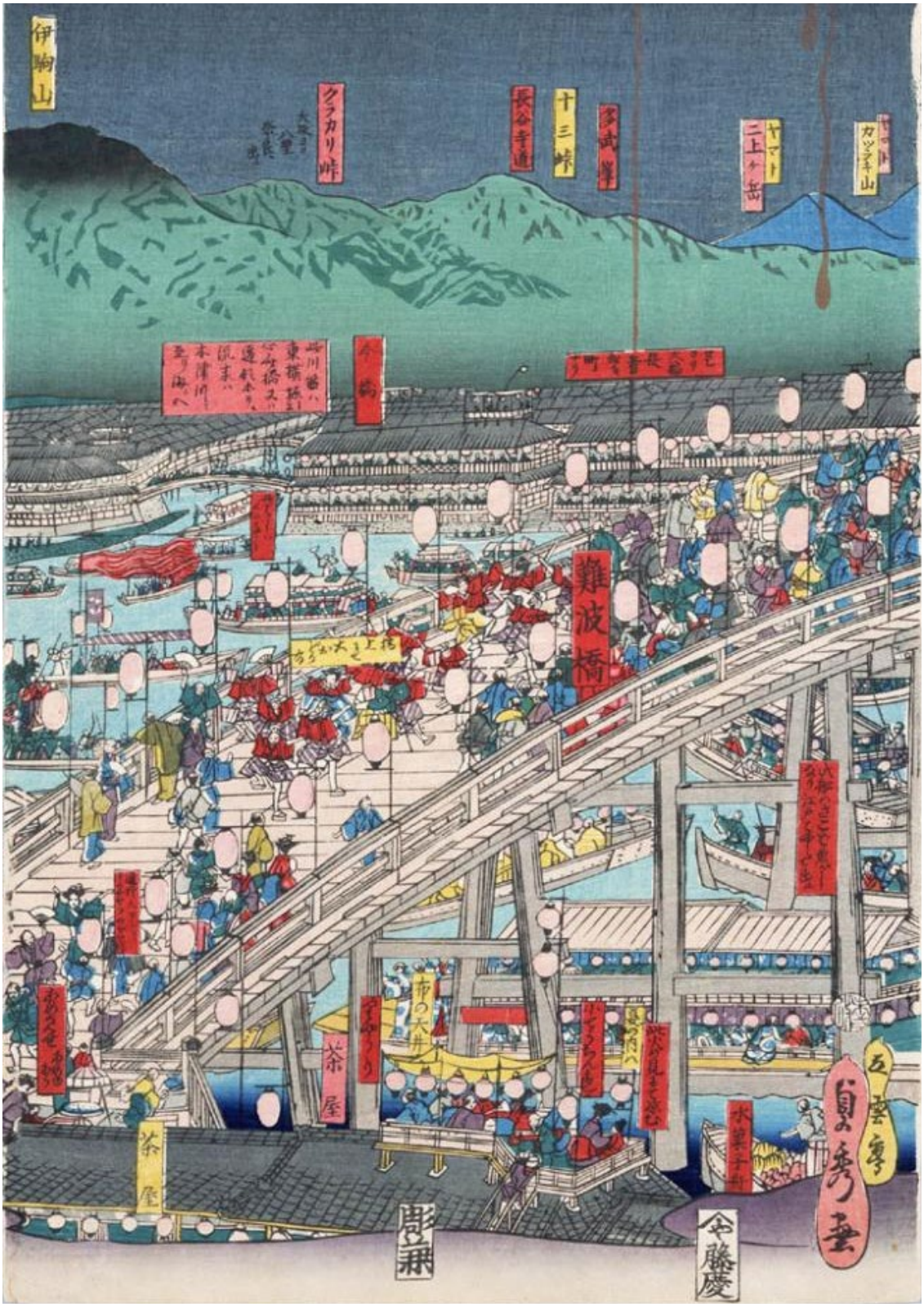


24th Congress of the International Organization for Mycoplasmology
(Associated with the Japanese Society of Mycoplasmology)
July 16th–20th, 2023, Osaka, Japan

Conference Program

IOM 2023 Osaka

Cover Art

Title: Naniwa Temma Matsuri
Artist: Sadahide Utagawa
Date of production: Edo period, 1859

“Naniwa Temma Matsuri” is a NISHIKIE, set of three panels. It depicts a summer festival of Osaka Temmangu shrine. This festival is thought to be held from 951 and one of the three big festivals in Japan. Related rituals are held from late June to 25th July. On 25th July, more than 100 boats go around from Tenjin-bashi bridge (drawn in middle and left panels) to the end of Ohkawa river. Dedication fireworks are also famous programs, around 3,000 fireworks will be set off this year. Naniwa-bashi bridge (drawn in right panel) locates on the front of congress venue. Current Naniwa-bashi bridge is iron bridge, nicely decorated and put four stone lions on the edges of bridge.

Table of Contents

Welcome Message from Local Organizing Committee

Chair of Local Organizing Committee

Makoto Miyata (Osaka Metropolitan University)

Dear IOM2023 congress participants,

Welcome to the scorching hot Osaka! After overcoming COVID-19 and the unstable international situations, we are finally able to hold the IOM congress in Osaka! This congress has various special meanings, such as the first onsite congress in five years, the first congress in Japan in 23 years, and the first congress in Asia in 15 years. I would like to express my sincere thanks to the IOM board, other committees, local organizing committee, and supporting organizations.

During COVID-19, we learned that online congresses work reasonably well. Participating and organizing an onsite IOM congress requires significant budget and efforts. In addition, congress presentations are not articles, and therefore are not official achievements. The IOM congress is definitely a treasure for Mycoplasmologists. However, what is the value of an onsite IOM congress that remains after taking the above into consideration? I think it is to build trust among Mycoplasmologists, to nurture young researchers, and to get new ideas in extraordinary.

In this congress, we are a little conscious of these values, and we have made some innovations, such as a longer sharing time and a joint reception party with the Japanese Society of Mycoplasmology. We hope you will enjoy the five days of Mycoplasma paradise with Japanese hospitality. <(_ _)>

Makoto Miyata

Chair of the local organizing committee

Local Organizing Committee Members

Chair of the 50th Japanese Society of Mycoplasmology Congress

Tadashi Ishida (Kurashiki Central Hospital)

President of the Japanese Society of Mycoplasmology

Koichi Izumikawa (Nagasaki University)

Local Organizing Committee Members

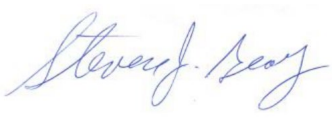
Akira Hasebe (Hokkaido University)
Hidetoshi Higuchi (Rakuno Gakuen University)
Hiroaki Ito (Kurashiki Central Hospital)
Satoshi Iwata (National Cancer Center Hospital)
Shigeyuki Kakizawa (National Institute of Advanced Industrial Science and Technology)
Shigeru Kamiya (Kyorin University)
Tsuyoshi Kenri (National Institute of Infectious Diseases)
Arihiro Kohara (National Institutes of Biomedical Innovation, Health and Nutrition)
Satoshi Kurata (Kyorin University)
Kazuhiro Matsuda (M Bio Technology Inc.)
Masaki Mizutani (National Institute of Advanced Industrial Science and Technology)
Naoyuki Miyashita (Kansai Medical University Hospital)
Shin-ichi Miyata (The Central Region Agricultural Research Center)
Daisuke Nakane (The University of Electro-Communications)
Kenro Oshima (Hosei University)
Kazunobu Ouchi (Kawasaki Medical School)
Takeshi Saraya (Kyorin University)
Yuko Sasaki (National Institute of Infectious Diseases)
Takashi Shimizu (Yamaguchi University)
Masato Tashiro (Nagasaki University)
Takuma Toyonaga (Osaka Metropolitan University)
Hidehiro Watanabe (Tokyo Medical University)
HN Wu (Osaka Medical Center for Maternal and Child Health)
Takeshi Yamamoto (Kurume University)
Itaru Yanagihara (Osaka Medical Center for Maternal and Child Health)

Welcome Message from IOM Chair

Chair of The International Organization for Mycoplasmology
Steven Geary (University of Connecticut)

Dear IOM Colleagues,

It is my pleasure to welcome all of you to the XXIV Congress of the International Organization for Mycoplasmology in Osaka, Japan. I want to thank Makoto Miyata (Chair of the local organizing committee, LOC) and all of the LOC members for their diligent and conscientious efforts developing this meeting. The virtual IOM Congress in Tel Aviv in 2021 was a tremendous success due to the extraordinary efforts of Ran Nir-Paz, his LOC, the IOM Board chaired by Glenn Browning, as well as the flexibility of the IOM community. After a five-year in-person meeting hiatus this congress promises to be very exciting both scientifically and socially. Meghan May, the Scientific Program Chair, has assembled a talented group of IOM scientists to serve on that committee. They have developed a highly stimulating program that is inclusive of all areas of interest to the entire organization membership. I also want to acknowledge and thank the IOM Awards Committee Chair, Amir Hadjinoormohammadi and all of the committee members for their efforts in reviewing candidates, abstracts, and presentations. Finally, the IOM Board discussed at length the proposal to move the future Congresses back to even number years as has been the case for all Congresses pre-covid. After considerable deliberation, the Board has voted to move the next Congress to July 2024. Details regarding the location and LOC Chair will be announced in Osaka but suffice it to be said, this has largely been made possible due to the collegiality and gracious dedication to the IOM of those hosting the next Congress. I look forward to seeing all of you again. Welcome to Osaka,



Steven Geary
Chair, The International Organization for Mycoplasmology

Supporting Organizations

The Japanese Society of Mycoplasmology (JSM)

Kurashiki Central Hospital
(Support for Welcome Reception)

Kurashiki Central Hospital

Osaka Metropolitan University

Osaka Metropolitan University

Osaka Convention & Tourism Bureau

OSAKA DOWNTOWN of Japan

The International Organization for Mycoplasmology (IOM)

IOM

JST CREST “Genome Programming” Project (Support for Sessions 6 and 7)

国立研究開発法人 科学技術振興機構 Japan Science and Technology Agency

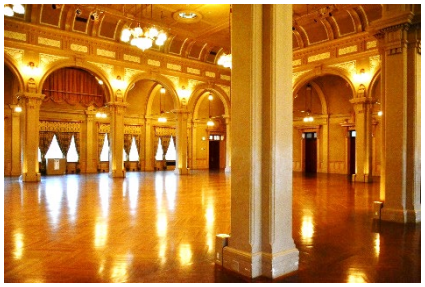
Pfizer

Pfizer

Venue At a Glance

Osaka City Central Public Hall
(Osaka-shi Chuo Kokaido)
1-1-27 Nakanoshima, Kita-ku, Osaka
TEL: +81-6-6208-2002(9:30-20:00)
https://osaka-chuokokaido.jp

Main Hall



Medium Hall

Room details

Main hall: the largest room with a stage for presentations.
Capacity: 810 + 351 people. (1st and 2nd floor)
Medium hall: the second largest room. Welcome party and poster exhibition will be held. Capacity: 300 people. (3rd floor)
Special room: nicely decorated with paintings of ancient Japanese stories.
IOM Board meeting will be held. Capacity: 32 people. (3rd floor)
Meeting room #3: normal meeting room. Capacity: 18 people. (Basement floor)
Meeting room #4: normal meeting room. Capacity: 30 people. (Basement floor)
Meeting room #8: normal meeting room. IRPCM Board meeting will be held.
Capacity: 18 people. (2nd floor)
Prayer room: Capacity: 8 people, available date 17th–20th July.

General Information

Registration Desk

Registration Desk will be located in 1F Entrance lobby on Sunday–Monday morning, in 3F Medium hall on Monday afternoon–Thursday. 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 Wi-Fi is provided in oral and poster rooms as below.
SSID:IOM Main Hall, Password: iomosaka
SSID:IOM Medium Hall, Password: iomosaka

Oral Presentation Instructions

All speakers

- Bring your PowerPoint file in a USB memory with you. Slide format 4:3 is better for the projector than 16:9. Check availability of your file before your session. PowerPoint in Windows 10 will be provided. Mac system is also available for backup. Please discuss with us in advance if you need special setting.
- Standard, clear, and not fast English is recommended. Nonnative speakers check pronunciation of individual words. We remember that many participants are not native for English speaking.

Speakers for special lectures

- You will get bells at 10 min before your assigned time.
- Presentation will be shared by IOM members through YouTube streaming and video. The links will provided later.

Speakers for standard sessions

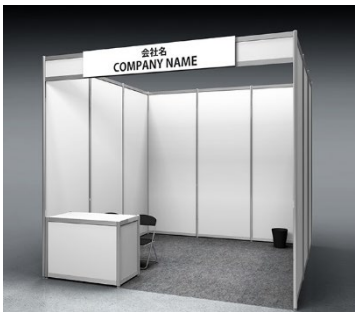
- 15 min including 3 min questions.
- You will get bells at 10, 12, and 14 min.
- Be ready in a next speaker's seat in the preceding talk.

Poster Presentation Instructions

• Posters are exhibited in the medium hall for three days (17–19 July).
• Poster panels are in portrait format (width: 900 mm; height: 2,100 mm)
• Exhibit your poster on an assigned panel.
• Include the abstract number in the upper left.
• Include the presenter’s photo in the upper right corner (recommended).
• Include the abstract for better understanding without presenter.
• In poster session, you try to explain to larger numbers of audience for efficiency.

Poster panels will be removed in 20th morning. Please remove your poster from panel before 6:15 pm on 19th.

Communications in congress



"Mycoplasma communications" booth will be set near the Registration Desk in 3F. Any printed information such as posters and fryers are welcome. We provide our twitter account, "@IOM_Osaka". Please mention this account when you tweet about IOM2023 congress. We set a Google drive to share photos in congress. Please upload and download your photos to share among participants. Be careful not to spread this address to much.

https://drive.google.com/drive/folders/1ASy_UhvmPLLIFRsAyShvKA-ZTWkmP51K?usp=sharing

Mobile Phones

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

Cameras

Recording and photograph of presentations without permission are forbidden.

Osaka City Central Public Hall

This building is designated a national important cultural property in Japan. Eating and drinking in Main hall and Special room are forbidden except drinking beverage in plastic bottle with screw cap and eating lunch box. Beverages and refreshments are

supplied and garbage boxes are set up in Medium hall. Vending machines are available on 1F and B1F.

Emergency Medical Services

Sources of first-aid and medical services can be contacted through the Registration Desk. Japanese medical doctors (IOM participants) will see you.
In case of a medical emergency, dial 119 to call for an ambulance. In Japan, transportation of patients by ambulance is free, but fees are charged for care.

Electricity

The voltage in western Japan is 100 V/60 Hz. Electrical outlets in Japan are type A (with two flat plugs), and type B (with two flat plugs and one ground pin).

Smoking Area

Smoking in Osaka City Central Public Hall and surrounding Nakanoshima area (Red area on below map) is forbidden. Official smoking area locates in Dojima Park (Green triangle on below map).

Currency and Exchange

Money Exchange Machine locates at KEIHAN YODOYABASHI Sta. (Blue triangle on above map). Available time 5:00–24:30

Weather

July in Osaka is hot and humid. Temperatures ranging around 25°C–33°C. Please be careful of heat shock when you walk around. Sunrise at around 5:00, Sunset at around 19:00.

Public Transport from YODOYABASHI Sta.

KEIHAN Electric Railway, bound for Kyoto area
Subway Osaka Metro MIDOSUJI Line, bound for Umeda (Northern central area in Osaka City), Namba (Southern central area in Osaka City)

IOM Board and Committee Rosters

IOM Board of Directors (2021–2023)

Chair: Steven J. Geary (University of Connecticut, USA)
Chair-Elect: Meghan A. May (University of New England, USA)
Past-Chair: Glenn F. Browning (University of Melbourne, Australia)
Secretary General: Pascal Sirand-Pugnet (INRAE-University of Bordeaux, France)
Treasurer: Maria Pieters (University of Minnesota, USA)
Membership-Secretary: Sabine Pereyre (University of Bordeaux, France)
Information Officer: Mitchell F. Balish (Miami University, USA)
At-Large Members: Yonathan Arfi (INRAE - University of Bordeaux, France)
At-Large Members: Birgit Henrich (University of Düsseldorf, Germany)
At-Large Members: Chih-Horng Kuo (Academia Sinica, Taiwan)
At-Large Members: Inna Lysnyansky (Kimron Veterinary Institute, Israel)
At-Large Members: Makoto Miyata (Osaka Metropolitan University, Japan)
IRPCM Liaison: Christine Citti (INRA-ENVT, France)

Scientific Program Committee

Chair of the Scientific Program Committee for 2023

Meghan A. May (University of New England, USA; vet/avian mycoplasmas)

Scientific Committee Members for 2023

Jörg Jores (University of Bern, Switzerland; vet/ruminant mycoplasmas)
Isaac Olorunshola (University of Ilorin, Nigeria; vet/ruminant mycoplasmas)
Amir Noormohammadi (University of Melbourne, Australia; vet/avian mycoplasmas)
Prescott Atkinson (The University of Alabama at Birmingham, USA; human mycoplasmas)
Tomohiro Oishi (Kawasaki Medical School Hospital, Japan; human mycoplasmas)
Inna Lysnyansky (Kimron Veterinary Institute, Israel; vet/ruminant mycoplasmas)
Rohini Chopra-Dewasthaly (University of Veterinary Medicine, Austria; vet/ruminant mycoplasmas)
Lucas Marques (Universidade Federal da Bahia, Brasil; human/vet ureaplasmas)
Saskia Hogenhout (John Innes Centre, UK; phytoplasmas/spiroplasmas)
Chih-Horng Kuo (Academia Sinica, Taiwan; phytoplasmas/spiroplasmas)
Sara Klose (University of Melbourne, Australia; veterinary/avian mycoplasmas/vaccines)
Arlind Mara (Geisei School of Medicine at Dartmouth, USA; human mycoplasmas/vaccines)

Award Committee

Chair of the Awards Committee for 2023

Amir H. Noormohammadi (The University of Melbourne, Australia; animal mycoplasmas)

Award Committee Members for 2023

Rohini Chopra Dewasthaly (University of Veterinary Medicine, Austria; animal mycoplasmas)
Meghan A. May (University of New England, USA; animal mycoplasmas)
Joachim Frey (University of Bern, Switzerland; animal mycoplasmas)
Inna Lysnyansky (Kimron Veterinary Institute, Israel; animal mycoplasmas)
Chih-Horng Kuo (Academia Sinica, Taiwan; phytoplasmas/spiroplasmas)
Tsuyoshi Kenri (National Institute of Infectious Diseases, Japan; human mycoplasmas)
Roger Dumke (Technical University Dresden, Germany; human mycoplasmas)
Laure Béven (French National Institute for Agriculture, Food, and Environment, France; phytoplasmas/spiroplasmas)

International Research Program on Comparative Mycoplasmology (IRPCM)

The IRPCM (International Research Programme on Comparative Mycoplasmology) is a permament standing committee of the IOM. Its objective as defined in the Constitution is to advance and disseminate knowledge on all aspects of mycoplasmas (members of the taxonomic class Mollicutes). In furtherance of this objecitve, the program shall aim to encourage individual and collaborative studies, including the effective exchange of scientific information, to reach this goal. Specifically, this will include:

- Developing a more complete understanding of the cellular and molecular biology of mycoplasmas, including those features that may be unique in the prokaryotic world to mycoplasmas;
- Elucidation of the diversity of mycoplasmas;
- Defining virulence factors of mycoplasmas pathogenic to humans, domestic and wild animals, and plants; and
- Identifying more effective means of diagnosis, prevention, and control of mycoplasma infections and disease.

IRPCM Board Members

Chair: Christine Citti (France)
Vice Chair: To be appointed
Past Chair: Glenn F. Browning (Australia)
Treasurer: Maria Pieters (USA)

IRPCM Ex Board Members

Daniel R. Brown (USA), Mitchel F. Balish (USA), Maria Pieters (USA)

IRPCM Team Leaders

Avian Mycoplasmas: Anneke Feberwee (Netherlands)
Chemotherapy of Mycoplasma Infections: Florence Tardy (France)
General Diagnostics and Cell culture: Team no longer active
Clinical Aspects of Human Mycoplasmas: Tsuyoshi Kenri (Japan)
Molecular Genetics and Cell Biology: Meghan A. May (USA)
Porcine Mycoplasmas: To be appointed
Ruminant Mycoplasmas: Nadeeka K. Wawegama (Australia)
New and Emerging Species: Ana S. Ramírez (Spain)
Plant and Arthropod Mycoplasmas: Chih-Horng Kuo (Taiwan)

Advisory Board Members

Amir H. Noormohammadi (Australia), Steven J. Geary (USA)

Schedule of IRPCM Team Meetings

Monday 07/17

12:45–13:45 Molecular Genetics and Cell Biology @ Main hall

13:45–14:45 Clinical Aspects of Human Mycoplasmas @ Meeting room#3 (B1F)
13:45–14:45 Avian Mycoplasmas @ Meeting room#4 (B1F)

Tuesday 07/18

12:45–13:45 Plant and Arthropod Mycoplasmas @ Main hall
12:45–13:45 Chemotherapy of Mycoplasma Infections @ Meeting room#4 (B1F)

13:45–14:45 New and Emerging Species @ Meeting room#3 (B1F)

Wednesday 07/19

12:45–13:45 Ruminant Mycoplasmas @ Main hall
12:45–13:45 Porcine Mycoplasmas @ Meeting room#3 (B1F)

International Committee on Systematics 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

Vicki Chalker
Mitchell F. Balish
Assunta Bertaccini
Alain Blanchard
Gail Gasparich
Ludwig Hölzle
Peter Kuhnert
Chih-Horng Kuo
Lucia Manso-Silvan
Meghan A. May
Ana Sofía Ramírez Corbera
Joachim Spergser
O. Brad Spiller
Emma Sweeney
Dmitriy Volokhov
Wei Wei

IOM Awards

IOM Emmy Klieneberger-Nobel Award

Made in honor of Emmy Klieneberger-Nobel and is given in recognition of outstanding contributions in research in the field of mycoplasmology.

Professor Glenn F. Browning (Australia)

Professor Browning is a veterinary graduate from the University of Sydney (1983) and has a PhD degree in veterinary virology at the University of Melbourne (1988). Glenn has worked as a veterinary clinician at the University of Sydney's Rural Veterinary Centre (1984), and as a veterinary researcher at the Moredun Research Institute in Edinburgh, Scotland (1988-1991). Glenn joined the University of Melbourne as an academic staff member in veterinary microbiology in 1991. He is currently a Redmond Barry Distinguished Professor and Director of the Asia-Pacific Centre for Animal Health, a multidisciplinary research centre within the university committed to improving animal health, welfare and production.

Glenn’s research has spanned from fundamental- to applied research including pathogenesis and epidemiology of infectious diseases, and development of vaccines and diagnostic assays. Glenn’s projects have led to the development of novel attenuated vaccines for the control pf bacterial and viral respiratory diseases of poultry, pigs and cattle, and of diagnostic assays to improve detection of a wide range of viral and bacterial diseases in animals. Glenn’s initiatives have led to the elucidation of reasons for emergence and spread of infectious diseases in livestock.

Glenn has also made major contribution to the community of mycoplasmologists by serving in various roles at the IOM board and its committee.

IOM Peter Hannan award

Given in recognition of outstanding research achievements in the field of applied clinical mycoplasmology and is made in honor of Peter C. Hannan. Dr. Hannan made major contributions to this specific discipline within mycoplasmology, particularly in the area of establishing methods for assessing the sensitivity of animal mycoplasmas to antimicrobial drugs.

Dr. Vicki Chalker (UK)

Vicki has worked on Mycoplasma and Chlamydia species for 23 years. She started her career on Mollicutes in dogs, cats, tortoises and animals with the Royal Veterinary College, UK where she worked on canine infectious respiratory disease, *Mycoplasma cynos* and other Mollicutes from dogs and any other animals processed in the lab. She implemented the first international PCR test for *Mycoplasma felis* and a PCR service to screen endangered tortoises for Mycoplasma species entering UK zoos. She then moved to the government Harrison-Pitcher laboratory to work on human Mollicutes. She worked with the late Dr. Dave Pitcher and became the former head of the UK national reference unit with remit for human mollicutes and other bacterial species, introducing services in respiratory mollicute detection for neonates and other patients and *Mycoplasma pneumoniae*. She led the development of UK tests for and the first laboratory service for sexually transmitted reference services for *Mycoplasma genitalium* and noted this pathogen could be of importance in rectal samples and has contributed to work on *Mycoplasma amphoriforme* and antimicrobial resistance and tested the first UK case for *Mycoplasma haemohominis*. She is currently the Chair of the International Committee on Systematics of Prokaryotes (ICSP) Subcommittee on the Taxonomy of Mollicutes and has led global studies on Mollicutes and established the Mycoplasma study group that contributed to the formation of ESCMAC (the European Study Group for Mycoplasma and Chlamydia). Vicki is currently the Chief Scientific Officer, National Health Service Blood and Transplant, UK.

IOM Robert F. Whitcomb award

Made in 2008 to honor Robert F. Whitcomb, whose lifetime contributions in plant and insect mycoplasmology were instrumental in the development of the discipline. The award is given in recognition of outstanding research achievements in the field of plant and insect mycoplasmology.

Professor Kenro Oshima (Japan)

Kenro Oshima received his PhD degree from The University of Tokyo in 1998. He joined Prof. Shigetou Namba's group as a postdoctoral fellow and started the research of phytoplasmas. He and his group determined the first complete genome sequence of phytoplasma and found that the phytoplasma has lost many genes of metabolic pathway in the process of evolution. They also discovered the virulence factors of phytoplasmas such as TENGU and PHYL and clarified its mechanism of action. He is a member of the International Phytoplasma Working Group and has participated in the revision of the '*Candidatus* Phytoplasma' species description guidelines in 2022. He was awarded as Kitamoto Award from Japanese Society of Mycoplasmology in 2018 and the Society Award from Phytopathological Society of Japan in 2021. Besides his scientific achievements, Kenro Oshima has contributed to IOM by serving as a member of the Scientific Committee (2008-2010) and the Award Committee (2014-2016).

IOM Derrick Edward Award

Made in memory of D.G. ff. Edward and is given in recognition of outstanding contributions in research in the field of mycoplasmology by young investigators entering the field of mycoplasmology.

Professor Chih-Horng Kuo (Taiwan)

Chih-Horng Kuo entered the field of mycoplasmology in 2010 when he joined Academia Sinica in Taiwan as a principal investigator. With his expertise in evolutionary and functional genomics, he has established extensive international collaborations to work on plant and arthropod mycoplasmas. For plant mycoplasmology, he and his collaborators investigated the evolution and function of phytoplasma effector genes that are key to pathogenicity. For arthropod mycoplasmology, he and his collaborators inferred the patterns of genome evolution in each of the *Spiroplasma* clades and utilized transcriptomics to compare gene expression regulation between pathogenic and nonpathogenic species. His studies also impacted the taxonomy of Mollicutes. For phytoplasmas, he led a project on genome-based delineation of species boundaries and helped to revise the '*Candidatus* Phytoplasma' species description guidelines. For arthropod mycoplasmas, the comprehensive genomic studies led to the union of *Mesoplasma* with *Entomoplasma*, and provided insights into the emergence of *Entomoplasma* from *Spiroplasma*, as well as the emergence of the *Mycoplasma mycoides* cluster from *Entomoplasma*. In addition to research, he has contributed to the communities through service, including his role as a Board Member of the International Organization for Mycoplasmology.

The Louis Dienes Award

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 the Ph.D. degree no more than five years previously.

Nominees

Arlind B. Mara O-14 The role of pulmonary interstitial macrophages in *Mycoplasma pneumoniae* induced inflammation

Sathya N. Kulappu Arachchige O-17 Global Transcriptional Changes in the Trachea After Vaccination and Infection with *Mycoplasma gallisepticum* in Immunosuppressed Chickens

Aizhen Guo O-23 The Secreted MbovP475 of *Mycoplasma bovis* Induces Macrophage Pro-inflammatory Response through CRYAB

Elhem Yacoub O-25 Studying Interactions between 12 *Mycoplasmopsis bovis* and Bovid Tissue Culture

Veronica Maria Jarocki O-41 Genomic analysis of *Mycoplasma bovis* causing bovine respiratory disease in Australian feedlot cattle

Xing Xie O-47 Inositol metabolism is the essential factor to the growth and virulence of *Mycoplasma hyopneumonia*

Grazieli Maboni P-006 Genome-Wide Association Study to Investigate Genetic Markers for Antimicrobial Resistance in *Mycoplasma cynos* and *Mycoplasma felis*

Grazieli Maboni P-030 Improving strain typing of *Mycoplasma ovipneumoniae* using multiplex PCR and Next Generation Sequencing

Yumiko Masukagami P-069 A new *Spiroplasma* species with a highly reduced genome discovered from a coral metagenome in Okinawa

Yuya Sasajima P-076 Cryo electron microscopy of cytoskeletal 'fibril' protein involved in *Spiroplasma* swimming

Daichi Takahashi P-079 Assembly properties of bacterial actin MreB5 essential for *Spiroplasma* swimming

Gang Zhao P-093 Novel Mycoplasma Nucleomodulin MbovP475 Decreased Cell Viability by Regulating Expression of CRYAB and MCF2L2

Masaki Mizutani P-096 Adaptive laboratory evolution of JCVI-syn3.0B to low temperature

The Harry Morton Student Award

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

Yan Xuan O-02 The Association between Genital Mycoplasmas Colonization and Adverse Pregnancy Outcomes—A Population-Based Retrospective Study from China

Calvin ChengYu Ko O-03 Diagnostic Investigation of *Mycoplasma hyorhinis* as a Potential Pathogen Associated with Neurological Clinical Signs and Central Nervous System Lesions in Pigs

Calvin ChengYu Ko P-029 Comparison of Different Laboratory Techniques to Evaluate Growth Activity in vitro of Different *Mycoplasma hyopneumoniae* Strains

Noriko Imaizumi O-15 Synergism of bovine mammary epithelial cells and bovine peripheral blood mononuclear cells enhances immunological responses to *Mycoplasmopsis bovis*

Pratima Verma O-26 Modulation of transcription factors involved during Phytoplasma-associated phyllody development in *Sesamum indicum* L. (Sesame) plants

Oki Matsumoto O-29 Comprehensive search for amino acid residues involved in target recognition of a phytoplasma effector protein by random mutagenesis-based screening

Chintha K. Premachandre O-39 Bovine Tracheal Organoids vs 2D Cell Cultures as Models to Study *Mycoplasma bovis* Cell Invasion

Shijie Geng O-46 Bioinformatic and Metabolomic Analyses Reveal the Potential Metabolic Functions of Genes Critical for Survival of *Mycoplasma bovis* in Cell Culture

Doukun Lu O-50 *Mycoplasma bovis* Nucleomodulin MbovP202 Inhibits Macrophage Proliferation by DNA methylation

Alexandra M. Burne P-001 *Tet44*: A New Ribosomal Protection Protein Conferring Tetracycline Resistance in Clinical Isolates of *Mycoplasma bovis*

Jeremy M. Miller P-007 Advancement in the Development of a Safe and Efficacious Subunit Vaccine against *Mycoplasma gallisepticum*

Barbaros Mulayim P-008 Comparison of Next-Generation Sequencing (NGS) Protocols for Diagnosis of Phytoplasmas

Rosemary Grace Ozyck P-010 Using Live Attenuated Vaccine, MslA 2204, Co-administered with a SiRNA Polyfunctional Nanoparticle Targeted Against Inflammatory Genes Up-regulated During *Mycoplasma gallisepticum* Infection

Yigit Sabri Unlu P-013 Development of Droplet Digital PCR (ddPCR) Technique for Quantitative Detection of Phytoplasmas

Kadir Boztas P-015 Visualization of Phytoplasma Infection in Plant Tissues

Kayhan Derecik P-018 In Silico Analysis of The Putative Phytoplasma Effector SAP55 that Mimics the Lipidation Mechanism of G Proteins

Busra Kara P-020 Genome Comparison of Spiralin, P58, P89 Regions of Naturally Infected and in vitro Cultures of *Spiroplasma citri* from Different Hosts in Turkey

Ali Ahsan P-058 Elucidating the Role of Fibril Protein in *Spiroplasma* Swimming using JCVI-syn3B

Muhammad Algiffari P-059 Elongated JCVI-syn3B Cell Caused by Expression of *Mycoplasma pneumoniae*-gliding Type Cytadherence Regulatory Locus

Minoru Fukushima P-063 Gliding Machinery of *Mycoplasma mobile* Visualized by Electron Tomography

Mone Mimura P-071 *Haloplasma* Motility Reconstituted in JCVI-syn3B by Combination of Two MreB Proteins

John W. Sanford P-075 Investigating the Glyco-Proteoform Landscape of Minimal Genome *Mycoplasma* spp.

Marina Marcos Silva P-077 Structural and functional studies of antibodies halting motility in *Mycoplasma pneumoniae* reveal the dynamic nature of the adhesion complex

Yoshiki Tanaka P-080 *Spiroplasma* swimming mechanism suggested by fluorescently labeled MreBs expressed in JCVI-syn3B

Haruka Yuasa P-081 Visualization of MreB4 and MreB5 Filaments Driving *Spiroplasma* Swimming by Using JCVI-Syn3B and Electron Microscopy

Shen-Chian Pei P-087 Genome analysis of '*Candidatus* Phytoplasma pruni' strain PR2021 associated with poinsettia

Xiao-Hua Yan P-102 Impact of Potential Mobile Units on Genome Stability in Phytoplasma Evolution

Yi-Ching Chiu P-105 Molecular characterizations of *Raphanus sativus* L. witches'-broom disease and the genetic status of the '*Candidatus* Phytoplasma aurantifolia' strains (16SrII-V) in Yulin, Taiwan

Masato Suzuki P-110 Target degradation specificity of a phytoplasma effector protein phyllogen is regulated by an ability to recruit host proteasome shuttle protein

Travel Award Recipients

To be announced

Keynote Speakers

Clifford Rosen

Professor, Tufts University, US

The central theme of the Rosen laboratory is understanding the metabolic and biochemical fate of marrow stromal cells as progenitor osteoblasts and/or adipocytes. In vivo, this translates into defining the relationship between marrow adipogenesis and osteoblastogenesis, and the interactions between whole body and skeletal metabolism. We use age, genetic, environmental, diet, and pharmacologic manipulations in order to understand the complex regulation of bone remodeling. We use a variety of techniques to address our research questions, including DXA imaging, NMR, microCT, MRI, osmium tetroxide staining, histomorphometry, immunohistochemistry, immunofluorescence, confocal microscopy, Seahorse extracellular flux analysis, and metabolic in vivo studies using Promethion technology.

Andrew Peters

Professor, The University of Edinburgh, UK

Andrew Peters is Professor of Tropical Veterinary Medicine at Edinburgh University and Director of SEBI-livestock, Centre for Evidence based Interventions, which focuses on improving data on livestock health and productivity in low and middle income countries. He also works as an independent consultant in development of veterinary medicines, with special emphasis on vaccines. He has long experience in development and regulation of veterinary medicines and diagnostics. He is a member of several public committees and charity trusts both in the UK and internationally. He is a veterinarian with PhD and DSc degrees in animal science.

Hiroyuki Noji

Professor, The University of Tokyo, Japan

Professor Hiroyuki Noji, a Professor of the Department of Applied Chemistry, The University of Tokyo, is a Single-Molecule Biophysicist. He has been studying the chemomechanical coupling mechanism of FoF1 ATP synthase using single-molecule techniques. He is also known as an inventor of the femtoliter chamber array system for single-molecule enzymatic assays that is currently applied in digital bioassays such as digital ELISA. Inspired by studies on synthetic cells including JCVI-syn3, he is also trying to reconstitute "life" from molecules on the chamber system. Professor Noji was trained under the supervision of Prof. Masasuke Yoshida and received his Ph.D from Tokyo Institute of Technology in 1997. After a postdoctoral Fellowship in the laboratory of Prof. Kazuhiko Kinosita, Jr., he was appointed as an Associate Professor at the Institute of Industrial Science, The Universitfy of Tokyo in 2001. In 2005, he moved to the Institute of Scientific and Industrial Research, Osaka University as a full professor. Since 2010, he is a Professor of Department of Applied Chemistry, The University of Tokyo.

Social Program

Welcome Reception (included)

Welcome Reception will be held on 16th July, from 18:00 (6:00 p.m.) in Medium hall (**Osaka City Central Public Hall**). Food, beverages, and alcohol will be served. Cheers with crackers, microphone relay including remote members on Zoom are scheduled.

https://us02web.zoom.us/j/87446304281?pwd=b1VtdzFHVkVzMjJkWnVlRnhITWI2QT09
ID: 874 4630 4281 Pass code: 901804

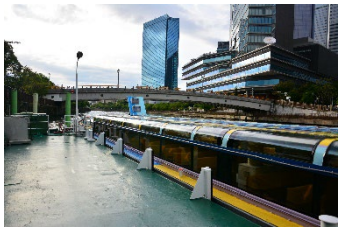
Banquet (optional)

Congress Banquet will be held at **TEN-NOU-DEN**, a traditional Japanese-style restaurant with a garden that you can see from the long corridor.
A Foreign Minister's Dinner at the G20 SUMMIT 2019 was held here. Kagamibiraki (Sake ceremony), speech around special lectures are scheduled.



Water Bus Tour (included)

Water bus tour is scheduled Mon and Tue afternoon during the convention. You can enjoy the view of Osaka from the river. The fee is included in the registration fee.



Pre-congress Symposium in JSM

Fiftieth Meeting of the Japanese Society of Mycoplasmology (JSM) will be held on 7/16 (Sun). For IOM participants, JSM prepares an English Session from 14:30 to 17:30 in Main hall. Please feel free to join.

Diverse Spectrum of Mycoplasma Infections
(Chair: Itaru Yanagihara/Tsuyoshi Kenri)

Potential role of mycoplasmas in the pathogenesis of human urologic diseases
Ourlad Alzeus G. Tangtengco (University of the Philippines Manila)

Laboratory detection of *Mycoplasma genitalium* infections and their clinical significance in women and neonates
Ken B. Waites (University of Alabama)

***Mycoplasma* (*Mycoplasmopsis*) *bovis* infections in veterinary medicine**
Satoshi Gondaira (Rakuno Gakuen University)

Phytoplasmas: Plant pathogenic mycoplasma-like organisms
Kensaku Maejima (The University of Tokyo)

Diagnostic methods and solutions of mycoplasma infections overlooked by having a diverse spectrum
Kazuhiro Matsuda (M Bio Technology Inc.)

Scientific Program At a Glance

	July 16th Sun.		July 17th Mon. (Holiday)	July 18th Tue.	July 19th Wed.	July 20th Thu.
9:30	9:50-10:30 JSM general meeting	9:30- IRPCM board meeting (Room #8)	**9:30-10:30 Opening ceremony & Group photo**	9:30-12:30 (with 15-minute break) **Oral Session 3** Vaccinology and Immunity Keynote: Andrew Peters	9:30-12:45 (with 15-minute break) **Oral Session 5** Virulence and Pathogenesis RFW Lecture: Kenro Oshima	9:30-12:45 (with 15-minute break) **Oral Session 7** Cell Biology and Genetics
10:00			10:30-12:45 **Oral Session 1** Clinical Microbiology / Mycoplasmology Keynote: Clifford Rosen			
	10:30-11:05 JSM Opening ceremony					
11:00	11:10-11:50 JSM Kitamoto award winner talk (in Japanese)					
12:00	12:00-13:00 JSM Luncheon seminar (in Japanese)	12:00- Taxonomy Committee Meeting (Room #8)	(In parallel) **IRPCM** 12:45- Genetics and Cell Biology (Main hall) 13:45- Human Mycoplasmas (Room #3) Avian Mycoplasmas (Room #4) *Lunch box provided	(In parallel) **IRPCM** 12:45- Plant and Arthropod Mycoplasmas (Main hall) Chemotherapy of Mycoplasma Infections (Room #4) 13:45- New and Emerging Mycoplasmas (Room #3) *Lunch box provided	(In parallel) **IRPCM** 12:45- Ruminant Mycoplasmas (Main hall) Porcine Mycoplasmas (Room#3) *Lunch box provided	Poster Award Talks *Lunch box provided
12:45-14:45 Lunch break	13:15-14:15 JSM Public lecture (in Japanese)					
	14:15- Coffee break	13:30- IOM board meeting (the special room)	13:00- **Poster room open** (Medium hall, 3F)			
15:00			**15:00-16:30 Water Bus Tour**	**15:00-16:30 Water Bus Tour**		**15:00-16:30 General Meeting & Closing Ceremony**
16:00	14:45-17:45 **Pre-congress symposium** (in English)				14:45-18:15 (with 15-minute break) **Oral Session 6** Development of Research Tools Keynote: Hiroyuki Noji	
17:00			16:30-19:30 **Oral Session 2** Phylogeny, Taxonomy, and Nomenclature in the Genomics Era PH Lecture: Vicki Chalker DE Lecture: Chih-Horng Kuo	16:30-19:30 (with 15-minute break) **Oral Session 4** Next Generation Vaccines and Host-Pathogen Interactions EKN Lecture: Glenn Browning		18:15 **Poster room closed**
18:00						
19:00	**18:00-20:30 Welcome party** (Medium hall, 3F)				**Banquet** (Move to the Banquet venue by bus)	
20:00			19:30-21:30 **Poster session with Food & Drink** (Medium hall, 3F) (20:00- IOM Board Dinner)	19:30-21:30 **Poster session with Food & Drink** (Medium hall, 3F)		
21:00						

Scientific Program Daily Overview

Speaker is marked by underline

* Nominee of The Louis Dienes Award

** Nominee of The Harry Morton Student Award

Monday 07/17

9:30–10:30 **Opening Ceremony and Group photo taking**
Greeting from chairs, Introductions of IRPCM and Taxonomy meeting, Instruction for this congress.

Session 1 Clinical Microbiology / Mycoplasmology
Chair: T. Prescott Atkinson, Meghan May

10:30–11:30 **Keynote-1 Post acute sequelae of SARS Cov2: A long way to go**
Clifford J Rosen

11:30–11:45 **O-1 Challenges in Distinguishing between Mycoplasma and COVID-19 Co-infection: Two Case Reports**
Kazuhiro Matsuda

11:45–12:00 **O-2 The Association between Genital Mycoplasmas Colonization and Adverse Pregnancy Outcomes—A Population-Based Retrospective Study from China**
**Yan Xuan, Tao Yan, Xiang Hong, Xu Zhou, Jun Zhao, Bei Wang

12:00–12:15 **O-3 Diagnostic Investigation of *Mycoplasma hyorhinis* as a Potential Pathogen Associated with Neurological Clinical Signs and Central Nervous System Lesions in Pigs**
**Calvin ChengYu Ko, Maria Merodio, Ethan Spronk, James Lehman, Huigang Shen, Ganwu Li, Rachel Derscheid, Pablo Piñeyro

12:15–12:30 **O-4 Prevalence of Macrolide and Fluoroquinolone Resistance in *Mycoplasma genitalium* in Metropolitan and Overseas France in 2021**
Sabine Pereyre, Cécile Laurier-Nadalié, Nadège Hénin, Amandine Dolzy, Marie Gardette, Cécile Bébéar

12:30–12:45 **O-5 Molecular genotyping of *Mycoplasma genitalium* strains in the United States**
Joshua T. Waites, Amy E. Ratliff, Donna M. Crabb, Barbara Van Der Pol, Jodie A. Dionne, William M. Geisler, Arthur H. Totten, Ken B. Waites, Li Xiao

12:45–13:45 **IRPCM** Molecular Genetics and Cell Biology

13:45–14:45 **IRPCM** Clinical Aspects of Human Mycoplasmas

13:45–14:45 **IRPCM** Avian Mycoplasmas

Session 2 Phylogeny, Taxonomy, and Nomenclature in the Genomics Era

Chair: Mitchell F. Balish, Ana S. Ramirez

16:30–17:30 **IOM Derrick Edward Award**
Mollicutes Taxonomy: A Perspective from Evolutionary Genomics
Chih-Horng Kuo

17:30–17:45 **O-6 Identification of a novel *Mycoplasma* species from an infection caused by a cat bite**
Danielle L. Gilbert, Donna M. Crabb, Johnathon Young, Juston Meier, Michele Granada, Casey Rice, Aneesa Afroze, Amy E. Ratliff, Thomas P. Atkinson, Ken B. Waites, Li Xiao

17:45–18:00 **O-7 Phytoplasma Taxonomy: Bridging the Gap with Taxogenomics**
Amit Yadav, Bhavesh Tiwarekar, Kiran Kirdat, Shivaji Sathe

18:00–18:15 **O-8 Examining Molecular Diversity of *Ureaplasma* Species Isolated from Neonates in the Global Multi-Country Child Health and Mortality Prevention Surveillance (CHAMPS) Network**
Jessica Lindsay Waller, Eungi Yang, Vasanta Chivukula, Will A. Overholt, Mahika Kadam, Maureen H. Diaz, Jonas M. Winchell

18:15–18:30 **O-9 Revised Minimum Standards for Valid Publication of Novel Species within the Class Mollicutes**
Meghan May, Vicki Chalker, Mitchell F. Balish, Alain Blanchard, Assunta Bertaccini, Joachim Frey, Gail E. Gasparich, Ludwig Holzle, Peter Kuhnert, Chih-Horng Kuo, Lucia Manso-Silvan, Ana S. Ramirez Corbera, Joachim Spergser, O. Brad Spiller, Emma L. Sweeney, Dmitriy Volokhov, Wei Wei

18:30–19:30 **IOM Peter Hannan Award**
“If you do not know the names of things, the knowledge of them is lost” Carl Linnaeus
Vicki Chalker, Mitchell F. Balish, Assunta Bertaccini, Alain Blanchard, Gail Gasparich, Ludwig Hölzle, Peter Kuhnert, Chih-Horng Kuo, Lucia Manso-Silvan, Meghan A. May, Ana Sofía Ramírez Corbera, Joachim Spergser, O. Brad Spiller, Emma Sweeney, Dmitriy Volokhov, Wei Wei

19:30–20:30 **Poster Session (20:00-20:30 Odd numbers, 20:30-21:00 Even numbers)(Dinner, Beverages provided)**

Tuesday 07/18

Session 3 Vaccinology and Immunity

Chair: Rohini Chopra-Dewasthaly, Jörg Jores

9:30–10:30 **Keynote-2 Current thoughts on Livestock *Mycoplasma* vaccines**
Andrew R Peters

10:30–10:45 **O-10 Evaluating the Dynamics and Efficacy of a Live, Attenuated *Mycoplasma anserisalpingitidis* Vaccine Candidate Under Farm Condition**
Dénes Grózner, Alexa Mitter, Dominika Buni, Katinka Bekő, Zsuzsa Kreizinger, Áron B. Kovács, Enikő Wehmann, Nikolett Belecz, Karola Költő, Veronika Hrivnák, Miklós Gyuranecz

10:45–11:00 **O-11 Clinical Efficacy of an Autogenous Killed Vaccine for *Mycoplasma ovipneumoniae* Used during a Pneumonia Outbreak in Saanen Goats in Northern Italy**
Marco Bottinelli, Davide Prataviera, Verdiana Righetti, Giorgia Nai, Monica Cagiola, Giulio Severi, Veronica Conci, Annalucia Tondo, Giovanni Martini, Matteo Cornaggia, Marianna Merenda

11:00–11:15 **O-12 Efficacy of a New Inactivated *Mycoplasma hyorhinis* Vaccine in Commercial Pigs**
Jia Wang, Yuan Gan, Yuanyuan Huang, Yanna Wei, Ting Yuan, Lei Zhang, Guoqing Shao, Zhixin Feng, Qiyan Xiong

11:15–11:30 Break

11:30–11:45 **O-13 Profiling *Mycoplasma hyosynoviae* Antibodies in Dams and Piglets**
Haley Schwecke, Emily McDowell, Amanda Sponheim, Joel Nerem, Robert Valeris-Chaci, Maria Pieters

11:45–12:00 **O-14 The role of pulmonary interstitial macrophages in *Mycoplasma pneumoniae* induced inflammation**
*Arlind B. Mara, Xin Li, Kavita Rawat, William T. King, Claudia V. Jakubzick

12:00–12: 15 **O-15 Synergism of bovine mammary epithelial cells and bovine peripheral blood mononuclear cells enhances immunological responses to *Mycoplasmopsis bovis***
**Noriko Imaizumi, Satoshi Gondaira, Tomochika Sugiura, Hidetoshi Higuchi

12:15–12: 30 **O-16 Insights from 16-year Mycoplasma Culture and PCR Data Analysis in a Canadian Provincial Laboratory**
Hugh Yuehua Cai, Pauline Nelson-Smikle, Fernando Munevar

12:45–13:45 **IRPCM** Plant and Arthropod Mycoplasmas
12:45–13:45 **IRPCM** Chemotherapy of Mycoplasma Infections

13:45–14:45 **IRPCM** New and Emerging Species

Session 4 Next Generation Vaccines and Host-Pathogen Interactions

Chair: Amir Noormohammadi, Steven J. Geary

16:30–17:30 **IOM Emmy Klieneberger-Nobel Award**
Mycoplasmas - Not That Simple, But Increasingly Understandable and Controllable
Glenn F. Browning

17:30–17:45 **O-17 Global Transcriptional Changes in the Trachea After Vaccination and Infection with *Mycoplasma gallisepticum* in Immunosuppressed Chickens**
*Sathya N. Kulappu Arachchige, Anna Kanci Condello, Nadeeka K. Wawegama, Glenn F. Browning

17: 45–18:00 **O-18 Genome engineering of the major goat pathogen *Mycoplasma capricolum* subsp. *capripneumoniae* as a first step towards the rational design of improved vaccines**
Géraldine Gourgues, Lucia Manso-Silvan, Gabrielle Guesdon, Catherine Chaberland, François Thiaucourt, Pascal Sirand-Pugnet, Vincent Baby, Alain Blanchard, Carole Lartigue

18: 00–18:15 Break

18: 15–18:30 **O-19 Quantification of the effect of vaccination on the control of horizontal transmission of *M. synoviae* under field conditions**
Christiaan ter Veen, Inge Santman-Berends, Marieke Augustijn, Anneke Feberwee

18: 30–18:45 **O-20 MyMIC: a Network for Standardization of Diagnostics, Antimicrobial Susceptibility Testing and Clinical Interpretation in Animal Mycoplasmas**
Anne V. Gautier-Bouchardon, Maryne Jaÿ, Sara M. Klose, Annet Heuvelink, Inna Lysnyansky, Miklós Gyuranecz, Ana S. Ramirez, Marco Bottinelli, Anne Ridley, Anneke Feberwee, Claire Becker, Florence Tardy

18: 45–19:00 **O-21 Fever-like temperature impairs the bovine ex vivo response to *Mycoplasmopsis bovis***
Thomas Démoulins, Thatcha Yimthin, Dorothea Lindtke, Sergi Torres-Puig, Lukas Eggerschwiler, Raphael Siegenthaler, Nicolas Ruggli, Fabien Labroussaa, Jörg Jores

19: 00–19:15 **O-22 *Mycoplasma hyrohinis* Hijacks Host Plasminogen/Plasmin System via Multiple Surface Moonlighting Proteins to Enhance Its Spread Across the Extracellular Matrix Barrier**
Qiyan Xiong, Qiyan Xiong, Jia Wang, Yao Li, Longji Pan, Yanfei Yu, Guoqing Shao, Zhixin Feng

19: 15–19:30 **O-23 The Secreted MbovP475 of *Mycoplasma bovis* Induces Macrophage Pro-inflammatory Response through CRYAB**
*Aizhen Guo, Shujuan Wang, Gang Zhao, Doukun Lu, Yingyu Chen, Xi Chen, Changmin Hu, Jianguo Chen

19:30–20:30 **Poster Session (20:00-20:30 Even numbers, 20:30-21:00 Odd**

numbers)(Dinner, Beverages provided)

Wednesday 07/19

Session 5 Virulence and Pathogenesis
Chair: Arlind Mara, Christine Citti

9:30–10:30 **IOM Robert F. Whitcomb award**
Molecular mechanisms of plant manipulation by phytoplasmas
Kenro Oshima

10:30–10:45 **O-24 Comparative Genomic Analysis of *M. agalactiae* Strain GM1309 Unravels its Peculiar Surface Architecture and Distinct Pathogenicity Traits**
Rohini Chopra-Dewasthaly, Maysa Santos Barbosa, Joachim Spergser

10:45–11:00 **O-25 Studying Interactions between 12 *Mycoplasmopsis bovis* and Bovid Tissue Culture**
*Elhem Yacoub, Antonio Ruzzini, Murray Jelinski

11:00–11:15 Break

11:15–11:30 **O-26 Modulation of transcription factors involved during Phytoplasma-associated phyllody development in *Sesamum indicum* L. (Sesame) plants**
**Pratima Verma, Suman Lakhanpaul, Ramya Parakkunnel

11:30–11:45 **O-27 The *Mycoplasma genitalium* biofilm-associated exopolysaccharide is a glucosamine polymer in the furanose configuration**
James M. Daubenspeck, Prescott T. Atkinson

11:45–12:00 **O-28 The MnuA Nuclease and 5'-Nucleotidase Impact *Mycoplasma bovis* Fitness in Mastitis**
Peleg Schneider, Yaa Amoah, Christine Citti, Emilie Dordet-Frison, Nahum Yehuda Shpigel, Inna Lysnyansky

12:00–12:15 **O-29 Comprehensive search for amino acid residues involved in target recognition of a phytoplasma effector protein by random mutagenesis-based screening**
**Oki Matsumoto, Yugo Kitazawa, Nozomu Iwabuchi, Kensaku Maejima, Masato Suzuki, Juri Matsuyama, Hiroaki Koinuma, Kenro Oshima, Shigetou Namba, Yasuyuki Yamaji

12:15–12:30 **O-30 The non-canonical host proteasome utilization by a phytoplasma effector: phyllogen acts as a ubiquitin-like mediator for proteasomal degradation of target proteins**
Yugo Kitazawa, Nozomu Iwabuchi, Kensaku Maejima, Momoka Sasano, Oki Matsumoto, Hiroaki Koinuma, Ryosuke Tokuda, Masato Suzuki, Kenro Oshima, Shigetou Namba, Yasuyuki Yamaji

12:15–12:45 **O-31 Monopodial to sympodial shift in *Phalaenopsis*: phytoplasma SAP11 effector breaks repression of axillary buds**
Jun-Yi Yang, Choon Meng Tan, Swee Suak Ko, Wen-Chieh Tsai, Yi-Ching Chiu

12:45–13:45 **IRPCM** Ruminant Mycoplasmas
12:45–13:45 **IRPCM** Porcine Mycoplasmas

Session 6 Development of Research Tools (Supported by JST CREST "Genome Programming" Project)

Chair: Chih-Horng Kuo, Pascal Sirand-Pugnet

14:45–15:45 **Keynote-3 Artificial cell reactor technology**
Hiroyuki Noji

15:45–16:00 **O-32 Minimal cell JCVI-syn3B as a chassis to investigate host-microbe interaction**
Daniela Matias de Carvalho Bittencourt, David M. Brown, Nacyra Assad-Garcia, Michaela R. Lynott, Lijie Sun, Luis Alberto M. Palhares de Melo, Marcelo Freire, John I. Glass

16:00–16:15 **O-33 Development of the Toolbox for *Mycoplasma* Genome Engineering**
Carole Lartigue, Thomas Ipoutcha, Fabien Rideau, Luis Garcia-Morales, Estelle Ruiz, Gabrielle Guesdon, Geraldine Gourgues, Alain Blanchard, Yonathan Arfi, Pascal Sirand-Pugnet

16:15–16:30 **O-34 Engineering a *Mycoplasma* Surface Display tool for Protein-Protein Interaction Screening**
Javier Gonzalez de Miguel, Irene Rodriguez Arce, Daniel Gerngross, Ariadna Montero Blay, Luis Serrano Pubul

16:30–16:45 Break

16:45–17:00 **O-35 Generating *Mycoplasma bovis* gene knock-out mutants using the CRISPR/Cas9 system of *Mycoplasma gallisepticum***
Nadeeka Kumari Wawegama, Sara Klose, Glenn Francis Browning

17:00–17:15 **O-36 Open Genome Browser for Mollicutes: A User-friendly and Powerful Web Platform for Comparative Genomics**
Thomas Roder, Hatice Akarsu, Fabien Labroussaa, Sergi Torres-Puig, Jörg Jores, Rémy Bruggmann

17:15–17:30 **O-37 Establishment of a *Mycoplasma hyorhinis* Challenge Model in Five-Week-Old Piglets**
Dorottya Földi, Zsófia Eszter Nagym, Nikolett Belecz, Levente Szeredi, József Földi, Anna Kollár, Miklós Tenk, Zsuzsa Kreizinger, Miklós Gyuranecz

17:30–17:45 **O-38 Homologous and heterologous plasmid-assisted expression of Ig cleavage systems of Mollicutes in an engineered *Mycoplasma feriruminatoris***
Sergi Torres Puig, Hatice Akarsu, Silvia Crespo-Pomar, Thatcha Yimthin, Thomas Démoulins, Isabelle Brodard, Valentina Cippà, Bettina Trüeb, Horst Posthaus, Nicolas Ruggli, Peter Kuhnert, Fabien Labroussaa, Jörg Jores

17:45–18:00 **O-39 Bovine Tracheal Organoids vs 2D Cell Cultures as Models to Study *Mycoplasma bovis* Cell Invasion**
**Chintha K. Premachandre, Pin Shie Quah, Bang Manh Tran, Elizabeth Vincan, Georgia Deliyannis, Glenn F. Browning, Paola K. Vaz, Nadeeka K. Wawegama

18:00–18:15 **O-40 The conjugative properties of *Mycoplasma agalactiae* are critically influenced by the expression of lipoprotein P48 at the surface of the recipient mating partner**
M'hamed Derriche, Laurent-Xavier Nouvel, Christine Citti, Eric Baranowski

Thursday 07/20

Session 7 Cell Biology and Genetics
(Supported by JST CREST "Genome Programming" Project)
Chair: Inna Lysnyansky, Steven P. Djordjevik

9:30–10:00 **Plenary**
Design, Construction, and Analysis of a Synthetic Minimal Bacterial Cell
John I. Glass

10:00–10:15 **O-41 Genomic analysis of *Mycoplasma bovis* causing bovine respiratory disease in Australian feedlot cattle**
*Veronica Maria Jarocki, Mauida Al Khallawi, Tony Batterham, Kiro Petrovski, Darren Trott, Steven Djordjevic

10:15–10:30 **O-42 Nucleotide-Binding Sites Visualized by CryoEM Suggest Rotational Movement of the Motor for *Mycoplasma mobile* Gliding**
Takuma Toyonaga, Takayuki Kato, Akihiro Kawamoto, Tomoko Miyata, Tasuku Hamaguchi, Keisuke Kawakami, Junso Fujita, Keiichi Namba, Makoto Miyata

10:30–10:45 **O-43 Horizontal transfer of phytoplasma effector phyllogen genes is driven by potential mobile units**
Nozomu Iwabuchi, Ryosuke Tokuda, Yugo Kitazawa, Takamichi Nijo, Masato Suzuki, Kensaku Maejima, Kenro Oshima, Shigetou Namba, Yasuyuki Yamaji

10:45–11:00 **O-44 Redundancy and essentiality of cytoskeleton protein components in *Spiroplasma* helicity and motility**
Yorick Dahan, Bastien Lambert, Alexandre Vilquin, Julien Pérochon, Marie-Pierre Dubrana, Sybille Duret, Pananghat Gayathri, Jean-Paul Douliez,

Laure Béven

11:00–11:15 Break

11:15–11:30 **O-45 Essential protein P116 extracts cholesterol and other indispensable lipids for Mycoplasmas**
Jesús Martín Romero, David Vizarraga Revuelto, Lasse Sprankel

11:30–11:45 **O-46 Bioinformatic and Metabolomic Analyses Reveal the Potential Metabolic Functions of Genes Critical for Survival of *Mycoplasma bovis* in Cell Culture**
**Shijie Geng, Chintha K. Premachandre, Shukriti Sharma, David P. De Souza
Sheik Nadeem Elahee Doomun, Jordi Hondrogiannis, Glenn F. Browning
Kelly A. Tivendale, Nadeeka K. Wawegama, Sara M. Klose

11:45–12:00 **O-47 Inositol metabolism is the essential factor to the growth and virulence of *Mycoplasma hyopneumonia***
*Xing Xie, Fei Hao, Qiyan Xiong, Maoda Pang, Yanna Wei, Rong Chen,
Zhenzhen Zhang, Wenbin Bao, Guoqing Shao, Daesub Song, Zhixin Feng

12:00–12:15 **O-48 *Mycoplasma pneumoniae* biofilms and the regulation of glycerol 3-phosphate oxidase activity by oxygen**
Mitchell F. Balish, Zoe E. Dapore, Ethan F. Boley, Davidson U. Nzenwata

12:15–12:30 **O-49 The Family of Type-II DNA-Methyltransferases of *Metamycoplasma hominis* including the first postulated type II MTase phase-variants**
Birgit Henrich, Lars Vogelgsang, Azlan Nisar, Sebastain Scharf,
Anna Rommerskirchen, Dana Belick, Alexander T. Dilthey

12:30–12:45 **O-50 *Mycoplasma bovis* Nucleomodulin MbovP202 Inhibits Macrophage Proliferation by DNA methylation**
**Doukun Lu, Gang Zhao, Jiongxi Chen, Shujuan Wang, Yingyu Chen, Xi Chen,
Changmin Hu, Jianguo Chen, Aizhen Guo

15:00–16:30 **General Meeting & Closing Ceremony**
Reports from IRPCM teams, Taxonomy meeting, LOC.
IOM Founders Award, Summary and start to Gran Canaria

Poster Presentation Overview

Category 1. Clinical Cases

P-001 *Tet44*: A New Ribosomal Protection Protein Conferring Tetracycline Resistance in Clinical Isolates of *Mycoplasma bovis*
**Alexandra M. Burne, Melanie Huhta, Mary B. Brown

P-002 Phenotypic and Genetic Characterization of an Atypical Virulent *Mycoplasma synoviae* Strain
Naola Ferguson-Noel

P-003 A Case Study of Swine Arthritis Caused by *Mycoplasma hyopharyngis*
Dorottya Földi, László Makrai, Gergely Tóth, Miklós Gyuranecz

O-3 Diagnostic Investigation of *Mycoplasma hyorhinis* as a Potential Pathogen Associated with Neurological Clinical Signs and Central Nervous System Lesions in Pigs
**Calvin ChengYu Ko, Maria Merodio, Ethan Spronk, James Lehman, Huigang Shen, Ganwu Li, Rachel Derscheid, Pablo Piñeyro

Category 2. Diseases

P-004 Genetic characterization of *Mycoplasma pneumoniae* strains isolated in Japan: Spread of p1 gene type 2c and 2j variant strains
Tsuyoshi Kenri, Tsutomu Yamazaki, Hitomi Ohya, Michio Jinnai, Yoichiro Oda, Sadasaburo Asai, Rikako Sato, Nobuhisa Ishiguro, Tomohiro Ohishi, Atsuko Horino, Hiroyuki Fujii, Toru Hashimoto, Hiroshi Nakajima, Keigo Shibayama

P-005 The identification of adhesive and proinflammatory function of MYPE6810, a membrane lipoprotein from *Mycoplasma penetrans*
Yuxin Li, Rong Li, Youyuan Ye, Yixue You, Peng Liu

P-006 Genome-Wide Association Study to Investigate Genetic Markers for Antimicrobial Resistance in *Mycoplasma cynos* and *Mycoplasma felis*
*Grazieli Maboni, Jeremy Chang, Walter Demczuk, Priyatharshini Ramesh, Irene Martin, Jeff Caswell, Michael Beeton

P-007 Advancement in the Development of a Safe and Efficacious Subunit Vaccine against *Mycoplasma gallisepticum*
**Jeremy M. Miller, Rosemary G. Ozyck, Edan R. Tulman, Steven M. Szczepanek, Jessica B. Malek, Lawrence K. Silbart Steven J. Geary

P-008 Comparison of Next-Generation Sequencing (NGS) Protocols for Diagnosis of Phytoplasmas
**Barbaros Mulayim, Isil Tulum

P-009 Abattoirs and Herds Survey to Monitor the Occurrence and Distribution of *Mycoplasma* Infections in Ruminants in Nigeria
Isaac Dayo Olorunshola, Oluwafemi Babatunde Daodu, Issa Atanda Muraina, Owen Gunner, Anne Ridley, David Sunday Adegboye, Andy Rowland Peters, Robin A.J. Nicholas

P-010 Using Live Attenuated Vaccine, MslA 2204, Co-administered with a SiRNA Polyfunctional Nanoparticle Targeted Against Inflammatory Genes Up-regulated During *Mycoplasma gallisepticum* Infection
**Rosemary Grace Ozyck, Rachelle Canete, Jessica Rouge, Jessica Beaudet Malek, Lawrence K. Silbart, Steven J. Geary

P-011 Status of Phytoplasma Causing Diseases in the Philippines
Marita Sanfuego Pinili, Darwin Magsino Landicho, Sotaro Chiba

P-012 Abstract withdrawal

P-013 Development of Droplet Digital PCR (ddPCR) Technique for Quantitative Detection of Phytoplasmas
**Yigit Sabri Unlu, Isil Tulum

P-014 *Spiroplasma eriocheiris* entered *Drosophila* Schneider 2 cells and relied on clathrin-mediated endocytosis and macropinocytosis
Panpan Wei, Mingxiao Ning, Meijun Yuan, Xiangqian Li, Hao Shi, Wei Gu, Peng Liu, Wen Wang, Qingguo Meng

P-015 Visualization of Phytoplasma Infection in Plant Tissues
**Kadir Boztas, Hamide Deniz Kocabag, Kayhan Derecik, Mona Gazel, Hikmet Murat Sipahioglu, Kadriye Caglayan, Isil Tulum

P-016 The Detection of Avian *Mycoplasma* spp. in Fecal Matter from Poultry
Mattie Capehart, Michael Davis, Marianne Dos Santos, Naola Ferguson-Noel

P-017 The Detection of *Mycoplasma synoviae* in Fecal Matter from Poultry
Michael Rose Davis, Mattie Capehart, Marianne Dos Santos, Naola Ferguson-Noel

P-018 In Silico Analysis of The Putative Phytoplasma Effector SAP55 that Mimics the Lipidation Mechanism of G Proteins
**Kayhan Derecik, Isil Tulum

P-019 Identification of antigenic *Mycoplasma bovis* proteins using North American Bison (Bison bison) convalescent sera
Bryan Seth Kaplan, Danielle E. Buttke, Rohana P. Dassanayake, Eduardo Casa, John D. Lippolis, Jarlath E. Nally

P-020 Genome Comparison of Spiralin, P58, P89 Regions of Naturally Infected and in vitro Cultures of *Spiroplasma citri* from Different Hosts in Turkey
**Busra Kara, Kadriye Caglayan, Isil Tulum

O-2 The Association between Genital Mycoplasmas Colonization and Adverse

Pregnancy Outcomes—A Population-Based Retrospective Study from China
**Yan Xuan, Tao Yan, Xiang Hong, Xu Zhou, Jun Zhao, Bei Wang

O-15 Synergism of bovine mammary epithelial cells and bovine peripheral blood mononuclear cells enhances immunological responses to *Mycoplasmopsis bovis*
**Noriko Imaizumi, Satoshi Gondaira, Tomochika Sugiura, Hidetoshi Higuchi

Category 3. Identification, Detection & Diagnosis

P-021 *Mycoplasma floridensis* sp. nov. from Cell Lines Developed for Diagnostic and Pathogen Discovery in Reptiles
Alexandra M. Burne, Robert J. Ossiboff, Mary B. Brown

P-022 Investigation on the Effects of Two Storage Methods of Diagnostic Samples on the Isolation of Avian *Mycoplasma* Strains
Veronica Conci, Marco Bottinelli, Micaela Picchi, Massimo Bottazzari, Ilenia Rossi, Salvatore Catania, Annalucia Tondo

P-023 The Development and Application of *Mycoplasma gallisepticum* Vaccine Strain Specific Quantitative PCR Protocols
Marianne Dos Santos, Naola Ferguson-Noel

P-024 The Occurrence of Infections with *Mycoplasma* spp. and *Ureaplasma* spp. in Clinical Cases of Respiratory Disorders in Polish Cattle Population
Katarzyna Dudek, Ewelina Szacawa, Dariusz Bednarek

P-025 A Visible Duplex Fluorescence-based LAMP assay for the Detection of *Mycoplasma bovis* and Infectious Bovine Rhinotracheitis Virus
Qing Fan, Zhixun Xie, Zhiqin Xie, Liji Xie, Sisi Luo, Meng Li, Yanfang Zhang, Jiaoling Huang, Tingting Zeng, Sheng Wang

P-026 Application of mucosal sIgA antibody in infection and immune evaluation of *Mycoplasma hyopneumoniae*
Zhixin Feng, Yun Bai, Yuan Gan, Yanna Wei, Ting Yuan, Lei Zhang, Li Wang, Bo Ni, Beibei Liu, Qiyan Xiong, Guoqing Shao

P-027 *Mycoplasma penetrans*, a Possible Urogenital Bystander More Frequent in HIV-Positive Men and Men Who Have Sex with Men
Marie Gardette, Arabella Touati, Cécile Laurier-Nadalié, Cécile Bébéar, Sabine Pereyre

P-028 Development and validation of three recombinase polymerase amplification (RPA) assays for direct and rapid detection of *Mycoplasma bovis*
Xiu jing Hao, Yang Han, Ying Zhang, Min Li

P-029 Comparison of Different Laboratory Techniques to Evaluate Growth Activity in vitro of Different *Mycoplasma hyopneumoniae* Strains
**Calvin ChengYu Ko, Pablo Pineyro, Chong Wang, Rachel Derscheid

P-030 Improving strain typing of *Mycoplasma ovipneumoniae* using multiplex PCR and Next Generation Sequencing
*Grazieli Maboni, Isaac Framst, Thomas Besser

P-031 *Mycoplasma wenyonii* circulates in cattle, deer and ticks
Laurent Xavier Nouvel, Marie-Claude Hygonenq, Chloé Saada, Laura Graziano, Nicolas Cèbe, Jean-Luc Rames, Vincent Bourret, Hélène Verheyden, Eric Baranowski, Renaud Maillard[1], Christine Citti

P-032 Serological and Molecular Detection of *Mycoplama gallisepticum*, *Mycoplasma synoviae* and *Mycoplasma meleagridis* in Free Range Chickens in Nigeria
Isaac Dayo Olorunshola, Oluwafemi Babatunde Daodu, Issa Atanda Muraina, Owen Gunner, Anne Ridley, David Sunday Adegboye, Andy Rowland Peters, Robin A.J. Nicholas

P-033 Evaluation of Commercial Customized Plates for *Ureaplasma* spp. and *Mycoplasma hominis* Antimicrobial Susceptibility Testing and Determination of Resistance Prevalence in France in 2020
Sabine Pereyre, Nadège Henin, Amandine Dolzy, Jennifer Guiraud, Marie Gardette, Cécile Bébéar

P-034 *Mycoplasma synoviae* Genotyping: Discrimination Power of Multiple Locus Variable-Number Tandem Repeat Analysis for Live Vaccine and Wild Strains
Elisabetta Stefani, Marco Bottinelli, Andrea Matucci, Michele Gastaldelli, Verdiana Righetti, Giorgia Nai, Veronica Conci, Annalucia Tondo, Zsuzsa Kreizinger, Miklós Gyuranecz, Salvatore Catania

P-035 Presence of Anti-*Mycoplasma bovis* Antibodies in Polish Cattle Suspected of *Mycoplasma bovis* Infection
Ewelina Szacawa, Katarzyna Dudek, Dariusz Bednarek

P-036 Screening of Cattle for the Presence of Anti-*Mycoplasma bovis* Antibodies in Poland
Ewelina Szacawa, Katarzyna Dudek, Dariusz Bednarek

P-037 Abstract withdrawal

P-038 A Multiplex Fluorescence-based Loop-mediated Isothermal Amplification Assay for Identifying *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and Avian orthoreovirus
Zhixun Xie, Qing Fan, Zhiqin Xie, Liji Xie Sisi Luo, Meng Li, Yanfang Zhang, Jiaoling Huang, Tingting Zeng, Mazhar Khan, Sheng Wang

Category 4. Epidemiology

P-039 Macrolide and Fluoroquinolone Resistance in *Mycoplasma genitalium* in Central Slovenia, 2017-2022
Darja Kese, Andreja Murnik Rauh, Mojca Maticic, Aljosa Obreza

P-040 Mollicutes study in freshwater fish
Ana Muniesa, Imanol Ruiz-Zarzuela, Juan C. Ramírez, José B. Poveda, Rubén S. Rosales, Ana S. Ramírez

P-041 Genetic Analysis of the *imp* Gene Encoding for the Immunodominant Membrane Protein of Phytoplasmas Associated with Lethal Yellowing Type Syndromes of Palms
Fabian Pilet

P-042 Detection of *Mycoplasma ovis* in Blood Samples from Small Ruminant Species in Cuba
Ana S. Ramirez, Roxana Marrero-Perera, Belkis Corona-González, Lisset Roblejo-Arias, Adrián A. Díaz-Sánchez, Rafael G. Matos-Rodríguez, Cristian Díaz-Corona, Yanet López-Dorta, Ernesto Vega-Cañizares, Evelyn Lobo-Rivero, Rubén S. Rosales, José B. Poveda

P-043 Intrauterine *Ureaplasma* is associated with small airway obstruction in extremely preterm infants
Xianya Zou, Hiroyuki Kitajima, Zhiqing Yuan, Yukiko Nakura, Itaru Yanagihara

Category 5. Chemotherapy & Resistance

P-044 Improved Antimicrobial Activity of Fatty Acylated Bovine NK-lysin-derived NK2A Peptide Against *Mycoplasma bovis*
Rohana Premachandra Dassanayake, William D. Boatwright, Bryan S. Kaplan, Erick M. Nicholson, Eduardo Casas

P-045 In Vitro Selection and Characterization of Resistance to Josamycin and Pristinamycin in *Mycoplasma genitalium*
Chloé Le Roy, Otgonjargal Byambaa, Carla Balcon[3], Cécile Bébéar, Sabine Pereyre

P-046 Apigenin suppresses mycoplasma-induced alveolar macrophages necroptosis via enhancing the methylation of TNF-α promoter by PPARγ-Uhrf1 axis
Xiuzhen Mei, Jian Wang, Zhixin Feng, Zhenzhen Zhang

P-047 Efflux transporters are involved in *Mycoplasma genitalium* tetracycline resistance
Li Xiao, Donna M. Crabb, Thomas P. Atkinson, Ken B. Waites, William M. Geisler

P-048 Antimicrobial Activity of Manuka Honey against Drug Resistant Mycoplasmas
Li Xiao, Donna M. Crabb, Melanie H. Fecanin, Thomas P. Atkinson, William M. Geisler

Category 6. Immunology & Vaccines

P-049 The Effect of Pegbovigrastim Administration on the Immune Response of

Calves Infected with *Mycoplasma bovis*
Katarzyna Dudek, Ewelina Szacawa, Magdalena Wasiak, Dariusz Bednarek, Michał Reichert

P-050 Non-replicating Vaccines for Better Control of *Mycoplasma gallisepticum* in Breeder Poultry Flocks
Kannan Ganapathy, Congriev Kumar Kabiraj

P-051 Evaluation of *Mycoplasma gallisepticum* (MG) ts-304 vaccine as a live attenuated vaccine in day-old specific pathogen-free (SPF) chicks
Anna Kanci Condello, Nadeeka K. Wawegama, Dilhani Ekanayake, Ling Zhu, Kelly A. Tivendale, Pollob K. Shil, Sameera Mohotti, Gregory J. Underwood, Philip Todhunder, Amir H. Noormohammadi, Glenn F. Browning

P-052 Identification of Vaccine Targets in Ruminant *Mycoplasma* Pathogens Using in Silico and Proteomic Approaches
Isaac Dayo Olorunshola, Stephen Fitzgerald, Kevin McLean, Tom McNeilly, Andy R. Peters

P-053 The molecular mechanism of *Mycoplasma bovis* lipid-associated membrane protein GLCP inhibiting host EBL cells apoptosis
Qiao Pan, Yujuan Zhang, Tong Liu, Qi Wu, Qingyuan Xu, Jiuqing Xin

P-054 RAMbo-V project: paving the way for the development of synthetic vaccines against *Mycoplasma bovis*
Fabien Rideau, Patrick Hogan, M'hamed Derriche, Géraldine Gourgues, Gwendoline Pot, Elisa Simon, Alain Blanchard, Renaud Maillard, Christine Citti, Laurent-Xavier Nouvel, Laure Béven, Carole Lartigue, Pascal Sirand-Pugnet[1], Yonathan Arfi, Eric Baranowski

P-055 Indigenous vaccine trials for mycoplasmosis in small ruminant of Pakistan
Muhammad Kamal Shah, Umer Sadique Khat, Shakeeb Ullah Khan, Muhammad Ijaz Ali, Hamayun Khan, Ali Zaman Paracha, Ata Ur Rehman

P-056 Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Activity in *Mycoplasma synoviae* and Vaccine Strain MS-H
Sahar Zare, Marc Serge Marenda, Sara Klose, Amir Hadji Noormohammadi

P-057 A secreted serine/threonine phosphatase encoded by Mbov_0725 of *Mycoplasma bovis* attenuate activation of MAPK p38 and ERK
Hui Zhang, Doukun Lu, Yiqiu Zhang, Yujia He, Shujuan Wang, Xi Chen, Yingyu Chen, Changmin Hu, Aizhen Guo

O-14 The role of pulmonary interstitial macrophages in *Mycoplasma pneumoniae* induced inflammation
*Arlind B. Mara, Xin Li, Kavita Rawat, William T. King, Claudia V. Jakubzick

O-17 Global Transcriptional Changes in the Trachea After Vaccination and Infection with *Mycoplasma gallisepticum* in Immunosuppressed Chickens
*Sathya N. Kulappu Arachchige, Anna Kanci Condello, Nadeeka K. Wawegama,

Glenn F. Browning

Category 7. Cell Biology, Physiology & Metabolism

P-058 Elucidating the Role of Fibril Protein in Spiroplasma Swimming using JCVI-syn3B
**Ali Ahsan, Hana Kiyama, Yuya Sasajima, Makoto Miyata

P-059 Elongated JCVI-syn3B Cell Caused by Expression of *Mycoplasma pneumoniae*-gliding Type Cytoadherence Regulatory Locus
**Muhammad Algiffari, Hana Kiyama, Daisuke Nakane, Tsuyoshi Kenri, Makoto Miyata

P-060 Electron Paramagnetic Resonance (EPR) Analysis on Nobel Protein Assemblies, Dimer of Hexameric F_1-like ATPases and Fibril Helical Filament, Involved in *Mycoplasma* Gliding and *Spiroplasma* Swimming
Toshiaki Arata, Kazunobu Sato, Takuma Toyonaga, Yuya Sasajima, Taiji Kanda, Isao Suetake, Akio Inoue, Takeji Takui, Toshimichi Fujiwara, Yoh Matsuki, Makoto Miyata

P-061 Importance of MreB5 ATPase activity in *Spiroplasma citri* helicity and motility
Yorick Dahan, Bastien Lambert, Sybille Duret, Marie-Pierre Dubrana, Vani Pande, Mrinmayee Bapat, Shrikant Harne, Jean-Paul Douliez, Pananghat Gayathri, Laure Béven

P-062 In silico analysis of cytoskeleton protein sequences in *Spiroplasma* species
Yorick Dahan, Bastien Lambert, Marie-Pierre Dubrana, Laure Béven

P-063 Gliding Machinery of *Mycoplasma mobile* Visualized by Electron Tomography
**Minoru Fukushima, Takuma Toyonaga, Yuhei O. Tahara, Daisuke Nakane, Makoto Miyata

P-064 Probing Interactions Between Adhesins and Attachment Organelle Core Proteins in *Mycoplasma pneumoniae*
Kristina A. Gara, Mitchell F. Balish

P-065 Cryo-EM observation of γ-polyglutamic acid hydrogels from *Bacillus subtilis* of Firmicutes
Tasuku Hamaguchi, Daisuke Unabara, Koji Yonekura

P-066 The analysis of *Spiroplasma eriocheiris* cell division proteins
Taishi Kasai, Yuhei O. Tahara, Makoto Miyata, Daisuke Shiomi

P-067 Mutational Analyses of MreB5 Essential for *Spiroplasma* Swimming Reconstructed in JCVI-syn3B
Hana Kiyama, Shigeyuki Kakizawa, Daichi Takahashi, Yuya Sasajima, Yuhei O. Tahara, Makoto Miyata

P-068 Morphology and motility of *Spiroplasma* species differing in their

cytoskeleton gene content
Bastien Lambert, Yorick Dahan, Alexandre Vilquin, Marie-Pierre Dubrana, Sybille Duret, Jean-Paul Douliez, Jean-Christophe Baret, Nicolas Martin, Laure Béven

P-069 A new *Spiroplasma* species with a highly reduced genome discovered from a coral metagenome in Okinawa
*Yumiko Masukagami, Jinyeong Choi, Maria E. A. Santos, Filip Husnik

P-070 Structure and Function of Gli123 Involved in *Mycoplasma mobile* Gliding
Daiki Matsuike, Yuhei O. Tahara, Takahiro Nonaka, Heng Ning Wu, Tasuku Hamaguchi, Hisashi Kudo, Yuuki Hayashi, Munehito Arai, Makoto Miyata

P-071 *Haloplasma* Motility Reconstituted in JCVI-syn3B by Combination of Two MreB Proteins
**Mone Mimura, Hana Kiyama, Shingo Kato, Yuya Sasajima, Atsuko Uenoyama, Shigeyuki Kakizawa, Andre Antunes, Tomoko Miyata, Fumiaki Makino, Keiichi Namba, Makoto Miyata

P-072 Using minimized mycoplasma JCVI-Syn3 to analyze *Ureaplasma* pathogenicity factors
Fumiko Nishiumi, Yukiko Nakura, Heng-Ning Wu, Shigeyuki Kakizawa, Yo Suzuki, John I. Glass, Itaru Yanagihara

P-073 Cell division in bacteria without a cell wall: Mechanism of action of the bacterial cell division proteins FtsZ and FtsA
Gayathri Pananghat, Joyeeta Chakraborty, Soumyajit Dutta, Vaishnavi Bahulekar

P-074 Impact of spiralin deletion on *Spiroplasma citri* membrane organization and composition, morphology and motility
Julien Pérochon, Yorick Dahan, Alexandre Vilquin, Julia Guéguéniat, Sybille Duret, Fabien Moroté, Marie-Pierre Dubrana, Touria Cohen-Bouhacina, Christine Grauby-Heywang, Laure Béven

P-075 Investigating the Glyco-Proteoform Landscape of Minimal Genome *Mycoplasma* spp.
**John W. Sanford, James M. Daubenspeck, Kevin Dybvig, T. Prescott Atkinson

P-076 Cryo electron microscopy of cytoskeletal 'fibril' protein involved in *Spiroplasma* swimming
*Yuya Sasajima, Takayuki Kato, Tomoko Miyata, Hana Kiyama, Akihiro Kawamoto, Fumiaki Makino, Keiichi Namba, Makoto Miyata

P-077 Structural and functional studies of antibodies halting motility in *Mycoplasma pneumoniae* reveal the dynamic nature of the adhesion complex
**Marina Marcos Silva, David Vizarraga Revuelto, Akihiro Kawamoto, Jesús Martín Romero, Fumiaki Makino, Tomoko Miyata, Makoto Miyata, Jaume Piñol Ribas, Keiichi Namba, Tsuyoshi Kenri, Ignacio Fita Rodriguez

P-078 Formation process of *Bacillus subtilis* L-form visualized by quick-freeze

deep-etch replica electron microscopy
Yuhei O. Tahara, Isil Tulum, Makoto Miyata

P-079 Assembly properties of bacterial actin MreB5 essential for *Spiroplasma* swimming
*Daichi Takahashi, Ikuko Fujiwara, Makoto Miyata

P-080 *Spiroplasma* swimming mechanism suggested by fluorescently labeled MreBs expressed in JCVI-syn3B
**Yoshiki Tanaka, Hana Kiyama, Makoto Miyata

P-081 Visualization of MreB4 and MreB5 Filaments Driving *Spiroplasma* Swimming by Using JCVI-Syn3B and Electron Microscopy
**Haruka Yuasa, Yuya Sasajima, Hana Kiyama, Daichi Takahashi, Takuma Toyonaga, Tomoko Miyata, Fumiaki Makino, Keiichi Namba, Makoto Miyata

O-46 Bioinformatic and Metabolomic Analyses Reveal the Potential Metabolic Functions of Genes Critical for Survival of *Mycoplasma bovis* in Cell Culture
**Shijie Geng, Chintha K. Premachandre, Shukriti Sharma, David P. De Souza Sheik Nadeem Elahee Doomun, Jordi Hondrogiannis, Glenn F. Browning Kelly A. Tivendale, Nadeeka K. Wawegama, Sara M. Klose

Category 8. Host-Pathogen Interaction (Virulence & Pathogenesis)

P 082 An atypical F-like ATPase is critical to the function of the antibody cleavage system MIB-MIP
Julien Berlureau, Laure Bataille, Robin Anger, Géraldine Gourgues, Carole Lartigue, Pascal Sirand-Pugnet, Yonathan Arfi

P-083 Novel In Planta Interaction of '*Candidatus* Phytoplasma solani' SAP11-like and SAP54-like Effectors With Plant Transcription Factors
Marina Drčelić, Bruno Polak, Andreja Škiljaica, Nataša Bauer, Martina Šeruga Musić

P-084 To Be or Not to Be! Complex Non-stochastic Dynamics of Variable Surface Lipoprotein Expression and Selection in *Mycoplasma agalactiae* in vivo
Reinhard Ertl, Rene Brunthaler, Melanie Stargardt, Debasmita Mishra, Joachim Spergser, Renate Rosengarten, Rohini Chopra-Dewasthaly

P-085 Characterisation of the Transcriptional Response of the Tracheal Mucosa of Chickens to Chronic *Mycoplasma synoviae* Infection
Kanishka I. Kamathewatta, Anna Kanci Condello, Sathya N. Kulappu Arachchige, Neil D. Young, Pollob K. Shil, Amir H. Noormohammadi, Nadeeka K. Wawegama, Glenn F. Browning

P-086 NOD2 activated by Duf16 in *Mycoplasma pneumoniae*
Hai Xia Luo, Yong Yu Wang, Min Li

P-087 Genome analysis of '*Candidatus* Phytoplasma pruni' strain PR2021 associated with poinsettia
**Shen-Chian Pei, Ting-Hsuan Hung, Chih-Horng Kuo

P-088 Immune Profiling of Experimental Murine Mastitis Reveals Conserved Response to Mammary Pathogenic *Escherichia coli*, *Mycoplasma bovis*, and *Streptococcus uberis*
Peleg Schneider, Hagit Salamon, Nathalie Weizmann, Einat Nissim-Eliraz, Inna Lysnyansky, Nahum Yehuda Shpigel

P-089 A feasibility study on cell growth inhibition of *Ureaplasma* species with *Lactobacillus acidophilus*
Hengning Wu, Michinobu Yoshimura, Yukiko Nakura, Fumiko Nishiumi, Xianya Zou, Masami Sato, Naomi Kokubo, Tomio Fujita, Shinsuke Fujiwara, Itaru Yanagihara

P-090 *Mycoplasma hyopneumoniae* membrane protein Mhp271 interacts with host UPR protein GRP78 to facilitate infection
Jiuqing Xin, Qiao Pan, Qingyuan Xu, Tong Liu, Yujuan Zhang

P-091 Hijacking of Host Plasminogen by *Mesomycoplasma* (*Mycoplasma*) *hyopneumoniae* via GAPDH: A Systematic Virulence Mechanism to Promote Adhesion and Extracellular Matrix Degradation
Yanfei Yu, Jiying Wang, Shiyang Li, Jia Wang, Qiyan Xiong, Zhixin Feng, Guoqing Shao

P-092 Abstract withdrawal

P-093 Novel Mycoplasma Nucleomodulin MbovP475 Decreased Cell Viability by Regulating Expression of CRYAB and MCF2L2
*Gang Zhao, Doukun Lu, Shujuan Wang, Aizhen Guo

O-23 The Secreted MbovP475 of *Mycoplasma bovis* Induces Macrophage Pro-inflammatory Response through CRYAB
*Aizhen Guo, Shujuan Wang, Gang Zhao, Doukun Lu, Yingyu Chen, Xi Chen, Changmin Hu, Jianguo Chen

O-25 Studying Interactions between 12 *Mycoplasmopsis bovis* and Bovid Tissue Culture
*Elhem Yacoub, Antonio Ruzzini, Murray Jelinski

O-39 Bovine Tracheal Organoids vs 2D Cell Cultures as Models to Study *Mycoplasma bovis* Cell Invasion
**Chintha K. Premachandre, Pin Shie Quah, Bang Manh Tran, Elizabeth Vincan, Georgia Deliyannis, Glenn F. Browning, Paola K. Vaz, Nadeeka K. Wawegama

O-41 Genomic analysis of *Mycoplasma bovis* causing bovine respiratory disease in Australian feedlot cattle
*Veronica Maria Jarocki, Mauida Al Khallawi, Tony Batterham, Kiro Petrovski, Darren Trott, Steven Djordjevic

O-47 Inositol metabolism is the essential factor to the growth and virulence of *Mycoplasma hyopneumonia*
*Xing Xie, Fei Hao, Qiyan Xiong, Maoda Pang, Yanna Wei, Rong Chen,

Zhenzhen Zhang, Wenbin Bao, Guoqing Shao, Daesub Song, Zhixin Feng

O-50 *Mycoplasma bovis* Nucleomodulin MbovP202 Inhibits Macrophage Proliferation by DNA methylation
**Doukun Lu, Gang Zhao, Jiongxi Chen, Shujuan Wang, Yingyu Chen, Xi Chen, Changmin Hu, Jianguo Chen, Aizhen Guo

Category 9. Mycoplasma Engineering & New Tools

P-094 New transformation method for JCVI-syn3B cells
Atsuko Uenoyama, Hana Kiyama, Makoto Miyata

P-095 Improved Transformation Efficiency in *Mycoplasma hominis* Allows Disruption of the MIB-MIP System Targeting Human Immunoglobulins
Jennifer Guiraud, Chloé Le Roy, Fabien Rideau, Pascal Sirand-Pugnet, Carole Lartigue, Cécile Bébéar, Yonathan Arfi, Sabine Pereyre

P-096 Adaptive laboratory evolution of JCVI-syn3.0B to low temperature
*Masaki Mizutani, Ryuichi Koga, Takema Fukatsu Shigeyuki Kakizawa

P-097 Reprogramming the synthetic cell JCVI-Syn3B for the production of active ingredients of interest to bio-based industry
Raquel Sampaio de Oliveira, Betúlia de Morais Souto, Betania Ferraz Quirino, John I. Glass, Elibio Rech, Daniela Matias de Carvalho Bittencourt

P-098 A proposal to use ciliate *Paramecium* as a natural host model
Takashi Shimizu, Masato Tachibana, Kenta Watanabe, Masahisa Watarai

P-099 Improved efficiency of mycoplasma genome transplantation by DNA repair
Kazuhito V. Tabata, Mana Ono, Nacyra Assad-Garcia, John I. Glass, J. Craig Venter, Masayuki Su'etsugu, Hiroyuki Noji

Category 10. Omics Studies

P-100 Remarkable Abundance of Potential Mobile Group II Introns in genome of PWB Phytoplasma Strain PR34
Kiran Kirdat, Bhavesh Tiwarekar, Shivaji Sathe, Amit Yadav

P-101 Genome analyses of '*Candidatus* Phytoplasma asteris' strains highlight heterogenicity and regularities
Rafael Toth, Anna Marie Ilic, Bruno Huttel, Michael Kube

P-102 Impact of Potential Mobile Units on Genome Stability in Phytoplasma Evolution
**Xiao-Hua Yan, Shen-Chian Pei, Chih-Horng Kuo

Category 11. Phylogeny & Taxonomy

P-103 Phylogenetic Comparison of *Mycoplasma gallisepticum* Sequenced Genomes for Relatedness to Commercial Avirulent Vaccine Strains
Spencer Leigh, Jeff Evans

P-104 New *Mycoplasma* Isolated from Portuguese Man o' War
Owen Brad Spiller, Ian C. Boostrom, Jordan A. T. Mathias, José B. Poveda, Rubén S. Rosales, Ana S. Ramírez

Category 12. Plant & Insect Mollicutes

P-105 Molecular characterizations of *Raphanus sativus* L. witches'-broom disease and the genetic status of the '*Candidatus* Phytoplasma aurantifolia' strains (16SrII-V) in Yulin, Taiwan
**Yi-Ching Chiu, Pei-Qing Liao, Yuh-Kun Chen, Chih-Horng Kuo, Jun-Yi Yang

P-106 Undetectable nature of phytoplasma in Cassava witches' broom diseased leaf tissues by conventional methods
Darwin M. Landicho, Ray Jerome M. Montañez, Marita S. Pinili, Sotaro Chiba

P-107 A genomics framework to phytoplasma taxonomy: The 16SrII and 16SrXXV phytoplasmas as a case study
Bianca Rodrigues Jardim, Lucy T. T. Tran-Nguyen, Cherie Gambley, Ali M. Al-Subhi, Xavier Foissac, Hong Cai, Jun-Yi Yang, Richard Davis, Lynne Jones, Brendan Rodoni, Fiona E. Constable

P-108 A large-scale investigation into phytoplasma diversity in Australia using metagenomics
Bianca Rodrigues Jardim, Cherie Gambley, Lucy T. T. Tran-Nguyen, Craig Webster, Monica Kehoe, Samantha Bond, Richard Davis, Lynne Jones, Nandita Pathania, Fiona E. Constable, Brendan Rodoni

P-109 Towards understanding of a versatile pathogen – quest for putative effectors in '*Candidatus* Phytoplasma solani' genomes
Martina Šeruga Musić, Bruno Polak, Marina Drčelić, Shen-Chian Pei, Chih-Horng Kuo

P-110 Target degradation specificity of a phytoplasma effector protein phyllogen is regulated by an ability to recruit host proteasome shuttle protein
**Masato Suzuki, Nozomu Iwabuchi, Yugo Kitazawa, Kensaku Maejima, Juri Matsuyama, Oki Matsumoto, Kenro Oshima, Shigetou Namba, Yasuyuki Yamaji

O-26 Modulation of transcription factors involved during Phytoplasma-associated phyllody development in *Sesamum indicum* L. (Sesame) plants
**Pratima Verma, Suman Lakhanpaul, Ramya Parakkunnel

O-29 Comprehensive search for amino acid residues involved in target

recognition of a phytoplasma effector protein by random mutagenesis-based screening

**Oki Matsumoto, Yugo Kitazawa, Nozomu Iwabuchi, Kensaku Maejima, Masato Suzuki, Juri Matsuyama, Hiroaki Koinuma, Kenro Oshima, Shigetou Namba, Yasuyuki Yamaji

Abstracts

Symposia

Session 1 Clinical Microbiology / Mycoplasmology

Keynote-1 Post acute sequelae of SARS Cov2: A long way to go

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Although the SARS CoV-2 pandemic has officially ended, it remains a part of our daily lives. Acute infections persist, but without adequate surveillance, and recording, it is unclear what those numbers represent in terms of the ongoing spread of the virus and its mutant variants. Left behind the headlines are the millions of sufferers of long Covid, or PASC, who continue to manifest an array of symptomatology across virtually all organ systems. Progress has been made in characterizing long Covid, although a true definition is lacking. Symptoms cluster around three major areas, cardiovascular, neurologic, and gastrointestinal. In respect to pathophysiology, three leading hypotheses center on: 1-viral persistence; 2-auto antibody excess possibly due to reactivation of latent viruses; or 3-endothelial/vascular dysfunction. There are currently no treatments for long COVID that have proven efficacy but trials are just beginning both nationally and local. But metabolic dysfunction accompanies these syndromes within the larger complex of symptoms. Latent virus may persist in fat tissue and enhance the chronic inflammation that occurs with obesity. It may also lead to immune fatigue which could potentially worsen some of the symptomatology of long covid. Future studies will be discussed and outlook for the future will be examined.

O-1 Challenges in Distinguishing between Mycoplasma and COVID-19 Co-infection: Two Case Reports

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BACKGROUND: The COVID-19 outbreak has posed significant challenges in differentiating it from mycoplasma infection. This can lead to misdiagnosis, delayed treatment, and improper use of medical resources. The accurate diagnosis of patients with long COVID is also difficult. CASES: This study presents two cases of mycoplasma and COVID-19 co-infection, demonstrating the difficulties in distinguishing between these infections. In the first case, a patient with respiratory problems was denied admission due to a negative PCR test for COVID-19 but was later diagnosed with mycoplasma infection and showed improvement after starting antibiotic treatment. In the second case, a patient diagnosed with RA was refused admission due to the COVID-19 outbreak but was later diagnosed with mycoplasma infection and positive for antibodies to *M. fermentans*. The patient showed significant improvement after starting antibiotic treatment and later tested positive for COVID-19 but recovered successfully. RESULT and DISCUSSION: The study emphasizes the importance of accurate diagnostic methods in distinguishing between mycoplasma and COVID-19 infections, particularly in cases of co-infection. Reliable antibody testing for mycoplasma infection was found to be crucial in these cases. The study highlights the need for international

collaboration in developing effective measures for infection control and healthcare. The establishment of an international consortium for infectious disease control and healthcare could facilitate collaboration and knowledge sharing among healthcare professionals and researchers worldwide. In conclusion, this study underscores the challenges in diagnosing and treating mycoplasma and COVID-19 infections and highlights the importance of accurate diagnosis and international cooperation in infectious disease management.

O-2 The Association between Genital Mycoplasmas Colonization and Adverse Pregnancy Outcomes—A Population-Based Retrospective Study from China

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The colonization of mycoplasmas varied based on the current improved healthcare. The purpose of this study was to investigate the associations between mycoplasma colonization and adverse pregnancy outcomes in perinatal women, and to explore whether the associations were robust after adjusting, especially the group B streptococcus (GBS) infection and interaction. We conducted a population-based retrospective study in a teaching hospital in southwest China from March 2019 to December 2020. The data and vaginal swabs were collected during their first time and second/third trimester reaching clinic. Mycoplasmas were tested using polymerase chain reaction method. We used the logistic regression models to estimate odds ratios (ORs) and their corresponding 95% confidence intervals (95% CIs). A total of 259 perinatal women were included in this study, 102 of which were colonized by mycoplasmas and 87 had at least one adverse pregnancy outcome. The colonized group showed 1.74 times higher odds than the non-colonized (OR: 1.74, 95% CI: 1.03~2.95) in all adverse pregnancy outcomes, especially in those who had PROM (OR: 1.84, 95% CI: 1.03~3.31). However, the regression models of preterm birth (OR: 2.83, 95% CI: 0.27~61.36), macrosomia (OR: 2.64, 95% CI: 0.63~13.13), fetal distress (OR: 0.92, 95% CI: 0.19~3.8) and other adverse outcomes (OR: 0.33, 95% CI: 0.05~1.31) suggested no significant statistical associations. Female genital mycoplasmas colonization was associated with increased adverse pregnancy outcomes, especially in PROM. All results were robust after adjustion especially interactions and GBS infection. For perinatal women, a comprehensive consideration was recommended to provide personalized pregnancy guidance.

O-3 Diagnostic Investigation of *Mycoplasma hyorhinis* as a Potential Pathogen Associated with Neurological Clinical Signs and Central Nervous System Lesions in Pigs

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Mycoplasma hyorhinis (*M. hyorhinis*) is a commensal of the upper respiratory tract in swine with the typical clinical presentations of arthritis and polyserositis in postweaning pigs. However, it has also been associated with conjunctivitis and otitis media, and recently has been isolated from meningeal swabs and/or cerebrospinal fluid of piglets with neurological signs. The objective of this study is to evaluate the role of *M. hyorhinis* as a potential pathogen associated with neurological clinical signs and central nervous system lesions in pigs. The presence of *M. hyorhinis* was evaluated in a clinical outbreak and a six-year retrospective study by PCR detection, bacteriological culture, in situ hybridization (RNAscope®), phylogenetic analysis, and with immunohistochemistry characterization of the inflammatory response associated with its infection. *M. hyorhinis* was confirmed by bacteriological culture and within central nervous system lesions by in situ hybridization on animals with neurological signs during the clinical outbreak. The isolates from the brain had close genetic similarities from those previously reported and isolated from eye, lung, or fibrin. The retrospective study confirmed by PCR the presence of *M. hyorhinis* in 9.9% of cases reported with neurological clinical signs and histological lesions of encephalitis or meningoencephalitis of unknown etiology. *M. hyorhinis* mRNA was confirmed within cerebrum, cerebellum, and choroid plexus lesions by in situ hybridization (RNAscope®) with a positive rate of 72.7% of the PCR-positive cases. Here we present strong evidence that *M. hyorhinis* should be included as a differential etiology in pigs with neurological signs and central nervous system inflammatory lesions.

O-4 Prevalence of Macrolide and Fluoroquinolone Resistance in *Mycoplasma genitalium* in Metropolitan and Overseas France in 2021

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Monitoring macrolide and fluoroquinolone resistance is required in *Mycoplasma genitalium* because these antibiotics are the first and second-line treatments. We assessed the prevalence of macrolide and fluoroquinolone resistance-associated mutations in *M. genitalium*-positive patients in metropolitan and overseas France in 2021. A one-month and a three-month systematic prospective collection of *M. genitalium*-positive specimens was proposed to metropolitan France and French overseas diagnostic laboratories, respectively. Resistance-associated mutations were detected using commercial kits and 23S rRNA and *parC* gene sequencing. A total of 903 *M. genitalium*-positive specimens were analyzed. In metropolitan France, macrolide resistance was 35.2%, mainly due to A2059G (*Escherichia coli* numbering), A2058T and A2058G 23SrRNA substitutions. Macrolide resistance was significantly higher in men than in women (54.8% versus 22.0%, respectively, $p<0.001$) and in men who have sex with men than in heterosexual men (63.2% versus 20.0%, respectively,

p<0.05). Macrolide resistance prevalence was 19.4% in overseas France, mainly due to A2059G, A2059C and A2058T mutations. This resistance rate was significantly lower than in metropolitan France, with no significant difference between men and women. Fluoroquinolone resistance rates were significantly higher in metropolitan France than in overseas territories (17.7% versus 5.3%, respectively, p<0.001). Fluoroquinolone resistance was significantly higher in men (24.1% and 11.3%) than in women (12.8% and 2.3%) in both metropolitan and overseas French territories, respectively. Macrolide and fluoroquinolone resistance rates are high but stable in metropolitan France. In overseas France, macrolide and fluoroquinolone resistance rates are significantly lower but have been alarmingly and significantly rising since our last study in 2018.

O-5 Molecular genotyping of *Mycoplasma genitalium* strains in the United States

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Mycoplasma genitalium (MG) is a sexually transmitted pathogen with increasing macrolide and quinolone resistance worldwide, making treatment challenging. Little is known about MG transmission patterns in the United States. We performed molecular genotyping on *mgpB* (MG191) and MG309 by PCR and sequencing on 88 clinical specimens collected between 1997-2022 from 72 patients with a broad range of demographic characteristics and clinical presentations. Macrolide and fluoroquinolone resistance-associated mutations were determined by sequencing 23S rRNA gene and *gyrA*, *gyrB*, *parC* and *parE* genes. Among the successfully sequenced specimens, 69 (79.3%) carried macrolide resistance-associated mutations and 26 (38.2%) had fluoroquinolone resistance-associated mutations. Macrolide and fluoroquinolone resistant rate was significantly higher in males and increased over time. Phylogenetic analysis of MG191 sequences identified 33 sequence types. Among them, 18 types have been found in Europe, 1 was reported in Australia, and 14 were novel. Strains from men who have sex with men (MSM) showed potential clonal groupings. Concordant genotypes were observed in most heterosexual couples. Chronologic samples from the same patients showed close phylogenetic clustering. There was a cluster of 11 strains from different patients that were mainly susceptible to macrolides (8) and fluoroquinolones (7) and were contained inearlier specimens (1997-2001). Strains from different patients in the same cluster could be differentiated by MG309 types. MG191 sequence type was not associated with gender, collection year, symptomatic status, antimicrobial resistance status, or MG309 types. These results suggest MG strain types in the U.S. are diverse and emergence of drug-resistant strains is recent.

Session 2 Phylogeny, Taxonomy, and Nomenclature in the Genomics Era

IOM Derrick Edward Award

Mollicutes Taxonomy: A Perspective from Evolutionary Genomics

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[2023 IOM Derrick Edward Award Lecture] Taxonomy is the science and art of biological classification. Through the establishment of a hierarchical scheme and a generally accepted nomenclature system, taxonomy provides the foundation for communication that involves biological entities. To establish a robust classification scheme, a polyphasic approach that considers evidence from different methods is essential. With the advancements of genomics in the past decade, enormous genotypic data sets have become available, which in turn greatly facilitated phylogenetic inference. Such changes have impacted the taxonomy of nearly all biological groups, and the class Mollicutes is no exception. In this lecture, recent works involving Mollicutes taxonomy, particularly those subgroups associated with plants or arthropods, are systematically reviewed from a perspective of evolutionary genomics. The utilities of such works in the inference of species boundaries to assist classification, as well as the development of molecular markers for identification to assist diagnostics, are emphasized. Notable examples include a revision of the '*Candidatus* Phytoplasma' species description guidelines in 2022. Furthermore, for arthropod-associated mollicutes, the evolutionary genomics studies have led to the union of *Mesoplasma* with *Entomoplasma*, and provided insights into the emergence of *Entomoplasma* from *Spiroplasma*, as well as the emergence of the *Mycoplasma mycoides* cluster from *Entomoplasma*. Finally, the critical issue of nomenclature, particularly several proposed changes to *Mycoplasma* taxonomy that have caused controversy and confusion, is to be discussed to build community consensus for the way forward.

O-6 Identification of a novel *Mycoplasma* species from an infection caused by a cat bite

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We isolated and characterized a novel *Mycoplasma* species from a patient with an upper extremity infection resulting from a cat bite. A 71-year-old male developed left thumb infection after bitten by an outdoor kitten who died shortly from a respiratory illness. The infection did not respond to broad-spectrum antibiotic therapy and bacterial cultures were negative. Broad-range PCRs were positive for an unknown *Mycoplasma* species and levofloxacin treatment yielded clinical improvement. The organism was isolated from wrist tissue in SP4 broth showing acidic color shift after 10 days of incubation. 16S rRNA gene sequencing showed 98-99% identity to two uncultured

Mycoplasma/bacterial spp. and < 94% identity to the known *Mycoplasma* species. The organism grows in Hayflick and SP4 media but not 10B. Colonies are round with rough surfaces and lack typical “fried egg” shape. Scanning electron microscopy revealed an oval cellular morphology. The minimum inhibitory concentrations are 64, 0.031, 0.008, and 0.008 µg/mL for erythromycin, clindamycin, tetracycline, and levofloxacin, respectively. Metabolic analysis on 28 substrates shows it only metabolizes sugars containing pure glucose unit(s) regardless of the glycosidic bond. The genome is 764.5 kb and G+C content is 27.24%. Genome BLAST Distance Phylogeny approach concluded it is a potential new species, with *Mycoplasma zalophi* CSL 4296 being the closest branch. The extracted *rpoB* gene sequence had a low similarity to the known species (< 76%) while *Mycoplasma* spp. still being the top matches. The 16S-23S ITS sequence was identical to an uncultured *Mycoplasma* spp. from Felis host with an abscess.

O-7 Phytoplasma Taxonomy: Bridging the Gap with Taxogenomics

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Phytoplasma taxonomy has undergone several transitions since its discovery in 1967, with multiple approaches being used for classification, including 16S rRNA gene sequencing and RFLP analysis. However, these methods have limitations, including the inadvertently creation of orphan species and unreliable publicly available sequences, including chimeric sequences of provisional phytoplasma species. The objective of the study was to identify chimeric sequences from a dataset of over 4500 phytoplasma 16S rRNA gene sequences using multiple tools. The tools ChimeraSlayer and UCHIME identified 12 and 11 chimeric sequences, respectively. Notably, these sequences included the reference 16S rRNA gene sequence of '*Ca*. Phytoplasma wodyetiae' strain Bangi-2 (KC844879) and '*Ca*. Phytoplasma allocasuarinae' (AY135523). The study's outcomes indicated the existence of chimeric 16S rRNA gene sequences, emphasizing the threat posed by such sequences in correctly assigning taxonomic status to phytoplasma strains. To address this issue, genomics presents a promising strategy for enhancing microbial taxonomy, with efforts underway to establish a stable nomenclature for uncultivated taxa. Two potential paths are being proposed which include allowing DNA sequence as type material and creating a parallel nomenclature system for uncultivated taxa. Both approaches aim to prioritize uncultivated taxa and establish a stable nomenclature. However, challenges persist, such as the heterogeneity of closely related phytoplasmas and the lack of a comprehensive MLST system. Researchers have attempted to address these issues by using genome sequences and Overall Genome relatedness Values (OGRI) values. To ensure a refined and stable taxonomy for phytoplasmas, the research community needs to consider and discuss these developments.

O-8 Examining Molecular Diversity of *Ureaplasma* Species Isolated from Neonates in the Global Multi-Country Child Health and Mortality Prevention Surveillance (CHAMPS) Network

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Ureaplasma species found within the female urogenital tract are transmissible to neonates during pregnancy and birth. Previous studies suggest *Ureaplasma* species may be an underrecognized infectious cause of stillbirth, preterm labor, and neonatal sepsis. Little is known about virulence factors and antimicrobial resistance (AMR) associated with *Ureaplasma* infections, but there are reports of resistance to various antibiotic types, including macrolides, the first line treatment for *Ureaplasma* infection. *Ureaplasma* species were previously identified by real-time reverse transcription polymerase chain reaction (RT-PCR) in post-mortem specimens from Child Health and Mortality Prevention Surveillance (CHAMPS) cases in 7 countries from May 2017 to January 2020. We performed additional characterization, including culture, species identifying real-time PCR for *U. urealyticum* and *U. parvum*, and whole genome sequencing (WGS). Ten isolates (5 *U. urealyticum*, 5 *U. parvum*) were recovered from 22 primary specimens, including lung tissue (n=5), blood (n=2), and cerebrospinal fluid (n=3), from 6 cases where *Ureaplasma* was detected but not attributed to the cause of death. Phylogenetic analysis of 8 high-quality genome assemblies revealed separation of the two species matching identification by real-time PCR. Analysis of potential macrolide, fluoroquinolone, and tetracycline resistance markers revealed one isolate harboring the *tetM* gene, a precursor for tetracycline resistance. Mutations in other genes known to confer resistance to various antibiotics were not identified, although unique polymorphisms were observed within these loci. Further genomic analysis may reveal important factors for invasive disease, including AMR, which could direct treatment and improve clinical outcomes.

O-9 Revised Minimum Standards for Valid Publication of Novel Species within the Class Mollicutes

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All proposed novel species must fulfill a minimum set of criteria in order to achieve acceptance as a taxonomically valid entity. These criteria are set by International Committee for the Systematics of Prokaryotes (ICSP) subcommittees composed of specialists with expertise on specific taxa, in compliance with the International Code of Nomenclature of Prokaryotes. Periodic revisions to these standards are critical so that novel taxa reflect current understandings of phylogeny and biology, and that accepted methodologies for characterization are updated as technology evolves. This is particularly important in the era of genomics, wherein both the tools and implications for findings evolve rapidly. The current minimum standards for valid publication of a novel species within the class Mollicutes (Phylum Mycoplasmatota) were published in 2007 and amended in 2012. The ICSP Subcommittee for the Taxonomy of Mollicutes have

developed an updated set of six standards requiring authors to report characteristics that will streamline understanding of the phylogeny and biology of novel species, recommend phenotypic evaluations that are diagnostically useful, and ensure compliance with the Nagoya Protocol. The proposed minimum standards require deposition of the 16S ribosomal RNA gene sequence and a reference genome sequence into major databases, description of cellular morphology, characterization of growth conditions, description of the isolation source and date, and deposition into two WFCC-registered culture collections, one of which must distribute cultures internationally. The majority of the committee are in agreement with these criteria and we are seeking wider opinion from the Mollicute community prior to publication and ratification.

IOM Peter Hannan Award

"If you do not know the names of things, the knowledge of them is lost" Carl Linnaeus

Vicki Chalker, Mitchell F. Balish, Assunta Bertaccini, Alain Blanchard,
Gail Gasparich, Ludwig Hölzle, Peter Kuhnert, Chih-Horng Kuo,
Lucia Manso-Silvan, Meghan A. May, Ana Sofía Ramírez Corbera,
Joachim Spergser, O. Brad Spiller, Emma Sweeney, Dmitriy Volokhov, Wei Wei
ICSP Subcommittee on the Taxonomy of Mollicutes

Antonie van Leeuwenhoek described both protozoa and bacteria in 1675 to 1677 as "animalcules" and the discovery of *Mycoplasma* came much later, in France in 1898. *Mycoplasma* have had numerous different names over the years with the term Mollicutes proposed in 1967. This lecture discusses Mollicute nomenclature and history. The International Committee on Systematics of Prokaryotes (ICSP) develops the International Code on which bacteriologists rely for the naming of prokaryotes. The ICSP Subcommittee on the Taxonomy of Mollicutes (ICSP-STM) advises on the nomenclature of Mollicutes, defining minimal standards to assign new species, and providing guidance on both the naming of new species and the renaming of known ones. Retention of historical names is of paramount importance to ensure nomenclature compatible with clinical, veterinary, agricultural, and industrial colleagues, as well as patients. Mollicutes have been named after people, higher species, clinical syndromes, phenotypic traits, morphology and more. The ICSP-STM takes the position that Mollicutes should retain conventional nomenclature, and considers the recent judicial commission decision fundamentally flawed, relying on intellectual taxonomic principles to the detriment of common sense for application. The ICSP-STM is committed to keep nomenclature aligned to the agreed code and existing scientific/clinical practice and retaining standards for authors naming Mollicutes. When considering naming new species, contacting ICSP-STM for advice and to engage with the Mollicutes research community prior to publication of changes.

Session 3 Vaccinology and Immunity

Keynote-2 Current thoughts on Livestock *Mycoplasma* vaccines

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For various reasons the development of safe and effective vaccines for livestock mycoplasma diseases has been problematic. *M. bovis* was first reported as a cattle pathogen in the early 1960s. However there have been few registered bacterin vaccines and in some countries, autogenous vaccines are used instead. Enzootic pneumonia of pigs was also first recognised in the 1960s and found to be caused by *M. hyopneumoniae*. Vaccines based on adjuvanted bacterins were developed in the 1990s and these became market leaders in the industry. However efficacy assessment has been based on reduction of lung lesions in vaccinated animals and they clearly do not completely control the disease or prevent transmission. There have been a number of attempts to develop more modern vaccines based on recombinant sub-unit and other approaches but to-date none has appeared on the market. Contagious bovine pleuro-pneumonia (CBPP) caused by *M. mycoides* subsp. *mycoides* has been eliminated from many of the wealthier countries but is still rife in many LMICs. In Africa the T1 based vaccines have been used for at least six decades with some success but have a very chequered reputation for both safety and efficacy. Attempts to produce effective sub-unit vaccines have not yet succeeded. In the search for better vaccines, there is usually a leap back to basic antigen discovery or immunology rather than building further on what is already available and at least partially successful. An argument is made for more attention to be paid to existing product improvement through better data collection, quality manufacturing and standardisation.

O-10 Evaluating the Dynamics and Efficacy of a Live, Attenuated *Mycoplasma anserisalpingitidis* Vaccine Candidate Under Farm Condition

Dénes Grózner, Alexa Mitter, Dominika Buni, Katinka Bekő, Zsuzsa Kreizinger, Áron B. Kovács, Enikő Wehmann, Nikolett Belecz, Karola Költő, Veronika Hrivnák, Miklós Gyuranecz
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Background: We developed a temperature-sensitive, live, attenuated vaccine candidate, clone MA271, by NTG mutagenesis. The aim of the present study was to monitor the dynamics and to measure the safety and efficacy of MA271 in geese breeder flocks under field condition. Methods: Two rearing flocks were vaccinated with MA271 at four-week of age and boostered at 24 weeks of age with cloaca inoculation (1 ml) and eye-dropping (50 µl). The geese then were transported to multi-aged breeding farms. Two breeding flocks served as controls. Results: Colonisation of the cloaca by MA271 showed 75% top prevalence between 4 and 6 weeks after the first vaccination. Then the prevalence decreased to 25% until the cooler, humid fall months which coincided with the booster vaccination, resulting 100% cloacal colonisation. No clinical signs were observed in the vaccinated birds. After transportation to five multi-aged breeding farms, the wild-type strain appeared beside MA271 in three flocks. In one flock the wild-type strain completely displaced MA271, while in one flock only MA271 was detected. Only wild-type strains were detected in the control flocks,

however, due to a HPAI outbreak both flocks were exterminated before the end of the study. Based on the available data the median percentage of infertile eggs were 3.7-5.1% in the MA271 vaccinated flocks, while 7.1-7.3% in the non-vaccinated flocks. Conclusions: MA271 is able to colonize the cloaca of geese under field condition as well. MA271 proved to be safe and presumably provides some protection against *M. anserisalpingitidis* induced reproduction losses.

O-11 Clinical Efficacy of an Autogenous Killed Vaccine for *Mycoplasma ovipneumoniae* Used during a Pneumonia Outbreak in Saanen Goats in Northern Italy

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Mycoplasma ovipneumoniae (MO) is a respiratory pathogen of small ruminants responsible for economic loss due to poor growth rates and productivity of affected animals. The pathogen is reported in many areas of the world, it is transmitted mainly horizontally, via the respiratory route, and it is a predisposing factor for other bacterial pneumonias. Several reports seem to indicate that disease caused by MO is more severe in goats compared to sheep, especially in younger stocks, and the variability in strain susceptibility to antimicrobials makes MO infection difficult to control. Currently, there is no commercial vaccine available for MO, probably due to production costs and for the high immunological variability of MO strains that may affect vaccination success. We present here a MO outbreak in a group of 110 Saanen goats resolved after the administration of an autogenous killed vaccine. Before vaccination, morbidity was around 55% and clinical signs (cough, nasal discharge, poor growth) were observed mainly in kids. The animals had received three antimicrobial treatments (penicillin, tylosin and enrofloxacin) without significant improvement of their health status. The autogenous killed vaccine was prepared using MO strains isolated from the lung of a dead kid of the affected farm. The vaccination protocol consisted in two consecutive injections to all brood goats before delivery and to all newborns starting at 15 days after birth. After 30-90 days post-vaccination no clinical signs in newborns were observed, nor mortality due to respiratory disease, and no antimicrobial therapy was needed in the following year.

O-12 Efficacy of a New Inactivated *Mycoplasma hyorhinis* Vaccine in Commercial Pigs

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Background: *Mycoplasma hyorhinis* is an emerging pathogen in recent years which causes polyserositis and polyarthritis in pigs. Previous studies revealed that different genotypes of *M. hyorhinis* were detected between pig herds, and different clinical and pathological symptoms also implied differences in virulence and tissue tropism between strains. At present, several vaccines have been developed against *M. hyorhinis* in previous studies. However, current vaccines did not clarify the cross-protection between different *M. hyorhinis* stains. In this study, we have evaluated the cross-protective efficacy of an inactivated *M. hyorhinis* vaccine. Methods: Two different sequence types *M. hyorhinis* strains (HEF-16 and JS-54) were inactivated and blended with oil adjuvant respectively. Pigs of two immunization groups were administered two inactivated vaccines respectively. Pigs were monitored daily for clinical changes after strain HEF-16 challenge. Pigs were euthanized 21 days after challenge and a gross pathological examination was used to assess the two inactivated vaccines. Results: The cross-protection group (Strain JS-54) demonstrated a significant reduction of clinical signs and pathological lesions, as well as the same strain immunization group (Strain HEF-16). Pigs of two immunization groups raised high antibody titer two weeks after vaccination, and an increase in average daily weight gain compared with the challenge group after *M. hyorhinis* challenge. Conclusion: we revealed a cross-protection between two different genotypes *M. hyorhinis* strains in commercial pigs. The data of the cross-protection group shows no significant difference compared to the same strain immunization group, and this will help in the prevention and control of *M. hyorhinis* infections.

O-13 Profiling *Mycoplasma hyosynoviae* Antibodies in Dams and Piglets

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Mycoplasma hyosynoviae (*M. hyosynoviae*) is a commensal bacterium that can cause growing pig lameness and leads to health and welfare issues. There is a need to generate information for improvement of *M. hyosynoviae*-associated disease control. The aim of this study was to determine the natural antibody profile of *M. hyosynoviae* in aclinically affected herd. A 9,000-head sow farm and five nursery/grow-finish

downstream sites with clinical history of *M. hyosynoviae*-associated disease were enrolled in this study. Blood (n=1,778) and colostrum (n=27) samples were conveniently collected from various age groups of dams, replacement gilts, newborn piglets, weaned piglets, nursery pigs, and finishing pigs. Samples were tested utilizing an *M. hyosynoviae* specific indirect ELISA assay. The percent pig positives for IgG per sample group were: dams at service 93.0%, midgestation 97.4%, farrowing 99.0%, weaning 100.0%, colostrum 100.0%, newborn piglets 86.4%, weaning piglets 92.0%, nursery seven wks 39.1%, nursery nine wks 28.3%, nursery ten wks 17.3%, nursery 12 wks3.7%, finishing 19 wks 9.7%, gilts five wks 66.0%, gilts ten wks 13.7%, gilts15 wks 1.0%, gilts 20 wks 11.7%, gilts 25 wks 12.0%, and gilts 30 wks 24.8%. In summary, 90% of dams were positive for *M. hyosynoviae* antibodies, while replacement gilts and piglets showed a similar pattern of seroconversion: a high percentage of positives early in life, to drop at ten weeks of age, remaining low until ~20 weeks when a slow increase in antibodies was detected. Results of this investigation aid pinpointing windows of immunological opportunity for improved disease control.

O-14 The role of pulmonary interstitial macrophages in *Mycoplasma pneumoniae* induced inflammation

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Experimental infection of mice with *Mycoplasma pneumoniae* results in characteristic pulmonary inflammation marked by infiltration of neutrophils in the airspaces and an accumulation of lymphocytes in the peribronchiolar and perivascular regions of the lung parenchyma. Little is known about how this inflammation develops, however. Single-cell-transcriptome analysis of pulmonary interstitial macrophages (IMs) revealed that these cells express several chemokines when activated by inflammatory stimuli. This led us to hypothesize that pulmonary IMs may play a role in the modulation of inflammation due to *M. pneumoniae*. Utilizing a novel mouse model that allows for selective and temporal depletion of pulmonary IMs, we show that IMs play a significant role in the development of perivascular and peribronchial inflammation during *M. pneumoniae* infection. Unlike wild type controls, IM-depleted mice developed less severe perivascular and peribronchial lesions. Furthermore, IM depleted animals showed a decrease in B cell infiltration and an increase in neutrophil infiltration in the airways. Similarly, mice with CXCL13 deficient leukocytes displayed a similar trend, suggesting that B cells recruited to the airspace during *M. pneumoniae* infection dampen neutrophil recruitment and concomitant severity of suppurative pneumonia. Ultimately, these data indicate that pulmonary IMs mediate pulmonary inflammation due to *M. pneumoniae* by controlling B cell recruitment. In turn, B cells appear to play a dampening effect on the suppurative pneumonia induced by *M. pneumoniae* infection.

O-15 Synergism of bovine mammary epithelial cells and bovine peripheral blood mononuclear cells enhances immunological responses to *Mycoplasmopsis bovis*

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Mycoplasmal mastitis, which is mostly caused by *Mycoplasmopsis bovis* (*M. bovis*), is a contagious infection with severe inflammation in the bovine mammary gland (MG). Although the symptoms of this disease are considered to result from the direct invasiveness of *M. bovis* and the characteristic immune responses of the host, the details of this pathophysiology remain incompletely understood. The aim of this study is to elucidate the immunological responses of MG to *M. bovis*, using co-culture system of bovine mammary epithelial cells (bMEC) and bovine peripheral blood mononuclear cells (PBMC). bMEC was isolated from MG, and PBMC were isolated from tail vein blood of Holstein dairy cows. bMEC and PBMC were cultured alone or co-culture with *M. bovis* (ATCC 25,523). After 24 hours of *M. bovis* stimulation, we evaluated the immune response-related mRNA expression in bMEC and PBMC, and the cytokine concentration in culture supernatant. Interleukin (IL)-1β, IL-6, and IL-8 mRNA expression levels tended to be higher in bMEC stimulated with *M. bovis*. The IL-6 mRNA expression level of bMEC stimulated with *M. bovis* under co-culture with PBMC tended to be higher than that in the absence of PBMC. IFN-γ and IL-12 mRNA expression levels tended to be higher in PBMC stimulated with *M. bovis*. Concentration of IFN-γ in culture supernatant tended to be higher under *M. bovis* stimulation compared with control. The immune responses of bMEC to *M. bovis* were enhanced under co-culture with PBMC, suggesting that the synergism of bMEC and PBMC forms the characteristic pathophysiology of mycoplasmal mastitis.

O-16 Insights from 16-year Mycoplasma Culture and PCR Data Analysis in a Canadian Provincial Laboratory

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For over 40 years, the Animal Health Laboratory (AHL) in Ontario, Canada has conducted culture isolation on *Mycoplasma*/*Ureaplasma*. In 2007, the laboratory implemented a Laboratory Information Management System for efficient data management and trend analysis. During the past 16 years, the AHL has cultured a total of 18,723 samples, primarily from food animals, and successfully isolated over 25 species of *Mycoplasma* and *Ureaplasma*. The most frequently identified species were *M. bovis* (7%) and *M. arginini* (5%). Notably, there was a large number of *Ureaplasma diversum* isolated in 2019 during abortion outbreaks. *M. bovis* was isolated only once since 2007 from semen sample indicating that the risk of transmitting through semen was likely low. Among non-food animals, the most commonly identified species were *M. canis* (8%), *M. edwardii* (4%), *M. cynos* (3%), *M. spumans* (2%), *Ureaplasma sp*. (2%), and *M. maculosum* (2%). Over the course of 16 years, the AHL conducted PCR tests on 70,853 samples targeting 15 different *Mycoplasma* species. In recent years, the PCR positive rate for *M. hyopneumoniae*, *M. bovis*, and *M. synoviae* has significantly increased. The predominant haemoplasmas identified were *M. haemofelis* and *M. haemocanis*. PCR assays were successfully used to assist eradicating *M. iowae* in a high-value turkey breeder facility. Overall, this study highlights the importance of using both culture and PCR assays for identifying emerging trends and

diagnosing *Mycoplasma*-related diseases. The combination of these methods allows for comprehensive monitoring and effective disease management.

Session 4 Next Generation Vaccines and Host-Pathogen Interactions

IOM Emmy Klieneberger-Nobel Award

Mycoplasmas - Not That Simple, But Increasingly Understandable and Controllable

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Although mycoplasmas are often described as the simplest free-living organisms, they have complex relationships with their hosts, exemplified by the major surface lipoproteins of the major poultry mycoplasmas and the different mechanisms used to generate phase variation in them. Mutagenesis has enabled identification of genes that influence virulence in mycoplasmas, many of which encode proteins on the cell surface. We have focussed on using bioinformatic, metabolomic and biochemical methods to elucidate the functions of these cell surface proteins. Mycoplasmosis is a consequence of the persistence of mycoplasmas in infected hosts and the response of the host to them. Studies of diseased tissues have enabled a more detailed exploration of the changes associated with infection and the impact of vaccination can have on these changes. Effective vaccination against some pathogenic mycoplasmas has been challenging, but we have demonstrated that attenuated live vaccines provide effective, persistent immunity and reduce transmission of the poultry mycoplasmas. These vaccines have now been broadly employed as an important tool in controlling mycoplasmosis. Improved serological diagnostic tests, with enhanced sensitivity and specificity, have also had a major impact on control and eradication programmes. Our understanding of the pathogenesis of mycoplasmoses, and their control, will be furthered by characterisation of the function of their genes of unknown or uncertain function, and by more detailed studies of the immune response to infection and vaccination. Application of this improved understanding should result in identification of approaches that will be effective in preventing disease caused by other animal mycoplasmas.

O-17 Global Transcriptional Changes in the Trachea After Vaccination and Infection with *Mycoplasma gallisepticum* in Immunosuppressed Chickens

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Immunosuppression could reduce the efficacy of live-attenuated vaccines against

Mycoplasma gallisepticum. Transcriptional profiles in the tracheal mucosae of four groups of chickens immunosuppressed by infection with either chicken anaemia virus (CAV), which mainly affects cell-mediated immunity (CMI), or infectious bursal disease virus (IBDV), which mainly affects humoral immunity (HI), before or after vaccination with the *M. gallisepticum* strain ts-304 vaccine, were explored at 2 weeks after subsequent challenge with virulent *M. gallisepticum* strain Ap3AS. Pair-wise comparisons of transcription between the four immunosuppressed groups and unvaccinated/unchallenged, unvaccinated/challenged, vaccinated/unchallenged and vaccinated/challenged groups, which had not been exposed to CAV or IBDV, detected significant differences in the unvaccinated/challenged and the four immunosuppressed groups compared to the other groups. Functional interrogation of differentially transcribed genes detected adverse effects of CAV and IBDV on activity of lymphocytes and macrophages, extracellular, intracellular and cytokine signalling, cell communication, cellular actin and cytoskeleton dynamics, phagocytosis, immunoglobulin production and class switching, endocytosis, apoptosis, and metabolism in the tracheal mucosa. These findings have led to the identification of mechanisms underlying the reduced efficacy of the attenuated *M. gallisepticum* strain ts-304 vaccine in immunosuppressed chickens, which was demonstrated previously. Differences in transcription were more pronounced in the two groups infected with IBDV before or after vaccination than in the two groups infected with CAV. This suggests that humoral immunity plays a major role in generation of the primary and secondary immune responses after vaccination and upon subsequent challenge, but that effective protective immunity also requires the CMI response.

O-18 Genome engineering of the major goat pathogen *Mycoplasma capricolum* subsp. *capripneumoniae* as a first step towards the rational design of improved vaccines

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Background- *Mycoplasma capricolum* subspecies *capripneumoniae* (*Mccp*) is the causative agent of contagious caprine pleuropneumonia (CCPP), a disease listed by the world organization for animal health (WOAH) threatening goat production in Africa and Asia. Although a few commercial inactivated vaccines are available, they do not comply with WOAH standards and their efficacy is questioned. One of the limiting factors to comprehend the molecular pathogenesis of CCPP and develop improved vaccines has been the lack of tools for *Mccp* genome engineering.
Results- In this study, synthetic biology techniques, recently developed for closely related mycoplasmas, were adapted to *Mccp*. CReasPy-cloning was used to simultaneously clone and engineer the *Mccp* genome in yeast, prior to whole genome transplantation into *M. capricolum* subsp. *capricolum* recipient cells. This approach was used to knock-out an S41 serine protease gene identified as a potential virulence factor, leading to the generation of the first site-specific *Mccp* mutants. This approach

was further extended to two other field strains of *Mccp* using CReasPy-Fusion, a method that allows to clone and edit bacterial genomes in yeast through cell-to-cell contact. Furthermore, the Cre-lox recombination system was applied to remove all DNA sequences added during genome engineering. Finally, the resulting unmarked S41 serine protease mutants were validated by genome sequencing and their non-caseinolytic phenotype was confirmed by casein digestion assay.
Conclusion- Synthetic biology tools were successfully implemented in *Mccp*. This innovation allows constructing targeted *Mccp* mutants at ease, which will be of great help to decipher *Mccp* pathogenicity determinants and develop novel vaccines.

O-19 Quantification of the effect of vaccination on the control of horizontal transmission of *M. synoviae* under field conditions

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Vaccination and biosecurity are important for the control of Ms. Besides reduction of economic impact, experimentally, Ms vaccination has shown to reduce horizontal transmission. In this study the effect of Ms live vaccination on horizontal transmission was quantified under field conditions. Four-year data from an Ms monitoring programme from non-vaccinated broiler and layer breeder farms and Ms-vaccinated broiler breeder farms was analysed. Flocks were monitored at 20 and 30 weeks of age and then every 12 weeks. Sixty blood samples or 24 tracheal swabs were tested by Ms RPA/ELISA or Ms DIVA PCR respectively. The Ms incidence rate was calculated, effects were analysed by logistic regression. The Ms incidence rate was 11.6 cases per 1000 weeks for non-vaccinated broiler breeders and dropped from 29.6 to 5.6 cases per 1000 weeks with successive Ms-vaccinated production cycles. In layer breeders it was 3.6 cases per 1000 weeks. Logistic regression showed a significant negative association with Ms in the fourth to sixth vaccinated flock when compared to non-vaccinated flocks (odds-ratio = 0.23, p = 0.05), which was even stronger when compared to the first vaccinated flock (odds-ratio = 0.12, p = 0.01). A significant negative association with Ms in layer breeders (odds-ratio = 0.28, p = 0.00) was observed compared to non-vaccinated broiler breeders. A controlled contact-structure within the layer breeder industry may explain this finding. The results suggest that vaccination and control of contacts can be successful tools in reducing between-farm transmission in a situation of single age and good biosecurity.

O-20 MyMIC: a Network for Standardization of Diagnostics, Antimicrobial Susceptibility Testing and Clinical Interpretation in Animal Mycoplasmas

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Mycoplasmas are causing various diseases and significant economic losses in avian, porcine and ruminants sectors. Their control that relies mainly on chemotherapy contributes largely to the general rise of antimicrobial resistance (AMR). Harmonized surveillance of AMR in animal mycoplasmas is hindered by the lack of i) standard procedures and guidelines (tests used for classic bacteria for in vitro antimicrobial susceptibility testing (AST), such as disk diffusion method, cannot be used for mycoplasmas), ii) approved quality control (QC) strains, iii) clinical breakpoints or epidemiological cut-off values (ECOFFs) as surrogates to interpret minimum inhibitory concentrations (MICs). MyMIC is one of the six new collaborative networks funded by the Joint Programming Initiative on Antimicrobial Resistance (https://www.jpiamr.eu/calls/network-call-2022/#supported-projects). It includes 22 laboratories from 18 countries working on animal mycoplasma diagnostics and AST. Its objective is to make an inventory of the methods used for identification, culturing, determination of MICs, and detection of resistance mechanisms by molecular or genomic tools. The MIC values of the main families of antimicrobials used against some key pathogenic *Mycoplasma* species, obtained by identical or comparable methods, will also be collected and aggregated to determine if this data can be used to define first tentative ECOFFs. This network will lead to the drafting of guidelines for standardized culture, identification and determination of MIC for livestock *Mycoplasma* species. Moreover, it will also enable to identify gaps and to start new research proposals for additional work to undertake to define QC strains, ECOFFs and clinical breakpoints.
Keywords: animal mycoplasmas, antimicrobial, standardization, diagnostics, network

O-21 Fever-like temperature impairs the bovine ex vivo response to *Mycoplasmopsis bovis*

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Although cattle are the mammalian species with most planetary 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 readouts: firstly, a novel 12-14 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, gamma-delta T cells, B and T cells; secondly, a multiplex immunoassay monitoring bovine chemokine and cytokine secretion levels. The experiments were conducted on 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 new key players such as strong IFN-gamma secreting NK cells, whose role was so far largely neglected. Additionally, we investigated the influence of the fever-like temperature. Strikingly, high fever temperature attenuated the capacity of most immune cell subsets to respond to *M. bovis*. Our experimental approach, phenotypically delineating the bovine immune system provided us with a deep insight of the bovine immune response towards *M. bovis* as well as new insights into the influence of high fever towards that immune response. A better understanding of host-pathogen interactions will foster the development of rationale vaccines.

O-22 *Mycoplasma hyrohinis* Hijacks Host Plasminogen/Plasmin System via Multiple Surface Moonlighting Proteins to Enhance Its Spread Across the Extracellular Matrix Barrier

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Background: The occurrence of disease in pigs infected by *Mycoplasma hyorhinis* often linked with the systemic invasion of the pathogen. However, the related pathogenesis is poorly understood. In the present study, we investigated whether and how *M. hyorhinis* employs the host's plasminogen/plasmin system to degrade the extracellular matrix (ECM) barrier to promote their dissemination. Methods: The ability of *M. hyorhinis* to bind plasminogen was detected as well as the activation of the latter. A transwell migration model was used to detect the breakthrough of the ECM barrier by *M. hyorhinis*. Furtherly, the plasminogen receptors of *M. hyorhinis* were identified. The interactions between those receptor proteins with host's plasminogen/plasmin system were confirmed. Results: *M. hyorhinis* bound plasminogen on its surface. The activation of the surface-bound plasminogen into plasmin was observed by using a chromogenic substrate. The plasmin modified-*M. hyorhinis* degraded and broke through the reconstituted ECM barrier. Several surface located moonlighting proteins were identified to be plasminogen receptors of *M. hyorhinis*, including glyceraldehyde-3-phosphate dehydrogenase (GAPDH), enolase, DnaK, elongation factor thermo unstable (EF-Tu), etc. The interactions between those receptor proteins and plasminogen were demonstrated by ELISA and Far-Western blot assay. The activation of the receptor protein-bound plasminogen was detected, and furtherly confirmed by ECM degradation detection. Conclusions: Our data imply that *M. hyrohinis* can hijack host plasminogen/plasmin system via multiple surface moonlighting proteins to

enhance its spread across the ECM barrier. The results may help to elucidate the mechanism of the systemic dissemination and disease induction of *M. hyrohinis*.

O-23 The Secreted MbovP475 of *Mycoplasma bovis* Induces Macrophage Pro-inflammatory Response through CRYAB

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Background: *Mycoplasma bovis* (*M. bovis*) is one of the main pathogens causing pneumonia, arthritis, mastitis and other diseases in cattle. The main reasons restricting the prevention and control of the disease are the unclear pathogenic mechanism of *M. bovis*. Secreted proteins are potential virulence factors of bacteria. Our previous study found that MbovP475 is a secreted nucleomodulin that decreases BoMac cell viability. The aim of this study was to explore the mechanism of proinflammatory response induced by MbovP475. Methods: we used RT-qPCR and western blotting assay to verify expression of pro-inflammatory cytokines and activation of the signaling pathways. Immunoprecipitation (IP), Indirect immunofluorescence assay (IFA), and coimmunoprecipitation (Co-IP) were used to screen and verify MbovP475-binding proteins. Then, the truncated MbovP475 was constructed to verify the MbovP475-binding protein is essential for MbovP475 to induce pro-inflammatory cytokines IL-1β, IL-6 and TNF-α Results: MbovP475 significantly upregulated expression of IL-1β, IL-6 and TNF-αin BoMac infected with *M. bovis* or treated with rMbovP475 protein. A western blotting assay confirmed that MbovP475 promoted the phosphorylation of NF-κB p65 in BoMac. Further, Co-IP assay identified the anti-inflammatory regulator CRYAB as an MbovP475-binding ligand. The conserved coiled-coil domain of MbovP475 at amino acids 329-368 is essential for its induction of inflammatory response because the truncated MbovP475 (Δ329-368aa) decreased the mRNA expression of pro-inflammatory cytokines compared with MbovP475. Conclusion: we revealed MbovP475 induced pro-inflammatory cytokine expression by interacting with CRYAB through activation of NF-κB pathway. These findings extend our understanding of *M. bovis* pathogenesis.

Session 5 Virulence and Pathogenesis

IOM Robert F. Whitcomb award

Molecular mechanisms of plant manipulation by phytoplasmas

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Phytoplasmas (genus '*Candidatus* Phytoplasma') are plant pathogens of the bacterial class Mollicutes. Phytoplasmas are transmitted by insect vectors and infect over 700 plant species, including many economically important crops. Infected plants show a wide range of symptoms, including witches' broom, phyllody, virescence, proliferation, and phloem necrosis. To investigate the features of phytoplasma, we determined the complete genomic sequence of '*Ca*. P. asteris OY-M'. Interestingly, the phytoplasmas have lost many metabolic genes, such as F-type ATP synthase, compared to other bacteria. Since the ATP synthase had been thought to be essential for life, the loss of its genes in the phytoplasma genome provided opportunities for reconsidering the question "what is life?". Instead, phytoplasmas harbor multiple copies of transporter-related genes, suggesting that they are highly dependent on metabolic compounds from their host. Since phytoplasmas reside within host cells, their secreted proteins encoded in the genomes are believed to directly function in the host cell as virulence factors. TENGU is a secreted protein that induces witches' broom symptoms by suppressing the auxin response of the host plant. In addition, PHYL was found to cause phyllody symptoms in flower organs. PHYL induces phyllody symptoms by degrading transcription factors involved in ABCE model that controls flower development. Further advances in our understanding of the molecular mechanisms underlying host manipulation by effectors will enable us to devise control strategies for phytoplasma diseases that target these virulence factors.

O-24 Comparative Genomic Analysis of *M. agalactiae* Strain GM1309 Unravels its Peculiar Surface Architecture and Distinct Pathogenicity Traits

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Vpma and other surface antigenic variations of *Mycoplasma agalactiae* play an important role in its host-cell interactions and pathogenesis. Vpma phenotypic profile of strain GM139 (goat-isolate, USA) was recently compared to type strain PG2 (sheep-isolate, Spain) to assess possible correlations between phase-variability, host/geographic distribution, and pathogenicity of these two strains. GM139 predominantly exhibited stable expression of a single VpmaV protein compared to the high-frequency variable expression of six Vpma proteins of PG2. The complete genome sequence of GM139 was obtained by hybrid assembly of Nanopore and Illumina reads, annotated via PGAP, and detailed analysis of the Vpma locus and comparisons with finished genomes of 3 *M. agalactiae* strains (PG2, 5632, GrTh01) were performed. Interestingly, GM139 presented a distinct *vpma* locus comprising 10 genes, none of which matched the six *vpma* genes of PG2, except a chimera between *vpmaV* and *vpmaZ* correlating well with the immuno-/Western blots and confirmed by LC-MS analysis of the corresponding positive band recognized by α-VpmaV (PG2) Ab; five *vpmas* were unique whereas the other 4 showed similarity to *vpmas* of 5632. Also, a larger Spma family of surface lipoproteins (25 kb-locus compared to 14 kb-locus in PG2), an intact *gsmA* gene (truncated in PG2, 5632, GrTh01) encoding phase-variable surface glucan, presence of ICE, and differences in transposases, have likely influenced pathogenicity traits like serum resistance, and host/geographic distribution,

segregating GM139 and PG2 onto well-separated clusters based on MALDI-ToF analysis. The study highlights the plasticity and dynamic evolution of *M. agalactiae* genomes, especially its surface antigenic architecture.

O-25 Studying Interactions between 12 *Mycoplasmopsis bovis* and Bovid Tissue Culture

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Background: *Mycoplasmopsis bovis* is one of the major causative agents of pneumonia, mastitis, and contributes significantly to multifactorial infections such as bovine respiratory disease. Due to antimicrobial resistance and the absence of effective vaccines, this bacterium continues to cause considerable economic losses and its mechanisms of pathogenicity remain understudied. We aimed to establish an in vitro cell infection assay to investigate bovine host-*M. bovis* interactions, assaying adhesion and invasion as virulence attributes. Methods: The Madin-Darby Bovine Kidney (MDBK) epithelial cell line was used to test a set of 12 *M. bovis* strains with various characteristics and origins (a type strain, three fluorescent strains, and eight Canadian clinical isolates). Gentamicin protection assays followed by a variety of processing methods – culture, qPCR, confocal and electron microscopy – were used to investigate the ability of *M. bovis* to adhere to live inside eukaryotic cells. Results: All 12 *M. bovis* strains could adhere, invade, persist, and multiply within or on the surface of MDBK cells despite being isolated from distinct hosts, anatomical sites and having different genetic backgrounds. qPCR and confocal microscopy showed that the number of cell-associated bacteria was dependent on incubation time and multiplicity of infection. The adhesion of *M. bovis* populations to the surface of MDBK cells could survive gentamicin treatment and was demonstrated by scanning electron microscopy. Conclusion: *M. bovis* is capable of adhering and entering non-phagocytic cells. The former may be underappreciated and have consequences related to treatment strategies for *M. bovis* in cattle.
Keywords: *Mycoplasmopsis bovis*/ MDBK cells/ host-pathogen interactions/ adherence/ invasion

O-26 Modulation of transcription factors involved during Phytoplasma-associated phyllody development in *Sesamum indicum* L. (Sesame) plants

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Background: Sesame (*Sesamum indicum* L.) is an important oilseed crop well known for its highly nutritious edible oil. Phytoplasma-associated phyllody is the leading biotic constraint drastically affecting sesame productivity, resulting in yield losses of up to 80%. Sesame phyllody is marked by the conversion of floral parts to proliferating leafy structures. Phytoplasma produces a novel effector protein (SAP54) that interacts with

members of the MADS-domain transcription factor (MTF) family; however, the exact mechanism of phyllody development in sesame has not been worked out yet. Methods: Healthy and infected sesame plants were subjected to RNA-Seq and differentially expressed genes (DEGs) were ascertained including Sesame TFs genes that were identified using Plant TFDB. Genome-wide identification and characterization of the Lateral Organ Boundary Domain (LBD) TF family, which was predominantly differentially expressed in phyllody-affected sesame were done followed by RT-PCR validation. Results: A total of 31 gene families out of the 52 TF families identified were differentially expressed comprising 85 upregulated and 53 downregulated genes. TF families having roles in development, growth and defensive responses were identified. A total of nine LBD genes were found to be differentially expressed out of which three were upregulated and six were downregulated. Conclusion: Differential expression of TFs especially LBD genes involved in cell proliferation and differentiation were observed in sesame phyllody development suggesting their significant role in defense response against Phytoplasma. Gene expression analyses of LBD genes in healthy and affected plants confirmed their role.
Keywords: *Sesamum indicum* L., Sesame, Phytoplasma, Phyllody, Transcription Factors (TFs)

O-27 The *Mycoplasma genitalium* biofilm-associated exopolysaccharide is a glucosamine polymer in the furanose configuration

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Background: Most bacterial biofilms are composed of an exopolysaccharide (EPS) and secreted proteins that form the structural matrix. A common bacterial EPS is the Poly-*N*-acetylglucosamine (PNAG), which is found in diverse species from *Bacillus subtilus* to *Escherichia coli*. Numerous species have evolved diverse synthetic pathways to synthesize a version of PNAG, indicating the importance of this molecule. *M. genitalium* synthesizes a molecule that is similar in composition to PNAG but distinct in structure. Method: *M. genitalium* cultures were grown attached in SP4. DNA, RNA and proteins were digested using the appropriate enzymes. Samples were analyzed by gas chromatography mass spectrometry (GC-MS), methylation analysis, and mass spectrometry. Results: The EPS component of the Mgen biofilm is composed of multiple non-acetylated glucosamine residues linked through a putative β-1-6 linkage and attached to the bacterial cell through a phospholipid linker. The GC-MS signatures are indicative of a furanose configuration, while methylation analysis indicates the non-acetylated glucosamine residues utilizing a β-1-6 linkage. These glucosamine residues are in the furanose (5 member ring) configuration instead of the common pyranose (6 member ring). The *M. genitalium* glucosamine EPS is unrelated to the polysaccharide synthesized by *Mycoplasma pneumoniae*, *M. genitalium* synthesizes a second capsule-like polysaccharide that is similar to the *M. pneumoniae* capsule. Conclusion: This is a uniquely structured molecule that is specific to *M. genitalium* and is not synthesized by the closely related species *M. pneumoniae*. This is the only example of a PNAG like molecule in the furanose configuration utilized for bacterial biofilms.

O-28 The MnuA Nuclease and 5'-Nucleotidase Impact *Mycoplasma bovis* Fitness in Mastitis

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Background: Nucleases and 5'-nucleotidases play an essential role in central pathways of cell biology and are often associated with bacterial virulence. In *Mycoplasma* spp., possessing limited metabolic capacities, the role of such enzymes is of great value. **Objective**: To investigate the potential role of the major membrane nuclease MnuA and 5'-nucleotidase (MBOVPG45_0690) on survival strategies and infectious potential of *Mycoplasma bovis* in mastitis. **Methods**: Genome-wide transposon mutagenesis of *M. bovis* PG45 was performed using pMT85 plasmid and mutants of interest were evaluated using in vitro and in vivo model systems. **Results**: The disruption of *mnuA* resulted in decreased nuclease activity, while disruption of MBOVPG45_0690 led to slower growth rate and smaller colony size. Using murine mastitis model, we demonstrated that the inactivation of the *mnuA* or MBOVPG45_0690 genes reduced the bacterial counts of *M. bovis* in the mammary glands. However, mammary inflammation, measured by the relative expression of major inflammatory genes, was significantly reduced only with Δ*0690*. Whole genome transcriptome of mouse mammary glands challenged with WT, Δ*mnuA* or Δ*0690* mutants displays differences in activation of genes and pathways involved in immune cells recruitment and function, among them myeloperoxidase and neutrophil extracellular traps (NETs). Advanced image analysis demonstrated differences in in-vivo NET formation between the WT and the Δ*mnuA* mutant. In addition, alterations in cellular physiology, fatty and amino acids metabolisms were identified in glands challenged with Δ*mnuA* or Δ*0690*. **Conclusion**: The results suggest that both enzymes play an important role in the pathogenesis of *M. bovis* mastitis.

O-29 Comprehensive search for amino acid residues involved in target recognition of a phytoplasma effector protein by random mutagenesis-based screening

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To gain a deeper understanding of protein function, it is crucial to identify how it interacts physically with its target. Phyllogen is an effector protein of phytoplasmas, a group of plant pathogenic bacteria, and induces phyllody phenotypes (transformation of floral organs into leaf-like structures) in plants through its expression. Phyllogen interacts with K domain of some plant MADS-box transcription factors (MTFs), leading to MTF degradation. While some amino acid residues of phyllogen have been identified as responsible for the interaction, the precise interface has remained elusive. In order

to comprehensively investigate the residues that mediate the interaction, we conducted random mutagenesis-based yeast two-hybrid screening of phyllogen. Through this approach, we discovered mutations on two residues: lysine for glutamic acid at the 37th amino acid residue (K37E) and threonine for alanine at the 78th amino acid residue (T78A), which increased the affinity of phyllogen for MTF. Notably, the introduction of K37E into phyllogen enhanced its phyllody-inducing activity. All the known residues involved in the interaction with MTFs, including the newly identified ones, were found to cluster together on the protein surface of phyllogen, indicating that they comprise the interaction interface. Furthermore, in silico structural prediction of the protein complex of phyllogen and K domain of MTF using ColabFold suggested that phyllogen interacts with the K domain of MTF through this interface. This study provides new insights into the mechanisms underlying the interaction between phyllogen and MTFs.

O-30 The non-canonical host proteasome utilization by a phytoplasma effector: phyllogen acts as a ubiquitin-like mediator for proteasomal degradation of target proteins

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Phytoplasmas (*Candidatus* Phytoplasma spp.) are Mollicutes, that infect over 1,000 plant species. Phytoplasmas transform host flowers into leaves (phyllody), by secreting an effector protein called as “phyllogen”. Phyllogen interacts with MADS-domain transcription factors (MTFs), plant proteins required for flower development, and induces the degradation of these MTFs via the host proteasome. Because the proteasome usually degrades ubiquitinated proteins, bacterial effectors reportedly utilize host proteasome by inducing ubiquitination of target proteins. On the contrary, phyllogen binds to RADIATION SENSITIVE 23 (RAD23), which interacts with ubiquitin and delivers ubiquitinated proteins to the proteasome. Here, the proteasome-utilizing mechanisms of phyllogen was elucidated. Co-immunoprecipitation assays showed that phyllogen mediates the interaction between MTF and RAD23, resulting in the formation of a ternary protein complex comprising these proteins. The ternary protein complex was formed sequentially, beginning with the interaction between MTF and phyllogen, followed by the recruitment of RAD23. Although ubiquitination of phyllogen, not MTF, was observed in the assay, a Lys-less non-ubiquitinated phyllogen mutant was found to function similarly to wild-type phyllogen. Moreover, an immunoprecipitation assay using purified non-ubiquitinated phyllogen, MTF, and RAD23 showed that phyllogen could mediate the interaction between MTF and RAD23 without ubiquitin, suggesting that phyllogen works independently of ubiquitination. Our findings indicate that phyllogen acts as a mediator between targets and RAD23 instead of ubiquitin. Consequently, the study concluded that phyllogen induces proteasome-dependent but ubiquitin-independent degradation of MTFs by functionally mimicking ubiquitin. This is a quite unique mechanism for bacterial utilization of the host proteasome.

O-31 Monopodial to sympodial shift in *Phalaenopsis*: phytoplasma SAP11 effector breaks repression of axillary buds

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There is a long-standing question regarding the evolutionary trend in growth patterns found in orchids. Despite the diversity of orchid species, they can be categorized simply as having monopodial or sympodial growth habits. In this study, we observed that the monopodial growth of *Phalaenopsis* can be altered by infection with '*Candidatus* Phytoplasma aurantifolia'. The phytoplasma SAP11 effector plays a key role in controlling the outgrowth of axillary buds by destabilization of the CYC/TB1-TCP transcription factors of *Phalaenopsis*. Thus, transgenic *Phalaenopsis* expressing SAP11 displayed multiple shoots, which resembles the growth pattern of sympodial orchids. Finally, we showed that CYC2 and CYC3 were predominantly expressed in the axillary buds of *Phalaenopsis*, and the knockdown of CYC2 and CYC3 by RNAi led to multiple branching *Phalaenopsis*. These findings suggest that CYC2 and CYC3 act as repressors to inhibit the outgrowth of axillary buds and control the growth pattern of *Phalaenopsis*.

Session 6 Development of Research Tools

Keynote-3 Artificial cell reactor technology

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Since we demonstrated the single-molecule enzymatic assay by micro-compartmentalization[1] (termed digital bioassay[2]), we have been pursuing the integration of complex biosystems on femtoliter reactor systems intending to realize more functional and autonomous microsystems that we term autonomous artificial cell reactors. Based on the femtoliter reactor array device (FRAD), we developed lipid bilayer chamber systems into which we reconstitute passive/active membrane transporters[3], and we fused a living bacterial protoplast to build a cyborg bacterial cell reactor[4]. Since then, we have been aiming to reconstruct cell systems that grow autonomously by taking a bottom-up approach. For this purpose, we developed a cell-free gene expression system from a single molecule template DNA (digital gene expression) on FRAD device and demonstrated a highly accurate screening method to obtain activity-enhanced enzyme sequences with a high enrichment factor, ten thousands[5]. We also implemented cell-free genome replisome termed RCR (reconstituted cycled replication) into FRAD or water-in-emulsion[6]. Currently, we are pursuing the possibility to use microdroplets in aqueous two-phase systems (ATPAS) as permeable and dynamic cell reactors for cell-free system reconstitution[7]. We will

introduce current findings on self-growing artificial cell reactors driven by internal DNA/RNA replication activity (Fig. 1), and discuss the perspectives of autonomous artificial cell reactor technology.

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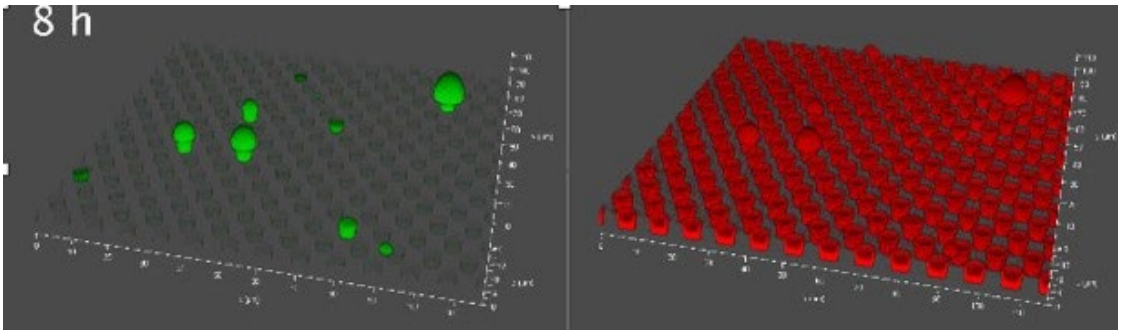

Fig. 1. Artificial cell reactor with self-growing activity. Fluorescent images of amplified DNA (left), and DEX-rich droplet (right), after 8 hr incubation.

O-32 Minimal cell JCVI-syn3B as a chassis to investigate host-microbe interaction

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As the smallest free growing organisms, mycoplasmas are used as model organisms to evaluate the genes necessary for life. However, because to their limited metabolic capabilities, most mycoplasmas are parasites exhibiting strict host and tissue specificities and are threats to mammalian cell cultures. In 2010, a 1.1 Mb synthetic

genome based on the genome of *Mycoplasma mycoides* subsp. *capri* (Mmc) was used to create strain JCVI-Syn1.0. A few years later, several strains of intermediate genome size were constructed until the development of JCVI-Syn3.0, with the minimized genome of 531 kb. In order to better understand the mechanisms behind mycoplasma host-microbe interactions, we applied an infectivity assay to evaluate the capacity JCVI-Syn1.0 and JCVI-Syn3.0, along with other synthetic minimized Mmc strains, to survive and grow in mammalian cell cultures. According with their behavior, we identified a cluster of eight non-essential genes (MMSYN1-0179 to MMSYN1-0186) that were likely involved in interactions between Mmc and cultured mammalian cells. We experimentally evaluated their contribution to Mmc pathogeny. Although these genes were not capable to restore the capacity of the minimal cell to infect mammalian cell cultures, our results indicated that they are essential for the attachment of synthetic Mmc to HeLa and HEK293 cells. They are likely required for Mmc attachment to goat cells in vivo. Furthermore, phagocytic activity of a human myeloid cell line in the presence of synthetic Mmc strains demonstrated the low immunogenicity of the JCVI-Syn3B, suggesting possible uses for the organism as a vehicle for delivering therapeutic molecules to patients.

O-33 Development of the Toolbox for *Mycoplasma* Genome Engineering

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Background- Due to the lack of efficient recombination and genome engineering tools, the production of mutants for functional genomics and applications such as the development of vaccine strains remains a bottleneck for most mycoplasma species.
Methods- Over the years, our team has developed many tools for the genome engineering of mycoplasmas. Some are designed for a direct use in mycoplasma cells and some others were developed following pioneering studies performed at the J. Craig Venter Institute, allowing the cloning and engineering of mycoplasma genomes in yeast before a whole genome transplantation into a suitable recipient mycoplasma.
Results- Among the tools we recently developed, the RecET system, derived from a *Bacillus subtilis* prophage, is an imported recombination system that was used to introduce small deletion, gene replacement and insertion in *Mycoplasma gallisepticum*. The recombinase-assisted genomic engineering (RAGE), was shown to be efficient for gene inactivation or large DNA replacement in *Mycoplasma pneumoniae*. The CRISPR-derived Base-Editor system was used to obtain gene knock-out by generating nonsense mutations in four major pathogenic mycoplasma species: *M. gallisepticum, Mycoplasma agalactiae, Mycoplasma bovis* and *Mycoplasma mycoides* subsp. *mycoides*. Regarding in-yeast genome engineering, 21 mollicutes genomes have already been cloned in yeast and the back-genome transplantation to generate a living bacterium has been achieved for 7 species, all related to the *M. mycoides* cluster.
Conclusion- Overall, our recent studies indicate that genome engineering in mycoplasma is now feasible in many significant species for which there is a need for improved control, including by building innovative vaccines.

O-34 Engineering a *Mycoplasma* Surface Display tool for Protein-Protein Interaction Screening

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Surface display technologies have been fundamental tools in the field of protein engineering and the discovery of high affinity interactions. Out of the commonly available display technologies (phage, yeast, bacteria), the latter offers unique advantages in terms of both library sizes and available methods for high-throughput characterisation. Their major limitation is the folding and exposition on the outside of the cell wall of the proteins of interest. *Mycoplasma* species has neither a cell wall nor multiple cellular membranes, allowing for direct anchoring of the tested library on the cell surface. It has been shown that *Mycoplasma pneumoniae* secretes correctly folded human proteins (enzymes, interleukins etc.). We propose a mycoplasma-based display assay for Protein-Protein Interaction screening combining different *Mycoplasma* species based on their specific properties. The model system *Mycoplasma pneumoniae* is used to express on its surface the protein against which we want to screen our library. A non-adherent *Mycoplasma* species is used to clone a mutant library of our protein of interest that will be present on its surface. We have already identified the sequences that allow exposure to the solvent in *Mycoplasma pneumoniae* as well as in the non-attached *Mycoplasma* species, and optimized promoters in both to maximize protein expression. Further work will be focused on the validation of this system with relevant protein interactions for treatment of lung disease such as Idiopathic Pulmonary Fibrosis.

O-35 Generating *Mycoplasma bovis* gene knock-out mutants using the CRISPR/Cas9 system of *Mycoplasma gallisepticum*

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Background: Limitations on genetic manipulation of mycoplasma species have been a significant barrier to the development of suitable vaccines. In recent years a few studies have successfully employed bacterial CRISPR/Cas systems delivered in transposons to achieve targeted genetic manipulation in mycoplasmas. In parallel, we have managed to achieve targeted mutagenesis in *Mycoplasma gallisepticum* using its endogenous CRISPR/Cas system. Unlike *M. gallisepticum*, the mycoplasmas in the hominis phylogenetic group (which includes *M. bovis*) lack a CRISPR/Cas system. Therefore, we aimed to use the CRISPR/Cas9 system of *M. gallisepticum* to generate targeted gene knock-out mutants of *M. bovis*. Methods: The endogenous CRISPR/Cas9 system of *M. gallisepticum* was delivered in a replicable *M. bovis oriC* plasmid that also contained CRISPR direct repeats bracketing target gene sequences, but without any antimicrobial resistance genes (pMBCRISPR). Electrocompetent *M. bovis* cells were transformed with the pMBCRISPR and clones were selected for

kasugamycin resistance, which was generated by mutations induced by a CRISPR spacer targeting the *ksgA* gene. The target gene was amplified and sequenced to confirm the mutations. Whole genome sequencing was performed to investigate whether there were any off target mutations in the mutants. Results: The pMBCRISPR construct induced mutations in the *ksgA* gene in *M. bovis* and all the mutants that were obtained were cured of the plasmid by the 3rd passage but retained the mutated phenotype. Conclusion: This genetic manipulation tool could be adapted to induce targeted mutations in genes responsible for invasion and/or immune evasion, which may be suitable *M. bovis* vaccine candidates.

O-36 Open Genome Browser for Mollicutes: A User-friendly and Powerful Web Platform for Comparative Genomics

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The ever-decreasing costs of next generation sequencing led to the generation and deposition of many Mollicutes genomes in public databases. While it is possible to execute basic BLAST searches and download any of these assemblies, many other analyses are cumbersome, time-consuming, or even require programming skills. We tested the self-hostable, customizable and open-source platform Open Genome Browser (OGB) for comparative genomics analysis of Mollicutes. Easily accessible through a user-friendly web-interface, this cloud-based software is modular and can be tailored to specific needs. We downloaded 1,337 Mollicutes genomes from GenBank and re-annotated 18% of these genomes using NCBI's PGAP pipeline, as these datasets were incomplete. Subsequently, all genomes were functionally annotated using the eggNOG database, ortholog inference was performed using FastOMA and genome completeness was assessed using BUSCO. Finally, all genomes were imported into OGB. We evaluated OGB by analyzing pan and core genomes of wild type strains belonging to different genera of Mollicutes. OGB's ability to correctly predict pan and core genomes was tested using genomes of defined mutant strains. As expected, OGB correctly detect these alterations. Additionally, we compared the completeness of KEGG-pathways between Mollicutes genera/species, studied lipoproteins, and confirmed the results using defined mutant strains. OGB is a value multiplier for the existing data as it drastically simplifies comparative genomics analyses without requiring programming skills, and nicely complements existing software such as Molligen. Moreover, it enforces systematic storage of genomic data, including annotations and metadata. It drastically simplifies common analyses, makes them reproducible and facilitates collaborative data sharing.

O-37 Establishment of a *Mycoplasma hyorhinis* Challenge Model in Five-Week-Old Piglets

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Background: *Mycoplasma hyorhinis* is distributed worldwide with high prevalence. Arthritis and polyserositis are the main lesions caused by the infection, which may result in significant economic losses. Our aim was to compare two challenge routes to induce *M. hyorhinis* infection. Methods: Five-week-old, Choice hybrid pigs were inoculated on two consecutive days by intravenous route (Group IV-IV) or by intravenous and intraperitoneal route (Group IV-IP). Mock infected animals were used as control (Control Group). The clinical signs were recorded for 28 days, after which the animals were euthanized. Gross pathological and histopathological examinations, PCR detection, isolation and genotyping of the re-isolated *Mycoplasma* sp. and culture of bacteria other than *Mycoplasma* sp. were carried out. ELISA test was used to detect anti-*M. hyorhinis* immunoglobulins. Results: Pericarditis and polyarthritis were observed in both challenge groups, however the serositis was more severe in Group IV-IV. Statistically significant differences were detected between the challenged groups and the control group regarding the average daily weight gain, pathological scores and ELISA titres. Additionally, histopathological scores in Group IV-IV differed significantly from the scores in the Control Group. All re-isolated strains were the same or a close genetic variant of the original challenge strain. Conclusions: Our results indicate that both challenge routes are suitable for modelling the disease. However, due to the evoked more severe pathological lesions and the application being similar to the natural route of infection the two-dose intravenous challenge is recommended by the authors.
Key words: challenge, ELISA, infection, *Mesomycoplasma hyorhinis*, PCR

O-38 Homologous and heterologous plasmid-assisted expression of Ig cleavage systems of Mollicutes in an engineered *Mycoplasma feriruminatoris*

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The *Mycoplasma* Immunoglobulin Binding/Protease (MIB/MIP) system are candidate virulence factors present in multiple pathogenic species of Mollicutes, including the

fast-growing species *M. feriruminatoris*. The MIB/MIP system cleaves the heavy chain of host immunoglobulins, hence affecting antigen-antibody interactions and facilitating immune evasion. In this work we analyzed the distribution and genetic relatedness between MIB/MIP systems of different species. Using proteomics and transcriptomics data we showed that the four copies of the *M. feriruminatoris* MIB/MIP system have different expression levels, are transcribed as operons and are controlled by four different promotors. We then removed the four existing gene copies of the MIB/MIP system of *M. feriruminatoris* using CReasPy cloning and genome transplantation. We developed *oriC*-based plasmids for *M. feriruminatoris* and shuttled in the different individual copies of the *M. feriruminatoris* MIB/MIP system and tested their functionality. We successfully cloned the different MIB/MIP copies of *M. feriruminatoris* under the control of different natural promoters as well as one MIB/MIP system of the closely related *Mycoplasma mycoides* subs. *capri*. Moreover, we showed immunoglobulin cleavage attributed to MIB/MIP activity in distantly related pathogenic porcine Mollicutes species. We were able to express the latter in *M. feriruminatoris*, but did not show activity, suggesting the presence of different protein export systems among Mollicutes. Since *M. feriruminatoris* is an interesting candidate for industrial purposes and as vaccine chassis, we confirmed its safety in domestic goats in vivo, which are the closest relatives to its native host the Alpine ibex.

O-39 Bovine Tracheal Organoids vs 2D Cell Cultures as Models to Study *Mycoplasma bovis* Cell Invasion

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Studies of the capacity of *Mycoplasma bovis* to invade cells and disrupt their function will help improve our understanding of its survival in host. Because of the constraints on studying the pathogenesis of *M. bovis* in experimentally infected cattle, better in vitro infection models are needed. Organoids are clusters of self-organised, differentiated cells that mimic the structure and function of an in-vivo organ. This study aimed to assess the feasibility of using organoids to study cell invasion by *M. bovis*. Bovine tracheal organoids were infected with the Australian wildtype strain of *M. bovis* 3683 and viable titres of *M. bovis* were determined 72 hours post infection (HPI). Immunofluorescence, immunohistochemistry and electron microscopy were performed to confirm *M. bovis* infection. Cell invasion assays in Madin-Darby bovine kidney (MDBK) cells were also performed with *M. bovis* PG45 and 3683, with the mycoplasmas visualised using immunofluorescence. *M. bovis* strain 3683 proliferated

in organoids, with the titre increasing by 1.6×10^6 CCU/mL by 72 HPI, similar to its kinetics during co-culture with MDBK cells. Histological examination confirmed that *M. bovis* could invade MDBK cells and infect the tracheal organoids. In MDBK cell invasion assays, 13% of *M. bovis* strain PG45 and 11% of *M. bovis* strain 3683 had invaded by 72 HPI. Bovine tracheal organoids can be successfully infected with an *M. bovis* wild-type strain, indicating that they can be used to investigate its pathogenesis in vitro, and may be a better alternative to cell culture monolayer systems, as they closely mimic host tissues.

O-40 The conjugative properties of *Mycoplasma agalactiae* are critically influenced by the expression of lipoprotein P48 at the surface of the recipient mating partner

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Background. Self-transmissible Integrative and Conjugative Elements (ICE) are key in massive horizontal chromosomal exchanges observed in several mycoplasma species. Understanding the factors controlling ICE transfer from donor to recipient cells is thus of utmost importance to further control horizontal gene transfer (HGT) in these organisms. By using the ruminant pathogen *M. agalactiae* as a model system, we demonstrated that a major antigenic lipoprotein at the surface of the ICE-recipient partner is critical for ICE transfer from donor cells. Methods. Large-scale random transposon mutagenesis was combined with high-throughput mating assays to map in the chromosome of the ICE-recipient partner those regions that are facilitating HGT. Results. Up to 20 mutants were selected that identified 12 regions whose disruption was associated with a reduced mating frequency. Remarkably, 3 mutants all characterized by a transposon inserted at different positions of the P48 lipoprotein gene, were found fully resistant to ICE transfer from donor cells. The central role of this surface expose lipoprotein in ICE transfer was confirmed by complementation studies. Preliminary results suggest that the initial contact between mating partners might be mediated by the interaction between P48 and the ICE-encoded lipoprotein CDS14 expressed at the surface of the donor partner. Whether P48 may facilitate HGT in other species is unknown but this putative ABC transporter substrate-binding protein was found highly conserved across Mollicutes. Conclusion. These data suggest that not only ICE-donor cells but also recipient mating partners may modulate ICE dissemination and provide interesting opportunities to control ICE-mediated HGT in these organisms.

Session 7 Cell Biology and Genetics

Plenary

Design, Construction, and Analysis of a Synthetic Minimal Bacterial Cell

John I. Glass

J. Craig Venter Institute

The minimal cell is the hydrogen atom of cellular biology. Such a cell, because of its simplicity and absence of redundancy would be a platform for investigating just what biological components are required for life, and how those parts work together to make a living cell. Our team built a minimal cell having about half of the genes that are in *Mycoplasma mycoides*. We constructed synthetic bacterium JCVI-Syn3.0 (531 kb, 474 genes). Synthetic bacterium JCVI-Syn3.0 retains almost all genes involved in synthesis and processing of macromolecules. Surprisingly, it also contained 149 genes with unknown biological functions, suggesting the presence of undiscovered functions essential for life. This minimal cell is a versatile platform for investigating the core functions of life, and for exploring whole‐genome design. Our research team now collaborates with research teams worldwide to use this versatile platform to investigate the core functions of life and to explore whole‐genome design. Since 2016, we have identified functions for about 65 of the original 149 genes of unknown function. These findings have been used to create flux balance analysis and kinetic whole cell computational models of our minimal cell that replicate laboratory observations about our minimal cell. Additionally, we have used JCVI-syn3.0, which has an abnormal cell division and cell morphology phenotype, and a JCVI-syn3.0 mutant containing an additional seven non-essential genes that has divides normally and looks like wild type *M. mycoides* to investigate how modern cell division and cell size control might have evolved.

O-41 Genomic analysis of *Mycoplasma bovis* causing bovine respiratory disease in Australian feedlot cattle

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Mycoplasma bovis affects cattle and causes diseases including bovine respiratory disease (BRD), arthritis, mastitis, and reproductive issues posing a serious economic threat to cattle production industries. Here, we sequenced 46 *M. bovis* BRD isolates from Australian feedlots to broaden our understanding of *M. bovis* diversity. The isolates shared a core genome of 90% (1474/1646 genes) and differed by an average of 145 SNPs across the different feedlot operations. A comparative genomic analysis using 93 Australian *M. bovis* genomes (mastitis=56; joints=11; respiratory=22; genitalia=6) found that isolates were all sequence type (ST) 52 (n=117) or ST52-like (n=22) (ST52 variants were not phylogenetically distinct), shared 1297 genes (76% core genome) and averaged 108 core SNPs. No significant differences were observed in virulence gene distribution across any pathologies. Likewise, genome-wide association studies found no genes with enough (>80%) specificity and sensitivity to differentiate *M. bovis* causing different pathologies. Finally, to put the Australian *M. bovis* isolates into a global context a comparative analysis was made using 583 additional *M. bovis* genomes from NCBI. Our results indicate *M. bovis* are very diverse, with a core genome consisting of only 17% of total genes. Australian isolates were most related to other ST52 isolates originating from China however isolates from the two regions formed distinct clades. These data suggest that *M. bovis* genomes: i) are

plastic and can readily gain or lose genetic content; ii) evolve as region-specific lineages that may reflect different management practices.

O-42 Nucleotide-Binding Sites Visualized by CryoEM Suggest Rotational Movement of the Motor for *Mycoplasma mobile* Gliding

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Mycoplasma mobile, a fish pathogen, exhibits gliding motility driven by a special motor. The motor is an F_1-like ATPase dimer, which forms a chain-like huge complex inside the cell. In the last IOM congress, we discussed the overall structure of the motor by combining the electron cryomicroscopy (cryoEM) structure at 5.7 Å resolution with the 3D structure predicted by AlphaFold2. In the present study, we improved single particle cryoEM on the motor using CRYOARM 300 equipped with a K3 camera and visualized the overall structure in detail at 3.2 Å resolution. This structure allowed us to construct the atomic model of the motor. The model contains ATP, ADP and phosphate at the catalytic sites, although the cryoEM analysis was performed under a nucleotide-free condition. The motor-core structure showed strong similarity to the structures of F-type ATPase in terms of (i) catalytically important residues (ii) nucleotide binding patterns in the three catalytic sites and (iii) conformational differences among three catalytic subunits. These observations suggest a rotational movement that is conserved in the rotary ATPase family, including F-type ATPases. Therefore, the rotational movement may drive motility in *M. mobile*. We propose that *M. mobile* has acquired its unique motility mechanism by appropriating the structure and mechanism of F-type ATPase.

O-43 Horizontal transfer of phytoplasma effector phyllogen genes is driven by potential mobile units

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Phytoplasmas are obligate intracellular plant pathogenic bacteria that can cause abnormal development of floral organs known as phyllody. One of conserved effector family produced by phytoplasmas, collectively called phyllogen, is responsible for inducing phyllody. While it has been suggested that phyllogen genes can be transferred horizontally between different phytoplasma species and strains (Iwabuchi et al., 2020), the underlying mechanisms and evolutionary history remain unclear. To shed light on this issue, we conducted an analysis of phyllogen flanking genomic regions in 17 phytoplasma strains related to six different species, including three newly sequenced strains. We found that many of the phyllogens were flanked by multicopy genes located

within potential mobile units (PMUs), putative transposable elements found in phytoplasmas. The multicopy genes displayed two distinct synteny patterns correlated with groups of the linked phyllogens. Notably, the low sequence identities and partial truncations observed in the phyllogen flanking genes suggested that PMU sequences are deteriorating, whereas the highly conserved sequences and functions of the phyllogens indicate that they are crucial for phytoplasma fitness. Additionally, we found that PMUs in strains related to '*Candidatus* P. asteris' were often located in different regions of the genome, despite of the similarity among their phyllogens. Our results strongly suggest that PMUs are the driving force behind the horizontal transfer of phyllogens among different phytoplasma species/strains. Overall, these findings have improved our understanding of how genes responsible for causing plant symptoms are shared among phytoplasmas.

O-44 Redundancy and essentiality of cytoskeleton protein components in *Spiroplasma* helicity and motility

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Understanding how *Spiroplasma* cell acquires, maintains and can even change its shape could help understanding how pathogenic *Spiroplasma* species adapt and resist to environmental conditions. *Spiroplasma* helical shape appears essential to their ability to swim in semi-viscous media. Indeed, motility of spiroplasmas is based on the propagation of a cell deformation called kink along the cell body, which triggers a helicity switch responsible for the displacement of the cell in the medium. The recent reconstruction of the *Spiroplasma citri* cytoskeleton in *Mycoplasma* highlighted that MreB isoforms, and more specifically MreB5, and fibril protein form internal polymers that play a major role in shape acquisition and kink propagation. However, the number of MreB isoforms vary from one species to another, making it difficult to understand the exact role of each isoform in *Spiroplasma* movement and helicity. In addition, the role of ATP hydrolysis by MreBs in *Spiroplasma* helicity and motility has not been demonstrated. Here we identified the different combinations of MreB isoforms, and the minimal cytoskeleton gene set allowing helicity and motility of natural strains by thoroughly examining 29 *Spiroplasma* genomes. Analyzing helicity and motility of different species including fibril-lacking ones showed that kinking capacity could be fibril-independent. In parallel, complementation assays of the non-helical, non-motile natural variant *S. citri* ASP-1 indicated that helicity and kink propagation were dependent on MreB5 nucleotide-bound state.

O-45 Essential protein P116 extracts cholesterol and other indispensable lipids for Mycoplasmas

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Mycoplasma pneumoniae, a human pathogen responsible of a high percentage of community-acquired human pneumonia worldwide, needs to extract cholesterol and other lipids from the host environment for survival and proliferation. We present here structural, functional and immunogenic results of the previously mostly uncharacterized protein P116 (MPN_213), an essential protein for the viability of *M. pneumoniae*. P116, distributed throuhgout the entire surface of the mycoplasma cell, is the target of a strong antigenic response in infected people. Antibodies generated against the ectodomain of P116 appear to delay the growth time of *M. pneumoniae*. The structure of a recombinant P116, spaning the whole ectodomain and determined by single-particle cryoEM, has a previously unseen fold containing a large hydrophobic cavity that is fully accesible from the external environement. The presence of unexplained density inside the cavity, Lipidomic analysis and Radioactivity transfer experiments, confirmed that P116 can bind a diversity of lipids, even extracting them from serum (FBS) or from Lipoproteins. Moreover, some of the ligands chararterized bound to P116 (such as cholesterol, sfingomielin (SM), phosphatidylcholine (PC) or oleic fatty acid), are essential for survival and abundant in the membranes of *M. pneumoniae* cells. The structure of an (almost) empty P116, where the cavity is mostly occluded, was also determined by cryoEM revealing the mechanism by which P116 can reversibly store or release its cargo of lipids. These findings provide an explanation for the essential character of P116 in *M. pneumoniae* , also revealing the unique properties of this protein.

O-46 Bioinformatic and Metabolomic Analyses Reveal the Potential Metabolic Functions of Genes Critical for Survival of *Mycoplasma bovis* in Cell Culture

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Mycoplasma bovis causes a range of chronic diseases in cattle, including mastitis, pneumonia, and arthritis. Although the genomes of a number of strains of *M. bovis* have been sequenced, the functions of many annotated genes in this pathogen are still uncertain. In this study, the genes disrupted in seven *M. bovis* PG45 transposon mutants with abrogated growth when co-cultured with Madin-Darby bovine kidney (MDBK) cells were subjected to bioinformatic analyses, and the metabolomes of the mutants were examined using gas chromatography-mass spectrometry (GC-MS). Bioinformatic analysis predicted that two of the disrupted genes encoded putative peptidases and that three encoded putative transporters. One deoxyribose-5-phosphate aldolase domain was also identified in one of the proteins, indicating that it may be involved in nucleotide metabolism. Consistent with the bioinformatic predictions, metabolomic analysis revealed significantly lower abundance of intracellular amino acids in the two mutants with disruptions in putative peptidase

genes, and lower abundance of intracellular sugar metabolites and amino acids in the three mutants with disrupted putative transporter genes. Glycerol-3-phosphate and metabolites associated with nucleotide metabolism were significantly less abundant in the mutant with a disruption of the putative deoxyribose-5-phosphate aldolase gene, indicating that the consumption of nucleotide metabolites might be affected. In summary, combined bioinformatic and metabolomic analyses revealed potential metabolic functions of several critical genes in *M. bovis*, which may yield novel insights into understanding the interplay between host cells and *M. bovis*, and the development of more effective vaccines.
Key words: metabolomics, metabolic function, *M. bovis*

O-47 Inositol metabolism is the essential factor to the growth and virulence of *Mycoplasma hyopneumonia*

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Background: The specific metabolic ability of pathogenic microorganisms has been regarded as "nutritional virulence" and is an important factor affecting their pathogenicity. The research on the pathogenic mechanism of *Mycoplasma hyopneumoniae* (Mhp) mostly focuses on the classic virulence factors, and the connection between its metabolic ability and pathogenicity is rarely reported. Methods: The clustered regularly interspaced short palindromic repeats-mediated interference (CRISPRi) system, inhibiting target gene expression on *Mycoplasma mycoides* significantly, combined with the pMD18-TO-gfp plasmid transient expressed in Mhp, was used to construct the gene expression inhibition and over-expression plasmid of Mhp *iolC*, and the *iolC* inhibition and its complementary strain were obtained through chemical transformation. Results: Only Mhp in swine-origin mycoplasmas containing the inositol metabolism, and supplemention a certain concentration of inositol can promote the growth of Mhp, virulent strains can use low levels of inositol more efficiently, i5-dehydro -2-deoxygluconokinase (*iolC*) is the key gene of inositol metabolism gene cluster. After the inositol metabolism was blocked, the morphology of Mhp solid clone, the biofilm formation ability, adhesion, oxidative stress damage to immortalized porcine bronchial epithelial cells, anti-macrophage phagocytosis to porcine macrophage, the pathogenicity in mice caused by Mhp infection were significantly decreased, and the expression of other virulence related genes of Mhp could be affected. Conclusion: Inositol metabolism is the essential factor to the growth and virulence of Mhp, revealing the pathogenic mechanism of Mhp from the perspective of metabolic adaptability, which provides a theoretical basis and ideas for

pathogenesis study of Mhp and other mycoplasmas.

O-48 *Mycoplasma pneumoniae* biofilms and the regulation of glycerol 3-phosphate oxidase activity by oxygen

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Biofilm towers formed by *Mycoplasma pneumoniae* in vitro and on bronchial epithelial cells have properties consistent with persistence and chronic disease. Some bacteria, including streptococci, attenuate production of virulence factors when growing in biofilm towers. Concordantly, our group has previously shown that the onset of tower formation by *M. pneumoniae* in vitro is accompanied by a sharp decline in the ability to produce several virulence factors, including the volatile molecule hydrogen peroxide. Hydrogen peroxide is produced during oxidation of glycerol 3-phosphate, a reaction catalyzed by the enzyme GlpO (glycerol 3-phosphate oxidase). These data suggest the hypothesis that *M. pneumoniae* decreases hydrogen peroxide production either to protect itself from damage or to limit damage to the host during long-term residence. We used minimum inhibitory concentration assays and scanning electron microscopy to test whether biofilm towers protected *M. pneumoniae* from peroxide. We also measured GlpO activity in extracts from *M. pneumoniae* cells grown in reduced oxygen environments. The results were that *M. pneumoniae* was exquisitely sensitive to hydrogen peroxide, that biofilm towers were not protective against hydrogen peroxide, and that growth in low oxygen distinctly reduced GlpO activity independent of substrate access. We propose that during chronic infection, low oxygen levels within biofilm towers signal *M. pneumoniae* to attenuate hydrogen peroxide production, protecting itself from self-inflicted damage.

O-49 The Family of Type-II DNA-Methyltransferases of *Metamycoplasma hominis* including the first postulated type II MTase phase-variants

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Bacterial virulence, persistence and defence are affected by epigenetic modifications, including DNA methylation. DNA-methyltransferases, if solitary, modulate a variety of cellular processes and influence bacterial virulence; if part of a restriction-modification (RM-) system, they act as a primitive immune system in methylating the own DNA, while unmethylated foreign DNA is restricted. In a bioinformatics approach, we identified a large family of type II DNA-methyltransferases in *Metamycoplasma hominis*, comprising six solitary methyltransferases and four RM-systems. Motif-specific 5mC and 6mA methylations were identified by tailored Tombo analysis on Nanopore-reads. Selected motifs with methylation scores > 0.5 fit with gene presence for DAM1 and DAM2, DCM2, DCM3 and DCM6, but not for DCM1, whose activity was strain-dependent. Activity of DCM1 for C(mC)WGG, and DAM1 and DAM2, both for G(mA)TC, was proven in methylation-sensitive restriction and finally for recombinant

rDCM1 and rDAM2 against a *dam*-, *dcm*-negative background. A hitherto unknown *dcm8*/*dam3* gene fusion containing a (TA) repeat region of varying length was characterized within a single strain, suggesting the expression of DCM8/DAM3 phase variants. The type II DNA MTases, characterized at the genetic and enzymatic levels in this study, can now be evaluated for their involvement in *M. hominis* pathophysiological effects, such as virulence and defence.

O-50 *Mycoplasma bovis* Nucleomodulin MbovP202 Inhibits Macrophage Proliferation by DNA methylation

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Background: *Mycoplasma bovis* (*M. bovis*) is an important pathogen of bovines mainly causing bovine bronchopneumonia and mastitis. The lack of vaccines and efficient therapeutic drugs results in huge losses of cattle industry. Nucleomodulin is a kind of secreted proteins which can internalize nuclei to regulate the gene expression of host cells and function as the critical virulence factors of pathogens. This study aims to explore the mechanism by which nucleomodulin MbvP202 inhibits cell proliferation. Methods: we used confocal microscopy to determine the subcellular localization of MbovP202. DNA methylation-interference assay and LC-MS/MS were used to verify DNA methylation by MbovP202. EMSA was used to verify MbovP202-binding DNA motif. CCK-8 was used to detect cell proliferation. Whole genome bisulfite sequencing (WGBS) combined with RNA-seq was used to analyze the relationship between DNA methylation and genes expression. Results: MbovP202 entered into BoMac nuclei guided by its N-terminal and C-terminal peptides. MbovP202 significantly increased 5-methylcytosine in BoMac genome and inhibited cell proliferation. EMSA assay identified that rMbovP202 specifically bound and methylated the mCATG site of dsDNA to prevent it from restriction endonuclease. However, WGBS results showed that MbovP202 increased not only CmATG site methylation but also mCG site methylation. Furthermore, Conjoint analysis of WGBS and RNA-Seq showed MbovP202 mainly regulated the expression of genes enriched in metabolic pathway. Conclusion: we revealed MbovP202 can methylate host DNA to affect expression of host metabolic genes, thereby inhibiting the proliferation of BoMac cells. The results would provide novel theoretical evidence for elucidating pathogenesis of *M. bovis*.

Poster Presentations

Category 1. Clinical Cases

P-001 *Tet44*: A New Ribosomal Protection Protein Conferring Tetracycline Resistance in Clinical Isolates of *Mycoplasma bovis*

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Background: *Mycoplasma bovis* is a ruminant pathogen that can result in substantial economic losses of affected herds. There are increasing global reports of antimicrobial resistance in *M. bovis*. Tet44 confers resistance to tetracycline in via ribosomal binding and was first described in *Campylobacter fetus* in 2010. Here we report the first detection of the *tet44* gene in *M. bovis*. **Methods**: Whole genome sequencing of 32 clinical isolates of *M. bovis* obtained from dairy calves in the U.S since 2020 was performed and the *tet44* gene was identified during in silico screening. To assess the historic incidence of *tet44*, we performed a BLAST nucleotide and protein search of the 279 genome assembly and annotation reports in GenBank. Screening of our existing collection of *M. bovis* clinical isolates obtained over the past 35 years by PCR to detect *tet44* is underway. **Results**: The *tet44* gene was not detected in any of the 279 GenBank genomes. In contrast, we found that 28/32 (87.5%) of current clinical isolates contained the *tet44* gene. Our BLAST search did find *tet44* in an uncultured mollicutes species and in *Acholeplasma laidlawii*; both had 98% identity to the *tet44* in *M. bovis*; **Conclusion**: Our data suggests that the acquisition of the *tet44* gene is a relatively new occurrence in *M. bovis*. The presence of *tet44* may contribute to the increased antimicrobial resistance in *M. bovis* and should be included when screening clinical isolates for tetracycline resistance.

P-002 Phenotypic and Genetic Characterization of an Atypical Virulent *Mycoplasma synoviae* Strain

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*Mycoplasma synoviae*is an infectious pathogen of poultry and although subclincal infection is common, virulent strains may result in respiratory signs and/or swollen joints and footpads. *M. synoviae* strains have been shown to vary widely in their virulence and in their ability to produce various clinical signs. In 2014 an *M. synoviae* (K6677) was isolated from a clinical case (complicated with infectious bursal disease (IBDV)) involving elevated mortality, severe bursal atrophy, airsacculitis and lymphocytic pneumonia. In vivo trials with K6677 have indicated that the strain is unusually virulent compared to *M. synoviae* type strains and other US-derived *M. synoviae* strains. Bird trials that included K6677 alone or in combination with respiratory viruses resulted in severe airsacculitis, tracheitis, synovitis, reduced weight gain and mortality. The strain K6677 was analyzed by DNA sequencing of portions of the *vlhA*, *nanA* and *ugpA* genes and the DNA sequences were compared to the

database at the Poultry Diagnostic and Research Center (PDRC), (University of Georgia, USA) as well as to publicly available sequences in NCBI Genbank database. Uncorrected pairwise distance (with pairwise gap removal) analysis following Clustal W alignments (using MegAlign software (in Lasergene; DNASTAR, Inc., Madison, WI) indicated that the percent identity of K6677 sequences ranged from 95–100% when compared to 66 *vlhA*, 45 *nanA* and 58 *upgA* sequence types identified. Whole genome analysis (Illumina) has revealed potential virulence factors present in K6677.

P-003 A Case Study of Swine Arthritis Caused by *Mycoplasma hyopharyngis*

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Background: *Mycoplasma hyopharyngis* is a rarely isolated, arginine hydrolysing *Mycoplasma* species mainly found in the upper respiratory tract of pigs. In one occasion, *M. hyopharyngis* was recovered from inflamed joint lesions from a herd in the United Kingdom. Methods: Joint samples from a Hungarian herd with history of arthritis were sent for bacteriology laboratory diagnostic examination. Bacteria other than mycoplasmas were cultured on blood and chocolate blood agar plates at 37°C in the presence of 5% CO_2. Identification of the cultured bacteria was accomplished by biochemical test and mass spectrometry. Culture of Mycoplasma sp. was carried out in MolliScience GM media at 37°C with 5% CO_2. Mycoplasmas were identified by sequencing the intergenic spacer region of the 16S-23S rRNA genes. Results: From the joint samples from which mycoplasmas were not cultured *Staphylococcus haemolyticus*, *S. hyicus* and *Aerococcus viridans* were recovered. In one joint sample besides *Trueperella pyogenes* and *Streptococcus agalactiae*, *M. hyopharyngis* was isolated. Conclusions: *M. hyopharyngis* is a neglected species, considered apathogenic with unknown prevalence. The role of *M. hyopharingis* in swine arthritis warrants further studies.
Key words: arthritis, *Mycoplasma hyopharnygis*, porcine

Category 2. Diseases

P-004 Genetic characterization of *Mycoplasma pneumoniae* strains isolated in Japan: Spread of p1 gene type 2c and 2j variant strains

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We characterized 118 *Mycoplasma pneumoniae* strains isolated from three areas of Japan during the period of 2019 and 2020. Genotyping of the *p1* gene in these strains revealed that type 2 lineage was dominant in this period (89/118, 75.4%). The most prevalent type 2 variant was 2c, while the second-most was 2j, a novel variant identified in this study. Type 2j *p1* is similar to type 2g *p1*, but cannot be distinguished from classical type 2 using the standard PCR-RFLP analysis with HaeIII digestion. Thus, we used MboI digestion in the PCR-RFLP analysis and re-examined the data from previous genotyping studies as well. This revealed that most strains reported as classical type 2 after 2010 in our studies were actually type 2j. The revised genotyping data showed that the type 2c and 2j strains have been spreading in recent years and were the most prevalent variants in Japan during the time-period of 2019 and 2020. We also analyzed the macrolide-resistance (MR) mutations in the 118 strains. MR mutations in the 23S rRNA gene were detected in 29 of these strains. The MR rate of type 1 lineage (14/29, 48.3%) was still higher than that of type 2 lineage (15/89, 16.9%); however, the MR rate of type 1 lineage was lower than that found in previous reports published in the 2010s, while that of type 2 lineage strains was slightly higher. Thus, there is a need for continuous surveillance of the *p1* genotype and MR rate of *M. pneumoniae* clinical strains.

P-005 The identification of adhesive and proinflammatory function of MYPE6810, a membrane lipoprotein from *Mycoplasma penetrans*

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Mycoplasma penetrans, a bacterium that infects HIV-positive patients, may contribute to the progression of AIDS, and it is named "AIDS-associated mycoplasma". Adhesion is the primary factor for mycoplasma to infect host cells. *M. penetrans* has the ability to adhere and penetrate into cells, cause genital and urinary diseases in humans, while its adhesion related protein is still a mystery. The P35 family lipoproteins encoded by the *mpl* genes of *M. penetrans* is the major lipoprotein on cell surface, can induce antibody production of host, and change its profiles frequently for immune escape. Hereon, we observed the cell morphology and gliding motility of *M. penetrans*, and found that *M. penetrans* has the terminal structure that similar to *M. pneumoniae*, can adhere and slide on the surface of coverslip. We isolated and identified the cytoskeletal proteins of *M. penetrans*. The MYPE6810, belonging to the P35 family lipoproteins of *M. penetrans*, was identified as the component of cytoskeleton, mainly existed in the membrane protein. Furthermore, both recombinant MYPE6810 (rMYPE6810) and *M. penetrans* could adhere to SV-HUC-1 cells, and the anti-rMYPE6810 serum partially inhibit the adhesion of *M. penetrans*. Meantime, the rMYPE6810 and *M. penetrans*

competed to bind SV-HUC-1 cell. On the other hand, the results of flow cytometry showed that both rMYPE6810 and *M. penetrans* can induce M1 polarization of RAW264.7 cells and to secrete proinflammatory factor IL-1β, IL-6 and TNF-α. Therefore, we concluded that the MYPE6810 protein may be one of adhesion related proteins of *M. penetrans* and have proinflammatory function.

P-006 Genome-Wide Association Study to Investigate Genetic Markers for Antimicrobial Resistance in *Mycoplasma cynos* and *Mycoplasma felis*

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Mycoplasma cynos and *Mycoplasma felis* are respiratory pathogens of dogs and cats, respectively. Treatment relies on the empirical use of antimicrobials, however, the in vitro activity against *M. cynos* and *M. felis* has never been studied, and the effectiveness of therapy is completely unknown. We aimed to evaluate the in vitro activity of antimicrobials and to implement the first genome-wide association study to identify genetic markers of antimicrobial resistance in *M. cynos* and *M. felis*. Oxford Nanopore and Illumina sequencing were used to generate hybrid whole genome assemblies of *M. cynos* (n=12) and *M. felis* (n=16). The minimum inhibitory concentration (MIC) of doxycycline, tetracycline, minocycline, enrofloxacin, marbofloxacin, and azithromycin were determined. Molecular markers in *gyrA* were associated with high MICs to enrofloxacin (4–16 µg/mL) and marbofloxacin (4–8 µg/mL) in *M. cynos* (n=3) and *M. felis* (n=6). SNPs found in the 23S rRNA gene were associated with decreased susceptibility to azithromycin (8–32 µg/mL) in *M. cynos* (n=4). Potentially new markers including Asp96 in GyrA, C1187T in 23S rRNA, and Val178Leu in L4 ribosomal protein were associated with high MICs to enrofloxacin, marbofloxacin, and azithromycin in *M. felis*. All isolates had low tetracycline MICs and no 16S resistance determinants were found. In summary, susceptibility to tetracyclines support their use to treat *Mycoplasma* infections in small animals. Decreased susceptibility to fluoroquinolones and macrolides was confirmed by detecting mutations in target genes. This pioneer study provides knowledge to direct effective therapeutic interventions for respiratory mycoplasmas in dogs and cats.

P-007 Advancement in the Development of a Safe and Efficacious Subunit Vaccine against *Mycoplasma gallisepticum*

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(i) Background: *Mycoplasma gallisepticum* is the etiologic agent of Chronic Respiratory Disease (CRD) in chickens, Infectious Sinusitis in turkeys, and Conjunctivitis in house

finches. Infections of chickens may result in reduced egg laying and downgrading of carcasses. Those two ailments have been estimated to cause an economic loss of over $700 million annually in the United States alone. A subunit vaccine could provide advantages over currently utilized vaccines including multi-strain protection, no risk of reversion to virulence, adaptability to emerging strains, and minimal to no pathologic changes in the host. (ii) Methods: We designed a subunit vaccine containing two primary adhesion proteins (GapA and CrmA) along with four early-expressed phase variable lipoprotein hemagglutination A proteins (VlhAs 3.03, 3.06, 4.07, 5.05). We vaccinated 5-week-old female white leghorn chickens in a prime-boost schedule. Groups of 10 chickens either received all six proteins, only VlhAs, only GapA and CrmA, or saline. Vaccine doses included 50 µg of each specified protein. All formulations were mixed with Addavax. Two weeks after challenge with *Mycoplasma gallisepticum* strain Rlow, tracheal sections were taken for bacterial recovery or fixed in formalin for histopathology. (iii) Results: Chickens that received formulations containing GapA and CrmA had reduced thickening of tracheal mucosa and submucosa. Chickens that received only GapA and CrmA had reduced bacterial recovery. (iv) Conclusion: Continued optimization of the vaccine formulation, adjuvant, dose, and schedule are needed. This research is uniquely poised to lead to substantial decreases in economic loss throughout the United States and the rest of the world.

P-008 Comparison of Next-Generation Sequencing (NGS) Protocols for Diagnosis of Phytoplasmas

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Phytoplasmas are a group of plant-pathogenic bacteria which are characterized by a remarkably small, AT-rich genome with repetitive regions and mobile units. Regardless of their small genome size, phytoplasmas can still adapt to a complex life cycle that involves two distinct host environment- plants and insects. However, the inability of invitro cultivation, the AT-rich and repetitive nature of phytoplasma genome hinder efforts to get a handle on their genomes. Traditional assembly workflows usually result in unresolved gaps, incomplete genome sequences or contamination of DNA from the host. The aim of this study is to utilize a universal workflow for both short (Illumina) and long read sequencing (Oxford Nanopore) to obtain complete assemblies of phytoplasma genomes. For this purpose, total DNA was isolated from *Candidatus* Phytoplasma mali infected daffodils. Sequencing was performed with both the Illumina NovaSeq 6000 (2x150 paired end) and the Oxford Nanopore Technologies (~10 h). The outputs of both reading platforms were de novo assembled as separate and hybrid. Raw reads were first mapped to different phytoplasma genomes by BBMap. Short reads were trimmed with Trimmomatic and assembled with metaSPAdes (kmer=93). The ONT sequencing data were base-called with quality filtering using GUPPY. Quality checked with Filtlong flowed by trimming step by Porechop. Long reads assembled with Canu flowed by visualization step by Nanoplot and Bandage Image tools. Finally, Unicycler was used for hybrid assembly. The availability of circular genome of phytoplasma from daffodils will be essential to reconstruct the lineage of *Ca*. P.mali.
Key words: Phytoplasma, Nanopore, hybrid assembly, comparative-genomics

P-009 Abattoirs and Herds Survey to Monitor the Occurrence and Distribution of *Mycoplasma* Infections in Ruminants in Nigeria

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Background: Mycoplasmas cause economically important ruminant diseases world-wide. Our preliminary clinical and serological work using BoviLAT and CapriLAT has shown that both both CBPP and CCPP are probably present in ruminants in Nigeria. Methods: Of the 342 cattle, and 319 goats tested in Nigeria, 42 cattle, 23 goats positive serum samples and six DNA from cultures positive were shipped to APHA for serological and molecular assays including: IDEXX CBPP and CCPP cELISAs, Immunoblot (IBT), CapriLAT, BoviLAT and CFT and PCR-DGGE, species specific-PCRs and Applied Biosystems™ VetMAX™ *M. agalactiae* & amp; *M. mycoides* Kit RT-PCR. Results: Two goat sera were positive for Mccp by ELISA, with a further two capriLAT positive. A further sample was positive for *M. agalactiae* by ELISA, while two were positive by Mmc and Mcc CFT tests, respectively. However, six samples were positive by *M. ovipenumoniae* ELISA, with a further three with elevated titres just below the cut-off. Although DGGE -PCR for some samples yielded bands close, but not exact matches, to *M. ovipneumoniae*, Mcc, *M. agalactiae*, *M. conjunctivae* and for the bovine samples *M. alkalescens* these pathogens were negated by a combination of specific PCR, product sequencing but all returned negative results. Mccp and *M. ovipneumoniae* serological positivity suggests mixed infections were present in the goats. Conclusion: Confirmed serological findings indicate likely exposure to Mccp and Mmm, further investigation includes identifying mycoplasmas diagnostic biomarkers and potential vaccine traits from Nigerian isolates anticipated.
Key words: Abattoirs, Herds, Mycoplasmas, Ruminants, Serological and molecular assays.

P-010 Using Live Attenuated Vaccine, MslA 2204, Co-administered with a SiRNA Polyfunctional Nanoparticle Targeted Against Inflammatory Genes Up-regulated During *Mycoplasma gallisepticum* Infection

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Background: *Mycoplasma gallisepticum* (*M. gallisepticum*) is the primary etiologic agent of Chronic Respiratory Disease (CRD) in chickens causing tracheitis, airsacculitis, reduced feed conversion, and resulting in major economic losses to the poultry industry. Currently, live attenuated vaccines (LAVs) are used to combat this infection. Over-attenuated vaccines have shortcomings including poor duration of immunity and incomplete protection. Conversely, under-attenuated vaccines provide durable immunity but often result in residual pathogenicity and infection of non-target species. Current work aims to retain the robust, long-lasting immunity of an under-attenuated strain while preventing a maladaptive inflammatory response. Methods: Immune modulation will be accomplished by administering a Polyfunctional nucleic-acid Nanoparticle (PFNP) combined with siRNAs targeting three inflammatory cytokines administered prior to vaccination. These genes (CCL5, IL-1B, and IL8-L1) are each upregulated during infection with wild-type MG Rlow and ostensibly contribute to the pathology. We hypothesize that simultaneous knock-down of all three genes will modulate the inflammatory response while preserving (or enhancing) vaccine efficacy. siRNAs were screened in vitro using the HD-11 chicken macrophage-like cells exposed to *M. gallisepticum* Lipid Associated Membrane Proteins (LAMPs) which are highly inflammatory due to TLR ligation. Results: We identified three unique siRNAs which knocked down gene expression by 54-70% individually and induced comparable activity when used in combination. Conclusion: Ultimately, we will vaccinate chickens with the isogenic mutant vaccine strain mslA 2204 supplemented with the PFNP and subsequently challenged with a pathogenic Rlow strain to assess protection. This approach has potential application to numerous LAVs beyond Mycoplasmas.

P-011 Status of Phytoplasma Causing Diseases in the Philippines

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Phytoplasma causing the little leaf and witches' broom diseases on cucurbits (Cucurbitaceae), cassava (*Manihot esculenta*), bamboo (*Bambusa* spp.), and even species of weeds are getting rampant in the Philippines. Field survey and collection conducted from 2021–2023 covering the selected provinces in Luzon, Visayas and Mindanao islands, revealed the high incidence of little leaf and witches' broom diseases from cassava varieties 'Rayong 72', 'Rayong 5', and Golden Yellow, vegetable crops particularly bitter gourd (*Momordica charantia*) and sponge gourd (*Luffa aegyptiaca*), Philippine bolo (*Gigantochloa levis*) and even species of weeds (*Passiflorabiflora*). Detection of phytoplasma genes i.e., 16S rRNA, chaperonin 60, and protein translocase subunit A from infected plants were confirmed using qPCR, LAMP and CPN60. Same positive results were obtained using the R16mF2n/R1 primers and Sca I. However, non-specific bands were amplified on cassava samples using the Sec

A primers. Gene sequences from infected samples confirmed the identity of '*Candidatus*' Phytoplasma luffae. In our observation, phytoplasma-infected cucurbits, bamboo and weed species can be easily detected using the above mentioned methods. However, in the case of CWB, the use of LAMP always generates false negative results despite of the severe symptoms expressed by the cassava. Thus, further improvement and development of detection tools must be done. Aside from cassava, this high incidence of phytoplasma detected from other plant species may pose risk in terms of spatial and temporal distribution. As weeds may serve as an efficient alternate host, an in-depth understanding on the etiology of diseases caused by phytoplasma is much needed.
Keywords: cassava witches' broom, little leaf, bamboo, Cucurbitaceae

P-012 Abstract withdrawal

P-013 Development of Droplet Digital PCR (ddPCR) Technique for Quantitative Detection of Phytoplasmas

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Phloem-limited agricultural pathogens are spreading at a dangerous rate, due to warming climates and increased global agricultural frameworks. Current treatment methods often do not specifically target these pathogens and are frequently preventative rather than curative. Phytoplasmas are one of the most aggressive phloem-limited pathogens that are obligate parasites of plants transmitted by sap-feeding insects which also serve as their hosts. Despite their agricultural importance and unique features, phytoplasmas remain one of the most poorly characterized plant pathogens mostly due to the lack of a successful in vitro culture, gene delivery, and mutagenesis systems. The aim of the study is to develop a universal detection methodology for phytoplasmas, evaluate the applicability of droplet digital PCR (ddPCR) as a quantitative detection tool, compare real time PCR (qPCR) and ddPCR sensitivity, repeatability and reproducibility. The initial trials were performed with 16S phytoplasma universal primers and 18S plant internal control primers for both detection platforms. For each reaction positive and negative controls (DNA from healthy plant samples) were included along with a no template control. ddPCR optimizations were set for both using EvaGreen, a DNA binding dye and TaqMan probes. TaqMan probes were designed with a 5' reporter dye, 6-carboxyfluorescein (FAM), and a 3' quencher, non-fluorescent quencher (NFQ). Under the optimized reaction conditions, consisting of 250/750 nM of primers/probe sets concentration, 58°C as annealing temperature and 45 PCR cycles ddPCR assay was 5-fold more sensitive than qPCR assay.
Keywords: Phytoplasma, ddPCR, qPCR, EvaGreen, probe

P-014 *Spiroplasma eriocheiris* entered *Drosophila* Schneider 2 cells and relied on clathrin-mediated endocytosis and macropinocytosis

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Spiroplasma eriocheiris causes great economic losses in the crustacean aquaculture industry. However, the mechanism of *S. eriocheiris* infecting host cells was poorly studied. We established a spiroplasma-infected *Drosophila* Schneider 2 cells model and investigated its pathogenic mechanism. Firstly, *S. eriocheiris* could induce S2-cell apoptosis and necrosis, seriously decreased cell viability and increased intracellular reactive oxygen species (ROS) production. Further research found that *S. eriocheiris* could invade S2 cells and copies of intracellular spiroplasma were sharply increased at 12 h infection. In addition, *S. eriocheiris* could cause S2 cells to form typical inclusion bodies, exhibited large vacuoles. Secondly, *S. eriocheiris* were internalized into S2 cells and strongly inhibited through blocking clathrin-mediated endocytosis using chlorpromazine and dynasore. Inhibitors of macropinocytosis, protein kinase C and myosin II resulted in a significant reduction of *S. eriocheiris* in S2 cells. In contrast, disruption of cellular cholesterol by methyl-β-cyclodextrin and nystatin had no effect on *S. eriocheiris* infection. These results suggested that the entry of *S. eriocheiris* into S2 cells relied on clathrin-dependent endocytosis and micropinocytosis, but not via the caveolae-mediated endocytic pathway. In addition, intracellular number of *S. eriocheiris* were dramatically reduced after S2 cells were treated with cytoskeleton-depolymerizing agents including nocodazole and cytochalasin B. Thus, cellular infection by *S. eriocheiris* was related to microtubules and actin filaments. This research had successfully shown for the first time that *S. eriocheiris* could invade *Drosophila* S2 cells and provided a process for *S. eriocheiris* infection.

P-015 Visualization of Phytoplasma Infection in Plant Tissues

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During the fieldwork carried out in the winter of 2019, daffodil plants (*Narcissus tazetta*) with stunning symptoms, typical phyllody and flower sterility were determined in Mersin-Türkiye. It is known that phytoplasmas, which cause disease in more than 700 plant species worldwide, spread to new areas with global warming-induced vector migrations, thus infecting different plant species encountered. In order to detect the presence of phytoplasma in daffodils, DNA samples obtained from symptomatic plants

were subjected to conventional PCR and Nested-PCR analyses with P1/P7 and R16F2n/R16R2 primers, respectively. Nested PCR products were subjected to conventional RFLP analyses with restriction endonucleases RsaI and SspI. Virtual RFLP patterns were generated by in silico digestion of the 16S rRNA gene assembly with 17 distinct restriction endonucleases. As a result of sequence analysis, we determined '*Candidatus* Phytoplasma mali' variants in daffodil at the nucleotide level. Moreover, intact phytoplasma-positive plant tissues were visualized using an inverted fluorescence microscope by using DAPI, PI and Cyto-9 staining in order to determine the distribution of phytoplasmas in phloem tissues. Next, detailed localization analyses were obtained by transmission electron microscopy (TEM). According to data until today, the presence of phytoplasma in *Narcissus tazetta* has been reported as 16SrXII-A and 16SrII-B subgroups. Although 16SrX-A subgroup phytoplasmas have been reported frequently from woody plants, to our knowledge this is the first report of '*Ca*. P. mali' (16SrX-A) infecting daffodil plants worldwide.
Keywords: daffodil, phyllody, PCR, fluorescence, TEM

P-016 The Detection of Avian *Mycoplasma* spp. in Fecal Matter from Poultry

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Mycoplasma synoviae and *Mycoplasma gallisepticum* are poultry pathogens of worldwide prevalence. Both species pose notable economic significance with *M. gallisepticum* being the most economically significant poultry mycoplasmal pathogen resulting in processing condemnations, reduced egg production and feed efficiency, and decreased hatchability. Both species are distributed worldwide, and there is not a reliable way of effectively eliminating either species from infected flocks. The current approaches to control avian mycoplasmosis include continuous surveillance and quarantine, medication, vaccination and/or elimination of infected breeding flocks. The transmission of *M. synoviae* among flocks is generally more rapid than the transmission of *M. gallisepticum*. In previous research, *M. synoviae* was detected from various fomites in the environment of naturally infected broiler breeders; *M. synoviae* was detected in dust, litter, and feather samples by real time PCR. In this research, the detection of *M. synoviae* and *M. gallisepticum* from cloacal swabs, fecal matter, and litter from infected broilers was compared using real time PCR and culture. A protocol to successfully facilitate the growth of *Mycoplasma* spp. in the presence of highly contaminated samples of feces was developed. The differences found in the level and duration of shed of *M. synoviae* compared to *M. gallisepticum* indicates that *M. synoviae* is more likely to be transmissible via fecal matter (as well as dust and litter) than *M. gallisepticum*.

P-017 The Detection of *Mycoplasma synoviae* in Fecal Matter from Poultry

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Mycoplasma synoviae is a poultry pathogen of worldwide prevalence causing severe

economic losses by decreasing production in both layers and breeding flocks. *Mycoplasma synoviae* causes subclinical upper respiratory disease, lameness, and reduced egg production and eggshell quality. There is not an effective and reliable treatment for mycoplasma, but some antibiotics have been shown to prevent clinical signs. The current approaches to control avian mycoplasmosis include continuous surveillance and quarantine, medication, vaccination and/or elimination of infected breeding flocks. *M. synoviae* strains have been shown to vary widely in their virulence, tissue distribution and rate of transmission. In previous research, *M. synoviae* was detected from various fomites in the environment of naturally infected broiler breeders; *M. synoviae* was detected in dust, litter, and feather samples by real time PCR. However, *M. synoviae* was not isolated from any of the environmental samples in that study. In this research, a methodology was developed to successfully isolate *M. synoviae* in highly contaminated samples (i.e., fecal matter) and compare the detection of different *M. synoviae* strains using culture confirmed through fluorescent antibody tests and real time PCR. The detection of living *M. synoviae* organisms is a valuable indicator of the importance of biosecurity (especially litter management) in the control of *M. synoviae*. Management of poultry litter is essential for the control of *Mycoplasma* from spreading across flocks.

P-018 In Silico Analysis of The Putative Phytoplasma Effector SAP55 that Mimics the Lipidation Mechanism of G Proteins

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Phytoplasmas, which phloem-limited plant pathogens manipulate host cell functions through effector proteins they secreted and cause various anomalies in plants. There are limited studies of phytoplasma effectors because of unculturable status of phytoplasmas and only four of them (TENGU, SAP05, SAP11 and SAP54) are well characterized. This ongoing study aims to identify phytoplasma effector proteins, their functions and virulence mechanisms. First, N-terminal signal peptides (SP) mediating the secretion of effector proteins were identified by SignalP-4.1 and TMHMM-v2.0. Next, candidate protein structures were predicted by ColabFold. Then, HMMER and MyHits Motif Scan were applied in order to examine the relationship of the orthologs and to predict the function of candidate effector proteins. The subcellular localization analyses were determined by using WoLF PSORT, TargetP-2.0 and LOCALIZER. Finally, phylogenetic analyses for the relevant proteins and gene organization were determined by MEGA-X and Geneious Prime. As a result of our evaluations, SAP55 was determined as one of the most potential effector candidates resembling a metalloendopeptidases-like structure and contains the peptidase family M41 like domain with a conserved HEXXH active site motif but it functions as an ATP-independent protease since it does not have an ATPase domain. In addition, lipidation predictions showed that SAP55 undergoes s-palmitoylation and prenylation via the CXCaaL motif located at its C-terminus, similar to that of G proteins. Our results showed that SAP55 which is originally a soluble protein, uses lipidation to interact with lipid rafts and to degrade relevant membrane proteins.
Key words: phytoplasma, signal peptide, effector, metalloendopeptidase, lipidation

P-019 Identification of antigenic *Mycoplasma bovis* proteins using North American Bison (Bison bison) convalescent sera

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Mycoplasma bovis is a bacterial pathogen that has recently emerged as the etiologic agent of severe respiratory disease in North American Bison (Bison bison). Commonly associated with bovine respiratory disease complex in cattle, *M. bovis* appears to be a primary pathogen in ranched bison. Epizootic pneumonia with high morbidity and mortality have been documented in bison herds from the United States and Canada. In bison, the disease is characterized by severe, chronic pneumonia commonly associated with polyarthritis, laryngitis, mastitis, and dissemination to other organs. Although modified-live and killed *M. bovis* vaccines are available for cattle, no commercial vaccine is licensed for use in bison. The goal of this study was to identify *M. bovis* proteins that are antigenic in bison. Whole cell lysates of multiple *M. bovis* isolates, representing the most common multilocus sequence types isolated from bison, were assessed using two-dimensional gel electrophoresis. Immunoblots probed using convalescent sera from multiple naturally infected animals identified *M. bovis* protein antigens recognized by bison antibodies. Protein spots were excised, digested with trypsin, then analyzed via mass spectrometry. Immunoblots showed conservation of targets between serum samples from different animals and longitudinal samples from the same animals. Discrete banding patterns were observed for each isolate evaluated, NADC1 and NADC55. Mass spectrometry of excised antigenic proteins identified multiple antibody targets from each isolate. The identification of antigenic proteins may be useful in the development of novel vaccines and improved diagnostics specific for use in bison.

P-020 Genome Comparison of Spiralin, P58, P89 Regions of Naturally Infected and in vitro Cultures of *Spiroplasma citri* from Different Hosts in Turkey

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Spiroplasma citri, the causal agent of Citrus Stubborn Disease, is spiral-shaped, no-cell wall bacteria belonging to the Mollicutes class. In the present study, *S. citri*, was detected and characterized from naturally infected citrus trees grown in different locations in Turkey and in vitro cultures obtained from periwinkle, sesame, turnip and cicadellids. In order to screen and confirm *S. citri*, PCR-based detection was performed by focusing on spiralin, P58 putative adhesin-like multigene, and P89 putative adhesin genes of *S. citri*. Detection rate of *S. citri* was consistently higher in the fruit columella than in the leaf midribs for naturally infected field samples. For cultured samples, we have found that primer pairs based on P89 were more sensitive in recognizing *S. citri*

than those based on the spiralin gene and P58 gene. Futhermore, isolates obtained with P58 F/R primer pairs showed 99.74% identity with *S. citri* GII3-3X strain which was originally isolated from the leafhopper Circulifer haematoceps, collected in Morocco (1980) and 99.34% identity with BLH-MB strain which was originally isolated from a Navel orange tree in Riverside, California (1972). Spiralin gene showed 99.34% identity only with the GII-3X strain where P89 F/R primer was not identical with either of the two strains. This study provides an overview of the characterization of gene regions of *S. citri* that are associated with vector transmission and disease spread in cultivated citrus growing regions and naturally infected citrus fruits.
Keywords: *Spiroplasma citri*, spiralin, P58, P89, adhesin

Category 3. Identification, Detection & Diagnosis

P-021 *Mycoplasma floridensis* sp. nov. from Cell Lines Developed for Diagnostic and Pathogen Discovery in Reptiles

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Background: Pathogen discovery in wildlife is increasing dramatically. Isolation as well determination of pathogenic potential of microbial agents, especially viruses, likely will require development of new cell lines from relevant hosts. As part of a program to develop cell lines of reptilian and amphibian origin, we identified a novel mycoplasma species as a cell culture contaminant. **Methods**: *Mycoplasma floridensis* sp. nov. was originally cultured from a primary lung cell line developed from a corn snake (*Pantherophis guttatus*). Whole genome sequencing was performed using Illumina with SPAdes assembly and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Whole genome-based taxonomic analysis was performed using the Type (Strain) Genome Server (https://tygs.dsmz.de), and phylogeny was inferred by 16S rRNA, digital DNA-DNA hybridization, genome BLAST distance hybridization, as well as the entire proteome. **Results**: The *M. floridensis* genome has a GC content of 25.6, with 797 genes encoding proteins, 3 noncoding RNAs, and 29 tRNAs. All genomic analyses confirmed the designation as a new species. The dDDH value for all *Mycoplasma* type species was <28%, below the suggested 70% cutoff for speciation. Independent of the whole proteome genome analysis, analysis of percentage of identity of 54 housekeeping proteins supported designation of *M. floridensis* as a new species. **Conclusion:** *M. floridensis* is a new species with as yet undetermined pathogenic potential. Both *Acholeplasma* spp. and *Mycoplasma* spp. can contaminate and induce cytopathic changes in cell lines, it is important that newly developed cell lines be rigorously screened for these agents.

P-022 Investigation on the Effects of Two Storage Methods of Diagnostic Samples on the Isolation of Avian *Mycoplasma* Strains

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Control of *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) in poultry production includes measures that prevent and contain their spread. Highly sensitive and specific diagnostic tools are fundamental for achieving this goal, even though due weight is not always given to the quality of the samples and the storage conditions before arrival at the laboratory. This is particularly important when viable strains are needed for antimicrobial sensitivity tests. We decided to investigate how isolation can be affected by two conservation methods: refrigeration and freezing. Forty tracheas of layer hens coming from two farms were enrolled. We decided to sample birds that died at the end of the production, so it was assumed mycoplasma cell load was very low, which certainly stresses test sensitivity and specificity. At time zero, all tracheas were sampled with sterile swabs, that subsequently underwent to a real-time PCR assay for MG, a real-time PCR assay for MS, and mycoplasma culturing. Then, half of the tracheas in equal numbers for the two farms were kept refrigerated at +5°C for 24 hours and sampled again with sterile swabs, used for mycoplasma culturing. The remaining tracheas were frozen at -24°C for 7 days, then thawed at room temperature and then sampled with sterile swabs at 0, 24, 48 and 72hours post-thawing. We noticed that, in conditions close to the test limit of detection, freezing preserved strain viability, while refrigeration allowed strain isolation after 24 hours only. Thus, under field conditions, prompt freezing of animal organs is advised.

P-023 The Development and Application of *Mycoplasma gallisepticum* Vaccine Strain Specific Quantitative PCR Protocols

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Vaccination is an increasingly important tool in the control *Mycoplasma gallisepticum* worldwide, and therefore techniques that allow differentiation of field strains of *M. gallisepticum* from the vaccine strains are increasingly critical. Various molecular techniques have been used for *M. gallisepticum* strain differentiation, including DNA sequencing of selected genomic targets (GTS). In previous research, whole genome sequencing and comparative genomics were used to develop PCR protocols F-strain vaccine from other live vaccine strains, reference strains and field strains. In this research, the F-strain-specific PCR protocols were adapted to quantitative real-time PCR, by including specific Taqman® probes. These PCR protocols have applications both in the poultry diagnostic laboratory and in research involving vaccine trials as they can be used to specifically detect and quantify *M. gallisepticum* F-strain without the need for DNA sequencing. These quantitative assays were used to evaluate the dynamics of the F-strain replication in different *M. gallisepticum* vaccine programs in layer chickens. The quantity of F-strain present in the trachea of vaccinated birds was assessed following secondary vaccination and in the face of virulent challenge. The vaccine programs investigated included combinations of commercial live F-strain vaccines and *M. gallisepticum* bacterins. The newly developed F-strain specific PCR protocols revealed that the colonization and persistence of the live vaccine similar in the vaccine programs evaluated.

P-024 The Occurrence of Infections with *Mycoplasma* spp. and *Ureaplasma* spp. in Clinical Cases of Respiratory Disorders in Polish Cattle Population

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Background: Mycoplasmas are known to be one of important respiratory pathogens in cattle. Methods: Total of 165 samples including nasal swabs (n=84), sera (n=75) and lungs (n=6) were collected from cattle in Poland in 2021. The animals were affected with clinical respiratory disorders. The sera were tested for the presence of specific anti-*Mycoplasma bovis* antibodies using indirect ELISA. *Mycoplasma* spp. and *Ureaplasma* spp. were identified from the nasal swabs and lungs using culturing (broth and agar) and polymerase chain reaction/denaturing gradient gel electrophoresis (PCR/DGGE) method. Results: The results showed the presence of specific antibodies to *M. bovis* in 12 sera, which accounted for 16% of all tested serum samples. Culturing of 84 nasal swabs and 6 lungs on agar showed the presence of specific colonies in the case of 9 swabs, which accounted for 10% of the analyzed samples. The analysis of 84 nasal swabs and 6 lungs using PCR/DGGE showed the presence of *Mycoplasma* and *Ureaplasma* DNA in 41 swabs and 3 lungs, which accounted for 48.9% of the tested samples, including mainly *M. bovis*, *M. dispar*, *M. bovirhinis* and *U. diversum*. Conclusion: Analysis of total of 165 samples confirmed the aetiological participation of *Mycoplasma* spp. and *Ureaplasma* spp. in the clinical cases of respiratory disorders in the Polish cattle population. Mostly isolated of the infectious agent in these cases was *M. bovis*, which was identified alone or in co-infection with at least one organism of the genus *Mycoplasma* and/or *Ureaplasma*.
Keywords: mycoplasmas, cattle, respiratory infections

P-025 A Visible Duplex Fluorescence-based LAMP assay for the Detection of *Mycoplasma bovis* and Infectious Bovine Rhinotracheitis Virus

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(Background) *Mycoplasma bovis* (MB) and infectious bovine rhinotracheitis virus (IBRV) are two important pathogens that cause bovine respiratory disease in the beef feedlot and dairy industries. The aim of this study was to develop and validate a duplex fluorescence-based loop-mediated isothermal amplification (DLAMP) assay for the simultaneous detection of MB and IBRV. (Methods) Two quencher-fluorophore composite probes complementary to the F1c segment of the inner primer FIP were added to the DLAMP system, and the composite probes were labelled with different fluorophores showing different colours at the corresponding wavelengths. Two primer sets and two composite probes were designed to detect and discriminate between MB

and IBRV based on the colour of the DLAMP products. The DLAMP reaction conditions were optimized for rapid and specific detection of MB and IBRV. (Results) The DLAMP assay developed here specifically detected MB and IBRV without cross-reaction with other common bovine pathogens. The detection limit of this DLAMP assay was as low as 673–724 copies/reaction for the recombinant plasmids containing the MB and IBRV target genes. When used on field materials, the results of the DLAMP assay were 100% in agreement with the results using World Organization for Animal Health (OIE)-recommended real-time PCR methods. (Conclusion) The DLAMP assay is simple, rapid, inexpensive, sensitive, and specific, and the results of amplification product can be observed by the naked eye. Therefore, the DLAMP assay is a useful tool for the clinical diagnosis of MB and IBRV and can be applied in rural areas.

P-026 Application of mucosal sIgA antibody in infection and immune evaluation of *Mycoplasma hyopneumoniae*

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Mycoplasma hyopneumoniae (*M. hyopneumoniae*) is the pathogen that causes Mycoplasma pneumonia of swine. There are some technical difficulties in clinical infection diagnosis and vaccine immune evaluation, which are also one of the main reasons for the widespread infection of *M. hyopneumoniae*. Respiratory mucosal sIgA antibody is the first antibody secreted by pigs in response to *M. hyopneumoniae* infection. In our previous report, a sIgA-ELISA method for *M. hyopneumoniae* specific mucosal antibody detection was developed and licensed in China, which has the role of early diagnosis and DIVA. This method was recently applied to the epidemiological surveillance, vaccine efficiency and eradication evaluation of *M. hyopneumoniae*. The results showed that the epidemiological survey data based on sIgA antibody from nasal swabs was close to that of nucleic acid detection of lung tissue, and maternal antibody and infectious antibody could be distinguished in suckling piglets. The response of sIgA against infection in nursery pigs was also significantly earlier than that of serum IgG antibody. The positive rate of sIgA antibody in the farms immunized with inactivated vaccine or treated with eradication strategies such as drug treatment was significantly lower than that in the farms non-immunized or non- eradication treatment, indicating a lower *M. hyopneumoniae* infection in these treated farms. In addition, it was also found that the positive rate of sIgA antibody and the titer dispersion in finishing pigs with an attenuated vaccine immunization were significantly lower than those of non-immunized pigs. In conclusion, the sIgA-ELISA method was implied to use for infection surveillance and immune evaluation of *M. hyopneumoniae*.

P-027 *Mycoplasma penetrans*, a Possible Urogenital Bystander More Frequent in HIV-Positive Men and Men Who Have Sex with Men

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Mycoplasma penetrans is a genital mycoplasma discovered in the 1990s. First studies suggested that this bacterium was associated with HIV-positive patients and progression to AIDS using indirect detection of anti-*M. penetrans* antibodies in serum samples. After the development of *M. penetrans*-specific PCR, only one study reported a significant higher prevalence of *M. penetrans* among HIV-positive patients compared to HIV-negative individuals. We aimed to assess the prevalence of *M. penetrans* in relation to HIV status and sexual behavior. Between February 1st and May 31st, 2021, the prevalence of *M. penetrans* was evaluated by real-time PCR in men screened for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Bordeaux University Hospital, France. Clinical data, HIV status, and sexual orientation were collated before anonymization. The prevalence of *Mycoplasma penetrans* was 2.1% (95% confidence interval, 1.1-3.9) among the 429 screened men. The prevalence was 3.5% (95%CI, 1.9-6.6) among men who have sex with men (MSM) and 5.3% (95%CI, 2.7-10.0) among HIV-positive patients, significantly higher than in HIV-negative individuals (0.4%, p=0.0016). All *M. penetrans*-positive patients were asymptomatic, and all were *C. trachomatis*-, *N. gonorrhoeae*-, *M. genitalium*- and *T. vaginalis*-negative. This study confirmed the higher prevalence of *M. penetrans* infection in urogenital samples from HIV-positive patients using a specific PCR method. The question of whether *M. penetrans* could act as a bystander or an opportunistic bacteria, more frequently circulating among MSM in connection with their sexual practices, and more specifically in HIV-positive MSM patients due to a higher susceptibility for an as yet unknown reason, may be raised.

P-028 Development and validation of three recombinase polymerase amplification (RPA) assays for direct and rapid detection of *Mycoplasma bovis*

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Mycoplasma bovis (*M. bovis*) is a major etiological agent of bovine mycoplasmosis, a triggering factor of various diseases, such as pneumonia, tenosynovitis and bovine endocarditis. it is imperative to develop a rapid detection assay for *M. bovis* diagnosis that can be used as a primary measure to prevent and control the infection. Recombinase polymerase amplification (RPA) is an efficient, sensitive and rapid isothermal amplification technology that does not require expensive equipment. In this study, we screened eight *M. bovis* specific genes to distinguish *M. bovis* and *Mycoplasma agalactiae* (*M. agalactiae*). Targeting the *M. bovis* lipoprotein LppA, three fast and reliable RPA assays were established, including a basic-RPA assay, an exo-RPA assay, and an nfo-RPA assay. All three assays showed high specificity towards *M. bovis*, displaying no cross-reaction with the other *Mycoplasmas* and pneumonia-related bacteria. Using the standard recombinant plasmid pMD18-LppA standard as template, the limit of detection (LOD) of basic-RPA, exo-RPA and nfo-RPA assays was 6, 6×10^2 and 6×10^3 copies/reaction, respectively, values higher than the LOD of traditional PCR (6×10^3 copies/reaction). These assays also showed excellent detection performance

on clinical samples. Indeed, the detection rates of the three RPA assays, especially nfo-RPA and exo-RPA, were higher than those of the PCR. Our study aimed to satisfy the detection of *M. bovis* under different settings, especially in feedlots, auction markets and slaughterhouses. Our findings provide a novel alternative for direct and rapid detection of *M. bovis*, showing great potential for early prevention and control of *M. bovis* infections.

P-029 Comparison of Different Laboratory Techniques to Evaluate Growth Activity in vitro of Different *Mycoplasma hyopneumoniae* Strains

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Mycoplasma hyopneumoniae (Mhp) is a slow-growing, fastidious porcine respiratory pathogen. Traditionally Mhp growth and metabolic activity have been evaluated by measuring colony-forming units (CFU) and/or color-changing units (CCU). This study compares and correlates different laboratory techniques, including CFU, CCU, flow cytometry (FlCy), ATP luminometry (ATPlu), and confocal microscopy (CoMi) to evaluate Mhp growth and metabolic activity. Mhp strain 232, J strain, and contemporary field strain 2010 were cultured in Friis medium and incubated at 37°C. At 0, 12, 24, 36, 48, 72, 96, 120, and 144 hours post-inoculation (HPI), aliquots from each replicate were collected for CFU, CCU, FlCy, and ATPlu evaluation. Replicates were also harvested at 48 HPI, aliquots inactivated by heat, and mixed proportionally by volume with live aliquots at 99%, 75%, 50%, 25%, and 1% for FlCy and CoMi analysis. Both direct quantitative methods CFU and FlCy demonstrated Mhp exponential growth with a detectable plateau at 48 HPI. Both CCU and ATPlu demonstrated an incremental growth by 72 HPI and a rapid decline of detectable metabolic activity by 120 and 144 HPI. Correlation of the four techniques ranged from 0.67 to 0.99. The two morphologic methods, FlCy and CoMi performed similarly. The similar detection rate over time and high correlation between traditional CFU-CCU and FlCy-ATPlu make the latest an interesting alternative for detecting and quantifying Mhp from approximately 21 days currently required by CFU-CCU to a few hours. CoMi is beneficial in providing visualization and timely quantitation with similar performance with FlCy for live Mhp cells.

P-030 Improving strain typing of *Mycoplasma ovipneumoniae* using multiplex PCR and Next Generation Sequencing

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Mycoplasma ovipneumoniae initiates fatal pneumonia outbreaks in bighorn sheep. *M. ovipneumoniae* strain-typing is based on an MLST process using Sanger sequencing, which is time-consuming, expensive and may fail to produce sequences for one or more genes. We aimed to improve this MLST technique by applying a new high-throughput and cost-effective strain-typing method based on real-time next generation

sequencing. Ten clinical samples (nasal swabs) were used for the establishment and validation of the method. We developed a novel multiplex PCR for four target genes to be used with the rapid barcoding amplicon kit from Oxford Nanopore Technologies (ONT). To lower cost, we assessed washing and reusing ONT flow cells. Finally, the method was applied to 68 clinical samples to assess its performance. We then compared results from ONT, Illumina, and Sanger sequencing. The developed ONT method provided full length sequences for all target genes. ONT sequences were of higher quality (99.5% >Q40) than Illumina (91% >Q40) and Sanger (93%<Q40). ONT correctly identified more target genes (94%) compared to Illumina (58%), which failed to recover full length *rpoB* sequences. Sanger sequencing correctly identified 85% of genes. Washed ONT flow cells showed no decrease in sequence quality. ONT run time of 4 hours was sufficient for MLST determination. The cost-per-sample of ONT was lower than Illumina and could be performed in-house within one day. Our improved method can be applied directly to clinical samples enabling accurate and cost-effective strain-typing. Besides *M. ovipneumoniae*, this workflow can be easily expanded to other *Mycoplasma* species.

P-031 *Mycoplasma wenyonii* circulates in cattle, deer and ticks

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Background. Hemoplasmas are non-cultivable mycoplasmas that infect red blood cells of mammals. Among them, *Mycoplasma wenyonii* (Mw) is associated with chronic anemia as well as acute signs such as fever, edema and drop in milk production in cattle. Mw was also detected in roe deer and ticks, but the interconnection between these three compartments has yet to be explored. Methods. Ticks and blood samples from roe deer and cattle were collected in the same geographical area of France. In parallel, some gorged female ticks were recovered from roe deer and reared in the laboratory to study trans-ovarial and trans-stadial transmissions. Hemoplasmas were detected by qPCR and Mw was further identified by amplicon sequencing. Whole genomic sequencing (Illumina and Oxford Nanopore Technologies) was performed with highly positive samples to produce Mw circular genome form different strains. Results. Mw was detected in cattle, deer and ticks. The assembly of entire Mw genomes was performed for bovine and deer strains. Comparative sequence analyses reveal that at least two different bovine strains circulate in France. Rearing of adult ticks collected on roe deer further revealed a trans-stadial transmission under laboratory conditions, suggesting a multiplication of Mw in ticks. Conclusions. These results showed the co-circulation in France of different Mw strains and provide a basis for understanding the eco-epidemiology of hemoplasmas, their interaction with vectors and their dynamics in the different compartments of agro-ecosystems.

P-032 Serological and Molecular Detection of *Mycoplama gallisepticum*,

Mycoplasma synoviae and *Mycoplasma meleagridis* in Free Range Chickens in Nigeria

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Background: *M. gallisepticum*, *M. synoviae* and *M. meleagridis* are mollicute pathogens of poultry, with the later predominately a pathogen of turkeys. This study was designed to investigate all three species among free-range chickens from two metropolitan slaughter slabs in Ilorin, Nigeria. Methods: Using diagnostic confirmatory support from APHA, UK. Of 602 serum samples screened with rapid serum agglutination test (RSAT), 33 were positive for both Mg and Ms. The 33 positive sera and 11 cultures with typical fried egg appearance suggesting mycoplasma organism were submitted to APHA for confirmation. Results: Rapid serum agglutination test for the three species were used with associated positive control sera, typically showing 2+ or 3+ reactions for the correct antigen, with no obvious reaction for the negative sera. ELISA data revealed 21 of the 33 samples to be positive for Mg, 19 positive for Ms and 14 for both. Two samples were also positive for Mm by ELISA. Of the six cultures tested to date, none is positive for Mg or Ms and we were unable to recover these pathogens (due to gross contamination). PCR-DGGE, confirmed the real-time PCR results, but a number of bands were observed which will need to be investigated further. Conclusion: Further serological tests confirmed antibody response to Mg and Ms in a high proportion indicating likely exposure of free- range chickens to these avian pathogens.
Key words: Serological and Molecular Detection, *Mycoplama gallisepticum*, *Mycoplasma synoviae*, *Mycoplasma meleagridis*, Chickens.

P-033 Evaluation of Commercial Customized Plates for *Ureaplasma* spp. and *Mycoplasma hominis* Antimicrobial Susceptibility Testing and Determination of Resistance Prevalence in France in 2020

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Determining MICs using broth dilution methods is labor-intensive in *Ureaplasma* spp. and *Mycoplasma hominis*. We evaluated the use of the commercial customized MICRONAUT-S plates (Biocentric-Bruker), designed on request for antimicrobial

susceptibility testing (AST), in comparison to MIC determination according to the CLSI guidelines used as reference. We then investigated the prevalence of resistance to tetracyclines, fluoroquinolones and macrolides in France in 2020. A total of 60 *Ureaplasma* spp. and *M. hominis* strains were used to compare both methods. For the resistance prevalence study, clinical specimens detected positive for *Ureaplasma* spp. and/or *M. hominis* were collected in France during one month and 151 *Ureaplasma* spp. and 50 *M. hominis* isolates were grown from them. MICs were determined using the MICRONAUT-S plates. The *tet(M)* gene and fluoroquinolone resistance-associated mutations were searched. All the MICs obtained with the MICRONAUT-S plates were in accordance with the CLSI reference method, with no more than a 2-fold dilution difference using an inoculum of 105 color-changing units/ml. Among the 50 *M. hominis* isolates, tetracycline, levofloxacin and moxifloxacin resistance rates were 8% (4/50), 2% (1/50) and 2% (1/50), respectively. No clindamycin resistance was observed. Among the 151 *Ureaplasma* spp. isolates, tetracycline and levofloxacin resistance rates were 2% (3/151) and 5.3% (8/151), respectively. No moxifloxacin nor erythromycin resistance was observed. All tetracycline-resistant isolates harbored the *tet(M)* gene and all levofloxacin-resistant isolates harbored a mutation in the *parC* gene. The customized MICRONAUT-S plates are useful for AST of human mycoplasmas. Tetracycline and fluoroquinolone resistance are limited in France in mycoplasmas.

P-034 *Mycoplasma synoviae* Genotyping: Discrimination Power of Multiple Locus Variable-Number Tandem Repeat Analysis for Live Vaccine and Wild Strains

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Mycoplasma synoviae (MS) infection generates relevant economic loss in the industrial poultry production. The disease is widespread in the European poultry sectors since MS can be transmitted both horizontally and vertically, which makes containment difficult. The application of strict biosecurity measures, antimicrobial treatments and vaccination of the animals are three options available for infection control. Currently, two MS live vaccines are licensed in Italy: MS1 (Nobilis MS live, MSD Animal Health Inc.) and MS-H (Vaxsafe MSH; Bioproperties Ltd., Ringwood, Victoria, Australia). The drawback of vaccination with live vaccines is that molecular tests with high discriminatory power are needed in order to identify properly MS strains collected on field and, consequently, to control MS infection. In pursuit of this aim, we developed a modified Multiple Locus Variable-Number tandem repeat Analysis (MLVA) protocol based on six tandem repeats loci (6 loci-MsMLVA). With this protocol, we characterized more than 100 Italian MS field-strains collected between 2012 and 2021, and 85 MS-H-like strains isolated from vaccinated animals. A total of 22 genotypes were identified among the MS field-strains and Genotype 3 (GT 3), the one of the reference MS-H

vaccine strain, was assigned to all the MS-H-like strains. No field strain co-clustered with the vaccine-like ones. Our data indicate that, compared to other commonly used genotyping methods such as gene-targeted sequencing analysis of vlhA gene (GTS-vlhA) or MLST for MS, the 6 loci-MsMLVA is a reliable, cost-effective method able to identify in a relatively short time MS-H-like strains collected in the field.

P-035 Presence of Anti-*Mycoplasma bovis* Antibodies in Polish Cattle Suspected of *Mycoplasma bovis* Infection

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Background: *Mycoplasma* (*M.*) *bovis* is an important pathogen of cattle causing mainly pneumonia, which is manifested with cough, dyspnea, fever and nasal discharge. The aim of the study was to determine the presence of anti-*M. bovis* antibodies in cattle suspected of *M. bovis* infection in Poland in 2022. Methods: One hundred and thirty-five sera samples were collected from animals suspected of *M. bovis* lung infection. The anti-*M. bovis* antibodies in sera samples were determined using commercial indirect ELISA. The samples originated from different regions of Poland i.e. the Eastern (13 samples), the Masovian Voivodeship (15 samples), the Central (25 samples), the Northern (45 samples), the North-western (35 samples) and the Southern (2 samples). Results: The sera samples from 24.44% (33/135 samples) examined cattle were positive for *M. bovis*. The seroprevalence was 14.8% (20 positive samples) in the Northern, 3.7% (5 positive samples) – in the Eastern, 2.22% (3 positive samples) – in the Central, 2.22% (3 positive samples) – in the North-western, 1.48% (2 positive samples) – in the Masovian Voivodeship region, and no specific antibodies were detected in the Southern region. Conclusion: The results showed that in cattle herds suspected of *M. bovis* infection specific antibodies were found and present in animals from almost every examined region of the country, with a difference in the proportion of positive samples per region ranging from 0 to 14.8%. The results confirmed that *M. bovis* is still widespread in cattle herds in Poland.
Keywords: *Mycoplasma bovis*, antibodies, ELISA, seroprevalence

P-036 Screening of Cattle for the Presence of Anti-*Mycoplasma bovis* Antibodies in Poland

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Background: *Mycoplasma* (*M.*) *bovis* is an emerging cattle pathogen that causes pneumonia, mastitis, arthritis and other diseases. This pathogen can infect cattle in all age groups of the animals and has a major impact on cattle welfare and production. *M. bovis* is detected in cattle almost all over the world. The aim of this study was the serological screening in clinically healthy cattle to estimate the prevalence of anti-*M. bovis* antibodies in Poland. Methods: Screening of cattle for the presence of anti-*M.*

bovis antibodies in Poland based on 400 bovine sera samples collected in 2022 was made. The animals were clinically healthy and originated from three of seven regions of Poland i.e. the North-western (160 samples), the Northern (160 samples) and the Central (80 samples). The examination of anti-*M. bovis* antibodies were made using the commercial indirect ELISA. Results: Sera samples from 13.25% (53/400 samples) examined cattle were positive for *M. bovis*. The seroprevalence in the North-western, the Northern and the Central regions was 7% (28 positive samples), 4% (16 positive samples) and 2.25% (9 positive samples), respectively. Conclusion: The results of this study indicate that anti-*M. bovis* antibodies can be detected in the absence of clinical disease. These antibodies may be present due to passive protection or as an active response to the infection. Serological screening may contribute to identify asymptomatic and carrier animals in the herds.
Keywords: *Mycoplasma bovis*, anti-*Mycoplasma bovis* antibodies, ELISA, serological screening

P-037 Abstract withdrawal

P-038 A Multiplex Fluorescence-based Loop-mediated Isothermal Amplification Assay for Identifying *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and Avian orthoreovirus

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Background. *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS) and Avian orthoreovirus (ARV) are three important pathogens of poultry, their infections causing significant economic losses in this industry. The loop-mediated isothermal amplification (LAMP) assay is a simple, rapid, sensitive, and cost-effective nucleic acid amplification method that does not require any specialized equipment. Methods. In this study, a multiplex fluorescence-based loop-mediated isothermal amplification (mLAMP) assay for detecting MG, MS and ARV was developed to simultaneously diagnose single and mixed infections in chickens. Three primer sets and composite probes were designed according to the conserved regions of each pathogen. Each composite probe was labelled with a different fluorophore, which was detached to release the fluorescence signal after amplification. The target viruses were distinguished based on the colour of the mLAMP products. Results. The mLAMP assay was shown to be sensitive, with detection limits of 805 copies of recombinant plasmids containing the MG target genes, 931 copies of MS and 1950 copies of ARV target genes. The assay exhibited high specificity and no cross-reactivity with other symptomatically related avian viruses. When used on field materials, the results of the mLAMP assay were in 100% agreement with those of the previously published real-time PCR assay on MG, MS, and ARV. Conclusion. The mLAMP assay is rapid, low cost, sensitive and specific,

Consequently, it will be a very useful screening assay for the surveillance of MG, MS, and ARV in underequipped laboratories as well as in field conditions.

Category 4. Epidemiology

P-039 Macrolide and Fluoroquinolone Resistance in *Mycoplasma genitalium* in Central Slovenia, 2017-2022

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Background: *Mycoplasma genitalium* (MG) is emerged cause of sexually transmitted diseases (STD), causing acute and chronic urogenital infections in men and women. The aim of this retrospective study was to investigate the prevalence of MG infections among patients attending clinics for STD in central Slovenia, and to determine its resistance to macrolides and moxifloxacin. Methods: From January 2017 to January 2023, a total of 14,427 clinical specimens from 9,630 outpatients (5,519 men and 4,111 women) were tested for MG infection using the Allplex STI PCR assay (Seegene Inc., Korea). Resistance-associated mutations in the 23S rRNA and *parC* genes were investigated using the Allplex MG&AziR assay and the Allplex MG&MoxiR PCR assay (Seegene Inc., Korea), respectively. Results: Out of 9,630 patients included, 342 (3.6 %) tested positive for MG infection. Mutations associated with macrolide resistance were detected in 109 (31.9 %) of the positive cases, with the A2059G mutation in the 23S rRNA gene being prevailing over others. Fluoroquinolone resistance conveying mutations were identified in 14 (4.1 %) cases. The most common mutation in the *parC* gene was G248T. In 64 (18.7 %) cases we also identified infections with MG strains that contained both macrolide and fluoroquinolone resistance associated mutations. Conclusion. The number of patients with detected MG infections progressively increased over the years of the study. We also found that the prevalence of strains with mutations associated with MG resistance to macrolides as well as to fluoroquinolones increased during the observed years in our community.

P-040 Mollicutes study in freshwater fish

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Background- Very little is known about the factors that may be associated with the

presence of Mollicutes in fish, so it is necessary to gain as much information as possible about the characteristics of the chosen sample. Due to the lack of epidemiological field studies and of knowledge about the factors linked to the presence of Mollicutes, we have designed an epidemiological survey, to discover factors related to the presence of Mollicutes in fish. Methods- A total of 318 samples were collected from Spanish aquaculture freshwater fish, including 150 from rainbow trout (*Oncorhynchus mykiss*) and 168 from tench (*Tinca tinca*). Skin mucus and intestine swabs were used to extract DNA, which was then subjected to a general Mollicutes real-time PCR. A questionnaire was administered to evaluate the presence/absence of Mollicutes in relation to various parameters including type of pond, presence of vegetation in the pond, at different stages of production, cold and warm seasons of the year and type of food. Data was analysed (SPSS). Results-. The prevalence of Mollicutes was 14.6%, with a statistically significant higher value in tench (18.5%) than in trout (8.0%) (p=0.019). Intestinal prevalence was found to be twice that of skin mucus in both species (p=0.029). However, no significant differences were observed regarding the ponds, stages of production, season, presence of vegetation in the pond, or type of food in either species. Conclusion- It was demonstrated that prevalence of Mollicutes is higher in both tench and in the intestine.

P-041 Genetic Analysis of the *imp* Gene Encoding for the Immunodominant Membrane Protein of Phytoplasmas Associated with Lethal Yellowing Type Syndromes of Palms

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Background. Different Phytoplasma species are associated with Lethal Yellowing Type Syndromes (LYTS) that dramatically affect palms in different parts of the world. Among genes involved in phytoplasma transmission are the genes encoding for the immunodominant membrane proteins (IDPs). Here we present some *imp* genetic data for three LYTS phytoplasmas. **Methods.** Draft genomes of '*Candidatus* Phytoplasma cocostanzania' (CPctz), '-palmae'(CPpal) and '-palmicola' (CPpml) were searched for IDPs genes based on the synteny usually observed. The unique target observed was amplified for some representative isolates of the three species. The nucleotide sequences were analyzed considering the complete sequences or by distinguishing the different domains (Signal Peptide, SP; Trans Membrane, TM; Extra Cellular, EC). **Results.** Among the different described IDPs, only *imp* gene was observed. Based on *imp* sequences, CPctz appears phylogenetically closer to the Caribbean phytoplasma CPpal than to the other African phytoplasma CPpml. The sequences appear to be highly conserved within each population, particularly the domains encoding SP and TM. Sequences coding for the EC domain are the most variable between populations and appeared to be submitted to positive selection while SP and TM are submitted to purifying selection when significative. **Conclusion.** In the absence of the other known IDPs, *imp* may play a predominant role in the interactions of LYTS phytoplasmas with their hosts. The positive selection on sequences encoding the extracellular domain of IMP suggests a capacity for adaptation of the phytoplasma to new insect vectors.

P-042 Detection of *Mycoplasma ovis* in Blood Samples from Small Ruminant Species in Cuba

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Background- The genus *Mycoplasma* is divided into two groups based on their affinity for mucous membranes or blood. The second group contains hematrophic mycoplasmas which are known to affect sheep and goats, including *Mycoplasma* (*M.*) *ovis* and *M. haemovis*. Transmission of these agents has been linked to arthropods such as fleas and ticks and fomites. These mycoplasmas colonize the surface of erythrocytes, leading to asymptomatic infections in the majority of chronically affected animals, although acute forms of hemolytic anemia caused by these microorganisms can cause death. Methods- In this study, the presence of *Mycoplasma ovis* was investigated in 23 tick and 264 blood samples from sheep and goats in the provinces of Havana and Mayabeque in western Cuba. DNA was extracted from the samples and a real-time PCR specific for *M. ovis* was performed. Results-. The results of the tick samples were all negative, whereas 85 of the blood samples tested were positive. Conclusion- This study showed that *M. ovis* is a prevalent pathogen in small ruminants in the western region of Cuba. The lack of *M. ovis* in ticks may indicate that other transmission pathways could be involved in the epidemiology of this agent, although the sample size was small.

P-043 Intrauterine *Ureaplasma* is associated with small airway obstruction in extremely preterm infants

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The long-term follow-up of lung function (LF) in extremely preterm (EP) infants revealed that increasingly emerging cases occurred with small airway obstructions (SAO) all over the world. Intrauterine *Ureaplasma* infection is associated with neonatal bronchopulmonary dysplasia (BPD) and other complications in preterm infants. We are aimed to scoop out the relationships between intrauterine *Ureaplasma* infection in EP infants and SAO at school age. 360 EP infants born from 1981 to 2004 were selected to investigate LF, placental pathology, placental *Ureaplasma* DNA (pU-DNA), and cord blood immunoglobulin M (C-IgM). Maternal amniotic inflammatory responses were classified from M0 to M3 according to the severity of pathological changes. Our results showed that infants in groups M2 and M3 presented with an elevated gastric white blood cell count and high C-IgM levels due to severe pathological chorioamnionitis, a high rate of premature rupture of the membranes in comparison with M0 infants. pU-DNA and C-IgM were significantly associated with the severity of maternal amniotic

inflammation ($p < 0.0012$, and $p < 0.0001$, respectively). The pU-DNA >1,000 units had an odds ratio of 12 (2.4-74) for SAO. While the odds ratio of surfactant treatment for SAO was 0.21 (0.075-0.58), indicating that even in preterm infants without respiratory distress syndrome, the surfactant administration may improve the subsequent childhood respiratory prognosis. Our study showed that intrauterine *Ureaplasma* was correlated with SAO at the school age.

Category 5. Chemotherapy & Resistance

P-044 Improved Antimicrobial Activity of Fatty Acylated Bovine NK-lysin-derived NK2A Peptide Against *Mycoplasma bovis*

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Mycoplasma bovis is one of the pathogens associated with bovine respiratory disease complex. Isolation of tetracycline and macrolides resistant *M. bovis* underscore the need to identify or develop novel antibiotics and/or alternatives to antibiotics since commercially available vaccines show poor efficacy. Previously, we have shown strong anti-*M. bovis* activity of bovine NK-lysin-derived antimicrobial peptides (AMPs) NK2A and NK2C, albeit at higher peptide concentrations. It is known that fatty acylation of AMPs and co-incubation of certain antibiotics with niclosamide (anti-helmintic dewormer) can improve antimicrobial activity. Therefore, this study assessed whether fatty acylated NK2A and/or NK2A co-incubated with niclosamide shows improved anti-*M. bovis* activity. Thirty-mer synthetic peptides corresponding to the functional region helix2-loop-helix3 of bovine NK2A and fatty acylated NK2A were chemically synthesized and evaluated for antibacterial activity against two *M. bovis* isolates using in vitro assays. CD spectrophotometry indicated both peptides have α-helical structures with fatty acylated NK2A more helical than NK2A. Similar antimicrobial activity was observed with NK2A alone or NK2A co-incubated with niclosamide where ~94% killing was observed at higher (50 μM) peptide concentration. *M. bovis* incubated with niclosamide or fatty acid alone (controls) showed no antimicrobial activity. In contrast, ~100% killing activity was observed when *M. bovis* was incubated with fatty acylated NK2A at 2 μM final concentration. Very low hemolysis (<6%) was observed when fatty acylated NK2A (2 μM) was incubated with cattle red blood cells. These findings suggest that anti-*M. bovis* activity of bovine NK-lysin NK2A can be improved by fatty acylation.

P-045 In Vitro Selection and Characterization of Resistance to Josamycin and Pristinamycin in *Mycoplasma genitalium*

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Macrolide resistance has emerged in *Mycoplasma genitalium* and pristinamycin, a streptogramin combination, is part of the recommended third-line treatment according to the European guidelines. No data regarding mechanisms of pristinamycin resistance have been available to date. In *M. pneumoniae*, a phylogenetically close species, cross resistance to pristinamycin and josamycin was associated with mutations in the 23S rRNA gene at position 2062 (*Escherichia coli* numbering). We aimed to investigate the in vitro development of macrolide resistance in *M. genitalium* in the presence of subinhibitory concentrations of josamycin and pristinamycin. Selection of macrolide-resistant mutants was performed by serial passages of *M. genitalium* reference strain G37 in SP4 broth medium containing subinhibitory concentrations of josamycin or pristinamycin. Resistant mutants were characterized by PCR amplification and DNA sequencing of 23S rRNA, L4 and L22 ribosomal protein genes. For each resistant-mutant, MICs of various antibiotics were determined. Mutants selected in the presence of josamycin harbored an A2059G mutation in 23S rRNA. Mutation A2062C and A2062G were selected in the presence of pristinamycin. No mutations in L4 and L22 gene were detected. The A2059G mutant shown an increase of the MICs of azithromycin (32 µg/mL), erythromycin (32 µg/mL), and clindamycin (16 µg/mL), but no changes in MICs of moxifloxacin, tetracycline and pristinamycin (0.125 µg/mL). MIC determination for A2062C/G mutants are in process but both mutants have a bad fitness. This study showed that macrolide-resistant mutants can be selected in vitro in *M. genitalium* with josamycin and pristinamycin. These laboratory-derived mutants could be predictive for mutations observed in clinical strains.

P-046 Apigenin suppresses mycoplasma-induced alveolar macrophages necroptosis via enhancing the methylation of TNF-α promoter by PPARγ-Uhrf1 axis

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Backgroud: Mycoplasma-associated pneumonia is characterized by severe lung inflammation and immunological dysfunction. However, current anti-mycoplasma agents used in clinical practice do not prevent dysfunction of alveolar macrophages caused by the high level of the cytokine tumor necrosis factor-α (TNF-α) after mycoplasma infection. Apigenin inhibits the production of TNF-α in variety inflammation associated disease. Purpose: This study aimed to investigate apigenin's effect on mycoplasma-induced alveolar immune cell injury and the mechanism by which it inhibits TNF-α transcription. Methods: We performed a mouse model of *Mycoplasma hyopneumoniae* infection to evaluate the effect of apigenin on reducing mycoplasma-induced alveolar immune cell injury. Furthermore, we carried out transcriptome analysis, RNA interference assay, methylated DNA bisulfite sequencing assay, and chromatin immunoprecipitation assay to explore the mechanism for apigenin in reducing TNF-α. Results: We discovered that *Mycoplasma hyopneumoniae* infection-

induced necroptosis in alveolar macrophages MH-S cells and primary mouse alveolar macrophages, which was activated by TNF-α autocrine. Apigenin inhibited *Mycoplasma hyopneumoniae*-induced elevation of TNF-α and necroptosis in alveolar macrophages. Apigenin inhibited TNF-α mRNA production via increasing ubiquitin-like with PHD and RING finger domains 1 (Uhrf1)-dependent DNA methylation of the TNF-α promotor. Finally, we demonstrated that apigenin regulated Uhrf1 transcription via peroxisome proliferator activated receptor gamma (PPARγ) activation, which acts as a transcription factor binding to the Uhrf1 promoter and protected infected mice's lungs, and promoted alveolar macrophage survival. Conclusion: This study identified a novel mechanism of action for apigenin in reducing alveolar macrophage necroptosis via the PPARγ/Uhrf1/TNF-α pathway, which may have implications for the treatment of mycoplasma pneumonia.

P-047 Efflux transporters are involved in *Mycoplasma genitalium* tetracycline resistance

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Mycoplasma genitalium (MG) is a urogenital pathogen and macrolides, fluoroquinolones, and tetracyclines are the current treatment options. CDC recommends a treatment strategy that includes an initial 7-day course of doxycycline to reduce the MG load, which raises a concern about the development of resistance to tetracycline drugs. While resistance to macrolide and fluoroquinolone resistance are extensively investigated, the tetracycline resistance mechanism in *M. genitalium* is still not clear. We collected three *M. genitalium* isolates from a patient showing elevated minimum inhibitory concentrations (MICs) for tetracycline (8-16 μg/mL, compared to 0.5 μg/mL for the reference strain G37) and doxycycline (0.5-2 μg/mL, compared to 0.125 μg/mL for strain G37). MICs for minocycline were also slightly elevated. Genome sequencing of these isolates did not find *tetM* or other mobile elements. There was no mutations in the 16S rRNA gene except a C1440T polymorphism which is unlikely to be related to tetracycline resistance, and no mutations in ribosomal protein genes *rpsJ* and *rpsC*, either. On the other hand, single nucleotide polymorphisms were identified in 32 out of 49 genes encoding transporters, permeases, or facilitators. RNA-seq analysis revealed that 33 of these genes were differentially expressed (12 up-regulated and 21 down-regulated). Inhibition of the efflux transporters with sub-MIC doses of efflux pump inhibitors reserpine and carbonyl cyanide 3-chlorophenylhydrazone decreased tetracycline MIC to 1 μg/mL. These results indicate that efflux transporters are involved in tetracycline resistance in *M. genitalium* and may provide a new consideration of improving treatment strategies for *M. genitalium* infection.

P-048 Antimicrobial Activity of Manuka Honey against Drug Resistant Mycoplasmas

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Mycoplasmas cause a variety of respiratory, urogenital, and systemic infections, which are worse in patients with immunodeficiencies. Due to the absence of a cell wall, treatment options are limited to macrolides, fluoroquinolones, and tetracyclines. Resistance to all three drug classes is emerging and multi-drug resistant mycoplasmal infections have been observed for which no effective treatments are available. Manuka honey, derived from the nectar of *Leptospermum scoparium*, exhibits broad-spectrum antimicrobial activity. We performed an in vitro antimicrobial susceptibility test on Manuka honey and its component methylglyoxal (MGO) against 19 strains of *Mycoplasma pneumoniae*, *M. hominis*, *M. genitalium*, *Ureaplasma parvum*, and *U. urealyticum* that were susceptible and resistant to macrolides, fluoroquinolones and/or tetracyclines. The effects of Manuka honey and MGO on mycoplasma metabolic activities and membrane status were also investigated. Results showed that the minimum inhibitory concentrations (MICs) of Manuka honey and MGO were 0.02-0.08 g/mL and 0.02-0.62 mg/mL on these strains, regardless of their antimicrobial susceptibility status. At MIC concentrations, Manuka honey accelerated cellular respiration in *M. genitalium* while MGO inhibited; Manuka honey did not induce membrane changes while MGO caused membrane depolarization. Results suggest that Manuka honey and MGO are effective to inhibit mycoplasmas, including the multi-drug resistant strains, with a possible mechanism of interfering the metabolic and membrane conditions. Manuka honey and its components could be targets for developing new drugs to treat mycoplasma infections.

Category 6. Immunology & Vaccines

P-049 The Effect of Pegbovigrastim Administration on the Immune Response of Calves Infected with *Mycoplasma bovis*

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Background: Pegbovigrastim has previously been used to stimulate immunity in calves, however, this is the first study to evaluate the effect of its administration alone on the immune response during *Mycoplasma bovis* infection. Methods: The experimental calves were administered with two injections of pegbovigrastim, 7 days apart. In addition, these calves and the positive control animals were experimentally infected with *M. bovis* field strain, whereas the negative control calves received PBS instead. Blood samples were collected for further analyses of the following parameters: a total number of leukocytes with differentiation of granulocytes, monocytes and lymphocytes, using a veterinary blood analyser, phagocytic and oxidative burst activities of granulocytes and monocytes as well as an expression of CD11b antigen on granulocytes and monocytes using a flow cytometer, and a concentration of selected cytokines analysed by separated ELISA kits. Results: In response to pegbovigrastim administration in the calves, a marked increase in the total number of leukocytes and their subsets was observed. Additionally, pegbovigrastim effectively increased the expression of CD11b antigen on monocytes, stimulated killing activity of the cells,

especially granulocytes, and modulated the cytokine concentration. The stimulating properties of pegbovigrastim were also expressed to varying degrees in the course of experimental infection of calves with *M. bovis*. Conclusion: The results of the study show a generally beneficial effect of pegbovigrastim on the immune response of the calves, despite ongoing *M. bovis* infection.
Keywords: calves, *Mycoplasma bovis*, pegbovigrastim, immune response

P-050 Non-replicating Vaccines for Better Control of *Mycoplasma gallisepticum* in Breeder Poultry Flocks

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Mycoplasma gallisepticum (MG) is an economically important pathogen. It causes huge economic loss in terms of production, treatment cost, lowering egg production and poor hatchability. With phase variation and immune evasion mechanisms, this delicate organism escapes host immunity, and establishes chronic infection. In recent years, control of MG depends on proper biosecurity, management and vaccination. Use of chemicals is substantially reduced due to antimicrobial resistance. Currently available vaccines for MG includes bacterin/inactivated, live attenuated and live recombinant fowl pox vectored vaccine. Disadvantages of live vaccines includes reliability of 'cold-chain', vaccine-related reactions and reversion to virulence. There is a need for safer vaccine in long-term birds, such as breeders and layers. Non-replicating vaccines for MG could overcome these constraints. Novel MG non-replicating vaccine candidates includes vector, subunit, DNA, nanoparticle, recombinant protein, multi-epitope, plant-based and inactivated antigens. Some of these antigens if used alongside an efficient immunostimulant adjuvant could provide an excellent alternative for better MG control. Recent advances in adjuvant technologies provides efficient administration of these antigens via respiratory routes for induction of mucosal, in addition to the cellular and humoral immunities. The non-replicating vaccine has the benefit of not being infectious and no risk of reversion to virulence, also easier to produce and induce potent immune response. More innovative research on non-replicating MG vaccines is proposed, for a better long-term MG control in breeder and layer flocks.

P-051 Evaluation of *Mycoplasma gallisepticum* (MG) ts-304 vaccine as a live attenuated vaccine in day-old specific pathogen-free (SPF) chicks

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Mycoplasma gallisepticum causes chronic respiratory disease in poultry. A novel

vaccine, Vaxsafe MG ts-304, has greater efficacy in chickens than the Vaxsafe MG (ts-11) vaccine and protects chickens when delivered by eye drop at 3 weeks of age. It would be best to apply this vaccine in the hatchery using mass administration methods, prior to transportation to the farm. This study assessed protection provided by Vaxsafe MG ts-304 against challenge with a virulent MG strain (Ap3AS) when it was delivered to 1-day-old chicks. Chicks were vaccinated with a single dose of the vaccine to assess the efficacy of a high dose (107.0 CCU) and a low dose (105.7 CCU) after eye drop or coarse spray (in water or gel) administration against experimental challenge with virulent *M. gallisepticum* strain Ap3AS at 7 weeks of age. The vaccine was able to colonise the palatine cleft of 1-day-old chicks after vaccination by eye-drop (at both doses), and chicks vaccinated by coarse spray (in water or gel) (at the high dose). The high dose of the vaccine, when delivered by eye-drop or coarse spray, induced a serological response and protective immunity (as measured by tracheal mucosal thickness and air sac lesion scores) against challenge. There was clear evidence that vaccination of day-old chicks by eye drop or coarse spray with a high dose of the Vaxsafe MG ts-304 vaccine resulted in significant protective immunity and that it is therefore a suitable live attenuated vaccine candidate for use in day-old chicks.

P-052 Identification of Vaccine Targets in Ruminant *Mycoplasma* Pathogens Using in Silico and Proteomic Approaches

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Background: Mycoplasmas cause economically important diseases world-wide. The two most pathogenic species in cattle are *Mycoplasma bovis*, which causes pneumo-arthritis (especially in calves) and mastitis, and *M. mycoides* subsp. *mycoides*, the causative agent of contagious bovine pleuropneumonia (CBPP). The two diseases are still endemic in most African low-income countries. Due to the increasing resistance of *M. bovis* to antimicrobials, vaccination is the preferred method of control, but effective vaccines are currently lacking. The purpose of this communication is to report experiences gained at the Moredun Research Institute, Edinburgh, U.K. as part of an International Veterinary Vaccinology Network (IVVN) training fellowship. During a 4-week tenure, research focused on: 1. Training in proteomic and sequencing - based approaches 2. Training in bioinformatic approaches for identifying potential *Mycoplasma* vaccine and sero-diagnostic candidates. Results: Initial training focused on current in vitro *Mycoplasma* culture techniques, isolation and purification of secreted proteins and whole genome sequencing (WGS) of isolates. Using *M. bovis* as a model, in silico predictions of secreted proteins were made from WGS data using computer-based homology modelling to identify novel vaccine candidates and diagnostic antigens. Mass-spectrometry analysis of secreted proteins from a UK *M. bovis* isolate was used to compliment and validate in silico predictions. Conclusion: Future work aims to apply the techniques and skills gained as part of this IVVN training fellowship to identify potential vaccine and sero-diagnostic candidates in *M. bovis* and other *Mycoplasma* spp. isolated from respiratory diseases in ruminants and chickens in

Nigeria.
Key words: Biomarkers, Vaccine targets, Ruminant *Mycoplasma*, in Silico, Proteomic.

P-053 The molecular mechanism of *Mycoplasma bovis* lipid-associated membrane protein GLCP inhibiting host EBL cells apoptosis

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To explore the molecular mechanism of *Mycoplasma bovis* (*M. bovis*) inhibiting host cell apoptosis, the lipid-associated membrane proteins (LAMPs) of *M. bovis* TJ strain were divided into 5 groups by AKTA molecular sieve and used to stimulate fetal bovine lung (EBL) cells in this study. Western blot and the CCK-8 test results demonstrated that group-D LAMPs could specifically inhibit EBL cells apoptosis. Subsequently, six candidate proteins of VSPHB0801-4, TPP, DJ-1, EF-TU, GAPDH and GLCP were screened by protein mass spectrometry analysis of the group-D LAMPs, which may inhibit the apoptosis of host EBL cells. GLCP was shown to inhibit apoptosis in EBL cells and was selected as the target protein to further investigate its molecular mechanism. The JC-1 probe and Western blot assays results showed that the recombinant protein GLCP could increase the mitochondrial membrane potential of EBL cells, up-regulate the protein Bcl-2 expression, and down-regulate the protein expression of Bax, Cytc, Apaf-1, Cleaved Caspase-9 and Cleaved PARP1, which are key molecules in mitochondrial-mediated apoptotic pathway. The above experimental results suggested that the recombinant protein GLCP inhibited the apoptosis of EBL cells mainly through the mitochondrial-mediated apoptosis pathway. In this study, *M. bovis* lipid-associated membrane protein GLCP was screened for the first time to inhibit the apoptosis of EBL cells, and its molecular mechanism of inhibiting host cell apoptosis was preliminarily elucidated, which provided a theoretical reference for the study of the mechanism of *M. bovis* persistent infection.

P-054 RAMbo-V project: paving the way for the development of synthetic vaccines against *Mycoplasma bovis*

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To meet the demographic pressure, the livestock industry must intensify production while striving for sustainable management. With the emergence and re-emergence of infectious diseases, including those caused by mycoplasmas, improving animal health and welfare is a key aspect of this challenge. While antibiotic treatments are facing an alarming rate of resistance, vaccines are only available for a very limited number of species and often provide insufficient protection. The RAMbo-V project intends to build on advances in bacterial genome engineering to pave the way for the development of

synthetic vaccines against *Mycoplasma bovis*, a livestock pathogen associated with emerging episodes worldwide. This will be done by adapting and improving genetic tools recently developed in other mycoplasma species and using a high-throughput strategy for large-scale mapping of highly conserved antibody epitopes in the *M. bovis* pan-proteome. To engineer an avirulent vaccine chassis and secure its genetic stability, virulence factors non-essential for laboratory growth will be deleted from the *M. bovis* genome together with mobile genetic elements and chromosomal regions contributing to horizontal gene flows. Finally, the surface of *M. bovis* will be redesigned to allow optimal expression of selected epitopes through the use of a transmembrane carrier and the locking of surface variation mechanisms. By exploring an innovative vaccine strategy, RAMbo-V will have a positive impact on animal production and its dependence on antimicrobials. RAMbo-V will thus contribute to the development of sustainable food systems that manage demographic pressure with environmental, societal, and ethical challenges.

P-055 Indigenous vaccine trials for mycoplasmosis in small ruminant of Pakistan

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Immunization is successful strategy to combat several infectious diseases of livestock throughout the world. Mycoplasmosis is a significant respiratory syndrome inflicting heavy production and economic losses across the globe. The current study was design to develop a saponized vaccine from the local field isolates of *Mycoplasma mycoides* subsp. *capri* (*Mmc*). The PCR confirmed isolates having 0.2 mg/mL protein content was inactivated with saponin at the dose rate of 3.0 mg/mL. This indigenous saponized vaccine was compared with commercially available lyophilized *Mmc* vaccine by inoculating in experimental animals (goats) to evaluate its immunogenic potential. A total of eighteen (18) goats were divided into three groups A, B and C. The groups A and B were further split into two sub-groups (3 animals each) that were served as unchallenged and challenged groups, while group C received normal saline. The unchallenged and challenged groups received saponized and lyophilized vaccine. The antibodies titer was monitored by indirect haemagglutination (IHA) 75 days post vaccination. The higher antibodies titer in challenged goats with maximum GMT (224) was recorded on 28th day with saponized vaccine as compared to low GMT value (192) with lyophilized vaccine. It is concluded from the findings that saponine is safe in food animals and can successfully be used as inactivated agent for production of local vaccine. It was further suggested that indigenous saponized vaccine prepared from the local field strain (*Mmc*) have promising immunogenic potential and can confer better protection as compared to the imported vaccine.

P-056 Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Activity in *Mycoplasma synoviae* and Vaccine Strain MS-H

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Mycoplasma synoviae is a poultry pathogen causing significant economic loss to the intensive chicken and turkey production due to reduced egg production, respiratory disease, lameness, and/or downgrade of eggshell quality. A live attenuated vaccine, MS-H, has been developed by random mutagenesis and currently used in over 50 countries around the world. The molecular mechanisms behind MS-H attenuation have been investigated extensively and appears to be related to a number of mutations including those found in both copies of the gene encoding Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a protein which is believed to catalyse glyceraldehyde 3-phosphate to 1,3-diphosphoglycerate by reduction of NAD+ to NADH in glycolysis pathway. In this study the activity of GAPDH copies in the vaccine strain MS-H was compared to that of the vaccine parent strain 7NS (which produces two identical copies of the protein). Recombinant GAPDH proteins were produced in E. coli and their enzymatic activity were evaluated through the level of NAD+ to NADH conversion. Mutation found in one of the MS-H GAPDH copies appeared to have completely abrogated its activity while mutation in the second copy appeared to have increased its activity. In-silico analysis of the structure of the two copies of the MS-H GAPDH and comparison with that of the 7NS provided explanation for altered activities of the MS-H GAPDH copies but further investigations are underway to explore the effect of these mutations on other potential function(s) of GAPDH in *M. synoviae* pathogenesis.

P-057 A secreted serine/threonine phosphatase encoded by Mbov_0725 of *Mycoplasma bovis* attenuate activation of MAPK p38 and ERK

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Background: Mycoplasmas are able to produce and release several components into their environment. In our previous study, one secreted protein, MbovP0725, is one putative hydrolases of the haloacid dehalogenase (HAD) superfamily and differentially expressed between wild-type strain HB0801 and its attenuated strain. However, its physiological roles have remained unclear. Methods: MbovP0725 protein sequence feature was analyzed using Clustalw and ESPript. The enzymatic activity of recombinant MbovP0725 was detected by the hydrolysis of pNPP. Transcriptomics and metabolomics analyses were performed to investigate the underlying mechanism of MbovP0725 on modulating metabolism and gene expression. MbovP0725 protein was introduced into BoMac cells to explore its function on inflammatory response. Results: MbovP0725 contains all four of signature motifs of HAD superfamily protein and is a secreted protein with serine/threonine phosphatase activity. MbovP0725 alters *M. bovis* gene expression involved in small molecule metabolic process and ion binding. Metabolomic profiling revealed significant differences between the wild-type HB0801

stain and the MbovP0725 mutant T8.66 strain in a number of metabolites of nucleotide and amino acid metabolism. rMbovP0725 protein inhibited lipopolysaccharide-induce IL-1β, IL-6 and TNF-α expression and attenuated MAPK signal pathway in BoMac cells. MbovP0725 transposon mutant strain T8.66 has reduced virulence as compared to wild type *M. bovis* due to its lower adhesion ability to epithelial cells. A pull down assay identified P38 and ERK1/2 as potential substrates for MbovP0725. Conclusion: Our findings define a virulence and metabolism related role of a HAD family phosphatase MbovP0725 and reveal an anti-inflammatory of an important intracellular pathogen.

Category 7. Cell Biology, Physiology & Metabolism

P-058 Elucidating the Role of Fibril Protein in *Spiroplasma* Swimming using JCVI-syn3B

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Spiroplasma swims by switching the handedness of the internal ribbon structure composed of fibril, and five MreB proteins belonging to actin superfamily. Previously, our group succeeded in reconstructing the helical cell morphology and the swimming in a synthetic minimal bacterium, JCVI-syn3B by expressing only MreB4 and MreB5 proteins (Kiyama H et al., Sci. Adv. 2022). In the present study, we focused on the role of fibril protein composed of 512 amino acids. Additional expression of fibril protein in the reconstituted system using the native promotor changed the helix width from 1.0 to 0.40 μm, which is closer to 0.23 μm, that of the original *Spiroplasma* species. To elucidate the role of individual parts, we introduced random mutagenesis and analyzed syn3B cells expressing fibril protein without MreBs. Five of 20 mutants with single amino acid substitution showed changes in cell helicity. Each K415E and V488I mutation caused a reduction in helical pitch from 0.87 μm of the wild type to 0.49 μm and 0.53 μm, respectively. I403T showed a decreased helix diameter of 0.37 μm. Gel electrophoresis showed increased protein amounts for B81G and K415E mutants. We will discuss these results based on the protein structure clarified by cryo-electron microscopy (Presentation in this congress by Sasajima et al.).

P-059 Elongated JCVI-syn3B Cell Caused by Expression of *Mycoplasma pneumoniae*-gliding Type Cytadherence Regulatory Locus

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M. pneumoniae and *M. genitalium* have the attachment organelle that have roles in gliding motility and cell division. The organelle is supported by a cytoskeleton-like

structure (core). The core is consist of a number of proteins including four that are coded in cyt~~o~~adherence regulatory locus (*crl*) on the genome. To clarify the function of proteins coded in *crl*, we expressed the *crl* proteins (P65, HMW2, P41, P24) of *M. pneumoniae* and their homologs of *M. genitalium* in a minimal synthetic bacterial cell, JCVI-syn3B (syn3B) that is non-motile and spherical. The *crl* was transcribed by a strong promoter *ldh* (lactate dehydrogenase) derived from syn3B. Replacement of RBS (ribosome binding site) was necessary for translation of *M. genitalium* genes. Syn3B cells expressing *crl* proteins showed elongated morphology. Based on the aspect ratio of cells, elongated cells that were 1.5 times longer than their short axis were 1%, 34%, and 21% for original syn3B, syn3B with *M. pneumoniae crl*, and syn3B with *M. genitalium crl*, respectively. Interestingly, the cells also showed elongated branching morphology as 0%, 4.1%, and 10.5% for original syn3B, syn3B with *M. pneumoniae crl*, and syn3B with *M. genitalium crl*, respectively. We will discuss the possible roles of *crl* proteins.
Keywords: syn3B, *crl*, microscopy, RBS.

P-060 Electron Paramagnetic Resonance (EPR) Analysis on Nobel Protein Assemblies, Dimer of Hexameric F_1-like ATPases and Fibril Helical Filament, Involved in *Mycoplasma* Gliding and *Spiroplasma* Swimming

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Spin-labeling EPR was pioneered in dynamics measurements of *Mycoplasma* membrane(1). Here, we applied it to *Mycoplasma* and *Spiroplasma* motility, to know the dynamic conformational changes or mechanical movements of cytoskeletal/motor protein molecules coupled with ATP hydrolysis. We developed multi-site mapping of 1.5-8 nm interspin distances in a protein using pulsed electron double resonance (DEER)(2). *Mycoplasma* mobile motor, a dimer of hexamers, (Gliα3Gliβ3)2ATPase(3), can be studied in solution for multi-site distance analysis using paramagnetic metal Mn^{2+}ATP bound to six catalytic sites as a substrate. We ensured Mn^{2+} substitution and characterized the Mn^{2+}-binding for catalytic sites by using continuous-wave EPR spectroscopy. The EPR spectrum of Mn^{2+}-nucleotide-ATPase complex was dominated by six lines arising from the hyperfine coupling to the Mn nucleus (I=5/2), and was extremely broadened by slower rotational motion and zero-field splitting due to a different ligand field in a catalytic site. Different amounts of spectral residuals of free Mn^{2+} would suggest different binding affinity of Mn^{2+} for protein-nucleotide complexes (AMPPNP>ATP(ADP.P)>ADP), that is also observed for cooperative binding of F_1-ATPase(4). Next, the cytoskeletal protein 'fibril' filaments from *Spiroplasma*, which were spin-labeled with nitroxyl maleimide(2) on two natural cysteines per monomer, and multi-spin interlabel distance measurements were performed to elucidate the filament structure in solution consistent with electron microscopy(5). This technique will examine whether fibril filament undergoes helical movements associated with MreB filaments.
(1)Rottem et al. BBA(1970); Tourtellotte et al. PNAS(1970). (2)Arata, IJMS.(2020). (3)Toyonaga et al. mBio.(2021). (4)Mao et al. FEBS.Lett.(2006). (5)Sasajima and Miyata, Front.Microbiol.(2021).

Keywords: motility, protein, ATP, spin-label, ESR

P-061 Importance of MreB5 ATPase activity in *Spiroplasma citri* helicity and motility

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Spiroplasmas are helical, motile Mollicutes. Their swimming movement is associated with a unique kink-based mechanism. Indeed, the movement of the cells in semi-viscous media results from a switch between two helical structures of the cell body with different handedness. *Spiroplasma citri* encodes 5 *mreB* genes in its genome. MreBs are actin-like proteins, found in most of the elongated bacteria, capable of polymerization in in vivo and in vitro experiments. Together with the protein fibril, they form the *Spiroplasma* internal cytoskeleton. We have previously highlighted the role of MreB proteins in maintenance of helicity and in kink propagation in *S. citri*. In particular, the lack of helicity and motility of the natural variant of *S. citri* strain ASP-1 was shown to be due to the disruption of *mreB5* gene. The complementation of this variant with the *mreB5* WT gene restored both helicity and swimming motility. MreB5 possesses an ATPase activity in vitro. The requirement of MreB5 ATPase activity for helicity, elongation and motility was here evaluated. The ASP-1 variant was complemented with different mutated forms of *mreB5*, among which one is deficient in its capacity to hydrolyse ATP and another in its nucleotide-binding capacity. Both mutations abolished MreB5 capacity to hydrolyse ATP, but they differentially affected cell morphology and motility. The comparison of their impact on cell curvature strongly suggests that determination of the membrane/cytoskeleton interaction site in vivo is dependent on the nucleotide-bound state of MreB5.

P-062 In silico analysis of cytoskeleton protein sequences in *Spiroplasma* species

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Spiroplasmas are pathogens or commensals in plants and arthropods. The genome size across *Spiroplasma* genus strongly varies, but all species share a characteristic helical morphology. This means that genetic determinants of the helical shape of these bacteria have been conserved despite the strong reduction of the genome size in some species. Actin-like proteins, MreBs, and the specific protein fibril form an internal cytoskeleton shown to be associated with the capacity to maintain the helical shape in a few species. These proteins are also involved in the kink-based swimming ability of *Spiroplasma* species. Also, the number of MreB isoforms differs from one species to another and some species lack an intact *fibril* gene. The exact contribution of the

different MreB isoforms in helicity and motility of *Spiroplasma* remains yet to be identified, as well as their regulation and mode of interaction. Nonetheless, five subgroups of MreB isoforms, MreB1-5, could be identified. Here, in light of the available genome sequences, the number of which has significantly increased in recent years, the cytoskeleton gene content in *Spiroplasma* species was updated. Phylogenetic analyses allowed to propose an evolutive scenario based on duplication and gene loss events. In addition, a thorough analysis based on multiple sequence alignments, on search for predicted functional domains allowed to identify (i) the minimal set of cytoskeleton proteins present in natural helical, motile spiroplasmas, and (ii) the sequence features specific to each MreB subgroup.

P-063 Gliding Machinery of *Mycoplasma mobile* Visualized by Electron Tomography

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Mycoplasma mobile, a fish pathogen glides by a special mechanism shared with *M. testudineum*, *M. agassizi*, and *M. plumonis*. The internal part of machinery is composed of a terminal structure "bell" and 28 chains extending from the bell. Each chain consists of 17 copies of gliding motors, which evolved from an F-type ATPase. However, its three-dimensional architecture is still unclear. In this study, we exposed the internal structure and applied it to tomography by using an electron microscope and obtained following results. (i) The bell is bowl-shaped. (ii) The bell has a honeycomb pattern with 8.4 nm periodicity on the surface. (iii) The chains are anchored to the entire rim of the bell with a uniform interval. (iv) A wedge structure approximately 65 nm long connects the bell and the chain. (v) The chains are connected by flexible filamentous structures laterally. (vi) Cylinder structure with a height of 13 nm and a diameter of 6 nm are found between gliding motors. The roles of individual structures will be discussed.

P-064 Probing Interactions Between Adhesins and Attachment Organelle Core Proteins in *Mycoplasma pneumoniae*

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Mycoplasma pneumoniae uses the attachment organelle (AO) to adhere to host cells and engage in gliding motility along surfaces. The AO is essential for virulence and contains some of the most immunodominant proteins of the cell, making it an appealing target for the development of therapeutic agents. Various AO-specific proteins localize to certain regions of the AO, but it is unclear how these proteins interact to form a cohesive and functional AO. Nap particles, consisting of a pair each of the homologous transmembrane proteins P1 and P40/P90, densely line the entire membrane of the AO and are the main factors in the functionality of the AO, while the proteins found in the dense internal core are necessary for the formation of the AO and its structural

integrity. Destabilization of the core protein HMW1 prevents localization of the components of the nap particle to the AO, suggesting there is a link between this protein and the membrane adhesins. We tested the hypothesis that the localization of the nap particle to the AO is driven by interactions between its components and core proteins. Specific charged domains of P1 and HMW1 appear to be the most likely to interact based on the organization of the AO and the topology of P1. Far-western blotting, affinity chromatography, and a bacterial two-hybrid screen were used to probe whether these two domains interacted. All were negative, suggesting that either the interaction is weak or indirect, or that other interactions are required for AO function in *M. pneumoniae*.

P-065 Cryo-EM observation of γ-polyglutamic acid hydrogels from *Bacillus subtilis* of Firmicutes

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Natto is a traditional fermented food in Japan, where the natural Firmicutes bacterium, *Bacillus subtilis*, present in straw ferments the soybeans, producing a unique aroma and viscosity. The viscosity of Natto is mainly derived from **γ-polyglutamic acid** (γ-PGA), which is thought to form a natural hydrogel. γ-Polyglutamic acid is a polypeptide chain in which the carboxyl group at the γ-position and the amino group at the α-position form a peptide bond, that not only contributes to health, but also has water cleansing properties, and its structural properties are very interesting. On the other hand, **cryo-electron microscopy** (cryo-EM) has made remarkable progress in recent years, mainly in the structural analysis of proteins, and is now taking second place to X-ray crystallography in the structural analysis of biological samples. Cryo-EM is characterized by its ability to rapidly freeze a sample and observe under a vacuum condition. Therefore, cryo-EM allows observation in a near-live state where water is present. This feature is expected to be applied not only to biological samples such as proteins and cells, but also to soft materials such as hydrogels and foods. In this study, we visualized the hydrogel structure of Natto by processing the sticky component with a Cryo-FIB-SEM and directly observing it with a cryo-EM.

P-066 The analysis of *Spiroplasma eriocheiris* cell division proteins

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In a bacterium surrounded by cell wall, the cell division is generally regulated by the tubulin homolog protein FtsZ. FtsZ assembles into the ring like structure (Z-ring) with the other cell division proteins including cell wall synthesis enzymes. FtsZ polymerizes into the filaments in the presence of GTP, and SepF stabilizes FtsZ filaments. Some species belonging to Mollicutes are lacking most genes involved in cell wall synthesis

including *ftsZ* gene. However, *Spiroplasma eriocheiris* still carries *ftsZ* and *sepF* genes. In the present study, we have analyzed the interaction between *S. eriocheiris* FtsZ (SeFtsZ) and SepF (SeSepF). We measured the GTPase activity of SeFtsZ. The rate of GTP hydrolysis was less than *E. coli* and *Bacillus subtilis* FtsZs. The SeSepF increased the GTPase activity of SeFtsZ. The SeFtsZ showed the GTP-dependent polymerization and the SeSepF promoted the polymerization of SeFtsZ. Next, we analyzed the interaction between SeFtsZ and SeSepF. The binding affinity of SeSepF to the SeFtsZ monomer was higher than the affinity to polymer. Finally, we examined the localization of SeFtsZ in *E. coli* L-form cell. GFP-SeFtsZ localized in the cytoplasm and formed the filaments and tubules. When GFP-SeFtsZ was co-produced with SeSepF, GFP-SeFtsZ localized to the cell membrane and formed the ring. These results suggested that SeFtsZ makes the narrow parts of cell membrane and promotes the cell division.

P-067 Mutational Analyses of MreB5 Essential for *Spiroplasma* Swimming Reconstructed in JCVI-syn3B

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Spiroplasma eriocheiris shows a unique swimming motility. In the last congress, we reported a reconstruction of the helical morphology and the swimming motility in *Mycoplasma mycoides* JCVI-syn3B (syn3B) by expressing two types of bacterial actin, MreB4 and MreB5. Next, we got interests in the mechanisms and evolution of *Spiroplasma* swimming. In this study, we introduced random mutations into MreB5 in MreB4–MreB5 combination to obtain insights for the role of each residue on MreB5. Mutations were induced at a ratio of 1–5 amino acids out of total 350. We used SP-4 medium made with horse serum and 0.3% agar to distinguish between non-motile and motile strains by the colony shape. The non-motile strains showed a typical fried egg colony shape, while the motile strains exhibited irregular outlines and rough surfaces. We observed 27 mutants from both types of colonies, in which 21 strains were motile. Six strains lost motility, as described below. i) Four mutants (I8T, S87P, T204P, and R247G) showed filamentous morphology with rare helical cells. ii) A mutant L265P showed a spherical cell morphology similar to the original syn3B. iii) A mutant lacking 27 amino acids at the C-terminus, including the membrane binding region, showed a filamentous morphology. These mutation points were distributed for the entire MreB5 structure. These results suggest molecular insights about the MreB5 role during *Spiroplasma* swimming and the importance of membrane binding at the C-terminus for swimming. We will also discuss an evolution of *Spiroplasma* swimming through experiments with ancestral MreBs.

P-068 Morphology and motility of *Spiroplasma* species differing in their cytoskeleton gene content

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Spiroplasma species are distinguished from other Mollicutes by a helical shape. In addition, the most studied species share a kink-based swimming motility, where a kink propagating along the cell body triggers a helicity shift. Their internal cytoskeleton is involved in their helicity and motility and is composed of actin-like proteins named MreBs and of a specific protein named fibril. Among *Spiroplasma* species, the number of MreB isoforms vary, and some species are deprived of a functional fibril gene. This raises the question whether or not there is a correlation between genetic content, morphological characteristics and swimming efficiency. Moreover, since no quantitative data regarding morphological features and motility are available for most species, the existence of a single kink-based swimming mechanism for all members of the genus *Spiroplasma*, and more specifically for spiroplasmas devoid of fibril, might be questioned. In this work, 2 strains lacking fibril were analyzed for the relative expression level of the cytoskeleton proteins, their helicity, cell length, mode of motility, and mean velocity in a semi-viscous medium under controlled conditions. Their morphology and motility features were compared to those of phylogenetically distant fibril-expressing spiroplasmas differing in their MreB content. An increase of the helical pitch could be associated with the absence of fibril. Regarding the swimming mechanism, all studied species, including fibril lacking ones, showed propagation of a kink along the cell body. Velocity of kink propagation and frequency of kink initiation did not depend on the presence of fibril, but most probably on the MreB content.

P-069 A new *Spiroplasma* species with a highly reduced genome discovered from a coral metagenome in Okinawa

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Corals are well-known as hosts of diverse symbiotic microorganisms. To better understand the interactions between corals and symbionts, we investigated *Palythoa tuberculosa* soft coral in Okinawa using a metagenomics approach with Illumina and Nanopore sequencing. The metagenome sequencing resulted in finding of a new species of *Spiroplasma* as one of the most abundant microbes in the *P. tuberculosa* population that was used for sequencing. To characterize this novel symbiont, we performed phylogenomic and comparative genomic analyses using its complete circular genome. The novel *Spiroplasma* species (tentatively named *Candidatus* Spiroplasma coralicola) seems to be related to *Ca.* S. holothuricola, a symbiont of deep-sea cucumber. Interestingly, *Ca.* S. coralicola and *Ca.* S. holothuricola contain reduced genomes of 0.68 Mbp and 0.42 Mbp, respectively, as well as low GC content of 36%. Both symbionts also show high mutation rate resulting in long branch lengths when compared to their related species from several arthropod hosts. These results suggest that *Ca.* S. coralicola has a long-term symbiotic relationship with corals (or potentially another organism in the metagenome). Most genes of *Ca.* S. coralicola are responsible for genetic information processing and carbohydrate metabolism similar to other free-living *Spiroplasma* species. However, symbiont might also play a role in the

protection of *Palythoa* coral against viruses based on its high density of clustered regularly interspaced short palindromic repeats (CRISPRs). Our results imply that there is likely a huge diversity of unexplored *Spiroplasma* species in marine invertebrates and characterizing their functions will be essential for understanding the diverse host-symbiont interactions in the marine environment.

P-070 Structure and Function of Gli123 Involved in *Mycoplasma mobile* Gliding

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Mycoplasma mobile is a fish pathogen that glides on solid surfaces by means of its own gliding machinery composed of internal and surface structures. We focused on the function and structure of Gli123, a surface protein that is essential for the localization of other surface proteins. The amino acid sequence of Gli123, which is 1128 amino acids long, contains lipoprotein-specific repeats. We isolated the native Gli123 protein from *M. mobile* cells and a recombinant protein, rGli123, from *Escherichia coli*. The isolated rGli123 complemented a non-binding and non-gliding mutant of *M. mobile* that lacked Gli123. Circular dichroism and rotary-shadowing electron microscopy (EM) showed that rGli123 has a structure that is not significantly different from that of the native protein. Rotary-shadowing EM suggested that Gli123 adopts two distinct globular and rod-like structures, depending on the ionic strength of the solution. Negative-staining EM coupled with single-particle analysis revealed that Gli123 forms a globular structure featuring a small protrusion with dimensions of 14.7, 14.1, and 15.7 nm. Small-angle X-ray scattering analyses indicated a rod-like structure composed of several tandem globular domains with total dimensions of approximately 34 nm length and 6 nm width. Both molecular structures were suggested to be dimers based on the predicted molecular size and structure. Gli123 may have evolved by multiplication of repeating lipoprotein units and acquired a role for Gli521 and Gli349 assembly.

P-071 *Haloplasma* Motility Reconstituted in JCVI-syn3B by Combination of Two MreB Proteins

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Previously, our group suggested that *Spiroplasma* swimming was evolutionally originated from differentiation and combination of two MreB proteins, bacterial actins (Kiyama H et al., Sci Adv 2022). In the present study, we focus on *Haloplasma contractile*, phylogenetically positioning closer to Firmicutes than *Spiroplasma* are. *H.*

contractile is halophilic, anaerobic, and hardly culturable, and contracts by changing its cell shape between straight and coiled. We completed the genome sequence and found seven *mreB* homologs, which we named as *hmreB*s *1–7*. These genes are coded in four regions as *hmreB 1–2*, *3–4–5*, *6*, and *7* on the genome. Next, we transformed JCVI-syn3B (syn3B) by the gene regions of *hmreB*. Either regions of *hmreB6* or *hmreB7* gave elongated cell shape to syn3B cells. Regions *hmreB 1–2* and *3–4–5* gave cell movements of coiling and wriggling, respectively. Deletion of *hmreB5* from the *3–4–5* construct retained movements. Cryo-electron tomography of the syn3B cells expressing *hmreB1-2* showed filament structures containing particles with a periodicity of ~5 nm, under the cell membrane. Sequence analyses showed that *hmreB*s can be divided into three phylogenetic groups. These results suggest that *Haloplasma* motility was caused by differentiation and combination of two MreB proteins, resulting in shear forces generated between different types of filaments as suggested for *Spiroplasma* swimming.

P-072 Using minimized mycoplasma JCVI-Syn3 to analyze *Ureaplasma* pathogenicity factors

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Ureaplasma parvum internalizes into HeLa cells, resulting in the cytoplasmic accumulation of galectin-3[1)]. *U. parvum* triggers ER stress and upregulates the unfolded protein response-related factors including BiP, P-eIF2 and IRE1 in the host cells. However, *U. parvum* does not induce the downstream apoptotic factors. We performed the vesicular trafficking inhibitory screening in yeast and identified a novel virulence factor, *U. parvum* vacuolating factor (UpVF). Transient expression of UpVF induced HeLa cell death with intracellular vacuolization. On the other hand, the stable transformant of UpVF-expressing HeLa and CaSki cells exhibited resistance to X-ray irradiation, cisplatin, and paclitaxel. The xenograft of UpVF-expressing CaSki cells in nude mice also revealed resistance to cisplatin and paclitaxel. These data indicated that UpVF acts as an enhancer of cervical cancer malignancy. However, no established methods are available for generating *Ureaplasma* knockout or transgenic strains for evaluating UpVF in the native organism. Therefore, we utilized the minimal cell JCVI-syn3B as a vector to express *Ureaplasma* proteins. JCVI-syn3B is a minimized version of *Mycoplasma mycoides* with a synthetic genome that as a result of gene removal has lost it capacity to attach to and parasitize mammalian cells. We found MBA-UpVF reduced HeLa cell survival compared with JCVI-syn3B expressing only MBA after 72 h of infection[2)]. JCVI-syn3B is a useful tool to investigate how one or a set of bacterial proteins interact with mammalian cells in the absence of other proteins that might affect that bacterial cell-mammalian cell interaction.
[1)]Nishiumi F, MicrobiologyOpen, 2017, [2)]Nishiumi F, Cell Microbiol, 2021

P-073 Cell division in bacteria without a cell wall: Mechanism of action of the

bacterial cell division proteins FtsZ and FtsA

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In bacterial cell division, FtsZ, a tubulin homolog, assemble into filaments that constitute the contractile ring. Typically FtsZ is attached to the membrane through adaptor proteins such as FtsA and SepF. Analysis of the divisome genes in mycoplasmas demonstrated that a minimum number of 5 genes constitute the division gene cluster in *Mycoplasma*. FtsZ/FtsA in mycoplasmas thus form an ideal model system for understanding bacterial cell division, in the absence of the cell wall. A comparative sequence analysis of 113 *Mycoplasma* genomes highlights special and conserved features of FtsZ sequences. The analysis highlights diversity in the possible modes of membrane attachment of the division ring, involving FtsZs with plausible direct membrane attachment bypassing the requirement of FtsA and SepF. Further experimental characterization of biochemical features of *Spiroplasma* FtsZ and FtsA and the possible combinations of membrane attachment through chimeric constructs with the *Mycoplasma* FtsZs provide valuable insights into division in a cell wall less mileu.

P-074 Impact of spiralin deletion on *Spiroplasma citri* membrane organization and composition, morphology and motility

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Spiroplasma membrane contains a high proportion of lipoproteins, among them spiralin. In the Citri clade, it accounts for around 25 % (w:w) of the total amount of membrane proteins. Helicity and motility of the *Spiroplasma* cell necessarily imply a high degree of membrane curvature and significant membrane deformations that put forward the physiological relevance of protein-lipid interactions in the membrane. In *S. citri*, spiralin has been shown to act as a lectin and is not essential for *Spiroplasma* helicity and motility, but both its abundance and its tension-active properties suggest this lipoprotein to exhibit an additional structural and mechanical function via its interactions with membrane lipids. In particular, detergent-resistant membrane domains (DRMs) enriched in spiralin, other lipoproteins and cholesterol were isolated from *S. citri* wild-type (WT) strain GII3 cells using Triton X-100 at high concentration. DRMs from the mutant spiralin-less strain GII3-9a3 were extracted at a lower Detergent/Protein ratio, suggesting a role of spiralin in membrane resistance to solubilization. In addition, the two strains were compared for their helicity, polar morphology and motility using darkfield and atomic force microscopies. Morphological features of the GII3-9a3 mutant significantly differed from those of the GII3 WT strain. Spiralin, by participating to the membrane lateral organization, could be involved in the lateral distribution of lipids allowing the correct attachment of the internal cytoskeleton at the membrane level and in the maintenance of the helical periodicity of *S. citri* cells.

P-075 Investigating the Glyco-Proteoform Landscape of Minimal Genome *Mycoplasma* spp.

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The surface of a *Mycoplasma* cell is a dynamic environment with many distinct proteoforms and glycans found across the membrane. Two organisms that are attractive models for minimal genome studies, the human urogenital pathogen *Mycoplasma genitalium* (Mgen) and the artificially genome reduced *Mycoplasma mycoides* JCVI-Syn3A (Syn3A) have evidence of protein glycosylation based on periodic acid staining of proteins from cell lysates and membrane fractions. Using high-resolution mass spectrometry, we are investigating the glyco-proteoform landscapes of these organisms. Interestingly, evidence of proteolytic processing exists in both Mgen and Syn3A in a similar manner reported to other, less genome reduced, *Mycoplasma* spp. We have found evidence of surface glycoproteins that suggests protein hexosylation reported in other distantly related spp. is active in JCVI-Syn3A and are currently confirming whether it is also active in Mgen. We are also currently investigating if a rhamnosylation system is active in these spp., as well as the less genome reduced organisms *Mycoplasma pneumoniae* and *Mycoplasma mycoides* subsp. *capri*. Protein glycosylation would contribute further antigenic variation in tandem with proteolytic processing and other described *Mycoplasma* immune evasion strategies. Additionally, glycosylation in these minimal spp., including the non-pathogenic Syn3A, implies a fundamental physiological role for survival, perhaps shielding their proteins from excreted proteases to suit their metabolic adaptation to parasitizing host cells. Finally, the evidence of glycosylation in minimal genome *Mycoplasma* spp. would imply essential enzymes in the pathway may be potential therapeutic targets against pathogenic *Mycoplasma*.

P-076 Cryo electron microscopy of cytoskeletal 'fibril' protein involved in *Spiroplasma* swimming

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Spiroplasma can swim by switching handedness of its helical cell body. The helix is formed by an internal ribbon composed of MreB an actin superfamily protein, and a *Spiroplasma*-specific fibril protein. The fibril filament is composed of repeated ring and cylinders. Here, the structure was clarified by single-particle cryo-electron microscopy at 3.6 Å resolution, and modeled for 512 amino acid residues. The N-terminal cylinder domain showed a structure similar to SAH nucleosidase, while the C-terminal ring domain did not show similarities to other proteins. The filaments were nonpolar and flexible possessing a helical pitch of 700 nm, consistent with cell pitch. Cryo-electron tomography of cells showed that several MreB filaments were aligned in the center of ribbon, and neighbored on each side by a few fibril filaments binding to the membrane

via electric charge. Evolution and role of the fibril filaments have been suggested.

P-077 Structural and functional studies of antibodies halting motility in *Mycoplasma pneumoniae* reveal the dynamic nature of the adhesion complex

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Mycoplasma pneumoniae is a bacterial wall-less human pathogen and the etiological agent of atypical pneumonia and tracheobronchitis in both adults and children. *M. pneumoniae* infectivity, gliding motility and adherence to host target respiratory epithelial cells, are mediated by proteins P1 and P40/P90 forming a trans-membrane complex that binds to sialylated oligosaccharides cell receptors. Here we report the Cryo-EM structure from P1 bound to the Fab fragment of monoclonal antibody (P1/MCA4), which stops gliding and induces detachment of motile *M. pneumoniae* cells. The epitope, centred on loop Thr1426-Asp1438 in the small C-terminal domain of P1, is inaccessible to antibodies in the “open” conformation of the adhesion complex, when ready for attachment to sialylated oligosaccharides receptors. Polyclonal antibodies against the C-terminal domain of P1 also halt gliding quickly, while on the contrary, polyclonal antibodies against the N-domain of P1 or against the whole ectodomain of P40/P90 have little or no effects on motility. Mutations in the highly conserved Engelman motifs found in the transmembrane helices of adhesins, can also alter adherence and/or motility. Altogether these results provide a deep insight into the adhesion/release cycle of the adhesion complex. The C-terminal domain of P1 experiences large conformational rearrangements, during the attachment/detachment cycle of the adhesion complex. These rearrangements are hindered by antibodies against the C-terminal domain thus interfering with gliding, which explains the specific neutralization mechanism deployed by antibodies against this domain suggesting new ways to confront *M. pneumoniae* infections.

P-078 Formation process of *Bacillus subtilis* L-form visualized by quick-freeze deep-etch replica electron microscopy

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Many bacteria continue to grow even though they lose cell walls composed of

peptidoglycan. The bacteria in this state were discovered by Dr. Emmy Klieneberger-Nobel in 1935 and named as L-form bacteria after the Lister Institute. The L-form is useful also for understand the origin and character of *Mycoplasma*. In this study, we visualized the conversion of *B. subtilis* to L-form and its recovery, by using quick-freeze deep-etch replica electron microscopy (QFDE). In QFDE, the sample is frozen and fixed in contact with a metal block cooled to liquid helium temperature, applied to sublimation of the ice to expose the sample surface. Then its surface is coated with platinum, and the platinum replica was observed by a transmission electron microscope. Previously we applied QFDE to walled form of *B. subtilis* and visualized a concentric circle and a mesh patterns on the polar and the body parts, respectively. In the present study, we analyzed L-form conversion induced by *murE* gene manipulation. In the conversion, the specific pattern of polar and body parts changed to coarse meshes, the cells were swollen, and the cell membrane was partly exposed. In the reversion, the cell surface was covered with fibrous structures and the morphology changed from spherical to rod-like. In both processes, division sites were observed as irregular formats.

P-079 Assembly properties of bacterial actin MreB5 essential for *Spiroplasma* swimming

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Spiroplasma forms a helical cell shape and swims in a viscous liquid using five classes of bacterial actin proteins MreB (MreB1–5). They polymerize into filaments that are assembled into higher-order structures like sheets and bundles. The polymerization dynamics of MreB5 is essential for both helicity formation and swimming motility of *Spiroplasma*. Here, we analyzed the assembly of MreB5 to consider molecular properties underlying the cellular dynamics on the swimming motility. Assembled structures of MreB5 and their assembly dynamics were analyzed by electron microscopy and static light scattering under different pH and ionic strength conditions to evaluate the electrostatic nature of MreB5 filaments. MreB5 formed sheet structures with distinct ends under extensive conditions. Under acidic and neutral pH conditions, MreB5 formed bundles in which the formation followed the sheet formation. Time-course light scattering measurements suggested that the bundle formation was mediated by electrostatic interactions, was enhanced by an unstructured C-terminal region, and was mediated by divalent cations. Our comprehensive biochemistry revealed the assembled structures of MreB5 and their formation dynamics and detailed properties. The asymmetry of the sheet structure may determine the direction of *Spiroplasma* swimming. Our analyses of MreB5 bundles may be important for the electrostatic nature of MreB5 filamentous structures underlying interactions with other factors.

P-080 *Spiroplasma* swimming mechanism suggested by fluorescently labeled MreBs expressed in JCVI-syn3B

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Spiroplasma swim by switching its handedness of helical cell body. The handedness switch is driven by an internal ribbon structure, composed of five MreBs, bacterial actin and *Spiroplasma* specific "fibril" protein. Previously, our group reconstituted *Spiroplasma* swimming in JCVI-syn3B, a minimal synthetic bacterium, by expressing MreB4 and MreB5 (Kiyama H et al., Sci Adv 2022). In the present study, we studied the behavior of MreB5 in the cells by fluorescence microscopy. MreB5 fused with mCherry immediately after tyrosine 218 of the total 350 amino acids (MreB5m) was induced through *tetO2* promoter, in a cell expressing MreB4 constitutively. Moving cells appeared when MreB5m amount in the culture reached 20% of MreB4. The movements were found specifically at fluorescent and helical cell parts, observations by TIRF (Total Internal Reflection Fluorescence) microscopy. Addition of 200 μM A22 inhibited the movement and helicity consecutively in 2 min, suggesting that the conformational change caused by ATP is essential both for motility and helicity. FRAP (Fluorescence Recovery After Photobleaching) observation showed MreB5 replacement is too slow to be linked to the handedness switching. To discuss the role of interaction between MreB5 and MreB4, MreB4 labeling is underway.

P-081 Visualization of MreB4 and MreB5 Filaments Driving *Spiroplasma* Swimming by Using JCVI-Syn3B and Electron Microscopy

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Spiroplasma eriocheiris swims in viscos liquid by a helicity switching mechanism. This swimming motility was reconstituted in a synthetic bacterium JCVI-Syn3B (syn3B) by expressing two distinct classes of bacterial actin proteins of *Spiroplasma*, MreB4 and MreB5 (Kiyama H et al., Sci Adv 2022). In the present study, using electron and fluorescence microscopy, we visualized filamentous structures in syn3B cells expressing MreB4 and MreB5, in which MreB5 was labeled by mCherry for identification. Negative staining electron microscopy of detergent treated and/or chemically fixed cells showed distinct alignments of filamentous structures. One was longitudinal to the cell axis and the other was perpendicular. The longitudinal one was applied to two-dimensional image averaging. The subunit repeat was estimated as 4.8 nm, which was shorter than that of the isolated MreB5 filaments, 5.2 nm. Fluorescence microscopy suggested that these alignments are not directly involved in cell movements. We succeeded to observe filamentous structures also without chemical fixation using cells with double mutations of MreB5, T109A/I129T. Next, we visualized syn3B cells capable for swimming by cryo-electron tomography. Two-dimensional averaging showed a 4.7 nm subunit repeat, consistent with that of a chemically fixed filamentous structure. We will discuss results by further structural analyses of these filamentous structures in the presentation.

Category 8. Host-Pathogen Interaction (Virulence & Pathogenesis)

P 082 An atypical F-like ATPase is critical to the function of the antibody cleavage system MIB-MIP

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Background – Mycoplasmas can cause chronic infections suggesting that they are able to evade their host's immune system. We have previously characterized a system called MIB-MIP, used by most animal-pathogenic mycoplasmas to selectively capture and cleave antibodies. A set of 7 genes encoding an atypical F_1-like X0 ATPase is genetically associated with MIB-MIP. This co-occurrence and conservation suggest that this ATPase could be involved in the antibody-cleavage process. **Methods** – To investigate this hypothesis, we generated a set of mutant strains of *Mycoplasma mycoides* subsp. *capri* GM12. We either performed a clean deletion of the F_1-like X0 ATPase loci without affecting the MIB-MIP part of the operon, or performed a single amino-acid substitution by replacing the putatively catalytic Lysine 152 of the Walker A domain of the beta-subunit. Mutants were validated through genome sequencing and mass spectrometry. We assessed the ability of the mutants to capture and cleave immunoglobulins. **Results** – We showed that the mutations introduced in the F_1-like X0 ATPase locus had no significant effect on the global proteome of the bacteria and that the MIB-MIP system was still expressed in similar amounts compared to the wild-type. However, a functional comparative study showed that the both the ΔF_1-like X0 ATPase and K152A mutants were no longer able to process antibodies. **Conclusion** – Our data confirm the involvement of the atypical F_1-like X0 ATPase in the MIB-MIP system and demonstrate that ATP binding is an essential step of the process. However, the exact function of the ATPase still remains unknown.

P-083 Novel In Planta Interaction of '*Candidatus* Phytoplasma solani' SAP11-like and SAP54-like Effectors With Plant Transcription Factors

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Phytoplasmas are obligate intracellular pathogens that significantly modulate development and behavior of their hosts by secreting effector proteins. Characterization of effectors and their host-cell targets was performed for only a few '*Candidatus* Phytoplasma' species so far. For '*Ca.*P. asteris' AY-WB strain it was shown that SAP11 alters host immune response by destabilizing TCP transcription factors in infected plants while SAP54 affects cellular processes crucial for plant development by degrading Type II MADS-domain transcription factors (MTFs). Our recent sequencing and comparative genome analyses of '*Ca.*P. solani' revealed the presence of SAP11-like and SAP54-like effectors. In order to investigate potential interactions of '*Ca.*P.

solani' SAP11-like effector with TCP2 and TCP4 (regulators of petal greening), and SAP54-like with MTFs AP1 and SEP3 (regulators of floral meristem identity and development) in planta bimolecular fluorescence complementation (BIFC) assay was conducted in agroinfiltrated *Nicotiana benthamiana* leaves and protein interactions were detected by confocal microscopy. Fluorescence signal of SAP11-like effector and TCP4 interaction was detected in cell cytoplasm, while interaction of SAP11-like and TCP2 fluoresced both in nuclei and cytoplasm of agroinfiltrated leaf epidermal cells. SAP54-like effector interacted with AP1 and SEP3 fluorescing predominantly in nuclei and cytoplasm of stomata. To the best of our knowledge, this is the first characterization of '*Ca.*P. solani' effectors interaction that indicates possible role in modulation of plant transcription factors, thus influencing plant development and appearance of symptoms in phytoplasma-infected plants.

P-084 To Be or Not to Be! Complex Non-stochastic Dynamics of Variable Surface Lipoprotein Expression and Selection in *Mycoplasma agalactiae* in vivo

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Mycoplasmas possess large multigene families encoding antigenic phase variants whose exact functions in pathogenicity are still under scrutiny. *Mycoplasma agalactiae*(MA) wild-type strain PG2 possessing a phase-variable family of six lipoproteins (Vpma-U, -Y, -W, - X, -Y, and -Z), and its corresponding Vpma-'Phase-Locked-Mutants' (PLMs), each expressing a single stable Vpma, were used for intramammary sheep-infections. RT-qPCR was performed to assess the Vpma variants present in the infected right udder-halves upon necropsy at D15-18 pi and compared to the starting inoculum for each of the 7 infection groups. Immunohistochemistry of udders and colony-immunoblots of MA reisolates were also made to verify their Vpma profiles. Interestingly, in all animals the starting Vpma variant (inoculum) had completely disappeared or reduced to ≤4% and replaced by completely new Vpmas. This contrasted with the invariable expression of a single stable Vpma in PLMs for several generations in vitro. Although there was no particular inter-group pattern of Vpma selection, all three sheep infected with the same PLM exhibited a similar preference for 'switch-over' Vpmas. Enrichment of VpmaW in all 6 different PLM-infection groups clearly point to its significance. Predominantly single 'switch-over' Vpma phenotypes were observed via colony-immunoblots where MA could invade into the uninoculated left udder-halves. The study reiterates that Vpma antigenic variation is imperative to the survival and spread of MA in an immunocompetent host. Moreover, similar selection of specific Vpma variants in multiple sheep implicate differential pathogenicity potential and complex non-stochastic dynamics of Vpma expression during infection.

P-085 Characterisation of the Transcriptional Response of the Tracheal Mucosa

of Chickens to Chronic *Mycoplasma synoviae* Infection

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Background: *Mycoplasma synoviae* (MS) causes infectious synovitis and respiratory tract disease in chickens, resulting in significant economic losses in the commercial poultry industry. Attachment to and colonisation of the trachea is vital for the progression and persistence of this disease. Respiratory tract infection is usually sub-clinical, but concurrent infection with the coronavirus infectious bronchitis virus (IBV) enhances the pathological effects of MS. This study aimed to explore the responses of the tracheal mucosa to chronic infection with MS, using a MS-IBV co-infection model. Methods: The transcriptional profiles of the tracheal mucosa were assessed 2 weeks after challenge in MS-IBV co-infected, MS infected, IBV infected and uninfected chickens using RNA sequencing. Results: In MS-IBV co-infected chickens, 827 genes were differentially expressed compared to uninfected chickens. Of these, 621 genes were upregulated, and 206 were downregulated. Upregulated genes and their functional categories were suggestive of uncontrolled and non-specific lymphoproliferation and an ongoing pro-inflammatory response. In particular, T cell proliferation and T-cell receptor signalling were upregulated. Genes associated with anti-inflammatory effects, pathogen removal, apoptosis, regulation of the immune response, airway homeostasis, cell adhesion and tissue regeneration were downregulated, but transcriptional changes associated with ciliary impairment were not evident. In contrast, only 1 gene was differentially expressed in MS infected chickens and only 8 genes in IBV infected chickens, compared to uninfected chickens. Conclusion: The transcriptional changes in the tracheal mucosa, 2 weeks after challenge with MS-IBV, indicate immune dysregulation, robust inflammation and lack of cytotoxic damage during chronic infection.

P-086 NOD2 activated by Duf16 in *Mycoplasma pneumoniae*

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Mycoplasma pneumoniae (Mp) is a smallest self-replicated prokaryotic microorganism lacks of cell wall. Mp is one of main pathogen responsible for community-acquired pneumonia (CAP) worldwide. Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) is an intracellular pattern recognition receptor that interacts with muramyl dipeptide (MDP) to recognize bacterial peptidoglycans and initiate innate immune responses. From our previous results we showed that NOD2 can be activated by *Mycoplasma ovipneumoniae*. Therefore, we hypothesis that mycoplasma might

contain a specific protein, which could interaction with NOD2. In this study, we used pull-down, MS Western blotting, immunofluorescence, RT-PCR, Co-IP analysis to identify the specific protein interaction with NOD2. We found out that Mp infection significantly increased the expression of NOD2 in RAW264.7 cells. The LRR domain of NOD2 can recognize ligands of bacteria. We purified LRR domain of NOD2 to screen Mp interaction protein by pull-down experiment. MS analysis found 12 Mp candidate proteins. Based on the bioinformatic analysis, P40/P90 adhesin, DUF16 protein, 30S-S17 protein, and P1 protein from Mp might interact NOD2. We construct the of eukaryotic expression vectors of P40/P90, DUF16, 30S-S17, and P1, then transfected into RAW264.7, it was found that the DUF16 transfection significantly increased the expression of NOD2 in RAW264.7 cells. Further pull-down and Co-IP experiments proved that NOD2 can interact with DUF16 protein. In this research, we found a novel NOD2 interaction protein in MP, expand the recognition mechanism of intracellular recognition receptor NOD2.

P-087 Genome analysis of '*Candidatus* Phytoplasma pruni' strain PR2021 associated with poinsettia

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Branching performance of ornamental plants is a crucial aspect in cutting production and marketing value. Previous study demonstrated that Poinsettia Branch-Inducing (POIBI) phytoplasmas are a key factor that promotes branching on poinsettia. Therefore, infecting poinsettia with POIBI phytoplasmas has become a common practice in commercial production. However, the mechanisms that POIBI phytoplasmas use to induce branching remains unknown. In this study, we performed whole genome sequencing of a POIBI phytoplasma from the poinsettia cultivar 'Princettia ROSA' to investigate the candidate genes. Based on sequence analysis, this strain PR2021 was classified as '*Candidatus* Phytoplasma pruni', and provides the first complete genome sequence for this species-level taxon. Examination of gene content revealed that PR2021 has two genes that encode SAP11 homolog, which have been experimentally demonstrated as effectors that induce branch proliferation in other phytoplasmas, thus likely explains the phenotype of POIBI phytoplasmas. To further investigate the diversity of POIBI phytoplasmas, we evaluated the branching phenotype among 10 poinsettia cultivars. Based on the phenotyping results, 6 cultivars that likely harbor POIBI phytoplasmas with strong or weak abilities for inducing branching were selected for whole genome shotgun sequencing. Upon the completion of genome assembly of these additional POIBI phytoplasma strains, we plan to conduct comparative analysis among different strains to identify the genetic variations that may explain the phenotypic variations. In addition to providing a basic understanding of POIBI phytoplasma biology, the knowledge produced in this work may help future poinsettia breeding programs for selecting POIBI phytoplasma strains with desired branch inducing ability.

P-088 Immune Profiling of Experimental Murine Mastitis Reveals Conserved

Response to Mammary Pathogenic *Escherichia coli*, *Mycoplasma bovis*, and *Streptococcus uberis*

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Background: Mastitis is an important disease in dairy animals that causes multibillion-dollar economic losses. The disease is caused by ascending bacterial infection through the teat canal and the most common mastitis-causing bacteria are Gram-negative coliforms, Gram-positive streptococci and staphylococci, and mycoplasmas. While field research of mastitis contributed a lot to the development of control strategies, little progress was made toward understanding the molecular mechanisms underlying the pathogenesis of mastitis. **Objectives**: To characterize immune response of mammary glands challenged with *Escherichia coli*, *Streptococcus uberis*, and *Mycoplasma bovis*. **Methods**: Using multiplex gene expression technology (Nanostring nCounter), multi-omics analysis of the normal and infected mammary glands was performed. **Results**: Transcriptomic analysis of over 700 immune genes revealed a core of 100 genes that are similarly regulated and functionally or physically interacting in *E. coli*, *M. bovis*, and *Strep. uberis* murine mastitis. Common significantly enriched gene sets include TNFα signaling via NF-κB, Interferon gamma and alpha responses, and IL6-JAK-STAT3 signaling. In addition, expression of genes, associated with neutrophil extracellular traps (NETs), was significantly enriched in glands challenged by the three pathogens. Moreover, ligand-receptor analysis revealed interactions shared by the three pathogens, including the interactions of the cytokines IL1β, IL1α, and TNFα with their receptors, and proteins involved in immune cell recruitment such as complement C3 and ICAM1 (with CD11b), chemokines CCL3 and CCL4 (with CCR1), and CSF3 (with CSF3R). **Conclusions**: Mammary infection with *M. bovis*, *E. coli*, and *Strep uberis* resulted in activation of conserved core of immune genes and pathways including NET formation.

P-089 A feasibility study on cell growth inhibition of *Ureaplasma* species with *Lactobacillus acidophilus*

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Ureaplasma species are one of the causative organisms of several disorders, including infertility, nongonococcal urethritis, miscarriage, and adverse birth outcomes. This

study examined the inhibition of *Ureaplasma* species. bacterial growth by *Lactobacillus acidophilus* WB2001. Co-incubation of *Ureaplasma* species with *L. acidophilus* WB2001 significantly inhibited cell growth in 10B medium. Moreover, the addition of *L. acidophilus* WB2001 supernatant to 10B medium nearly completely inhibited *Ureaplasma* species cell growth. In the culture supernatant produced by *L. acidophilus* WB2001, results demonstrated that the products of lactic acid and hydrogen peroxide in it inhibited the growth of *Ureaplasma* species. In contrast, the growth of *L. acidophilus* WB2001 was unaffected by *Ureaplasma* cells or their culture supernatant. In addition, in vitro co-infection of HeLa cells with *L. acidophilus* WB2001 inhibited the internalization of *Ureaplasma parvum*. These findings may suggest a novel prophylactic approach to prevent *Ureaplasma* species infections and the subsequent complications during pregnancy.
Keywords: *Ureaplasma* species, *L. acidophilus* WB2001, 10B medium, lactic acid.

P-090 *Mycoplasma hyopneumoniae* membrane protein Mhp271 interacts with host UPR protein GRP78 to facilitate infection

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The unfolded protein response (UPR) plays a crucial role in *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) pathogenesis. We previously demonstrated that *M. hyopneumoniae* interferes with the host UPR to foster bacterial adhesion and infection. However, the underlying molecular mechanism of this UPR modulation is unclear. Here, we report that *M. hyopneumoniae* membrane protein Mhp271 interacts with host GRP78, a master regulator of UPR localized to the porcine tracheal epithelial cells (PTECs) surface. The interaction of Mhp271 with GRP78 reduces the porcine beta-defensin 2 (PBD-2) production, thereby facilitating *M. hyopneumoniae* adherence and infection. Furthermore, the R1-2 repeat region of Mhp271 is crucial for GRP78 binding and the regulation of PBD-2 expression. Intriguingly, a coimmunoprecipitation (Co-IP) assay and molecular docking prediction indicated that the ATP- rather than the substrate-binding domain of GRP78 is targeted by Mhp271 R1-2. Overall, our findings identify host GRP78 as a target for *M. hyopneumoniae* Mhp271 modulating the host UPR to facilitate *M. hyopneumoniae* adherence and infection.

P-091 Hijacking of Host Plasminogen by *Mesomycoplasma* (*Mycoplasma*) *hyopneumoniae* via GAPDH: A Systematic Virulence Mechanism to Promote Adhesion and Extracellular Matrix Degradation

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(i) Background *Mesomycoplasma hyopneumoniae* is the etiological agent of mycoplasmal pneumonia of swine, which causes substantial economic losses to the world's swine industry. Moonlighting proteins are increasingly shown to play a role in the pathogenic process of *M. hyopneumoniae*. In this study, we explored the mechanism by which Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key enzyme in glycolysis exerts its function. (ii) Methods Flow cytometry and colony blotting analysis were used to explore the surface exposure of GAPDH. Fluorescent microsphere analysis and qPCR were used to evaluate the adhesion function. The interaction between GAPDH and plasminogen was evaluated by surface plasmon resonance. The key site for GAPDH binding to plasminogen was demonstrated by amino acid mutation. The interaction between *M. hyopneumoniae* GAPDH and extracellular matrix (ECM) was analyzed by scanning electron microscopy and comparative proteomics. (iii) Results GAPDH displayed higher abundance in a highly virulent strain compared to an attenuated strain and was partly displayed on the surface of *M. hyopneumoniae*. rGAPDH was able to bind PK15 cells, while the adherence was significantly blocked by anti-rGAPDH antibody. In addition, rGAPDH could interact with plasminogen. The interaction could further shift plasminogen to active plasmin to degrade ECM, and the major affected component was collagen. The affinity of plasminogen to the rGAPDH C terminal mutant (K336A) was significantly decreased. (iv) Conclusion GAPDH might be an important virulence factor that facilitates adhesion and dissemination of *M. hyopneumoniae* by hijacking host plasminogen to break through the tissue ECM barrier.

P-092 Abstract withdrawal

P-093 Novel Mycoplasma Nucleomodulin MbovP475 Decreased Cell Viability by Regulating Expression of CRYAB and MCF2L2

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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. *Mycoplasma bovis* is a major veterinary pathogen that secretes several potential virulence factors. The aim of this study was to determine whether any of their secreted proteins might function as nucleomodulins. After an initial in silico screening, the nuclear localization of the secreted putative lipoprotein MbovP475 of *M. bovis* was demonstrated in bovine macrophage cell line (BoMac) experimentally infected with *M. bovis*. Through combined application of ChIP-seq, Electrophoretic mobility shift assay (EMSA) and surface plasmon resonance (SPR) analysis, MbovP475 was determined to bind the promoter regions of the cell cycle central regulatory genes CRYAB and MCF2L2.

MbovP475 has similar secondary structures with the transcription activator-like effectors (TALEs). Screening of various mutants affecting the potential DNA binding sites indicated that the residues 242NI243 within MbovP475 loop region of the helix-loop-helix domain were essential to its DNA binding activity, thereby contributing to decrease in BoMac cell viability. In conclusion, this is the first report to confirm *M. bovis* secretes a conserved TALE-like nucleomodulin that binds the promoters of CRYAB and MCF2L2 genes, and subsequently down-regulates their expression and decreases BoMac cell viability. Therefore, this study offers a new understanding of mycoplasma pathogenesis.

Category 9. Mycoplasma Engineering & New Tools

P-094 New transformation method for JCVI-syn3B cells

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JCVI-syn3B can be used to study protein functions derived from Mollicutes and others. For protein expression in JCVI-syn3B, the plasmid harboring the target gene is needed to be constructed in *E. coli*. However, the plasmid construction sometimes causes problems such as unpredictable recombination and poor *E. coli* growth due to gene sequences and toxicity of its product. In this study, we succeeded to transform target genes without construction in *E. coli*. Target genes of *Mycoplasma mobile* and vectors harboring Cre/*loxP* system were assembled with “NEBuilder HiFi DNA assembly”. The assembled product was amplified by PCR and the resulting linear fragments were transformed into JCVI-syn3B cells. However, few transformants were obtained, probably due to the linear DNA form. Next, we tried the transformation of circular DNA. Since the products of “NEBuilder HiFi DNA assembly” is circular DNA, they were directly transformed into JCVI-syn3B cells without PCR amplification. Finally, 10-141 transformants were obtained from 450 µL of culture in which the target gene was correctly recombined into JCVI-Syn3B genome, in most cases. The inserted DNA fragments ranged 0.7 to 6.6 kbp, and two fragments could be assembled simultaneously. This method can be useful for Mollicutes genome manipulation.

P-095 Improved Transformation Efficiency in *Mycoplasma hominis* Allows Disruption of the MIB-MIP System Targeting Human Immunoglobulins

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The pathogenicity of *Mycoplasma hominis* (Mho) is poorly understood, mainly due to the absence of efficient genetic tools. A polyethylene glycol-mediated transformation

protocol for the Mho M132 reference strain was recently developed using the pMT85-Tet plasmid. However, the transformation efficiency (TE) measured was low (10-9 transformants/UFC/µg), limiting our ability to generate a large mutant library. In this study, we improved the TE through the design of Mho-specific pMT85 derivates. Using the Gibson assembly method, we first replaced the streptococcus-derived *tet(M)* gene of the original pMT85-Tet plasmid by that of an Mho clinical isolate. Then, the spiroplasma-derived *spiralin* gene promoter driving the *tet(M)* gene expression was substituted by one of three different regulatory regions (RRs): the Mho arginine deiminase RR, the Mho elongation factor *tufA* RR, or the 68-bp SynMyco synthetic RR. Only the SynMyco-based construction led to a 100-fold increase of the TE using the Mho M132 strain. Using this construction, we could also transform the PG21 reference strain and three other clinical strains. The precise transposon position was determined for 128 M132-transformants. Although the transposons were likely randomly inserted, the targeted-CDSs mostly encoded lipoproteins, proteins involved in DNA repair or in gene transfer. Interestingly, one transposon integration site was located in the *Mycoplasma* Immunoglobulin Protease gene. Phenotypical characterization of the mutant showed a complete disruption of the human antibody-cleavage ability of the Mho transformant. Overall, the development of an Mho-optimized plasmid significantly improves our ability to generate random transposon insertion libraries in Mho, to further understand Mho pathogenicity.

P-096 Adaptive laboratory evolution of JCVI-syn3.0B to low temperature

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JCVI-syn3.0B is an artificial minimal genome bacterium derived from *Mycoplasma mycoides*. Recently, JCVI-syn3.0B has been used as a recipient to investigate functions of introduced genes, such as *Spiroplasma* swimming and *Ureaplasma* adhesion. In order to better reproduce the function of the introduced proteins, the growth temperature of transformed JCVI-syn3.0B should be similar to that of original bacterium. In the present study, we performed adaptive laboratory evolution of JCVI-syn3.0B to lower temperatures. First, we measured the growth of JCVI-syn3.0B in SP4 medium at the optimal temperature 37°C and also at lower temperatures. JCVI-syn3.0B grew 3–4 times slower at 30°C than 37°C, and no significant growth was observed at 27°C. Next, we continuously cultivated JCVI-syn3.0B at 30°C and measured the growth. The strains passaged 20 times grew twice as fast as the original strain. Interestingly, the 20-passaged strains could also be cultured at 25°C. To understand the adaptation mechanism, we sequenced genomes of four independent lineages of the 30°C-optimized strains and compared them with the original genome. The 30°C-optimized strains accumulated several mutations in their genomes, which included amino acid substitutions in elongation factor, RNA polymerase subunit beta, and CTP synthase. These mutations are candidates for contributing to the growth at lower temperature.

P-097 Reprogramming the synthetic cell JCVI-Syn3B for the production of active ingredients of interest to bio-based industry

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JCVI-Syn3B is a minimal cell that encodes the essential genes of *Mycoplasma mycoides* subspecies *capri*. Its genome has less than 500 genes, the majority of which are essential. Besides serving as a research platform to understand the universal principles of life, the cell encoded by minimal genome could be used as a safe and efficient “cell factory” for producing a wide variety of compounds. In searches for hemicellulose degrading enzymes, BGL11 a multifunctional enzyme with high β-xylosidase activity, was identified in a goat rumen metagenomic library screening. The capacity of BGL11 to hydrolyze multiple substrates may be a useful characteristic for industrial application. Although recombinant BGL11 showed activity towards different substrates, the enzyme was most effective in degrading xylobiose. Here we show that JCVI-Syn3B was capable of expressing and secreting active BGL11 (80 kDa). We cloned a synthetic BGL11 gene that was codon optimized for mycoplasma expression into the plasmid Pmod2-loxpurolox-sp-cre. The BGL11 gene also contained a signal peptide sequence for protein secretion. JCVI-Syn3B cells were transformed with the Pmod2-loxpurolox-sp-cre-BGL11 plasmid and the presence of BGL11 was confirmed by PCR. BGL11 activity in the SP4 growth media was measured using nitrophenyl-beta-D-glucopyranoside (pNPG) as substrate. The amount of pNP released was measured by reading absorbance at 405 nm. The absorbance of the samples was compared to a standard curve prepared with pNP and the activity of BGL11 produced by JCVI-Syn3B∴BGL11 clones was confirmed. This result shows the ability of the minimal cell to produce a functional enzyme of bio-based industry interest.

P-098 A proposal to use ciliate *Paramecium* as a natural host model

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Mycoplasma is widely isolated from humans, animals, and plants, causing disease and economic problems in humans, industrial animals and plants. Mycoplasmas are highly host-specific, and it is believed that each *Mycoplasma* species establishes infection among specific host. However, how mycoplasmas are maintained in the natural environment is largely unknown. The (Japanese) National BioResource Project (NBRP) is a national institution that collects, preserves, and provides bioresources that serve as the basis and foundation for life science research, and maintains many resources including animals, plants, and microorganisms. Among them, we maintain a collection of 1000 strains (24 species) of ciliate *Paramecium* consisting of various morphologically indistinguishable mating types and sibling groups (syngens).

Paramecium has been used in various studies as a model material for eukaryotic cells and symbiosis with microorganisms. We have proposed the use of *Paramecium* as a natural host model for pathogenic bacteria, and indeed it has been used in studies as a natural host model for the *Francisella* and *Legionella* species. In this presentation, we propose the use of *Paramecium* as a natural host for *Mycoplasma* species and will introduce the handling and availability of *Paramecium* species.

P-099 Improved efficiency of mycoplasma genome transplantation by DNA repair

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The genome transplantation method published in 2007 is the only method to exchange genomes in vitro. However, because genome transplantation requires a large amount of genomic DNA, genomic DNA preparation for genome transplantation is required almost every time. Therefore, there is a need for an efficient genome transplantation method that can reduce the amount of genomic DNA used. On the other hand, the RCR method developed by Su'etsugu et al. allows exponential amplification of circular DNA in vitro. This method uses several DNA repair enzymes in addition to DNA polymerase to amplify a large amount of supercoiled DNA. In this study, we hypothesized that genomic DNA prepared for genome transplantation contains many nicks and cleavages. Therefore, we expected that treating genomic DNA used for genome transplantation with these repair enzymes would repair the genomic DNA and improve the efficiency of genome transplantation. The experimental results showed that genomic DNA treated with DNA repair enzymes significantly improved the efficiency of genome transplantation. In particular, we were able to achieve the same results as before even when the amount of genomic DNA was reduced to one-twentieth. This result enables efficient genome transplantation even with a small amount of genomic DNA.

Category 10. Omics Studies

P-100 Remarkable Abundance of Potential Mobile Group II Introns in genome of PWB Phytoplasma Strain PR34

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'*Candidatus* Phytoplasma' have undergone reductive evolution, losing non-essential genes but still retained highly repetitive regions, sequence-variable mosaics, and potential mobile units in their genomes, facilitating genome rearrangements. Parthenium phyllody phytoplasma strain PR34, endemic to Indian subcontinent, was

genome sequenced, revealing randomly distributed high copy number full-length and truncated group II introns. Group II introns are mobile genetic elements containing self-splicing RNAs and intron-encoded protein (IEP) capable of splicing themselves and retrohoming into an intron less allele. The genome of PR34 contained 22 homologs of IEPs corresponding to full length (6), truncated (7), and pseudogenes (9), harbouring RT, X, and En domains, suggesting they are potentially functional and mobile. Phylogenetic analysis revealed IEPs of phytoplasma were similar to those found in *Clostridium difficile* and *Lactococcus lactis* than those in *Acholeplasma* or *Anaeroplasma*. The multiple copies of IEP homologs are sporadically present in *Acholeplasma*, one species of *Anaeroplasma* and *Haloplasma*, and entirely absent in Entomoplasmatales. The *ltrA* gene, encoding the IEP in phytoplasma, has a high G+C content than the rest of the genome, possibly acquired through horizontal transfer. All group II introns in strain PR34 are classified as RNA class IIA1 and IEP class ML, except for one suggesting a horizontal gene transfer event may have occurred between mitochondria and phytoplasma during evolution. The full-length group II intron is rare in the class Mollicutes, but are present in all 16Sr group II phytoplasma genomes. Presence of Group II introns have significantly altered the genomic architecture of PWB phytoplasma.

P-101 Genome analyses of '*Candidatus* Phytoplasma asteris' strains highlight heterogenicity and regularities

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Genome research is of particular importance in phytoplasma research, as it provides insights ranging from metabolism to pathogen-host interaction. The first complete phytoplasma genome was obtained for '*Candidatus* Phytoplasma asteris'. This was followed by another six complete genomes, which form the most comprehensive phytoplasma database today. In Germany, '*Ca.* P. asteris', associated with carrot reddening, occurs frequently, but little information on any associated strains is currently available. Complete genomes of the M8 and M33 strains were determined by applying SMRT and paired-end Illumina sequencing. After taxonomic binning, Canu hybrid assembly enabled the complete de novo reconstruction of circular chromosomes (773 kb and 657 kb) and plasmids (5.6 and 5 kb). RAST-annotated genomes were manually curated, completeness was supported by BUSCO analysis and the CDS underwent comparative analysis against complete asteris genomes. Results for M8 and M33 strains highlight their assignment to two different phylogenetic branches present in '*Ca.* P. asteris'. The splitting of the taxon into two clades is supported by conserved gene synteny, ANI and single-gene analyses. Furthermore, genetic relatedness is not reflected by the geographical origin of strains, thereby suggesting a seed transmission impact. Apart from resulting taxonomical questions relating to the two clades, we re-analysed the encoded genetic repertoire of '*Ca.* P. asteris' genomes. Despite the typical limited functional gene set, characterised by reductive evolution, our analysis highlighted a stable environment of transport and metabolic pathways separated from the coding of effector proteins involved in virulence and paralog genes resulting in genome size differences.

P-102 Impact of Potential Mobile Units on Genome Stability in Phytoplasma Evolution

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Phytoplasmas are uncultivated bacteria that impact a wide range of crops. Similar to other obligate symbionts, phytoplasmas have small genome sizes and lack certain genes necessary for an independent life. Intriguingly, phytoplasmas differ from most other obligate symbionts by harboring large mobile genetic elements known as potential mobile units (PMUs). To obtain a more comprehensive view of PMUs, we examined 20 complete genome sequences available from 11 '*Candidatus* Phytoplasma' species for in-depth analysis. The results revealed that the number of PMU-associated genes ranges from 13 to 118 per genome. Moreover, 82 intact PMUs were identified, which can be classified into three major types based on the order of core genes as established in our previous studies. Further examination indicated that the three types of PMUs exhibit different phylogenetic distributions, with type A PMUs being widely distributed, while types B and C mostly restricted to specific lineages. Interestingly, while most phytoplasma lineages have unstable chromosomal organization as indicated by GC-skew patterns and orientation of coding sequences, '*Ca*. P. aurantifolia' and an unnamed species-level taxon appear to maintain stable chromosomal organization as *Acholeplasma*. This new finding indicates that while PMUs may promote genome instability as previously hypothesized, such impact is not universal across all phytoplasma lineages. Future studies that improve the taxon sampling of complete genome sequences, as well as detailed investigation of the effector genes associated with PMUs, are needed to better understand the evolution and functions of these pathogens.
Keywords: phytoplasma, genome, molecular evolution, mobile genetic elements

Category 11. Phylogeny & Taxonomy

P-103 Phylogenetic Comparison of *Mycoplasma gallisepticum* Sequenced Genomes for Relatedness to Commercial Avirulent Vaccine Strains

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The use of live avirulent *Mycoplasma gallisepticum* (MG) strains as vaccines has proven effective in controlling the pathology associated with virulent MG infection. However, over time virulent isolates identified by specific genetic markers as being identical to vaccines strains have been found, suggesting that attenuated vaccine strains could revert to virulent phenotypes. In this study, the genomic sequences of MG vaccine strains including F, 6/85, ts-11, other MG isolates, and field isolates identified as either being 6/85 or ts-11-like were compared. Phylogenetic trees were constructed

to show their evolutionary relationship using both the complete genome sequence and selected genomic regions. The results demonstrate that the virulent 6/85-like strains were generally closely related to the 6/85 vaccine strain and very closely related to each other. However, the phylograms suggest that the 6/85 vaccine strain and virulent strains come from a common ancestor rather than the 6/85 vaccine strain being the ancestor. Analysis of the ts-11 vaccine strain and the draft genomes of ts-11-like strains appear to show a high degree of similarity between the strains. Select region sequence analysis of the ts-11-like strains show them to be identical to ts-11, suggesting that the ts-11-like strains originated from the vaccine strain. Comparable results were obtained for the known related MG genomes such as F99 and its commercial vaccines. This demonstrates that the 6/85-like field strains are not revertants of the vaccine strain, but suggests that the sequenced ts-11-like field strains are vaccine strain revertants.

P-104 New *Mycoplasma* Isolated from Portuguese Man o' War

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Background- There are no previous records of colonization by Mollicutes in Portuguese man o' war (*Physalia physalis*) (Hydrozoa: Physaliidae), an ocean aquatic invertebrate. Methods- Specimens of Portuguese man o' war were studied for the presence of mollicutes. Two batches were homogenated and 1 ml was inoculated in liquid SP4-II medium with 3% NaCl and incubated at 25ºC for 24 h and filtered and incubated again. DNA was extracted from the resultant bacterial growth one week later. Samples were Mollicutes real time PCR positive. Partial 16S rRNA sequence was amplified and Sanger sequenced. Genomic DNA was extracted and subjected to short read (Illumina) and long read sequencing (Oxford Nanopore) prior to Unicycler (v0.4.9) assembly and analysis by Kraken, Resfinder, Prokka annotation, Mauve and Blast were performed. Results-. All samples grew in broth medium and were positive for mollicutes. Amplicon sequence from 16S rRNA PCR corresponded to *Mycoplasma* sp. A single closed genome was identified from each of the two samples that were nearly identical (860,889 bp and 861,079 bp; 48 single nucleotide polymorphisms excluding the additional 190 nucleotides) with 27.6% GC content. Kraken analysis mapped 92.6% of the reads as “unclassified” with 3.58% mapping to Mollicutes and 2.65% to *Mycoplasma* (closest homology to *M. mobile, M. pulmonis*, and *M. crocodyli* at 0.18% read identities each). A single 16S rRNA sequence was identified in the sealed genome by Prokka with the closest homology (87.5%) to *Mycoplasma todarodis* isolated from common cephalopods. Conclusion- A new putative *Mycoplasma* species is presented.

Category 12. Plant & Insect Mollicutes

P-105 Molecular characterizations of *Raphanus sativus* L. witches’-broom disease and the genetic status of the ‘*Candidatus* Phytoplasma aurantifolia’

strains (16SrII-V) in Yulin, Taiwan

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Daikon is an edible root vegetable, as well as a traditional medicinal plant belonging to the Brassicaceae family. In the summer of 2021, commercial production of daikon in Yunlin County was damaged by an undocumented disease in Taiwan. Symptoms consisted of early-bolting, witches'-broom, phyllody, virescence, and root growth retardation significantly affected the yield and economic value of daikon. Observed under the transmission electron microscope are pleomorphic phytoplasma-like bodies in the sieve elements of the symptomatic daikon. The 16S rRNA-based phylogenetic analysis and the iPhyClassifier-based virtual RFLP study demonstrated that the causal agent associated with the diseased daikon is a '*Candidatus* Phytoplasma aurantifolia' strain belonging to the 16SrII-V subgroup. The disease symptoms such as phyllody, virescence, and witches'-broom observed in diseased daikon can be explained by the identification of PHYL1 and SAP11 effectors, which are identical to those of peanut witches' broom phytoplasma. Further classifications of *tufB*, *rluA*, *degV*, *dnaD* and *Imp* revealed that the phytoplasma identified in this study presents a very close phylogenetic relationship with other 16SrII-V subgroup phytoplasma strains associated with peanut, mungbean, soybean, Ixeris chinensis, *Desmodium triflorum*, *Emilia sonchifolia*, *Nicotiana plumbaginifolia Viv*., *Digera muricata* L., *Parthenium hysterophorus* L., *Scaevola taccada*, *Celosia argentea* L., and *Eclipta prostrata* in Yunlin, Taiwan. These results not only present the local status of the widespread occurrence of the 16SrII-V subgroup phytoplasma strains, but also indicate that phytoplasma diseases have become a threat to agriculture in Yulin, Taiwan, and strategies are required to prevent the spread of the emerging bacterial disease.

P-106 Undetectable nature of phytoplasma in Cassava witches' broom diseased leaf tissues by conventional methods

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Cassava Witches' Broom Disease (CWBD) has spread to major cassava producing areas, and it threatens the livelihood of cassava farmers in the Philippines. CWBD is believed to be caused by a pathogenic phytoplasma (*Candidatus* Phytoplasma sp.), *Ca*. P. luffae in the Philippines, but there is still no evidence regarding vector transmission and proof of etiology by Koch's postulate. Verifying the causal agent may be the key in optimizing an effective management strategy. However, another serious

problem has arisen recently: although we attempted to identify CWB's pathogen using conventional nested PCR, real-time PCR, and LAMP targeting phytoplasma, no positive detection was revealed in most cassava plant tissues showing typical CWBD symptoms. Amplification and sequencing of 16S rRNA, *secA*, and *groL* genes revealed that the presence of *Ca*. P. luffae in bitter gourd and luffa plants showing typical phytoplasma-associated symptoms, and the methods applied for cassava samples were not a problem. In addition, amplicon sequencing reveals the bacterial community composition in CWBD-affected cassava leaf tissues. Very low abundance and prevalence of phytoplasma was observed in cassava, whereas a high abundance in luffa and bitter gourd. Only one of six tested cassava microbiome samples generated phytoplasma reads (8.3% and 0.06% of bacterial and total reads, respectively). Overall, due to the undetectable nature of phytoplasma in CWBD-showing cassava in recent years, diagnosis and management strategies must be further developed based on thorough investigations on the unidentified pathogen.

P-107 A genomics framework to phytoplasma taxonomy: The 16SrII and 16SrXXV phytoplasmas as a case study

Bianca Rodrigues Jardim[1,2], Lucy T. T. Tran-Nguyen[3], Cherie Gambley[4], Ali M. Al-Subhi[5], Xavier Foissac[6], Hong Cai[7], Jun-Yi Yang[8,9], Richard Davis[10], Lynne Jones[10], Brendan Rodoni[1,2], Fiona E. Constable[1,2]
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Twenty-four 16SrII subgroups are classified based on restriction fragment length polymorphism of their 16S rRNA gene, and two species are described ('*Candidatus* Phytoplasma aurantifolia' and '*Ca*. Phytoplasma australasia'). In this study, the taxonomic boundaries between 42 phytoplasma strains classified into seven 16SrII subgroups, five 16SrII taxa with unassigned subgroup classifications, and one 16SrXXV-A phytoplasma strain was investigated using whole genome analyses. Based on phylogenomic analyses as well as whole genome average nucleotide and average amino acid identity (ANI and AAI), eight distinct 16SrII species were identified, six of which were novel descriptions. Strains within the same species had ANI and AAI values of >97%, with an alignment factor of >80% in the ANI analysis. Species also had distinct biological and/or ecological features. Members classified within the subgroups 16SrII-A, 16SrII-D, and 16SrII-V and the Sweet Potato Little Leaf phytoplasmas fulfilled criteria to be included as members of a single species, but with subspecies-level relationships with each other. The 16SrXXV-A taxon was described as a novel

phytoplasma species and, based on criteria used for other bacterial families, was also classified as a distinct genus from the 16SrII phytoplasmas. This study, therefore, indicates that novel phytoplasma genus names are needed, with the genus '*Ca*. Phytoplasma' being an inaccurate designation. Instead, the phytoplasmas should be encompassed in the family '*Ca*. Phytoplasmatales', which is yet to be formally designated. As more phytoplasma genome sequences become available, the classification system of these bacteria can be further refined at the genus, species, and subspecies taxonomic ranks.

P-108 A large-scale investigation into phytoplasma diversity in Australia using metagenomics

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Phytoplasmas are unculturable, phytopathogens that impact the yield of many crop plant species. In this study, phytoplasma detection based on PCR and Sanger sequencing was coupled with metagenomic sequencing to determine the diversity of phytoplasma taxa and associated diseases in Australian vegetable-growing regions. Metagenomic sequencing was performed on 195 phytoplasma-positive samples classified as crop (n=155), weed (n=24), ornamental (n=7), native plant (n=6), or insect (n=3) species. Of the 195 draft phytoplasma genomes, 178 met our genome quality criteria for comparison using Average Nucleotide Identity. Ten distinct species were identified and could be classified within phytoplasma groups already known to be present in Australia (16SrII, 16SrXII (PCR only), 16SrXXV, and 16SrXXXVIII), with the 16SrII group members most commonly detected (n=153). This study significantly improved our understanding of phytoplasmas in Australia and reported several phytoplasma geographic- and host-range expansions, with >20 plant species reported as new phytoplasma hosts in Australia. The metagenomic-based analyses also permitted the identification of five novel subspecies of '*Ca*. Phytoplasma australasiaticum' (syn. '*Ca*. Phytoplasma australasia'), one novel 16SrII phytoplasma species, and mixed infections. While 16S rRNA-based PCR and Sanger sequencing was suitable for triage, the metagenomic provided higher resolution phytoplasma identification, permitted the identification of mixed infections, and host family/genus/or species identifications when unknown based on visual inspection. In addition to improving our understanding of the phytoplasma taxa affecting crop production in Australia, the study also significantly expands the genomic sequence data available in

public sequence repositories, contributing to improving phytoplasma molecular epidemiology studies, taxonomic investigations, and diagnostics.

P-109 Towards understanding of a versatile pathogen – quest for putative effectors in '*Candidatus* Phytoplasma solani' genomes

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The rise of genomics technologies prompted new possibilities in finding potential effectors by which phytoplasmas successfully manipulate their hosts. '*Candidatus* P. solani' is a cosmopolitan pathogen with a wide host range, transmitted by different insect-vectors. In our previous study, sequencing of '*Ca.* P. solani' SA-1 strain identified 38 potential effectors. However, the within-species diversity remains unclear. In order to get better insight into the '*Ca.* P. solani' effector pool and diversity, we sequenced two *'Ca.* P. solani' strains (ST19 and STOL) by using Illumina MiSeq and ONT MinION platforms. De novo assembly was performed, followed by prediction and annotation of putative effector genes. Whole genome alignment and comparative analyses were also performed. For selected putative effectors, including homologues of SAP11 and SAP54, specific primers were designed. Thirty isolates from our lab collection of '*Ca*. P. solani' originating from different hosts was used in assessment of variability of selected effectors. Prediction of putative effector genes in the draft genomes with total size of 707,036 bp (ST19) and 656,141 bp (STOL) and comparative analyses revealed the presence of 22 and 20 putative effector genes, respectively, including SAP11-like and SAP54-like, as well as species- and strain-specific ones. Assessment of gene presence confirmed that SAP11-like is conserved among '*Ca*. P. solani' strains, while SAP54-like is more diverse. This study sets a base for functional studies of putative '*Ca.* P. solani' effectors and their interactions with host targets, which can facilitate deciphering the pathogenicity strategies of this successful pathogen.

P-110 Target degradation specificity of a phytoplasma effector protein phyllogen is regulated by an ability to recruit host proteasome shuttle protein

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A phytoplasma effector phyllogen causes phyllody in plants by inducing proteasomal degradation of host MADS-domain transcription factors (MTFs) that determine floral organ identities. For delivery of the MTFs to the proteasome, phyllogen directly interacts with the MTFs (MTF/phyllogen complex), then with a proteasomal shuttle protein RAD23 (MTF/phyllogen/RAD23 ternary complex). (Kitazawa et al., this conference). Since phyllogen selectively binds to specific MTFs and all the reported phyllogens lacking phyllody-inducing activity are defective in both MTF-binding affinity

and MTF degradation activity, it has been considered that the MTF-binding affinity determines the degradation specificity of phyllogen. However, the present study discovered another mechanism determining the degradation specificity through detailed analyses of the function of $PHYL_{RYD}$, a phyllogen of '*Candidatus* Phytoplasma oryzae' rice yellow dwarf (RYD) strain. In planta protein expression assay revealed that $PHYL_{RYD}$ could degrade only a narrower range of MTFs than other phyllogens and its phyllody-inducing activity was significantly attenuated. Interestingly, yeast two-hybrid and co-immunoprecipitation assays elucidated that $PHYL_{RYD}$ retained binding affinity to the MTFs that $PHYL_{RYD}$ could not efficiently degrade. However, $PHYL_{RYD}$ exhibited impaired RAD23-recruitment ability when binding to the non-degradable MTF, while it could efficiently recruit RAD23 when binding to the degradable MTF, indicating that the MTF degradation specificity of $PHYL_{RYD}$ is determined by the ability to form the MTF/phyllogen/RAD23 ternary complex. This study demonstrated that the target specificity of phyllogen is regulated by not only the MTF-binding affinity but also RAD23-recruitment ability of the MTF/phyllogen complex.

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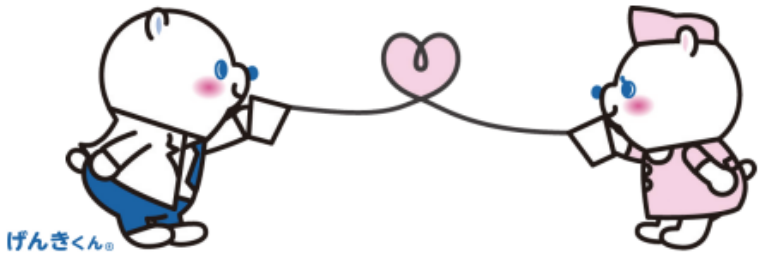

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