STAR IDAZ | International Research Consortium on Animal Health

Roadmap Lead Summaries

Disease/pathogen	Contagious bovine pleuropneumonia (CBPP) / *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*)					
Roadmap type	Development of CBPP vaccines					
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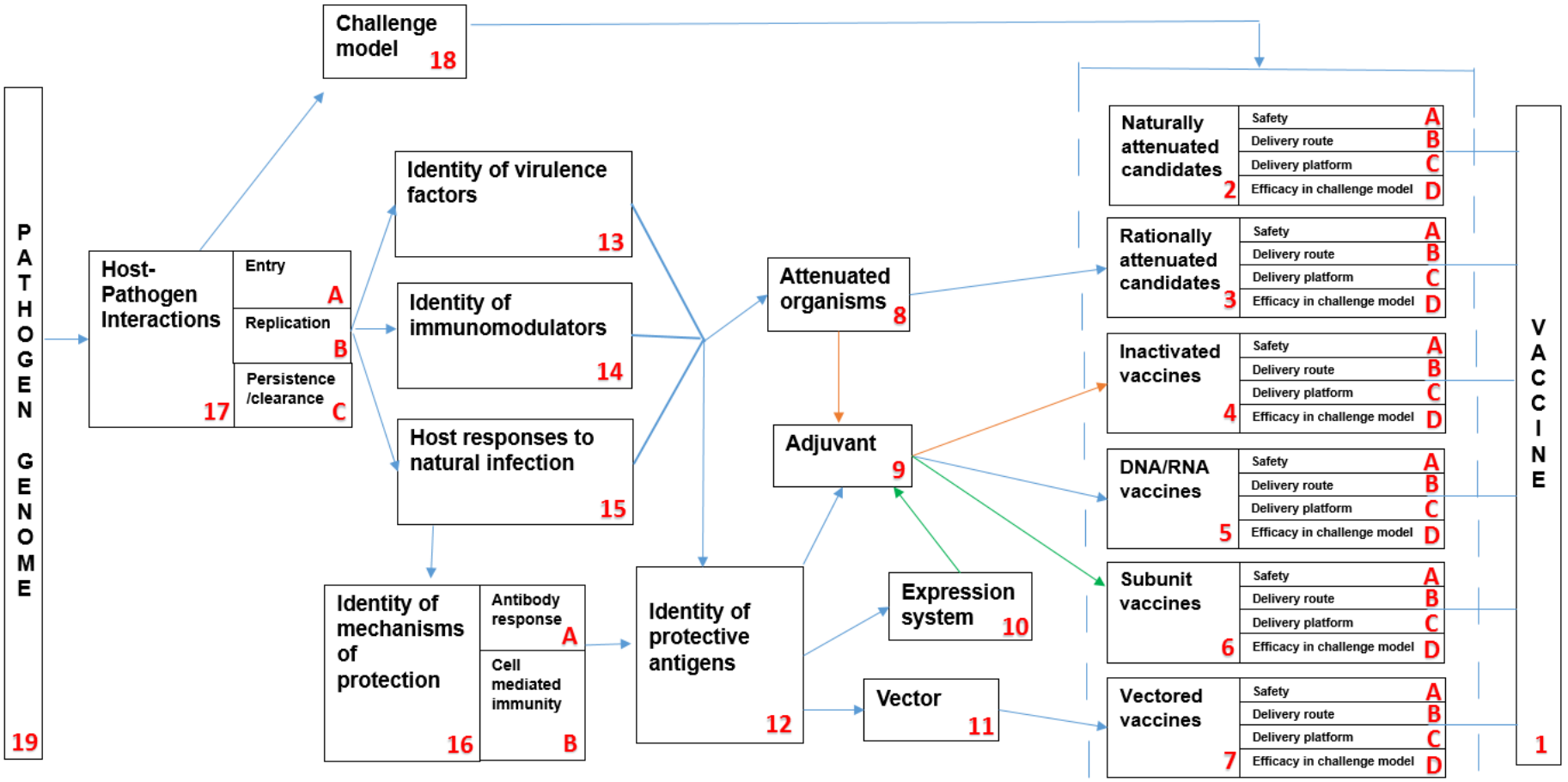
Roadmap for Vaccine Development



Lead Summary 1 - Vaccine

TITLE	Vaccine to CBPP – contagious bovine pleuropneumonia – a disease of ruminants in the genus *Bos* and *Bubalis*
Research Question(s)	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Use existing live attenuated vaccines (LAVs) to control CBPP caused by *Mycoplasma* subsp. *mycoides* (*Mmm*) in Africa to improve cattle productivity and gain access to new markets. a. Could better implementation of current LAVs (T1/44 or T1sr) help bring CBPP under control? 2. Is it possible to develop more efficacious and safer vaccines than the current LAVs.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Better diagnostic tools of the different clinical phases of CBPP are needed to support LAVs vaccination campaigns. 2. Issues such as need for a cold chain, short duration of immunity after a single LAV dose, the incidence of side effects, access to vaccine, diluent composition, short shelf life after reconstitution and policies restrict the routine use of current LAVs. 3. No new attenuated vaccines which improve on the LAVs developed in the 1950s. 4. No inactivated or subunit commercial vaccines have been developed. 5. Incomplete knowledge on acquired and innate immune responses that mediate or contribute to immunity and to disease. 6. Incomplete knowledge on subunit antigens associated with immunity or those that contribute to disease. 7. Cattle breed, age and health status influence immunity and huge differences can occur between individuals in response to infection. 8. Lack of *in vitro* tests that correlate/associate with different clinical manifestations of and immunity to CBPP. 9. Currently, the only way to assess vaccine improvements is via laboratory or field challenge trials. 10. Field trials to assess vaccine efficacy are not ideal in early phase vaccine R&D. 11. Difficulty in obtaining sequential data from natural outbreaks because of long and variable incubation periods.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Resolve conflicting data on the quality, safety, use and efficacy of existing LAVs. 2. Establish if there is a dose response in cattle breeds between route of immunization and interval between primary and booster vaccinations to obtain the best safety, efficacy and duration of immunity in different age groups of cattle using existing LAVs.

	3. Standardize cattle breed specific laboratory challenge models that reproduce CBPP caused by natural infection or are acceptable surrogates of natural infection. 4. Develop disease related metrics that do not rely on postmortem data to assess vaccine efficacy. 5. Determine the impact of different clinical phases of CBPP on the epidemiology of disease. 6. Develop assays and biomarkers for improved diagnosis of disease and immunity to CBPP. 7. Use assays and biomarkers to guide and design improved use of LAVs, new generation vaccines and diagnostic tests, including DIVA vaccines. 8. Develop an antigen map of *Mmm* through different phases of CBPP and stages of disease. 9. Explore the protective capacity of candidate vaccine antigens using different vaccine platforms.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Develop community agreed minimum and optimal vaccine TPPs to prioritize and guide vaccine R&D. 2. Harmonize regional, national, and continental wide vaccination policies and campaigns for better manufacture and delivery of LAVs. 3. Improve fragmented veterinary services and vaccine supply chain. 4. Incentivize use of GMP and AU-PANVAC certified LAV vaccines. 5. Assess vaccine effectiveness and implement pharmacovigilance in disease control. 6. Attract animal health companies to help deploy LAVs and develop new generation vaccines.
State of the Art	*Existing knowledge including successes and failures*
	1. Epidemiological data show that CBPP continues to spread within sub-Saharan Africa, with some reports suggesting that *Mmm* may also be circulating in parts of Asia. 2. The incubation period for naturally infected animals can range from 3 weeks to 6 months with clinical manifestations ranging from hyperacute through acute, subacute and persistence of chronic forms after the clinical phase. 3. The disease is mainly localized in the lungs, where it causes a highly characteristic “marbling” of the lungs in the acute stages and lesions known as a “sequestra” that contain viable *Mmm* in the chronic form of CBPP. 4. *Mmm* is transmitted by close contact between animals within 50m. 5. The role of sequestra in transmission remains controversial. 6. The immune response to experimental infection differs when challenged by different routes such as intubation or by contact.

	7. Despite genotypic and phenotypic differences between *Mmm* strains, they all appear to fall into a single immunity group. 8. Most countries eliminated CBPP by adopting integrated control strategies, e.g., biosecurity measures, control of animal movement, vaccination, diagnosis, slaughter and compensation. 9. Animals that develop sequestra have significantly higher antibody titres against *Mmm* surface proteins than those that did not. 10. Due to the weak economies in Africa, control of CBPP via restricting cattle movement, slaughter and compensation are not suitable options. 11. The attenuated *Mmm* strains T1/44 and T1sr are recommended by WOAH for vaccination. 12. LAVs are given either sub-cutaneous in the neck (T1sr) or the tip of the tail (T1/44). 13. Side effects are only associated with T1/44, but their incidence decreases on boosting, or by including skimmed milk in the vaccine during freeze drying. 14. Vaccine efficacy of LAVs ranges from ~40-60% and increases on boosting. 15. T1/44 is more efficacious than T1sr. 16. Duration of immunity with T1/44 is 1 year and for T1sr, 6 months. 17. Control by vaccination alone requires >80% vaccine coverage and booster doses. 18. Elimination of disease with current LAVs will need booster vaccination and removal of infected cattle. 19. 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. 20. Mycoplasma specific IgA is present in bronchoalveolar lavage (BAL) and the sera of less severely affected cattle, and antiserum from convalescent animals induce mycoplasma killing by macrophages. 21. There is no correlation between antibody titres and severity of clinical disease and lesions. 22. Attempts to vaccinate with immunogenic purified proteins may lead to disease exacerbation suggesting a type III hypersensitivity reaction leading to damage by immune complexes. 23. Higher levels of IFN-g in convalescent and fully recovered animals suggested that cell mediated immunity (CMI) 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. 24. 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. 25. Simple and inexpensive changes to the current vaccine, such as the use of HEPES buffer systems and the inclusion of pH indicators together with restrictions in the use of 1 M MgSO4 as a vaccine diluent, can increase vaccine yields 10-fold and stability several 100-fold, producing a vaccine which should improve its effectiveness in the field. 26. Antibody dependent growth inhibition assays can be used in diagnosis. 27. There are encouraging results on the development of a subunit vaccine using a combination of four Mmm antigens.
Projects	*What activities are planned or underway?*
	1. The standing group of experts (SGE-CBPP) under GF-TADs aims to strengthen regional cooperation and dialogue on CBPP control in Africa through regular information exchange, technical support for reviewing national and regional CBPP control strategies, and technical formulation of disease control policies. The group will enhance collaboration on laboratory diagnosis, applied research, awareness-raising campaigns, cross-border surveillance, and risk management measures along the beef and dairy value chains. The SGE-CBPP is composed of experts from various sectors, including veterinary services, academia, research institutions, NGOs, and the private sector. 2. STAR-IDAZ-IRC: Veterinary Mycoplasmas research report, 2023. https://www.star-idaz.net/report/2023-veterinary-mycoplasmas-research-report. 3. STAR-IDAZ-IRC and USDA-ARS: Report of the gap analysis meeting for CBPP, 2023. https://www.star-idaz.net/report/report-of-the-gap-analysis-meeting-for-cbpp/ 4. DISCONTOOLS report: CBPP, 2023. https://www.discontools.eu/database/39-contagious-bovine-pleuro-pneumonia.html. 5. Institutes with ongoing CBPP research: ILRI, GALVmed, CIRAD, INRA, USDA-ARS, Uni Connecticut, KALRO, VIDO. 6. VIDO is in early stages of planning for an efficacy trial using the four Mmm antigens purified as inclusion bodies.

Lead Summary 2 - Naturally attenuated candidates

TITLE	Naturally attenuated candidates
Research Question(s)	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Live attenuated organism-based vaccines can provoke robust and long duration of immunity against disease.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Need to improve the control of CBPP using existing LAVs as it is unlikely that effort will be put into identifying new "natural" LAVs. a. Issues such as need for a cold chain, short duration of immunity after a single vaccine dose, the incidence of side effects, access to vaccine, diluent composition and short shelf life after reconstitution and policies restrict the routine use of current LAVs. 2. Lack of *in vitro* tests that correlate/associate with different clinical manifestations of and immunity to CBPP.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Resolve conflicting data on the quality, safety and use of T1/44 and T1*sr* vaccines. 2. Develop disease related metrics that do not rely on postmortem data to assess vaccine efficacy. 3. Establish if there is a dose response in cattle breeds between route of immunization and interval between primary and booster vaccinations to obtain the best safety, efficacy and duration of immunity in different age groups of cattle. 4. Field evaluation of improved vaccination regimens and protocols. 5. Develop tests to allow differentiation of infected from vaccinated animals (DIVA). 6. Develop biomarkers for improved diagnosis of different clinical phases of CBPP. 7. Increase quality and quantity of immune response in the lungs.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Agreement on whether to use T1/44 or T1sr would simplify developing solution routes. 2. Need to increase vaccine production as 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. 3. Harmonize regional, national, and continental wide vaccination policies and campaigns for better manufacture and delivery of LAVs. 4. Improve fragmented veterinary services and vaccine supply chain. 5. Incentivize use of GMP and AU-PANVAC certified vaccines and pharmacovigilance in disease control.

State of the Art	*Existing knowledge including successes and failures*
	1. Several attenuated *Mmm* strains have been isolated by passage but only T1/44 and T1sr are recommended by WOAH for vaccination. 2. Deployment of a bivalent CBPP-rinderpest vaccine led to control of CBPP in many countries, but regular CBPP vaccination was discontinued after the rinderpest eradication campaign leading to re-emergence of CBPP. a. Sustained vaccination can result in control of CBPP; modelling data suggest need >80% vaccine coverage and booster doses. 3. T1/44 and T1sr are produced as freeze-dried vaccines by seven certified laboratories in Africa. a. The minimal required titre is 10^7 mycoplasmas per vaccine dose, but higher titres of at least 10^8 are recommended. b. T1/44 induces higher efficacy and a longer duration of immunity than T1sr. c. Vaccine efficacy for T1/44 ranges from ~40-60% after a single dose given subcutaneously in the neck and efficacy levels increase on boosting. d. "Willems reaction" can occur in ~10% of animals 2 to 3 weeks post vaccination with T1/44 but is not evident on boosting. The percentage of reactors varies greatly from one region to another. e. Recommended to vaccinate animals over 6 months old except in case of a severe outbreak, as calves less than 3 months can develop arthritis or valvular heart disease. 4. T1/44 was attenuated by passaging a mild field strain 44 times in embryonated eggs. T1/44 retains low level of virulence. 5. Strain T1sr is a direct derivative of T1/44, adapted to streptomycin resistance by four serial passages in growth medium with increasing concentrations of streptomycin. T1sr has no residual virulence. • Contavax (KEVAVAPI): Freeze-dried vaccine. Stored at -20°C the shelf life is 2 years. At 2°C to 8°C the shelf life is one month. Once the vaccine has been reconstituted, it must be kept cool and used immediately. 50-100 dose pks with diluent. • BoviVax (MCI): Freeze-dried vaccine. No shelf-life data. Once the vaccine has been reconstituted, it must be kept cool and used immediately. 50-100 dose pks with diluent.
Projects	*What activities are planned or underway?*

	1. The standing group of experts (SGE-CBPP) Africa aims to strengthen regional cooperation and dialogue on CBPP control in Africa through regular information exchange, technical support for reviewing national and regional CBPP control strategies, and technical formulation of disease control policies.

Lead Summary 3 - Rationally attenuated candidates

TITLE	Rationally attenuated candidates
Research Question(s)	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Live attenuated organism-based vaccines can provoke robust and long duration of immunity against disease. 2. Develop through genetic manipulation a new live attenuated vaccine that is thermotolerant, safer and more efficacious than T1/44 (and T1sr) and allows differentiation of infected and vaccinated animals (DIVA).
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Over attenuation can lead to non-protective strains and under-attenuation can result in residual safety issues. 2. Lack of *in vitro* assays to guide down-selection of candidate attenuated strains prior to *in vivo* safety and efficacy testing. 3. Regulatory restrictions for approval of vaccines derived using GMO technology.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Knockout candidate genes that contribute to disease and test mutant strains. 2. Knockout candidate DIVA genes in attenuated strains of *Mmm*. 3. Identify candidate genes for manipulation using comparative *Mmm* and mycoplasma genomics.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Standardize cattle breed specific laboratory challenge models that reproduce CBPP caused by natural infection or are acceptable surrogate models. 2. Knowledge on the *Mmm* targets of host immune responses that contribute to disease and immunity. 3. A list of *Mmm* candidate virulence/disease causing genes. 4. A list of *Mmm* antigens that could serve as a candidate DIVA test. 5. Develop a suite of methods to identify and down-select disease causing *Mmm* molecules. 6. Rapid and accurate methods for *Mmm* genome manipulation at single and multiple loci. 7. Methods to assess safety and efficacy of novel candidate vaccines.
State of the Art	*Existing knowledge including successes and failures*
	1. Capsular glycan has been identified as a virulence factor, but it is also the target of *Mmm* killing mAbs. 2. Knockout of glycerophosphate oxidase does not attenuate *Mmm*. 3. Whole genome transplantation technology has not so far been successful for *Mmm.*

	4. CRISPR-*cas* genome editing of *Mmm* is possible.
Projects	*What activities are planned or underway?*
	ILRI/UConn/NADC teams have developed a transposon generated mutant library and are currently sequencing clones to identify mutants for potential use as rationally attenuated vaccine candidates.

Lead Summary 4 - Inactivated vaccines

TITLE	Inactivated vaccines
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. A bacterin vaccine should be intrinsically safer than T1/44 and bypasses the need to identify antigens associated with immunity. a. Attempts to develop an efficacious bacterin has not so far been successful.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. It is not clear why *Mmm* bacterins have failed as the vaccine for CCPP is based on a bacterin. a. Could be due to antigenic differences between *in vitro* cultured *Mmm* and *Mmm* during an infection. b. Sub-optimal adjuvants and antigen doses were tested in previous studies. 2. Lack of *in vitro* assays that correlate/associate with immunity to CBPP.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. More comprehensive proteomic comparison of *in vitro* versus *in vivo Mmm*. 2. Testing of new generation adjuvants may provide better immunostimulatory signals. 3. Increase quality and quantity of immune response in the lungs.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Standardized cattle breed specific laboratory challenge models that reproduce CBPP caused by natural infection or are acceptable surrogate models. 2. Knowledge on the *Mmm* targets of host immune responses that contribute to disease and immunity.
State of the Art	*Existing knowledge including successes and failures*
	1. Aluminium hydroxide used in a bacterin with no success. 2. Montanide gave limited efficacy.
Projects	*What activities are planned or underway?*

Lead Summary 5 - DNA/RNA vaccines

TITLE	DNA/RNA vaccines
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Assess if DNA/RNA platform technologies can be used to develop safer and more efficacious vaccines than current LAVs.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Lack of *in vitro* assays to guide immunogenicity and efficacy testing. 2. DNA/RNA based antigen delivery systems have not been optimized for use in cattle. 3. Regulatory restrictions may limit use of nucleic acid-based vaccines. 4. Cost could limit the use of these technologies.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Assess DNA and RNA (mRNA and sa-RNA) formulations, route and dose of immunization with a control antigen in small-scale immunogenicity studies in target species. 2. Assess ability to meet minimal and desired TPPs. 3. Assess ability to scale-up and manufacture.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Standardize cattle breed specific laboratory challenge models that reproduce CBPP caused by natural infection or are acceptable surrogate models. 2. Need optimized methods for antigen delivery via these platforms to prime antibody and T-cell responses.
State of the Art	*Existing knowledge including successes and failures*
	1. Experimental vaccine antigens have been identified.
Projects	*What activities are planned or underway?*

Lead Summary 6 - Subunit vaccines

TITLE	Subunit vaccines
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Assess if subunit platform technologies can be used to develop safer and more efficacious vaccines than current LAVs. 2. Identify and test candidate subunit vaccine antigens.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Develop a thermotolerant subunit vaccine with DIVA properties. 2. Subunit-based vaccines still at the experimental stage. 3. Lack of *in vitro* assays to guide immunogenicity and efficacy testing.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Reverse vaccinology approaches to antigen identification are being tested. 2. Identify antigens for a DIVA vaccine. 3. Assess formulations, route and dose of immunization in small-scale immunogenicity studies. 4. Assess ability to meet minimal and desired TPPs. 5. Assess ability to scale-up and manufacture.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Standardized cattle breed specific laboratory challenge models that reproduce CBPP caused by natural infection or are acceptable surrogates. 2. Better understanding of immune correlates of protection. 3. Assess ability to meet minimal and desired TPPs. 4. Assess ability to scale-up and manufacture.
State of the Art	*Existing knowledge including successes and failures*
	1. A combination of four proteins for an experimental vaccine has been identified. This does not preclude the need for identification of additional antigens.
Projects	*What activities are planned or underway?*
	1. Safety of a subunit experimental vaccine has been established. 2. Antigens suitable for a DIVA test have been identified.

Lead Summary 7 - Vectored vaccines

TITLE	Vectored vaccines
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Vectored antigen delivery systems may provoke stronger immune responses and be easier to manufacture and deliver than protein-based vaccines.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Lack of *in vitro* assays to guide immunogenicity and efficacy testing. 2. Regulatory restrictions for approval of vaccines derived using GMO technology. 3. There are many viral vectored systems that could be used.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Assess formulations, route and dose of immunization in small-scale immunogenicity studies. 2. Assess ability to meet minimal and desired TPPs. 3. Assess ability to scale-up and manufacture.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Identification of Mmm antigens that are targets of protective adaptive and innate immune responses to infection. 2. Standardized cattle breed specific laboratory challenge models that reproduce CBPP caused by natural infection or are acceptable surrogates.
State of the Art	*Existing knowledge including successes and failures*
	1. Experimental vaccine antigens have been identified.
Projects	*What activities are planned or underway?*

Lead Summary 8 - Attenuated organisms

TITLE	Attenuated organisms
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Live attenuated organism-based vaccines can provoke robust and long duration of immunity against disease. 2. Overcomes the need to identify protective antigens but requires knowledge on how to attenuate *Mmm*.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?* 1. Monitoring production quality, consistent potency, free of other contaminating microorganisms 2. Antibiotics cannot be used at the same time as the vaccine 3. Lack of *in vitro* assays that correlate/associate with immunity to CBPP.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Develop a suite of methods to identify and down-select disease causing *Mmm* molecules.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Standardize cattle breed specific laboratory challenge models that reproduce CBPP caused by natural infection or are acceptable surrogate models. 2. Knowledge on the *Mmm* targets of host immune responses that contribute to disease and immunity. 3. A list of *Mmm* candidate virulence/disease causing genes. 4. A list of *Mmm* antigens that could serve as a candidate DIVA test. 5. Rapid and accurate methods for *Mmm* genome manipulation at single and multiple loci. 6. Methods to assess safety and efficacy of novel candidate vaccines.
State of the Art	*Existing knowledge including successes and failures*
Projects	*What activities are planned or underway?*

Lead Summary 9 - Adjuvant

TITLE	Adjuvant
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Use adjuvants to generate an optimal immune response to different types of antigen delivery systems in cattle.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. While there are some guidelines on the use of different types of adjuvants, immunogenicity studies are needed. 2. Insufficient knowledge on molecular adjuvants to skew immune responses. 3. Lack of *in vitro* assays that correlate/associate with immunity to CBPP.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Further studies with Montanide and other adjuvants with the subunit vaccine.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Standardized cattle breed specific laboratory challenge models that reproduce CBPP caused by natural infection or are acceptable surrogates. 2. Identity of protective mechanisms and immune correlates of protection *in vitro* and *in vivo*. 3. Assess ability to meet minimal and desired TPPs. 4. Assess ability to scale-up and manufacture.
State of the Art	*Existing knowledge including successes and failures*
	1. Experimental vaccine antigens have been identified.
Projects	*What activities are planned or underway?*

Lead Summary 10 - Expression system

TITLE	Expression system
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Identify heterologous gene expression systems that can express recombinant *Mmm* antigens which resemble native immunogens.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Lack of *in vitro* reagents and assays to guide immunogen design. 2. Expression of proteins that resemble native antigens.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Express antigens in a non-virulent strain of mycoplasma.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Identity of a range of candidate vaccine antigens.
State of the Art	*Existing knowledge including successes and failures*
	1. A few experimental vaccine antigens have been identified.
Projects	*What activities are planned or underway?*

Lead Summary 11 - Vector

TITLE	Vector
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Vectored antigen delivery systems may provoke stronger immune responses and be easier to manufacture and deliver than protein-based vaccines.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Lack of *in vitro* assays to guide vector selection.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Structured assessment of available vector platforms for suitability to vector candidate vaccine antigens.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Standardized cattle breed specific laboratory challenge models that reproduce CBPP caused by natural infection or are acceptable surrogates.
State of the Art	*Existing knowledge including successes and failures*
	1. A few experimental vaccine antigens have been identified.
Projects	*What activities are planned or underway?*

Lead Summary 12 - Identity of protective antigens

TITLE	Identity of protective antigens
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Identify *Mmm* antigens that are the targets of innate and acquired protective immune responses 2. Use protective antigens as experimental vaccines.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Lack of assays to define candidate protective antigens.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Computational approaches to identify candidate genes. 2. Develop assays to identify candidate antigens.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Standardized cattle breed specific laboratory challenge models that reproduce CBPP caused by natural infection or are acceptable surrogates. 2. A detailed antigen map of *Mmm* through different phases of CBPP using sera and cells from different tissues and stages of disease in different breeds of cattle.
State of the Art	*Existing knowledge including successes and failures*
	1. Some experimental vaccine antigens have been identified.
Projects	*What activities are planned or underway?*
	The UConn and VIDO groups has some early data on some models that could be of utility here, in both identifying protective immune responses, as well as to serve as screening models for protective antigens

Lead Summary 13 - Identity of virulence factors

TITLE	Identity of virulence factors
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Identify *Mmm* factors that contribute to virulence and pathology resulting in disease. 2. Deletion of virulence factors could result in attenuated *Mmm* vaccines. 3. Antigenic virulence factors are candidate vaccine antigens.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Lack of assays to measure virulence factors.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Computational approaches to identify candidate genes. 2. Develop assays to measure virulence. 3. Map immunity/pathology enhancing antigens.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Standardized cattle breed specific laboratory challenge models that reproduce CBPP caused by natural infection or are acceptable surrogates. 2. Develop or re-purpose *in vitro* models that may identify putative virulence factors.
State of the Art	*Existing knowledge including successes and failures*
	1. Precision cut lung slices and cell culture experiments have been used to characterize Mmm by comparing to other mycoplasmas. 2. Some *in vitro* assays, e.g., measurement of hydrogen peroxide exist.
Projects	*What activities are planned or underway?*
	See ILRI/UConn/NADC comments above regarding transposon mutant library, and activities to screen for attenuated mutants

Lead Summary 14 - Identity of immunomodulators

TITLE	Identity of immunomodulators
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Identify *Mmm* molecules that may modulate the immune response to promote infection and survival. 2. Deletion of immunomodulatory factors may help in the design of attenuated and subunit *Mmm* vaccines.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Lack of assays to measure immunomodulatory molecules.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Computational approaches to identify candidate genes. 2. Develop assays to measure immunologically active molecules.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Computational approaches to identify candidate genes. 2. Develop assays to measure immunologically active molecules.
State of the Art	*Existing knowledge including successes and failures*
Projects	*What activities are planned or underway?*

Lead Summary 15 - Host responses to natural infection

TITLE	Host responses to natural infection
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Identify innate and acquired immune responses to *Mmm* infection as these should help in understanding mechanisms that lead to disease and immunity.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. The immune response to experimental infection differs ~~significantly~~ when challenged by different routes such as intubation or by contact.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Develop *in vitro* and *in vivo* options for evaluating host responses to infection.
Dependencies	*What else needs to be done before we can solve this need?*
	1. A better understanding of host responses and host-pathogen interactions.
State of the Art	*Existing knowledge including successes and failures*
	1. Several genomics approaches are being used to map this process.
Projects	*What activities are planned or underway?*
	The UConn group is working with ILRI to conduct transcriptomic and proteomic work on both pathogen and host isolated from experimentally challenged cattle both in situ (lung tissue) and peripheral/systemic responses.

Lead Summary 16A - Identity of mechanisms of protection (antibody responses)

TITLE	Identity of mechanisms of protection (antibody responses)
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Identify antigen specific antibody responses to *Mmm* molecules as this should help in understanding mechanisms that lead to disease and immunity.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Lack of assays to measure functionally relevant immune responses. 2. Lack of disease stage biomarkers.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Develop *in vitro* and *in vivo* options for evaluating host responses to infection. 2. Passive antibody transfer experiments.
Dependencies	*What else needs to be done before we can solve this need?*
State of the Art	*Existing knowledge including successes and failures*
Projects	*What activities are planned or underway?*

Lead Summary 16B - Identity of mechanisms of protection (cell mediated immunity)

TITLE	Identity of mechanisms of protection (cell mediated immunity)
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Identify antigen specific cell mediated immune responses to *Mmm* molecules as this should help in understanding mechanisms that lead to disease and immunity.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Lack of assays to measure functionally relevant immune responses. 2. Lack of disease stage biomarkers.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Develop *in vitro* and *in vivo* options for evaluating host responses to infection. 2. Passive transfer and cell depletion experiments.
Dependencies	*What else needs to be done before we can solve this need?*
State of the Art	*Existing knowledge including successes and failures*
Projects	*What activities are planned or underway?*
	Work by UConn/ILRI group is looking into defining *in situ* immune responses from experimentally infected animals to identify responses that contribute to pathology vs clearance

Lead Summary 17A - Host-pathogen interactions (entry)

TITLE	Host-pathogen interactions (entry)
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Characterise host and *Mmm* molecules involved in establishing an infection as an improved understanding of this process should help design better vaccines.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Lack of high-throughput assays to measure bacterial proliferation in the host or host-like conditions. 2. Lack of standardized assays.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Knowledge on colonization to prevent infection and disease. 2. Understanding and preventing the establishment of disease is likely to be more promising in moving towards any intervention.
Dependencies	*What else needs to be done before we can solve this need?*
State of the Art	*Existing knowledge including successes and failures*
	1. Mmm is less capable of invading macrophages than *M. bovis*. 2. Mmm specific antisera promotes phagocytosis and killing.
Projects	*What activities are planned or underway?*

Lead Summary 17B - Host-pathogen interactions (replication)

TITLE	Host-pathogen interactions (replication)
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Characterise host and *Mmm* molecules involved in bacterial proliferation as an improved understanding of this process should help design better vaccines.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Lack of standardized assays to measure bacterial replication.
Solution Routes	*What approaches could/should be taken to address the research question?*
Dependencies	*What else needs to be done before we can solve this need?*
State of the Art	*Existing knowledge including successes and failures*
Projects	*What activities are planned or underway?*

Lead Summary 17C - Host-pathogen interactions (persistence/clearance)

TITLE	Host-pathogen interactions (persistence/clearance)
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Characterise host and *Mmm* molecules involved in maintaining and clearing of an infection as an improved understanding of these processes should help design better vaccines.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. Lack of standardized assays to measure sites of bacterial persistence and clearance.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Understanding the dynamics needed to maintain or clear an infection is important in developing a response to improve bacterial clearance.
Dependencies	*What else needs to be done before we can solve this need?*
State of the Art	*Existing knowledge including successes and failures*
Projects	*What activities are planned or underway?*

Lead Summary 18 - Challenge model

TITLE	Challenge model
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Accelerate vaccine R&D by developing a laboratory-based infection model in cattle to reproduce CBPP.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. The incubation period for naturally infected animals ranges from 3 weeks to 6 months with clinical manifestations ranging from hyperacute through acute, subacute and persistence of chronic forms after the clinical phase. 2. Cattle breed and age influence immunity and huge differences can occur between individuals in response to infection. 3. Lack of *in vitro* diagnostic tests that correlate with immunity.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Assess different aerosol delivery-based systems.
Dependencies	*What else needs to be done before we can solve this need?*
	1. Develop community agreed minimum and optimal standards and methods to measure vaccine efficacy. 2. Develop vaccine TPPs to prioritize and guide vaccine R&D for laboratory and field challenge.
State of the Art	*Existing knowledge including successes and failures*
	1. Intubation – successfully infects 50% of the inoculated animals. Failure is that large numbers of cattle are needed, and the method does not reproduce disease as in the field. 2. Nebulizer – has been used with cattle but the method still needs improvement. 3. Laboratory mice – disease produced is systemic without lung lesions and does not serve the intended purpose 4. The disease is mainly localized in the lungs, where it causes a highly characteristic “marbling” of the lungs in the acute stages and lesions known as a “sequestra” that contain *Mmm* in the chronic form of CBPP. 5. *Mmm* is transmitted by close and repeated contact between animals within 50m. 6. The immune response to experimental infection differs significantly when challenged by different routes such as intubation or by contact.
Projects	*What activities are planned or underway?*
	1. Use of different neubulizer designs to replace intubation.

Lead Summary 19 - Pathogen genome

TITLE	Pathogen genome
Research Question	*What are we trying to achieve and why? What is the problem we are trying to solve?*
	1. Use a genomics approach to acquire knowledge on the cell-biology of *Mmm* (*Mycoplasma mycoides* subsp. *mycoides*) and host-pathogen interactions to aid the development of improved live attenuated and subunit vaccines (and diagnostics) for the control (and eradication) of CBPP.
Challenge(s)	*What are the scientific and technological challenges (knowledge gaps needing to be addressed)?*
	1. *Mmm* contains many hypothetical genes and genes of unknown function. 2. To target vaccine R&D from an understanding of *Mmm* at a molecular level and strain differences as well as the host responses to infection and those which contribute to immunity to CBPP and those that contribute to disease.
Solution Routes	*What approaches could/should be taken to address the research question?*
	1. Complete whole genome sequencing and annotation to define the pan and core genome of *Mmm*. 2. Develop a database of the transcriptome, proteome, glycome, other 'omes of *Mmm*. 3. Map gene expression regulatory circuits. 4. Create a comparative genomics database of *Mmm*, the mycoides cluster and other mycoplasma species. 5. Use computational tools to predict protein function/s and to identify candidate virulence factors, immunomodulators and vaccine antigens. 6. Undertake forward and reverse vaccinology approaches to identify candidate vaccine antigens. 7. Target *Mmm* molecules based on antigen homologs.
Dependencies	*What else needs to be done before we can solve this need?*
	1. A collection of well characterized laboratory and recent field strains of *Mmm* and related mycoplasmas. 2. Assays and methods to unravel the cell-biology of *Mmm* and host-pathogen interactions. 3. Assays and methods to select and down-select candidate subunit vaccine antigens. 4. Assays, methods and biomarkers to design improved LAVs. 5. A library *Mmm* mutants to link genotype to phenotype. 6. A challenge model that resembles natural infection.
State of the Art	*Existing knowledge including successes and failures*

	1. *Mmm* belongs to the "mycoides cluster" consisting of four additional species (*Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp)*, the agent of CCPP), *Mycoplasma capricolum* subsp. *capricolum* (*Mcc*), *Mycoplasma leachii* and *Mycoplasma mycoides* subsp. *capri* (*Mmc*). 2. The five mycoplasma species share phenotypic and genotypic characteristics that cause cross-reactions in conventional diagnostic techniques. 3. The closest relative to *Mmm* is *Mmc*, which is usually found in goats. 4. *Mmm* is a new pathogen that evolved about 300 years ago. 5. Whole genome transplantation for *Mmm* has not been successful so far. 6. CRISPR-cas base editing technology has been developed for *Mmm*. 7. Reverse vaccinology and other approaches have identified candidate vaccine antigens.
Projects	*What activities are planned or underway?*
	1. The U Connecticut group is working with ILRI to conduct transcriptomic and proteomic work on both pathogen and host isolated from experimentally challenged cattle both in situ (lung tissue) and peripheral/systemic responses. 2. Work by U Connecticut/ILRI group is looking into defining *in situ* immune responses from experimentally infected animals to identify responses that contribute to pathology vs clearance. 3. The U Connecticut group has some early data on some models that could be of utility here, in both identifying protective immune responses, as well as to serve as screening models for protective antigens. 4. ILRI/U Connecticut/USDA-ARS teams have developed a large transposon generated mutant library and are currently sequencing to identify mutants of vaccine and biological interest.