STAR-IDAZ
International Research
Consortium on Animal Health

USDA ars
Agricultural Research Service

Report of Gap Analysis Meeting for CBPP

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Sanger Institute, Hinxton, UK

Organised by USDA-ARS and STAR-IDAZ IRC

Workshop Report

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Wellcome Sanger Institute, near Cambridge, UK

Contents

Organising Bodies

This meeting was organised by the Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA), in collaboration with the STAR-IDAZ International Research Consortium on Animal Health (IRC).

The Agricultural Research Service, United States Department of Agriculture

The Agricultural Research Service (ARS) is the principal in-house research agency of the United States Department of Agriculture (USDA) and aims to extend the nation's scientific knowledge with research projects in agriculture, human nutrition, food safety, natural resources, and the environment. ARS supports more than 2,000 scientists organised into approximately 660 permanent research projects at over 90 locations across the country and five laboratories overseas. ARS conducts innovative research to find solutions to problems of high national priority that impact the American people daily. ARS often undertakes high-risk research endeavours to make significant breakthroughs in important problem areas, including biodefence initiatives to detect, prevent, and mitigate the impact of especially dangerous infectious diseases that pose a threat to animals and public health.

The STAR-IDAZ IRC

The STAR-IDAZ IRC is a global initiative aiming to coordinate research programmes at the international level and to contribute to the development of new and improved animal health strategies for priority diseases, infections, and issues. The partners, research funders and programme owners together form the Executive Committee, which is supported by a Scientific Committee of 16 experts and an EU-funded Secretariat (SIRCAH2 – Horizon Europe Grant Agreement Number 101082377 and UK Research and Innovation (UKRI) through the UK government's Horizon Europe funding guarantee grant numbers 10055666 and 10058793). Target deliverables of the STAR-IDAZ IRC include candidate vaccines, diagnostics, therapeutics, other animal health products and procedures, and key scientific information/ tools to support risk analysis and disease control. To achieve these goals, the IRC partners agree to coordinate and align their research programmes to address identified research needs relating to the priority topics and to share results. Research gaps identified by expert Working Groups are organised into research roadmaps for the development of candidate vaccines, diagnostics, therapeutics and disease control strategies, providing a structure to plot identified research gaps and focus future investment.

Organising Committee

The CBPP gap analysis meeting was organised by: Roxann Motroni (Chair of the Scientific Committee), Susan Noh, Eduardo Casas, Mark Ackerman, Georgina Grell, Madeline Newman.

Scientific Committee

USDA ARS and STAR-IDAZ IRC wish to thank each of the following CBPP experts, who helped organise the workshops:

Rohana Dassanayake
Steve Geary
Bryan Kaplan
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Primary goals, purpose and deliverables of the report

The primary goals of this gap analysis workshop were:

1) to develop a publishable gap analysis report and
2) facilitate research collaborations.

The purpose of the gap analysis report is to: 1) provide current scientific knowledge of contagious bovine pleuropneumonia (CBPP); 2) identify potential threats to livestock worldwide; 3) identify research needs and priorities; 4) offer an in-depth analysis of available countermeasures to contain and mitigate threats; and 5) deliver specific recommendations for research and countermeasure development.

The eventual deliverables were to be: 1) Publishable gap analysis report in peer-reviewed literature;
2) Presentation of the outcome of the gap analysis at the IOM in Japan on 16-20 July 2023; and
3) Creation of research collaborations amongst the CBPP and associated community.

Executive summary

Contagious bovine pleuropneumonia (CBPP), one of the great historical plagues of cattle, remains a major problem in sub-Saharan Africa affecting over 20 countries. While attempts to control the disease over the last two decades have been inadequate, the COVID-19 pandemic further hampered these efforts with few countries now carrying out surveillance or vaccinating the numbers of cattle required to protect the national herds. Under this dire situation, this workshop was held to identify research gaps that should be addressed to improve control and eventually to eradicate CBPP. The subject area covered was divided into six overlapping research areas. During the **Epidemiology and Control** session, it was unanimously agreed that improving knowledge of the distribution, prevalence, and socio-economic impact of disease was a major priority. Linked to this was the need to generate experimental evidence of the effectiveness of control methods using a combination of strategies including vaccination, antibiotics and movement control while recognising that an overarching continental rather than national strategy was a prerequisite for this transboundary disease. The priorities agreed in the **Diagnostics sessions** were the development of improved tests with emphasis on rapid, robust and sensitive pen-side tests for both antigen and antibody detection. Abattoir surveillance of the characteristic CBPP lesions would greatly accelerate the detection of infected cattle and enable trace-back to affected herds and would require training of field staff as well as village leaders and herd owners. The urgent need for a better *in vivo* infection model was identified in the **Immunology** session and was considered crucial for vaccine testing and immunological investigations. Research was required to identify the correlates of protection for vaccine potency which would enable the definition of the type of immune response which would trigger protection. *In vitro* models like tissue explants could also be developed for elucidating the early stages of infection. The obvious requirement prioritised during the **Vaccine and Therapeutics** session was the development of safer more potent vaccines with DIVA capability while simultaneously improving the efficacy of the existing T1/44 vaccine in the short term by increasing its stability both before and after reconstitution. The combined use of vaccines with effective antibiotics to better control CBPP outbreaks while carefully monitoring for AMR emergence was highlighted. Improving immunological and molecular understanding of attenuation and protection were seen as important research aims. The research gaps identified during the **Bacteriology and Antimicrobial Resistance** session included the further evaluation of biofilms in their ability to persist in the environment while resisting the host and effect of antibiotics. Equally, identifying and characterising virulence factors and elucidating their mode of action by comparative genomics using phenotypic variants was considered an important research aim. With the resurgence of interest in using antibiotics, it was considered essential to increase understanding of AMR in the field with the establishment of standardised protocols, strain banks and assessing antibiotic quality while instigating research to identify genes involved in AMR. Finally, key objectives identified in the **Pathology and Pathogenesis** session included extensively characterising the kinetics of naturally occurring CBPP gross and microscopic lesions to better understand etiopathogenesis using immunohistochemistry and in situ hybridisation and as a baseline for experimental model development; elucidating the kinetics of mycoplasma colonisation of the lung; and attempting to identify biomarkers that predict disease progression and pathology. All sessions emphasised the need for training and capacity building of African laboratories.

Abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
BR	Basic research
CFT	Complement fixation test:
CBPP	Contagious bovine pleuropneumonia
cELISA	Competitive enzyme linked immunosorbent assay
DIVA	Differentiating infected from vaccinated individuals
ERFAN	Enhancing research for African network
LAMP	Loop-mediated isothermal amplification
LT	Long term goal
Mmm	*Mycoplasma mycoides* subsp. *mycoides*
MICs	Minimum inhibitory concentrations
PPR	Pestes des petit ruminants
ST	Short term goal

Photographs of meeting

Delegates at the workshop

Breakout groups

General introduction

In 1993, the ad hoc OIE (now the World Organisation for Animal Health, WOAH) Group on contagious bovine pleuropneumonia (CBPP) surveillance systems met in Paris to draw up plans to eradicate one of the last great historical cattle plagues from all parts of the world. This initiative was stimulated by the alarming spread of CBPP in sub-Saharan Africa and its unexpected re-emergence in southern Europe in the 1980s after an apparent absence of over a decade. At one-point CBPP simultaneously affected France, Portugal and Spain then spread to Italy in 1990 where it had a significant economic impact. An unusual feature of the new European isolates was that, compared to African strains and a European isolate from 1967, their genome was about 10% smaller. This 8.8k base deletion covered genes involved in glycerol oxidation which greatly reduced hydrogen peroxide production, a well-known virulence factor. Surprisingly, perhaps, these strains were still capable of causing severe lung damage and mortality in the early phases of the disease outbreaks in southern Europe; later outbreaks were less severe. The origin of these re-emergent strains is still unresolved but genetic and biochemical analysis showed that they had definitely not originated in Africa (Cheng et al., 1995; Houshaymi et al., 1997). The disease was eventually eradicated from Europe by movement control and test and slaughter with the last case seen in northern Portugal in 1999.

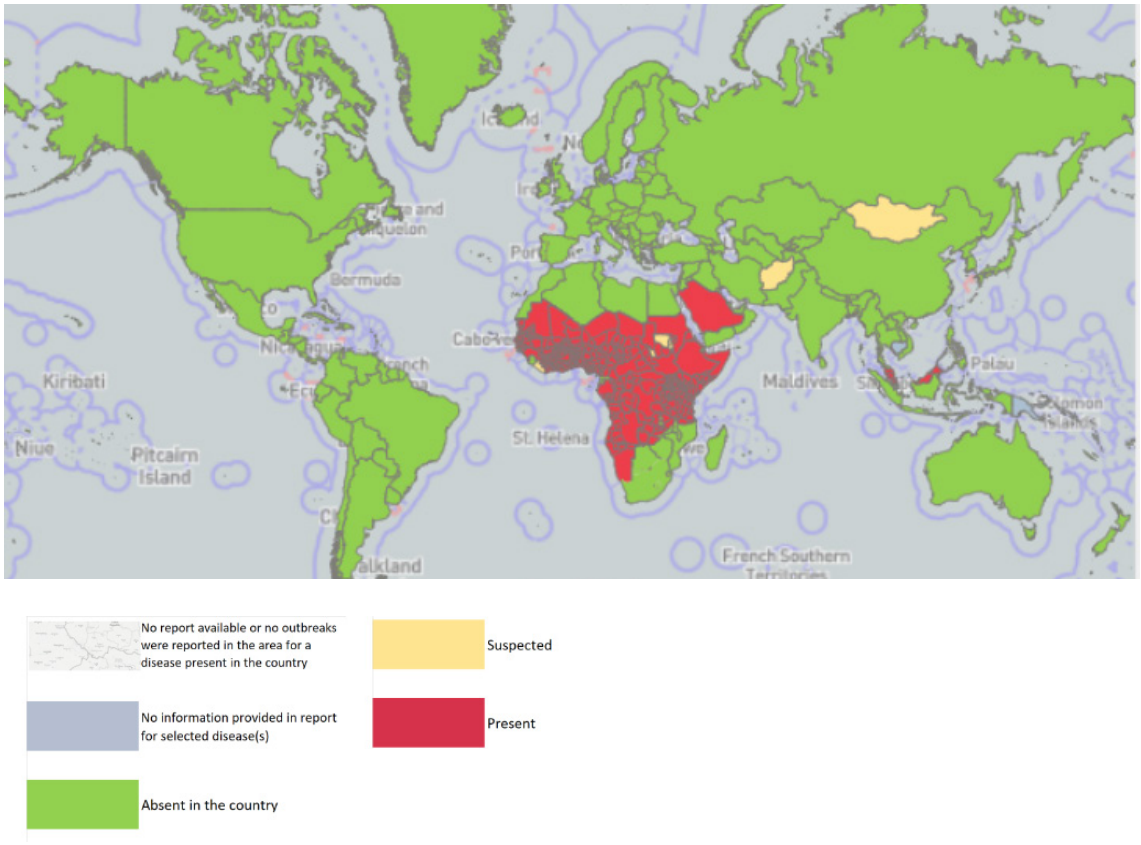
The causative agent of CBPP, *Mycoplasma* subsp. *mycoides* (*Mmm*), is a member of the *Mycoplasma mycoides* cluster consisting of five important ruminant pathogens which share immunological, genetic and biochemical features. Until 2012, the term "SC" (small colony) was appended to its species name but was removed when it became clear that there were sufficient differences with the subspecies previously referred to as LC (large colony) to reclassify. CBPP was once thought to have existed "since time immemorial" but detailed descriptions of the disease do not start to appear until the middle ages when it was first distinguished from other bovine lung diseases. Indeed, genetic analysis confirms this relatively recent emergence was possibly initiated by changes in livestock husbandry (Dupuy et al., 2012).

CBPP, known originally as "pulmona", first appeared in central Europe in the 16th century and was differentiated from other respiratory diseases by French workers. It spread throughout the continent by wars and trade during the early 19th century, predominantly by movements of Swiss and Dutch cattle. The Netherlands became a hot bed of infection with high mortality and morbidity rates. Britain too became infected through the importation of cattle from mainland Europe, resulting in huge losses: 187,000 dying in 1860 alone. CBPP was then exported from Britain and the Netherlands via infected cattle to Australia and South Africa, respectively. From the latter, CBPP-affected cattle were trekked north by Boer farmers and/or British troops (Provost et al., 1987).

The disease was introduced into the USA in imported cattle from Great Britain on several separate occasions between 1843 and 1859. It first arrived in Brooklyn, New York, then New Jersey and later in Massachusetts where it was quickly eradicated. However, the disease had broken out from New Jersey and spread to Ohio then to Illinois, Missouri and Kentucky in traded cattle in the 1880s. Eventually by co-operation between stock owners and USDA the disease was stamped out in most states but stubbornly persisted in the large herds near Chicago and New York before it was finally eradicated in the early 1890s. Ironically, considering Britain had exported CBPP to the USA in the first place and was heavily infected itself in late 19th century, a trade war nearly broke out between the two countries in 1879 when British veterinary officials suspected live cattle imported from North America of being infected with CBPP on arrival in Liverpool. However, these diagnoses were contested by both the US authorities and some experts in Britain who believed it was a general non-contagious and less fatal bronchitis, known today as "shipping fever", which had developed during a stressful transatlantic voyage. While never satisfactorily resolved, the dispute accelerated US attempts to eradicate CBPP which it did in 1892. Salmon, writing in 1896, declared prophetically: *"…and it is not probable that it will ever be seen in this country again"*. Britain, however, suffered for a few more years before final eradication in 1898.

In Africa, however, despite the great advances in diagnostic technology and improved epidemiological tools over the last 30 years, it is fair to say that eradication is as far off as ever with the disease endemic in over 20 countries and probably persisting in parts of Asia including Pakistan and Malaysia (Anjum et al., 2019; Zarina et al., 2016). The COVID-19 pandemic of 2020 has also had a dramatic negative impact on CBPP control with national surveillance and vaccination rates decreasing significantly,

leaving exposed many vulnerable and susceptible herds. Prior to the pandemic, a number of studies had attempted to estimate the losses incurred by CBPP. In 2006, Tambi et al (2006) calculated losses of US$47 million over 14 countries, while in Nigeria alone, one of the largest cattle raising countries in Africa, FAO (2004) estimated losses to be over US$2 billion. More recently and at a much more local level, Kairu-Wanyoike et al. (2017) showed that cattle owners in Kenya could lose more than twice their annual income, as a result of vaccination, postvaccine reactions, treatment costs, morbidity and mortality. With the reduction in vaccination and surveillance seen since the pandemic, it can be expected that losses will rise significantly.



Countries affected or suspected of being affected by CBPP between 2005-2023
(Patrick Bastiaensen, WOAH)

The impact of CBPP in Africa has not always been so bleak. South Africa, Zimbabwe (then known as Rhodesia), Tanzania and Botswana were all able to eradicate CBPP by mixed policies of movement controls, vaccination and slaughter. Indeed, in the 1960s and 70s, a huge international effort code named Joint Project 16 (JP16) mainly aimed at rinderpest, resulted in a dramatic reduction in clinical CBPP. Unfortunately, the eradication of rinderpest negatively impacted CBPP control particularly in the horn of Africa as the bivalent rinderpest/CBPP vaccine was no longer been administered. Inevitably, after an absence of 25 years, CBPP returned to Tanzania in 1994 spreading throughout the country where it remains endemic costing the country at least $3 million per year, though these estimates are over 30 years old. Furthermore, an incursion of CBPP in 1996 into Botswana led to the slaughter of over 300,000 cattle at the cost of between $100-400 million enabling the re-establishment of its disease-free status and protecting the lucrative EU export market. The situation in many other African countries is deteriorating.

The Workshop

This workshop, attended by delegates from 28 institutions, was brought together to review, stimulate and exchange ideas amongst current and retired experts, personnel from control authorities experienced in the field, as well as those interested in becoming involved in the fight to control CBPP; it is hoped the outcome of the meeting will kick-start new research.

The 3-day meeting was divided into six main research areas, although there was much overlap between the topics: Epidemiology and Control; Diagnostics; Immunology; Vaccines and Therapeutics; Bacteriology and Antimicrobial Resistance; and Pathology and Pathogenesis. Prior to the workshop a questionnaire was completed by the delegates and other interested parties to identify research gaps and priorities. At the workshop, delegates were divided into four working groups of similar size to discuss each of the subject areas following the relevant presentations using the results of the survey as a starting point. The make-up of the working groups was varied during the workshop to enable free discussion. The chair of each working group presented their research gaps for further discussion amongst all delegates. A consensus was then reached by the delegates on the priorities to go forward as recommendations.

Research gaps were identified and assessed as to whether they were short term (ST), long term (LT) or basic research (BR). The narrative that leads to the listing of the research gaps broadly reflects on the presentations and discussions given during that particular session. The research gaps identified by STAR-IDAZ and USDA-ARS Insight Editing Report, WOAH Standing Group of Experts, and DISCONTOOLS project and CBPP research community (Jores et al., 2020) will be found in the appendices.

Session 1: Epidemiology and Control

CBPP is primarily a disease of sub-Saharan Africa extending from Senegal and the Gambia in the west through Somalia in the east, and as far south as Namibia, Zambia and Tanzania. Currently it has been reported that more than 20 countries are affected, though data on prevalence and impact has been seriously lacking compounded by the COVID-19 pandemic. Ethiopia, Ghana, Tanzania and Angola are reported to have the highest prevalence rates. Occasional outbreaks are seen in Saudi Arabia following importation of cattle for festivals from neighbouring African countries like Ethiopia and Sudan. Unlike Europe where a single strategy of movement control, test and slaughter was imposed enabling the eradication of CBPP at the end of the 20thcentury, the lack of a sustained co-operation between countries in sub-Saharan Africa seriously inhibits control of this transboundary disease. In addition, a panoply of other factors compounds control efforts including: a need for an improved safer and more robust vaccine and greater national coverage; a lack of a versatile field diagnostic test; a greater awareness of the socio-economic impact of CBPP; poor private and public funding; climate change; and even terrorist activities. Moreover, the COVID-19 pandemic has exacerbated the problem as reflected in the drastic reduction in the submission of country reports of CBPP from 55 in 2017 to 16 in 2022 resulting in the reporting of only 20 outbreaks in 2022 from two countries compared to a peak of 273 in 2019 prior to the beginning of pandemic. Moreover, the numbers of countries reported to be vaccinating against CBPP has dropped drastically from 25 in 2015 to only four in 2022. Clearly this alarming situation will have serious impacts on attempts to control CBPP. In addition to Africa, recent but unconfirmed cases of CBPP have been reported in Pakistan and Malaysia but WOAH has not been officially notified.

Due to this lack of data, prevalence and distribution studies are urgently required locating the disease both nationally and within countries in order to protect clean zones and limit losses in high prevalence areas. Despite the difficulties in controlling CBPP in Africa, the disease does have some advantages compared to other diseases: it is not very contagious (R0 values are low at 2 though estimates vary and further studies would be valuable), lesions are pathognomonic at post-mortem examination by experienced vets; identification of the causative organism, *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*) is relatively easy by polymerase chain reaction (PCR); there is only a single serotype, vaccines are cheap and largely effective if given regularly and on a large-scale; antibiotics are effective presently with no signs of the development of antimicrobial resistance (AMR) and generally the environment and wildlife appear to play little role in the epidemiology of the disease. Consequently, mixed control strategies can be implemented. On the other hand, the T1/44 vaccine has well-known flaws including

some adverse effects at the inoculation site (Willem's reaction), short-term protection and lack of thermostability. Antibiotics rarely eliminate the pathogen completely and more recently there is concern over the role of buffalo as a reservoir of infection in Kenya. Encouragingly, with such a low R value any attempts to reduce transmission such as vaccination, culling, isolation or antibiotic treatment should lead to R0 >1, which will help lead to eradication.

Research gaps in epidemiology and control

- Investigate role for testing to detect carrier animals, for control strategy and understand the importance of carrier animals in terms of transmission.
- Improve knowledge of the distribution, prevalence, and socio-economic impact of disease (**ST**).
- Generate experimental evidence of efficacy for control strategies, based on combination of approaches, in an appropriate area (**LT**).
- Develop improved diagnostic tools for early and accurate detection including training staff to use these methods to enable capacity building (**BR**).
- Carry out epidemiological studies to track cattle movements in different regions of Africa, to better understand disease spread (**ST**).
- Assess the effectiveness of antibiotic treatments under controlled conditions including their role in continued transmission of the pathogen (**ST**).
- Standardise and improve control and quality of vaccines and antimicrobials, including monitoring for risk of AMR (**ST**).
- Investigate the potential for wildlife reservoirs such as buffalo in Kenya (**ST**).
- Obtain more data on transmission patterns between different phases of infection (especially chronic) and include up to date accurate assessment of R0 value (**ST**).

LT = long term goal; BR = basic research; ST - short term goal

Session 2: Diagnostics

It could be argued that the diagnostic tests available today, including the pen-side agglutination tests, are sufficient to detect infected herds thus greatly improving the speed of control and eradication. These tests are preferable because of the distances between outbreaks and the diagnostic laboratory. The technology for these tests is not complex and in fact similar tests for the closely related disease contagious caprine pleuropneumonia (CCPP) were developed and manufactured in East Africa. Serious consideration should be given to producing these and other diagnostic tests in countries affected by CBPP. The development of sensitive molecular-based field tests such as loop-mediated isothermal amplification (LAMP) promises the possibility of detecting disease even in individually infected animals which could be important for movement control and trade purposes. The combined use of complement fixation tests (CFT) and competitive enzyme-linked immunosorbent assay (ELISA) for serological detection of CBPP greatly increases sensitivity but has cost implications. Unfortunately, the unavailability of some of these tests commercially, such as cELISA, possibly because of cost and other supply problems, compromises these research gains. However, traditional tests like CFT and even laboratory-based PCRs are more than sufficient if used correctly to detect and confirm CBPP outbreaks. Indeed, PCR has become the method of choice for identifying the causative mycoplasma because of the well-known shortcomings of classical culturing and serological identification. Kits for both conventional and real-time PCRs are available, although these have not yet been validated for diagnostic purposes. Immunoblotting is a highly specific and sensitive test used extensively in Portugal (1990-1999) to confirm suspect cases but is technically demanding and is not suitable for mass screening. Standardisation and harmonisation of these tests across Africa is a key priority with the associated requirement for training, proficiency testing and reference materials. The ability to differentiate naturally infected from vaccinated cattle (DIVA), not possible at present, would also be a great benefit.

Despite the benefits of laboratory testing for control, there is reluctance among cattle owners to subject their animals to testing because of the fear of quarantine and market closures compounded by lack of financial compensation. Funding, of course, is a continuing problem with urgent requests for reducing costs of commercial tests, though it is more likely that it will be governments who will be the chief buyers rather than individuals.

A major success of the Italian CBPP eradication campaign (1990-1993) was the use of abattoir surveillance and this offered a cheap and relatively unequivocal method of detecting infected cattle with the possibility to trace back to the affected farm. The success of such trace backs, however, is highly dependent on the establishment of an animal identification systems. While it requires training of staff, in particular meat inspectors, to recognise and differentiate the characteristic lesions from those caused by other respiratory pathogens, it would be eminently suitable to the African setting. This is covered more fully under **Pathogenesis and Pathology**. The development of syndromic surveillance systems, the process of collecting, analysing and interpreting health-related data to provide an early warning of veterinary health threats such as those caused by CBPP, would also be an advantage but would require significant inputs in information technology (IT).

While whole genome sequencing is available widely, it has still to make some impact on CBPP diagnosis where it is hampered by the lack of strains isolated in Africa and elsewhere. Its use should allow the identification of virulence traits and molecular markers of AMR. The use of antibiotics to help control CBPP has been much discussed and as a prerequisite would require careful monitoring of the development of AMR in *Mmm* strains. Thus, standardised protocols and controls to determine the *in vitro* MIC values of *Mmm* to antimicrobials used in the field are needed.

Research gaps in diagnostics

- Development of improved diagnostic tools for CBPP in Africa with emphasis on rapid, robust and sensitive pen-side/abattoir tests for both antigen and antibody. Assess possibility of pooling samples for more economical testing (**ST**).
- Develop mass screening test for obtaining seroprevalence data across Africa if supply difficulties for cELISA cannot be resolved though back-up test is desirable (**ST**).
- Create standardise bank of sera that can be used for verification of CBPP diagnosis (**ST**).
- Develop and promote use of DIVA tests to differentiate vaccinated from field infected cattle (**BR**).
- Develop tests for detecting all stages of the disease in particular carrier animals (**BR**).
- Produce antigen/nucleic acid detection kit test for the determination of accurate prevalence data (**ST**).
- Continue search for new disease targets to improve diagnostic tests in the long term (**BR**).

LT = long term goal; BR = basic research; ST - short term goal

Session 3: Immunology

Despite years of research much of our understanding of the relationship between the immune response of the host with *Mmm* remains largely unresolved. Cattle breed and age have long been known to influence protection and huge differences occur between individuals in response to infection with *Mmm*. Furthermore, mycoplasma specific IgA has been shown to be present in bronchoalveolar lavage (BAL) and the sera of less severely affected cattle, and antiserum from convalescent animals induced mycoplasma killing by macrophages. In addition, animals that developed sequestra have significantly higher antibody titres against *Mmm* surface proteins than those that did not. However, crucially what is clear is that there is no correlation between antibody titres and severity of clinical disease and lesions. Worryingly, attempts to vaccinate with immunogenic purified proteins often leads to disease exacerbation suggesting a type III hypersensitivity reaction leading to damage by immune complexes. Research showing higher levels of IFN-g in convalescent and fully recovered animals suggested that cell mediated immunity (CMI) clearly plays a role in the immune response to CBPP, though differences in the CMI between cattle with and without lesions have yet to be seen in experimental and natural infections

Research here is impeded by the difficulty in obtaining sequential data from natural outbreaks because of the long and variable incubation period. This problem is compounded by a lack of a reliable challenge model. Unhelpfully, the immune response to experimental infection differs significantly if animals are challenged by different routes such as intubation or by contact.

A number of *in vitro* models using caprine and bovine respiratory explants have been developed to help understand the early immune events. These show that *Mmm* preferentially attaches to the lower respiratory tract, is cytotype specific and can locate inside non-phagocytic cells. Furthermore, the pathogen shows a higher tropism to the ciliated bronchial epithelial cells leading to ciliostasis and tissue destruction within 24 hours.

Research gaps in immunology

Urgent need to improve and standardise *in vivo* infection models by comparing infection parameters such as aerosols, contact, intubation (**ST**).

- Continue development of *in vitro* models (tissue explants and other cellular systems) to understand early stages of infection (**BR**).
- Identify and differentiate B and T cell epitopes in cattle of different infection statuses (**BR**).
- Study and characterise adverse vaccine reactions to understand immune-pathogenesis (**BR**).
- Reach definitions of acceptable efficacy and protection for CBPP, that are sufficient to enable other control methods to have an impact (**ST**).
- Identify correlates of protection for vaccine development and vaccine potency testing using transcriptomics and other tools to include protective antigens, role of antibodies, T-cell responses, biosignatures, *in vitro* assays, memory cells (generation and function) (**BR**).
- Further study host resistance linked to susceptibility and the immune response which has implication for CBPP control (**BR**).

LT = long term goal; BR = basic research; ST - short term goal

Session 4: Vaccines and Therapeutics

The T1/44 vaccine has been in constant use in Africa for over 70 years and was mainly responsible for the large reduction in CBPP cases in the 1970s/1980s when the disease was probably restricted to only a few East African countries. However, for many reasons including wars, drought, civil unrest etc there has been a steady increase in countries affected as national vaccine coverage and surveillance has decreased significantly. Ironically, the eradication of rinderpest also affected CBPP control as the bivalent rinderpest/CBPP vaccine was discontinued meaning less vaccination for CBPP. The T1/44 vaccine has many well-known faults including short duration of immunity, occasional side effects and the need for a cold chain. In combination with movement controls and slaughter of affected herds, restoring vaccine coverage levels across sub-Saharan Africa to previous levels would certainly help curtail the disease. Of greater concern is the significant drop in vaccine production and usage following the COVID-19 pandemic. Today only four of seven certified laboratories send their vaccines to be quality controlled by AU-PANVAC (Pan-African Veterinary Vaccine Centre of the African Union) and it is estimated that only 16% of the vaccine required to vaccinate 80% of Africa's 370 million at-risk cattle population was produced in 2022.

A new vaccine with none of the disadvantages of T1/44 would help but achieving the 80% coverage required to have an impact on CBPP control would remain a problem. Work has been reported on the development of new vaccines that includes sub-unit, attenuated and killed vaccines with variable reports of success. Using reverse vaccinology, the team at VIDO (Vaccine and Infectious Disease Organization), Canada identified immunogenic recombinant proteins that provided similar protection to T1/44 in cattle using an improved aerosol challenge model. The vaccine is going through further trials in Kenya to achieve acceptance.

Antibiotic therapy is not officially approved for CBPP in Africa though antibiotics are widely available and frequently used by livestock owners when respiratory disease is seen. Recognition over the last two decades that vaccination will not bring about eradication has led to a reconsideration of the use of antibiotics (begun at the FAO CBPP meeting in Rome in 2005 entitled: *CBPP: antibiotics to the rescue!*) ideally in a targeted setting. Work to date has shown African strains of *Mmm*, unlike *Mycoplasma bovis* common in intensive cattle operations in Europe and North America, have not yet shown signs of AMR and antibiotics have been effective in reducing disease in experimental trials and during a natural disease outbreak in Namibia. In Kenya, more effective control of CBPP in a naturally affected herd was achieved by a combination of targeted treatment of a macrolide antibiotic a few weeks before vaccination. Further trials and more data gathering are needed.

Research gaps in vaccines and therapeutics*

*Some of the research gaps identified under **Immunology** are appropriate here as well

- Improve existing vaccine responses by comparing and optimising new vaccination protocols (such as prime/boost), use of new adjuvants, improving thermostability and extending lifespan of the vaccine after reconstitution (**ST**).
- Produce better, safer more robust next generation vaccines with improved protection rate and longer duration of immunity, ideally for at least 2 years with DIVA compatibility. The availability of genome engineering techniques to generate attenuated mutants must be accelerated (**BR**).
- Assess and compare the different experimental challenge models to simulate more closely natural field infections with standardisation of scoring schemes for use in vaccine studies (**ST**).
- Carry out more studies including modelling on the use of antibiotics in combination with vaccination to control CBPP while continuously monitoring for AMR of strains. The use of syndromic diagnosis may improve the effectiveness of their use (**ST**).
- Improve immunological and molecular understanding of the attenuation of vaccine strains and their protection. Include improved understanding of the immunology of infected vs vaccinated animals/recovered vs disease (chronic infection, death) (**BR**).
- Identify correlates of protection for vaccine development and vaccine potency testing using transcriptomics and other tools. To include protective antigens, role of antibodies, T-cell responses, biosignatures, in-vitro assays, memory cells (generation and function (**BR**).
- Continue to improve media formulations to enable greater mycoplasma yields for more efficient and economic vaccine production (**ST**).

LT = long term goal; BR = basic research; ST - short term goal

Session 5: Bacteriology and Antimicrobial Resistance

While it is not generally accepted that the persistence of mycoplasmas in the African environment contributes significantly to the epidemiology of CBPP, it is clear that these wall-less bacteria are tougher than they look. Research carried out 15 years ago showed that mycoplasmas including *Mmm* can form a true biofilm which greatly increases their persistence in the environment and renders them more resistant to disinfectants and other antimicrobial compounds (McAuliffe et al., 2008). Mycoplasma biofilms are quite distinct for mycoplasma aggregates and colonies as they are organised with channels, towers and protective carbohydrate covering. Furthermore biofilm-grown mycoplasmas have altered gene expression making them potentially more virulent in the host. Further work is needed to confirm and extend these findings, particularly *in vivo*, to establish whether mycoplasmas exist in biofilms in the natural state.

Galactan, a capsular polysaccharide of *Mmm*, has been of interest to researchers for over 60 years; it is also present in large quantities in a cell-free form. Galactan appears to be both cytotoxic to host cells and have anti-inflammatory properties as it can inhibit phagocytosis. Consequently, it has been the target of vaccine development, some of which have shown potential to protect against CBPP.
The extracellular material can be harvested for attachment to latex beads which is the basis of the pen-side agglutination test, a very useful test easily capable of detecting infected herds in remote areas.

AMR surveillance of animal mycoplasmas has been hampered for many years because of lack of harmonised methods for antimicrobial susceptibility testing (AST) and interpretative criteria, resulting in a severe shortage of data. Recently, attempts have been made in Europe to address this deficiency by participating in an existing clinical surveillance network system to monitor antibiotic sensitivity in the field. Results show that *M. bovis*, a cattle respiratory pathogen, has high MICs for all classes of antibiotics except the fluoroquinolones probably because of their use in intensive production of European livestock; members of the *M. mycoides* cluster including *Mmm* on the other hand, show predominantly low MICs because CBPP is more common in smaller herds kept extensively where the development of AMR is less of a risk. Further work is required to establish breakpoints for animal mycoplasmas to provide clinical guidance for treatment. This work should be extended to Africa to monitor *Mmm* strains to provide an early warning of the emergence of AMR. The establishment of strain banks and surveillance networks would complement this approach.

Early use of proteomics by Krasteva et al (2014, 2015) to define major protein determinants in *Mmm* by comparing a virulent strain and some vaccine strains showed that while many proteins were shared, a significant number were exclusive to each strain, some of which were immunogenic, protective or conversely contributed to immunopathology. This study called for further systematic investigations of *Mmm* protein expression and function including inter strain comparisons. Other studies have shown that *Mmm* cells secrete extracellular vesicles of highly variable size during culture. Analysis of their membrane-associated proteins revealed several that had previously been linked to pathogenicity and host pathogen interaction, suggesting that this secretion pathway may play an active role in *Mmm* infectivity.

Research gaps in bacteriology and AMR

- Increase understanding of AMR in *Mmm* with particular emphasis on the use of antibiotics in Africa using standardised protocols, data collection and combined with assessment of antibiotic quality in the field. Include identification of AMR genes (**ST**).
- Assess the importance of biofilms with emphasis on identifying markers particularly *in vivo*; identifying genes responsible for biofilm formation using transcriptomics; and develop MIC tests using biofilm-grown *Mmm* to determine more realistic MICs. Consider knocking out genes for biofilm formation (**BR**).
- Identify and characterise virulence factors, their genes and elucidate mechanisms of action by comparative genomics using phenotypic variants and/or across natural diversity (**BR**).
- Focus on the processes surrounding adhesion through examination of surface proteome and protective antigens (**BR**).
- Examine and compare contents of extracellular vesicles in *Mmm* with whole cells using proteomics (**BR**).
- General research on the molecular mechanisms of pathogenicity of *Mmm* and immune evasion to cover virulence factors, adhesion molecules (**BR**).
- Characterise *Mmm* tropisms, colonisation, persistence and immune activation of ciliated and non-ciliated epithelial and submucosal cell subsets of respiratory tract (including upper nasal area) from young and adult animals. In parallel conduct *in vitro* experiments using primary cell cultures and ex vivo respiratory explants (**BR**).

LT = long term goal; BR = basic research; ST - short term goal

Session 6: Pathogenesis and Pathology

A WOAH group of experts on surveillance concluded that the most efficient method of detecting CBPP is through effective meat inspection at abattoirs followed by laboratory testing of suspect samples. These were the major tools in the eradication of the disease in Italy in the 1990s, which was accomplished in 3 years involving the slaughter of over 24,000 cattle and buffalo. Lesions of this largely unilateral pulmonary disease, in the most part, are pathognomonic. In Africa, post-mortem examinations can be carried out in bush abattoirs and even at festivals where cattle are slaughtered, enabling trace back to affected farms. Nonetheless, mobile technology can also be used to send images from remote locations to diagnostic laboratories, so called telediagnosis, greatly saving time. Rigorous training of meat inspectors, field vets and other interested parties to recognise CBPP lesions in abattoirs and places of slaughter is essential. This training should be given not just to veterinary officials but also to traditional leaders and cattle breeders. Connecting with other networks such as the ERFAN (Enhancing Research for Africa) Network, a network of 30 African institutions focused on diseases including CBPP, could support the implementation of training.

It is essential to be able to differentiate CBPP lesions from other diseases like bovine mycoplasmosis caused by *M. bovis* which is often found in pneumonic cattle worldwide though rarely in association with *Mmm*. Cross-reactions were seen with the less specific CFT during the final months of the CBPP eradication campaign in Portugal and required immunoblotting to confirm results. Both mycoplasma diseases can be classified together as being characterised by inflammation of the respiratory tract with tissue damage. This compares with mycoplasma infections of other species where there is lymphocytic inflammation in the respiratory tract with hyperplasia, such as those caused by *M. hyopneumoniae* and *M. gallisepticum*, and diseases causing systemic infection affecting many sites, like other *Mycoides* group members including *Mm capri*. However, the kinetics of histopathologic lesions of naturally occurring CBPP has not been fully characterised and those published were completed some time ago. Thus, they do not include immunohistochemistry or other advanced assays such as RNAScope. Histopathologic findings are needed for: a) the creation of experimental models that best fit naturally occurring disease; b) an understanding of the kinetics and localisation of inflammatory and immune cell infiltration, epithelial lesions (in upper respiratory tract, trachea, and lung bronchi and bronchioles), and microbial colonisation.

A recently discovered potent mechanism by which pathogenic mycoplasmas combat the host immune system, known as the MIB/MIP-ATPase, which binds, disrupts and cleaves most classes of antibody, has now been found in all sequenced *Mmm* strains. Its main action is to prevent immune agglutination and may be responsible for the inadequacies of the host immune system and ineffectiveness of vaccines. In response, the host has developed countermeasures and combats the pathogen by stimulating the innate immune system when it detects broken antibodies.

Research gaps in pathology and pathogenesis

- Verify surface-localised virulence factors, both *in vivo* and ex vivo (**BR**).
- Extensively characterise CBPP lesions using histological and immunohistochemistry of the development (kinetics) of naturally occurring disease for a better understanding of etiopathogenesis along with investigations into the kinetics of microbial colonisation within the lung (**ST**).
- Generate *Mmm* mutants based on an attenuated *M. mycoides* subsp. *capri* model or CRISPR system and their testing *in vivo* as live-attenuated vaccine candidates (**BR**).
- Increase current knowledge of both local and systemic immunology following natural infection and vaccination against *Mmm*, taking advantage of multi-omics technology (**BR**).
- Study responses of respiratory epithelia to *Mmm* infection (e.g., cilia loss, necrosis/apoptosis, metaplasia) in young and adult animals (**ST**).
- Identify biomarkers that predict progression of diseases and to measure pathology (**LT**).
- Further investigate CBPP immunopathology by studying effects on host response of LppQ and other purified surface exposed proteins (**BR**).

LT = long term goal; BR = basic research; ST - short term goal

Non research gaps

During the course of the workshop, it became clear that many of the requirements discussed and identified by the participants were not gaps that could be addressed by research. These were considered essential measures to be put in place through regulations, procedure and policy to enhance and achieve control and eradication. These are listed here:

- Need for sustained co-operation between countries in sub-Saharan Africa to enable control of this transboundary disease.
- Standardisation and harmonisation of diagnostic tests across Africa with the associated requirement for training, proficiency testing and reference materials.
- General training, replacement and re-allocation of experienced staff following the pandemic, to underpin any attempts to rejuvenate and accelerate control efforts.
- Establishment of an animal identification system to enable detection of affected herds following identification in the abattoir.
- Training of staff, in particular meat inspectors, to recognise and differentiate the characteristic lesions from those caused by other respiratory pathogens,
- Standardisation of protocols and establishment of controls to determine the *in vitro* MIC values of *Mmm* to antimicrobials used in the field; the establishment of strain banks and surveillance networks for AMR; harmonisation methods and interpretative criteria for AST.
- Consideration should also be given to connecting with existing networks such as ERFAN led by Dr M Scacchia (WOAH-CBPP Reference Laboratory, Teramo), a network already connecting 30 African institutions on diseases including CBPP disease recognition and AMR.

References

Anjum, A., Aslam, A., Akhtar, R., Yaqub, T., Khan, M.-R., Sultan, R., Usman, S., Durrani, A.Z., Usman, M., 2019. Molecular Detection and Pathological Investigation of Contagious Bovine Pleuropneumonia in Selected Districts of Punjab, Pak. J. Zool. 52.

Cheng, X., Nicolet, J., Poumarat, F., Regalla, J., Thiaucourt, F., Frey, J. 1995. Insertion element IS1296 in Mycoplasma mycoides subsp. mycoides SC identifies a European clonal line distinct from African and Australian strains.Microbiology 141, 3221-3238

Dupuy, V., Manso-Silván, L., Barbe, V., Thebault, P., Dordet-Frisoni, E., Citti, C., Poumarat, F.,Blanchard, A., Breton, M., Sirand-Pugnet, P., Thiaucourt, F., 2012. Evolutionary History of Contagious Bovine Pleuropneumonia Using Next Generation Sequencing of Mycoplasma mycoides Subsp. mycoides "Small Colony." PLOS ONE 7, e46821.

FAO, 2004. Towards sustainable CBPP control programmes for Africa, in: Proceedings of the 3rd Meeting of the FAO-OIE-OAU/IBAR-IAEA Consultative Group on CBPP. FAO, Rome, Italy, pp. 1–46.FA0 (2004)

Houshaymi, B., Miles, R J., Nicholas R A J. 1997 Oxidation of glycerol differentiates African from European isolates of Mycoplasma mycoides subsp. mycoides SC (small colony) Vet. Rec 140, 182-183

Jores, J., Baldwin, C., Blanchard, A., Browning, G.F., Colston, A., Gerdts, V., Goovaerts, D., Heller, M.,Juleff, N., Labroussaa, F., Liljander, A., Muuka, G., Nene, V., Nir-Paz, R., Sacchini, F.,Summerfield, A., Thiaucourt, F., Unger, H., Vashee, S., Wang, X., Salt, J., 2020. Contagious Bovine and Caprine Pleuropneumonia: a research community's recommendations for the development of better vaccines. Npj Vaccines 5, 1–9.

Kairu-Wanyoike, S.W., Taylor, N.M., Heffernan, C., Kiara, H., 2017. Micro-economic analysis of the potential impact of contagious bovine pleuropneumonia and its control by vaccination in Narok district of Kenya. Livest. Sci. 197, 61–72.

Krasteva, I., Inglis, N.F., Sacchini, F., Nicholas, R., Ayling, R., Churchward, C.P., March, J., Lainson, A.,Mclean, K., Hughes, V., Imrie, L., Manson, E., Clark, J., Pini, A., Smith, D.G.E., 2015. Proteomic characterisation of two strains of Mycoplasma mycoides subsp. mycoides of differing pathogenicity. J. Proteomics Bioinform. S13

Krasteva, I., Liljander, A., Fischer, A., Smith, D.G.E., Inglis, N.F., Scacchia, M., Pini, A., Jores, J., Sacchini,F., 2014. Characterisation of the *in vitro* core surface proteome of Mycoplasma mycoides subsp. mycoides, the causative agent of contagious bovine pleuropneumonia. Vet. Microbiol.168, 116–123.

McAuliffe, L., Ayling, R.D., Ellis, R.J., Nicholas, R.A.J., 2008. Biofilm-grown Mycoplasma mycoides subsp. mycoides SC exhibit both phenotypic and genotypic variation compared with planktonic cells. Vet. Microbiol. 129, 315–324.

Provost, A.; Perreau, P.; Bréard, A.; Le Goff, C.; Martel, J.L.; Cottew, G.S. Contagious bovine pleuropneumonia Rev. Sci. Tech. Off. Int. Epizoot. 1987, 6, 625–79.

Tambi, N.E., Maina, W.O., Ndi, C., 2006. An estimation of the economic impact of contagious bovine pleuropneumonia in Africa. Rev. Sci. Tech. Int. Off. Epizoot. 25, 999–1011.

Zarina, M., Zamri-Saad, M., Latiffah, H., Shahrom, M.S., Norlida, O., 2016. Seroprevalence and detection of Contagious Bovine Pleuropneumonia (CBPP) in northeast states of Peninsular Malaysia. Pertanika J. Trop. Agric. Sci. 39, 257–265.

Appendices

I Meeting programme

Monday 26 June 2023

8:00-8:30	Arrival Refreshments	
8:30-8:45	Welcome and Introductions of Goals of Workshop	Dr. Roxann Motroni, National Program Leader for Animal Health, USDA-ARS
Overview		
8:45-9:10	The World Organisation of Animal Health and CBPP	Dr. Patrick Bastiaensen, Programme Officer WOAH, Regional Representation for Eastern Africa
9:10-9:15	Q&A	
9:15-9:40	Contagious Bovine Pleuropneumonia in Africa – An Overview of Challenges	Dr. William Amanfu, Former Senior Officer, FAO/UN
9:40-9:45	Q&A	
9:45-10:10	Bacterial Vaccines: An Overview of Old Versus Emerging Vaccine Discovery and Platform Technologies	Dr. Paul Langford, Professor of Paediatric Infectious Diseases, Imperial College London
10:10-10:15	Q&A	
10:15-10:30	Break	
Scientific Session 1: Epidemiology and Control Scientific Steering Committee Lead: Geoffrey Muuka (Central Veterinary Research Institute, Zambia)		
10:30-10:55	Epidemiology of contagious bovine pleuropneumonia	Dr. Chandapiwa Marobela-Raborokgwe, Botswana National Veterinary Laboratory
10:55-11:00	Q&A	
11:00-11:25	CBPP control strategies	Dr. Francois Thiaucourt, Former Head of CBPP World Reference Laboratory, CIRAD (Retired)
11:25-11:30	Q&A	
11:30-13:00	Gap Analysis/Discussion	Moderator: Geoffrey Muuka
13:00-14:00	Lunch	
Scientific Session 2: Diagnostics Scientific Steering Committee Lead: Elise Schieck (ILRI, Kenya) Scientific Steering Committee Lead: Musa Mulongo (ILRI, Kenya) Scientific Steering Committee Lead: Lucia Manso-Silvan (CIRAD, France)		
14:00-14:25	CBPP diagnosis: overview and gap analysis	Dr. Lucia Manso-Silvan, Veterinary Researcher, CIRAD, France
14:25-14:30	Q&A	
14:30-14:55	Use of CBPP diagnostic test to inform CBPP control programs: myth and reality	Dr. Rose Matua, Kenyan Department of Veterinary Services, Kenya

14:55-15:00	Q&A	
15:00-15:25	The immune system and mycoplasma: the case of CBPP	Dr. Jan Naessens, International Livestock, Research Institute, Nairobi, Kenya
15:25-15:30	Q&A	
15:30-16:00	Break	
16:00-17:30	Gap Analysis/Discussion	Moderator: Elise Schieck
19:00	Dinner	Hinxton Hall

Tuesday 27 June 2023

8:00-8:15	Arrival Refreshments/Recap	
Scientific Session 3: Immunology Scientific Steering Committee Lead: Rohana Dassanayake (USDA-NADC, USA) Scientific Steering Committee Lead: Bryan Kaplan (USDA-NADC, USA)		
8:15-8:40	*In vivo* and *in vitro* immune response to Mycoplasma mycoides subsp. mycoides infection	Dr. Flavio Sacchini, WOAH Reference Laboratory for CBPP, Istituto Zooprofilattico Sperimentale, Teramo, Italy
8:40-8:45	Q&A	
8:45-9:15	Immune responses associated with protection to CBPP and induced by immunised	Dr. Philippe Totte, CIRAD, Montpellier, France
9:15-9:20	Q&A	
9:20-9:45	Developing and Deploying Tools to Understand Natural and Vaccine-Induced Immune Responses in Cattle	Prof. Jayne Hope, Personal Chair of Immunology, The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh
9:45-9:50	Q&A	
9:50-10:05	Break	
Scientific Session 4: Vaccines and Therapeutics Scientific Steering Committee Lead: Elise Shieck (ILRI, Kenya) Scientific Steering Committee Lead: Musa Mulongo (ILRI, Kenya) Scientific Steering Committee Lead: Lucia Manso-Silvan (CIRAD, France)		
10:05-10:30	Background scientific knowledge on the development of CBPP vaccines and antibiotic therapy for the disease (virtual presentation)	Dr. Mamadou Niang, FAO, Regional Office for Africa, Ghana
10:30-10:35	Q&A	
10:35-11:00	Efficacy of a novel recombinant vaccine for control of CBPP tested using an improved challenge model	Dr. Jose Perez-Casal, Research Scientist, Vaccine and Infectious Disease Organisation
11:00-11:05	Q&A	
11:05-11:30	Generating evidence required to modify the “test and slaughter” policy in the control of CBPP outbreaks in Africa	Dr. Hezron Wesonga, Senior Research Scientist, Kenya Agricultural and Livestock Research Organisation, Kenya

11:30-11:35	Q&A	
11:35-12:00	PANVAC	Dr. Charles Bodjo, African Union Pan African Veterinary Centre (AU-PANVAC), Ethiopia
12:00-12:05	Q&A	
12:05-12:30	Beyond humans: Advancement in vaccines for animal health	Dr. Jon Cuccui, Associate Professor Glycoengineering and Glycobiology; Department of Infection Biology; London School of Hygiene and Tropical Medicine, UK
12:30-12:35	Q&A	
12:35-13:30	Lunch	
13:30-15:00	Gap Analysis: Immunology	Moderator: Rohana Dassanayake and Bryan Kaplan
15:00-15:30	Break	
15:30-17:00	Gap Analysis: Vaccines and Therapeutics	Moderator: Lucia Manso-Silvan and Musa Mulongo
19:00	Dinner	

Wednesday 28 June 2023

8:00-8:30	Arrival Refreshments/Recap	
Scientific Session 5: Bacteriology and AMR Scientific Steering Committee Lead: Edan Tulman (University of Connecticut, USA) Scientific Steering Committee Lead: Steven Szczepanek (University of Connecticut, USA)		
8:30-8:45	Mycoplasmas: wolves in sheep's clothing	Dr. Robin Nicholas, Consultant, UK (formerly head of Mycoplasma Group, APHA, Weybridge, UK)
8:45-9:10	Antimicrobial resistance in mycoplasmas: prospects and challenges (Virtual Presentation)	Dr. Florence Tardy, ANSES (France), former head of the Animal Mycoplasmoses Unit, Lyon and now Head of Antibioresistance, bacteriology and mycoplasma unit, Ploufragan, France
9:10-9:15	Q&A	
9:15-9:40	Multi-OMICs Analysis of the Mycoplasma Mycoides Cluster	Dr. Joerg Jores, Institute of Veterinary Bacteriology, Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern and Multidisciplinary Center for Infectious Diseases (MCID), University of Bern, Switzerland
9:40-9:45	Q&A	
9:45-10:10	Genome engineering in Mycoplasma mycoides for building attenuated mutants with a special focus targeting capsule biosynthesis	Prof. Alain Blanchard University of Bordeaux-INRAE

10:10-10:15	Q&A	
10:15-10:30	Break	
Scientific Session 6: Pathogenesis and Pathology Scientific Steering Committee Lead: Flavio Sacchini (IZSAM, Italy) Scientific Steering Committee Lead: Hezron Wesonga (KALRO, Kenya)		
10:30-10:55	Contagious bovine pleuropneumonia, pathology	Dr. Massimo Scacchia, Head of Department, WOAH Reference Laboratory for CBPP, Istituto Zooprofilattico Sperimentale, Teramo, Italy
10:55-11:00	Q&A	
11:00-11:25	Comparison of pathologic findings in mycoplasmal infections of domestic animals	Prof. Jeff Caswell, Ontario Veterinary College, University of Guelph, Canada
11:25-11:30	Q&A	
11:30-11:55	From antigenic variation to direct targeting of immunoglobulin, the many layers of mycoplasmas survivability onion	Dr. Yonathan Arfi, Assistant Professor, University of Bordeaux, France
11:55-12:00	Q&A	
12:00-13:00	Lunch	
13:00-14:30	Joint Gap Analysis	Moderator: Edan Tulman, Steve Szczepanek, Flavio Sacchini, Hezron Wesonga
14:30-15:00	Wrap Up/Next Steps	
15:00	Adjourn	

II Delegates

Chandapiwa Marobela, Botswana National Veterinary Laboratory
Jose Perez-Casal, University of Saskatchewan
Sanne Charles Bodjo, AU-PANVAC
Jeffrey Caswell, University of Guelph
Steven Szczepanek, University of Connecticut
Edan Tulman, University of Connecticut
Godwin Egwu, University of Abuja, Nigeria
Patrick Bastiaesen, WOAH
Elise Schieck, ILRI
Vish Nene, ILRI
Hezron Wesonga, KALRO
Musa Mulongo, CGIAR
Rose Matua Alumiria, Kenyan Department of Veterinary Services
Philippe Totte, CIRAD
Geoffrey Muuka, Ministry of Fisheries and Livestock, Zambia
William Amanfu, Consultant
Lucia Manso-Silvan, CIRAD
Alain Blanchard, INRAE
Yonathan Arfi, INRAE
Carole Lartigue-Prat, INRAE
Jayne Hope, The Roslin Institute
Flavio Sacchini, IZS Teramo
Massimo Scacchia, IZS Teramo
Baptiste Dungu, UKIKIN
Joerg Jores, University of Bern
Francois Thiaucourt, Consultant
Mamadou Niang, FAO
Nick Juleff, Bill & Melinda Gates Foundation
Stephen Wilson, GALVmed
Jan Naessens, ILRI
Robin Nicholas, Consultant
Maddy Newman, Secretariat to STAR-IDAZ IRC
Georgina Grell, CABI
Paul Langford, Imperial College London
John Cuccui, LSHTM
Andy Peters, University of Edinburgh
Roxann Motroni USDA-ARS
Mark Ackerman USDA-ARS
Susan Noh USDA-ARS
Rohanna Dassanayake USDA-ARS
Eduardo Casas USDA-ARS
Bryan Kaplan USDA-ARS
Kelley Black USDA-APHIS
Ping Wu USDA-APHIS

III Research gaps identified by other studies

Main research gaps identified by Insight Editing 2023*

Biology of the pathogen

Development of a reproducible and robust cattle challenge model, ideally aerosol-based, utilising a low passage field isolate, cattle over one year of age, and with a defined dose of *Mmm*; and applicable to low-resource settings

Continuing collection of complete *Mmm* genome sequences, especially from virulent African strains

Diagnosis

Development and validation of rapid, cost-effective diagnostic assays able to be performed in the field without laboratory equipment

Increased training (on PCR, ELISA, etc.) for veterinary personnel in low- and middle-income countries

Pathogenesis

Verification of surface-localised virulence factors, both *in vivo* and ex vivo

Generation of *Mmm* mutants based on attenuated *Mmm* subsp. *capri* model and their testing *in vivo* as live-attenuated vaccine candidates

Extension of current immunological knowledge, both local and systemic, following natural infection or vaccination against *Mmm*, taking advantage of multi-omics technology to generate valuable insights

Characterisation of the Willems reaction following vaccination against *Mmm* to support rational design of vaccines without this drawback

Assessment of the potential role of anti-LppQ antibodies in immunopathology

Understanding of responses of respiratory epithelia to *Mmm* infection (e.g., cilia loss, necrosis/apoptosis, metaplasia)

Studies to assess the importance of *Mmm* biofilm formation in the field

Immunology

Standardisation of studies analysing risk factors for CBPP transmission and maintenance, including the incorporation of local sociocultural and/or climatic variables

Characterisation of the local IgA response and its role in protection

Studies to understand the role of maternal immunity in early protection/interference with vaccination

Immunogenetics of resistance/susceptibility to *Mmm*

Assessment of whether blood sampling is useful in understanding T cell responses to *Mmm* or rather lymph node T cells should be assayed as standard

Identification of pathogenic versus protective antigens/antibodies in cattle

Epidemiology

Increased surveillance for *Mmm* in Africa, the Middle East and Southeast Asia

Communication and outreach methods for promoting vaccine usage among farmers in endemic rural areas

Effective outreach and financial assistance programmes for farmers in low- and middle-income countries

Potential transmission by chronically infected animals under different farm management systems

Control

Design of an improved vaccine with an excellent safety profile: in the absence of/while awaiting significant advances in knowledge on pathogenicity and immunology of *Mmm*, existing lead candidates should be prioritised for further testing, optimisation, and commercialisation

Evidence-based requirements for new vaccines to include a duration of immunity of around 18 months, minimal/no induction of vaccine site reaction, suitability for needle-free or mucosal delivery, DIVA compatibility, and being cheap, safe and easy to produce

Standardisation of vaccine challenge studies towards a protocol that might include: control unvaccinated group, adjuvant-only group, conventional vaccine group, and two-test vaccine groups (one for challenge and another for duration of immunity studies); the challenge should then be conducted by a standardised aerosol method and disease severity/pathogen titre comprehensively assessed

Standardisation of immunological readouts for vaccine studies to increase value; assays could include specific immunoglobulin titre, specific T cell proliferation including information on T cell phenotype and cytokine production (ideally by multi-colour flow cytometry), and lung IgA response.

***2023 Veterinary Mycoplasmas Research Report Insight Editing London**

WOAH Standing Group of Experts (SGE) 2021*

Main research gaps identified by SGE

Collaboration with researchers to generate evidence:

Effect of antibiotics on sequestra

Effect of antibiotics on live vaccines

Applied research to test effective antibiotic treatment/vaccination protocols

Understanding drivers for farmers' behaviour

Alternative vaccines - vaccine candidates (other than the T1 based strains)

Understanding the socio-economic burden of CBPP

*** Report of the virtual meeting of the OIE ad hoc Group on the Evaluation of Contagious Bovine Pleuropneumonia Status of Members 5 – 7 October 2021**

Main research gaps identified by EU Discontools (2023)*

Training on disease recognition, reporting, telediagnosis, AI & smart applications for reporting and early application of control measures are needed to make syndromic surveillance more effective

Diagnostic capacity building through training towards quality assurance, networking and organisation of inter-lab ring trials

Availability of diagnostic kits (i.e., alternative, strictly specific fully validated cELISA) is urgently needed for use in international trade, as well as for field surveillance and prevalence studies

Direct molecular assays must also be fully validated by international standards and inter-laboratory assays. Affordable freeze-dried products should be favoured to minimise cold chain requirements

Availability of reference standards and qualified sample panels from free vs experimentally/naturally infected animals are needed for validation of new diagnostics

Market studies are needed to assess the demand and affordability of new diagnostics and the involvement of manufacturing companies is required to target and optimise the development of inventions into successful commercial products, particularly regarding pen-side tests for rapid screening in the field & DIVA assays

Standardised MIC protocols & controls for assessment of AMR acquisition by *Mmm* strains, & molecular detection methods

Repositories for animal health data collection, storing, updating and sharing dedicated to CBPP are needed (genotyping, "omics", AMR, relevant epidemiological information…).

A whole genome analysis approach for finest *Mmm* strain genotyping and comparative "omics" analyses should be developed; scripts and data repositories should be made publicly available

Research towards the development of sensitive assays for individual diagnosis is needed (detection of silent carriers); also, affordable pen-side tests & DIVA strategies.

***Summary of unpublished report on CBPP by DISCONTOOLS (2005)**

Main research gaps identified by CBPP Research Community (Jores et al 2020)*

Development of a robust challenge model

Ex vivo and *in vivo* verification of surface-localised virulence factors using harmonised caprine models and *M. mycoide*s subsp. *capri*. Generate *M. mycoides* subsp. *mycoides* mutants based on mutations that led to attenuation in a M. mycoides subsp. capri model and test them as a live vaccine *in vivo*

Extend and revisit immunological knowledge based on correlates of protection using the novel challenge model (see Priority 1): characterise the innate and adaptive immune responses (local and systemic) after infection of vaccinated and naïve animals

Characterise the Willems reaction and the mycoplasma factors that drive it.

***Jores et al (2020) Contagious bovine and caprine pleuropneumonia: a research community's recommendations for the development of better vaccines. Npj Vaccines 5, 66**

doi: 10.1038/s41541-020-00214-2

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