrick Edward Award Lecture**
Chairs: Cécile Bébéar & Alain Blanchard

Patrick Meyer Sauteur
Mycoplasma pneumoniae: delayed re-emergence, new diagnostics, and evaluation of antibiotic treatment

Scientific Program Tuesday 09

12:10 – 13:00 **Short Talks 1**

Chairs: Sathya Kulappu Arachchig & Calvin Ko

Lars Vogelgsang

O-32 From sequence to activity: Insights into the HgaI-RM-system of *Mycoplasma hominis*

Maverick Monié—Ibanes

O-24 Airways and gut microbiome disturbance and resilience dynamics during polymicrobial bovine respiratory disease

Beatriz Almeida Sampaio

O-34 Assessment of immune response in goats using recombinant P40 and MAG_1560 as vaccine candidates for *Mycoplasma agalactiae*

Jitra-Marie Jittasevi

O-09 Deciphering genome transplantation mechanisms as a step towards understanding basic principles of life

Hani Alnakhli

O-35 Development of a subunit vaccine against *Mycoplasma gallisepticum* utilizing diverse surface proteins

Elisabet Frutos Grilo

O-08 Mycoplasma-derived vesicles: novel therapeutic vehicle for lung disorders

Zhixin Feng

O-36 Mycoplasma shares a similar immune escape mechanism to mediate persistent infection

13:00 – 14:00 **Lunch**

14:00 – 15:00 **IRPCM**

15:00 – 16:30 **Seminar 2 – Genomics and beyond**

Chairs: Veronica Jarocki & Shigeyuki Kakizawa

15:00 **Anna-Marie Ilic**

O-54 Power of codon usage analysis for phylogenetic reconstruction of the *Mollicutes*

15:15 **M'hamed Derriche**

O-12 Host environmental factors promote horizontal dissemination of integrative conjugative elements (ICE) in ruminant mycoplasmas

Scientific Program Tuesday 09

15:30 **Sabine Pereyre**
O-33 Circularization, transcription and transfer capacity of integrative and conjugative elements of *Mycoplasma hominis*

15:45 **Sergi Torres-Puig**
O-31 Prepare for landing: use of a double site-specific recombination system to stably insert foreign DNA sequences in the chromosome of *Mycoplasma feriruminatoris*.

16:00 **Patrick Hogan**
O-29 Improved CRISPR-base editor tools for genome edition in *Mycoplasma bovis*: Application to surface proteins

16:15 **Javier Gonzalez de Miguel**
O-07 Engineering Mycoplasma for the treatment of Idiopathic Pulmonary Fibrosis

16:30 – 17:00 **Coffee Break**

17:00 – 17:30 **Short Talks 2**
Chairs: Laure Beven & Amir Hadjinoormohammadi

Manon Vastel
O-02 β(1-6)glucan homopolymer in mycoplasma capsules

Doukun Lu
O-64 Identification of nucleomodulins of *Mycoplasmopsis bovis* by Magnetic bead enrichment and proximity-based biotinylation approaches

Sathya N. Kulappu Arachchige
O-39 Assessment of tracheal mucosal thickness is the most powerful and reproducible method for evaluation of the efficacy of vaccines against *Mycoplasma gallisepticum* in poultry

Salim Chibani
O-49 Breaking down the complexity of *Mycoplasma hominis*

Mitchell Balish
O-16 Synergistic interactions among antimicrobial agents targeting *Mycoplasma pneumoniae* biofilms *in vitro*

17:30 – 19:00 **Poster Session 1**

Scientific Program Wednesday 10

09:00 – 10:30 **Symposium 3**
Omics and high-throughput approaches
Chairs: Christine Citti & Sergi Torres Puig

09:00 **Keynote Lecture**
Héctor Argüello-Rodríguez
Disclosing pig-pathogen interactions through meta-omics tools

09:50 **Chih-Horng Kuo**
O-53 Assessing the recent revisions of *Mollicutes* taxonomy based on genome-scale analyses of phylogenetic inferences and gene content comparisons

10:10 **Glenn Browning**
O-03 Characterisation and comparison of the genomes of feline mycoplasmas

10:30 – 11:00 **Coffee Break**

11:00 **Yoshihito Kitaoku**
O-55 Mycoplasmogenesis: Towards Definition of the Obscured Contour

11:20 **Alessandra Tata**
O-47 Ambient mass spectrometry-based culturomics: a novel, rapid, and accurate approach for monitoring the metabolic behavior of Mycoplasma: a proof-of-concept demonstration on *Mycoplasma gallisepticum*

11:40 – 13:00 **Short Talks 3**
Chairs: Maureen Diaz & Sabine Pereyre

John W. Sanford
O-11 A Surface post-translational modification system is conserved in *Mycoplasma genitalium*, *Mycoplasma mycoides*, and JCVI-Syn3A

Imdad Ullah
O-04 Molecular detection and isolation of *Mycoplasma bovis* strains from tissue samples of *Bos granniens* (Yak)

Marina Marcos Silva
O-30 Heterologous expression of *Mycoplasma pneumoniae* major adhesins in the close related species *Mycoplasma genitalium*

Michele Gastaldelli
O-21 Isolation of a potentially novel Mycoplasma species related to *M. bovirhinis* from dairy cattle affected by BRD in Northern Italy

Scientific Program Wednesday 10

Morgan Hunte

O-66 In vivo disease severity in a mouse model of *Mycoplasma pneumoniae* is influenced by the adherence state of bacteria during inoculum preparation.

Jan Werner Böhm

O-51 Analysis of the Genomic Core of *Phytoplasmas* and other *Mollicutes*

Li Xiao

O-22 Development of a real-time PCR assay for simultaneously detecting *Mycoplasma genitalium* and its mutations associated with fluoroquinolone resistance in clinical specimens

Anne Bouchardon

O-06 Can BactoBox® be used for Mycoplasma viable cell counting?

Yukiko Nakura

O-48 Identification of an endonuclease and N6-adenine methyltransferase from *Ureaplasma parvum* SV3F4 strain

Chloé Ambroset

O-52 Hotspot of recombination in *Metamycoplasma equirhinis*: evidence of multiple horizontal gene transfer events originating from bacteria sharing the same ecological niche

13:00 – 14:00 **Lunch**

14:00 – 15:00 **IRPCM**

15:00 – 16:30 **Seminar 3 - Cell Biology**

Chairs: Birgit Henrich & Eric Baranowski

15:00 **Laure Beven**

O-15 Unlocking Spiroplasma helix secrets: fibril's role in morphological resilience

15:15 **Aizhen Guo**

O-10 Cyclic adenylate synthase MbovP496 regulates *Mycoplasmopsis bovis* growth by affecting potassium ion transport channels

Scientific Program Wednesday 10

15:30 **Hengning Wu**
O-62 Structural analysis and Molecular dynamics simulations of urease from *Ureaplasma parvum*

15:45 **Federico Gabriel Mirkin**
O-61 The structural landscape of the secretome of phytoplasmas, master modulators of plant architecture

16:00 **Prescott Atkinson**
O-63 An ABC transporter pair are essential for the synthesis of the *Mycoplasma genitalium* biofilm structural exopolysaccharide

16:15 **Shigeyuki Kakizawa**
O-14 Serum and albumin free media for Mycoplasmas

10:30 – 11:00 **Coffee Break**

17:00 – 17:30 **Short Talks 4**
Chairs: Rohini Chopra- Dewasthaly & Rubén Rosales

Theresa Wagner
O-13 Mycoplasma extracellular vesicle interaction with the bovine host

Maryne Jaÿ
O-26 Recent updates for VIGIMYC, a settled 20-year old surveillance network dedicated to ruminant mycoplasmas

Hezron O. Wesonga
O-17 Recent observations on use of tulathromycin and oxytetracylines in the treatment of contagious bovine pleuropneumonia in Kenya

Musa Mulongo
O-45 Report of CBPP expert Workshop on measurement of efficacy in Clinical Trials

James Miser Akoko
O-27 Development of a scalable CBPP control strategy for pastoral production systems: preliminary findings

17:30 – 19:00 **Poster Session 2**

Scientific Program Thursday 11

09:00 – 10:30 **Seminar 4 - Immunology**

Chairs: Florence Tardy & Miklós Gyuranecz

09:00 **Thomas Démoulins**
O-43 Temperature impacts the bovine *ex vivo* immune response towards *Mycoplasmopsis bovis*

09:15 **Rohini Chopra-Dewasthaly**
O-46 A window into the immune responses of sheep experimentally infected with *M. agalactiae* – Role of Vpma phase variation and differential responses elicited by individual "Phase-Locked" Vpma Expression Variants

09:30 **Sahar Zare**
O-42 Could the GAPDH-enhanced MS-H strain redefine vaccine efficacy?

09:45 **Thatcha Yimthin**
O-44 Immune responses elicited by Janus-faced *Mycoplasma mycoides* - glycans make the difference

10:00 **Manoel Neres Santos Junior**
O-41 Microorganism-host interaction and the immunomodulatory mechanisms of synthetic genome *Mycoplasma mycoides* subsp. *capri* (Mmc) strains in caprine peripheral blood mononuclear cells culture

10:15 **Lucas Miranda Marques**
O-40 Development and immunogenicity of a multiepitope recombinant antigen for the diagnosis of *Mycoplasma genitalium*

10:30 – 11:00 **Coffee Break**

11:00 – 13:00 **General Meeting & Closing Ceremony**

13:00 **Lunch**

Poster Presentation Overview

Session 1

Animal Mollicutes

O-04 Harry Morton Student Award Candidate

Molecular detection and isolation of *Mycoplasma bovis* strains from tissue samples of *Bos granniens* (Yak)

Imdad Ullah, Farhan Anwar Khan, Muhammad Saeed

P-01

A pathogen-specific T-cell response is induced after *Mycoplasma hyorhinis* lung infection

Moritz Bünger, Melissa Stas, Armin Saalmüller, Joachim Spergser, Andrea Ladinig

P-02

Comparative genome analysis of *Mycoplasma* cavipharyngis, an apathogenic *mycoplasma* related to the pathogenic cluster of hemotrophic mycoplasma

Janina Kramer, Bruno Huettel, Christina Zübert, Ludwig Hoelzle, Michael Kube

P-03

Assessing the effectiveness of disinfectants against agents of contagious agalactia contained in organic matrices

Anne Ridley, Issa Muraina

P-04

Comparison of DNA extraction procedures for detection of *Mycoplasma bovis* directly from extended bovine semen straw samples using a commercial *M. bovis* PCR

Emma Taylor, Alannah Deeney, Anne Ridley, Georgia Mayne

P-05

Elucidation of the Surfaceomes of *Mycoplasma bovis* Isolates from Australian Feedlot Cattle

Veronica Jarocki, Darren Trott, Kiro Petrovski, Mauida Al Khallawi, Steven Djordjevic

P-06

In vivo intravaginal application of probiotic *Lactobacillus* spp. in sheep: influence on the presence of *Mycoplasma* spp. in the vaginal microbiota

Marion Toquet, Jesús Gomis, Estrella Jiménez-Trigos, Esther Bataller, Marta Barba, Antonio Sánchez, Pedro González Torres, Ángel Gómez Martín

P-07

Description and comparison of the microbiota of raw milk in healthy and subclinical mastitis-affected small ruminants: presence of *Mycoplasmataceae*

Marion Toquet, Jesús Gomis, Esther Bataller, Estrella Jiménez-Trigos, Nuria Mach, Xavier Nouvel, Eric Baranowski, Ángel Gómez Martín

P-08

Presence of *Mycoplasma bovis* subtype ST-1 with a variable susceptibility to fluoroquinolones in Spain

Juan Carlos Corrales, Antonio Sánchez, Xóchil Pérez, Joaquín Amores, Antón Esnal, Patricia Panales, Isabel Pérez, Christian de la Fe

P-09

Determining antibiotic susceptibility of *Mycoplasma hyorhinis* circulating in Spain

Juan Carlos Corrales, Xóchil Hernández, Antonio Sánchez, Joaquín Amores, Isabel Pérez, Christian de la Fe

P-10

Evolution of antimicrobial susceptibility of *Mycoplasma agalactiae* and *Mycoplasma mycoides* subsp. *capri* in Spanish isolates collected between 2020 and 2023

Joaquín Amores, Xóchil Hernández, Antonio Sánchez, Juan Carlos Corrales, Isabel Pérez, Christian de la Fe

P-11

Genetic Analysis of *Mycoplasma gallisepticum* Isolates – Implications for Epidemiology

Naola Ferguson-Noel, Marianne Dos Santos

P-12

Virulence of *Mycoplasma tullyi* using the *Galleria mellonella* infection model

Alexandra Burne, Brittany Whitcher, Dina Michaels, Mary Brown

P-13

Outbreak of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* in captive peregrine falcons

Rubén S Rosales, Ana S Ramírez, Ruth Blanco, Pedro Salinas-Casas, José B Poveda

Cell Biology and Metabolism

O-08 Louis Dienes Award Candidate

Mycoplasma-Derived Vesicles: Novel Therapeutic Vehicle for Lung Disorders

Elisabet Frutos Grilo, Giulia Gaudino, Matilde D'Angelo, Luis Serrano

O-11 Harry Morton Student Award Candidate

A Surface Post-Translational Modification System is Conserved in *Mycoplasma genitalium*, *Mycoplasma mycoides*, and JCVI-Syn3A

John W. Sanford, James Daubenspeck, Kevin Dybvig, T. Prescott Atkinson

O-13

Mycoplasma extracellular vesicle interaction with the bovine host

Theresa Wagner, Thomas Démoulins, Jörg Jores

P-14

L-Asparagine is the essential factor for the susceptibility of Chinese local pigs to *Mycoplasma hyopneumoniae*

Xing Xie, Long Zhao, Fei Hao, Lulu Xu, Qiyan Xiong, Yanna Wei, Lei Zhang, Rong Chen, Yanfei Yu, Yun Bai, Yuan Gan, Yongjie Liu, Guoqing Shao, Zhixin Feng

Chemotherapy and Resistance

O-16

Synergistic Interactions among Antimicrobial Agents Targeting *Mycoplasma pneumoniae* Biofilms In Vitro

Rasha A. Fahim, Natalie E. Young, Kavita Shrestha, Mitchell F. Balish

P-15

Antimicrobial Susceptibility and genetic mechanisms of resistance of *Ureaplasma* isolates between 2012 and 2023 in the United States

Joshua Waites, Donna Crabb, Amy Ratliff, Melanie Fecanin, Ken Waites, Li Xiao

P-16

Development of molecular assays for the detection of antibiotic susceptibility in *Mycoplasma iowae*

Dominika Buni, Enikő Wehmann, Dorottya Földi, Krisztián Bányai, Krisztina Bali, Janet Bradbury, Marco Bottinelli, Salvatore Catania, Inna Lysnyansky, Miklós Gyuranecz, Zsuzsa Kreizinger

P-17

Antimicrobial susceptibility profiles of *Mycoplasma hyosynoviae* strains isolated from swine across Europe between 2018 and 2023

Ulrich Klein, Dorottya Földi, Zsófia Eszter Nagy, Lilla Tóth, Nikolett Belecz, Karola Költő, Marianna Merenda, Salvatore Catania, Joachim Spergser, Philip Vyt, Ute Siesonop, Zsuzsa Kreizinger, Miklós Gyuranecz

P-18

Antimycoplasmal activity of *Peganum harmala* on *Mycoplasma hominis* Tunisian strains

Boutheina Ben Abdelmoumen Mardassi, Béhija Mlik, Nadine Khadraoui, Imen Chniba, Nadia Fares, Olfa Tabbene, Rym Essid, Selim Jallouli

P-19

Antimicrobial susceptibility and genetic mechanisms of resistance of *Mycoplasma hominis* isolates between 2012 and 2023 in the United States

Li Xiao, Amy Ratliff, Donna Crabb, Joshua Waites, Ken Waites, Melanie Fecanin

P-20

Use of antibiotics in the control of contagious bovine pleuropneumonia (CBPP): current status.

Angela Makumi, Arshnee Moodley, Elise Schieck, Tore Tollersrud

Diagnostic and Epidemiology

O-23 Harry Morton Student Award Candidate

Development of a viability-qPCR detection system and in vitro growth evaluation of *Mycoplasma hyopneumoniae*

Calvin Ko, Pablo Piñeyro, Phillip Gauger, Rachel Derscheid, Shawn Rigby

P-21 **Harry Morton Student Award Candidate**

Evaluation of diagnostic methods for the detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infections in poultry

Congriev Kumar Kabiraj, Nicholas Evans, Kannan Ganapathy

P-22

Duplex droplet digital PCR for the detection and quantification of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*

Zhixun Xie, Zhiqin Xie, Qing Fan, Liji Xie, Sisi Luo

P-23

Innovative MG and MS ELISA tests, based on highly specific recombinant proteins, for the diagnosis and the monitoring of vaccination

Emmanuelle Cambon, Stéphanie Lesceu, Catherine Lefebvre, Chloé Redal, Jean- Emmanuel Drus, Marina Gaimard

P-24

New qPCR assays for CCPP and CBPP diagnosis

Anne Boissiere, Marjolaine Durand, Phonsiri Saengram, Lucia Manso-Silvan

P-26

Rapid detection of human pathogenic Mycoplasma species using a multiplex real-time PCR assay

Alvaro Benitez, Jonas Winchell, Maureen Diaz

P-27 **Harry Morton Student Award Candidate**

***Mycoplasma* spp. presence in vagina, prepuce and raw milk microbiota of sheep: preliminary metagenomic study**

Raquel Toledo Perona, Jesús Gomis Almendro, Marion Toquet, Antonio Contreras de Vera, Juan José Quereda Torres, Pedro González Torres, Ángel Gómez Martín

P-28

The unexpected outcome: incidental detection of *M. gallinarum* septicemia in turkeys affected by respiratory syndrome

Marco Bottinelli, Ilenia Rossi, Verdiana Righetti, Beatrice Colò, Davide Prataviera, Massimo Zago, Elena Rinaldi, Elisabetta Stefani

P-29

Genotyping of *Mycoplasma hyorhinis* through Multi-Locus Sequence Typing

Giorgia Nai, Verdiana Righetti, Davide Prataviera, Massimo Bottazzari, Michele Gastaldelli, Elisabetta Stefani, Marianna Merenda

P-30

Establishment and preliminary application of antibody-sandwich ELISA for detection of *Mycoplasma ovipneumoniae* antigen

Wenwen Zhang, Xiaonan Wang, Siyu Chen, Yiyi Shan, Yi Chen, Wenliang Li, Zilong Cheng, Leilei Yang, Jinquan Wang, Maojun Liu

P-31

Seroprevalence of contagious bovine pleuropneumonia in Poland in 2023

Katarzyna Dudek, Dariusz Bednarek

P-32

A three-year study on the seroprevalence of contagious agalactia in Poland

Katarzyna Dudek, Dariusz Bednarek

P-33

Validation of a multiplex PCR for detection of Mycoplasma species in dogs with respiratory diseases

Maryne Jaÿ, Maxime Bruto, Florine Dieu, Salomé Grizard, Chloé Ambroset, Emilie Krafft, Claire A.M. Becker,

P-35

Detection of hemoplasmas and direct sequencing in blood from cattle reveals a diversity amongst *Mycoplasma wenyonii* species

Chloé SAADA, Hortensia ROBERT, Lucie ROQUES, Marie-Claude HYGONENQ, Renaud MAILLARD, Christine CITTI, Laurent Xavier NOUVEL

P-36

Preventive Medicine for Mycoplasma infectious diseases: proposal for continuous and centralized management of databases

Kazuhiro Matsuda

P-37

Enhanced surveillance of *Mycoplasma pneumoniae* epidemic and macrolide resistance in England from December 2023 to March 2024

Baharak Afshar, Anna Lewis, Hannah Bransbury-Hare, Kanchanamala Gunasekara, KayDee Hosten-Sandy, Rebecca Thombre, Sendurann Nadarajah, Nita Doshi

Poster Presentation Overview

Session 2

Genome Engineering and Synthetic Biology

O-07 Harry Morton Student Award Candidate

Engineering Mycoplasma for the treatment of Idiopathic Pulmonary Fibrosis

Javier Gonzalez de Miguel, Irene Rodriguez Arce, Luis Serrano

O-09 Harry Morton Student Award Candidate

Deciphering genome transplantation mechanisms as a step towards understanding basic principles of life

Jitra-Marie Jittasevi, Géraldine Gourgues, Dominick Matteau, Sébastien Rodrigue, Carole Lartigue

O-29 Harry Morton Student Award Candidate

Improved CRISPR-base editor tools for genome edition in *Mycoplasma bovis*: Application to surface proteins

Patrick Hogan, Fabien Rideau (Institut Bergonié), Carole Lartigue, Alain Blanchard, Pascal Sirand-Pugnet, Laure Beven, Eric Baranowski, Yonathan Arfi

O-30 Harry Morton Student Award Candidate

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Alexandra Burne, Dina Michaels, Mary Brown

ABSTRACTS

Oral Presentations

Animal Mollicutes

O-01

Development of a model of *Mycoplasma bovis* mastitis in sheep

Nadeeka Wawegama (1), Anna Kanci Condello (1), Ayesha Salgadu (1), Paola Vaz (1), Dinusha Elapathage (1), Muhammad Joan Ailia (1), Sagar Regmi (1), Reza Sanaei (2), Joanne Allen (1), Liz Dobson (2), Stuart Barber (1), Glenn Browning (1)

(1) Asia Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Science, The University of Melbourne, Parkville, Australia
(2) Melbourne Veterinary School, Faculty of Science, University of Melbourne, Victoria, Australia

Nadeeka Wawegama <nadeekaw@unimelb.edu.au>

Mycoplasma bovis is a pathogen of cattle, causing pneumonia, arthritis and mastitis. The aim of this study was to develop a small ruminant model to evaluate the pathogenesis of mastitis caused by *M. bovis*. The left mammary glands of five *M. bovis*-free lactating ewes (infected group) were inoculated with an Australian isolate of *M. bovis* and the left glands of another five *M. bovis*-free lactating ewes (control group) were inoculated with culture medium. Ewes in both groups were milked twice daily and the milk volume and quality were recorded daily. Milk was cultured from all ewes on sheep blood agar and in mycoplasma broth every second day until necropsy, at 21 days post-infection (PI). The milk production in the infected left glands dropped significantly, from an average of 250 ml/day to 40.4 ml/day, from day 2 PI, and the milk quality changed from creamy and clotted on day 1 PI to watery and purulent on day 7 PI, then to watery and greenish with clots from day 14 PI until necropsy. The milk consistency in the uninfected right glands of the infected ewes and both glands of the control ewes was normal and continued to produce an average milk volume of 260 - 300 ml/day throughout the experiment. The mean somatic cell counts (SSC) of the milk from the infected left glands was significantly higher than that from the uninfected glands from day 1 to day 20 PI, with the mean SCCs of the infected glands increasing from 64 cells/µl to >4000 cells/µl from day 1 PI. At necropsy, the infected left glands were reduced in size, with loss of mammary tissue, and filled with caseous material. Microscopically, the normal architecture of the mammary tissue was lost, with alveolar tissues and the lobular ducts filled with inflammatory cells. This small ruminant mastitis model appeared to induce mastitis typical of the naturally occurring disease associated with infection with *M. bovis* in cattle and should be applicable for testing the safety and efficacy of attenuated vaccine candidates to control disease caused by this pathogen.

O-02

β(1-6)glucan homopolymer in mycoplasma capsules

Manon Vastel (1, 2), Séverine Ferre (2), Véronique Tocqueville (2), Chloé Ambroset (1), Anne V. Gautier-Bouchardon (2), Florence Tardy (1, 2), Patrice Gaurivaud (1)

(1) ANSES, Lyon Laboratory, Joint Research Unit on Animal Mycoplasmoses, Lyon, France
(2) ANSES, Ploufragan-Plouzané-Niort Laboratory, Mycoplasmology, Bacteriology and Antimicrobial resistance Unit, Ploufragan, France

Manon Vastel <manon.vastel@anses.fr>

β(1-6)glucopyranose (β(1-6)glucan) polysaccharidic capsules have been previously described in three ruminant mycoplasma species (*Mycoplasma* (*M.*) *agalactiae*, *M. feriruminatoris* and *M. mycoides* subsp. *capri serovar capri*) and associated to pro-inflammatory properties (Rubin-Bejerano et al., 2007). The aim of this study was to determine if other *Mycoplasma* species from different animal hosts (ruminants but also poultry, swine and primates) were also able to secrete capsular β(1-6)glucan. A first *in vitro* screening conducted using the non-specific Dubois' method for polysaccharides quantification evidenced a polysaccharidic capsule in various mycoplasmas infecting ruminants, primates and poultry but not swine. Amongst them, only *M. iowae* strain I695 was shown by dot blot to produce a capsule containing β(1-6)glucan. Genome comparisons evidenced in *M. iowae* strain I695 (accession number of the genome: CP033512.2) a β(1-6)glucan biosynthesis pathway similar to that previously described in *M. agalactiae* strain L14628 (Gaurivaud et al., 2016). Furthermore, an antigenic variation resulting in sectored colonies using immunoblotting assays was observed in both strains. In *M. agalactiae* L14628 this variation is due to a nine-G repetition at the beginning of the gene coding the glycosyltransferase responsible for the secretion of the β(1-6)glucan (Gaurivaud et al., 2016). In *M. iowae* I695 the presence of a nine-TA repetition at the beginning of the glycosyltransferase gene might similarly be responsible for the antigenic variation.
The potential secretion of a β(1-6)glucan capsule was screened *in vitro* by colony blotting and by a PCR assay targeting the gene coding the glycosyltransferase in different *M. iowae* isolates. Preliminary results indicate that out of 21 isolates only the strain I695 produces a β(1-6)glucan capsule *in vitro* and is equipped with the gene coding the glycosyltransferase. The absence of a glycosyltransferase gene has so far been confirmed by WGS of three isolates (with available genome in Genbank for one of them). This suggests that the β(1-6)glucan capsule production is not a characteristic of the *M. iowae* species, in contrast to what was observed in *M. agalactiae*. The role of the capsule in serum-killing susceptibility of the isolates will be further assessed.

O-03

Characterisation and comparison of the genomes of feline mycoplasmas

Glenn Browning (1), Sara Klose (1), Alistair Legione (1), Rhys Bushell (1), Marc Marenda (1), Isobel Monotti (2), Takanori Sugiyama (2), Paola Vaz (1)

(1) Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Science, The University of Melbourne, Parkville, Australia
(2) uVet Veterinary Hospital, Melbourne Veterinary School, Australia

Glenn Browning <glenfb@unimelb.edu.au>

Although several mycoplasma species have been implicated as causes of disease in cats, there has been limited recent characterisation of any of the species involved. Here we characterised the genomes of several isolates of different mycoplasma species recovered from cats. The first isolate was recovered from a purulent wound infection that had resulted from a dog bite and that had failed to respond to treatment with amoxycillin/clavulanate. Using a combination of short read Illumina and long read Nanopore sequencing, a complete genomic sequence was assembled. The species was identified as *Mycoplasma edwardii*, a species that has previously been recovered from mucosal surfaces of dogs. A second isolate from a surgical wound infection, as well as three isolates from respiratory tract infections, were also characterised using a combination of short read Illumina and long read Nanopore sequencing. All four isolates were found to be *Mycoplasma felis*. Comparison of these genomes of feline isolates with the genome of an equine isolate of this species, the only genome of *M. felis* characterised previously, revealed multiple horizontal genetic transfer events and genomic rearrangements, as well as the presence of a phage genome in one isolate. The production of phage particles by this isolate was confirmed by electron microscopy. Several genes were found in all the feline isolates, but not the equine isolates, and, conversely, several genes were only found in the equine isolate. Further studies comparing the biology of equine and feline isolates, as well as isolates infected with phages, will assist in determining whether equine and feline isolates of *M. felis* are adapted to their respective hosts, and whether the *M. felis* phage has any effect on the virulence of *M. felis*.

O-04 Harry Morton Student Award Candidate

Molecular detection and isolation of *Mycoplasma bovis* strains from tissue samples of *Bos granniens* (Yak)

Imdad Ullah (1), Farhan Anwar Khan (1), Muhammad Saeed (1)

(1) The University of Agriculture Peshawar, Pakistan

Imdad Ullah <imdad75@aup.edu.pk>

Mycoplasma bovis (*M. bovis*) is one of the causative agents of bovine respiratory disease syndrome (BRDS) in adult animals and calf pneumonia (CP) in the young calves. BRDS results in the enormous economic impact on the cattle industry in terms of production loss i.e. milk and meat. Treatment cost and welfare of the animals are also counted as the important out comes of this disease. Yaks (Bos grunniens), member of bovidae family, is also susceptible to mycoplasmosis. Yaks are indispensable for the livelihood of high mountainous inhabitants; however very limited information is available regarding *M. bovis* infection in yaks. In this study, samples (nasal swabs, lung tissues and milk) were collected from *M. bovis* suspected Yaks (n=200) at Yarkhoon-Broghil (n=100) and Laspur-Shandur (n=100) valleys of district Chitral. For the identification and isolation of the *M. bovis* these samples were cultured in PPLO media. Purified fried egg colonies of *M. bovis* were isolated from the agar plate by day 5th post incubation. Fifty-five samples from Broghil area were culture positive while 42% were positive from Laspur. PCR analyses revealed *M. bovis* in 14% cultured positive samples from Broghil, whereas in Laspur only 7% were found positive for M.bovis. Eleven (11) strains of *M. bovis*, successfully isolated from tissue samples, were confirm by PCR using specie specific primers for *M. bovis*. Lungs from the *M. bovis* infected animals were showing supporative to caesonecrotic bronchopneumonia. The results of the present study highlighted the presence of mycoplasma infections, especially *M. bovis*, in Yaks population. This study demand for devising a vibrant control strategy for *M. bovis* infections in Yaks.

O-05

***Mycoplasma equirhinis*, a neglected player in equine respiratory disorders?**

Matthieu Martineau (1), Éléna Kokabi (1), Chloé Ambroset (2), Maryne Jaÿ (2), Albertine Leon (1), Florence Tardy (3)

(1) LABÉO, Pôle Recherche, Développement et Innovation, Normandie Univ, UNICAEN, UNIROUEN, Inserm UMR 1311 DYNAMICURE
(2) ANSES, Lyon Laboratory, VetAgro Sup, UMR Animal Mycoplasmosis, University of Lyon, Lyon, France
(3) ANSES, Ploufragan-Plouzané-Niort Laboratory, Mycoplasmology, Bacteriology and Antimicrobial Resistance Unit, Ploufragan, France

Florence TARDY <Florence.TARDY@anses.fr>

Respiratory disorders are responsible for poor performance in racehorses and for overall significant economic problems in the equine industry. Bacteria belonging to the *Mycoplasma* (*M.*) genus have been regularly reported in up to 15% of clinical specimens in different countries, but their clinical contribution remains unclear.

To assess their role, a detection / isolation protocol combining culture and post-enrichment PCRs was optimized and used on 1,948 respiratory samples collected from 1,764 horses in France over the 2020-2022 period. The prevalence of mycoplasmas was refined to 16.1%, with a predominance of *M. equirhinis* (*Mequi*) species (85.3%). We further used a clinical scoring, a risk factor analysis as well as a test for association with other bacteria and viruses to improve our understanding of the clinical role of *Mequi*. In contrast to the pattern of primary pathogens, *Mequi* prevalence remained steady whatever the clinical score. Nonetheless, in presence of *Streptococcus equi* subsp. *zooepidemicus* (*S. zoo*) or viruses like EHV-5 the prevalence of *Mequi* was significantly increased. CO-infection between *Mequi* and *S. zoo* was associated with, on average, a significantly higher load of *S. zoo*. These results indicate that *Mequi* is not a primary pathogen but could contribute to clinical signs in association with other microbes. In bronchoalveolar lavages, the detection of *Mequi* was associated with neutrophil-mediated inflammation, suggesting it could participate in an inappropriate immune response, similar to that observed in equine asthma. This is consistent with the fact that *Mequi* was more frequently isolated in racehorses and stabled horses, two favouring factors for asthma.

Through comparison of 24 genomes, we further showed that *Mequi* is a highly homogeneous species, with only a few mobile genetic elements like the *M. arginini*-like ICE (Integrative Conjugative Element) and a *M. arthritidis*-like prophage. The repertoire of genes putatively associated to virulence was rather limited to cytoadherence and immune escape, similarly to its closest phylogenetic neighbour, *M. hominis*. In conclusion, *Mequi* could play a part in the equine respiratory disease complex, in association with other microbes, essentially through dysregulation of the host immune response, as is well known for other mycoplasma species in other respiratory complexes.

O-06

Can BactoBox® be used for Mycoplasma viable cell counting?

Anne V. Gautier-Bouchardon (1), Séverine Ferré (1), Véronique Tocqueville (1), Maryne Jaÿ (1), Florence Tardy (1)

(4) ANSES, Ploufragan-Plouzané-Niort Laboratory, Mycoplasmology, Bacteriology and Antimicrobial Resistance Unit, Ploufragan, France

Anne V. Gautier-Bouchardon <anne.bouchardon@anses.fr>

Optical density measurements at a wavelength of 600 nm (OD600) are often used as a proxy to estimate the number of cells in bacterial suspensions. However, it is not applicable to *Mycoplasma* spp. because of the small size and pleomorphic nature of their cells. In consequence, mycoplasma cell counts rely on long and tedious methods such as counting colony forming units (CFU/mL) on agar plates or estimating colony changing units (CCU/mL) by following up the extinction of growth by serial broth dilutions in microtiter plates. Count results are then only available within 2-7 days (agar) and up to 1-3 weeks (broth) depending on mycoplasma species and strains. Impedance-based flow cytometry offers a sensitive methodology to detect and count bacterial cells. The BactoBox® (SBT instruments) claims to provide a simple portable solution for bacterial cell count (https://sbtinstruments.com/bactobox/resources). Whether this technique applies also to mycoplasmas has yet to be assessed. SBT contacted us within the framework of the MyMIC network to propose BactoBox® as a potential help in inoculum calibration for antimicrobial susceptibility testing (AST). To determine whether this technology could be used to quickly and reliably enumerate mycoplasma suspensions, tests were carried out in parallel with traditional cell counting techniques. Strains of different mycoplasma species isolated from ruminants (*Mycoplasma* (*M.*) *bovis*, *M. agalactiae*, *M. mycoides* subsp. *capri*, *M. capricolum* subsp. *capricolum*, *M. putrefaciens*), pigs (*M. hyopneumoniae*, *M. hyorhinis*, *M. flocculare*) and poultry (*M. gallisepticum*, *M. synoviae*, *M. meleagridis*, *M. iowae*, *M. pullorum*, *M. glycophilum*, *M. iners*, *M. gallinarum*) were tested. For most of the species tested, the results in CFU/mL obtained in a few minutes with the BactoBox® were comparable to those obtained on agar medium in several days. However, cell counts obtained during late growth phase or with lysed mycoplasma cells showed that the BactoBox® was not able to discriminate living from dead mycoplasmas. Due to conductivity issues for low dilutions, the lowest count determined by BactoBox® was at 10^5 CFU/mL. Despite these limitations, and provided that BactoBox® counts are standardized per species, it could really allow a gain of time in the preparation of strains' suspensions for AST.

Cell Biology and Metabolism

O-08 Louis Dienes Award Candidate

Mycoplasma-derived vesicles: novel therapeutic vehicle for lung disorders

Elisabet Frutos Grilo (1), Giulia Gaudino (1, 2), Matilde D'Angelo (1), Luis Serrano (1)

(1) Centre de Regulació Genòmica, Spain
(2) Unisa University, Italy

Elisabet Frutos Grilo <elisabet.frutos@crg.eu>

The use of extracellular vesicles (EVs) as advanced drug delivery systems has allowed the development of therapies based on a continuous or regulated targeted release of therapeutic molecules to a desired site. These therapeutic agents, including drugs or small interfering RNAs, can be encapsulated within the lumen of EVs or engineered onto their surfaces. Bacterial vesicles, non-replicating proteo-liposomes ranging from 20 to 250 nm in size, have garnered significant attention due to their versatile applications. Our research aims to leverage *Mycoplasma pneumoniae* (MPN) to produce vesicles for delivering target molecules into lung cells to address respiratory diseases. These conditions constitute a diverse group of disorders affecting the pulmonary system, exerting substantial impacts on individuals' health and quality of life. Encompassing both acute respiratory infections and chronic ailments such as asthma, cystic fibrosis, idiopathic fibrosis, chronic obstructive pulmonary disease, and lung cancer, these disorders present significant therapeutic challenges. MPN is a bacterium that lacks a cell wall, its membrane composition closely resembles that of mammalian cells, and it thrives in the lung's ecological niche. Consequently, MPN is an excellent candidate for target therapy in the lung and a promising candidate for use as a “live therapeutic” agent. MPN has been recently attenuated with the removal of virulence factors, yielding a non-pathogenic platform, named CV8. In this study, we not only elucidate the production and characteristics of vesicles derived from MPN but also explore their potential application in delivering specific molecules to lung cells, utilizing CV8-derived vesicles. These findings underscore the biotechnological promise of these vesicles as a versatile pharmacological platform, paving the way for further investigation into their therapeutic potential.

O-10

Cyclic adenylate synthase MbovP496 regulates *Mycoplasmopsis bovis* growth by affecting potassium ion transport channels

Aizhen Guo (1,2,3,4), Jiongxi Chen (1,2,3,4), Doukun Lu (1,2,3,4), Yingyu Chen (2), Xi Chen (2), Changmin Hu (2), Jianguo Chen (2)

(1) National Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, China
(2) College of Veterinary Medicine Huazhong Agricultural University, Wuhan, China
(3) Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan, China
(4) International Research Center for Animal Disease, Ministry of Science and Technology, Huazhong Agricultural University, Wuhan, China

Aizhen Guo <aizhen@mail.hzau.edu.cn>

Background: Intracellular homeostasis of c-di-AMP is crucial for maintaining normal bacterial physiology. Diadenylate cyclases (DAC) and phosphodiesterases (PDE) are the main proteins involved in c-di-AMP synthesis and degradation. In our previous report, we identified three PDEs in *Mycoplasmopsis bovis* (*M. bovis*) that are involved in c-di-AMP degradation and influence its growth. In this study, we further investigated the synthesis function and its mechanism of a DAC named MbovP496. **Methods**: Bioinformatic prediction and High Performance Liquid Chromatography (HPLC) were employed to determine the enzymatic activity and functional sites of MbovP496. Growth curve experiments were conducted in PPLO and co-inoculation with embryo bovine lung (EBL) cell line to comparatively investigate K+ tolerance of Mbov_0496 knock-out *M. bovis* mutant T5.415, its complementary CT5.415 and wild type HB0801(WT). Scanning electron microscope (SEM) was used to observe *M. bovis* morphology under various K+ concentrations. HPLC and Flame Atomic Absorption Spectrometry (FAAS) were used to measure intracellular c-di-AMP and K+ concentrations respectively. Pull-down assay, Bio-Layer Interferometry (BLI), Isothermal Titration Calorimetry (ITC), and RNA-seq were utilized to construct a regulation network. **Results**: MbovP496 belongs to DisA_N superfamily DAC. Then recombinant MbovP496 (rMbovP496) was expressed and confirmed the ability to synthesize c-di-AMP by using ATP. It is a thermostable alkaline synthase with functional motifs (DGA and RHR). Further, the T5.415 exhibited more sensitive to high K+ environment than CT5.415 and WT shown by significant shrinkage under SEM. Correspondingly, transcriptomics data revealed that the genes such as Mbov_0415 and Mbov_0421 responsible for K+ transport activity were upregulated. In addition, the K+ uptake transporter gene Mbov_0421 was confirmed to significantly upregulated in T5.415, compared to in CT5.415 and WT strains. The pull-down, BLI, ITC were used to determine that MbovP421 can bind to c-di-AMP through the sites of K172, R174, S179. **Conclusion**: We revealed MbovP496 can regulate c-di-AMP homeostasis to influence *M. bovis* adaptation to environmental K+ and its growth. This study offers a new understanding of the survival strategy of *M. bovis* under adverse condition, and the proteins of the DisA_N superfamily could be promising targets for development of novel anti-*M. bovis* drugs.

O-11 Harry Morton Student Award Candidate

A surface post-translational modification system is conserved in *Mycoplasma genitalium*, *Mycoplasma mycoides*, and JCVI-Syn3A

John W. Sanford (1), James Daubenspeck (1), Kevin Dybvig (1), T. Prescott Atkinson (1)

(1) University of Alabama at Birmingham, USA

John W. Sanford <jsanfor4@uab.edu>

Mycoplasma metabolism primarily revolves around parasitizing host cells and evading host immune responses. Previous work has demonstrated that the surface of mycoplasma cells is highly dynamic, with phase variation and proteolytic processing increasing the variety of epitopes in a mycoplasma population. We have previously identified a surface protein hexosylation system in the murine pathogens *Mycoplasma arthritidis* and *Mycoplasma pulmonis*. Unlike canonical bacterial glycosylation systems, hexoses are cleaved from exogenous oligosaccharides and attached through *N*- and *O*-linkages to glutamine, asparagine, serine, threonine, and tyrosine residues. This hexosylation system is highly stochastic, lacking any glycosylation motifs and instead being governed by spatial availability of the recipient amino acid. We have been unable to find evidence of a complete glycosylation knockout and therefore hypothesized that this system would be retained in minimal genome mycoplasmas. To test this hypothesis, we screened *Mycoplasma genitalium* (Mgen), a urogenital pathogen with the smallest natural mycoplasma genome; JCVI-syn3A (syn3A), a non-pathogenic mycoplasma with a synthetic near-minimum genome; and syn3A's parental organism *Mycoplasma mycoides* subsp. *capri*, for evidence of protein glycosylation using paired SDS-PAGE staining for protein and glycoprotein content. Upon finding evidence of glycosylation in all three organisms, we searched for evidence of hexosylation using high-resolution mass spectrometry. All three organisms have hexosylated surface proteins, including known moonlighting proteins such as EF-Tu. Furthermore, EF-Tu can be hexosylated at numerous residues in Mgen and syn3A, including Thr159 and Tyr161 residues, indicating this process remains stochastic in these minimal organisms. The presence of a gene or metabolic pathway retained in syn3A's artificial genome reduction and Mgen's natural genome streamlining strongly implies an essential or quasi-essential physiological function, even under axenic culturing conditions. While we conjecture that a highly variable hexosylation system would play a role in epitope masking, we also hypothesize that this hexosylation system is needed for axenic growth. Perhaps hexoses are shielding mycoplasma surface proteins from self-digestion from secreted proteases. Further work will be needed to identify what gene is responsible for this process. We hypothesize that this hexosyltransferase is among the genes annotated with an unknown function conserved between Mgen and syn3A.

O-12

host environmental factors promote horizontal dissemination of integrative conjugative elements (ICE) in ruminant Mycoplasmas

M'hamed Derriche (1), Laurent Xavier Nouvel (1), Eveline Sagné (1), Elisa Simon (1), Hortensia Robert (1), Gwendoline Pot (INRAE), Maria Gaudino (1), Gilles Meyer (1), Yonathan Arfi (2), Christine Citti (1), Eric Baranowski (1)

(1) IHAP, ENVT, INRAE, Université de Toulouse, Toulouse, France
(2) INRAE, BFP, UMR 1332, Univ. Bordeaux, Villenave d Ornon, France

M'hamed Derriche <mhamed.derriche@envt.fr>

Advances in understanding ICE horizontal transfer in ruminant mycoplasmas have been facilitated by the establishment of axenic, optimal conditions. However, these provided only limited information on the influence of host factors on this event which is known to play an important role in chromosomal transfer. To address this issue, ICE-transfer frequencies were analyzed in *M. agalactiae* under replicative environments of increasing complexity, from axenic and cell culture growth conditions to *ex vivo* infections using Precision-Cut Lung Slices (PCLS). Remarkably, co-incubation of mating partners with epithelial cells resulted in increasing ICE-transfer frequency by ca 200-fold when compared to axenic, optimized mating conditions. ICEs are known to integrate randomly in the mycoplasma chromosome and a similar number of different integration sites were found in axenic and cell conditions by whole-genome sequencing of transconjugants, thus ruling out the amplification of a few individual transconjugants with high fitness values in cell culture conditions. The positive effect of host-related environmental factors on the conjugative behavior of *M. agalactiae* has also been observed upon ex vivo infections of bovine PCLS. As expected, only sporadic transconjugants could be selected when mating partners were incubated alone in the culture medium without host cells. This study reveals that the conjugative properties of mycoplasma cells may be influenced by their replicative environment, with enhanced mating frequency upon co-incubation with host cells. Most importantly, these results also suggest that the frequency of horizontal gene transfer *in vivo* is likely to be underestimated.

O-13

Mycoplasma extracellular vesicle interaction with the bovine host

Theresa Wagner (1), Thomas Démoulins (1), Jörg Jores (1)

(1) Institute of Veterinary Bacteriology, Vetsuisse Faculty – University of Bern, Switzerland

Theresa Wagner <theresa.wagner@unibe.ch>

In host-pathogen interactions of many pathogenic bacteria the secretome is crucial. One such path of interaction are extracellular vesicles (EV). EVs are small membrane enclosed spheres shed by cells across all domains of life. While EV release has been reported in Mollicutes, the proteomic composition and functional significance of these EVs remain unexplored. Here, we aim to establish and optimize a robust isolation protocol for EVs of different bacteria of the class Mollicutes, applicable to different species, including clinically relevant recent field isolates. EVs will be subjected to morphological characterization using nanoparticle tracking analysis and electron microscopy, and their proteomic content will be described. To elucidate their role in host pathogen-interaction, EVs will be exposed to bovine peripheral blood mononuclear cells (PBMCs), employing a comprehensive bovine ex vivo platform recently developed in our research group. This platform offers the advantage to investigate the response of most bovine immune cell subsets without the need for animal experiments. The platform combines two independent complementary readouts: firstly, a multi-color multiparameter flow cytometry assay measuring maturation (modulation of cell surface marker expression) and activation (intracellular cytokine detection) of monocytes, conventional and plasmacytoid dendritic cells, natural killer cells, γδ T cells, B and T cells; and secondly, a multiplex immunoassay monitoring bovine chemokine and cytokine secretion levels. By studying the immune response to both bacterial cells and EVs derived from the parental strain, our study aims to enhance our understanding of Mollicutes-derived EVs with the bovine host. Ultimately, this research holds promise for advancing our knowledge of host-pathogen interactions and is expected to have implications for veterinary medicine and beyond.

O-14

Serum and albumin free media for Mycoplasmas

Wichittra Arai (1), Masaki Mizutani (1), Minoru Moriyama (1), Yoko Hagiwara (1), Takema Fukatsu (1, 2, 3), Shigeyuki Kakizawa (1)

(1) Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan
(2) Department of Biological Sciences, Graduate School of Science, the University of Tokyo, Tokyo, Japan
(3) Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan

Shigeyuki Kakizawa <s.kakizawa@aist.go.jp>

Pathogenic bacteria often require nutrients from their hosts, and some of them require serum in their cultivation medium. Mycoplasmas typically lack lipid biosynthesis systems and depend on serum-derived lipids for growth. However, serum-containing media are difficult to use for the secretome analysis due to their high protein content, such as albumin. In addition, serum is usually expensive and has lot-to-lot variation, and reducing serum usage is also associated with enhancing animal welfare. Here, we developed serum-free and albumin-free media for Mycoplasmas. We added three types of lipids and two types of lipid carriers as alternatives to serum. This new media supported the growth of the minimal cells JCVI-syn3.0, JCVI-syn3B, JCVI-syn1.0, *Mycoplasma pneumoniae, M. gallisepticum, M. synoviae, Spiroplasma chrysopicola*, and *S. eriocheiris*. Similar growth speeds of JCVI-syn1.0 and JCVI-syn3B were observed both in SP4-FBS (fetal bovine serum) and serum-free SP4 media. We also tested colony formation on solid media and recovery from -80°C freeze stocks, obtaining positive results. Proteomics with mass spectrometry and RNA-seq analysis were conducted on JCVI-syn3B cells cultivated in both the original serum-containing SP4-FBS medium and the serum-free medium. The results indicated that the expression levels of most genes remained largely unchanged at both RNA and protein levels. This suggests that the newly developed serum-free medium could potentially serve as a viable alternative to the original serum-containing medium. These newly developed media would be useful for research, detection, and vaccine production for those mycoplasmas.

O-15

Unlocking Spiroplasma helix secrets: fibril's role in morphological resilience

Bastien Lambert (1, 2), Yorick Dahan (1), Alexandre Vilquin (3, 4), Maxime Bouttier (5), Marie-Pierre Dubrana (1), Marion Decossas (5), Olivier Lambert (5), Jean-Christophe Baret (2), Nicolas Martin (2), Laure Béven (1)

(1) University Bordeaux, INRAE, BFP, UMR 1332, Villenave d'Ornon, France
(2) University Bordeaux, CNRS, Centre de Recherche Paul Pascal, UMR5031, Pessac, France
(3) UMR CNRS Gulliver 7083, ESPCI Paris, PSL Research University, 75005 Paris, France
(4) University Bordeaux, CNRS, LOMA, UMR 5798, F-33400 Talence, France
(5) University Bordeaux, CNRS, CBMN UMR 5248, Bordeaux INP, Pessac, France

Laure Beven <laure.beven@inrae.fr>

The helical shape and movement of *Spiroplasma* rely on an internal cytoskeleton primarily composed of two proteins: fibril (Fib) and MreB. *Spiroplasma*'s kink-based movement involves the inversion of helical chirality throughout the cell body. Helicity and motility are thus intricately linked in these bacteria. Fibril, unique to *Spiroplasma*, forms a ribbon within the cell, regularly interacting with the inner leaflet of the plasma membrane. For this reason, it has long been suspected to play a role in maintaining *Spiroplasma*'s helical shape. However, the precise contribution of fibril to helicity remains unclear, given that MreB proteins alone can induce membrane curvature, shaping the cell into a helix. Phylogenetic analysis indicates a gene gain of fibril in the Citri and Apis clades during evolution, raising questions about its selective advantage for Spiroplasma and how species that later underwent gene inactivation compensate for the lack of fibril expression. We conducted a systematic study to explore correlations between fibril expression levels, measured through proteomics, and morphological or motility characteristics across different *Spiroplasma* species. Statistical analysis of shape parameters (curvature angles, helix pitch, lengths, small and large cell diameters) and motility parameters (kink propagation speed, average velocity, trajectories) of 11 species *via* video-microscopy, coupled with intracellular cytoskeleton organization analysis *via* cryo-electron microscopy in three species, sheds light on the impact of fibril presence in the cytoskeleton. Notably, while the fibril is dispensable for the regular interaction of the cytoskeleton with the membrane, it facilitates the preservation of a consistent helix pitch, which varies among species based on genetic background and MreB content. Fibril likely aids in regaining helicity after the passage of kinks. Consequently, we propose a pivotal role for fibril in morphological resilience, enabling *Spiroplasma* to deform during kink propagation and regain a helical shape thereafter. This resilience correlates with the ability to maintain the orientation of the cell body in a semi-viscous environment and undertake longer linear trajectories. The presence of fibril could thus confer a selective advantage during the colonization of the host by *Spiroplasma*.

Chemotherapy and Resistance

O-16

synergistic interactions among antimicrobial agents targeting *Mycoplasma pneumoniae* biofilms *in vitro*

Rasha A. Fahim (1), Natalie E. Young (1), Kavita Shrestha (1), Mitchell F. Balish (1)

(1) Miami University, USA

Mitchell F. Balish <balishmf@miamioh.edu>

Chronic, recurrent, and antibiotic-resistant infections caused by *Mycoplasma pneumoniae* are a major concern. Even more worrisome is that these infections persist even after antibiotic treatment. *M. pneumoniae* forms protective biofilms *in vitro*, which suggests that biofilms may play a crucial role in the persistence of the infection. M. pneumoniae becomes overwhelmingly resistant to antibiotics when it grows as biofilm towers, making characterization of this lifestyle a priority. Because developing *M. pneumoniae* biofilms become increasingly resistant to antibiotics, making monotherapy ineffective, combination therapy might be a relevant approach toward their eradication. In this study, we aimed to assess the efficacy of combination therapy using US FDA-approved antimicrobial agents against *M. pneumoniae* compared to their individual effects on both inhibiting biofilm formation and eradicating pre-existing biofilms. The antibacterial and antibiofilm effects of erythromycin, doxycycline, and moxifloxacin were assessed using a broth dilution minimum inhibitory concentration (MIC) assay and a minimum biofilm eradication concentration assay (MBEC), respectively. Fractional inhibitory concentration indices (FICIs) were calculated from checkerboard assays that were employed to evaluate synergy between antibiotic pairs. Biofilm mass was quantified using a crystal violet assay to compare the effectiveness of each antibiotic alone and in combination both to prevent biofilm formation and to eradicate pre-formed biofilms. Scanning electron microscopy (SEM) was also used to monitor changes in biofilm mass and morphology following treatment. These experiments were performed on *M. pneumoniae* strains M129 and 19294 in addition to the macrolide-resistant strain 54505. The MICs of the three tested antibiotics matched the guidelines when included during inoculation, representing the susceptibility of M. pneumoniae cells not growing as biofilm towers. However, when antibiotics were included after biofilm tower formation, the MBEC could not be reached, even with the maximum clinically achievable concentrations. Nonetheless, most of the combinations showed synergistic interactions in reducing biofilm density, whereas some showed additive interactions. All the tested combinations significantly increased the disruption of pre-formed towers compared to their individual effect at concentrations matching their maximum plasma concentrations. These results suggest that antibiotic combinations are a potential means of combating *M. pneumoniae* chronic and persistent infections.

O-17

Recent observations on use of tulathromycin and oxytetracylines in the treatment of contagious bovine pleuropneumonia in Kenya

Monicah Maichomo (1), Stephen Ngulu (2), Peter Mwai (3), George Njogu (4), Ednah Masila (1), James Akoko (5), Elise Schieck (5), Tore Tollersrud (5), Musa Mulongo (5), Harry Oyas (4), Hezron O. Wesonga (1)

(1) Kenya Agricultural & Livestock Research Organization, Kenya
(2) Conservation and One Health, Kenya
(3) County Directorate of Veterinary Services, Kenya
(4) Directorate of Veterinary Services, Kenya
(5) International Livestock Research Institute, Kenya

Hezron O. Wesonga <hezron.wesonga@gmail.com>

Contagious Bovine Pleuropneumonia (CBPP) is a severe lung disease of cattle, caused by *Mycoplasma mycoides* subspecies *mycoides* (Mmm). Outbreaks are associated with high mortality which without intervention can reach 100%. In endemic areas, the disease is controlled by mass vaccinations, and in CBPP-free areas by slaughtering infected animals followed by prophylactic vaccination in a process referred to as “test and slaughter”. Conclusive diagnosis by serology, may take up to 30 days, during which time the disease has spread to almost the entire herd. Treatment with antimicrobials is not recommended because of the perception that it may induce formation of lung sequestra but farmers still treat while awaiting confirmation of diagnosis. An on-station study replicated in Kenya and Zambia, confirmed effectiveness of treatment using tulathromycin and oxytetracycline. This observation prompted a follow up on use of antibiotics in firld CBPP outbreaks confirmed by CFT (BoraR test kit), on ranches and pastoralist herds with naïve cattle in Laikipia County of Kenya; carried out in the years 2022 and 2023. On one Ranch (A) with 600 Boran cattle, no intervention was made while awaiting diagnosis. This ranch lost half of the herd through death and the rest were sold for slaughter. Another Ranch (B) used oxytetracline and Tylosin on 3,500 and lost 1,500 head of Boran cattle. The ranch carried out prophylactic vaccination in addition to movement restrictions. It took up to 2 months to obtain the vaccine, arriving when the morbidity was high. Additional 5 ranches reported outbreaks and use of Oxytetracycline and Tylosin on sick animals, with relapse of cases. One of the 5 additional ranches (Ranch D) applied tulathromycin metaphylactic treatment followed by vaccination 21 days later. Whereas other ranches lost 50-100% of their animals, ranch D lost through slaughter, 2.8% from 4 of their herds affected in the outbreak. These findings suggest that metaphylactic treatment with tulathromycin followed with vaccination 21 days later appeared to control the disease. Farmers appear more receptive to this new method compared to the currently recommended “test and slaughter”. However, further studies are required to clarify if treatment can prevent CBPP transmission.

Diagnostic and Epidemiology

O-18

Mycoplasma pneumoniae epidemic in France, 2023-2024

Jennifer Guiraud (1), Cécile Laurier (1), Carla Balcon (1), Nadège Hénin (2), Marie Gardette (1), Sabine Pereyre (1, 2), Cécile Bébéar (1, 2)

(1) CHU Bordeaux, Bacteriology Department, National Reference Centre for Bacterial Sexually Transmitted Infections, Bordeaux, France
(2) University Bordeaux, CNRS, UMR 5234, Fundamental Microbiology and Pathogenicity, Bordeaux, France

Jennifer Guiraud <jennifer.guiraud@u-bordeaux.fr>

Since November 2023, an unusual increase in *Mycoplasma pneumoniae* respiratory infections, mainly community-acquired pneumonia, has been observed in France. Although studies conducted in South East Asia have reported a predominance of the P1 type 1 and a prevalence of macrolide resistance close to 100%, few data are currently available in Europe. We aimed to investigate the clinical manifestations in patients with *M. pneumoniae* infections, the P1 type and the antibiotic resistance profile of circulating strains during the current outbreak.
Our laboratory centralized the diagnostic of macrolide resistance in *M. pneumoniae*-positive specimens isolated from in-patients for French microbiology laboratories. Macrolide resistance-associated mutations in the 23S rRNA were detected using a FRET-based real-time PCR and sequencing in case of a mutated profile. On a subsample of 58 M. pneumoniae strains (i) 16S rRNA, *rplD*, *rplV*, *gyrA* and *parC* genes were amplified and sequenced to look for resistance-associated mutations; (ii) P1 adhesin subtyping was performed. A total of 853 *M. pneumoniae*-positive specimens were analyzed between September 2023 and March 2024. The sex ratio M/F was 1.20 and the average age was 28.4 years. A total of 75.2% of samples were isolated from the ENT site, 20.1% from the upper respiratory tract and 4.7% from the lower respiratory tract. Regarding the clinical manifestations, 85.0% of patients were hospitalized for pneumonia, 3.1% for skin disorders and 1.1% for neurologic disorders/meningoencephalitis. Regarding the antimicrobial treatment, 71.9% of patients received macrolides, 3.0% fluoroquinolones and 2.1% tetracyclines, respectively. For note, 22.3% of patients received no antibiotic or an inactive antibiotic against *M. pneumoniae* such as beta-lactams. Among amplified samples, the prevalence of macrolide resistance-associated mutations was 1.53% (12/780), with a predominance of mutation A2063G (75.0%; 9/12). P1 subtype 2 was the predominant P1 subtype (68%). Macrolide-resistant *M. pneumoniae* strains mainly belonged to the P1 subtype 1 (83.3%). No resistance-associated mutation was found in the 16S RNA, the rplD and rplV genes, or the gyrA and parC quinolone resistance-determining regions. This study reports the low prevalence of macrolide resistance in *M. pneumoniae* and highlights the relative predominance of the P1 subtype 2 among the current French surge.

O-21

Isolation of a potentially novel *Mycoplasma* species related to *M. bovirhinis* from dairy cattle affected by BRD in Northern Italy

Michele Gastaldelli (1), Annalucia Tondo (1), Giorgia Nai (1), Kinga Mária Sulyok (2), Marianna Merenda (1), Miklós Gyuranecz (2), Salvatore Catania (1)

(1) Mycoplasma Unit, WOAH reference laboratory for avian mycoplasmosis (M. gallisepticum, M. synoviae), SCT-1 Verona, Istituto Zooprofilattico Sperimentale delle Venezie
(2) HUN-REN Veterinary Medical Research Institute, Hungary

Michele Gastaldelli <mgastaldelli@izsvenezie.it>

Bovine respiratory disease (BRD) is an important cause of cattle morbidity and mortality and it consequently determines heavy economic losses in dairy herds and beef cattle production. Among the etiological agents of this syndrome are bovine viral diarrhea virus, infectious bovine rhinotracheitis, bovine respiratory syncithial virus, bovine coronavirus, *Hystophilus somni*, *Manhemia haemolytica* and *Mycoplasma bovis*. In addition, some studies have suggested a potential involvement of other mollicutes like *Mycoplasma dispar* and *Mycoplasma bovirhinis*. In this study we report the isolation of a M. bovirhinis-related strain in association with BRD from two independent dairy farms and, in one of them, for two consecutive years. In both cases, the animals presented a febrile state with pulmonary involvement, ruminal blockage and a decrease in milk production. Even though clinical signs improved after antimicrobial treatment, total recovery was not achieved. The analysis of nasal swabs for the common viral and bacterial forms involved in BRD yielded negative results. Therefore, further bacteriological investigations were conducted, which led to the isolation of a specific mycoplasma strain from more than 50% of the sampled subjects. The sequence analysis of the genes *16s rRNA* (1189 nt) and *rpoB* (1193 nt) revealed that the closest related species was *M. bovirhinis*. However, the percent identity was respectively below 99% and 84%. In addition, the phylogenetic analysis revealed a clear segregation of our isolates respect to the *M. bovirhinis* clade. These data suggest that this strain could belong to a different, novel species in potential association with BRD pathogenesis.

O-22

Development of a real-time PCR assay for simultaneously detecting *Mycoplasma genitalium* and its mutations associated with fluoroquinolone resistance in clinical specimens

Li Xiao (1), Amy Ratliff (1), Donna Crabb (1), Hong Wang (1), William Geisler (1), Ken Waites (1)

(1) University of Alabama at Birmingham, Birmingham, AL USA

Li Xiao <lixiao@uabmc.edu>

Mycoplasma genitalium (MG) is a sexually transmitted pathogen that causes urethritis and cervicitis, and has been associated with pelvic inflammatory disease, infertility, and pregnancy complications. CDC 2021 Treatment Guidelines for Sexually Transmitted Infections recommends MG treatment regimens that include azithromycin or moxifloxacin. However, MG resistance to macrolides and fluoroquinolones is increasing. Fluoroquinolone resistance in MG is mainly associated with parC and gryA mutations in the quinolone resistance determining region (QRDR). There are no FDA-cleared assays for detection of MG QRDR mutations in the United States. We developed a real-time PCR based assay (MGFQ PCR) using hybridization probes and melting curve analysis for simultaneously detecting MG and QRDR mutations associated with fluoroquinolone resistance in clinical specimens. The assay has a limit of detection of 1 and 6 genome per test for parC and gyrA, respectively, and no cross reaction with other organisms. It detects all currently reported QRDR mutations related to fluoroquinolone treatment failure but avoids the most frequent synonymous mutation in parC. The assay was validated against 257 urine, cervical, vaginal, and endotracheal aspirates specimens, showing a sensitivity of 98.5% and specificity of 95.9% for MG detection compared to a reference method MGMR PCR, and a 100% sensitivity and specificity for identifying QRDR mutations compared to Sanger sequencing. Overall, MGFQ PCR is a sensitive and specific assay suitable for clinical diagnosis of MG and QRDR mutations associated with fluoroquinolone resistance. UAB Diagnostic Mycoplasma laboratory is now using this new MGFQ PCR together with the previously validated MGMR PCR that detects mutations associated with macrolide resistance to guide antimicrobial treatment for patients with MG infection.

O-23 Harry Morton Student Award Candidate

Development of a viability-qPCR detection system and in vitro growth evaluation of *Mycoplasma hyopneumoniae*

Calvin Ko (1), Pablo Piñeyro (1), Phillip Gauger (1), Rachel Derscheid (1), Shawn Rigby (2)

(1) Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, USA
(2) Flow Cytometry Facility, Office of Biotechnology, Iowa State University, USA

Calvin Ko <calvinko@iastate.edu>

Mycoplasma hyopneumoniae (*M. hyopneumoniae*) causes significant economic losses to the pork industry and is a fastidious pathogen with extremely slow *in vitro* growth. The most common *M. hyopneumoniae* quantification technique is a broth-based method called color changing units (CCU) that is indirect, subjective, and very time-consuming. Viability real-time polymerase chain reaction (v-qPCR) is a modified molecular method aiming to provide rapid, sensitive, and specific detection of only viable bacteria. The main objective of this study is to evaluate and compare multiple quantification techniques for *M. hyopneumoniae*, propose and validate an accurate real-time method, and utilize this method as a reference to develop a v-qPCR assay for this fastidious organism. First, *M. hyopneumoniae* (strains 232, J, and 2010) *in vitro* growth curves were compared between CCU, colony forming units (CFU), flow cytometry, and ATP luminometry. Secondly, we established a confocal microscopy protocol to directly enumerate *M. hyopneumoniae* in culture media and utilized it to validate flow cytometry in a controlled condition. Finally, we developed and optimized the rapid v-qPCR assay in *M. hyopneumoniae* with reported analytical sensitivity and specificity. *M. hyopneumoniae* growth curves compared in CFU, CCU, flow cytometry, and ATP luminometry had similar growth trends and high correlation in assays analyzing similar biological outcomes. For flow cytometry and confocal microscopy quantification, Pearson correlation was significant at $p < 0.05$ in strains 232 ($p = 0.0002$) and J ($p = 0.0411$) with an R2 of 0.9946 and 0.7981, respectively. SEM from confocal microscopy were overall higher than flow cytometry. *M. hyopneumoniae* v-qPCR assay was further optimized and compared with flow cytometry live cell counts with high fitted goodness (R2 for 232: 0.9726; J: 0.8628; 2010: 0.9933, all $p < 0.05$) and detection limit Ct value with a mean of 31.05 and an SEM of 0.1063. Here we present the first PMA-based v-qPCR validated in *Mycoplasma* species. This can potentially aid swine practitioners in understanding infection dynamics by rapidly and effectively characterizing live *M. hyopneumoniae* cells. We also demonstrated confocal microscopy and flow cytometry to fast and accurately discriminate and quantify viable and non-viable *M. hyopneumoniae*.

O-24

Airways and gut microbiome disturbance and resilience dynamics during polymicrobial bovine respiratory disease

Maverick Monié—Ibanes (1), Fatima Z. Sikt (1), Marie-Claude Hygoneq (1), Annabelle Meynadier (2), Christine Citti (1), Eric Baranowski (1), Eric Richard (3), Gwendoline Pot (1), Hortensia Robert (1), Joshua Malsa (1), Laurent Xavier Nouvel (1), Gilles Meyer (1), Mariette Ducatez (1), Nuria Mach (1)

(1) IHAP, Université de Toulouse, INRAE, ENVT, Toulouse, France
(2) École Nationale Vétérinaire de Toulouse, Toulouse, France
(3) Normandie Université, UniCaen, Caen, France

Maverick Monié--Ibanes <maverick.monie-ibanes@envt.fr>

The challenge of Bovine Respiratory Disease (BRD) to cattle health and welfare demands a comprehensive understanding of its interplay with the gut-lung axis. In this study, we longitudinally tracked the nasopharyngeal and gastrointestinal microbiota of 30 calves from day 7 to 5 months of age. Our aim was to shed light on the microbial temporal profiles associated with BRD pathogens across a range of disease severity. The study revealed the presence of pathogenic taxa such as Mycoplasma bovis, Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni, alongside viruses like influenza D virus (IDV), bovine parainfluenza virus type 3 (BPIV3), and bovine coronavirus (BCoV), in both symptomatic and asymptomatic calves throughout the initial 10-day of life. During this initial window, we observed a substantial interindividual variation in the microbiota of both the airways and gastrointestinal tracts. This variation was notably distinct from the microbial profiles encountered at subsequent time points, suggesting a distinct and critical microbial development phase early in life. Notably, elevated *M. bovis* and BCoV loads significantly shaped the gut and respiratory tract microbiome structures within this timeframe, regardless of the calves' clinical health status. In symptomatic calves, however, an elevated prevalence of the Mycoplasma genus was observed within the respiratory microbiome, accounting for over 50% of the genera identified. After this initial period of microbial instability, the microbiomes of the respiratory and gastrointestinal tracts, irrespective of symptom presentation, exhibited ecostatic communities characterized by significant consistency intra- and inter-individually. These results emphasize the resilience and dynamism of the airways and gut microbiome under polymicrobial challenges, highlighting the critical role of microbiome balance early in life. The potential of maintaining this homeostasis from an early age to prevent viral and bacterial co -infections within bovine cohorts emerges as a promising therapeutic strategy with significant implications for the management of BRD.

O-25

Artificial Intelligence-Based Diagnosis of Phytoplasma Diseases: A Case Study on Tomato Plants Infected by Potato Purple Top Phytoplasma

Wei Wei (1), Jonathan Shao (2), Yan Zhao (1)

(1) Molecular Plant Pathology Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD, United States
(2) Statistics and Bioinformatics Group - Northeast Area, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD, United States

Wei Wei <wei.wei@usda.gov>

Phytoplasmas are graft-and insect-transmissible bacteria that cause diseases and significantly impact crop quality and yield, leading to economic losses and jeopardizing food security in affected regions. Early and accurate diagnosis of phytoplasma infections is essential for preventing disease spread and mitigating their adverse effects on agriculture and the environment. Artificial intelligence (AI) has demonstrated promise in automated disease detection and diagnosis using image recognition techniques. However, the application of AI for phytoplasma detection in plants remains underexplored. The present study addressed the gap by focusing on tomato plants infected by potato purple top (PPT) phytoplasma as a case study. A dataset comprising over 3000 healthy and over 3000 PPT phytoplasma-infected tomato images was collected for training convolutional neural network (CNN) AI models. Machine-learning techniques were employed, and the dataset was augmented using rotations, shifts, and flips to enhance model performance. Three models were developed to distinguish between healthy and PPT phytoplasma-infected plants, including a custom model (CNN-PPT), and two models using transfer learning techniques, Google Inception V3 and VGG-16 architecture. For performance evaluation, an additional dataset of 1600 images (800 healthy and 800 infected) was collected as an unseen dataset. Our custom CNN model, Inception V3, and VGG-16, implemented in TensorFlow, effectively differentiated healthy and infected tomato plants, achieving an accuracy rate of 90, 95, and 99%, respectively. Additional transfer-learning algorithms are currently being tested, aiming at further improving the performance of the AI models, especially our custom model CNN-PPT.

O-26

Recent updates for VIGIMYC, a settled 20-year old surveillance network dedicated to ruminant mycoplasmas

Maryne Jaÿ (1), Nathalie JARRIGE (2), Sandra Lacôte (1), Stéphanie Lefebvre (1), Florine Dieu (1), François Poumarat (1), Patrice GAURIVAUD (1), Chloé Ambroset (1), Claire A.M. Becker (1), Fabien Labroussaa (1), Florence TARDY (3)

(1) ANSES, Lyon Laboratory, VetAgro Sup, UMR Animal Mycoplasmosis, University of Lyon, Lyon, France.
(2) Epidemiology and Surveillance Support Unit, ANSES, Lyon Laboratory, University of Lyon, Lyon, France
(3) ANSES, Ploufragan-Plouzané-Niort Laboratory, Mycoplasmology, Bacteriology and Antimicrobial Resistance Unit, Ploufragan, France

Maryne Jaÿ <maryne.jay@anses.fr>

For over 20 years, the so-called VIGIMYC network has enabled the passive surveillance of ruminant mycoplasmas over the French territory, including WOAH regulated diseases. VIGIMYC has grown overtime and, since 2013, around 500 documented clinical isolates have been collected each year from veterinary diagnostic laboratories (VDLs) and identified at the (sub)species level. New initiatives have recently been launched to improve the service provided by VIGIMYC and further extend its impact within and across borders. First, since 2018, we have investigated the antimicrobial resistance (AMR) trends of major pathogenic mycoplasma species on collected isolates. While the Minimum Inhibitory Concentrations (MICs) of small ruminant mycoplasmas remain predominantly low, Mycoplasmopsis (M.) bovis (formerly Mycoplasma bovis) shows moderate or high MICs for all the tested antimicrobials except fluoroquinolones. Surprisingly, we observed a decrease of spectinomycin MICs in *M. bovis* lately, which could be associated to the subtypes currently circulating in France. Consolidated MIC data from VIGIMYC will contribute to establishing future tECOFF within the MyMIC network (https://www.jpiamr.eu/projects/mymic/). Since 2020, annual surveys were performed to get insight on positivity testing rates of major pathogenic mycoplasma, reaching a proxy of their prevalence. Collected data complemented relative proportions of identified mycoplasmas and allowed monitoring diagnostic volume. Important differences were observed, cattle being the most monitored species by far and *M. bovis* detection rate in respiratory samples has increased from 14 to 19% between 2020 and 2022. VIGIMYC also provides a solid technical and scientific support to VDLs. In 2023-2024, proficiency tests and trainings were organized to help partner VDLs in learning and maintaining adequate diagnostic practices. Lastly, a selection of clinical isolates are stored yearly enabling the set-up of a collection of more than 2600 isolates to date, which are increasingly being used for whole genome sequence comparison studies that provide a better understanding of the diversity and evolution of mycoplasmas. VIGIMYC is a dynamic network aiming to improve diagnostic and AMR surveillance for several mycoplasmoses in France. Its output reaches far beyond the French borders and the issue of diagnostic, with an increasing contribution to AMR surveillance and follow up of mycoplasma genomic evolution within Europe.

O-27

Development of a scalable CBPP control strategy for pastoral production systems: preliminary findings

Angela Mukami (1), Bernard Bett (1), Elise Schieck (1), Hezron O. Wesonga (1), Hussein Abkallo (1), James Akoko (1), Musa Mulongo (1)

(1) International Livestock Research Institute, Kenya

James Akoko <J.Akoko@cgiar.org

Background. Contagious Bovine Pleuropneumonia (CBPP) caused by *Mycoplasma mycoides* subsp. *mycoides* (Mmm), remains a major impediment to cattle production and trade in Sub-Saharan Africa. Enforcement of control measures for CBPP, such as regulating livestock movements, treatment, 'test and slaughter', as well as achieving the desired vaccination coverage, has been a challenge in pastoralist regions. The objective of this study is to generate data in Kenya for developing a scalable CBPP control strategy in the pastoral production systems of Sub-Saharan Africa. **Methods**. The study will be conducted in three phases. The first is a scoping phase that involves reviewing records of i) outbreaks documented in the Veterinary reports, ii) meat inspection data from slaughterhouses, as well as carrying out iii) current outbreak investigations, and iv) evaluation of the status of lung lesions in selected Counties. In the second phase, mathematical models will be developed and used to simulate the effects of CBPP vaccination, treatment, and transmission. The selection of antibiotics for CBPP treatment will precede a field trial combining all parameters for a scalable control strategy. The third phase will be the rolling out of the successful intervention strategy. **Results**. Out of the 8 Counties targeted for the scoping survey, data has been collected from 4 and preliminary findings indicate that occurrence of CBPP is common in 3 of them; associated with frequent clinical disease outbreaks. Affected areas reported CBPP-characteristic lung lesions from slaughterhouses. Farmers confirm CBPP when they open carcasses and thereafter employ a wide range of antibiotic combinations for treatment. Despite the treatment, they still suffer substantial losses due to animal deaths, the purchase of drugs, restricted animal movement, and reduced market prices. Challenges noted, which require follow-up, include the low frequency of vaccination in certain areas, and inadequate maintenance of vaccination records in regions with high vaccination rates. **Conclusion**. The project is at its initial stage of phase 1, and we have already identified areas with CBPP. Therefore, we look forward to carrying out the study for the successful introduction of a scalable control strategy. The key findings will be shared with the veterinary service providers and policymakers.

O-28

Harnessing clinical laboratory data sources for surveillance of *Mycoplasma pneumoniae* infections in the United States

Maureen Diaz (1), Stephanie Dietz (2), Sarah Jiudice (3), Chris Edens (1)

(1) Division of Bacterial Diseases, U.S. Centers for Disease Control and Prevention, USA
(2) Detect & Monitor Division, Office of Public Health Data, Surveillance, and Technology, Centers for Disease Control and Prevention, USA
(3) bioMérieux

Maureen Diaz <mdiaz1@cdc.gov>

Mycoplasma pneumoniae (Mp) commonly causes mild respiratory illness but may lead to severe pneumonia. Mp infection is often diagnosed based on clinical presentation and treated empirically without confirmatory laboratory testing, especially in outpatient settings. Respiratory pathogen panels (RPP) for detection of common pathogens, including Mp, are widely available in clinical laboratories; however, with no systematic surveillance, the true burden of Mp infections in the United States is unknown. In response to reports of increasing Mp infections in several countries during fall 2023, we analyzed clinical laboratory test data to investigate trends in Mp infections in the United States. Mp test results from September 25, 2023 through April 1, 2024, were obtained through CDC's National Syndromic Surveillance Program (NSSP) from Quest and Labcorp, two major clinical laboratories. As an additional source of data, we acquired aggregated data from the BIOFIRE® Syndromic Trends (Trends) program, an opt-in network of BIOFIRE® FILMARRAY® clinical diagnostic systems at facilities across the United States, from January 2016 through April 2024. Overall, Mp was detected in 62 (0.28%) of 22,415 tests in NSSP commercial laboratory data and 1,044 (0.27%) of 393,718 tests in BIOFIRE Trends. Mp test positivity increased from September 2023 through April 2024 in both data sources, reaching a maximum of 0.80% in week 9 of 2024 (Trends) and 0.87% in week 12 (NSSP). Higher test positivity (0.84%; 382/45,209) was observed among children and adolescents when restricting Trends data to pediatric facilities as determined from facility name, with a maximum of 4.1% (63/1,480) in week 9. In NSSP, test positivity was 0.58% in children ≤ 17, with the highest positivity (0.88%) in the 5-17 year age group. Mp detection decreased dramatically in April 2020 at the start of the COVID-19 pandemic and remained low (0.04%) through August 2023 despite a high number of tests reported in Trends (n=1,365,338). Laboratory test results from large laboratory networks can be useful for monitoring trends in Mp infections in the United States. These data may help identify changes in affected populations and improve public health response.

Genome Engineering and Synthetic Biology

O-07 Harry Morton Student Award Candidate

Engineering Mycoplasma for the treatment of idiopathic pulmonary fibrosis

Javier Gonzalez de Miguel (1), Irene Rodriguez Arce (1), Luis Serrano (1)

(1) Centre de Regulació Genòmica, Spain

Javier Gonzalez de Miguel <javier.gonzalez@crg.eu>

Mycoplasma pneumoniae (*Mpn*) is a mild human pathogen capable of establishing infection in the lung tissue. After years of extensive characterisation, this knowledge was leveraged by our laboratory to create a chassis for therapeutic use. In recently published work, our group has demonstrated its capacity to treat different pre-clinical models of infection, even being able to express mammalian proteins. This work also showed that *Mpn* may not be able to secrete protein at physiologically relevant levels without overly increasing the bacterial load. Consequently, all therapeutic molecules may need to be screened for enhanced properties prior to pre-clinical testing. This process is time-consuming in *Mpn*, therefore we have developed a protein screening platform in the fast-growing *Mycoplasma feriruminatoris* (*Mfr*). The focus now is to screen therapeutic molecules in Mfr to later on use them in *Mpn* for the treatment of lung disease, including Idiopathic Pulmonary Fibrosis (IPF). IPF is a disease characterised by the deposition of extracellular matrix components into the interstitial space in lung tissue. Currently, there are no available treatments and survival upon diagnosis is up to two years. Dysregulation of the immune system is a key hallmark of IPF. Interleukin signaling has been suggested to be involved in several of the pathophysiological events of this disease. The aim of this project is to develop enhanced immune signalling proteins in Mfr and extend the therapeutic use of Mpn to the treatment of IPF. The first part of the project was to develop a cassette for secretion in Mfr. This involved combining systems biology data along with in silico analyses to develop both promoter and secretion sequences. The ability of Mfr to secrete biologically active immunomodulatory molecules was then confirmed. The goal now is to enhance several proteins with potentially therapeutic effects in IPF. Screening is currently being performed in in in vitro models. The combination of different proteins will be assessed in a model of bleomycin-induced IPF. Their individual effects will be assessed in ex vivo Precision Cut Lung Slices isolated from affected mice and their combined effect in an in vivo context.

O-09 Harry Morton Student Award Candidate

Deciphering genome transplantation mechanisms as a step towards understanding basic principles of life

Jitra-Marie Jittasevi (1), Géraldine Gourgues (1), Dominick Matteau (2), Sébastien Rodrigue (2), Carole Lartigue (1)

(1) Université de Bordeaux, INRAE, BFP, UMR 1332, F-33140 Villenave d'Ornon, France
(2) Département de biologie, Université de Sherbrooke, Sherbrooke, QC, Canada

Jitra-Marie Jittasevi <jitra-marie.jittasevi@inrae.fr>

Building a cell from the ground up would help identify the minimal set of elements necessary for a cell-like compartment to become a functional living entity. This requires an extensive understanding of the contribution of each component to the cell function. This project focuses on genetic information and its processing by a compartment capable of gene expression, using an original approach named whole genome transplantation (WGT). This technique consists in isolating a whole bacterial genome belonging to Species A (donor genome) and installing it in the cytoplasm of Species B (recipient cell), resulting in cells genotypically and phenotypically identical to Species A. WGT is currently performed on Mollicutes, the simplest living forms capable of autonomous replication outside of a host, an ideal model for studying essential requirements for life. Understanding what defines the compatibility between the donor genome and recipient cell may lead to identifying key elements that enable booting up a living cell and to understanding the rules that regulate the interactions between genetic material and the compartment which expresses it. We hypothetize that the ability of the recipient transcription machinery to “interpret” the data encoded on the donor chromosome is essential for successful boot-up. Our approach consists in engineering a recipient cell, Mycoplasma capricolum (Mcap), to preload it with transcription factors belonging to a donor genome, Mesoplasma florum (Mflorum). The coding sequences of the five subunits of the Mflorum RNA polymerase were cloned into plasmids along with their native promoters. Transformation of Mcap suggests that the Mflorum genes can be expressed individually in Mcap and that their presence does not impact the recipient’s survival. In the next phase, the 5 subunits will be simultaneously expressed in Mcap. The resulting cells will be used as recipients for WGT assays and structural analysis of their transcription machinery will be performed.

O-29 Harry Morton Student Award Candidate

Improved CRISPR-base editor tools for genome edition in *Mycoplasma bovis*: Application to surface proteins

Patrick Hogan (1), Fabien Rideau (Institut Bergonié), Carole Lartigue (1), Alain Blanchard (1), Pascal Sirand-Pugnet (1), Laure Beven (1), Eric Baranowski (3), Yonathan Arfi (1)

(1) Université de Bordeaux, INRAE, BFP, UMR 1332, F-33140 Villenave d'Ornon, France
(2) Institut Bergonié, France
(3) IHAP, ENVT, INRAE, Université de Toulouse, Toulouse, France

Patrick Hogan <patrick.hogan@inrae.fr>

Among the minimal bacteria belonging to the genus *Mycoplasma*, several of them are recognized pathogens for a wide diversity of animals. *Mycoplasma bovis* is one of the most significant species infecting dairy and fattening cows, causing mastitis and pneumonia, respectively. Attempts to control the disease have led to mass culling and costs of hundreds of millions in treatments and compensation worldwide, emphasizing the need for improved vaccines. Within the RAMbo-V consortium (Rational Approach to a *Mycoplasma bovis* Vaccine), we believe that advances in genome engineering tools provide a unique opportunity to pave the way towards producing a vaccine strain of *M. bovis* expressing at its surface a specific set of conserved antigenic structures. Advances in CRISPR-based tools, such as SpyCas9 recognizing PAM variants and base editors, have allowed for an increase in potential uses. These technologies paired with an algorithm predicting the complete array of potential targets within a given CDS library of *M. bovis* has allowed for a more in-depth look at the possibilities of these technologies and the feasibility of gene knockouts. Here we report the use of multiple tools in *M. bovis* that led to the knockout of genes of interest such as the vsp (variable surface proteins) locus. VSPs are abundant and phase-variable components of the *M. bovis* membrane, which may participate in surface crowding and masking of stably expressed proteins, in addition to their putative biological functions. Targeting of the Xer1 tyrosine recombinase that permits spontaneous non-coordinate phase-variable expression results in the generation of phase-locked mutants. The development of genome engineering tools will permit targeting virulence factors and optimized antigenic presentation. Such tools will be useful to build a vaccine chassis that would allow presentation of selected epitopes at the cell surface, leading to an improved immune response.

O-30 Harry Morton Student Award Candidate

Heterologous expression of *Mycoplasma pneumoniae* major adhesins in the close related species *Mycoplasma genitalium*

Marina Marcos Silva (1), David Vizárraga (1), Ignacio Fita (1), Oscar Quijada Pich (2,3), Jaume Piñol Ribas (2)

(1) Instituto de Biología Molecular de Barcelona (IBMB-CSIC), Parc Científic de Barcelona, Baldiri Reixac 10, 08028 Barcelona, Spain
(2) Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
(3) Laboratori de Recerca en Microbiologia i Malalties Infeccioses, Institut d'Investigació i Innovació Parc Taulí (I3PT), Hospital Universitari Parc Taulí, Universitat Autònoma de Barcelona, 08208 Sabadell, Spain

Marina Marcos Silva <marina.marcos@uab.cat>

Mycoplasma pneumoniae (*Mpn*) is a human pathogen that causes primary atypical pneumonia. Elucidation of the tridimensional structure of P1 and P40/P90, the major adhesins of this bacterium, revealed important clues about the function of these proteins essential for infection. The lack of routine tools to introduce target mutations in *Mpn* hinders the construction and evaluation of strains carrying selected cytoadhesin variants. To circumvent these problems, we used *Mycoplasma genitalium* (*Mge*) as a surrogate host to express and characterize *Mpn* adhesins. To this end, target mutations were introduced in *Mpn* adhesins and used to construct *Mge* strains carrying *Mpn* adhesin variants for structure-function studies. The next *Mge* mutant strains were obtained by gene replacement methods and transposition: i) a non-adherent *Mge*-ad- strain bearing a deletion of the P140 and P110 coding genes; ii) the *Mge*-Mpn strain is a *Mge*-ad- derivative complemented with the wild-type genes coding for P1 and P40/P90; iii) the *Mge*-Mpn-CS and *Mge*-MpnΔSII strains are *Mge*-ad- derivatives complemented with the wild-type allel coding for P1 and P40/P90 bearing mutations in the cleavage site or a deletion of the insertion loop II (SII), respectively. Adhesin expression was assessed by SDS-PAGE and immunoblotting. Cell morphology was examined by SEM and motility by microcinematography. Cytadherence properties were assessed by flow cytometry and epifluorescence using human red blood cells (hRBCs). Expression of P1 and P40/P90 adhesins in *Mge*-Mpn was confirmed by SDS-PAGE and Western-blotting. These analyses showed that the proteolytic cleavage of P40/P90 was preserved in all mutant strains, suggesting that this adhesin has an auto-processing capacity. *Mge*-Mpn cells were elongated and prone to form small clusters. Introduction of P1 and P40/P90 coding genes restored adhesion properties for hRBCs and motility of *Mge*-ad- cells, suggesting that P1 and P40/P90 are properly folded and functional. However, *Mge*-Mpn cells showed hybrid motile properties, with a gliding velocity comparable to that of *Mge* cells. Adhesion and motile properties of *Mge* cells expressing *Mpn* adhesins indicate that *Mge* is a suitable model to perform structure-function relationship studies. This model could be also helpful to identify mycoplasma factors involved in tissue tropism.

O-31

Prepare for Landing: use of a double site-specific recombination system to stably insert foreign DNA sequences in the chromosome of *Mycoplasma feriruminatoris*

Sergi Torres Puig (1), Fabien Labroussaa (2), Jana Inäbnit (1), Jörg Jores (1), Paraskevi Pramateftaki (1), Thomas Démoulins (1)

(1) Institute of Veterinary Bacteriology, Vetsuisse Faculty – University of Bern, Switzerland
(2) ANSES, Lyon Laboratory, VetAgro Sup, UMR Animal Mycoplasmosis, University of Lyon, Lyon, France

Sergi Torres Puig <sergi.torrespuig@unibe.ch>

Mollicutes are the smallest cultivable free-living prokaryotes, bearing streamlined genomes resulting of reductive evolution. A limited number of *Mollicutes* have been used as model organisms for the development of the "minimal cell" and therefore have been amended for genome engineering, including genome transplantation. However, many species still cannot be genetically altered due to the lack of genetic modification tools. Since genome transplantation is technically challenging, time-consuming, and often requires multiple modification cycles, we aimed at facilitating foreign gene insertion by creating a site-specific insertion site in the chromosome of *Mycoplasma feriruminatoris* consisting of a combined Cre-lox/Flp-FRT system. We introduced an antibiotic marker flanked by FRT and a modified *loxP* sequence into the genome of *M. feriruminatoris*, creating a selectable "landing pad". In parallel, we developed a shuttle fosmid able to specifically integrate into the *M. feriruminatoris* genome by expression of the Cre recombinase, allowing the insertion of any genes of interest in the chromosome of the bacterium. By using an inducible promoter controlling the Flp/FRT recombination system, the fosmid backbone together with all the resistance markers can be meticulously excised from the engineered mycoplasma chromosome, allowing only the gene(s) of interest to remain. We used this selective DNA integration/excision technology to test several promoters for heterologous expression in *M. feriruminatoris* and we also developed a versatile system to stably couple any molecules of interest at the surface of the bacterium. These presented tools will increase the available synthetic genomics tools for these mycoplasmas, facilitating the expression and display of recombinant antigens and settling the foundations for a future synthetic vaccine chassis based on *M. feriruminatoris*.

Human Mollicutes

O-32 Harry Morton Student Award Candidate

From sequence to activity: Insights into the HgaI-RM-System of *Mycoplasma hominis*

Lars Vogelgsang (1), Manuel Dolgopolow-Schmidt (1), Dana Bäcker (1), Birgit Henrich (1)

(1) Institute for Medical Microbiology and Hospital Hygiene, University Clinic of the Heinrich-Heine University of Duesseldorf, Duesseldorf, Germany

Lars Vogelgsang <lars.vogelgsang@hhu.de>

Type-II restriction-modification systems (RM-systems) are active bacterial defense mechanisms against foreign DNA, consisting of a restriction endonuclease (REase) and a methyltransferase (MTase). The REase cuts the unmethylated foreign DNA at a sequence motif that is protected by methylation of the host genome, either at 5mC (by deoxycytidine MTase (Dcm)) or at 6mA (by deoxyadenine MTase (Dam)). In addition to the 2023 published family of Dcm-MTases, an HgaI-homolog RM-system was detected in *Mycoplasma hominis* with the more rare constellation of two dcm-MTase genes, formerly only reported for the HhaI-homolog type-II RM-system of *M. hominis*. A qPCR screening of 115 randomly selected *M. hominis* isolates revealed a prevalence of the HgaI-system of 7.89% (n=9/115), with two isolates also carrying the HhaI-RM-system. Notably, in all HgaI-positive isolates, the HgaI-RM cassette comprised a XRE-gene in addition to the HgaI-RM genes and localized between MHO3120 (atpD) and MHO3110 (*lmp*-related protein). Intraspecies conservation of the HgaI-RM enzymes was high in *M. hominis* (>99% identities). Homologues were also found in bacteria of mammals, poultry, fish and plants, with an interspecies conservation ranging from 48-72% for the MTases and 29-53% for the REases. Functionality of the HgaI-system was suggested for *M. hominis*, as 1.) conserved domains of type II MTases, which are crucial for DNA binding and methyl group transfer, were present in M1.HgaI and M2.HgaI; and 2.) transcripts of all HgaI-genes detected, which were up to 7-fold (R.HgaI) higher than transcripts of the housekeeping genes. As final proof of HgaI-RM activity, methylation sensitive restriction analysis with HgaI-enzyme was conducted, demonstrating that only the unmethylated sequence motif 5'-GACGC-3'/5'-GCGTC3' was cut in the HgaI-negative isolates, whereas DNA of the HgaI-positive isolates was protected. Cutting frequency was low. This corresponded to the reduced motif presence in the genome, which comprised on average one HgaI-motif per 9641 bp compared to one Dcm1-motif (canonical 5'-CCAGG-3') per 5553 bp; both of identical GC content. This study clearly demonstrates activity of an HgaI-like RM-system in *M. hominis* and provides the basis for future studies to analyze its effects on pathogenicity and defense.

O-33

Circularization, transcription and transfer capacity of integrative and conjugative elements of *Mycoplasma hominis*

Bachir Boureima Abdou (1), Alicia Silvant (1,2), Chloé Le Roy (1), Jennifer Guiraud (3), Léa Bientz (1,2), Véronique Dubois (1,2), Cécile Bébéar (1,2,3), Sabine Pereyre (1,2,3)

(1) Univ. Bordeaux, CNRS, UMR 5234 MFP, ARMYNE team, Bordeaux, France
(2) Bordeaux University Hospital, Bacteriology Department, Bordeaux, France
(3) Bordeaux University Hospital, National Reference Centre for Bacterial Sexually Transmitted Infections, Bordeaux, France.

Sabine Pereyre <sabine.pereyre@u-bordeaux.fr>

Integrative and conjugative elements (ICEs) are modular mobile genetic elements that can disseminate through excision, circularisation, and transfer. In *Mycoplasma hominis*, a human urogenital pathogen, 45% of isolates harbor integrative and conjugative elements (ICEs) integrated in the chromosome. These ICEs, of 27-30 kbp, harbor 25-30 CDSs. In this study, we aimed to assess the ICE circularization capacities under different environmental conditions and investigate the transcription of the ICE CDSs. The second part of our study focused on transfer capacities of *M. hominis* ICE through mating experiments. Using qPCR and RT-qPCR experiments, we assessed the circularization and transcription of the *M. hominis* 4788 strain ICE (ICEHo-4788) under different conditions: exponential/stationary growth phase, exposure to mitomycin C, a DNA-damaging agent, to -80°C cold shock stress, and growth in HeLa cell culture. Functional studies involved mating experiments between donor strains carrying one ICE and recipient strains under both axenic and cell culture conditions. Upon axenic growth, a circularization peak occurred at 12h of culture, with a 9.4-fold increase of circular forms compared to the reference condition. Exposure to mitomycin C and to cold-shock stress resulted in 3.3- and 3.0-fold rise in circular forms, respectively. In cell culture conditions, maximum circularization was observed at 72h and 7 days post-infection, with 10- and 23-fold increases, respectively. Regarding transcription, RT-qPCR experiments showed that all ICEHo-4788 CDSs were transcribed. Moreover, all RT-PCRs targeting CDS intergenic region produced amplicons, suggesting that ICEHo-4788 is transcribed as a single polycistronic mRNA. No transcriptional variations were observed in ICE CDSs upon growth nor under mitomycin C exposure and cold shock stress. However, significant increases in ICE CDSs transcription were observed in cell culture conditions at 7 days post-infection with 5- to 23-fold increases depending on the CDSs. Regarding mating experiments, no ICE transfer was achieved. However, a “Mycoplasma chromosomal transfer” was observed, wherein the recipient strain transferred several regions of its chromosome to the donor strain, thus creating mosaic genomes. This study emphasizes the influence of environmental conditions on the circularization dynamics and transcription of ICEHo-4788. Additionally, genetic material exchange was evidenced in *M. hominis*.

Immunology and Vaccines

O-34 Harry Morton Student Award Candidate

assessment of immune response in goats using recombinant P40 and MAG_1560 as vaccine candidates for *Mycoplasma agalactiae*

Beatriz Almeida Sampaio (1), Maysa Santos Barbosa (2), Manoel Neres Santos Júnior (2), Bruna Carolina de Brito Guimarães (1), Camila Pacheco Gomes (1), Thiago Macêdo Lopes Correia (2), Lucas Santana Coelho da Silva (1), Rohini Chopra-Dewasthaly (3), Guilherme Barreto Campos (2), Jorge Timenetsky (4), Bruno Lopes Bastos (2), Lucas Miranda Marques (2)

(1) Universidade Estadual de Santa Cruz, Brazil
(2) Universidade Federal da Bahia, Brazil
(3) Institute of Microbiology, University of Veterinary Medicine Vienna, Austria
(4) Universidade de São Paulo, Brazil

Beatriz Almeida Sampaio <beatrizalmeida2808@gmail.com>

Contagious agalactia (CA) is a listed disease by the World Organization for Animal Health (WOAH, founded as OIE), classically caused by *Mycoplasma agalactiae*. It affects goats and sheep worldwide and is considered endemic in some countries surrounding the Mediterranean, South America, and Western Asia. CA is characterized by agalactia, mastitis, keratoconjunctivitis, and arthritis, causing severe economic losses due to livestock incapacitation. In previous studies within our research group, recombinant proteins demonstrated antigenicity and immunogenicity by recognizing sera from goats naturally infected with *M. agalactiae* and stimulating IgG production in rabbits. As an alternative to existing immunoprophylactic measures, which raise concerns about safety and efficacy, the present study aimed to develop and evaluate a recombinant subunit vaccine's immune response in goats against *M. agalactiae*. Goats were divided into three groups (A, B, and C) and immunized with a solution of antigenic recombinant proteins (group A: P40 and MAG_1560), formalin-inactivated *M. agalactiae* (group B), or negative control (group C: tris-buffered saline). All solutions were emulsified in Freund's adjuvant. The animals were evaluated for 181 days for IgG antibody production by ELISA, and in vitro analysis of peripheral blood mononuclear cells (PBMCs) was performed for the expression of IL-1β and IFN-γ genes by real-time PCR. *M. agalactiae*-specific antibody response was observed in the sera of animals immunized with recombinant proteins for six months. Analysis of cytokine gene expression revealed an increase in mRNA expression of the pro-inflammatory cytokine IL-1β over time in response to the stimulation of recombinant proteins in both experimental groups. Over time, compared to before immunization, in group A, IFN-γ mRNA levels were maintained or increased when stimulated by the proteins, while in group B, the levels decreased. Taken together, the recombinant proteins induced the production of antibodies, as well as the expression of cytokines that play a role in the adaptive immune response, thereby demonstrating the immunogenic potential and promising data for the development of a recombinant vaccine capable of inducing responses against *M. agalactiae.*

O-35 Harry Morton Student Award Candidate

Development of a subunit vaccine against *Mycoplasma gallisepticum* utilizing diverse surface proteins

Hani J. Alnakhli (1), Kannan Ganapathy (1), Nicholas J. Evans (1)

(1) Department of Infection Biology and Microbiome, University of Liverpool, UK

Hani J. Alnakhli <h.alnakhli@liverpool.ac.uk>

The control of *Mycoplasma gallisepticum* (MG) in poultry farming remains a considerable challenge, traditionally relying on live attenuated vaccines, bacterins, and stringent biosecurity measures. Despite these efforts, concerns about the efficacy, safety, and potential for attenuated vaccine reversion underscore the urgent need for alternative strategies. The variability of live vaccines and bacterins' inability to induce robust immunity have propelled the search for a non-replicating vaccine that minimizes live vaccine risks. Given the limitations of current vaccines, we explore developing a novel subunit vaccine leveraging diverse MG surface proteins, including cytoadhesion proteins 1 and 2, accessory cytoadhesion proteins 3 and 4, and phase-variable proteins 5 through 8. These antigenic targets were selected through an in-depth analysis of their roles and immunogenic potential, informed by literature and protein sequence repositories. This study's approach started with producing targeted proteins, facilitated by gene synthesis within expression plasmids from Twist Bioscience. These genes underwent transformation, expression, and purification via affinity chromatography using six-histidine tags. We customized the purification process for each protein to address specific challenges and optimize yield. Concentration assessments with NanoDrop and Qubit assays supported further validation. The integrity of our proteins was confirmed by 12% SDS-PAGE and Western blot, using anti-His tags and anti-MG antibodies to evaluate purity and cross-reactivity. ELISA assays employing antisera quantified immunoreactivity, highlighting these proteins as likely expressed during MG infection and interacting with the host immune system. Building on this foundation, our next steps involve detailed in vitro and in vivo studies to evaluate our vaccine candidates' protective and immune-stimulating properties. We aim to explore various adjuvants and administration methods to enhance host immune responses, enabling MG protection. This effort will validate our approach and improve the vaccine's effectiveness against MG in poultry. Overcoming vaccines' current limitations should facilitate more efficacious future vaccines, increasing animal health and welfare and ensuring future food security.

O-36

Mycoplasma shares a similar immune escape mechanism to mediate persistent infection

Zhixin Feng (1), Qiyan Xiong (1), Yanfei Yu (1), Jian Wang (1), Rong Chen (1), Zhenzhen Zhang (1), Xin Xie (1), Guoqing Shao (1)

(2) Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences, Nanjing, China

Zhixin Feng <fzxjaas@163.com>

So far, more than 240 species of mycoplasma have been found in humans and animals, but most of them have similar infectious behavior and pathogenic characteristics, among which persistent infection in the host is one of the common characteristics of many human and animal mycoplasmas, and it is also the main reason why mycoplasma is difficult to eradicate. There are many reasons for mycoplasma to be continuously infected or carried in the host, and escaping the host immune monitoring is one of the main factors. Our study found that a variety of human and animal mycoplasmas can survive in the host body for a long time by breaking through the mucosal barrier system, inhibiting complement activation, escaping phagocytic cells, and antigenic variation, respectively. *Mycoplasma hyopneumoniae*, *Mycoplasma hyorhinis* and other mycoplasmas can hijack fibrinolysis system to degrade cell junction and extracellular matrix, and break through respiratory mucosal barrier to achieve extrapulmonary infection. The ENO, GAPDH and other proteins of Mycoplasma play the role of PlgRs, and the key domains and binding sites of these proteins are highly homologous in a variety of human and animal mycoplasmas. *Mycoplasma pneumoniae*, *Mycoplasma genitalium*, *Mycoplasma hyopneumoniae*, *Mycoplasma bovis*, *Mycoplasma gallisepticum* and other mycoplasmas can hijack H factor to inhibit the activation of complement through C3 pathway, and found that the key moonlighting protein EF-TU is highly homologous on these mycoplasmas. *Mycoplasma hyopneumoniae*, *Mycoplasma bovis*, *Mycoplasma ovipneumoniae*, and *Mycoplasma synoviae* can secrete nucleases, such as Nfo, to degrade METS and NETS, thereby escaping capture and phagocytosis by macrophages and neutrophils. *Mycoplasma hyorhinis* and other variable lipoprotein families of mycoplasmas can undergo antigenic variation through ON/OFF switching, size variation, domain shuffling, antigenic drift and other ways to avoid recognition by the immune system. Our study confirms that many mycoplasmas in humans and animals share similar immune escape mechanisms to enable persistent infection or carriage. In addition, this may also be related to the metabolic mechanism of mycoplasma and the mechanism of host immune down regulation induced by chronic inflammation, which is worthy of more systematic and in-depth study.

O-37

Efficacy evaluation in pigs of a combined inactivated vaccine against *Mycoplasma hyopneumoniae* and *Mycoplasma* hyorhinis

Jia Wang (1), Qiyan Xiong (1), Jiayu Chen (1,2), Yuan Gan (1), Yanna Wei (1), Guoqing Shao (1), Zhixin Feng (1)

(1) Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences, Nanjing, China
(2) College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China

Qiyan Xiong <qiyanxiongnj@163.com>

Background: *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* are both mycoplasmas in pigs and are highly prevalent worldwide, causing major economic losses to the swine industry. Co-infection with *M. hyopneumoniae* and *M. hyorhinis* could lead to more severe clinical and pathological signs than a single infection. Therefore, a combined vaccine against *M. hyopneumoniae* and *M. hyorhinis* has been developed and evaluated its protective efficacy against infection. **Methods**: The *M. hyopneumoniae* and *M. hyorhinis* cultures were inactivated, mixed, and blended with adjuvant to produce a w/o/w type vaccine. Pigs of two immunization groups were inoculated with the combined vaccine and challenged with *M. hyopneumoniae* and *M. hyorhinis* respectively 4 weeks after the second vaccination. Pigs were monitored daily for clinical changes after challenge. All the pigs were euthanized 21 days after challenge with *M. hyorhinis* or euthanized 28 days after challenge with *M. hyopneumoniae*. A gross pathological examination was used to assess the efficacy of the combined vaccine. **Results**: The pigs of the vaccination group demonstrated a significant reduction of clinical signs, polyserositis and arthritis after *M. hyorhinis* challenge, while the challenged group exhibited varying degrees of clinical and severe pathological changes. Regarding the *M. hyopneumoniae* postmortem examination results, the lung lesion score in the vaccinated group was significantly lower compared to that of the challenged group. However, it was noted that some pigs in the vaccinated group still exhibited mild to moderate lung lesions. Based on the scoring system used in the evaluation, the vaccine demonstrated a protection rate of 65.38% against *M. hyopneumoniae* infection and 75.00% against *M. hyorhinis* infection. The pathogen loads of *M. hyopneumoniae* and *M. hyorhinis* in the immunized groups were lower than those in the challenge groups. The antibody detection results showed that the pigs generated significant antibodies against *M. hyopneumoniae* and *M. hyorhinis* after immunization, which were maintained throughout the experimental period. **Conclusion**: We successfully developed a combined vaccine against *M. hyopneumoniae* and *M. hyorhinis*, which induced specific serum antibodies and significantly reduced the lesions after challenge. The development of combined vaccine contributes to improved pig health, reduced morbidity, and enhanced productivity in commercial pig populations.

O-38

Mycoplasma nucleomodulin MbovP475 promotes inflammatory cytokines transcription by Remodeling chromatin of macrophages

Gang Zhao (1), Aizhen Guo (2), Doukun Lu (2), Elise Schieck (3), Shujuan Wang (2)

(1) Ningxia University, China
(2) Huazhong Agricultural University, China
(3) International Livestock Research Institute, Nairobi, Kenya

Gang Zhao <zhaogang@nxu.edu.cn>

Background: *Mycoplasma bovis* (*M. bovis*) causes pneumonia and mastitis in cattle, abnormal inflammatory response is one of its pathogenic mechanisms. But there are few reports on the important virulence factors of *M. bovis* causing a pro -inflammatory response, and it does not reveal the mechanism of pro-inflammatory response. Our previous study verified that *M. bovis* MbovP475 was nucleomodulin and promoted the transcription of pro-inflammatory cytokines in BoMac. Nucleomodulins are secreted bacterial proteins whose molecular targets are located in host cell nuclei. They are gaining attention as critical virulence factors that either modify the epigenetics of host cells or directly regulate host gene expression. **Methods**: The IP-MS and Co-IP method were used to identify and verify the protein-protein interaction. Nano-LC ESI-TOF and Mod Spec analysis were used to verify that MbovP475 regulate histone post-translational modification (PTMs). Then ChIP-seq and DLo-Hi-C techniques were used to reveal that MbovP475 remodels chromatin accessibility and 3D structure. **Results**: The IP-MS revealed that Histone H4 was the abundant protein with highest intensity-based absolute quantification (iBAQ=33.69%). We confirmed that MbovP475 interacted with histone H4 but not H3 or H2B. Mod Spec analysis shown MbovP475 increased the H2AK36ac, H3K4me2, H3K4me3, H3R42me2, H3K79ac, H3K122ac, H3.1K36ac, H3.3K27ac, and H4K20ac, and decreased the H2A1K13ac, H3K56me1, and H4K20un. Further, we confirmed MbovP475 enhanced the H3K122ac, H3K4me3, and H3K27ac by comparing the above modification in BoMac cells infected with wild strains HB0801, MbovP475 knock-out strains T9.55, and complementary strains CT9.55. The mRNA-Seq, ChIP-seq, and DLo-Hi-C results indicated the transcription of inflammatory cytokines was associated the upregulation of H3K27ac, and the upregulation of H3K27ac also changed the promoter-enhancer interaction (Chromatin loop) by remodeling the chromatin 3D structure. After analysis of chromatin loops, we found the loops changes increased the transcription NF-κB2. **Conclusion**: This study reveals the nucleomodulin MbovP475 possesses the ability to promotes the inflammatory cytokine transcription by remodeling the chromatin via regulating the PTMs. The results will be of significance in elucidating *M. bovis* pathogenesis and revealing novel target for developing novel vaccine.

O-39 Louis Dienes Award Candidate

Assessment of tracheal mucosal thickness is the most powerful and reproducible method for evaluation of the efficacy of vaccines against *Mycoplasma gallisepticum* in poultry

Sathya N. Kulappu Arachchige (1), A. M. Hasanthi Abeykoon (1), Mark A. Stevenson (1), Nadeeka K. Wawegama (1), Glenn F. Browning (1)

(1) Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Science, The University of Melbourne, Werribee, Victoria, Australia

Sathya N. Kulappu Arachchige <skulappuarac@unimelb.edu.au>

Mycoplasma gallisepticum is the primary causative agent of chronic respiratory disease (CRD) in poultry. Lesions associated with CRD are seen mainly in the trachea and air sacs. In many countries vaccination of flocks with live-attenuated vaccines is the most effective control measure against this disease. Vaccination and experimental infection of poultry are used to evaluate the efficacy of novel *M. gallisepticum* vaccines. In such studies, a vaccine is considered compliant with the test for immunogenicity if the lesions in the vaccinated-and-challenged (or test) birds are significantly less severe than those in the unvaccinated-infected (or positive) control group and the test is considered valid if the degree of discrimination between the lesions in the positive and unvaccinated-uninfected (or negative) control groups is significant. This study systematically reviewed and critically appraised the designs of *M. gallisepticum* vaccination-challenge studies and compared the parameters used to evaluate histopathological changes in the trachea and gross air sac lesions. A search of four electronic databases, with subsequent manual filtering, identified 13 eligible papers published between 1962 and 2021. Analyses included in the study were assessed using the data presented in the papers and raw data obtained from 7/13 eligible studies. Analysis of data at group and individual bird level revealed that the proportion of experiments that detected a significant difference between the negative and positive control groups in tracheal mucosal thicknesses (TMT) was significantly greater than the proportion of experiments that detected a significant difference in air sac lesion scores (ALS) between the control groups. In addition, a significantly greater proportion of experiment detected a significant difference in TMT between test and positive control groups at group and individual bird level than in ALS. Our analyses of the published data further revealed that assessment of TMT has a greater statistical power than assessment of ALS, enabling detection of a significant difference between the positive and negative control groups and between the test and positive control groups with a smaller number of birds, thus enabling the size of experimental studies for efficacy studies to be reduced, with consequent improved animal welfare.

O-40

Development and immunogenicity of a multiepitope recombinant antigen for the diagnosis of *Mycoplasma genitalium*

Lucas Santana Coelho da Silva (1), Lucas Miranda Marques (2), Camila Pacheco Gomes (1), Manoel Neres Santos Junior (2), Maysa Santos Barbosa (2), Beatriz Almeida Sampaio, Bruna Carolina Brito Guimarães (2), Leonardo Silva Santos (2), Guilherme Barreto Campos (2), Bruno Lopes Bastos (2)

(1) Universidade Estadual de Santa Cruz, Brazil
(2) Universidade Federal da Bahia, Brazil

Lucas Miranda Marques <lucasm@ufba.br>

Mycoplasma genitalium, an important pathogen associated with sexually transmitted infections, has been increasingly linked to antibiotic resistance in recent years. Nevertheless, the cost-effectiveness and efficacy of diagnostic tests employing nucleic acid amplification for mycoplasmas still need to be more adequately assessed in randomized trials. Therefore, the present study aimed to develop a recombinant antigen as an alternative for diagnosing this pathogen. This study constructed a multi-epitope protein for *M. genitalium* using bioinformatics methods and expressed in an *Escherichia coli* model. The production of antibodies post-immunization was evaluated, and the reactivity of the protein with sera from volunteers and animals experimentally infected with the bacteria was analyzed. Satisfactory expression and purification of the recombinant proteins were achieved. Furthermore, the kinetics of antibody production in goats inoculated with the recombinant protein revealed a significant increase in specific antibodies after immunization with Mg01 compared to pre-immune serum and the negative control. Antigenicity was also confirmed with sera from animals experimentally infected with the bacteria. This is the first study to propose a diagnostic method for *M. genitalium* based on a recombinant antigen rigorously predicted by bioinformatics, aiming to reduce cross-reactions with other pathogens. This research represents an initial and highly relevant phase for future validation of an immunodiagnostic assay. Additionally, specific antibodies against the Mg01 protein were generated, enhancing the potential for applying this technology in the diagnostic market. The recombinant antigen exhibited the ability to recognize antibodies against natural infection, rendering this invention even more promising for application in laboratory routines. Further studies are warranted to evaluate a larger cohort of individuals and to standardize the diagnostic test for *M. genitalium*.

O-41 Louis Dienes Award Candidate

Microorganism-host interaction and the immunomodulatory mechanisms of synthetic genome *mycoplasma mycoides* subsp. *capri* (Mmc) strains in caprine peripheral blood mononuclear cells culture

Manoel Neres Santos Junior (1), Beatriz Almeida Sampaio (1), Guilherme Campos (2), Bruno Lopes Bastos, Marcelo Freire (3), John Glass (3), Daniela Matias de Carvalho Bittencourt (4), Elibio Rech (4), Lucas Marques (2)

(1) Multidisciplinary Health Institute, Federal University of Bahia, Brazil
(2) Universidade Federal da Bahia, Brazil
(3) John Craig Venter Institute - West Coast Campus, JCVI/La Jolla, United States
(4) EMBRAPA Genetic Resources and Biotechnology, Brazil

Manoel Neres Santos Junior <neresmanoel@gmail.com>

The comprehension of the pathogen-host interaction persists as a challenge within the scientific community. Concurrently, the advent of synthetic genome bacterial strains has broadened the scope for investigating aggression and defense mechanisms in this domain. This study aimed to assess the microorganism-host interaction and the immunomodulation mechanisms of the synthetic genome *Mycoplasma mycoides subsp. capri* (*Mmc*) strains in cell culture. Four previously described synthetic strains were utilized: JCVI-syn1.0, (1,079kb) based on the chemically synthesized genome; JCVI-syn3A, derived from JCVI-syn1.0 but with a reduced genome size of 531kb; and two other strains containing the mCherry (mCh) genes (JCVI-Syn3-mch179-180 and JCVI-Syn3-mch179-186). Additionally, a wild-type *Mmc* strain was included. These strains were used to infect cultures of peripheral blood mononuclear cells (PBMCs) from goats. Following the assessment of cell viability using alamarBlue™, cytokine production (IL-1, TNF-α, and IL-4) was examined via ELISA. Nitric oxide (NO) levels were evaluated using Griess assay. After ensuring animal health, PBMC was utilized for culture (1x10^6 cells/mL). Inoculated cultures were exposed to varied bacterial concentrations for 6-24 hours. Viability testing revealed that synthetic strains, except JCVI-Syn3-mch179-180 and JCVI-Syn3-mch179-186 at 12 hours, inhibited PBMC proliferation ($p<0.05$). All strains studied induced an increase in NO production at all time points evaluated. All strains induced IL-1β at 6 and 12 hours, with JCVI-syn1.0 showing higher induction at 6 hours (1x10^4 and 1x10^5 cells/mL) and 12 hours (only 1x105 cells/mL). At 18 and 24 hours, other synthetic strains and wild-type followed the observed profile. The wild-type strain induced significant TNF-α production at 6 hours (1x10^4 and 1x10^5 cells/mL) compared to controls and synthetic strains. After 12 hours, JCVI-syn3A and Syn1.0 strains also induced TNF-α production. At 18 hours, all treatments with synthetic strains at 1x10^5 cells/mL stimulated TNF-α production. IL-4 production lacked significance at 6-18 hours but increased significantly after 24 hours for all strains except JCVI-syn3A. This study demonstrates synthetic *Mmc* strains' potential in modulating the host-pathogen interaction and immune response in PBMC. They inhibit proliferation and induce pro-inflammatory response. Further studies, including animal models, are crucial for understanding these mechanisms and developing therapeutic strategies against *Mmc* and related pathogens.

O-42 Harry Morton Student Award Candidate

Could the GAPDH-enhanced MS-H strain redefine vaccine efficacy?

Sahar Zare (1), Marc S. Marenda (1), Pollob Shil (1), Sathya N. Kulappu Arachchige (1), Chathuri H. Rodrigo (1), Amin Kawarizadeh (1), Amir H. Noormohammadi (1)

(1) Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, The University of Melbourne, Victoria, Australia

Sahar Zare <szare@student.unimelb.edu.au>

Mycoplasma synoviae poses a significant threat to poultry production, causing adverse effects on egg production, respiratory health, and overall economic losses. The live attenuated vaccine, MS-H, developed through random mutagenesis of the virulent strain 86079/7NS, is widely employed to control the disease. Investigations of the genetic basis and molecular biology underlying MS-H attenuation revealed mutations in a gene encoding the Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key enzyme in the glycolysis pathway. This study explores the involvement of GAPDH in MS-H pathogenicity and immunogenicity. The pathogenicity of a GAPDH-complemented MS-H transformant carrying a WT copy of *gapdh* on an *OriC* plasmid (MS-H+GAPDH) was compared to the wild-type parent strain 86079/7NS containing an empty plasmid (7NS+EP), the original MS-H strain, MS-H strain with an empty plasmid (MS-H+EP), and two other MS-H transformants: obgE-complemented MS-H (MS-H+obgE) and oppF-complemented MS-H (MS-H+oppF). Six groups of 20 specific-pathogen-free (SPF) chickens were inoculated with one of these strains at four weeks of age via aerosol and eye drop routes. Postmortem examinations were conducted at two- and three-weeks post-challenge, with samples collected for serological responses, colonisation assessment, and histopathological analysis. All strains colonised the upper respiratory tract but only 7NS+EP and MS-H+GAPDH could colonise the lower respiratory tract. Significant higher pathogenicity (measured by gross air sac lesions and microscopic tracheal mucosal thickness) was observed in the 7NS+EP group compared to other groups. ELISA assays revealed an early antibody response in MS-H+GAPDH group, with significantly higher antibody levels at two weeks post-inoculation compared to other groups except 7NS+EP. In three weeks post-inoculation 7NS+EP group showed significantly higher antibody response than other groups. Overall, GAPDH presence in MS-H appears to enhance colonization ability without exacerbating pathological lesions. Moreover, it may have modulated strain immunogenicity, accelerating the onset of the host immune response against *M. synoviae.*

O-43

Temperature impacts the bovine ex vivo immune response towards *Mycoplasmopsis bovis*

Thomas Démoulins (1), Thatcha Yimthin (1), Dorothea Lindtke (2), Lukas Eggerschwiler (2), Raphael Siegenthaler (2), Fabien Labroussaa (1), Joerg Jores (1)

(1) Institute of Veterinary Bacteriology, Department of Infectious Diseases and Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland
(2) Animals Group, Agroscope, Posieux, Switzerland

Thomas Démoulins <thomas.demoulins@unibe.ch>

Although cattle are the mammalian species with most global biomass associated with a huge impact on our planet, their immune system remains poorly understood. Notably, the bovine immune system has peculiarities such as an overrepresentation of gamma delta T cells that requires particular attention, specifically in an infectious context. In line of 3R principles, we developed an *ex vivo* platform to dissect host-pathogen interactions. The experimental design was based on two independent complementary readouts: firstly, a novel 12-14 color multiparameter flow cytometry assay measuring maturation and activation of most of immune cell subsets; secondly, a multiplex immunoassay monitoring bovine chemokine and cytokine secretion levels. The experiments were conducted on fresh primary bovine blood cells exposed to *Mycoplasmopsis bovis* (*M. bovis*), a major bovine respiratory pathogen. Besides reaffirming the tight cooperation of the different primary blood cells, we also identified novel key players such as strong IFN-gamma secreting NK cells. Additionally, we compared the host-pathogen interactions at different temperatures, including commonly used 37 °C, ruminant body temperature (38-38.5 °C) and fever (≥39.5 °C). Strikingly, working under ruminant physiological temperature influenced the capacity of most immune cell subsets to respond to *M. bovis* compared to 37 °C. Under fever-like temperature conditions the immune response was impaired compared to physiological temperature. Our experimental approach, phenotypically delineating the bovine immune system provided a thorough vision of the immune response towards *M. bovis* and the infuence of temperature towards that immune response.

O-44 Harry Morton Student Award Candidate

Immune responses elicited by Janus-faced *Mycoplasma mycoides* - glycans make the difference

Thatcha Yimthin (1), Thomas Démoulins (1), Sergi Torres-Puig (1), Lukas Eggerschwiler (2), Raphael Siegenthaler (2), Nicolas Ruggli (3), Jörg Jores (1)

(1) Institute of Veterinary Bacteriology, Department of Infectious Diseases and Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland
(2) Animals Group, Agroscope, Posieux, Switzerland
(3) Institute of Virology and Immunology, Bern and Mittelhäusern, Switzerland

Thatcha Yimthin <thatcha.yimthin@unibe.ch>

Mycoplasmas are minute cell wall less bacteria that encompass a number of livestock pathogens. Mycoplasma vaccines, if available at all, have low efficacy and often induce only short-term immunity. The development of rationale vaccines would benefit from a better understanding of the interplay between mycoplasmas and host cells. *Mycoplasma mycoides* subsp. *capri* GM12 (*Mmc*-GM12) is a highly virulent strain, causing severe septicemia in goats. This strain is amendable to novel genome editing techniques, making it an excellent model to study host-pathogen interactions. In line with 3R principles, we developed a blood-based *ex vivo* platform to compare the response of many different immune cell subsets towards the (i) highly virulent wild-type (GM12), (ii) a capsular polysaccharide-deficient and highly adherent mutant strain (GM12::YCpMmyc1.1-Δ*glf*) and (iii) the fully attenuated CPS producing mutant strain GM12::YCpMmyc1.1-Δ68, which all have been tested in vivo earlier. Ruminant peripheral blood mononuclear cells (PBMCs) were stimulated with GM12 and its different isogenic mutant strains. GM12 and GM12::YCpMmyc1.1-Δ68 had only moderate effects on PBMCs apoptosis and surface marker expression, while stimulation with the mutant strain GM12::YCpMmyc1.1-Δ*glf*, which exposes surface proteins including lipoproteins due to the lack of CPS, led to high apoptosis and strongly suppressed MHC expression on antigen-presenting cells, suggesting immunosuppressive effect. Moreover, exposure to GM12::YCpMmyc1.1-Δ*glf* produced a variety of pro -inflammatory cytokines/chemokines that could promote a robust T-cell mediated and inflammatory response. Since macrophages stand as one of the primary immune defense cells against mycoplasma infection, we generated monocyte-derived macrophages (MDMs) and investigated their interactions with GM12. Interestingly GM12 showed the capacity to replicate and grow inside the MDMs. In conclusion, we showed that a CPS-deficient strain induces cell apoptosis, pro-inflammatory cytokine induction and immunosuppression, while the presence of CPS does not. We provided evidence that *Mmc*-GM12 can survive and persist within macrophages *in vitro*. We hypothesize that different populations of the strain exist *in vivo* and induce different immune responses, while the CPS-equipped strain escape clearance via its stealth mode.

O-45

Report of CBPP expert workshop on measurement of efficacy in clinical trials

Elise Schieck (1), Musa Mulongo (1)

(1) International Livestock Research Institute (ILRI), Kenya

Musa Mulongo <m.m.mulongo@cgiar.org>

The measurement of vaccine efficacy and protection against CBPP has been dominated by the Hudson & Turner composite scoring system (Hudson and Turner, 1963). This system is prone to amplify small variations in pathology, even when control groups in vaccine efficacy trials are not developing disease, and this has further complicated the reporting of the outcome of vaccine protection data. Despite its weaknesses, this system has been used to report vaccine efficacy in experimental trials, often compounding scientific interpretation of efficacy. In addition, there is no consensus in the mycoplasma community the meaning of "protection" in experimental vaccine or therapeutic studies. To respond to these challenges, a workshop organized by Star-IDAZ, CABI, USDA and TAHSSL (ILRI) was held on April 9th and 10th in Frankfurt, Germany to discuss CBPP animal trials and their evaluation. Consensus was reached that although the Hudson & Turner composite score has supported CBPP research for more than six decades, it is time to rethink how CBPP vaccine protection is evaluated and reported, and that this evaluation should be based on the specific demonstrable claims that are made regarding a vaccine candidate. Further, the need for improved lesion scoring methods was discussed and it was agreed that the recently published lung lesion scoring system (Di Provvido et al., 2018) is very promising and was recommended for further evaluation. Recent advances in infection models were also discussed. Finally, it was agreed that the lack of a correlate of protection is still hampering the advancement of CBPP research and vaccine development, and that there is a need to characterize both natural infection and experimental infections including at the histopathological level.

O-46

A window into the immune responses of sheep experimentally infected with *M. agalactiae* – Role of Vpma phase variation and differential responses elicited by individual "Phase-Locked" Vpma expression variants

Rohini Chopra-Dewasthaly (1), Martina Baumgartner (2), Astrid Stütz, Andreas Dagn, Christian Lohinger, Martina Flöck, Martina Zimmermann, Munkhtsetseg Kargl, Reinhard Ertl, Joachim Spergser, Renate Rosengarten

(1) Institute of Microbiology, University of Veterinary Medicine Vienna, Austria
(2) Clinic for Ruminants, University of Veterinary Medicine Vienna, Austria

Rohini Chopra-Dewasthaly <rohini.chopra-dewasthaly@vetmeduni.ac.at>

Mycoplasma lipoproteins are well-known immunomodulins. In *M. agalactiae*, Vpma family of surface-lipoproteins undergo high-frequency phase variations and help the pathogen to survive and persist in the host. However, not much is known about the immune-responses elicited by individual Vpma variants, and the effect of Vpma-phase variation, per se We addressed these points during experimental intramammary sheep-infections with phase-variable wild-type strain PG2 expressing all six lipoproteins (Vpma-U,-Y,-W,-X,-Y,and-Z) and its corresponding Vpma-'Phase-Locked-Mutants'(PLMs), each expressing a single stable Vpma. Host responses were evaluated in milk, sera and blood, and correlated with clinical outcomes and bacterial loads. To summarize, especially considering reduced infectivity of PLMU compared to PLMY in earlier studies, the number of leucocytes, lymphocytes and segmented-neutrophils was 2-fold higher in the blood of PLMU-infected sheep as compared to PLMY, PLMW and PG2 groups. The stronger lymphopenia & neutropenia of PLMY (PLMW & PG2) correlated with the earlier and higher SCC in milk starting 2h pi (PG2>PLMY>PLMU). Contrastingly, PG2 exhibited earlier and/or stronger neutropenia and lymphopenia compared to PLMU/PLMY co-challenge infection group, together with significantly higher eosinophil and serum SAA concentrations from 8h pi to Day5pi. An earlier increase of IFN-gamma and IL1-beta in milk of PLM-infected sheep, whereby the concentration of IL1-beta was significantly higher compared to PG2 suggests that Vpma phase variation might be playing a role in the early phase of infection, though insufficient to clear the pathogen. Furthermore, PLMU showed higher titers of MA-specific antibody compared to PLMY, and in-line with seroconversion most PLM groups predominantly retained their initial Vpma until D2-D9 pi and started to switch between D9-D11pi, as observed in colony-and Western-blots with milk and sera containing Vpma-specific Abs. Vpma switching was also observed by qPCR-analysis of infected udders, whereby VpmaW was the most preferred switch-over phenotype, and almost in all PLM groups, the starting Vpma disappeared to less than 4% at the end. Preliminary results of lymphocyte proliferation assays reveal that PLMY antigen elicits a much stronger immune response compared to PLMU. Overall, results correlate with transcriptomic analysis showing that PLMs induce a more dynamic host response, and that PLMU, is distinct in its host interactions.

Omics Studies

O-47

Ambient mass spectrometry-based culturomics: a novel, rapid, and accurate approach for monitoring the metabolic behavior of Mycoplasma*:* a proof-of-concept demonstration on *Mycoplasma* gallisepticum

Alessandra Tata (1), Andrea Massaro (1), Annalucia Tondo (1), Beatrice Colò (1), Carmela Zacometti (1), Davide Prataviera (1), Roberto Piro (1), Salvatore Catania (1)

(1) Istituto Zooprofilattico sperimentale delle Venezie, Italy

Alessandra Tata <atata@izsvenezie.it>

Critical and comprehensive knowledge of the nutritional factors influencing mycoplasma growth is a prerequisite for any study leading to the understanding of the metabolism and *in vitro* behavior of these bacteria. Recently, metabolomics, the qualitative and quantitative measurement of metabolites within a system, aided the characterization of the metabolism of different mycoplasma species, revealing fundamental differences in active metabolic pathways and improving the genome annotation. However, not much attention has been given to the impact of different culture media on the metabo-lomic study outcomes. Hence, knowledge of the influence of various culture media on the metabolomic data of mycoplasmas should be assessed. Moreover, the well-established analytical techniques (e.g., gas-chromatography-mass spectrometry, liquid chromatography-mass spectrometry,nuclear magnetic resonance) employed in metabolomics are lengthy and laborious, and therefore, more effective alternatives are needed for real-near-time and cost-effective monitoring. We additionally highlight the need for rapid, sustainable, and accurate methods capable of revealing changes to metabolic behaviors in the presence of 'dynamic' and 'living' entities. In the present study, we aimed at assessing the utility of an ambient mass spectrometry technique, namely, direct analysis in real time high resolution mass spectrometry (DART-HRMS), for temporally monitoring the metabolic behavior of *Mycoplasma gallisepticum* cultured on three different media. This study highlights the considerable potential of DART-HRMS analyses to assist scientists in obtaining new insights into mycoplasma metabolomics and establishing how the culture medium affects certain metabolic pathways, thereby showing the adaptive plasticity of mycoplasmas.

O-48

Identification of an endonuclease and N6-adenine methyltransferase from *Ureaplasma parvum* SV3F4 strain

Yukiko Nakura (1), Hengning Wu (1), Yuya Fujisawa (2), Zenzaburo Tozuka (3), Alexey Fomenkov (4), Shin-ichiro Kajiyama (2), Shinsuke Fujiwara (5), Kiyoshi Yasukawa (6), Richard Roberts (4), Itaru Yanagihara (1)

(1) Osaka Women's and Children's Hospital, Japan
(2) Graduate School of Biology-Oriented Science and Technology, Kindai University Kindai University
(3) Graduate School of Pharmaceutical Sciences, Osaka University, Japan
(4) New England Biolabs Inc.
(5) School of Biological and Environmental Sciences, Kwansei-Gakuin University, Japan
(6) Graduate School of Agriculture, Kyoto University, Japan

Yukiko Nakura <nakurayu@wch.opho.jp>, Hengning Wu <whn163@wch.opho.jp>

Ureaplasma species are one of the causative organisms of several disorders, including infertility, nongonococcal urethritis, miscarriage, and adverse birth outcomes, and there is growing evidence that the methylation status of the bacterial genome affects virulence and bacterial survival against antimicrobial agents. However, the role of methyltransferases in *Ureaplasma* remains unclear, as little is known about the epigenetics of *Ureaplasma* spp. Here, we report a novel endonuclease and N6-adenine DNA methyltransferase (m6A methyltransferase) in the *Ureaplasma parvum* SV3F4 strain. Our previous study found that the SV3F4 strain carries 17 unique genes compared to the two genomes of *U. parvum* strains, OMC-P162 and ATCC 700970. Among these 17 unique genes, the UP3_c0261 and UP3_c0262 genes were individually expressed and purified in *Escherichia coli*. The UP3_c0261 recombinant protein showed endonuclease activity recognizing and cleaving a GTNAC motif, resulting in a 5 base 5’ extension. The UP3_c0261 protein digested a polymerase chain reaction (PCR) product harboring the GTNAC motif and was designated UpaF4I. Treatment of the PCR product with the recombinant protein UP3_c0262 completely blocked the restriction enzyme activity of UpaF4I. Analysis of the treated PCR product harboring a modified nucleotide by UP3_c0262 with HPLC-MS/MS and MS/MS showed that UP3_c0262 was an m6A methyltransferase modifying the A residues in both DNA strands of the GTNAC motif. Whole genome methylation analysis of SV3F4 showed that all of the GTNAC motifs were m6A modified. These results suggest the UP3_c0261 and UP3_c0262 genes may act as a novel Type II restriction-modification system in the *Ureaplasma* SV3F4 strain.

O-49

Breaking down the complexity of *Mycoplasma hominis*

Boutheina Mardassi (1), Salim CHIBANI (1), Behija Mlik (1), Elhem Yacoub (1), Nadine Kadhraoui, Safa Boujemaa (1), Sarra Abassi (1)

(1) Institut Pasteur de Tunis, Tunisia

Chibani Salim <salim.chibani92@gmail.com>

Mycoplasma hominis (MH) is an underestimated commensal bacterium belonging to the Mollicutes class causing a wide range of urogenital infections and infertility. In this study we perform genomic comparison between MH Tunisian strains and others from different origins. This will provide an insight into the genomic evolution and distinctive features that underpin the pathogenicity of MH. For this purpose, whole genome sequencing of 62 clinical MH strains was performed using illumina Miseq. Denovo assembly was performed using Fq2dna pipeline. Prokka was used for annotation and Treewas was applied to detect genes associated with pathotypes. For genomic comparaison between a variety of MH strains, energy Metabolism was investigated using Kegg Pathway, classification of gene families was done using bacterial pangenome pipeline. Single nucleotide polymorphism (Snp) analysis was done using BCF tools. Virulence factors were annotated using virulence factors database VFDB. Average genome size of Tunisian MH strains is between 656421 and 752582 bp. TreeWas identified 4 virulence genes associated with the pathotype gynecological infection and five genes associated with infertility. All strains harbored an efflux pomp that belongs to the family of multidrug resistance ABC transporters. Both MH Tunisian, Russian, Newengland and French strains have a conserved thymidine hydrolysis pathway which is activated under arginine deprivation. Snp analysis revealed high level of transition substitutions among MH Tunisian strains causing infertility as well as French, Russian and USA strains. This is explained by the presence of unique genes involved in recombination and repair. The highest number of insertions and deletions was found to occur among MH French strains. Russian strains of MH appear to have more Snp and an elevated number of postranslational modifications genes. We identified Heat shock thermosensor HrcA among MH Tunisian strains and cold shock dead box RNA helicase among European MH strains. Hemolysin C family proteins have been found in all MH strains at the exception of German strains. These findings shed the light on the complexity of MH genome and suggest that it can thrive inside the host and resist to all kind of environmental challenges through sophisticated virulence mechanisms.

O-50

Genomic insights into the sugar beet pathogen '*Candidatus Phytoplasma solani*' driving the outbreak in Germany

Michael Kube (1), Rafael Toth (1), Bruno Huettel (2), Jan Werner Böhm (1), Omid Eini (3), Mark Varrelmann (3)

(1) University of Hohenheim, Stuttgart, Germany
(2) Max Planck Genome Center, Cologne, Germany
(3) Institute of Sugar Beet Research, Göttingen, Germany

Michael Kube <michael.kube@uni-hohenheim.de>

Few phytoplasma diseases in Europe are associated with dramatic economic losses. The rapid spread of the cixiid *Pentastiridius leporinus* L. has changed this situation. The cixiid was identified in the 1990s as a vector of the phytopathogenic bacteria '*Candidatus* Arsenophonus phytopathogenicus' (*Morganellaceae*) and '*Candidatus* Phytoplasma solani' (*Acholeplasmataceae*) to sugar beet (*Beta vulgaris* subsp. *vulgaris*) in Burgundy (France) and, at least since 2008, in Baden-Württemberg (Germany). The pathogens cause the so-called Syndrome Basses Richesses (SBR) of sugar beet, leading to losses in sugar content of 2-4% in absolute terms but also biomass (Gatineau et al., 2002; Pfitzer et al. 2022). Sugar beet cultivation is facing a serious threat to its economic sustainability due to losses caused by SBR. The disease was mainly related to '*Ca*. A. phytopathogenicus' infection, while a mixed infection with the phytoplasma resulted in the formation of more severe symptoms. Until 2018, '*Ca*. A. phytopathogenicus' was widely detected in symptomatic sugar beets, while the stolbur pathogen was detected in less than 15% in most areas but with the rapid spread of *P. leporinus* in Germany, a drastic increase in mixed infections was observed (Duduk et al. 2023), often resulting in complete losses of sugar beet in the field due to primary pathogens and several secondary microbial pathogens. In addition, the polyphagous vector transmitted the phloem restricted pathogens to other host plants, including potato. Genetic marker analysis revealed the emergence of a previously unknown 16SrXII subgroup P type strain. This prominent *P. leporinus*-transmitted strain is considered to be the driver of the current epidemics in Germany. Functional reconstruction and comparative genomic analyses suggest that the success of this prominent stolbur strain is likely to depend on transmission efficiency and virulence factors rather than metabolic capabilities. The ongoing epidemic spread of the vector and its pathogens in Europe must be considered one of the major phytoplasma challenges for many crops in agriculture in this decade.

O-51

Analysis of the genomic core of *Phytoplasmas* and other *Mollicutes*

Jan W. Böhm (1), Michael Kube (1), Bernd Schneider (2), Bruno Huettel (3)

(1) University of Hohenheim, Stuttgart, Germany
(2) Julius Kühn-Institut, Germany
(3) Max Planck Institute for Plant Breeding, Cologne Germany

Jan W. Böhm <jan.boehm@uni-hohenheim.de>

Phytoplasmas (Mollicutes) are bacterial pathogens associated with various plant diseases worldwide. During their intracellular life cycle, they colonise the plant phloem and are transmitted by phloem-sucking insects. Both environments provide sufficient nutrients, which is thought to enable genome reduction. In contrast, the presence of mobile elements leads to further expansion of these genomes. With an increasing number of complete phytoplasma genomes available, ten 16S ribosomal groups are covered. Our group recently determined '*Candidatus* Phytoplasma fraxini' AshY1 (16SrVII-A) covering another group and enabling a comprehensive core content analysis. The objective of this study is to identify conserved features within complete phytoplasma genomes and higher taxonomic levels for its application in taxonomy and diagnostics. By analysing the deduced protein content in relation to the orthology inference of uniformly annotated genomes, the core content was delineated. Findings show that phytoplasmas have a core content of 227 orthogroups and a high single copy proportion. Seven transport systems like the Sec-dependant secretion system, P-type ATPases, ABC-transporters, and channel proteins for nutrient uptake, ion-pool regulation and protein secretion are encoded in all genomes observed. In contrast, metabolic features within the glycolysis are lacking throughout the core set. This emphasises the differences between phytoplasma species in their ability to perform the whole glycolysis or only encoding the absolute necessity. In contrast, analysis highlights the importance of carboxylic acid metabolism within phytoplasmas. Extending the analysis to complete genomes of the family of *Acholeplasmataceae* displays a core content of >200 orthogroups with a single copy share of >60%. The core content is further reduced to less than 70 with a single copy share of >30%, by taking complete genomes of the class mollicutes into account. Single-copy orthologs within each taxonomic cluster are suggested as candidate sequences for phylogenetic analysis and species delineation through multilocus sequencing analysis (MLSA) and application as internal controls in diagnostics.

O-52

Hotspot of recombination in *Metamycoplasma equirhinis*: evidence of multiple horizontal gene transfer events originating from bacteria sharing the same ecological niche

Maxime Bruto (1), Albertine LEON (2), Matthieu MARTINEAU (2), Florence TARDY (3), Fabien Labroussaa (1), Chloé Ambroset (1)

(1) ANSES, Lyon Laboratory, VetAgro Sup, UMR Animal Mycoplasmosis, University of Lyon, Lyon, France
(2) LABÉO, Pôle Recherche, Développement et Innovation, Normandie Univ, UNICAEN, UNIROUEN, Inserm UMR 1311 DYNAMICURE, France
(3) Mycoplasmology, Bacteriology and Antibioresistance unit, Anses, laboratoire de Ploufragan-Plouzané-Niort, France

AMBROSET Chloe <chloe.ambroset@anses.fr>

Mycoplasma genomes were long thought to be shaped exclusively by successive gene losses. In-depth analyses of their genomes have clearly demonstrated the contribution of horizontal gene transfer (HGT) to the evolution and plasticity of many mycoplasma species. Therefore, HGT may be used by mycoplasmas as a mean to adapt to new environmental stresses or ecological niches. A recent effort to sequence and analyze the genomes of 24 *Metamycoplasma (M.) equirhinis*, a species associated with the equine respiratory disease complex in horses, revealed that all these genomes are overall syntenic except for a few regions, one of which is adjacent to the CRISPR/Cas locus (Martineau *et al.*, IOM submission number 80). This locus turned out to be highly variable between the 24 genomes with eight different genetic patterns resulting from rearrangements affecting i) the presence and/or general genetic organization of the CRISPR/Cas locus, but also ii) the genomic region downstream of the CRISPR/Cas locus, which was defined as a hot spot of genetic diversity. Remarkably, all the genes downstream of the CRISPR/Cas system were horizontally acquired and appeared to originate from bacteria belonging to the *Bacillota* and *Fusobacteriota* phyla, many of them being commonly found in the respiratory tract of horses. *In silico* analyses further revealed that some of the coding sequences present in these regions shared high homologies with previously described defense mechanisms such as restriction-modification or toxin-antitoxin (TA) systems. In particular, the presence of at least one candidate TA system was identified in half of the genomes and shared high amino acid homology with one of the TA systems functionally characterized in *Mycoplasma mycoides* subsp. *capri* (1). The functional characterization of these systems is currently ongoing using different heterologous expression systems. These results suggest that *M. equirhinis* can acquire and potentially exchange genetic material with other phylogenetically distant bacteria that share the same ecological niche, i.e. the equine respiratory tract. The coding sequences located in this hotspot of recombination have putative functions linked to bacterial defense systems.

1. Hill V, Akarsu H, Barbarroja RS, Cippa VL, Kuhnert P, Heller M, et al. Minimalistic mycoplasmas harbor different functional toxin-antitoxin systems.PLoSGenet.2021;17(10)

Phylogeny and Taxonomy

O-53

Assessing the recent revisions of Mollicutes taxonomy based on genome-scale analyses of phylogenetic inferences and gene content comparisons

Chih-Horng Kuo (1), Hsi-Ching Yen (1), Xiao-Hua Yan (1)

(1) Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan

Chih-Horng Kuo <chk@gate.sinica.edu.tw>

The taxonomy of Mollicutes provides a foundation that is critical for communication and research concerning mycoplasmology. In recent years, extensive taxonomic revisions were made in this class. Particularly, the creation of multiple novel genera that impact the classification of *Mycoplasma*, *Entomoplasma*, and other related lineages have resulted in debates and confusion among the international mycoplasmology community and other stakeholders. In this work, we conducted genome-scale analyses to evaluate these taxonomic revisions. Based on 182 representative genome sequences of Mollicutes available from public databases, we inferred the core genome phylogeny and quantified the gene content divergence of these representatives. Based on our findings, the recent revisions that reclassified *Mycoplasma* species belonging to the hominis group to three novel genera (i.e., *Mesomycoplasma*, *Metamycoplasma*, and *Mycoplasmopsis*) and several *Entomoplasma* species to four other genera (i.e., *Edwardiiplasma*, *Mesoplasma*, *Tullyiplasma*, and *Williamsoniiplasma*), appeared to be questionable as no robust pattern of overall gene content differentiation was observed between those emended or novel genera. Considering that the aforementioned revisions were mainly based on only a handful of shared sequence polymorphisms as synapomorphic characteristics, further investigations, particularly those utilizing polyphasic approaches, are necessary for improving the taxonomy of Mollicutes and providing better support to mycoplasmology.

O-54 Harry Morton Student Award Candidate

Power of codon usage analysis for phylogenetic reconstruction of the *Mollicutes*

Anna-Marie Ilic (1), Michael Kube (1)

(1) Integrative Infection Biology Crops - Livestock (460K), Stuttgart, Germany

Anna-Marie Ilic <annamarie.ilic@uni-hohenheim.de>

Background: Phylogeny of Mollicutes is a dynamic field that is constantly evolving as research progresses. The extensive generation of sequence data has revealed unexpected relationships between many species by providing deeper insights into genetic diversity. Furthermore, it enabled phylogenetic analyses of unprecedented quality and subsequently led to overdue revisions in bacterial taxonomy. The need to consider as many informative sites as possible is reflected in the use of average nucleotide identity (ANI) and average amino acid identity (AAI) analyses, alongside other phylogenetic markers. Uncertainty and thus problems in the assignment of taxonomical ranks arise with rapidly evolving genomes, such as the obligatory bacterial parasites from the phytoplasma group. Here, we propose the complementary use of the qualitative scoring calculation of the percentage of conserved proteins (POCP) and signature-based approaches such as codon usage and tetranucleotide frequency analysis. **Material and Methods**: Using genome sequences for 149 Mollicutes, with RefSeq entries prioritised for automatic and comparable curation if available, the study of their evolutionary relationships was conducted based on multiple approaches. These included the analysis and calculation of POCP, codon usage and tetranucleotide frequency, followed by comparison with previously applied methods. The software packages were applied with the recommended default parameters. **Results**: The results are consistent with the ANI and AAI analyses as well as with the most recent taxonomic revisions. Using the taxonomy recommended by the International Committee on Systematics of Prokaryotes, the POCP analysis of the species within the different families shows only weak significance. In contrast, the codon usage analysis shows species-specific patterns and provides a stable phylogenetic signal. Tetranucleotide frequency analysis additionally allows differentiation below the species level. Threshold values are proposed using the phytoplasma group in example. **Conclusion**: Codon usage analyses of genome sequences should be integrated as an important addition to future phylogenetic analyses, as their lineage-specific analyses enable reliable phylogenetic clustering.

O-55

Mycoplasmogenesis: Towards definition of the obscured contour

Yoshihito Kitaoku (1), Shingo Kato (2), André Antunes (3), Mone Mimura (4), Hana Kiyama (4), Daichi Takahashi (1), Masaki Mizutani (5), Masafumi Hayashi (6), Taishi Kasai (6), Robert C. Robinson (1, 7), Samson Ali (1), Akihiro Narita (8), Shigeyuki Kakizawa (5), Taku Oshima (9), Daisuke Shiomi (6), Makoto Miyata (4)

(1) Okayama University, Japan
(2) JCM, RIKEN, Japan
(3) MUST, Macao
(4) Osaka Metropolitan University, Japan
(5) AIST, Japan
(6) Rikkyo University, Japan
(7) VISTEC, Thailand
(8) Nagoya University, Japan
(9) Toyama Prefecture University, Japan

Yoshihito Kitaoku <ykitaoku@okayama-u.ac.jp>

Recent discoveries of free-living mycoplasmas and their peripheral species, along with the accumulation of completed genomes, prompted us to revisit the phylogenetics and the evolutionary origin of mycoplasmas. Completion of the genome sequence for *Haloplasma contractile* SSD-17B, the rooting species of mycoplasmas, allowed us to trace the bacterial lineage leading the genesis of mycoplasmas: “Mycoplasmogenesis”. Highlights of our analysis includes: (1) Completion of the *Haloplasma* genome disclosed the positively biased GC-/AT-skew toward the leading strand with accompanied CDS in contrast to mycoplasmas with reduced genomes. The trajectories of the mobile genetic elements streamlined through the host-associated life styles revealed the yet unknown process of the genome reduction. (2) One of the characteristic features of mycoplasmas is the *Spiroplasma* swimming based on the MreB filaments. The mechanism of the dynamically regulated polymerization/de-polymerization cycle is still yet to be explored. Comparison of MreB clusters for individual species shed light on the process of the gene multiplication and formation of gene clusters with somewhat conserved fundamental elements. The predicted operonic regulation suggests the functional disparity of the paired MreBs in operons. (3) Comparative genomics of mycoplasma genomes revealed plasticity in a good agreement with JCVI Syn3.0, providing implications for further design of minimal genomes. Further details of the streamlined genome in relation to the host-associated life styles suggests the dependency of mycoplasma genomes on the growth conditions. (4) Accumulation of Erysipelotrichales genomes with the indicated phylogenetic coherence to mycoplasmas in the previous reports suggested the multiple events of cell wall loss in phylogenetically distant two lineages, i.e. Myco-/Enteroplasmatales and Haloplasmatales. Phylogenetic trees constructed for 16S/23S rRNA sequences of the Terrabacteria group, with the accompanied signature analysis, further supports this observation. The syntenic conservation of division and cell wall (*dcw*) cluster genes, together with the gradual decomposition of the constituting elements in the intermediate species, provides a footprint for the loss of the peptidoglycan cell wall, allowing tracking of the process.

The obscured contour of the rooting species and the definition of mycoplasmas will be discussed.

O-56

Species diversity within family *Mycoplasmataceae* – a fresh perspective on the expansion of genera *Mycoplasma* and *Ureaplasma* with 140 novel species entities

Joachim Spergser (1), Ana S. Ramirez (2)

(1) Institute of Microbiology, University of Veterinary Medicine Vienna, Austria
(2) Unidad de Epidemiología y Medicina Preventiva, IUSA, Veterinary Faculty, Universidad de Las Palmas de Gran Canaria, Canary Islands, Spain

Joachim Spergser <joachim.spergser@vetmeduni.ac.at>

Mycoplasmas are widespread in nature with currently 145 established and validly named *Mycoplasma* (n = 136) and *Ureaplasma* (n = 9) species detected in or isolated from a broad range of animal hosts including humans. To further explore the species diversity within family *Mycoplasmataceae*, a total of 960 unidentified *Mycoplasma* and *Ureaplasma* isolates predominantly recovered from wildlife were investigated and thoroughly characterized by applying a polyphasic approach. Cryopreserved isolates were cultivated, and colony-derived cultures phenotypically grouped by MALDI-ToF mass spectrometry. In addition, the 16S rRNA gene, the 16S-23S intergenic spacer, and a fragment of the *rpoB* were sequenced, similarities determined (BLASTn), and phylogenetic trees constructed. Representative strains of putatively novel species were then whole genome sequenced (Illumina), phylogenomic trees constructed and genomic similarity metrics (e.g., ANI, TETRA, dDDH) determined to consolidate species delineation. By using this polyphasic approach including mass spectrometric, phylogenetic, and genomic characterization, a total of 140 novel species entities within genera *Mycoplasma* and *Ureaplasma* were identified. Most of these novel species (n = 129) were affiliated to the Hominis group, 11 to the Pneumoniae group but none to the Spiroplasma group. Within the Hominis group, 47 novel species were phylogenetically positioned within the *M. synoviae*, 32 within the *M. bovis*, 20 within the *M. hominis*, 14 within the *M. neurolyticum*, and 13 within the *M. gypis* cluster. Furthermore, one new species was each assigned to the *M. equigenitalium*, *M. pulmonis*, and *M. sualvi* cluster. Within the Pneumoniae group, most of the novel species (n = 9) belonged to the *Ureaplasma* cluster and the remaining (n = 2) were assigned to the *M. pneumoniae* and *M. fastidiosum* cluster, respectively. Altogether, results of this comprehensive study highlight the exceptional species diversity within *Mycoplasmataceae* by almost doubling the number of taxa within genera *Mycoplasma* and *Ureaplasma*. In addition, genome sequences generated from representatives of new taxa constitute an invaluable source for comparative genomics to better understand the biology and evolution of members of these genera.

O-57

***Mycoplasma agassizii*: Coevolution with Ancient Land Tortoises or Introduction to Wild Populations?**

Alexandra Burne (1), Bonnie Raphael (2), Dina Michaels (1), Mary Brown (1)

(1) University of Florida, USA
(2) Turtle Survival Alliance, USA

Mary Brown <mbbrown@ufl.edu>

While traditionally thought to be host specific, new and emerging mycoplasmas are increasingly being detected in novel hosts, and particularly in wildlife species. *Mycoplasma agassizii* was first identified in free-ranging desert tortoise populations in the 1990s and has subsequently been isolated from a wide range of hosts in the Testudinidae family. A long standing question, especially in light of captive release issues in desert tortoises, has been "was the pathogen introduced into wild populations". To better understand the evolutionary relationship between host and pathogen, we performed whole genome sequence analysis of isolates of *M. agassizii* from four free-ranging hosts with highly basal (*Gopherus* species, N=3) and recent (*Astrochelys radiata*, N=1) lineages. Additionally, an isolate of *M. agassizii* from a clinically ill captive Burmese black mountain tortoise (*Manouria emys*), a sister clade of *Gopherus*, was included. Phylogenomics shows little divergence among the *M. agassizii* isolates, regardless of host. However, comparative system analysis identified variable gene arrangement and copy number of genes. Comparative genome analysis of *M. agassizii* obtained from wild animals in geographically disparate locations supports the hypothesis that *M. agassizii* was present in ancestral tortoise species and has co-evolved with Testudines hosts.

Plant and Insect Mollicutes

O-60

'Candidatus Phytoplasma solani' predicted effectors SAP11-like and SAP54-like are individually sufficient to alter phenotype of transformed Arabidopsis plants

Marina Drčelić (1), Bruno Polak (1), Nataša Bauer (1), Andreja Škiljaica (1), Martina Seruga Music (1)

(1) University of Zagreb, Faculty of Science, Department of Biology, Croatia

Martina Seruga Music < martina.seruga.music@biol.pmf.hr>

In phytopathogenic bacteria, effectors are usually small proteins that influence plant development and physiology by modifying signaling pathways and interactions with both plant and insect hosts. Only a few phytoplasma effectors have been studied in detail such as SAP11, SAP54, SAP5, TENGU and PHYL1 from '*Ca.* P. asteris' strains, and SAP11-like from 'Ca. P. mali'. In our previous studies of 3 'Ca. P. solani' strains we identified 38, 22 and 20 putative effector genes, respectively, including homologues of SAP11 and SAP54. In the scope of this work, we focused on the effect of SAP11-like and SAP54-like effectors on transformed *Arabidopsis thaliana* plants. SAP11-like and SAP54-like coding sequences (without the signal peptide sequence) were codon-optimized for expression in *A. thaliana,* synthesized by commercial service and used as templates for PCR in subsequent cloning reactions. For overexpression of 6xHis-taged SAP11-like and SAP54-like in *A. thaliana*, appropriate plasmids were prepared by using InFusion cloning. Genes were cloned into pGWB529 plasmid and transformed to *Agrobacterium tumefaciens* GV3101 pMP90 by electroporation. A. thaliana plants were transformed by floral dip. Transgenic A. thaliana lines were selected and homozygous lines with one T-DNA insertion regenerated. Stable transgene integration and expression of *SAP11-like* and *SAP54-like* genes were verified by PCR and qPCR, respectively. Successfully transformed *A. thaliana* plants that overexpressed SAP11-like or SAP54-like genes were compared to the wild type. SAP11-like transformed lines exhibited significantly altered leaf morphology with extremely crinkled and rolled leaves, while SAP54-like transformed lines showed yet undescribed unusual phenotype with hairy leaves and stems, additional cauline leaves and the appearance of hairy leafy structures surrounding the inflorescence. To the best of our knowledge, this is the first report of the change of morphology in transformed Arabidopsis plants due to '*Ca.* P. solani' predicted effectors. Detection of potential protein targets of SAP11-like and SAP54-like effectors in *A. thaliana* are in progress.

Structural Biology

O-61 Louis Dienes Award Candidate

The structural landscape of the secretome of phytoplasmas, master modulators of plant architecture

Federico Gabriel Mirkin (1), Sam T Mugford (1), Jay K. Goldberg (1), Yeshveer Singh (1), Qun Liu (1), Saskia A. Hogenhout (1)

(1) John Innes Centre, Norwich, UK

Federico Gabriel Mirkin <federico-gabriel.mirkin@jic.ac.uk>

Parasites possess remarkable abilities to influence host development and behaviour, crucial for their survival and dissemination. This manipulation, especially evident in obligate parasites, showcases extended phenotypes where an organism's genetic impact extends beyond itself, affecting others in the ecosystem.
Phytoplasmas, spread by insect vectors, often trigger profound developmental alterations in plants, such as witches' brooms, phyllody, neoteny, and extended lifespan. We and others uncovered that these pathogens secrete effectors that specifically target critical plant transcription factors (TFs) involved in developmental regulation. These effectors facilitate the degradation of entire TF families, frequently creating short circuits between plant pathways and bypassing the ubiquitination process, leading to disrupted plant growth and distinct developmental anomalies. Our research also uncovered that plants displaying the alterations are more appealing to insect vectors responsible for transmitting phytoplasmas across vast distances. However, it remains unclear whether the phytoplasma effectors that have been functionally characterised to date adequately represent the full spectrum of diversity among phytoplasma effectors. To investigate this, we assessed the structural landscape of the secretome of over 230 phytoplasma species. Our investigation has revealed that an important proportion of phytoplasmas secrete proteins bearing structural similarities to SAP11, SAP54/PHYL1, or Tengu. These proteins, characterised by their small, helical structures, interact with helical structures within TF oligomerization domains, thus promoting the degradation of these TFs. Furthermore, bacteria distributed across the phytoplasma phylogeny encode effectors that are distinguished by a distinct globular bimodal architecture, akin to SAP05. This effector binds to the Zinc-finger domains of plant TFs and tethers them to a 26S proteasome component, thereby facilitating TF degradation. This process leads to neoteny, stem and leaf proliferations, and increased longevity. Within the structural clusters of SAP05-like proteins, there exist subclusters showcasing unique SAP05-like folds, and these may have scaffolding abilities as well. Beyond the structural clusters that include functionally characterised effectors, many other structural clusters were identified. This study revealed that phytoplasmas conceal a treasure trove of effector structures and functions that extend beyond the scope of known effectors. Studying them may unveil the true extent of the phytoplasma extended phenotype.

O-62

Structural analysis and Molecular dynamics simulations of urease from *Ureaplasma parvum*

Hengning Wu (1), Junso Fujita (2,3,4), Yukiko Nakura (1), Masao Inoue (5), Koichiro Suzuki (6), Toru Ekimoto (5), Mitsunori Ikeguchi (5,7), Keiichi Namba (2,3), Itaru Yanagihara (1)

(1) Department of Developmental Medicine, Research Institute, Osaka Women's and Children's Hospital, Osaka, Japan
(2) Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan
(3) JEOL YOKOGUSHI Research Alliance Laboratories, Osaka University, Osaka, Japan
(4) Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan
(5) Graduate School of Medical Life Science, Yokohama City University, Yokohama, Japan
(6) The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan
(7) RIKEN Center for Computational Science, Yokohama, Japan

Hengning Wu <whn163@wch.opho.jp>

Ureaplasma species are the major causative organism of human chorioamnionitis and generate ATP using urease. *Ureaplasma* species urease consists of 7 genes, including three enzyme subunits, UreA, UreB, and UreC, and four accessory proteins, UreD, UreE, UreF, and UreG. Accessory proteins assemble the active metallocentre of urease and produce a fully activated mature urease. However, the structure and precise enzymatic character of *Ureaplasma* urease remain unclear. We purified urease from *Ureaplasma parvum* OMC-P162 strain (UPU), and the UPU showed the highest activity at pH 7.2 and 7.4. At pH 7.4, the *Km* and *Vmax* of the reaction mediated by the urease were estimated to be 4.3 mM and 3,333 µmol NH3/min/mg protein, respectively. The UPU activity and bacterial growth were abolished with 2-Mercaptoethanol (2-ME), a known urease inhibitor, in a dose-dependent manner. Mass spectrometric analysis showed that the UPU complex included three subunits (UreA, UreB, and UreC). We further analyzed the structure of UPU complex using Cryo-EM at the atomic level. UPU complex showed a trimer of heterotrimer (3x3 structure) at a resolution of 2.03 Å, and the shared typical architecture of the urease active site included two Ni2+ ions coordinated by His, Asp, and carbamylated Lys residues. 2-ME bridged two Ni2+ and overlapped with urea, which inhibited the activity. All-atom molecular dynamics simulations for *Sporosarcina pasteurii* and *Klebsiella aerogenes* ureases were also performed for comparison. The principal component analysis revealed that the domain motions differ among the three ureases. In the *U. parvum* urease, the flap and UreB are more separated than in the other two species, which may affect the entrance and/or exit of substrates and products. Our results provide a structural basis for UPU activity.

O-63

An ABC transporter pair are essential for the synthesis of the *Mycoplasma genitalium* biofilm structural exopolysaccharide

Kevin Dybvig (1), James Daubenspeck (1), T. Prescott Atkinson (1)

(1) University of Alabama at Birmingham, USA

T. Prescott Atkinson < tatkinso@uabmc.edu>

Bacteria synthesize biofilms to protect themselves from host immune function, a harsh environment, or antibiotics. The exopolysaccharide (EPS) that is most associated with bacterial biofilm structure is a linear polymer of glucosamine residues connected through a β-1-6 linkage, with varying degrees of acetylation, poly-N-acetylglucosamine (PNAG). In a classic example of convergent evolution, this molecule has evolved countless times in species from *Bacillus subtilus* to *Escherichia coli* as a structural component of biofilms, this includes *Mycoplasma genitalium* (Mgen). In Mgen, the molecular composition of PNAG is the same but instead of sugars in a pyranose configuration, the Mgen EPS is in the furanose conformation. 97 distinct Mgen transposon mutants from the library obtained from John Glass (JCVI) were screened. Cultures were grown attached in T-75 tissue culture flasks and observed for biofilm formation. 26 putative mutants were identified that formed aberrant biofilms. These were analyzed by gas chromatography to determine the concentration of GlcNAc in the sample. We identified four mutants as essential for the synthesis of GlcNAc or the PNAG polymer. MG_289, MG_290, and MG_291 are a heterodimeric pair of ABC transporters and an accessory protein. These glycosyltransferases synthesize and transport the EPS polymer out of the bacteria. This is the second set of glycosyltransferases identified, that by homology are ABC transporters. The first example was in *Mycoplasma pulmonis*, where capsular EPS was synthesized by the ABC transporter pair MYPU_7410 and MYPU_7420. These are the only 2 known examples of a novel enzyme class that appears to be unique to Mollicutes. We also identified MG_011 a mutant that does not synthesize the Mgen PNAG. This enzyme appears to be involved in glucosamine synthesis. Mgen has the smallest genome of any known free-living organism, the number of functions hidden in that tiny genome continues to astound.

Virulence and Pathogenesis

O-64

Identification of nucleomodulins of *Mycoplasmopsis bovis* by magnetic bead enrichment and proximity-based biotinylation approaches

Doukun Lu (1,2), Gang Zhao (3), Jiongxi Chen (1,2), Menghan Zhang (1,2), Yingjie Fu (1,2), Yingyu Chen (2), Changmin Hu (2), Jianguo Chen (2), Elise Schieckd (4), Aizhen Guo (1,2)

(1) State Key Laboratory of Agricultural Microbiology, Hubei Hongshan Laboratory, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China
(2) International Research Center for Animal Disease, Ministry of Science and Technology, Huazhong Agricultural University, Wuhan, China
(3) Key Laboratory of Ministry of Education for Conservation and Utilization of Special Biological Resources in the Western China, Ningxia University, Yinchuan, China
(4) International Livestock Research Institute, Nairobi, Kenya

Doukun Lu <doukunlu@163.com>

Background: Mycoplasmopsis bovis (*M. bovis*) is a significant bovine pathogen associated with various diseases, including bovine bronchopneumonia and mastitis resulting in substantial economic losses within the livestock industry. However, the development of effective control measures for *M. bovis* is hindered by a limited understanding of its virulence factors and pathogenesis. Nucleomodulins are newly identified secreted proteins of bacteria that internalize the host nuclei to regulate host cell gene expression and serve as critical virulence factors. Previously one nucleomodulin MbovP475 of *M. bovis* was identified to function as a powerful virulence factor. This study aimed to identify novel *M. bovis* nucleomodulins by using high throughput methods. **Methods**: Magnetic bead enrichment (MBE) and proximity-based biotinylation (PBB) approaches combined with mass spectrometry were employed to screen potential nucleomodulins of *M. bovis*. Overlapping PCR was used to construct corresponding recombinant plasmids expressing potential EGFP labeled nucleomodulins. Opera Phenix was utilized to confirm the cellular location of EGFP fusion proteins. **Results**: Through the MBE approach, a total of 289 proteins were identified including 66 highly abundant proteins. In parallel, the PBB approach identified 28 proteins. Finally, 7 nucleomodulins were verified to be nucleus location with Opera Phenix, including the known nucleomodulin MbovP475 and 6 novel ones. Among these novel proteins, there were 4 ribosomal proteins (MbovP599, MbovP678, MbovP710, and MbovP712), 1 transposase (MbovP790), and 1 conserved hypothetical protein (MbovP513). Among them, 1 unique nucleomodulin was identified with the MBE approach, 2 unique nucleomodulins with the PBB approach, and 4 common nucleomodulins by both. Using bioinformatic tools like SignalP 6.0 and cNLS Mapper, we predicted that only MbovP475 possesses a classical signal peptide; meanwhile, all 7 proteins under investigation were predicted to contain nuclear localization signals. **Conclusion**: A total of 7 nucleomodulins candidates were confirmed to enter the nucleus of BoMac cell with 6 novel and one known (MbovP475) nucleomodulins. These findings establish a foundation for further research on *M. bovis* nucleomodulin-host interactions and identification of new virulence factors.

O-66 Harry Morton Student Award Candidate

In vivo disease severity in a mouse model of *Mycoplasma pneumoniae* is influenced by the adherence state of bacteria during inoculum preparation

Jeremy Miller (1), Rosemary Grace Ozyck (1), Morgan Hunte (1), Edan Tulman (1), Steven Szczepanek (1), Steven Geary (1)

(1) University of Connecticut, USA

Morgan Hunte <Morgan.Hunte@UConn.edu.>

Mycoplasma pneumoniae (Mp) is the most common cause of primary atypical pneumoniae in humans. In the US alone, this pathogen is responsible for approximately two million cases of community-acquired pneumoniae and over 100,000 hospitalizations every year. In conjunction with its significant morbidity, the organism is becoming increasingly macrolide resistant. To address these issues, researchers require the most up-to -date animal models available. We hypothesized that the state of adherence at the time of Mp inoculum preparation may contribute to variability in degree of disease in the model host, and thus may have serious implications for in vivo host-pathogen interaction studies. We tested whether Mp inoculum prepared with adherent, non-adherent, or a mix of two resulted in differing levels of severity in a BALB/c mouse model. Mp PI1428 was grown in FC media in T175 flasks. Supernatants were collected as “non-adherent” Mp and pooled. Remaining adherent Mp was scraped as “adherent” Mp and pooled. Other flasks were scraped and collected in their entirety as “mixed” populations and pooled. All fractions were quantified by OD620. Three groups of fifteen mice per group were infected intranasally with 1E+08 CFU Mp. The remaining inocula were titered back to confirm comparable infectious dose. Four days post infection, lungs were removed for histopathology and bacterial recovery. Lung lesion scores were assessed using a blinded in-house lesion scoring system. No statistically significant difference was seen in bacterial recovery across the three groups, however the non-adherent group had significantly higher lung lesion scores than the adherent group ($p = 0.0303$). The mixed population was not statistically different from the other two groups. Our data show that non-adherent Mp causes more severe lung pathology in a female BALB/c mouse model. Future studies will focus on proteomic and RNA expression differences between adherent and non-adherent Mp. This work contributes to the further optimization of the in vivo Mp infection model.

O-67

Virulence of two recent Dutch *Mycoplasma synoviae* isolates in broilers and demonstration of M. synoviae in lesions by an in situ hybridization test

Anneke Feberwee (1), Hanneke Bataille (1), Naomi de Bruijn (1), Jeanine Wiegel (1), Manon Holstege (1), Christiaan ter Veen (1), Sjaak de Wit (1)

(1) Royal GD, Deventer, The Netherlands

Anneke Feberwee <a.feberwee@gddiergezondheid.nl>

The virulence of two recent *Mycoplasma synoviae* (MS) field isolates was investigated in SPF (EXP.1) and commercial broilers (EXP.2). Both experiments consisted of two groups that were intratracheally (i.t.) inoculated at day 5 (D5) with an MS joint strain or MS airsac strain respectively and an i.t. sham inoculated group (NC). EXP.2 was expanded with an MS joint and MS airsac group which were vaccinated at hatch with live IB H120 vaccine (MS joint or MS airsac + H120) and an IB H120 and i.t. sham inoculated group (IBH120).Each group consisted of 15 birds. Mortality, clinical signs and growth were evaluated during 35 days. At D35 post mortem was performed including airsac lesion (ASL) scores. On air sacs of SPF birds histological examination (HE) and an MS in situ hybridization (ISH) test was performed. In EXP.1+2 all MS inoculated groups showed mild respiratory signs, mortality was low. Mean ASL scores were significantly higher for MS joint and MS air sac compared to NC and IBH120. In EXP.2 the mean ASL score in MS airsac + H120 was significantly higher compared to MS joint + IBH120. Significant reduced growth was observed in (i) EXP.1 for both MS inoculated groups compared to NC (ii), and in EXP.2 for both MS + IBH120 inoculated groups compared to IBH120. Hydrops ascites was observed in all MS inoculated groups. In EXP.1 a significant higher percentage of birds developed hydrops ascites in the MS air sac group, compared to the NC. Histologically, animals had a mild tracheitis and airsacculitis, whereas ISH demonstrated presence of MS bacteria on trachea epithelium and in macrophages within the exudate. It can be concluded that the two recent MS isolates induce airsac lesions and reduce growth in broilers. The effect on airsac lesions was increased by a mild IB vaccine strain. MS bacteria were demonstrated by ISH staining in the lesions. Hydrops ascites by MS is a new finding. Parametric or non parametrics tests were appropriate, including multiple testing issues.

O-69 Harry Morton Student Award Candidate

Interactions of *Mycoplasma penetrans* with urethral epithelial tissue culture cells

Elyse M. Levenda (1), Mitchell F. Balish (1)

(1) Miami University, USA

Elyse M. Levenda <levendem@miamioh.edu>

Mycoplasma penetrans, previously only found in individuals with chronic immunodeficiencies, has recently been found as the predominant microbe in the urethras of some immunocompetent males with idiopathic urethritis. From these patients four new strains, including U4, were isolated, all containing a 23S rRNA mutation conferring macrolide resistance. Numerous aspects of *M. penetrans* infection and its invasion of the human urethra are unknown. HeLa cells, derived from a cervical tumor, have been widely used to study *M. penetrans*. Unfortunately, rapidly proliferating HeLa cells have significant differences from slow-growing epithelial cells, making it unlikely that they model a physiologically relevant response to an *M. penetrans* infection, leaving a need to establish a more representative tissue culture model. Our goal is to develop a model for *M. penetrans* using PURL cells, immortalized keratinocytes derived from a male urethra. To begin to develop this model we used a combination of confocal, light, and immunofluorescence microscopy to monitor and characterize the infection of PURL cells with *M. penetrans* strain U4. Light microscopy was used to investigate damage to PURL cells by *M. penetrans*. Cell-free spent media was used to test whether contact is necessary for this damage or whether secreted factors are sufficient. Scanning electron microscopy and immunofluorescence microscopy were used to test for biofilm tower formation by *M. penetrans in vitro*, which was used to inform studies of interactions with host cells. Gentamicin protection assays and confocal microscopy were used to evaluate the invasion of PURL cells by *M. penetrans*. Microscopy indicated that *M. penetrans* does not form biofilm towers and loses adherence to glass after a few days *in vitro*. However, the bacteria were found to cause severe, contact-dependent damage to PURL cells in a dose-dependent manner. Invasion of PURL cells by *M. penetrans* appeared to be infrequent, unlike for HeLa cells, and typically was observed only after 48 hours post-infection. Overall, the infection of PURL cells by *M. penetrans* differed from the infection of HeLa cells by *M. penetrans* substantially. These results suggest that PURL cells will be able to give us greater insights into *M. penetrans* infection associated with urethritis.

O-71 Harry Morton Student Award Candidate

The invertebrate *Galleria mellonella* confirms differences in virulence potential between *Mycoplasma alligatoris* and *Mycoplasma crocodyli*, two closely related Crocodilian pathogens

Alexandra Burne (1) Daniel Brown (1), Dina Michaels (1), Mary Brown (1)

(1) University of Florida, USA

Alexandra Burne <burnea@ufl.edu>

The highly virulent reptilian pathogen, *Mycoplasma alligatoris*, was first isolated from an epizootic of farmed American alligators that resulted in over 80% mortality of the population. Experimental infections of the natural host revealed *M. alligatoris* preferentially colonizes extrapulmonary sites with a predilection for blood, likely contributing to the multisystemic clinical presentation in alligators. *Mycoplasma crocodyli* was isolated during an outbreak of polyarthritis in farmed Nile crocodiles in Zambia a year prior to the original isolation of *M. alligatoris* in Florida. Experimental infections of *M. alligatoris* in crocodilians outside of the natural host suggest fulminant disease is restricted to members of the Alligatoridae family. While *M. crocodyli* is predicted to be less virulent in general, no experimental infection outside of the natural host has been done to confirm this. Previously, we validated the invertebrate *Galleria mellonella* as an alternative model to virulence testing of *M. alligatoris*. To confirm the pathogenic potential irrespective of host, we conducted a dose response of *M. crocodyli* and *M. alligatoris* in the *G. mellonella* model. Additionally, previous genomic comparisons of these mycoplasmas showed that *M. alligatoris* contains an extracellular sialidase not present in *M. crocodyli*. *In vitro* studies have confirmed that sialidase is a virulence effector of *M. alligatoris*, likely contributing to the invasiveness of the pathogen. We confirmed in the *G. mellonella in vivo* model system that mutants of *M. alligatoris* lacking extracellular sialidase activity (*nanI*) or an essential enzyme in sialic acid catabolism (*nanE*) result in a marked decrease in virulence, confirming earlier *in vitro* studies that sialidase is a potent virulence effector of *M. alligatoris*.

Poster Presentations

Animal Mollicutes

P-01

A pathogen-specific T-cell response is induced after *Mycoplasma hyorhinis* lung infection

Moritz Bünger (1), Melissa Stas (2), Armin Saalmüller (3), Joachim Spergser (3), Andrea Ladinig (2)

(1) Clinical Centre for population medicine in fish, pigs and poultry, University of Veterinary Medicine Vienna, Austria
(2) Clinical Centre for population medicine in fish, pigs and poultry, University of Veterinary Medicine Vienna, Austria
(3) Centre of Pathobiology, University of Veterinary Medicine Vienna, Austria

Background: Mycoplasma hyorhinis is a pathobiont that resides in the upper airways of swine which mainly causes polyserositis and polyarthritis. It has also been linked to lung lesions similar to those caused by M. hyopneumoniae even though its role in the pathogenesis of lung lesions is controversially discussed. Little is known about the host-pathogen interaction of *M. hyorhinis*. **Material and Methods**: PBMCs were isolated from a 6-week old piglet originating from a farm with a history of putatively *M. hyorhinis* associated pneumonia. The *M. hyorhinis* infection of this piglet was limited to the respiratory tract as it was only showing mild pneumonia and no signs of other *M. hyorhinis* associated diseases; *M. hyorhinis* was successfully isolated from the lungs, but not from serosal cavities, meninges, or synovia. CellTrace Violet-labeled PBMCs were incubated with either the heat-inactivated bacteria or its fractioned proteins (hydrophilic, amphiphilic, and insoluble) for 96 hours. In order to identify the phenotype and activation status of cytokine producing cells, intracellular cytokine staining was performed after re-stimulation with heat-inactivated bacteria for 18 hours. The proliferative response of major T cell subsets and the cytokine production was investigated via flow cytometry. **Results**: Our results indicate that the major T cell subsets display distinct proliferative responses following in vitro restimulation. CD4 and γδ T cells responded to most of the antigen preparations whereas the CD8 T cells did not proliferate to any of them. In CD4 cells, production of IFNg, TFNa and IL17A were also detected. **Conclusion**: Apparently, a T cell response was induced by an *M. hyorhinis* infection of the lungs. In PBMCs, CD4 T cells seem to be the main responders which also release the cytokines IFNg, TFNa and IL17A. As a next step, we will characterize and compare the T cell response as well as the humoral immune response following nasal colonization, lung infections and systemic infections in a field trial.

P-02

Comparative genome analysis of *Mycoplasma cavipharyngis*, an apathogenic mycoplasma related to the pathogenic cluster of hemotrophic mycoplasma

Janina Kramer (1), Bruno Huettel (2), Christina Zübert (3), Ludwig Hoelzle (1), Michael Kube (3)

(1) Department of Livestock Infectiology and Environmental Hygiene, University of Hohenheim, Stuttgart, Germany
(2) Max Planck Genome Center, Cologne, Germany
(3) Department of Integrative Infection Biology Crops-Livestock, University of Hohenheim, Stuttgart, Germany

Mycoplasma cavipharyngis, originally isolated from the nasopharynx of guinea pigs, is a cultivable, glucose-fermenting mycoplasma exhibiting a genetic similarity of 96.5% to *Mycoplasmoides fastidiosum*. Phylogenetic analysis based on 16S rRNA sequences identified *M. cavipharyngis* and *M. fastidiosum* as forming a distinct cluster within the pneumoniae group of mollicutes. This so-called *M. fastidiosum* cluster forms a sister lineage to the cluster of hemotrophic mycoplasma (HM; hemoplasma), thereby representing the closest relatives to HM. While HM are highly specialized, to date uncultivable bacteria causing infectious hemolytic anemia in a wide range of mammalian worldwide, *M. cavipharyngis* and *M. fastidiosum* are generally considered as non-pathogenic species with an available *in-vitro* cultivation system. This study presents the first complete genome of *M. cavipharyngis* strain 117C. Further, metabolic functions and the origin of apathogenicity were determined using comparative genome analysis. Genome sequencing of *M. cavipharyngis* was conducted using a long-read Pacific Biosciences single-molecule real-time sequencing technology. Genome assembly was achieved using Canu v2.2 and automatically annotated with RAST, followed by a manual curation. Initial genome comparisons were performed by a pan-genome analysis to identify unique and shared features for the complete genomes of *M. cavipharyngis*, *M. fastidiosum* and all available complete HM genomes. The circular genome of *M. cavipharyngis* comprised over 1034 kb, with an rRNA organization in one contiguous operon and a complete set of tRNAs. Among approximately 800 coding sequences, more than 30% represented proteins with unknown function. First results of comparative genome analysis revealed differences between the genomes of *M. cavipharyngis* and *M. fastidiosum*, as well as the HM cluster, particularly in the encoded carbohydrate metabolism, in several transport systems such as PTS, and in the presence of some putative membrane and secretory proteins contributing to the bacterial secretome. In contrast, the majority of the unique protein-coding sequences found in HM encode hypothetical proteins, which may also encode for unknown virulence factors. This first complete genome of *M. cavipharyngis* and its functional reconstruction may contribute to further decipher the mechanisms of hemoplasmas pathogenicity and provide insight into the genomic features underlying their lack of *in-vitro* cultivation.

P-03

Assessing the effectiveness of disinfectants against agents of contagious agalactia contained in organic matrices

Anne Ridley (1), Issa Muraina (1)

(1) Animal and Plant Health Agency, UK

The recovery of *Mycoplasma agalactiae* in faeces of infected small ruminants and its environmental survival in biological matrices has been demonstrated previously. The present concern is whether this organism, or other agents of contagious agalactia, when present in organic matrices may have reduced susceptibility to commonly used farm disinfectants. *Mycoplasma agalactiae* strain 112SR14, isolated in GB from an imported goat, and *M. mycoides* subsp *capri* strain F30 were used to spike a sterile suspension of sheep faeces (10%) and, independently, 5% dry yeast, at between 1 × 106 and 1 × 109 CFU/ml. The spiked matrices were exposed for 1 minute and 30 minutes to three disinfectants belonging to different classes (potassium peroxymonosulphate, chlorocresol and gluteraldyde/QAC). Each disinfectant was tested in triplicate over two experiments at concentrations between 4x and 0.004x Defra General Orders (GO) concentrations, diluted in WHO Standard Hard Water in a 96 micro-well plate. All three disinfectants were effective, irrespective of the interfering matrix, at Defra GO concentrations. However, the chlorocresol product was consistently efficacious at more dilute concentrations for both matrices and exposure times, while the potassium peroxymonosulphate product was markedly more effective at the longer exposure time. The interfering soiling material reduced the effectiveness of disinfectants, compared with a saline control, at least in this micro-scale laboratory suspension model. A disinfectant suspension "foot-dip" model sterilised sheep faeces was added to 2% at different GO concentrations disinfectant at day 0, 1, 2, 3, 5,(7) before efficacy testing. Disinfectant samples tested for efficacy after 1- and 30-minute contact times in quintuplicate on each test day. The disinfectant was stored at 4oC. After seven days, despite storage at 4o+/- 1oC) of simulated boot-dip usage the test glutaraldehyde/QAC product remained effective, even at 1 minute contact time. further variables, including investigation of different strains, temperature and disinfectant products will be tested in a laboratory setting before assessing the impact on disinfectant efficacy of the more variable external environment afforded by a farm setting. emphasized text.

P-04

Comparison of DNA extraction procedures for detection of *Mycoplasma bovis* directly from extended bovine semen straw samples using a commercial *M. bovis* PCR

Emma Taylor (1), Alannah Deeney (1), Anne Ridley (1), Georgia Mayne (1)

(1) Animal and Plant Health Agency, UK

Mycoplasma bovis is a global pathogen of cattle but was only detected for the first time in New Zealand in 2017, triggering a response under their Biosecurity Act as an "unwanted organism". Consequently, the Ministry of Primary Industries (MPI) now require all bovine semen destined for export to New Zealand to be screened with an *M. bovis*-specific real-time PCR (rtPCR) compliant with amended import health standard (IHS) test requirements aimed at preventing the accidental importation of *M. bovis*. The standard stipulates that semen samples cannot be centrifuged prior to DNA extraction. To comply with these strict requirements, one of the listed tests, was validated together with different DNA preparation steps and compared with existing in-house procedures. DNA was extracted from semen straws using the current in-house semi-automated platform procedures for processing culture, tissue and body fluid sample submissions and was compared with the stipulated test requirements. DNA from centrifuged and unspun semen samples spiked with *M. bovis* were also compared.The rtPCR had a sensitivity and specificity of 100% (95% confidence interval = 79.41% to 100% and 78.20% to 100%, respectively) when testing DNA from other *Mycoplasma* species or bovine semen spiked with the latter with a high level of repeatability for within- and between- run replicates. The consistent limit of detection was 0.001 pg/µl *M. bovis* DNA and between 5.3 x 102 to 7.5 x 102 CFU/ml *M. bovis* when artificially spiked in semen. DNA extracted using the KingFisher Flex was detected with lower Ct values than the Maxwell RSC, but the comparable improvements in sensitivity was mainly associated with non-centrifuged samples ($p < 0.001$). None of the procedures tested impeded the detection sensitivity of *M. bovis* in the presence of competitor organisms *Acholeplasma laidlawii*, *Mycoplasma bovigenitalium* and *Ureaplasma diversum*, confirming *M. bovis* specificity of the polC target. Under the experimental conditions applied this rtPCR test efficiently detected *M. bovis* in extended bovine semen straw samples from DNA extracted using both semi-automated extraction platforms, irrespective of prior centrifugation of extended semen.

P-05

Elucidation of the surfaceomes of *Mycoplasma bovis* isolates from australian feedlot cattle

Veronica Jarocki, Darren Trott (1), Kiro Petrovski (1), Mauida Al Khallawi (1), Steven Djordjevic (2)

(1) University of Adelaide, Australia
(2) University of Technology Sydney, Australia

Bovine respiratory disease (BRD) is a leading cause of morbidity and mortality in cattle worldwide, imposing significant economic burdens on the livestock industry. *Mycoplasma bovis* is recognised as a primary pathogen contributing to the complexity and severity of BRD. Current vaccines have limited efficacy against *M. bovis*, partly due to the pathogen's genetic diversity and ability to evade the host immune response. Thus, there is an urgent need for more effective vaccines that can provide broad protection against diverse *M. bovis* strains. This study aims to address this gap by characterising the surfaceome of *M. bovis* isolates, facilitating the identification of potential targets for novel vaccine strategies. Specifically, this work involved trypsin surface protein shaving and cell surface biotinylation, followed by mass spectrometry and bioinformatics analysis to characterise of the surfaceome of four *M. bovis* isolates obtained from Australian feedlot cattle afflicted with BRD. These isolates were selected following an extensive phylogenomic analysis of 46 *M. bovis* isolates and included two strains representing clonal clusters and two outlier strains. Our findings included the identification of 137, 138, 57, and 116 surface proteins for the isolates from South Australia, Queensland and two from New South Wales, respectively. All isolates shared 46 surface proteins, encompassing several moonlighting proteins (i.e., proteins with well-defined canonical functions in the bacterial cytoplasm), as well as other proteins involved in adhesion, invasion, immune evasion, and modulation of host cell signalling. Among these, nine were previously characterised *M. bovis* virulence factors. The comparative analysis sheds light on a core subset of proteins shared across isolates, alongside the discovery of unique proteins in individual strains, hinting at the possibility of strain-specific immune responses. Continued exploration of these surface proteins' functions could markedly advance our capabilities in designing effective vaccines against *M. bovis*, thus improving the management of bovine respiratory disease.

P-06

In vivo intravaginal application of probiotic *Lactobacillus* spp. in sheep: influence on the presence of *Mycoplasma* spp. in the vaginal microbiota

Marion Toquet (1), Jesús Gomis (1), Estrella Jiménez-Trigos (1), Esther Bataller (1), Marta Barba (2), Antonio Sánchez (3), Pedro González Torres (1,4), Ángel Gómez Martín (1)

(1) Microbiological Agents Associated with Animal Reproduction (ProVaginBIO) Research Group, Departamento Producción y Sanidad Animal, Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, CEU Universities, Carrer Tirant lo Blanc, 7, 46115 Alfara del Patriarca, Valencia, Spain
(3) Agrifood Research and Technology Centre of Aragon (CITA), Calle Corinto nº3, 44159, Teruel, Spain
(4) Ruminant Health Research Group, Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de Murcia, Campus de Espinardo, 30100 Murcia, Spain
(5) Microomics Systems S.L., Barcelona, Spain

Antimicrobial effects against *Mycoplasma (M.) bovis* and *M. agalactiae* of an inoculum (L2) made from *Lactobacillus* spp. have recently been reported in vitro. Therefore, the aim of this study was to apply intravaginally L2 in ewes and monitor the effects on the microbiota. A double dose of L2 was inoculated in 60 ewes (P group) from two dairy commercial ovine flocks. A group of sheep remained in each flock without inoculation as a control group (C group, n = 60). The first inoculation occurred just before the intravaginal sponge insertion (T0) while the second dose was inoculated 14 days after just after the sponge removal (T1). The pregnancy was diagnosed by ultrasound around day 50 after artificial insemination (T2). At each sampling time point, vaginal swabs were taken for 16S rRNA gene metabarcoding. Globally, the genus Ureaplasma was one of the most abundant especially during the pregnancy at T2. In the first flock, Lactobacilalles genera *Aerococcus* and *Streptococcus*, showed a significant decrease at T1 ($P < 0.05$) and increase at T2 in the P group ($P < 0.001$ and $P < 0.01$ respectively). Meanwhile in the C group, a significant increase of *Mycoplasma* spp. ($P < 0.001$) was observed at T2. Thus, the abundance of *Mycoplasma* was significantly greater in the C group than in the P group at T2 ($P < 0.001$) while the abundance of *Aerococcus* was significantly greater in the P group ($P < 0.05$). In the second flock, there was a significant increase in both groups of Lactobacillales genera *Aerococcus* ($P < 0.05$), *Alloiococcus* ($P < 0.01$) and *Streptococcus* ($P < 0.01$) at T2. There was also a significant increase in the C group of *Ureaplasma* ($P < 0.05$). Additionally, the conception rate was 10.7% higher in the P group of the first flock, while the conception rate (over 80%) in the second flock was the same. Our results suggest that the intravaginal use of probiotics could modulate the vaginal microbiota by preventing the proliferation of *Mycoplasma* spp. and promoting the presence of Lactobacillales genera, which could lead eventually to improvements in fertility in sheep.

P-07

Description and comparison of the microbiota of raw milk in healthy and subclinical mastitis-affected small ruminants: presence of *Mycoplasmataceae*

Marion Toquet (1), Jesús Gomis (1), Esther Bataller (1), Estrella Jiménez-Trigos (1), Nuria Mach (2), Xavier Nouvel (2), Eric Baranowski (2), Ángel Gómez Martín (1)

(1) Microbiological Agents Associated with Animal Reproduction (ProVaginBIO) Research Group, Departamento Producción y Sanidad Animal, Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, CEU Universities, Valencia, Spain
(2) IHAP, Université de Toulouse, INRAE, ENVT, Toulouse, France

Microbiome studies have demonstrated the complex interplay of factors in diseases, including how pathogens and commensal microbes interact. Nevertheless, there is a lack of studies about the milk microbiota in mastitis-affected small ruminants, notably goats, and the role of mycoplasmas. Therefore, this study aimed to characterize and compare the microbiota of healthy and mastitis milk samples to get insights into the bacterial ecology surrounding mastitis in caprine and ovine animals. A total of 104 small ruminants from 13 flocks were sampled: four healthy animals and four animals with subclinical mastitis by flock. The milk samples were analysed using 16S rRNA gene metabarcoding. Overall, the animal species (sheep/goat) did not significantly affect the microbiome diversity. However, the health status of the samples (healthy/mastitis) had a notable impact. Healthy samples showed significantly greater microbial richness than mastitis samples ($P < 0.01$). The microbial evenness was significantly higher ($P < 0.001$) in healthy goat samples than in those with mastitis. Regarding the β diversity, there were significant differences in the bacterial community structure between healthy and mastitis samples ($P < 0.01$). The dominant phyla across all samples were Proteobacteria, followed by Firmicutes, Actinobacteria, Bacteroidota, and Fusobacteria. In healthy milk samples, the most prevalent genera were *Sphingomonas, Pseudomonas*, unknown *Caulobacteraceae* genus, *Curvibacter*, and *Staphylococcus*. The most abundant genera in mastitis samples were *Sphingomonas*, *Staphylococcus, Pseudomonas, Streptococcus*, and *Mycoplasma*. Conversely, the subclinical mastitis samples predominantly included *Sphingomonas* sp., *Staphylococcus (S.)* sp., *S. equorum*, *S. muscae*, *S. simulans*, *Pseudomonas* sp., *Mycoplasma (M.). agalactiae*, *Streptococcus* sp., *Streptococcus ruminantium*, and *Serratia* sp. Some of these microorganisms were solely responsible for the mastitis, such as *M. agalactiae*, which represented over 98% of the microbiota in two animals affected by subclinical mastitis, while in other samples, various predominated. Additional members of *Mycoplasmataceae* included *M. bovigenitalium*, *M. ovis*, and *Ureaplasma* sp., with the latter being the most frequent, while the others appeared sporadically. This work was supported by the Generalitat Valenciana (Spain) [GVA/2020/026] and the Spanish Ministry of Science and Innovation [PID2020-119462RA-I00/AEI/10.13039/501100011033]. Ángel Gómez-Martín is supported by a “Ramón y Cajal” contract of the Spanish Ministry of Science and Innovation [RYC2021-032245-I].

P-08

Presence of *Mycoplasma bovis* subtype ST-1 with a variable susceptibility to fluoroquinolones in Spain

Juan Carlos Corrales (1), Antonio Sánchez (1), Xóchil Pérez (1), Joaquín Amores (1), Antón Esnal (1), Patricia Panales (1), Isabel Pérez (1), Christian de la Fe (1)

(1) Grupo de Investigación Sanidad de Rumiantes, Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain

Mycoplasma bovis (Mb) is one of the most important infectious agents involved in the bovine respiratory complex. The endemic presence in Spain of the ST-2 and ST-3 subtypes, with phenotypic differences linked to their susceptibility to fluoroquinolones, from the samples analyzed between 2016 and 2022, paved the way to develop control strategies focused on the use of targeted therapies prior diagnosis of the subtype involved. Surprisingly, microbiological studies carried out in 2023 showed for the first time the presence of Spanish isolates of *M. bovis* classified in another subtype (ST-1), recovered from calves with respiratory symptoms in different areas of the country. For the development of minimum inhibitory concentration (MIC) assays with *M. bovis* isolates, a number of antimicrobials commonly used against mycoplasmosis were selected, including several macrolides, quinolones, tetracyclines, a pleuromutilin and a lincosamide. All the *M. bovis* isolates belonging to ST-1 (n = 16) were sequenced to compare nucleotide changes in the QRDR region of 4 genes (gyrA, gyrB, parC and parE). The MIC results obtained has revealed minimal antimicrobial susceptibility against macrolides, lincomycin or tetracyclines but important phenotypic differences when using fluoroquinolones (FLQ), without any geographical correlation between the MIC profiles, not even for a set of isolates recovered at the same time in the same herd. Genetic sequencing showed the presence of cumulative mutations in 2 isolates with high MICs to FLQ. In conclusion, our study revealed the extended circulation of a third polC ST biotype of *M. bovis* in Spain. Circulating isolates are now divided in to three groups, ST-1 to ST-3, all being resistant to macrolides, lincosamides. Most ST-3 isolates circulating in Spain are resistant to FLQ, while ST-2 remained resistant and ST-1 susceptibility seems to be variable. The presence of this subtype complicates the diagnostic control options proposed for this microorganism.

P-09

Determining antibiotic susceptibility of *Mycoplasma hyorhinis* circulating in Spain

Juan Carlos Corrales (1), Xóchil Hernández (1), Antonio Sánchez (1), Joaquín Amores (1), Isabel Pérez (1), Christian de la Fe (1)

(1) Grupo de Investigación Sanidad de Rumiantes, Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain

Mycoplasma (M.) *hyorhinis* is an infectious agent of swine. It colonizes ciliated cells of the upper respiratory cells and most of the hosts remain asymptomatic. However, it has been associated with severe respiratory symptoms (such as pneumonia), and systematic clinical signs such as arthritis, conjunctivitis, polyseritis and abortion. The increase of antimicrobial resistances detected in other mycoplasma species causing veterinary diseases, leads us to question the situation in this agent. Thus, the minimum inhibitory concentration (MIC) against 13 antimicrobials was studied in a set of 20 isolates of *M. hyorhinis* collected from different regions of Spain. Quinolones, macrolides, tetracyclines, valnemuline and lincomycin were included in the study. Furthermore, we performed an MLST analysis in order to detect possible geographical correlation with the circulating isolates. Quinolones exhibit the lowest range of effectiveness (0.0625-8 µg/ml) and marbofloxacin showed the lowest MIC 90 (2µg/ ml). Tetracyclines showed a range of 0.125-8 mg/ml, but doxycycline demonstrated a MIC 90 of 0.5 µg/ml, lower than the lowest quinolone. All macrolides tested, showed a higher MIC, with intervals of effectiveness of 0.125->16 µg/ml, and MIC 90 of 16 µg/ml in all of them. Finally, valnemuline demonstrated to be very effective, with a range of 0.0312-4 µg/ml and a MIC 90 of 0.5 µg/ml, meanwhile lincomycin resulted ineffective (CMI90 16 µg/ml). The MLST performed included the genes gltX, dnaA, rpoB, gyrB and adk. No geographical association or association with antibiotic effectiveness was established using this genetic approach. However, we were able to detect 4 different groups of strains circulating in Spain.

P-10

Evolution of antimicrobial susceptibility of *Mycoplasma agalactiae* and *Mycoplasma mycoides* subsp. *capri* in Spanish isolates collected between 2020 and 2023

Joaquín Amores (1), Xóchil Hernández (1), Antonio Sánchez (1), Juan Carlos Corrales (1), Isabel Pérez (1), Christian de la Fe (1)

(1) Grupo de Investigación Sanidad de Rumiantes, Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain

Contagious agalactia (CA) is a WHOA notifiable disease affecting small ruminants. It may present as an epidemic form, affecting a high percentage of the animals in the flock, decreasing milk production and causing arthritis, conjunctivitis, pneumonia and abortion. In endemic areas, it is presented as a chronic disease with continuous clinical and/or subclinical mastitis and punctual other symptoms, with a high economic impact. Since vaccines do not result fully effective, most of the control measures are focused on the detection of positive animals and culling or the use of antibiotics. Considering the endemic situation of Spain, we performed a study to determinate the antimicrobial effectiveness on M. agalactiae and *Mycoplasma mycoides* subsp. *capri* (Mmc), the most prevalent CA-causal agents, using strains collected between 2020 and 2023. Finally, the study included 100 isolates of M. agalactiae and 71 isolates of Mmc recovered in this period. Results obtained for M. agalactiae showed an important increase in quinolones effectiveness comparing the period 2020-2021 with the period 2022-2023 where the range for enrofloxacyn, marbofloxacyn or danofloxacyn decreased, and the MIC90 decreased from 2 µg/ml to1 µg/ml and 4 µg/ml to 1 µg/ml, respectively. For doxiciclyne and oxitetracycline we observed the same situation, with a reduction in both MIC range and MIC90 from 4 and 2 µg/ml to 0.5 and 1 µg/ml, respectively. The same situation was registered for Mmc where both MIC ranges and MIC 90 were lower for the quinolones and tetracyclines studied. Considering those results, we decided to study the quinolone resistant determine region (QRDR) of both species in isolates collected in 2023. Only one mutation was found in the gene parC of M. agalactiae involving an aminoacidic change (Thr80Ile) located in 2 different resistant isolates, a mutation previously described. No QRDR mutation was detected in Mmc isolates. In conclusion, The results show a reduction in the MIC of M. agalactiae and Mmc to certain antimicrobials, including quinolones, which may be related to their more consistent use on the affected farms.

P-11

Genetic Analysis of *Mycoplasma gallisepticum* Isolates – Implications for Epidemiology

Naola Ferguson-Noel (1), Marianne Dos Santos (1)

Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA

Avian Mycoplasma spp. are pathogens that continue to result in significant morbidity and mortality in poultry and considerable economic loss to the global industry. *Mycoplasma gallisepticum* and *Mycoplasma synoviae* are members of a select group of poultry pathogens that are highly monitored nationally and internationally on a continuous basis to ensure adequate control of the disease. Continuing advances in the high throughput sequencing technology and with the growing number of bacterial genomes in the public domain, predictions can be made about how an organism behaves from its gene sequences. In this research several M. gallisepticum isolates were sequenced and analyzed in order to identify genetic differences and epidemiological patterns over time. The isolates consisted of vaccine-like isolates as well as "wild-type" field strains isolated from commercial and non-commercial (backyard/pet) chickens, turkeys, and wild birds across the United States from 1984 – 2024. Publicly available genomes were also included in comparisons. Whole genome sequencing was performed using Illumina and the collective contigs for each strain were annotated using fully annotated Mycoplasma reference genomes. In addition, DNA sequences of portions of the mgc2 gene and the16S-23S rRNA intergenic spacer region (IGSR) from these isolates were compared. The analyses revealed a wide spectrum of genetic differences among the isolates and using phylogenetic analysis, the isolates could be divided into several clades the major ones including vaccine and vaccine-like isolates and a house-finch isolate group. Aside from this, most of the M. gallisepticum could not be resolved into distinct clades. Several potential virulence factors were identified among the genomes, M. gallisepticum isolates vary widely in their relative pathogenicity and several factors including the host can influence the degree of virulence observed. More research is necessary to clarify the likely role of these mutations in different host species.

P-12

Virulence of *Mycoplasma tullyi* using the *Galleria mellonella* infection model

Alexandra Burne (1), Brittany Whitcher (1), Dina Michaels (1), Mary Brown (1)

(1) University of Florida, USA

Mycoplasmas are important bacterial pathogens of both veterinary and human medicine, causing acute and chronic infections in a wide range of hosts. Several new *Mycoplasma* spp. have been discovered in wildlife hosts, creating a need for determining their pathogenic potential. Experimental infection of the natural host is often not feasible; therefore, an alternative model is needed. *Galleria mellonella*, the greater wax moth, is a well-defined invertebrate model system that has successfully confirmed the pathogenic potential of a number of bacterial and fungal species. The aim of this study was to determine the pathogenic potential of *Mycoplasma tullyi*, a putative pathogen of penguins, using *G. mellonella* as an alternative host system. G. mellonella larvae (n=24) were injected with a low and high dose of three strains of *M. tullyi* and monitored for 28 days for successful pupation and emergence, as well as mortality events. *M. tullyi* infection was confirmed by culture of hemolymph extracted from larvae. Here, we report that *M. tullyi* isolates are pathogenic in the *G. mellonella* model, suggesting that they may also have pathogenic potential in penguins. This study supports that the *G. mellonella* model is a significant tool to determine the pathogenic potential of new *Mycoplasma* spp. in wildlife, providing new knowledge for wildlife conservation in both wild and captive animals.

P-13

Outbreak of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* in captive peregrine falcons

Rubén S Rosales (1), Ana S Ramírez (1), Ruth Blanco (2), Pedro Salinas-Casas (1), José B Poveda (1)

(1) Instituto Universitario de Sanidad Animal y Seguridad Alimentaria, University of Las Palmas de Gran Canaria, Spain
(2) APB Immunological SL

Various mollicutes species are often found in birds of prey, though their exact impact on clinical disease remains unclear. Species like *Mycoplasma buteonis*, *M. falconis*, *M. gypis*, *M. corogypsi*, *M. neophronis*, and *M. seminis* are commonly detected in these birds. Among the most clinically relevant avian mycoplasmas, *M. gallisepticum* and *M. synoviae*, only the previous one has been isolated from a peregrine falcon (*Falco peregrinus*), while limited serological evidence for both avian pathogens have been found in prairie falcons (*Falco mexicanus*). The present study reports an outbreak involving both of these pathogenic species in captive-bred peregrine falcons in spring 2023 in Spain. Thirteen three-week-old chicks from a breeding nucleus displayed chronic mycoplasma respiratory disease symptoms, ultimately dying despite initial tylosin treatment. Swabs from seven falcons at the same centre were submitted to our laboratory and processed in APB® broth for isolation. *M. synoviae* was detected in all samples via culture and qPCR, while three of the samples also showed *M. gallisepticum* presence. Antimicrobial susceptibility testing indicated a high susceptibility to tylvalosin (<0.06 µg/ml) and tulathromycin (0.016 µg/ml) for both mycoplasmas. Intramuscular administration of tulathromycin (0.025 ml/kg) weekly for four weeks led to clearance of both mycoplasmas from the falcons. Post-treatment, fertility improved, resulting in seven fertile eggs and four healthy chicks up to date. Based on the anamnesis performed, the origin of the outbreak can be clearly linked to *M. gallisepticum*/ *M. synoviae*-contaminated animals (day-old chicks and quails) used for the feeding of the falcons. Both mycoplasmas are commonly associated with reproductive disorders, reduced hatchability, embryonic development issues, and weak chicks in poultry, sometimes without evident symptoms in adults, similar to the clinical outcome observed in the falcons studied. This study provides insights into the clinical role of *M. synoviae* and *M. gallisepticum* in birds of prey, proving the ability of both mycoplasmas to induce some of the classical clinical signs observed in chickens in peregrine falcons, while also providing insights into a potential effective treatment strategy for this type of outbreaks.

Cell Biology and Metabolism

P-14

L-Asparagine is the essential factor for the susceptibility of chinese local pigs to *Mycoplasma hyopneumoniae*

Xing Xie (1), Long Zhao (1,2), Fei Hao (1), Lulu Xu (1, 2), Qiyan Xiong (1), Yanna Wei (1), Lei Zhang (1), Rong Chen (1), Yanfei Yu (1), Yun Bai (1), Yuan Gan (1), Yongjie Liu (2), Guoqing Shao (1), Zhixin Feng (1)

(1) Key Laboratory for Veterinary Bio-Product Engineering, Ministry of Agriculture and Rural Affairs, Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences, Nanjing, China
(2) Joint International Research Laboratory of Animal Health and Food Safety, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China

Background: Swine Mycoplasma pneumonia caused by Mycoplasma hyopneumoniae (Mhp) is the most prevalent and frequently occurring chronic respiratory disease in pigs worldwide. Local pig breeds in China are more susceptible to Mhp, and understanding the reasons for their susceptibility is crucial for the prevention and control of swine mycoplasma pneumonia, as well as for promoting the healthy development of the swine industry. **Methods**: Growth speed, titres and pathogenic biological characterisitics of different Mhp strains cultured in the serum medium from different Chinese local pig breeds and introduced pig breeds were compared. combined with the pathogenicity of highly virulent Mhp strain, which were respectively challenged to infect introduced pig breeds and Chinese local pig breeds, the most susceptible Chinese local pig breeds and the least susceptible introduced pig breeds to Mycoplasma hyopneumoniae were determined. Non-targeted metabolomics analysis of serum samples from two representative pig breeds were performed. **Results**: Regardless of the highly or low virulent strains, growth rate, titres, cytotoxicity and adhesion ability to immortalized pig bronchial epithelial cells of Mhp strains cultured with Chinese local pig serum medium are significantly stronger than those strains cultured in introduced pig serum medium. Expression level of L-Asparagine in Bama miniature pigs was 15-18 times higher than in Large white pigs. Additionally, the contents of inositol and Arachidonic acid in Bama miniature pigs were significantly upregulated as well. A certain concentration of L-Asparagine significantly increased the growth titer, metabolic capacity and pathogenicity of Mhp. **Conclusion**: Bama minature pig and Large white pig are representative pig breeds that are susceptible and non-susceptible to Mhp respectively. L-Asparagine is the essential factor and biomarker for the susceptibility of local pigs to Mhp.

Chemotherapy and Resistance

P-15

Antimicrobial susceptibility and genetic mechanisms of resistance of *Ureaplasma* isolates between 2012 and 2023 in the United States

Joshua Waites (1), Donna Crabb (1), Amy Ratliff (1), Melanie Fecanin (1), Ken Waites (1), Li Xiao (1)

(1) University of Alabama at Birmingham, Birmingham, AL USA

Ureaplasma species are implicated in nongonococcal urethritis, endometritis, chorioamnionitis, spontaneous abortion, arthritis, and urinary calculi infections in adults, and prematurity, low birth weight, bacteremia, meningitis, and lung infections in neonates. Macrolides, tetracyclines and fluoroquinolones are used in treating Ureaplasma infections. There are limited data about the antimicrobial susceptibility of Ureaplasma species in the US in the recent decades. We analyzed the antimicrobial susceptibility data of 415 Ureaplasma isolates from various sample types that were submitted to University of Alabama at Birmingham Diagnostic Mycoplasma Laboratory from different regions of the United States for routine antimicrobial susceptibility testing during 2012-2023 and investigated the genetic mechanisms of antimicrobial resistance. Results showed that the minimum inhibitory concentration (MIC) distribution for erythromycin, tetracycline, and levofloxacin were mainly bell-shaped with a range of 0.063-256, 0.016-64, and 0.063-32 mg/L, respectively. MIC50 for erythromycin, tetracycline, and levofloxacin were 2, 0.25 and 1 mg/L, respectively and the numbers for MIC90 were 4, 1, and 2 mg/L. According to the cutoffs of Clinical and Laboratory Standards Institute, there were 61 (14.7%) isolates resistant to one or more drugs, and resistance rates for erythromycin, tetracycline, and levofloxacin were 2.4% (10/415), 6.5% (27/413), and 6.7% (28/415), respectively. Four isolates (9.6%) were resistant to two drugs. Mutations in domain V of 23S rRNA, mainly A2058G (E.coli numbering), and/or in ribosomal protein L4 were identified in erythromycin-resistant isolates. Tet(M) was detected in all isolates with tetracycline MIC > 2 mg/L, while no known 16S rRNA mutations were found. For fluoroquinolone-resistant isolates, C248T (S83L) mutation in parC was identified in most isolates. In summary, erythromycin, tetracycline, and levofloxacin are still likely to be effective in treating most Ureaplasma infections in the United States, but cautions need to be made for tetracyclines as doxycycline is widely used for post-exposure prophylaxis for bacterial sexually transmitted infection prevention.

P-16

Development of molecular assays for the detection of antibiotic susceptibility in *Mycoplasma iowae*

Dominika Buni (1), Enikő Wehmann (1), Dorottya Földi (1), Krisztián Bányai (1), Krisztina Bali (1), Janet Bradbury (2), Marco Bottinelli (3), Salvatore Catania (3), Inna Lysnyansky (4), Miklós Gyuranecz (1), Zsuzsa Kreizinger (1)

(1) HUN-REN Veterinary Medical Research Institute, Hungary
(2) University of Liverpool, United Kingdom
(3) Istituto Zooprofilattico Sperimentale delle Venezie, Italy
(4) Kimron Veterinary Institute, Israel

Mycoplasma iowae is an economically important pathogen of turkey causing reduced hatchability, late embryo mortality and leg deformities, chondrodystrophy and skeletal lesions in poults. Currently no vaccine is available against this pathogen, thus appropriate antibiotic therapy is the only way to reduce economic losses. The quick and efficient determination of the antibiotic susceptibility profile of the pathogens is essential in choosing the appropriate treatment. The aim of the study was to develop molecular biological assays to determine the antibiotic susceptibility of *M. iowae* by identifying point mutations associated with elevated minimum inhibitory concentration (MIC) values. The whole genome sequences of 99 *M. iowae* strains were determined using Illumina and Oxford Nanopore platforms, and the MIC values were assessed using the broth micro-dilution method. Bimodal distribution of the MIC values was observed in the case of fluoroquinolones, macrolides and lincosamides which enabled further analyses in search for point mutations showing correlations with elevated MIC values. Mismatch amplification mutation assays (MAMAs) were designed for the differentiation of the point mutations in the corresponding regions. Antibiotic resistance-associated SNPs were identified in the gyrA, parC and 23S rRNA genes. In the gyrA gene a SNP at nucleotide position 279. (according to E. coli numbering) was identified, causing serine and phenylalanine exchange; while in the parC gene a SNP at nucleotide position 2021. resulting in isoleucine and serine change was associated with elevated MIC values. The MAMAs developed for the differentiation of these SNPs showed 10^3 template copy number/µl sensitivity. Although a resistance-associated mutation at nucleotide position 2059. in the 23S rRNA gene was also detected, all four nucleotides could be present at this position hindering the development of the molecular detection system. The developed MAMA methods are time efficient and cost-effective systems that can also be used on clinical samples to identify *M. iowae* strains with elevated MIC values in the case of fluoroquinolones. Therefore, the use of the designed molecular assays could support targeted antibiotic therapy in outbreaks.

P-17

Antimicrobial susceptibility profiles of *Mycoplasma hyosynoviae* strains isolated from swine across Europe between 2018 and 2023

Ulrich Klein (1), Dorottya Földi (2), Zsófia Eszter Nagy (2), Lilla Tóth (2), Nikolett Belecz (2), Karola Költő (2), Marianna Merenda (3), Salvatore Catania (3), Joachim Spergser (4), Philip Vyt (5), Ute Siesonop (6), Zsuzsa Kreizinger (2), Miklós Gyuranecz (2)

(1) Huvepharma NV, Belgium
(2) HUN-REN Veterinary Medical Research Institute, Hungary
(3) Istituto Zooprofilattico Sperimentale delle Venezie, Italy
(4) University of Veterinary Medicine Vienna, Austria
(5) Dialab, Belgium
(6) University of Veterinary Medicine Hannover, Germany

Mycoplasma hyosynoviae is a facultative pathogen bacterium present in pig farms world-wide. This bacterium colonizes the tonsils, is shed via nasal secretions and is transmitted through close contact. *M. hyosynoviae* causes arthritis in pigs mainly older than 10-weeks of age. Improvement of housing conditions and antibiotic therapy are the only options to alleviate the clinical signs and to reduce economic losses. The aim of this study was to determine minimal inhibitory concentrations (MIC) of antibiotics potentially used for the treatment of *M. hyosynoviae* infections in swine. One hundred and six *M. hyosynoviae* isolates (20 Austrian, 20 Belgian, 25 German, 21 Hungarian and 20 Italian) collected between 2018 and 2023 from various tissue samples were examined by broth micro-dilution tests for ten antimicrobial agents. The majority of the isolates originated from joints (n=39) or tonsils (n=41). The type strain (NCTC10167) was used as quality control. Low concentrations of six of the examined antibiotics (tiamulin ≤0.039 µg/ml, tylosin 0.5 µg/ml, tilmicosin 1 µg/ml, tylvalosin ≤0.039 µg/ml, lincomycin ≤0.25 µg/ml and doxycycline 0.312 µg/ml) inhibited the growth of the clinical isolates. Enrofloxacin, oxytetracycline and florfenicol inhibited the growth of the clinical isolates at moderate concentrations (enrofloxacin MIC90 0.625 µg/ml, oxytetracycline MIC90 2 µg/ml and florfenicol MIC90 2 µg/ml) and tulathromycin could inhibit these isolates at high concentrations only (MIC90 64 µg/ml). The MIC distributions for all tested antibiotics were monomodal, except for tulathromycin where the MIC values showed a bimodal right shift. The statistical analysis revealed significant differences in association with the countries of origin in case of enrofloxacin, where the Hungarian isolates showed the lowest MIC values and the German isolates the highest MIC values among the tested countries. The results show that the European *M. hyosynoviae* isolates are generally susceptible to the tested antibiotics with the exception of tulathromycin. The country specific differences indicate the importance of regular susceptibility testing of isolates on Pan-European level.

P-18

Antimycoplasmal activity of *Peganum Harmala* on *Mycoplasma hominis* tunisian strains

Boutheina Ben Abdelmoumen Mardassi (1), Béhija Mlik (1), Nadine Khadraoui (1), Imen Chniba (1), Nadia Fares (2), Olfa Tabbene (2), Rym Essid (2), Selim Jallouli (2)

(1) Institut Pasteur de Tunis, Tunisia
(2) Biotechnology Center, Borj Cedria Tunis, Tunisia

Mycoplasma hominis is an opportunistic pathogen that can cause various gynecological infections such cervicitis, infertility, and less frequently, extra-genital infections. Previous studies on the antimicrobial susceptibility of Mycoplasma hominis Tunisian strains have highlighted a significant resistance, even multi-resistance to the most used antibiotic in the therapy of consequential infections. To address this concern, the present study attempted to develop an alternative based on phytotherapy. Peganum harmala seed extract was tested as an antibacterial agent against multidrug-resistant M.hominis clinical strains. Peganum harmala plant was collected from the North West region of Tunisia, air-dried, grounded and extracted by different solvents.The crude methanolic extract was further partitioned with n-HEX, DCM, EtOAC and n-BuOl. Antibacterial activity was evaluated against M. hominis ATCC 23114 and 20 M. hominis clinical strains.The antimycoplasmal activity was tested by the microdilution method and the minimal inhibitory concentration (MIC) values were determined. Phytochemical analysis and hemolytic activity on human erythrocytes were also performed. The active fraction was then subjected to purification and the chemical identification of the active compound was investigated. Among the tested fractions, the n-BuOH extract was the most active fraction since it exhibited an inhibitory effect against M. hominis ATCC 23114 and 80% of the tested clinical strains with MIC between 125 and 1000 µg/ml. The phytochemical analysis of the n-BuOH revealed its metabolic abundance in polyphenols, flavonoids and condensed tannin with levels of 257.37 mg AGE/g, 172.27 mg EC/g and 58.27 mg EC/g, respectively. In addition, P. harmala n-BuOH extract exhibited potent bactericidal activity against all M. hominis isolates with minimum bactericidal concentration (MBC) values ranging between 125 and 4000 µg/ml. Further, the active fraction exhibited weak cytotoxicity effect at active concentrations when tested on human erythrocytes.The active compound was identified by GC-MS as an indole alkaloid harmaline. In summary, P .harmala extract demonstrated an interesting anti-mycoplasmal activity against M. hominis Tunisian strains. Therefore, it could be considered as a potential candidate for the treatment of consequential infections. However, further studies are necessary to evaluate its mechanism of action in mycoplasmas.

P-19

Antimicrobial susceptibility and genetic mechanisms of resistance of *Mycoplasma hominis* isolates between 2012 and 2023 in the United States

Li Xiao (1), Amy Ratliff (1), Donna Crabb (1), Joshua Waites, Ken Waites (1), Melanie Fecanin (1)

(1) University of Alabama at Birmingham, Birmingham, AL USA

Mycoplasma hominis is associated with bacterial vaginosis, pelvic inflammatory disease, postpartum/ postabortal fever, spontaneous abortion, and neonatal bacteremia, meningitis, abscesses and various systemic infections in immunocompromised persons M. hominis is intrinsically resistant to 14- and 15-membered macrolides. Treatment options for M. hominis infections are limited mainly to lincosamides, tetracyclines and fluoroquinolones. There is no systematic study about the antimicrobial susceptibility of M. hominis in the US in recent years. We analyzed the antimicrobial susceptibility data of 259 M. hominis isolates from various sample types that were submitted to UAB Diagnostic Mycoplasma Laboratory from different regions of US for routine antimicrobial susceptibility testing during 2012-2023 and investigated the genetic mechanisms of antimicrobial resistance. The minimum inhibitory concentration (MIC) distributions for clindamycin, tetracycline, and levofloxacin were all basically bell-shaped with a range of 0.008-128, 0.016-128, and 0.031-128 mg/L, respectively. MIC50 values for clindamycin, tetracycline, and levofloxacin was 0.031, 0.125 and 0.25 mg/L, respectively and MIC90 values were 0.063, 0.5, and 4 mg/L. According to the Clinical and Laboratory Standards Institute breakpoints, there were 38 (14.7%) isolates resistant to one or more drugs, and resistance rates for clindamycin, tetracycline, and levofloxacin were 2.7% (7/259), 6.2% (16/259), and 19.8% (28/259), respectively. Eight isolates (3.1%) were resistant two or more drugs. A2058G (E.coli numbering) mutation in one or both 23S rRNA operons and/or mutations in ribosomal protein L4 and L22 were identified in clindamycin-resistant isolates. In most tetracycline-resistant isolates, tet(M) was detected, and two isolates harbored 16S rRNA mutations at position 965 (E.coli numbering) in one operon. For fluoroquinolone-resistant isolates, mutations in ParC (S91I and E95K/G/R) were most frequent, followed by GyrA mutations S153L/A and ParE mutation D426N. No plasmid mediated fluoroquinolone resistant genes were detected. In a summary, clindamycin and tetracycline are still effective in treating M. hominis infections in the US but cautions need to be made for levofloxacin. Since doxycycline is widely used for post-exposure prophylaxis for bacterial sexually transmitted infection prevention, the possible antimicrobial susceptibility changes also need to be watched.

P-20

Use of antibiotics in the control of contagious bovine pleuropneumonia (CBPP): current status

Angela Makumi (1), Arshnee Moodley (1), Elise Schieck (1), Tore Tollersrud (1)

(1) International Livestock Research Institute (ILRI), Kenya

Livestock plays a significant role in rural livelihoods and the economies of developing countries. The dependance on livestock for livelihood and foods is estimated at 1.3 billion people worldwide and 300 million people in sub-Saharan Africa. Livestock are, however, affected by diseases which cause dire consequences to the livelihoods of farmers. Of importance are transboundary diseases such as Contagious Bovine Pleuropneumonia (CBPP) caused by *Mycoplasma mycoides* subsp. *mycoides* (Mmm). These diseases are characterized by respiratory symptoms and pleurisy and pneumonia lesions. Several strategies employed to control these diseases include vaccination, treatment, movement control, and stamping-out through slaughter, however because of the insidious nature of these diseases control becomes difficult to implement. Vaccines used for disease control CBPP includes a live attenuated vaccine however, these vaccines have many limitations such a low efficacy, adverse reactions and short-term immunity therefore requiring revaccination. Because of this, antibiotics are widely used by farmers to treat infections, though not recommended based on the on suspicion that it may increase development of resistant strains, mask clinical disease occurrence, promote formation of sequestra, and increase the number of carriers within a herd. Evidence is currently emerging that when antibiotics are applied under controlled conditions, they could play an effective role in CBPP control. The goal of our research will focus on conducting efficacy of antibiotics (existing and new generation) including their treatment regimens to provide clear conclusions with regards to their possible use. This will be done by through pilot studies as proof of concepts to compare the control alternatives, particularly to demonstrate that the combination of vaccination and controlled use of antibiotics can be applied to full effect for CBPP. The data generated from this work will contribute towards (i) Understanding the resistance patterns of Mmm (ii) establishing Minimum Inhibitory Concentration (MIC) across existing and new generation antibiotics (iii) possibly setting Tentative Epidemiological Cut-Off Value (ECOFF) and (iv) allow for the definition of policy guidelines in the regulated treatment and control of CBPP.

Diagnostic and Epidemiology

P-21 **Harry Morton Student Award Candidate**

Evaluation of diagnostic methods for the detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infections in poultry

Congriev Kumar Kabiraj (1), Nicholas Evans (1), Kannan Ganapathy (1)

(1) University of Liverpool, UK

Mycoplasmas are a major threat to the poultry industry globally. Chickens are commonly infected with *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS), resulting in substantial financial losses. Clinically, MS causes musculoskeletal disease, whilst both MG and MS are responsible for respiratory disease, causing a significant decrease in egg production and quality. Appropriate and rapid diagnosis is a prerequisite for effective control of poultry mycoplasmosis to minimise economic losses and maintain the welfare and health of flocks. Different surface proteins of mycoplasmas with known and putative functions act as virulence factors that have a significant contribution to host-pathogen interactions. Recombinant protein technology is an important alternative to using purified antigen fractions to generate consistent production of useful amounts of multiple antigens. As potential diagnostics, a pool of immunodominant surface proteins from MG and MS was selected for expression, and in silico analysis was performed to determine the desired sequences. The selected proteins were expressed using an Escherichia coli expression system and purified by the immobilised metal affinity chromatography technique. Purified proteins were identified and visualised using SDS-PAGE and Western blot assays. Indirect ELISAs have been developed as a method of validation using the expressed recombinant proteins as a coating antigen against specific chicken antisera. The optimum concentration of coating antigen, positive and negative sera, and conjugated secondary antibody dilutions was achieved by checkerboard titration for each antigen. For specificity and sensitivity, the recombinant proteins were cross-compared with MG and MS antisera. No cross-reactivity was found using heterologous chicken antisera, suggesting the expressed proteins have highly species-specific domains for MG and MS, respectively. The recombinant proteins will be compared with crude antigens (whole and sonicated) by ELISA. Other rapid diagnostic techniques will be assessed and evaluated, such as the dot-blot assay and the rapid immunofiltration assay (RIFA). The results to date demonstrated that the purified recombinant proteins could be potential diagnostic candidates for the effective diagnosis of MG or MS in poultry.

P-22

Duplex droplet digital PCR for the detection and quantification of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*

Zhixun Xie (1), Zhiqin Xie (1), Qing Fan (1), Liji Xie (1), Sisi Luo (1)

(1) Guangxi Veterinary Research Institute, Nanning, China

Mycoplasma gallisepticum (MG) and *Mycoplasma synoviae* (MS) are two important poultry pathogens, which have caused great economic losses to the poultry industry in the world. MG and MS cause similar clinical signs including respiratory disease, synovitis and airsacculitis in commercial poultry. In addition, MG can lead to delayed growth, incomplete development, high elimination rates and reduced egg production. MS can cause tenosynovitis or bursitis, and unilateral or bilateral swelling of the tibia tarsometatarsal joint in chicks. In this study, a duplex droplet digital polymerase chain reaction (ddPCR) for the detection and quantification of MG and MS was developed to simultaneously diagnose single and mixed infections in chickens. Two primer sets and composite probes were designed for the conserved regions of the PvpA and VlhA genes based on separately published sequences. Each composite probe was labelled with a different fluorophore. The final concentrations of primers and probes were 1.2 and 0.35 μM, respectively. The annealing temperature was 55°C. The ddPCR assay was shown to be sensitive, with detection limits of 5.1 and 3.9 copies of recombinant plasmids containing the MG and MS target genes, respectively. The assay also exhibited high specificity and no cross-reactivity with other avian pathogens. The ddPCR assay was more sensitive than real-time PCR (qPCR). Consequently, the duplex ddPCR assay is a sensitive technique for the detection and absolute quantification of MG and MS. This assay has potential for application to the clinical diagnosis of MG and MScoinfection in clinical samples. This work was supported by the Guangxi BaGui Scholars Program Foundation (2019A50).

P-23

Innovative MG and MS ELISA tests, based on highly specific recombinant proteins, for the diagnosis and the monitoring of vaccination

Emmanuelle CAMBON (1), Stéphanie LESCEU (1), Catherine LEFEBVRE (1), Chloé REDAL (1), Jean- Emmanuel DRUS (1), Marina GAIMARD (1)

(1) Innovative Diagnostics

Introduction. Avian mycoplasmosis is caused by several pathogenic bacteria of which *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS). MG causes chronic respiratory disease and infectious sinusitis in poultry. MS infection affects mainly joints and bones of chickens, chronically weakening the birds and reducing egg production. They both lead to large financial losses. Mycoplasma control and eradication programs require accurate and reliable tests. Especially, serology is commonly used for diagnosis, and vaccination monitoring. As a consequence, IDvet has developed the ID Screen® MG Indirect ELISA, and the ID Screen® MS Indirect ELISA for the detection of antibodies in serum and plasma. **Material and Methods.** The ID Screen® MG Indirect ELISA, and the ID Screen® MS Indirect ELISA, based on recombinant protein were used. The specificity was evaluated with negative samples coming from Germany (SPF chickens), France (SPF chickens and commercial broilers from disease free flocks) and Malaysia (breeder flocks). The sensitivity of both indirect ELISA was also evaluated with panel of vaccinated and/or infected animals. **Main results**. The specificity of the ID Screen® MG Indirect ELISA, and the ID Screen® MS Indirect ELISA was evaluated at 100%. No false positive was identified in the tested panels. The ID Screen® MG Indirect ELISA, and the ID Screen® MS Indirect ELISA also demonstrate high sensitivity, with early detection of IgM antibodies (7 days post-infection). The test also demonstrates a good capacity to detect the different circulating strains, belonging to MS or MG. **Conclusion**. The ID Screen® MG Indirect ELISA, and the ID Screen® MS Indirect ELISA are suitable serological tests for the detection of antibodies in serum and plasma, in case of diagnosis or monitoring of vaccination. These indirect ELISA based on specific recombinant protein offer high specificity, without cross reaction with other pathogens.

P-24

New qPCR assays for CCPP and CBPP diagnosis

Anne Boissiere (1), Marjolaine Durand (2), Phonsiri Saengram (3), Lucia Manso-Silvan (1)

(1) 1 CIRAD, UMR ASTRE, F-34398 Montpellier, France
(2) iSPHERES, Jacou, France
(3) CIRAD, UMR AGAP, F-34398 Montpellier, France

Ruminant mycoplasmosis such as contagious caprine pleuropneumonia (CCPP) and contagious bovine pleuropneumonia (CBPP) are important diseases worldwide. They are both listed by the World Organization for Animal Health to be of major economic significance. CCPP rife in Africa, in the Middle East and along eastern European borders and is found in small ruminants and in wildlife. While CBPP is found in cattle and is of consequence in Africa. Regarding the diseases, CCPP is caused by *M. capricolum* subsp. *capripneumoniae* (Mccp) while CBPP is caused by *Mycoplasma mycoides* subsp. *mycoides* (Mmm). Both bacteria belong to the *Mycoplasma mycoides* cluster, a group of five very closely related ruminant pathogens sharing many genetic and phenotypic features (Manso-Silvan et al., 2009). Within this cluster, it is also found M. *mycoides* subsp. *capri* (Mmc) and M. capricolum subsp. capricolum (Mcc) which are the causative agents of CA (contagious agalactia) and which affects mainly small ruminants. Today, reliable and frugal diagnostic tests are needed since two new genomes of *Mycoplasma mycoides capri* (Mmc KerTCR and Wi) was recently published. Indeed, targeted sequences usually used for qPCR diagnosis can be found in Mmc and showed that most of them are no more specifics. Here, we would like to talk about the tests which can be used for diagnosis and to present two new specific and sensitive qPCR assays, to detect Mmm and Mccp in culture and in field samples using either sybrgreen or taqman technology.

P-26

Rapid Detection of Human Pathogenic *Mycoplasma* Species Using a Multiplex Real-Time PCR Assay

Alvaro Benitez (1), Jonas Winchell (1), Maureen Diaz (1)

(1) Division of Bacterial Diseases, U.S. Centers for Disease Control and Prevention, USA

Mycoplasmas are prevalent in nature as parasites of mammals, reptiles, fish, arthropods, and plants. As a conditional pathogenic organism, they are associated with numerous diseases in humans, including pneumonia, meningitis, arthritis, and chronic urogenital tract infection. Conventional tests for Mycoplasma detection, including culture and serological assays, lack specificity and sensitivity and are not suitable for diagnosis of acute infection. We developed a single-tube multiplex real-time PCR assay capable of detecting all human pathogenic Mycoplasma spp. along with a human internal control. The assay consists of one set of common primers and six uniquely labeled hydrolysis probes that bind within the 23S spacer region for specific detection of the following species groups: M. fermentans, M. primatum, M. spermatophilum, M. lipophilum and M. agalactiae (FAM); M. hominis, M. buccale, M. salivarium, M. orale, M. faucium, M. arginini and M. arthriditis (VIC); M. pneumoniae, M. amphoriforme, M. genitalium and M. pirum (ABY); M. penetrans and *M. hyorhinis* (Quasar 670); and human RNase P internal control (Texas Red). Evaluation of the assay with 32 Mycoplasma isolates, including all 18 targeted species, and 31 closely-related non-Mycoplasma isolates demonstrated 100% specificity. The assay has a 7-log dynamic range and lower limit of detection between 12.5 and 250 fg per reaction. Diagnostic sensitivity and specificity for M. pneumoniae was 100% as evaluated by testing respiratory clinical specimens previously identified as positive (n=30) or negative (n=51) for M. pneumoniae using a validated, laboratory developed test. Co-detection of another Mycoplasma in the species group detected by VIC-labeled probe was also identified in 14/30 (47%) specimens tested; this group includes species commonly found in the oral cavity (M. orale and M. salivarium). A Mycoplasma species other than M. pneumoniae was detected in 32/51 (63%) M. pneumoniae-negative specimens; results were confirmed by rpoB sequencing. This rapid, high-throughput test for Mycoplasma spp. detection may aid in investigations of outbreaks with unknown etiologies. Application of this test for surveillance could provide insight into the prevalence and potential role of Mycoplasma spp. in causing human disease.

P-27 **Harry Morton Student Award Candidate**

Mycoplasma spp. presence in vagina, prepuce and raw milk microbiota of sheep: preliminary metagenomic study

Raquel Toledo Perona (1), Jesús Gomis Almendro (1), Marion Toquet (1), Antonio Contreras de Vera (2), Juan José Quereda Torres (1), Pedro González Torres (1,3), Ángel Gómez Martín (1)

(1) Universidad CEU Cardenal Herrera, Spain
(2) Universidad de Murcia, Spain
(3) Microomics Systems S.L.

The genus Mycoplasma is responsible for important diseases in sheep, with significant health and economic implications for the small ruminant sector. However, little is known about the role of mycoplasma species in the microbiota of different anatomical locations in sheep. Due to the presence of pathogenic, apathogenic, and opportunistic species, molecular techniques such as metagenomics could help detect these species and elucidate their interaction in the mammary gland and reproductive tract. Therefore, the objective of this study was to describe the resident mycoplasma species in the ovine vaginal, preputial and dairy microbiota. A total of twenty-two animals (eighteen ewes and four rams), from two herds in Spain (herd A = dairy; herd B = meat) with unknown mycoplasmosis antecedents. Despite of, Q fever abortions were diagnosed. In each herd, between eight and ten females, one week after delivery, and two males, were sampled. In total, forty samples (eighteen vaginal swabs, eighteen milk samples and four preputial swabs) were obtained. The mean relative abundance of the Mycoplasma genus was 1.30% in vaginal swabs (herd A = 0.11%; herd B = 2.46%) and 4.30% in preputial swabs (herd A = 0.00%; herd B = 8.54%). This was higher compared to milk samples (0.40%) (herd A = 0.43%; herd B = 0.34%). Mycoplasma bovigenitalium was detected in low abundance in the vagina in herd A (0.11%) and herd B (0.08%), but in high abundance in preputial swabs (8.54%) from herd B. Mycoplasma hyopharyngis was also detected in the vaginal samples from herd A (1.43%). In milk samples, the species Mycoplasma ovis in herd A (0.43%), and Mycoplasma arginini and Mycoplasma hyopharyngis (0.18% and 0.15%, respectively) in herd B, were detected. Metagenomics allows detecting species of mycoplasmas not previously contemplated in the mammary gland, vagina and prepuce of sheep. This preliminary study evidences the presence of Mycoplasma spp. in the ovine microbiota that could play a role, still unknown, in maintaining bacterial balance. Á. Gómez-Martín and J.J. Quereda are supported by a “Ramón y Cajal” contract (RYC2021-032245-I; RYC-2018-024985-I) (PID2020-119462RA-I00/AEI/10.13039/501100011033). A research grant supports R. Toledo-Perona (CIACIF/2021/245) and CEU-UCH supports M. Toquet.

P-28

The unexpected outcome: incidental detection of *M. gallinarum* septicemia in turkeys affected by respiratory syndrome

Marco Bottinelli (1), Ilenia Rossi (1), Verdiana Righetti (1), Beatrice Colò (1), Davide Prataviera (1), Massimo Zago (2), Elena Rinaldi (1), Elisabetta Stefani (1)

(1) Mycoplasma Unit, WOAH reference laboratory for avian, SCT-1 Verona, Istituto Zooprofilattico Sperimentale delle Venezie, Italy
(2) FIleni Alimentare S.p.A.

Mycoplasma gallinarum (Mgl) is classified among non-pathogenic avian mycoplasmas, although it has been associated with respiratory infections in broiler chickens, and it has been reported to be able to induce airsacculitis with concurrent administration of Newcastle disease-infectious bronchitis vaccine. While isolated from turkeys, there have been no documented reports of associated diseases in this species. Five deceased 30 days-old meat turkeys with severe respiratory disease were referred to IZSVe for diagnostic investigation. The animals exhibited symptoms such as productive cough, swollen head, and sub-mandibular edema. Upon necropsy, all animals exhibited catarrhal sinusitis, moderate splenomegaly and varying degrees of fibrinous airsacculitis, with three displaying fibrinous pneumonia. Additionally, two animals showed fibrinous pericarditis with accumulation of clear yellowish exudate in the pericardium. Bacteriological analysis revealed Escherichia coli in the pericardium and spleen, while the lungs tested negative. Despite efforts, Ornithobacterium rhinotracheale was not recovered from the pericardium using a dedicated medium (blood agar supplemented with gentamycin and polymyxin B); however, α-haemolytic microcolonies adhering to the surface of the agar plate, were observed after 72 hours of incubation under a 5% CO2 atmosphere. Subsequently, an 0.5x0.5 cm agar cube was collected and placed into 2 ml of Avian Mycoplasma Experience broth, and after 4 days of incubation at 37°C, turbidity was observed. Denaturing gradient gel electrophoresis of the DNA extracted from both agar colonies and broth confirmed the presence of Mgl alone. After evaluation of its biofilm forming ability, this strain was classified as low-biofilm producer. Real-time PCR tests for M. gallisepticum, M. synoviae, and Avian Flu virus performed on tracheal swabs returned negative results. With over 8000 Mycoplasma-positive avian samples analyzed at IZSVe since 2008, Mgl accounted for 4.2%. Among turkey positive-samples, Mgl was found in the 0.5%, with half originating from backyard birds. Despite the uncommon occurrence of finding Mgl in industrial poultry, investigations into viruses that could have suppressed the immune system were not conducted, leaving uncertain the role of this mycoplasma in the pathogenesis of the described respiratory infection. However, it is important to highlight the importance of culture-based diagnostic methods for the diagnosis of animal mycoplasmoses.

P-29

Genotyping of *Mycoplasma hyorhinis* through Multi-Locus Sequence Typing

Giorgia Nai (1), Verdiana Righetti (1), Davide Prataviera (2), Massimo Bottazzari (1), Michele Gastaldelli (1), Elisabetta Stefani (1), Marianna Merenda (1)

(1) Mycoplasma Unit, WOAH reference laboratory for avian mycoplasmosis, SCT-1 Verona, Istituto Zooprofilattico Sperimentale delle Venezie, Italy
(2) Istituto Zooprofilattico sperimentale delle Venezie, Italy

Mycoplasma hyorhinis (MHR) colonizes the upper respiratory tract of pigs and spreads throughout the body causing polysierositis, polyarthritis, conjunctivitis, otitis, and meningitis. This microorganism is also potentially involved in exacerbating the clinical symptoms of Porcine Respiratory Disease Complex (PRDC), a condition associated with significant economic losses in swine farms. The implementation of typing methods can be a valuable aid in better understanding the dynamics of MHR spread in the swine industry. Among the available methods, the protocol of Multi-locus Sequence Typing (MLST) developed by Tocqueville and Trüeb has the advantage of allowing the unique identification of MHR strains through the global public database PubMLST (https://pubmlst.org/). This database assigns a sequence type (ST) to each strain based on the sequence of 6 housekeeping genes (allelic profile). In this study, MHR strains isolated over the years at the Mycoplasma Unit of IZSVe from different organic matrices and from pig farms in Northern Italy were genotyped using the aforementioned method. The analysis of our samples with those deposited in the database revealed a lack of genetic correlation among isolates from the same geographical area. Furthermore, as already reported in the literature, no relation between STs and sampling matrix was observed. Interestingly, repeated sampling carried out on farrow-to-finish breeding farms revealed a farm-specific genetic signature among MHR isolates, which was maintained over time. This result can be explained by the limited introduction of new animals and the coexistence of animals of various categories (breeders, weaning, and fattening), which apparently allows for continuous circulation of the same MHR in such types of farms. In conclusion, the use of MLST methodology represents a valuable tool for investigating the intra-farm and inter-farm spread dynamics of the swine industry, with the aim of improving the management and control of clinical forms associated with MHR.

P-30

Establishment and preliminary application of antibody-sandwich ELISA for detection of *Mycoplasma ovipneumoniae* antigen

Wenwen ZHANG (1), Xiaonan WANG (1,2), Siyu CHEN (1; 2), Yiyi SHAN (1), Yi CHEN (1,2), Wenliang LI (1), Zilong CHENG (1), Leilei YANG (1), Jinquan WANG (1), Maojun LIU (1)

(1) Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences / Key Laboratory of Veterinary Biological Engineering and Technology, Ministry of Agriculture and Rural Affairs, Nanjing, China
(2) College of Veterinary Medicine, Xinjiang Agricultural University, Urumqi, China

In order to establish a rapid quantitative antigen detection method for Mycoplasma ovipneumoniae, a sandwich ELISA method based on monoclonal antibody was established through monoclonal antibody screening and condition optimization. The exact reaction conditions and methods are as follows: 105μg/mL monoclonal antibody 1A11 was coated on 96-well microplates at 37°C for 1h and then kept at 4℃ overnight. After blocked with 10g/L BSA at 37℃ for 1h. The samples were added and incubated at 37℃ for 1.5 h. Then the detecting antibody (3.81μg/mL) was added and the plate was incubated at 37℃ for 2h. Following that, the HRP-labeled sheep anti-rabbit antibody (1:4000) was added and incubated at 37℃ for 1.5 h. Finally, the substrate was added and incubated at 37°C in dark for 10min. The coefficient of variation between and within batches were lower than 10%. The method showed good specificity, repeatability, and sensitivity. The content of M. ovipneumoniae antigen detected by this method was positive correlation with its titer (CCU/mL). This study provided a new method for the quantitative detection of M. ovipneumoniae antigen, which can be used as an alternatives to colour change unit(CCU) test of M. ovipneumoniae cultures in the laboratory and in the production process of M. ovipneumoniae vaccine.

P-31

Seroprevalence of contagious bovine pleuropneumonia in Poland in 2023

Katarzyna Dudek (1), Dariusz Bednarek (1)

(1) Department of Cattle and Sheep Diseases, National Veterinary Research Institute, Pulawy, Poland

Contagious bovine pleuropneumonia (CBPP) is highly contagious disease caused by *Mycoplasma mycoides* subsp. *mycoides*. Although CBPP currently affects sub-Saharan Africa, ongoing monitoring of the disease in other countries is important to its control. A total of 800 sera were collected in 2023 from cattle originating from all six regions of Poland, i.e. eastern (80 sera), central (80 sera), southern (80 sera), north-western (160 sera), northern (240 sera) and south-western (160 sera). Due to the recent lack of availability of ELISA tests for serological diagnosis of CBPP all over the world, the sera were analyzed only with a complement fixation test (CFT) using a commercially available kit. Samples with doubtful results in the first CFT examination were re-analyzed using the same method. One hundred eighty-nine sera gave doubtful results in the first CFT examination. These samples originated from eastern (29 sera), central (14 sera), southern (21 sera), north-western (36 sera), northern (58 sera) and south-western (31 sera) regions of Poland. The second CFT examination resulted in a total of nine doubtful results originating from the eastern (7 sera) and northern (2 sera) regions which were finally reduced to negative in the third CFT examination. The CFT may give false positive results in individuals due to occurrence of cross-reactions with other species of mycoplasmas. Further screening of cattle in Poland is required to confirm a country free of CBPP.

P-32

A three-year study on the seroprevalence of contagious agalactia in Poland

Katarzyna Dudek (1), Dariusz Bednarek (1)

(1) Department of Cattle and Sheep Diseases, National Veterinary Research Institute, Pulawy, Poland

Mycoplasma agalactiae is considered the main etiological agent of contagious agalactia (CA). Due to the risk of spreading the disease from other European countries, constant serological screening of approximately 200 animals (sheep and goats) per year has been carried out in Poland for many years. The study was performed on a total of 607 sera collected in 2020-2022 in the number of 206 (2020), 200 (2021) and 201 (2022) sera. The sera originated from sheep and goats in all six regions of Poland, including eastern (116 sera), central (50 sera), northern (128 sera), north-western (213 sera), southern (50 sera) and south-western (50 sera). The samples were examined using commercially available ELISA according to the manufacturer's requirements. The positive or doubtful results in the first ELISA examination were re-tested using the same method. The first ELISA showed one positive serum (2022, eastern region) and six doubtful sera (one in 2021 – northern region; five in 2022; two - eastern region; two - northern region; one - central region). The remaining 600 sera were negative. The second ELISA examination gave five doubtful and two negative sera originating from the northern (two doubtful sera; one each in 2021 and 2022; one negative serum in 2022), the eastern (two doubtful and one negative sera in 2022) and the central (one doubtful serum in 2022) regions of Poland. A few doubtful sera finally reported in this study should be treated as false results due to cross-reactions with other mycoplasma species, especially since the animals from which the samples came were clinically healthy. Constant screening of small ruminants for this disease should continue.

P-33

Validation of a multiplex PCR for detection of *Mycoplasma* species in dogs with respiratory diseases

Maryne Jaÿ (1), Maxime Bruto (1), Florine Dieu (1), Salomé Grizard (2), Chloé Ambroset (1), Emilie Krafft (3), Claire A.M. Becker (1),

(1) UMR Anses VetAgro Sup Mycoplasmoses Animales, VetAgro Sup, Université de Lyon, Marcy l'Etoile, France
(2) VetAgro Sup, Université de Lyon, Marcy l'Etoile, France
(3) Université de Lyon, VetAgro Sup, campus vétérinaire, Département des animaux de compagnie, de loisir et de sport, USC 1233 RS2GP INRAE, Marcy l'Etoile, France

Canine infectious respiratory disease complex (CIRDC) is an acute and highly contagious disease. Various primary and secondary pathogens have been involved, including several mycoplasma species namely Mycoplasmopsis canis, Mycoplasmopsis cynos, Mycoplasmopsis maculosa, Metamycoplasma spumans, Mesomycoplasma molare, Mycoplasmopsis opalescens, Mycoplasmopsis edwardii and Mycoplasmopsis arginini but their pathologic implication remains to be clarified. These different species are generally found in the upper or lower respiratory tract of diseased dogs but can also be harboured by asymptomatic or recovering carriers. Their detection and identification from respiratory samples remain limited. The current method solely consists of an rRNA 16S universal PCR followed by sequencing, which is tedious, long and not suitable for the detection of co-infections. To better assess their role and implications in dogs affected with CIRDC, we aimed to develop a multiplex PCR to specifically (co-)detect these eight mycoplasma species in respiratory samples. Genome comparison was done on all genome sequences available in public database using mmseqs2. To better reflect the genetic diversity in certain mycoplasma species understudied, whole genome sequencing was performed on several additional mycoplasma strains recently isolated. This bioinformatics pipeline allowed us to design eight species-specific primer pairs, primarily targeting housekeeping genes. The sizes of each specific PCR amplicons, ranging between 73 to 1129 bp, separated by ~120bp from each other, were chosen to be easily distinguished using a capillary electrophoresis system. We first evaluated each PCR individually for both inclusivity and exclusivity criteria using an appropriate selection of field and reference strains per targeted and non-targeted species. Importantly, these validation steps were carried out in the same conditions that were later used during the multiplexing step. Then, we evaluated the functionality and specificity of the multiplex PCR using that same selection of strains. Finally, the evaluation of our newly developed multiplex PCR is ongoing for the detection of canine mycoplasmas in several respiratory samples isolated from dogs admitted to our local veterinary teaching hospital. This study will allow us to better monitor the prevalence of the different mycoplasma species in dogs, which will improve our understanding of their role in dogs affected with CIRDC.

P-35

Detection of hemoplasmas and direct sequencing in blood from cattle reveals a diversity amongst *Mycoplasma wenyonii* species

Chloé SAADA (1), Hortensia ROBERT (1), Lucie ROQUES (1), Marie-Claude HYGONENQ (1), Renaud MAILLARD (1), Christine CITTI (1), Laurent Xavier NOUVEL (1)

(1) IHAP, Université de Toulouse, INRAE, ENVT, Toulouse, France

Hemoplasmas are non-cultivable mycoplasmas that infect red blood cells of mammals. Several are found in cattle, such as *Mycoplasma wenyonii* (Mw) which is associated with chronic anemia, fever, oedema and drop in milk production or 'Candidatus M. haemobos' (CMh) which impact on health is unclear. These infections are increasingly reported worldwide, yet their detection is mainly based on PCR of conserved genes like 16S and provides only partial insights into the diversity of circulating species and strains. Our inability to cultivate these bacteria limits genomic data availability for better identification, typing and understanding of their biology. In this study, we collected 583 blood samples from cattle across diverse farms and geographical regions in France. Using 16S qPCR, we detected hemoplasma DNA in 69% of samples. By employing whole-genome sequencing via Illumina and Oxford Nanopore Technologies on 6 highly positive samples, we successfully generated circular genomes for different strains. Comparative sequence analyses revealed the presence of at least two distinct Mw strains circulating within France. Subsequently, we developed a set of specific PCR probes to identify and differenciate Mw strains and CMh. Remarkably, Mw was detected in about 36% of the samples, while CMh was present in 55%, and co-infection by both species was observed in nearly 25% of cases. Interestingly, among Mw-positive animals, over 55% exhibited co-infection with multiple Mw strains. Our findings unraveled the co-circulation of CMh and diverse Mw strains in France. Notably, we report the novel observation of co-occurring Mw strains within the same animal for the first time. These results raised the question on the potential implication of specific strains, alone or in combination, in the developpement of clinical signs in cattle. This emphases the need of more comprehensive molecular epidemiology approaches for understanding hemoplasma infections.

P-36

Preventive Medicine for *Mycoplasma* Infectious Diseases: Proposal for continuous and centralized management of databases

Kazuhiro Matsuda (1)

(1) M Bio Technolgy Inc.

Mycoplasma pneumonia is known to be responsible for 10-30% of community-acquired pneumonia in adults, and even higher in 20-50% of pediatric pneumonia. Not only that, but it is also known to progress to asthma and various immunologically intractable diseases such as IgA nephropathy, Guillain-Barre syndrome, and Stevens-Johnson syndrome as complications. Due to the diverse spectrum nature of mycoplasma infections, conventional diagnostic methods have limited sensitivity and specificity, making it difficult to conduct clinical research on these immunologically intractable diseases that can be incorporated into epidemiological surveys and clinical practice guidelines. We have identified a unique antigen on the mycoplasma cell membrane, and have also succeeded in chemically synthesizing a structure identical to that antigen. Using this technology, we are proceeding with translational research from basic research to early clinical trials for vaccines and antibody drugs, as well as a technology that can measure extremely small amounts of antibodies (MID Prism®), which makes it possible to understand the state of infection. It is expected that this will lead to early diagnosis that has not been possible until now in disease areas related to mycoplasma, as well as innovative drug discovery modalities such as vaccines. It is hoped that epidemiological studies using this sophisticated antibody measurement method will be actively pursued in order to put it into practical use. Particularly in the field of pediatric medicine, there is a strong need for early diagnosis and treatment to prevent complications and complications from becoming chronic. As can be seen from the complications of autoimmune diseases, searching for causes and differential diagnosis are extremely important. If an infectious disease is involved, the understanding of the pathophysiology and treatment policy will be different, and we believe that this test will be required as a first-choice test in clinical practice guidelines. In order to more accurately understand the state of infection, investigations involving clinical specimens, serum banks, and gene banks are urgently needed. A comprehensive approach to infectious disease management is also required, as infectious disease conditions range from acute to chronic stages. I would like to propose the need for a comprehensive global medical approach.

P-37

Enhanced surveillance of *Mycoplasma pneumoniae* epidemic and macrolide resistance in England from December 2023 to March 2024

Baharak Afshar (1), Anna Lewis (1), Hannah Bransbury-Hare (1), Kanchanamala Gunasekara (1), KayDee Hosten-Sandy (1), Rebecca Thombre (1), Sendurann Nadarajah (1), Nita Doshi (1)

(1) UK Health Security Agency, UK

Background: *Mycoplasma pneumoniae* is a common cause of community-acquired pneumonia (CAP) transmitted by aerosol or close contact. Major increases and decreases in M. pneumoniae infection have occurred periodically in the United Kingdom. Mycoplasma pneumoniae is found in all age groups, however it is one of the most common etiological agents of CAP found in children with pneumonia. There has been an upsurge in the number of samples received for M. pneumoniae detection by PCR and macrolide resistance testing at the reference laboratory, Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU), UK Health Security Agency (UKHSA), since December 2023. This study provides an overview on the number of laboratory-confirmed M. pneumoniae cases and the prevalence of macrolide resistance amongst confirmed cases in England from April 2023 to March 2024. **Methods**: A total of 1,668 samples were tested using an in-house qPCR assay based on the detection of M. pneumoniae P1 adhesion gene, at the national reference laboratory, RVPBRU, UKHSA. M. pneumoniae PCR positive samples were subjected to macrolide resistance testing using a PCR and sequencing method for determination of known point mutations in domain V of 23S rDNA conferring macrolide resistance (MR) to M. pneumoniae. **Results**: Of 1,668 samples tested from April 2023 to March 2024, 1,055 (63.25 %) were M. pneumoniae PCR positive of which 732 (69%) PCR positive samples were from children (≤18 years). The data showed a significant increase in the number of confirmed M. pneumoniae cases from December 2023 (142/220) to March 2024 (282/346) with the highest number of cases detected in January 2024 (332/527). Most of the M. pneumoniae PCR positive samples were from December 2023 - March 2024 (1,017 (96%)). Macrolide resistance was detected in 3.6% of samples tested (38/1,055). The most common point mutation was A2063G mutation (87.3%) followed by A2064C and A2064G mutation. **Discussion**: A nationwide epidemic of M. pneumoniae infections has occurred since December 2023 in England, particularly among children. Although resistance against macrolides has been low, continuous surveillance of *M. pneumoniae* infections and antimicrobial resistance is important to monitor the surge and provide information for public heath actions and recommendations.

Genome Engineering and Synthetic Biology

P-38 Louis Dienes Award Candidate

Molecules secretion and exposure optimization in *Mycoplasma pneumoniae* chassis

Yamile Ana (1), Daniel Gerngross (1), Luis Serrano (1)

(1) Centre for Genomic Regulation (CRG), Spain

Respiratory diseases, including non-communicable chronic conditions such as lung cancer and fibrosis, along with lower respiratory infections, are in the top five death- leading causes. The development of more effective therapies is necessary to improve live quality and reduce the public spending as well. Synthetic biology has contributed with promising live therapeutics through the generation of genetically modified bacterial chassis to treat human diseases. Serrano's group has developed a non-pathogenic *Mycoplasma pneumoniae* chassis (Mycochassis) based on a profound characterization of this biological system. The advantages of employing this mild human pathogen as a therapy vector rely on its respiratory tissue tropism, the lack of cell wall and almost null recombination capacity, and in terms of safety, it has its own genetic code since TGA stop codon codifies for a tryptophan. Mycochassis secretes active biomolecules and has shown therapeutic effects in *ex vivo* and *in vivo Staphylococcus aureus*, and *in vivo Pseudomonas aeruginosa* infection models. However, one of the limitations of this chassis is its limited protein production capability, becoming difficult to reach the level required for physiological action in situ. To solve this issue we are following two avenues. The first one is to improve the stability and activity of the biomolecules secreted as we did with IL-10. *Pseudomonas aeruginosa* infected mice treated with Mycochassis expressing this IL-10variant, showed a potent anti-inflammatory effect increasing mice survival, compared to the wild type molecule. The second strategy is to enhance secretion levels by improving the secretion signal. We have engineered different variants of our mpn142 secretion signal generating variants that improve two to three times the extracellular protein abundance. Additionally, we are exploring peptide sequences that will expose on Mycochassis surface proteins with different purposes. To select the best sequences we looked into protein anchoring orientation, and a high retention/release ratio of the protein. The accomplishment of these expression systems prototypes in Mycochassis provides the possibility to cover different therapeutic mechanisms and the opportunity to make complex strategies directed to generate more optimal approaches for disease handling.

P-39

Engineered Mycochassis to secrete gamma-interferon

Marcel Cardona-i-Collado (1,2), Javier Delgado Blanco (2), Irene Rodriguez-Alce (2), Luis Serrano (1,2,3)

(1) Universitat Pompeu Fabra (UPF), Barcelona, Spain
(2) Centre de Regulació Genòmica (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain
(3) ICREA, Barcelona, Spain

Lung cancer is the leading cause of cancer incidence and mortality worldwide, accounting for approximately 2 million diagnoses and 1.8 million deaths. Despite the increasing importance of immunotherapy, systemic administration is associated with severe autoimmune side effects leading to therapeutic failure. Therefore, the use of therapies based on bacteria that colonize the tumor and release different therapeutic molecules in situ has aroused great interest in the synthetic microbiology community. Mycoplasma pneumoniae (Mpn) is a mild human pathogen characterized by its pulmonary tropism. Our group has recently developed an attenuated Mpn strain (Mycochassis), with the same colonization capacity as the wild strain in murine and porcine lungs. Our Mycochassis is capable of secreting functional biological products of clinical interest for the treatment of lung cancer, including interleukins, chemokines and nanobodies. Interferons (IFN) comprise a group of molecules mainly related to the activation of the immune system, triggering responses against infections and other diseases. Among them, IFN gamma (IFN-y) plays an important role in the antitumor response, making it of great interest as a therapeutic for lung cancer. In this study, we demonstrate the ability of our Mycochassis to secrete both human and murine functional IFN-y. Furthermore, we have designed new variants in silico using FoldX/Modelx software, increasing IFN-y activity in vitro when evaluated in the Hek- (human) or B16 (murine) reporter cell line and other relevant cell line models. Further studies will evaluate the potential of this strategy in a murine model of lung adenocarcinoma.

P-40

Highly efficient DNA transformation of different *Mollicutes* species using the Nucleofector® technology

Fabien Labroussaa (1, 2), Anya Schnyder (2), Marilou Bourgeon (2), Jörg Jores (3), Sergi Torres Puig (2)

(1) ANSES, Lyon Laboratory, VetAgro Sup, UMR Animal Mycoplasmosis, University of Lyon, France
(2) Institute of Veterinary Bacteriology, Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Switzerland
(3) Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland

Transformation of *Mollicutes* species in laboratory conditions is an absolute prerequisite for genome modification purposes, including the study of genes of interest. Several transformation protocols were developed. Most of them rely on electroporation-based methods or chemically assisted protocols using calcium chloride and polyethylene glycol. However, even the implementation of published efficient transformation methods is generally time-consuming, species- or strain-specific and can be subject to inter-laboratory variability. Here, we developed a simple and reliable method for the transformation of several *Mollicutes* species based on the Nucleofector® technology. This technology, initially developed for the transfection of eukaryotic cell, relies on specific combinations of optimized electrical parameters and cell type-specific solutions for the transfer of DNA into cells. Using the 4-D Nucleofector® X-unit apparatus, we first tested all possible combinations of nucleofection solutions and preset programs for the transformation of a restriction enzyme (RE)-deficient strain of *Mycoplasma capricolum* subsp. *capricolum* (*Mcap*ΔRE). This first round of optimization enabled us to select the most effective program (i.e. FF137) associated to the P3 nucleofection solution, which together allowed the transformation of the pIVB3 replicative plasmid in *Mcap*ΔRE at high efficiency (~5x10-4 transformants/µg/10^7cells). We then tested several additional physiological parameters including the medium composition, pH and state of the *Mcap*ΔRE culture, the effect of washing cells prior to transformation as well as the quantity of recipient cells and plasmid amount needed. This second round of optimization improved the transformation efficiency up to ~2x10^-3 transformants/µg/10^7cells, which is at least comparable to the highly efficient PEG transformation protocol used for genome transplantation experiments. Finally, we successfully used this optimized protocol to transform *Mycoplasma mycoides* subsp. *capri* GM12, *Mycoplasma feriruminatoris* IVB14/OD_0535, *Mycoplasmoides gallisepticum* S6 without any modifications. *Mesomycoplasma hyorhinis* IVB16/0007 will be tested next. Originally thought as an alternative method for the transplantation of whole mycoplasma genomes, the Nucleofector® technology was used to transform several species of *Mollicutes* belonging to different genera. This method offers several advantages, as it is fast, efficient and required less cells and plasmid than traditional DNA transformation protocols. Additionally, it has potential to be directly picked up to transform other *Mollicutes* species of interest.

Human Mollicutes

P-42

The heterogeneity of *M. hominis* is characterized by the interplay of type II DNA MTases and mobile genetic elements

Birgit Henrich (1), Lars Vogelgsang (1), Bäcker Dana (1)

(1) Institute for Medical Microbiology and Hospital Hygiene, University Clinic of the Heinrich-Heine University of Duesseldorf, Duesseldorf, Germany

Bacterial type-II DNA MTases fulfil many jobs: Solitary MTases are involved in the modulation of cellular processes and virulence. As part of a restriction modification (RM) system, they protect against invading foreign DNA by methylating the host DNA at motifs that are recognized by the restriction enzyme and cut if the foreign DNA is unmethylated. In *M. hominis*, a genetically heterogeneous, facultative-pathogen of the human urogenital tract, DNA MTases were characterized, whose presence varied isolate-specifically. The aim of this study was to find out whether the spectrum of mobile genetic elements (MGE), which are also involved in genetic intraspecies diversity, appears to be influenced by the type and extent of MTases. 115 clinical *M. hominis* isolates were screened by Realtime PCR for the repertoire of mobile genetic elements (ISMhom-1, MhoV1, *tet*(M) transposon and the integrative and conjugative elements ICEHo-I and –II) and DNA type II MTases (seven solitary and five RM-systems) and correlations calculated. Presence of ISMhom-1, the smallest MGE of 1.26 kb encoding a transposase, was negatively correlated and declined with increasing numbers of RM-systems suggesting their defence of ISMhom-1 uptake; whereas in isolates carrying only solitary MTases, especially dam2 or dcm5A, ISMhom-1 presence was calculated positive. Of the larger and more complex MGEs, no statistically significant correlation was calculated between presence of RM-systems and prophage MHoV or the tetracycline resistance-mediating *tet*(M) transposon. Both MGEs were not detected in MTase-free isolates, and only presence of MHoV1 was positively correlated with solitary MTases (except dam1). The two integrative and conjugative elements of M. hominis, ICEHo-I and ICEHo-II, both encode a T4SS secretion system with individual cargo genes. Presence of RM-systems seems to promote presence of ICEHo-II (+/- ICEHo-I), but, especially RM.Sau3AI, to impede exclusive ICEHo-I. Presence of solitary MTases versus ICEHo elements was reversed: in RM-free isolates, they correlated positive with presence of exclusive ICEHo-I, but negative with presence of ICEHo-II. Although the general dogma of RM-systems to serve as a defence mechanism for the uptake of MGEs as foreign DNA was generally found in *M. hominis*, the ICEHo-II seems to be the exception that proves the rule.

Immunology and Vaccines

P-43

Deep sequencing and variant frequency analysis for the quality control of T1 vaccine against contagious bovine pleuropneumonia

François Thiaucourt (1, 2), Antoni Exbrayat (1, 2), Etienne Loire (1, 2), Anne Boissière (1, 2), Nick Nwankpa (3, 4), Lucía Manso-Silván (1, 2)

(1) CIRAD, UMR ASTRE, Montpellier, France
(2) ASTRE, Univ Montpellier, CIRAD, INRAE, Montpellier, France
(3) Pan-African Veterinary Vaccine Centre of the African Union (AU -PANVAC), Bishoftu, Ethiopia
(4) National Veterinary Research Institute (NVRI), Vom, Nigeria

Vaccination is the most cost-effective tool to control contagious bovine pleuropneumonia. The vaccines currently used in Africa are derived from a live strain called T1, which was attenuated by passage in embryonated eggs and broth culture. The number of passages is directly correlated to the degree of attenuation of the vaccinal strains and inversely correlated to their immunogenicity in cattle. Current quality control protocols applied to vaccine batches allow the assessment of identity, purity, and titers, but cannot assess the level of genetic drift form the parental vaccine strains. Deep sequencing was used to assess the genetic drift generated over controlled in vitro passages of the parental strain, as well as on commercial vaccine batches. Signatures of cloning procedures were detected in some batches, which imply a deviation from the standard production protocol. Deep sequencing is proposed as a new tool for the identity and stability control of T1 vaccines.

P-44

BoviScan: a Phage Immuno-Precipitation Sequencing library to study the humoral response to *Mycoplasma bovis*

Yonathan Arfi (1), Eric Baranowski (2), Christine Citti (2), Pascal Sirand-Pugnet (1)

(1) INRAE, BFP, UMR 1332, Univ. Bordeaux, Villenave d Ornon, France
(2) IHAP, ENVT, INRAE, Université de Toulouse, Toulouse, France

Phage Immuno-Precipitation Sequencing (PhIP-Seq) is an emerging technique that enables the medium-to-high throughput characterization of the antibody repertoire of an individual against a library of epitopes. PhIP-Seq is based on a library of bacteriophages displaying antigens of choice, which are then bound by antigen-specific immunoglobulins from the individual's samples. Antibody-bound viruses are immune-precipitated, and the corresponding epitopes are identified through sequencing. Here, we describe the design and synthesis of BoviScan, a T7 bacteriophage library that encodes the pan-proteome of 295 *M. bovis* strains. A set of 557 genome assemblies was used to extract 421,793 predicted ORFs. The amino-acid sequence encoded by each ORF was then split into 56 amino-acids segments (or "tiles"), with a 26 amino-acids overlap between each tile. To reduce redundancy, the 5,140,114 tiles generated were then clustered at 98% sequence identity, forming a set of 37,027 unique tiles. A set of 258 control tiles (antigens for which specific antibodies are available) were added, yielding the final set of 37,285 tiles. A pool of synthetic DNA oligonucleotide encoding each tile was ordered from Twist, and subsequently converted into double-stranded DNA. After sequencing of the DNA pool for quality control, it was cloned by restriction-ligation into the left and right arms of the T7 genome. The resulting assembled viral genomes were rescued by in vitro packaging, and after expansion the final BoviScan library was recovered. Sequencing of the library showed that more than 99.85% of the expected tiles are present, with a uniform abundance (95% of tiles within 1 log). We are currently adapting the immune-precipitation protocol to bovine immunoglobulins, and will use BoviScan to study a broad range of samples (serum, milk, BAL fluid, etc…) from a broad range of animals (infected naturally, infected in laboratories, non-infected, vaccinated, etc…).

P-45 **Harry Morton Student Award Candidate**

Protection of day-old chicks with the Vaxsafe MG304 live-attenuated *Mycoplasma gallisepticum* vaccine: a transcriptional profile

Kanishka Kamathewatta (1), Anna Kanci Condello (1), Dilhani Ekanayake (1), Kelly Tivendale (1), Nadeeka Wawegama (1), Glenn Browning (1)

(1) Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Science, The University of Melbourne, Parkville, Victoria, Australia

The capacity to administer vaccines to chicks at one day of age, before they leave the hatchery, has considerable advantages in the commercial poultry industry. We evaluated the efficacy of the live-attenuated Vaxsafe MG304 vaccine in day-old chicks using differential gene transcription analysis of the tracheal mucosa. Infected unvaccinated chickens had differing transcriptional profiles correlated with their tracheal mucosal thicknesses (TMTs). In infected unvaccinated chickens that had a low TMT 4,920 genes were differentially transcribed compared to uninfected chickens, while in infected unvaccinated chickens that had a high TMT 669 genes were differentially transcribed compared to uninfected chickens. In the infected unvaccinated chickens with low TMTs, functions associated with mitochondrial dysfunction and DNA damage response were enriched with up-regulated genes, while functions associated with tissue repair and apoptosis were enriched with down-regulated genes. In the infected unvaccinated chickens with high TMTs, functions associated with the cytokine response, cell proliferation and the lymphocyte response were enriched with up-regulated genes, while functions associated with extracellular matrix organisation were enriched with down-regulated genes. At two weeks after challenge, the transcriptional profiles of the chickens that had been vaccinated with a high dose or a low dose of Vaxsafe MG304 were similar to those of the uninfected chickens. In infected unvaccinated chickens that had a low TMT, 4,631 genes were differentially transcribed compared to infected chickens that had been vaccinated with a high dose of Vaxsafe MG304, and 3,298 genes were differentially transcribed compared to infected chickens that had been vaccinated with a low dose of Vaxsafe MG304. In infected unvaccinated chickens that had a high TMT, 569 genes were differentially transcribed compared to infected chickens that had been vaccinated with a high dose of Vaxsafe MG304, and 20 genes were differentially transcribed compared to infected chickens that had been vaccinated with a low dose of Vaxsafe MG304. This study has demonstrated that Vaxsafe MG304 is efficacious when administered to day-old chicks, protecting against both of the different pathological transcriptional changes induced by infection with *M. gallisepticum*.

P-46

***Mycoplasma arthritis* in antibiotic-free pigs: a case study for vaccination trials**

Marianna Merenda (1), Marco Bottinelli (1), Beatrice Colò (2), Micaela Picchi (1), Elena Rinaldi (1), Claudio Tonelli (3), Denis Vio (4)

(1) Mycoplasma Unit, WOAH reference laboratory for avian mycoplasmosis, SCT-1 Verona, Istituto Zooprofilattico Sperimentale delle Venezie, Italy
(2) Istituto Zooprofilattico sperimentale delle Venezie, Italy
(3) Private Practice Veterinarian
(4) Laboratorio di Patologia e Benessere della Specie Suina, SCT4, Istituto Zooprofilattico Sperimentale delle Venezie, Italy

Porcine infectious arthritis attributed to bacteria such as Mycoplasma hyorhinis (MHR) and *Mycoplasma hyosynoviae* (MHS) poses significant challenges in swine production. This study reports a severe case of arthritis in weaning and growing pigs from a farrow-to finish pig farm in Northern Italy. The pigs were certified as antibiotic-free (ABF) from birth to slaughter. Five carcasses and 9 joints were conferred to IZSVe for diagnostic investigations. Culturing revealed the presence of MHR and MHS in synovial fluids, including a coinfection in one joint, with no other bacteria detected. The histopathological examination revealed severe chronic fibrinous arthritis, with the microscopic findings suggestive of a mycoplasma infection. Despite low Minimum Inhibitory Concentration (MIC) values for commonly used antibiotics in mycoplasma treatment, piglets couldn't be treated with antibiotics due to the ABF certification. To mitigate MHR/MHS spread, sows were treated with tiamulin pre- and post-farrowing. Although clinical improvement was observed, in order to reduce antibiotic use in the farm we proposed to produce autogenous vaccines from the isolated strains. Therefore, three autogenous killed vaccines were formulated: MHR, MHS, and a bivalent MHR-MHS vaccine. A vaccination trial, comprising three vaccinated groups (MHR, MHS and MHR-MHS) and a control group (non-vaccinated animals) was designed. The protocol involved two injections administered three weeks apart to sows pre-delivery and piglets 21 days post-birth. The efficacy of these vaccines is currently under evaluation, considering clinical assessments of lameness cases, documentation of deceased and/or discarded pigs, as well as the total antimicrobial drug consumption on the farm. In conclusion, even though obtaining viable mycoplasma strains is challenging, these represents a valuable resource since they can be used for the production of autogenous vaccines, a promising alternative to antimicrobial treatments that aligns with the "One Health" vision.

P-47

Serological monitoring of *Mycoplasma hyopneumoniae* vaccination uptake using a new ELISA kit

Emmanuelle CAMBON (1), Stéphanie LESCEU (1), Fabien DONNET (1), Han SMITS (2), Istvan KISS (2), Loïc COMTET (1), Philippe POURQUIER (1), Rafael FORERO (1), Roman KREJCI (2)

(1) Innovative Diagnostics
(2) Ceva Animal Health

Introduction. Mycoplasma hyopneumoniae (M.hyo) is the primary pathogen of Enzootic pneumonia. Humoral response to the infection can be measured by different types of ELISA1. The aim of this study was to assess the potency of different M.hyo vaccines using serology and propose the optimal sample testing for semi-quantitative evaluation. **Materials and Methods**. In total, 250 samples from pigs vaccinated with Hyogen® alone or mixed with Circovac® (both Ceva) or other commercially available vaccines A or B were analyzed using the ID Screen® Mycoplasma hyopneumoniae Competition ELISA test (IDvet). To highlight post vaccination monitoring and immune response, serial dilutions of positive vaccinated sample were tested. Standardized values were calculated and the percentage of positive samples for each dilution was compared among differently vaccinated groups of pigs. Positivity at dilutions 1:1 and 1:5 was evaluated to better objectivate seroconversion. Prevalence of true positive responders (Prev. Index) was determined as follows: Prev. Index = nP dil1/n with nP dil1 the number of positive samples at the dilution 1:1 (S/N %≤50%). Magnitude of the humoral immune response (Mag. Index) was determined as follows: Mag. Index = nP dil2/nP dil1 with nP dil2 the number of positive samples at the dilution 2 (S/N % ≤ 50 %). **Results**. With only two dilutions per sample, only a semi quantitative interpretation is possible. The first dilution (1:1) measures the overall immune response in a population, and the second dilution (1:5) determines its strength. Analysis with the Mhyo cELISA allows to measure the seroconversion with pig´s samples vaccinated with vaccine Hyogen (Prev Index=79% and Mag. Index=54%). Such strong seroconversion is not observed with the other commercial vaccines (Prev Index=45%, 32% and Mag. Index=16%, 0 respectively for vaccines A and B). **Conclusions.** Having results in two dilutions (1:1 and 1:5) of each serum sample enables to define the prevalence of true positive responders but also to estimate the magnitude of the humoral immune response. In this study Hyogen® provided stronger serological response than the other tested vaccines. The IDvet Mhyo cELISA can be used for this vaccine uptake monitoring. **Bibliography.** 1. Thacker E.L., 2004; JSHP, 12, 5

P-48 **Harry Morton Student Award Candidate**

Quality control of CBPP vaccines: Current status and challenges

Juliet Masiga (1)

(1) International Livestock Research Institute (ILRI), Kenya

Contagious bovine pleuropneumonia (CBPP) is a serious and very painful respiratory disease that affects cattle and is a major threat to livestock trade across sub-Saharan Africa. One of the most effective control measures against CBPP is vaccination, and the production of quality vaccines is important to ensure good immune responses. Current quality control (QC) methods for vaccines against CBPP are inadequate, and maintenance of good cold chains systems for vaccine transportation has various challenges and this could, impede on the efficacy of the vaccine leading to use of ineffective vaccine. Surveillance and monitoring of vaccine quality in the field is limited and, in most cases, non-existent. Strengthening CBPP vaccine QC is crucial to safeguard the efficacy and potency of the vaccine. Priorities should be placed on ensuring quality control protocols are followed at all stages of production and implementing pre- and post-vaccination monitoring systems to assess vaccine quality, safety, and protective immunity. Positive developments have been made towards improving QC methods, but there still exists significant gaps that hinder achieving batch to batch consistent for CBPP. The paucity of data on improving QC methods and field monitoring of CBPP vaccines is hindering the success of control programs. The aim of this study is to evaluate the quality of vaccines during vaccination and seek improve quality control procedures for CBPP vaccines, by developing better in vitro potency assays that correlate with in vivo protection. Overcoming these quality control deficiencies is crucial for CBPP control programs to have their desired preventive impact and protect cattle productivity which millions of farmers/pastoralists in Africa depend upon.

P-49

Yet another potential microbial player in autoimmunity: multicentric analysis of the association between *mycoplasma hominis* serostatus and rheumatoid arthritis (Italy, Vietnam)

GL Erre (1, 4), NDT Phan (2), N Diaz (3), A Congiargiu (3), N Mundula (4), AA Mangoni (5), Phuong PhanThi Minh (2), PL Fiori (3), P Rappelli (3), C Cacciotto (6), A Alberti (6), D Dessì (3),

(1) Dipartimento di Medicina, Chirurgia e Farmacia, Università degli Studi di Sassari, Italy
(2) Hue University of Medicine and Pharmacy, Hue, Vietnam
(3) Dipartimento di Scienze Biomediche, Università degli Studi di Sassari, Sassari, Italy
(4) UO Reumatologia, Azienda Ospedaliero-Universitaria di Sassari, Sassari, Italy
(5) Department of Clinical Pharmacology, College of Medicine and Public Health, Flinders University and Flinders Medical Centre, Adelaide, Australia
(6) Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Italy

Resident mucosal pathogens may induce immune tolerance breach, specific autoimmune response, and development of rheumatoid arthriemphasized texttis (RA) in susceptible individuals. Despite a number of studies linking infections by bacteria belonging to the Class of Mollicutes to autoimmune disorders onset and progression, the role of *Mycoplasma hominis*, a common urogenital mucosa colonizing bacterium, in inducing a specific humoral response in RA has been seldom addressed. Beyond infections, there is some evidence suggesting a potential role of *M. hominis* in inducing specific autoimmune disorders in susceptible subjects. Firstly *M. hominis*, consistently with a molecular mechanism conserved among many Mycoplasma species, is equipped with a surface nuclease which induces both Neutrophil Extracellular Traps (NETs) extrusion and degradation in vitro. Indeed, NETs appear to contribute to inflammation and citrullination at mucosal sites, with anti-citrullinated protein autoantibodies (ACPA) being a hallmark feature of RA. Furthermore, *M. hominis* energy metabolosim relies mainly on the activity of a Arginine Deiminase pathway, which leads to the formation of citrulline and could therefore contribute to ACPA generation, manipulating immune response, disrupting host immune tolerance, and favouring the development of autoimmune diseases including RA. A furher clue in this direction comes from the *M. hominis*-induced IL-23 production by human dendritic cells. In this study we compared the prevalence of humoral immune response against *M.hominis* in RA patients and healthy controls (HC). In particular, sera from two separate cohorts from two distinct geographical settings (Italy, Vietnam) were tested. The titer of sera anti-Lipid-Associated Membrane Proteins (LAMPs) antibodies was significantly higher in RA patients than HC in both cohorts Accordingly, we found a significantly higher seroprevalence of anti-M. hominis LAMPs antibodies in RA patients compared to HC in both cohorts. Notably, neither titers nor positivity of anti-LAMP were significantly associated with RA-specific variables. Results highlight the potential relevance of *M. hominis* as an infectious trigger in RA onset and progression. Further studies aimed at shedding light on the possible mechanisms involved are needed to clarify the role of this Mycoplasma species in RA, adding knowledge to the causal relationships between Mollicutes and autoimmune disorders.

Omics Studies

P-50

Insights from the *Mycoplasma gallinarum* genome

Spencer Leigh (1), Jeff Evans (1)

(1) USDA-ARS Poultry Research Unit, USA

Mycoplasma gallinarum is a common mycoplasma isolate from poultry species that is rarely associated with disease. Unlike other avian mycoplasma species, *M. gallinarum* has also been isolated from mammalian species including cattle, pigs, and sheep and grows using a wide array of metabolic substates. Because of its commensal nature, little research has been performed to study *M. gallinarum*. As part of this work, 5 *M. gallinarum* isolates were obtained, and their genomes were sequenced using Oxford Nanopore MinION sequencing with V14 chemistry, assembled use the Flye assembler, and annotated using Prokka. Assembled genome sizes range from 845 kb to 924 kb: within the typical range of mycoplasma species. The genomes maintain a high degree of synteny, and the assembled genomic data also correlates well in both size and gene organization with the two available draft genomes in the NCBI database. Major differences include the number and location of insertion sequences (between 16 and 49 transposases or transposase fragments), the presence or absence of a DNA restriction-modification system, and the presence or absence of genes encoding hypothetical proteins, some of which are conserved in other mycoplasma species that are commensal in avian hosts. While no surface protein families are present, two copies of the lipoprotein 17-related variable surface protein are present. Additionally, multiple strains contain a 27 kb insert with similarities to integrative conjugative elements found in some mycoplasma from mammalian hosts but not found in poultry mycoplasma. These results suggest that *M. gallinarum* strains retain their genomic organization and homogeneity across both time and geographic distance. The results further suggest that *M. gallinarum* can move between mammalian and avian hosts.

P-51

Insights into the *Mycoplasma hyorhinis* pangenome

PAU OBREGON-GUTIERREZ (1), Joaquin Nogales (1), Eva Huerta (1), Alejandro Rubio (2), Antonio José Pérez-Pulido (2), VIRGINIA ARAGÓN (1), FLORENCIA CORREA-FIZ (1), Marina Sibila (1)

(1) Centre de Recerca en Sanitat Animal -IRTA, Spain
(2) Andalusian Centre for Developmental Biology, Spain

Background. Mycoplasma hyorhinis, a common colonizer of the swine upper respiratory tract, is one of the main causative agents of fibrinous polyserositis when it spreads systemically. Nevertheless, the pathogenicity and the virulence mechanisms of this pathogen are still unclear and need deeper understanding. The goal of this study was to characterize a pangenome of *M. hyorhinis*, and search for virulence markers in the accessory genes. **Methodology.** *M. hyorhinis* strains were isolated from the nasal cavity of healthy animals (N=10) or systemic lesions (N=8) to respectively separate commensal and pathogenic isolates. Genomes were sequenced using MiniON nanopore and/or Illumina MiSeq sequencing platforms and assembled de novo. Publicly available strains isolated from pigs (N=87) that came from a variety of countries and several body sites (systemic lesion, nasal cavity or lung) were added to the pangenome analysis. Comparative genomic studies according to strain, origin (country) and clinical source were performed through in-house bioinformatic pipelines. **Results.** All *M. hyorhinis* strains shared a core-genome (genes present in all strains) of 589 genes, while 449 were found to be accessory-genes. The mean number of genes found per strain was 662.4, while the mean number of shared genes between strains was 634.9. Despite no differences in the number of genes were detected between commensal and pathogenic strains, further phylogenetic analyses suggested possible clusters of commensal strains and disease-associated strains. The lack of information in some publicly available data regarding the health status of the animals limited our analyses. Nevertheless, we compared the genomes of strains with clear metadata and identified genes and variants, including lipoproteins, as putative markers for pathogenicity. **Conclusion.** Pangenome analysis allowed to deeper characterize the genomic variability in a wide number of *M. hyorhinis* strains. The increase in the number of sequenced strains with complete metadata would help overcoming the limitations of this study. A better understanding of the pathogenicity mechanisms of this bacterium could reveal associated virulence markers.

Phylogeny and Taxonomy

P-52

The revised taxonomy of the Hominis group is questionable

Alain Blanchard (1), Vincent Baby (2), Pascal Sirand-Pugnet (1)

(1) INRAE, BFP, UMR 1332, Univ. Bordeaux, Villenave d Ornon, France
(2) Université de Montréal, Faculté de médecine vétérinaire, Saint-Hyacinthe, Québec, J2S 2M2, Canada

The phylogenetic analysis of Mycoplasmatota spp. has shown that Mollicutes are splitted into different clades. One of the largest clades is the Hominis group that includes more than 90 distinct species with some of them being of significant importance. In 2018, Gupta et al (Antonie Van Leeuwenhoek 111:1583-1630) proposed a new classification of the phylum Mycoplasmatota, including the Hominis group, based on phylogenetic analyses but also on the identification of conserved signature indels (CSI). In this new taxonomic scheme, the clades within the Hominis group are given new genus names, Mycoplasmopsis, Mesomycoplasma and Metamycoplasma for clades I-III, respectively; each is characterized by 4 CSIs. Using a dataset including 103 sequenced genomes from Mycoplasmatota, a phylogenetic tree was built using 29 core proteins, corresponding to 4,507 positions in the final alignment using the HiPhyGeny pipeline. Groups of orthologous proteins were identified based on sequence identity. In addition, the CSIs proposed for the Hominis group were verified. Our results indicate that the branching between clade I (Mycoplasmopsis) and II (Mesomycoplasma) is not strongly supported by our analysis. The phylogenetic position of M. arginini was found in a different clade as compared to Gupta et al, this discrepancy being due to a mistake in the choice of the corresponding genome. Within the core proteome of these clades, only the clade III was found to show potential specific proteins, but all of unknown biological function. The examination of Hominis-specific CSIs revealed several inconsistencies, i.e. species that do not share this CSI although they belong to this clade or a species that show this CSI without belonging to this clade. For the clades I and II, inconsistencies were found for all CSIs. This is not the case for clade III, for which at this stage of the analysis, there are only a few inconsistencies to report. Overall, our analyses indicate that new taxonomy of the Hominis group, as proposed Gupta et al in 2018 and followed by the International Committee on Systematics of Prokaryotes and the major international databases including NCBI, was premature and needs to be reevaluated.

P-53 **Harry Morton Student Award Candidate**

Genome-scale analysis of the hominis group mycoplasmas and the taxonomic implications

Xiao-Hua Yan (1), Hsi-Ching Yen (1), Chih-Horng Kuo (1)

(1) Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan

Based on the conventional classification of mycoplasmas, the majority of described species belong to the hominis group, which includes many important pathogens of diverse hosts such as *Mycoplasma hominis* (humans), *Mycoplasma agalactiae* (ruminants), *Mycoplasma hyopneumoniae* (swine), *Mycoplasma synoviae* (birds), and *Mycoplasma mobile* (fish). In recent taxonomic revisions, these species were reclassified to three novel genera, namely *Mesomycoplasma*, *Metamycoplasma*, and *Mycoplasmopsis*. However, the reclassification was mainly based on only four shared sequence polymorphisms in conserved genes as synapomorphic characteristics for each group. The biological significance of such sequence polymorphisms remains largely unknown and the extensive changes in Mollicutes taxonomy have resulted in debates and confusion. To provide scientific evidence for facilitating community discussion on this issue, we utilized 95 genome sequences belonging to this group for the inference of molecular phylogeny and comparisons of overall gene content differentiation. Our results indicated that the monophyly of those three novel genera, a major argument for the taxonomic revisions, was not supported by the core genome phylogeny. Specifically, the phylogenetic placements of *Mycoplasma* (‘*Mycoplasmopsis*’) *arginini*, *Mycoplasma* (‘*Mycoplasmopsis*’) *iguanae*, and *Mycoplasma* (‘*Mesomycoplasma*’) *mobile*, were inconsistent with those reported in the previous study that utilized fewer marker genes. More importantly, no robust pattern of gene content differentiation was observed among those three novel genera, suggesting that the reclassification based on phylogeny may not reflect divergence in biological functions. Taken together, the results from this work provided useful information for future re-evaluation of the Mollicutes taxonomy.

Plant and Insect Mollicutes

P-54 **Harry Morton Student Award Candidate**

Investigation into the putative effectors of poinsettia-associated '*Candidatus Phytoplasma pruni*'

Shen-Chian Pei (1), Hsin-Ying Chang (1), Hsin-Yi Chang (1), Ai-Ping Chen (1), Shu-Jen Chou (1), Ting-Hsuan Hung (2), Chih-Horng Kuo (1)

(1) Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan
(2) National Taiwan University, Taiwan

Phytoplasmas are plant-pathogenic bacteria with a broad range of hosts and geographic distribution, posing threats to agriculture worldwide. Intriguingly, in one notable case, the unusual ability of these bacteria for altering plant development has been harnessed by humans to benefit horticulture. In this case, '*Candidatus* Phytoplasma pruni' has been utilized by the poinsettia industry to induce bushy growth and dwarfism for increasing the market value of these ornamental plants. Unfortunately, with limited understanding of '*Ca*. P. pruni' and its interactions with the host, the branch-inducing effect varies among cultivars and may be affected by environmental factors. To better understand these questions, we assembled and analyzed the complete genome sequence of '*Ca*. P. pruni' PR2021 associated with poinsettia cultivar "Princettia Pink". Based on bioinformatic analysis, a total of 20 genes that encode putative secreted proteins were predicted. Among these 20 candidates, two are homologs of SAP11, which is a known effector that has been experimentally characterized in other '*Ca*. P.' species, making these prime targets for investigation. However, based on RT-qPCR assessments of seven poinsettia cultivars, the branching performance of these cultivars did not correlate with either the phytoplasma titer or the *in planta* mRNA expression level of one SAP11 homolog, and the second SAP11 homolog had no detectable expression. These results suggested that the molecular interactions between '*Ca*. P. pruni' and their poinsettia hosts likely involve novel effectors that are yet to be identified. Our on-going work include pan-genome analysis of additional '*Ca*. P. pruni' strains, dual RNA-Seq analysis to investigate gene expression of both '*Ca*. P. pruni' and the infected poinsettia, and experimental characterization of other putative effector genes of '*Ca*. P. pruni'. With these efforts, we aim to infer the molecular mechanisms of '*Ca*. P. pruni' for manipulating poinsettia development. Such knowledge may be used in the future to establish more controllable and reliable systems for modulating poinsettia development.

Virulence and Pathogenesis

P-56

A novel lncRNA-MMTP regulates *mycoplasma*-induced alveolar macrophages necroptosis via enhancing the expression of TNF-α by TF-II/c-fos axis

Zhenzhen Zhang (1), Xiuzhen Mei (1), Jian Wang (1), Beibei Liu (1), Ting Yuan (1), Yuzi Wu (1), Rong Chen (1), Yanna Wei (1), Qiyan Xiong (1), Zhixin Feng (1), Guoqing Shao (1)

(1) Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences, Nanjing, China

Mycoplasma hyopneumoniae (MHP) is the primary pathogen of porcine mycoplasmal pneumonia, causing chronic pulmonary lesions in swine populations. Alveolar macrophages play a key role in respiratory immune defense. Preliminary studies have shown that MHP infection promotes the transcription of TNF-α in mouse alveolar macrophages (MH-S), with subsequently autocrine TNF-α inducing necroptosis of the cells. However, the mechanism by which MHP induces the production of TNF-α in porcine alveolar macrophages is not clear. Long non-coding RNAs, although not encoding proteins, play a key role in transcription, post-transcription, and epigenetic regulation. The abnormal expression of lncRNAs is related to a variety of diseases, including inflammatory diseases related to pathogen infection. In this study, we identified a lncRNA, named lncRNA-MMTP, which is significantly upregulated in MHP-infected porcine alveolar macrophages. This study for the first time reveals the significant role of lncRNA-MMTP in the death of alveolar macrophages induced by MHP infection. Mechanistic studies found that lncRNA-MMTP enhances c-Fos promoter activity by recruiting transcription factors TFII-I and STAT3, thereby upregulating the transcription of TNF-α, and subsequently causing necroptosis of alveolar macrophages. Our findings not only provide a new molecular mechanism for the alveolar inflammatory response caused by MHP, but also offer new molecular targets for the inflammatory regulation mechanisms following pneumonia pathogen infection.

P-57

Ureaplasma parvum infection could alter the oxidative stress state and contribute to miscarriage

Fernanda Karoline Almeida Freire (1), Guilherme Campos (1), Lara Fabiana Luz Malheiro (1), Liliany Souza de Brito Amaral (1), Thiago Macedo Lopes Correia (1), Lucas Miranda Marques (1), Caline Novais Teixeira Oliveira (1), Andrea Pires dos Santos (2)

(1) Universidade Federal da Bahia, Brazil
(2) Purdue University, India

Spontaneous abortion, or miscarriage, is a complex phenomenon influenced by multiple factors, among which genital infections have been identified as significant contributors. This study aimed to investigate markers of oxidative stress in the context of spontaneous abortion associated with *Ureaplasma parvum* (UP) infection. The study comprised 89 women who experienced spontaneous abortion (abortion group) and 20 women who underwent full-term delivery (control group). Participants were further categorized based on UP infection status into four groups: term delivery without UP infection, term delivery with UP infection, abortion without UP infection, and abortion with UP infection. Placental samples were collected and analyzed for markers of oxidative and nitroxidative damage, levels of antioxidant enzymes, and gene expression. Results revealed that in the abortion group without UP infection, there was a decrease in TBARS levels, accompanied by increased protein carbonylation and nitrite levels. While superoxide dismutase (SOD) levels showed no significant difference, catalase (CAT) levels were elevated, and glutathione peroxidase (GPx) levels were reduced. In the abortion group infected with UP, a further reduction in TBARS levels, elevation in protein carbonylation, and increased nitrite levels were observed. Additionally, SOD levels were elevated, CAT levels were notably increased, and GPx levels were decreased. Furthermore, the SOD/CAT ratio was reduced while the SOD/GPx ratio was increased. Analysis of gene expression revealed that in the abortion group without UP infection, there was a decrease in the Cu Zn SOD/CAT and Mn SOD/CAT gene expression ratios, alongside an increase in Cu Zn SOD/GPx and Mn SOD/GPx gene expression ratios. Conversely, in the abortion group infected with UP, there was an increase in the Cu Zn SOD/CAT and Mn SOD/CAT gene expression ratios, coupled with a reduction in Cu Zn SOD/GPx and Mn SOD/GPx gene expression ratios. In conclusion, this study elucidates significant alterations in the redox status, including oxidative/nitroxidative damage, and notable variability in antioxidant capacity between abortion groups infected with UP and those without UP infection. These findings suggest that bacterial infection may exacerbate the oxidative imbalance, thereby contributing to the occurrence of spontaneous abortion.

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Enolase acts as an adhesin in *Mycoplasms ovipneumonia* adhesion to goat tracheal epithelial cells

Maojun Liu (1,2), Mengjie Zhang (1,2), Wenliang Li (1), Leilei Yang (1), Wenwen Zhang (1), Zilong Cheng (1)

(1) Key Laboratory of Veterinary Biological Engineering and Technology, Ministry of Agriculture, Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences, Nanjing, China
(2) College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China

Mycoplasma ovipneumoniae is a pathogen that mainly poses a great threat to ovine animals. At present, there are few studies on the adhesion of Mo enolase to host cells. Therefore, this study mainly focused on the adhesion of Mo Enolase, and explored its interaction with host proteins. **1 Prokaryotic expression of Mo Enolase protein**. The purpose of this study was to obtain the prokaryotic expression protein of Mo Enolase, and to establish an indirect ELISA antibody detection method. In this experiment, Mo IK3-3 strain was used as the gene template, and the recombinant plasmid of Mo Enolase was constructed. After inducing expression, the supernatant was purified to obtain the protein, and its immunoreogenicity was analyzed by Western blot.Mouse anti-serum was prepared with rEno. **2 Analysis of the adhesion and adhesion inhibition effect of Mo Enolase protein on goat tracheal epithelial cells**. The purpose of this study was to investigate the protein localization of Mo Eno to study its adhesion to host cells and the inhibitory effect of antibodies against adhesion, and to explore the surface interacting proteins with host cells. The distribution of Eno in Mo was analyzed by Western blot, the adhesion of Mo rEno to goat tracheal epithelial cells was detected by indirect immunofluorescence, and the effect of anti-rEno serum on Mo adhesion to goat tracheal epithelial cells was detected by qPCR. The interaction between Mo rEno and goat tracheal epithelial membrane protein was analyzed by Far-Western blot and pull-down. The results showed that Western blot localization analysis showed that Eno was distributed on both the cytoplasm and cell membrane of Mo. The immunofluorescence results showed that Mo rEno could adhere to goat tracheal epithelial cells, and mouse anti-rEno serum could significantly inhibited the adhesion of Mo to goat tracheal epithelial cells. Far-Western blot and pull-down analysis showed that rEno could bind to fibronectin(Fn), plasminogen(Plg), tubulin(α-tubulin) and β-actin. The results showed that Mo Eno could be involved in the adhesion of Mo to host cells.

P-59

***Mycoplasma bovis* chaperone ClpB is involved in adhesion and biofilm formation and activity of its secreted virulence factor nuclease**

Hui zhang (1), Doukun Lu (2), Gang Zhao (3), Xi Chen (2), Yingyu Chen (1), Changmin Hu (2), Aizhen Guo (2)

(1) College of Animal & Veterinary Sciences, Southwest Minzu University, Chengdu, China
(2) State Key Laboratory of Agricultural Microbiology, Hubei Hongshan Laboratory, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China
(3) Key Laboratory of Ministry of Education for Conservation and Utilization of Special Biological Resources in the Western China, School of Life Sciences, Ningxia University, Yinchuan, China

Background. Bacterial ClpB is an ATP-dependent molecular chaperone that belongs to the Hsp100/Clp subfamily of the AAA+ ATPases, which cooperates with the DnaK chaperone system in the reactivation of aggregated proteins and promotes bacteria survival under stress conditions. However, the exact role of ClpB in the ruminant pathogen *Mycoplasma bovis* (*M. bovis*) pathogenicity remains poorly characterized. **Methods**. The amino acid sequence of ClpB was analyzed, and the recombinant ClpB protein was purified and characterized. The ATPase activities and its chaperone activity in vitro by DnaK, DnaJ, GrpE-dependent reactivation of heat-aggregated firefly luciferase were measured, respectively. ClpB mutants T9.210 of *M. bovis* and the complemented strains CT9.210 were constructed to investigate the function of ClpB. We also investigate the involvement of ClpB protein from *M. bovis* adhesion, biofilm formation, and generating an immune response in bovine macrophages (BoMac) using qRT-PCR and western blot assays. Furthermore, the role of ClpB on the activity of the *M. bovis* secreted virulence factor nuclease was explored. **Results**. In the genome of *M. bovis*, the gene encoding ClpB has been annotated as non-essential and dispensable for growth under co-culture with host cells conditions. After purify recombinant ClpB, it exhibits ATPase activity and found to be capable of resolubilizing protein aggregates with DnaK, DnaJ and GrpE. The ClpB mutants exhibits no defective growth or morphology under 37 ℃ conditions, but had a decreased growth during heat stress at 42 ℃. Moreover, ClpB mutants of *M. bovis* showed weakened adhesion and biofilm formation ability. The qRT-PCR results showed that the expression of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α was significantly downregulated in BoMac infected with the mutant strain T9.210, compared with that in wildtype strain HB0801 and complementary strain CT9.210. Western blot results showed that the phosphorylation level of p38, ERK and P65 was significantly downregulated in BoMac infected with the mutant strain T9.210. Interestingly, disruption of ClpB results in decreased nuclease activity in culture supernatants. **Conclusion**. These results suggest that ClpB plays important roles in the stress tolerance for achieving the full virulence of *M. bovis* during survival and infection.

P-60 **Harry Morton Student Award Candidate**

Efforts towards the purification of the putative F_1-like X_0 ATPase involved in the MIB-MIP system

Julien Berlureau (1), Clémence Duguet (1), Carole Lartigue-Prat (1), Pascal Sirand-Pugnet (1), Yonathan Arfi (1)

(1) INRAE, BFP, UMR 1332, Univ. Bordeaux, Villenave d Ornon, France

Mycoplasmas are obligate parasites of vertebrates and, although they are not necessarily pathogenic, they must survive and multiply in organisms with highly complex immune defense mechanisms. Several immune evasion strategies have been identified in mycoplasmas that enable them to persist in their host, including the MIB-MIP system used to counter the humoral component of adaptive immunity. This system based on the proteins MIB and MIP, which respectively capture and cleave antibodies, has already been well characterized. However, the mechanism(s) by which the cleaved antibodies are released, and MIB-MIP reset for further capture/cleavage cycles, remain unknown. Data obtained *in vivo* suggest that this step may involve a third partner. A putative atypical F_1-like X_0 ATPase. Here we report our attempts to purify this ATPase complex from *Mycoplasma mycoides* subsp. *capri* in sufficient amount to use in Cryo-EM imagery, and other *in vitro* assays. The purification process includes solubilization of the membrane components of this complex (proteins MMCAP2_0581 and MMCAP2_0577) which are thought to form the X0 domain, with a range of SMA, DIBMA and detergents, with or without the presence of a cross-linking agent. Despite being able to purify the membrane protein 0577, several challenges remain regarding both the purification yield we would like to achieve and the co-purification of other proteins of this putative ATPase complex. Furthermore, we present the construction of two functional mutants which might help in purification strategies. We hypothesize that preventing the cleavage and/or the release of the cleaved antibody could be the key in maintaining the assembly of the F_1-like X_0 ATPase, which we supposed to be triggered under certain conditions. In this context we are developing a mutant of the MIP MMCAP2_0582 for which the protease doesn't have catalytic activity towards antibodies, and a mutant of MMCAP2_0575 unable to bind ATP for hydrolysis.

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The invertebrate *Galleria mellonella* differentiates virulence potential between species and strains of *Mycoplasma agassizii* and *Mycoplasma testudineum*

Alexandra Burne (1), Dina Michaels (1), Mary Brown (1)

(1) University of Florida, USA

Both *Mycoplasma agassizii* and *Mycoplasma testudineum* are etiological agents of upper respiratory tract disease in captive-raised and free-ranging Chelonian species. Both species have been isolated from clinically ill free-ranging and captive tortoises, but *M. agassizii* is associated with more severe clinical signs. While limited experimental infections have confirmed the pathogenicity and examined transmission dynamics, ethical considerations are a factor in continued research regarding host-pathogen interactions due to the federal protection status of many Chelonian species. Additionally, there is a lack of commercially available cell lines for temperature-restricted pathogens isolated from reptiles. To address these challenges, we established an alternative model of pathogenicity testing using the invertebrate *Galleria mellonella*. To assess the efficacy of this model we selected strains of *M. agassizii* and *M. testudineum* isolated from desert and gopher tortoises. A dose-response was run using low, medium, and high infection doses. Larval mortality, pupation, and emergence events were collected over 28 days. We found that, independent of strain, *M. agassizii* is more pathogenic in *G. mellonella* larva compared to *M. testudineum*. Interestingly, we also found that the pathogenicity of *M. agassizii* strain PS6 and 723 is highly dependent on dose when comparing differences within strains. Overall, *G. mellonella* is a tractable alternative model for assessing differences in virulence potential of *M. agassizii* and *M. testudineum* and an important tool to understand how different species and strain impact clinical outcomes.

P-62 **Harry Morton Student Award Candidate**

Macrophages join forces with neutrophils in *Mycoplasma bovis* mastitis

Yaa Serwaah Amoah (1), Peleg Schneider (1), Yeshurun Levi (1), Tal Levi (1), Inna Lysnyansky (2), Nahum Yehuda Shpigel (1)

(1) The Koret School of Veterinary Medicine. The Robert H. Smith Faculty of Agriculture, Food and Environment. The Hebrew University of Jerusalem, Rehovot, Israel
(2) Mycoplasma Unit, Kimron Veterinary Institute, Bet Dagan, Israel

Yaa Serwaah Amoah <yaa.amoah@mail.huji.ac.il>

Mycoplasma bovis (*M. bovis*) is an important etiological agent of bovine mastitis imposing considerable burdens on the health and productivity of dairy animals. The current lack of understanding regarding its interaction with the host immune system is one of the major obstacles impeding progress in the development of effective control measures to treat and prevent *M. bovis* mastitis. The overall goal of this study was to characterize the pathogenic mechanisms of *M. bovis* intramammary infection. Utilizing a recently established *M. bovis* murine mastitis model, our study demonstrated that *M. bovis* mastitis is characterized by the substantial recruitment of blood neutrophils into alveolar and ductal milk spaces, formation of neutrophil extracellular traps (NETs), and recruitment of monocyte-derived macrophages. The latter represents a unique feature not observed in mastitis induced by *Escherichia coli*, *Streptococcus uberis*, or *Staphylococcus*. Furthermore, in order to overcome the limitations of the *M. bovis* murine mastitis model stemming from its slow growth, we have recently established a novel murine mastitis model using the fast-growing specie *M. feriruminatoris*. Our findings reveal that low challenge dose (10 4 cfu) of *M. feriruminatoris* ascending infection elicited acute mastitis associated with massive neutrophil recruitment. However, contrary to previous findings, we did not observe recruitment of monocyte-derived macrophages as described above. Comparison of whole-genome transcriptomic response between *M. bovis* and *M. feriruminatoris* will enable us to unveil the molecular mechanism underlying the recruitment of monocyte-derived macrophages and its significance in the pathogenesis of the disease.

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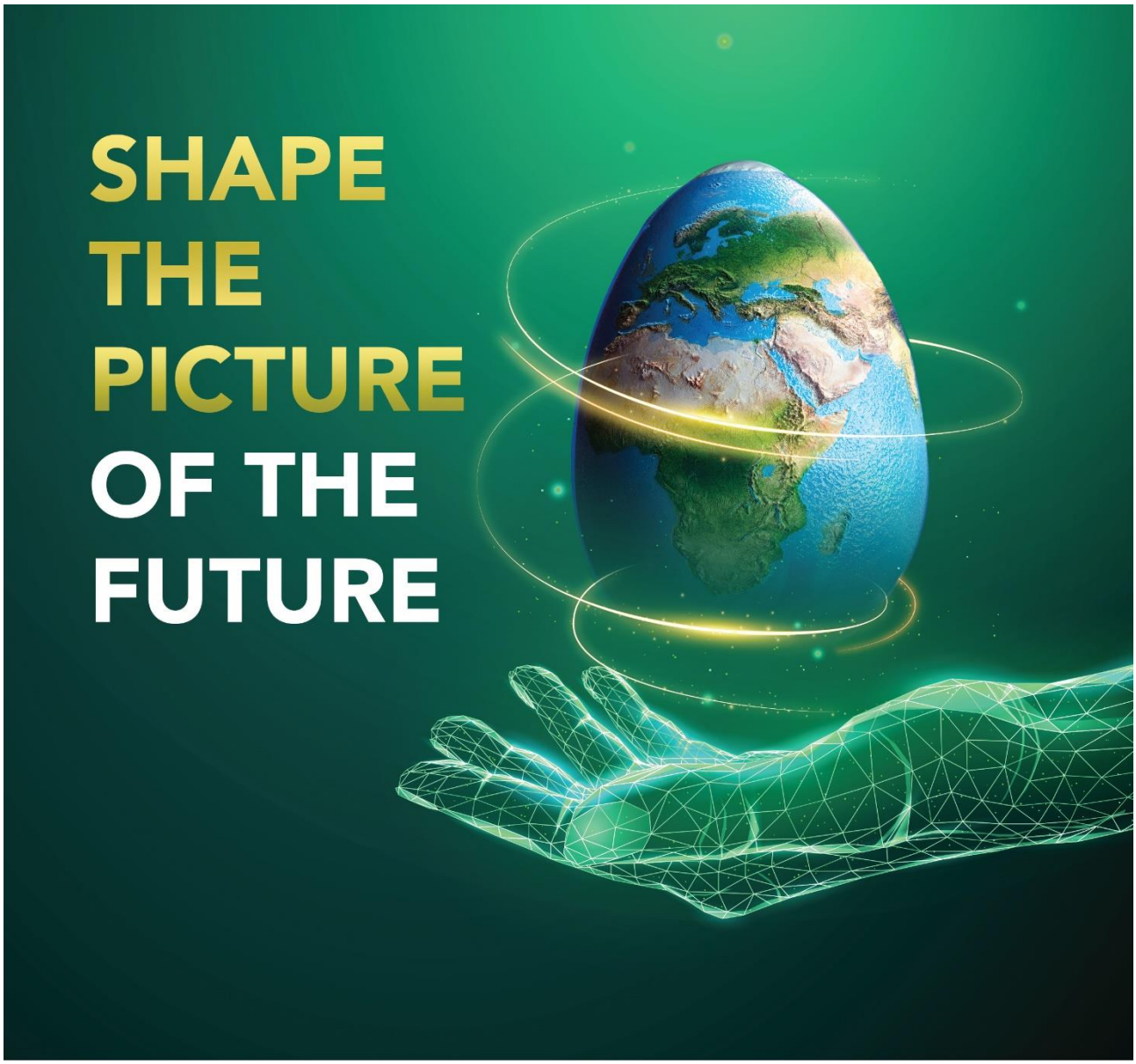

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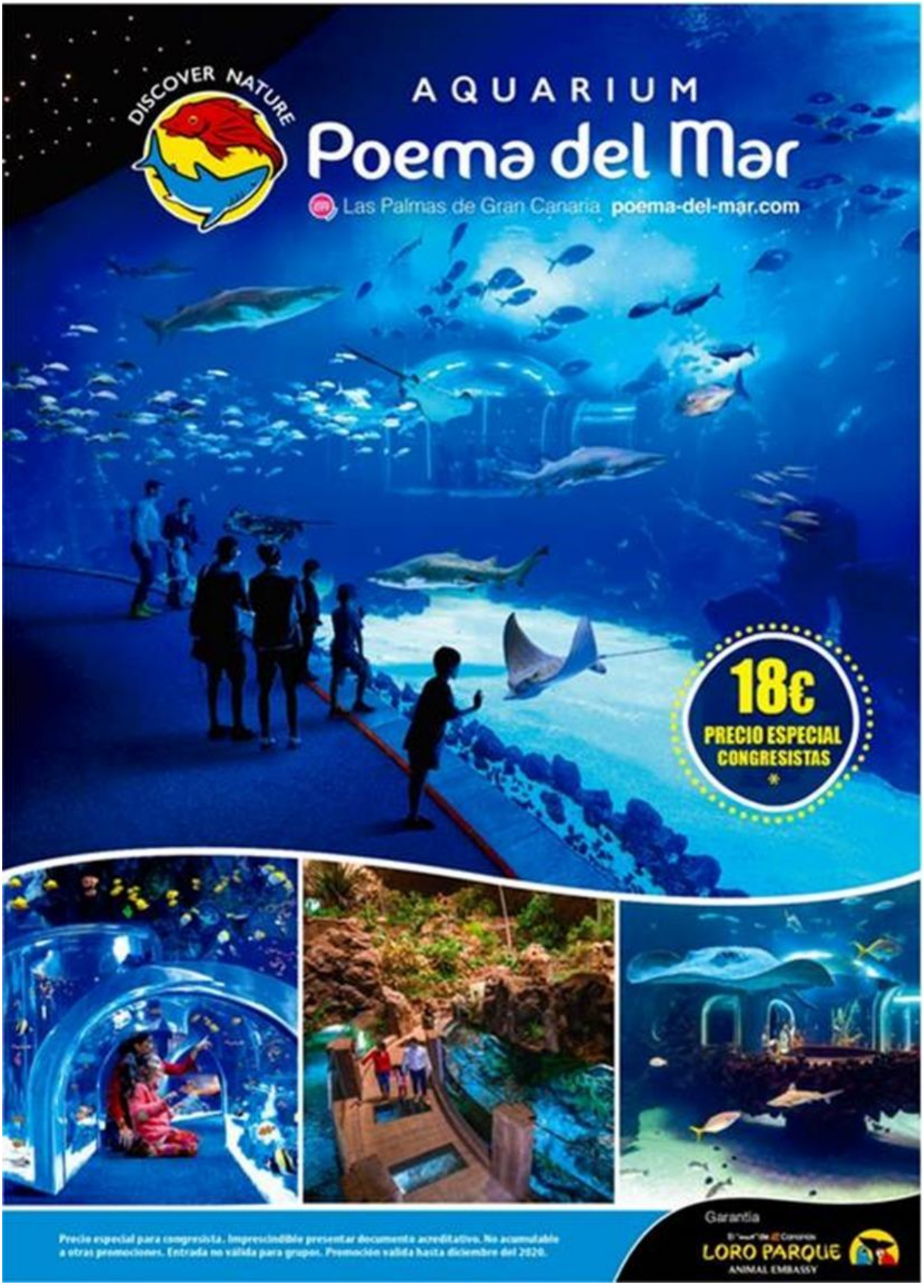
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