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(54) Title: VACCINES COMPRISING ATTENUATED MYCOPLASMA BOVIS STRAINS AND METHOD FOR THE ATTENUATION

(57) Abstract: The present invention relates to new attenuated mycoplasma bovis bacteria strains passaged at least 110 times. Moreover, the present invention also provides immunogenic compositions comprising live bacteria of any of those attenuated *M. bovis* bacteria strain, their manufacture and use for the treatment and prophylaxis of *M. bovis* infections and combinations with other veterinary vaccines or medicaments.

IMPROVED MODIFIED LIVE VACCINE OF MYCOPLASMA BOVIS, METHODS OF PRODUCING MODIFIED LIVE MYCOPLASMA BOVIS VACCINES, COMBINATION VACCINES AND METHODS OF TREATMENT

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RELATED APPLICATIONS

This application claims the priority benefit of U.S. Provisional Patent Application Serial Number 61/172,543, filed on April 24, 2009.

SEQUENCE LISTING

10 The instant application contains a Sequence Listing which has been submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 20, 2010, is named 100118PC.txt and is 103,856 bytes in size.

BACKGROUND OF THE INVENTION

15 *Mycoplasma bovis* (*M. bovis*) is considered to be one of the more pathogenic species of Mycoplasma and causes significant economic losses worldwide. Mycoplasmas cause severe clinical signs in cattle of all ages. *M. bovis* is the most frequent *Mycoplasma* pathogen found to cause pneumonia, mastitis, and arthritis in cattle and its etiological role has also been associated with otitis, keratoconjunctivitis, synovitis, and reproductive disorders in cows and bulls. In 20 general, Mycoplasmas are difficult to treat since they lack a cell wall or membrane, which tends to make them resistant to several classes of commonly used broad-spectrum antibiotic treatments. Mycoplasmas differ from viruses in that Mycoplasmas are larger than most viruses and damage tissue cells by attaching to the surface of cells and destroying them, rather than by entering the cells. Animals infected with *M. bovis* have depressed immune responses and may 25 exhibit signs of *M. bovis* infection such as fever, depression, anorexia, labored breathing, nasal and ocular discharge, coughing, sneezing, gasping, grunting, lameness and swollen joints, mastitis, middle ear infections, abortions, recumbence and death. The organism persists in unsanitary, warm, moist environments. Mycoplasmas may survive in milk, and even seem to thrive in the presence of large numbers of leukocytes, which are produced in response to the 30 infection.

US 6,548,069 discloses a vaccine composition that incorporates a whole cell inactivated bacterin containing at least two killed *M. bovis* strains and that an isolate may rapidly alter its

5 antigens in culture. The patent teaches that high passage strains of greater than about 50 passages may lose infectivity and elicit a poorer immune response when used in a bacterin. It teaches use of a *Mycoplasma* strain which has been passed no more than about ten times or less before mass scale production because the antigens are believed to retain their natural state and thus will elicit a protective immune response against the infectious microorganism.

10 Killed *M. bovis* is not as effective or efficient as desired in lessening the severity of clinical symptoms associated with a *Mycoplasma bovis* infection. Even passage at a low level does not produce a *Mycoplasma* vaccine with high efficacy such that clinical symptoms are greatly reduced in animals when compared to animals not receiving such a vaccine. The few low 15 passage, inactivated, *M. bovis* vaccines that are available do not show a large reduction in the severity of clinical symptoms in animals when compared to animals not receiving such vaccine.

15 The nature of the market requires that farmers be able to effectively immunize their animals for a wide variety of conditions in an efficient way. Other conditions that would be suitable for efficient immunization include, but are not limited to, Bovine viral diarrhea virus (BVDV), *Parainfluenza-3 virus* (PI-3), *Bovine Respiratory Syncytial Virus* (BRSV), *Bovine Herpesvirus* (BHV-1), *Bovine rotavirus*, Breda virus, a calici-like virus, *Adenovirus*, *Astrovirus* and *Parvovirus*, *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*), *Pasteurella multocida*, *Actinomyces (Arcanobacterium) pyogenes*, *. Haemophilus somnus* (reclassified as *Histophilus somni*), *Chlamydiae*, Bovine genital campylobacteriosis, *Leptospirosis*, Brucellosis, 20 *Clostridia*, *Escherichia coli*, *Cryptosporidium parvum*, *Mycobacterium avium paratuberculosis*, *Salmonella*, *Mycobacterium avium paratuberculosis*, *Cryptosporidiosis*, mastitis, *Dermatophytes*, lower respiratory tract infections, *Trichomoniasis*, *Neospora Canum*, *Babesiosis* and the like.

25 There remains a need for an immunogenic composition effective for eliciting an immunological response against *M. bovis* for lessening the severity of or reducing the incidence of signs of *M. bovis* infection, and for reducing or eliminating the incidence of signs of *M. bovis* infection.

## Summary of the Invention

The present invention provides an immunogenic composition or vaccine which uses high passage attenuated *M. bovis* strains, such that signs of *M. bovis* infection and/or the *M. bovis* infection itself and/or incidence or severity, are reduced in animals receiving the immunogenic composition or vaccine as compared to those animals with infection by wild-type *M. bovis* strains. The immunological composition of the present invention provides rapid onset of protection and long-lasting protection to an animal in need thereof.

According to a first aspect of the present invention, there is provided an immunogenic composition comprising an attenuated, avirulent *Mycoplasma bovis* bacterium strain, wherein

- 5 the bacterium is passaged more than 110 times, wherein the attenuated, avirulent *Mycoplasma bovis* strain is selected from the group consisting of: the attenuated *Mycoplasma bovis* bacteria strain deposited with the ATCC under accession number PTA-9666 and PTA-9667, any attenuated descendant *Mycoplasma bovis* bacteria strains thereof when used as live vaccine, reduces the mortality rate in a group of animals of at least 56% as compared to a non-vaccinated
- 10 control group of animals after the administration of a single dose of said vaccine.

According to a second aspect of the present invention, there is provided a method of producing an immunogenic composition in accordance with the first aspect of the present invention, comprising admixing said bacteria of an attenuated, avirulent *Mycoplasma bovis* strain with a pharmaceutical acceptable carrier.

- 15 According to a third aspect of the present invention, there is provided a method for the treatment or prophylaxis of infections caused by *Mycoplasma bovis*, comprising, administering an effective amount of the immunogenic composition in accordance with the first aspect of the present invention to an animal, wherein said treatment or prophylaxis is selected from the group consisting of reducing signs of *Mycoplasma bovis* infection, reducing the severity of or
- 20 incidence of clinical signs of *Mycoplasma bovis* infection, reducing the mortality of animals from *Mycoplasma bovis* infection, and combinations thereof.

According to a fourth aspect of the present invention, there is provided a method for reducing the incidence of mortality of animals resulting from infection by *Mycoplasma bovis* comprising administering the immunogenic composition in accordance with the first aspect of the present invention to an animal.

5 According to a fifth aspect of the present invention, there is provided a method of producing an immunogenic composition comprising attenuating *Mycoplasma bovis*, comprising,

- a. passaging *Mycoplasma bovis* bacteria more than 135 times to produce a cultured *Mycoplasma bovis* bacteria;
- b. obtaining the cultured *Mycoplasma bovis* bacteria;
- 10 c. testing the cultured *Mycoplasma bovis* bacteria obtained under step b) for their pathogenicity and immunogenicity; and
- d. propagating the non-pathogenic, but immunogenic *Mycoplasma bovis* bacteria to obtain the attenuated *Mycoplasma bovis* bacteria.

The invention provides for attenuated and avirulent strains or isolates of *M. bovis* which have been passaged at least 110 times that provoke or elicit an immune response when administered to an animal. According to another aspect, the present invention also relates to attenuated *M. bovis* bacteria having the same characteristics as the *M. bovis* bacteria strain deposited with the ATCC under accession numbers PTA-9666 and PTA-9667.

15 An *M. bovis* strain of the present invention, attenuated through multiple passage or serial attenuation as described above, may be used as a medicine, preferably as a veterinary medicine. Further, the attenuated *M. bovis* strains of the present invention may be used for the preparation of veterinary compositions, for the prophylaxis or treatment of infections caused by *M. bovis* in animals susceptible to infection by *M. bovis*.

Such an immunological composition would be suitable as either a one dose or two dose or multi-dose (initial dose followed by booster(s)) immunization regimen, an immunological composition suitable and convenient for administration by several routes, and an immunological composition that is compatible with other immunogens and immunological compositions for preparation of combination vaccines.

In another embodiment of the present invention, the *M. bovis* strains of the present invention may be combined with other medicaments, therapies or vaccines.

The present invention also provides for a method of making the immunogenic composition of the present invention. The method comprises obtaining a virulent strain of *M. bovis* and passaging said strain at least 110 times, such that it becomes attenuated and avirulent. The high passage strain may then be mixed with an additional components including but not limited to, pharmaceutically acceptable carriers, diluents, other medicaments, therapeutic compositions or vaccines, and combinations thereof.

The present invention also provides for a method of treatment or prophylaxis of animals having an *M. Bovis* infection to reduce signs of *M. bovis* infection, reduce the severity of or incidence of clinical signs of *M. bovis* infection, reduce the mortality of animals from *M. bovis* infection, and combinations thereof.

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#### BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1** is a graph illustrating lung lesion scores for animals receiving an *M. bovis* isolate passaged 135 times in accordance with the present invention isolate;

**Fig. 2** is a graph illustrating total lameness scores for animals receiving an *M. bovis* isolate 10 passaged 135 times in accordance with the present invention;

**Fig. 3** is a graph illustrating total joint swelling for animals receiving an *M. bovis* isolate passaged 135 times in accordance with the present invention;

**Fig. 4** is a graph illustrating arthritis scores for animals receiving an *M. bovis* isolate passaged 135 times in accordance with the present invention;

**15 Fig. 5** is a graph illustrating a comparison of serology for Live Vac I, II, III and No Vaccine Group (SQ+IN only); and

**Fig. 6** is a graph illustrating a comparison of serology for Live Vac I using various routes of administration.

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#### DETAILED DESCRIPTION OF THE INVENTION

##### Definitions

For purposes of the present invention, the terms “isolate” and “strain” are used interchangeably and that differences between individual strains or isolates may be detected using DNA fingerprinting (i.e. different strains or isolates will have differing fingerprints). For 25 purposes of the present invention, the terms “vaccine” and “composition” are used interchangeably.

The following terms and expressions are used herein:

“Attenuation” means reducing the virulence of a pathogen. In the present invention “attenuation” is synonymous with “avirulent”. In the present invention, an attenuated bacterium 30 is one in which the virulence has been reduced so that it does not cause clinical signs of a *M. bovis* infection but is capable of inducing an immune response in the target mammal, but may

also mean that the clinical signs are reduced in incidence or severity in animals infected with the attenuated *M. bovis* in comparison with a “control group” of animals infected with non-attenuated *M. bovis* and not receiving the attenuated bacterium. In this context, the term “reduce/reduced” means a reduction of at least 10%, preferably 25%, even more preferably 50%, 5 still more preferably 60%, even more preferably 70%, still more preferably 80%, even more preferably 90% and most preferably of 100% as compared to the control group as defined above. Thus, an attenuated, avirulent *M. bovis* strain is one that suitable for incorporation into an immunogenic composition comprising a modified live *M. bovis* bacterium.

“Diluents” may include water, saline, dextrose, ethanol, glycerol, and the like. Isotonic 10 agents may include sodium chloride, dextrose, mannitol, sorbitol, and lactose, among others. Stabilizers include albumin and alkali salts of ethylenediamintetraacetic acid, among others.

An “effective amount” for purposes of the present invention, means an amount of an immunogenic composition capable of inducing an immune response that reduces the incidence of or lessens the severity of *M. bovis* infection in an animal. Particularly, an effective amount refers 15 to colony forming units (CFU) per dose. The preferred immunogenic composition or vaccine of the present invention has at least 1.0 E7 CFU of the live bacteria of the attenuated, avirulent *M. bovis* bacteria per dose, more preferably 8.4 E7 CFU or 9.4 E7 CFU of the live bacteria of the attenuated, avirulent *M. bovis* bacteria per dose. An example (but not meant to be limited to such example) of an effective amount would be an *M. bovis* vaccine administered via a simultaneous 20 intranasal and subcutaneous route administered 2 times with a 2 week interval at a high dose level (1E9 CFU).

“High passage strain” as well as the term “passaged at least 110 times” for purposes of the present invention, refers to an *M. bovis* strain that has been passaged more than 110 times, more preferably, more than 115 times, more preferably, more than 120 times, even more 25 preferably, more than 125, even more preferably, more than 130 times, more preferably, more than 133 times, even more preferably, more than 135 times, and still more preferably between 135 and 145 times, specifically more preferably more than 140 times, and more preferably more than 145 times, and further more preferably more than 150 times.

The term “having the characteristics as the *M. bovis* bacteria strain deposited with the 30 ATCC under accession numbers PTA-9666 and PTA-9667” means that such a bacteria strain is attenuated, and is capable to reduce the mortality and euthanization rate in a group of animals of

at least 83% as compared to a non-vaccinated control group of animals. Furthermore, the term also means that such a bacteria strain is attenuated, and is cable to reduce the mortality and euthanization rate in a group of animals at least 56% when compared to a non-vaccinated control group of animals after the administration of a single dose of said vaccine. Additionally, the term 5 also means that such a bacteria strain is attenuated, and is cable to reduce the mortality and euthanization rate in a group of animals at least 65% when compared to a non-vaccinated control group of animals after the administration of a single dose of said vaccine.

An “immunogenic or immunological composition” refers to a composition of matter that comprises at least one antigen, which elicits an immunological response in the host of a cellular 10 and/or antibody-mediated immune response to the composition or vaccine of interest. Usually, an “immunological response” includes but is not limited to one or more of the following effects: the production or activation of antibodies, B cells, helper T cells, suppressor T cells, and/or cytotoxic T cells and/or gamma-delta T cells, directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably, the host will display either a therapeutic or 15 protective immunological response such that resistance to new infection will be enhanced and/or the clinical severity of the disease reduced. Such protection will be demonstrated by either a reduction or lack of clinical signs normally displayed by an infected host, a quicker recovery time and/or a lowered duration or bacterial titer in the tissues or body fluids or excretions of the infected host.

20 The term “Immunogenic testing” means infecting cattle with the passaged *M. bovis* bacteria and monitoring the development of the humoral antibody response against *M. bovis* in the infected cattle.

The term “Improved efficacy such that clinical signs associated with *M. bovis* infection and/or the *M. bovis* infection itself were reduced in comparison to currently available vaccines 25 when vaccines are exposed to *M. bovis* or suffer infection by wild-type *M. bovis* strains” refers to a reduction in either the incidence of or severity of clinical signs of *M. bovis* infection when comparing vaccines made from strains passaged as taught by the present invention with *M. bovis* vaccines that were available prior to this invention. In this context, animals not vaccinated, or vaccinated with *M. bovis* vaccines available prior to the present invention will have clinical signs 30 of *M. bovis* infection that are at least 30%, and possibly up to more preferably at least 40%, still more preferably at least 50%, even more preferably at least 60%, still more preferably at least

70%, even more preferably at least 75%, still more preferably at least 80%, even more preferably at least 85%, still more preferably at least 90%, and most preferably at least 95% more severe or prevalent than in animals receiving an administration of an *M. bovis* immunogenic composition in accordance with the present invention.

5 The term “in need of such administration” or “in need of such administration treatment”, means that the administration or treatment is associated with the boosting or improvement in health or any other positive medicinal effect on health of the animals which receive the immunogenic composition in accordance with the present invention.

10 The term “Long-lasting protection” refers to improved efficacy that persists for at least 3 weeks, but more preferably at least 6 months, still more preferably at least 1 year, even more preferably at least 2 years for beef animals, and at least 6 months, more preferably at least 1 year, still more preferably at least 2 years, still more preferably at least 3 years, and even more preferably at least 4 years for dairy animals. For both dairy animals and beef animals, it is most preferred that the long lasting protection shall persist until the average age at which beef animals 15 are marketed for meat and the age at which dairy animals conclude their productive life of milking.

20 The term “Lung Pathology Assessment” refers to observation of the lungs after necropsy, including, but not limited to, assessment of consolidation, lesions, and nodular formations as well as assessment of the thoracic cavity including pleuritis and fluid accumulation.

The term “Mortality” refers to death caused by *M. bovis* infection. This includes the situation where the infection is so severe that an animal is euthanized to prevent suffering and provide a humane ending to their life.

25 The term “Signs of *M. bovis* infection” refers to the manifestations of infection or disease caused by *M. bovis* including both the clinical symptom(s) and pathology typically experienced by cattle infected with wild type *M. bovis*. These manifestations of infection or disease may take many forms including, but not limited to, fever, depression, anorexia, labored breathing, nasal and ocular discharge, coughing, sneezing, gasping, grunting, lameness and swollen joints, middle ear infections, discharge from inflammation of the inner ear, abortions and 30 other reproductive disorders, recumbence, respiratory infection, head tilt, ataxia, arthritis,

mastitis, otitis, keratoconjunctivitis, synovitis, pleuritis, lung lesions, lung consolidation and nodular formation in the lungs, increased synovial fluid, thickened joint capsules, and death.

The term a “veterinary acceptable carrier” “pharmaceutically acceptable carrier” or “carrier” includes any and all solvents, dispersion media, coatings, adjuvants, stabilizing agents, 5 diluents, preservatives, antibacterial and antifungal agents, isotonic agents, adsorption delaying agents, and the like. In some preferred embodiments, and especially those that include lyophilized immunogenic compositions, stabilizing agents for use in the present invention include stabilizers for lyophilization or freeze-drying.

The present invention provides an immunogenic composition or vaccine which 10 overcomes the problems inherent in previous vaccines and provides a safe, efficacious vaccine utilizing high passage attenuated *M. bovis* strains, such that signs of *M. bovis* infection and/or the *M. bovis* infection itself and/or incidence or severity, are reduced in animals receiving the immunogenic composition or vaccine comparison with infection by wild-type *M. bovis* strains. Additionally, the lethal effect of *M. bovis* is reduced when the immunogenic composition of the 15 present invention is administered to an animal (e.g., calves given a vaccine in accordance with the present invention are at a lower risk of developing signs of *M. bovis* infection, as well as death associated with *M. bovis* infection, and any clinical signs that result would be less severe or prevalent than in animals not receiving any vaccine, but were infected with *M. bovis* or receiving a vaccine not in accordance with the present invention).

20 Additionally, herds would experience a smaller number of infected and deceased animals in a herd when animals are administered the vaccine in accordance with the present invention as compared to non-vaccinated but infected animals, and preferably even as compared to animals vaccinated with conventionally available vaccine(s). The high passage, attenuated strains of the present invention provide added efficacy when compared to other vaccines currently on the 25 market.

The present invention provides for attenuated and avirulent strains or isolates of *M. bovis* which have been passaged at least 110 times that provoke or elicit an immune response when administered to an animal. Advantageously, such attenuated and avirulent strains or isolates of *M. bovis* which have been passaged at least 110 times provokes or elicits an immune response 30 that protects the animals receiving the immunogenic composition of the present invention and reduces the risk of the animals dying or having to be euthanized as a result of *M. bovis* infection.

The immunogenic composition also has the benefit of reducing the number of animals in a herd experiencing death or euthanasia as a result of *M. bovis* infection. Further, the composition has been shown to lessen the incidence and severity of clinical signs of *M. bovis* infections in individual animals and herds.

5 In one embodiment, an immunogenic composition is disclosed which comprises one or more high passage *M. bovis* strain(s) which have been passaged at least 110 times and a pharmaceutically acceptable carrier. The immunogenic composition of the present invention elicits an immune or immunogenic response against *M. bovis* infection in animals, and preferably cattle. Generation of the immunogenic response has the effect of lessening the incidence and 10 severity of clinical signs of *M. bovis* infection as well as reducing mortality and euthanization as a result of *M. bovis* infection.

Another embodiment provides for a method of making the immunogenic composition of the present invention. The method comprises obtaining a virulent strain of *M. bovis* and passaging said strain at least 110 times, such that it becomes attenuated and avirulent. The high 15 passage strain may optionally then be mixed with additional components including but not limited to, adjuvants, pharmaceutically acceptable carriers, diluents, and combinations thereof. The method generally comprises (a) passaging *M. bovis* bacteria more than 110 times to produce a cultured *M. bovis* bacteria; (b) obtaining the cultured *M. bovis* bacteria; and (c) propagating the non-pathogenic, but immunogenic *M. bovis* bacteria to obtain the attenuated *M. bovis* bacteria.

20 In preferred forms of this method, an additional step of testing the cultured *M. bovis* bacteria obtained under step (b) for its pathogenicity and immunogenicity. Preferably, this step is done prior to step (c). Pathogenicity testing comprises infecting cattle with the passaged *M. bovis* bacteria and monitoring the infected cattle for developing clinical symptoms of an *M. bovis* infection.

25 The present invention also provides for a method of reducing the incidence of death or euthanasia resulting from *M. bovis* infection in an individual cow or within a herd of cattle. The method comprises administration of a high passage strain of *M. bovis*, passaged at least 110 times, to a bovine. The immunogenic composition of the present invention has been shown to reduce mortality in cattle and in herds when compared to those animals not receiving a vaccine 30 as well as compared to those strains passaged less than 110 times. Preferably, mortality in cattle and in herds is reduced by at least 10%, more preferably, mortality is reduced by at least 20%,

even more preferably, mortality is reduced by at least 25%, more preferably, mortality is reduced by at least 30%, even more preferably, mortality is reduced by at least 40%, still more preferably, mortality is reduced by at least 50%, even more preferably, mortality is reduced by at least 56%, still more preferably mortality is reduced by at least 60%, even more preferably, mortality is reduced by at least 70%, still more preferably, mortality is reduced by at least 75%, even more preferably, mortality is reduced by at least 80%, still more preferably, mortality is reduced by at least 83%, and, most preferably, mortality is reduced by at least 90% as compared to those animals not receiving a vaccine.

Additionally, mortality in cattle and in herds is reduced by at least 10%, more preferably, mortality is reduced by at least 20%, even more preferably, mortality is reduced by at least 25%, more preferably, mortality is reduced by at least 30%, even more preferably, mortality is reduced by at least 40%, still more preferably, mortality is reduced by at least 48% as compared to those strains passaged less than 110 times as compared to other *M. bovis* stains, including for example, *M. bovis* bacteria strains deposited with the ATCC under accession numbers PTA-8694; PTA 15 8695; or PTA 8696.

Another embodiment includes a method for the treatment or prophylaxis of infections caused by *M. bovis*. The method comprises administering an effective amount of the immunogenic composition of the present invention to an animal, wherein said treatment or prophylaxis is selected from the group consisting of reducing signs of *M. bovis* infection, 20 reducing the severity of or incidence of clinical signs of *M. bovis* infection, reducing the mortality of animals from *M. bovis* infection, and combinations thereof.

A further embodiment includes a method of reducing the incidence and/or severity of clinical symptoms of *M. bovis* infection. The method generally comprises administration of a high passage strain of *M. bovis*, passaged at least 110 times, to animals, preferably cattle. More 25 particularly, the method may be used to reduce lung consolidation due to *M. bovis* infection. The effectiveness of the administration of a high passage strain of *M. bovis* to an animal in need thereof may be verified in a number of conventional ways, including lung pathology assessment. Preferably, lung pathology assessment, specifically the percentage of lung consolidation attributed to lesions due to *M. bovis* as customarily scored for various species may be made post-30 necropsy. Still more preferably, such lung pathology will be reduced when compared to the non-vaccinated group, by at least 33%, more preferably at least 50%, even more preferably at least

70%, still more preferably at least 80%, even more preferably at least 90%, and most preferably by at least 95%.

A surprising result of the present invention found that further passaging of the *M. bovis* strain led to increased efficacy of the vaccine. When results of efficacy studies for previous high passage strains, i.e. those passaged less than 110 times (e.g., *M. bovis* bacteria strains deposited with the ATCC under accession numbers PTA-8694; PTA 8695 or PTA 8696), were compared to the results of those studies using the higher passage strain of the present invention, it was surprisingly found that there was a reduction in mortality or euthanasia for humanitarian reasons for cattle resulting from *M. bovis* infection using the strain of the present invention.

It was an additionally surprising result that administration of one dose of the higher passaged strain of the present invention was as or more efficient and efficacious as the two dose administration of the previous high passage strain, passaged less than 110 times, in reducing of the number of cattle experiencing death or euthanasia as a result of *M. bovis* infection. While a reduction in mortality of about 56%, as compared to a non-vaccinated animals, was provided by a single administration of the attenuated *M. bovis* strains of the present invention passaged more than 110 times, two administrations of attenuated *M. bovis* strains passaged not more 106 times were needed to obtain the same reduction in mortality.

In a preferred embodiment, the high passage strain of *M. bovis* is passaged at least 110 times *in vitro* in cell culture, more preferably, between 110 times and 200 times, even more preferably, between about 110 and 180 times, still more preferably, at least 115 times, even more preferably at least 120 times, still more preferably, between 120 and 170 times, even more preferably at least 125 times, still more preferably between 125 and 160 times, even more preferably, at least 130 times, still more preferably, between 130 and 150 times, even more preferably at least 131 times, more preferably, at least 132 times, even more preferably, at least 133 times, more preferably, at least 134 times, and even more preferably, at least 135 times, and most preferably between 135 and 145 times.

The strains of *M. bovis* useful in the vaccine or immunogenic composition may be any strain or isolate of *M. bovis* having the same properties like inventive strains as described herein. Representative strains include those deposited with the ATCC in Manassas, VA on December 18, 2008, under the terms of the Budapest Treaty and designated as ATCC deposit numbers PTA-9666 and PTA-9667. These strains are pathogenic prior to passaging, but after passaging

the strain as described above, and particularly after passaging more than 110 times, the resultant passaged strains were attenuated, avirulent, and produced an immune response in an animal receiving an administration of the immunogenic composition of the strain. In particular, these strains led to increased efficacy and show a reduced mortality in cattle and in herds when used as 5 a modified live vaccine. Advantageously, the vaccine or immunogenic composition of the present invention utilizing such deposited strains exhibited effective cross-protection against *M. bovis* strains other than the strain passaged to attenuation and then used as an antigenic component.

10 Combination Compositions

In another embodiment of the present invention, the *M. bovis* strains of the present invention may be combined with other medicaments, therapies or vaccines. Conditions that would be suitable for efficient immunization include, but are not limited to, Bovine viral diarrhea virus (BVDV), *Parainfluenza-3 virus* (PI-3), *Bovine Respiratory Syncytial Virus* (BRSV), 15 *Bovine Herpesvirus* (BHV-1), *Bovine rotavirus*, Breda virus, a calici-like virus, *Adenovirus*, *Astrovirus* and *Parvovirus*, *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*), *Pasteurella multocida*, *Actinomyces (Arcanobacterium) pyogenes*, *Haemophilus somnus* (reclassified as *Histophilus somni*), *Chlamydiae*, Bovine genital campylobacteriosis, *Leptospirosis*, Brucellosis, *Clostridia*, *Escherichia coli*, *Cryptosporidium parvum*, 20 *Mycobacterium avium paratuberculosis*, *Salmonella*, *Mycobacterium avium paratuberculosis*, *Cryptosporidiosis*, mastitis, *Dermatomycoses*, lower respiratory tract infections, *Trichomoniases*, *Neospora Canum*, *Babesiosis* and the like.

*Bovine viral diarrhea virus* (BVDV) type 1 (BVDV-1) and type 2 (BVDV-2) cause bovine viral diarrhea (BVD) and mucosal disease (MD) in cattle (Baker, 1987; Moennig and 25 Plagemann, 1992; Thiel et al., 1996, hereby entirely incorporated by reference). The division of BVDV into 2 serotypes is based on significant differences at the level of genomic sequences (summarized in Heinz et al., 2000, hereby entirely incorporated by reference) which are also obvious from limited cross neutralizing antibody reactions (Ridpath et al. 1994, entirely incorporated by reference). Inactivation of the RNase activity residing within the E<sup>ms</sup> results in 30 an attenuated apathogenic BVDV which may be used as a modified live vaccine (WO 99/64604, hereby entirely incorporated by reference). The international patent application WO2005/111201

(hereby entirely incorporated by reference) provides a further generation of an attenuated BVDV suitable for MLV vaccines, which comprises a multiple modified BVDV having at least one mutation in the coding sequence for glycoprotein E<sup>rns</sup> and at least another mutation in the coding sequence for N<sup>pro</sup>, wherein said mutation in the coding sequence for glycoprotein E<sup>rns</sup> leads to 5 inactivation of RNase activity residing in E<sup>rns</sup> and/or said mutation in the coding sequence for N<sup>pro</sup> leads to inactivation of said N<sup>pro</sup>. Furthermore, various conventional attenuated BVDV viruses are known in the art, which are also suitable candidates for vaccine development.

*Parainfluenza-3 virus* (PI-3) is an RNA virus classified in the paramyxovirus family. Infections caused by PI-3 are common in cattle. Although PI-3 is capable of causing disease, it is 10 usually associated with mild to subclinical infections. The most important role of PI-3 is to serve as an initiator that may lead to the development of secondary bacterial pneumonia. Clinical signs include pyrexia, cough, serous nasal and lacrimal discharge, increased respiratory rate, and increased breath sounds. The severity of clinical signs worsen with the onset of bacterial pneumonia. Fatalities from uncomplicated PI-3 pneumonia are rare. Lesions include 15 cranoventral lung consolidation, bronchiolitis, and alveolitis with marked congestion and haemorrhage. Inclusion bodies may be identified. Most fatal cases will also have a concurrent bacterial bronchopneumonia.

*Bovine Respiratory Syncytial Virus* (BRSV) is an RNA virus classified as a pneumovirus in the paramyxovirus family. In addition to cattle, sheep and goats may also be infected by 20 respiratory syncytial viruses. This virus was named for its characteristic cytopathic effect—the formation of syncytial cells. BRSV is distributed worldwide, and the virus is indigenous in the cattle population. BRSV infections associated with respiratory disease occur predominantly in young beef and dairy cattle. Passively derived immunity does not appear to prevent BRSV infections but will reduce the severity of disease. Initial exposures to the virus are associated 25 with severe respiratory disease; subsequent exposures result in mild to subclinical disease. BRSV appears to be an important virus in the bovine respiratory disease complex because of its frequency of occurrence, predilection for the lower respiratory tract, and its ability to predispose the respiratory tract to secondary bacterial infection. In outbreaks, morbidity tends to be high, and case fatality may be 0-20%. Signs include increased rectal temperature 40-42°C, depression, 30 decreased feed intake, increased respiratory rate, cough, and nasal and lacrimal discharge. Generally, respiratory signs predominate. Dyspnea may become pronounced in the later stages of

the disease. Subcutaneous emphysema is sometimes reported. Secondary bacterial pneumonia is a frequent occurrence. A biphasic disease pattern has been described but is not consistent. Gross lesions include a diffuse interstitial pneumonia with subpleural and interstitial emphysema along with interstitial edema. These lesions are similar to and must be differentiated from other causes 5 of interstitial pneumonia. Histologic examination reveals syncytial cells in bronchiolar epithelium and lung parenchyma, intracytoplasmic inclusion bodies, proliferation and/or degeneration of bronchiolar epithelium, alveolar epithelialization, edema, and hyaline membrane formation.

***Bovine Herpesvirus (BHV-1)*** is associated with several diseases and symptoms in cattle:  
10 Infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV), balanoposthitis, conjunctivitis, abortion, encephalomyelitis, and mastitis. Only a single serotype of BHV-1 is recognized; however, three subtypes of BHV-1 have been described on the basis of endonuclease cleavage patterns of viral DNA. These types are referred to as BHV-1.1 (respiratory subtype), BHV-1.2 (genital subtype), and BHV-1.3 (encephalitic subtype). Recently, BHV-1.3 has been  
15 reclassified as a distinct herpesvirus designated BHV-5. BHV-1 infections are widespread in the cattle population. In feedlot cattle, the respiratory form is most common. The viral infection alone is not life-threatening but predisposes cattle to secondary bacterial pneumonia, which may result in death. In breeding cattle, abortion or genital infections are more common. Genital infections may occur in bulls (infectious pustular balanoposthitis) and cows (IPV) within 1-3  
20 days of mating or close contact with an infected animal. Transmission may occur in the absence of visible lesions and through artificial insemination with semen from subclinically infected bulls. Cattle with latent BHV-1 infections generally show no clinical signs when the virus is reactivated, but they do serve as a source of infection for other susceptible animals and thus perpetuate the disease. The incubation period for the respiratory and genital forms is 2-6 days. In  
25 the respiratory form, clinical signs range from mild to severe, depending on the presence of secondary bacterial pneumonia. Clinical signs include pyrexia, anorexia, coughing, excessive salivation, nasal discharge that progresses from serous to mucopurulent, conjunctivitis with lacrimal discharge, inflamed nares (hence the common name “red nose”), and dyspnea if the larynx becomes occluded with purulent material. Pustules may develop on the nasal mucosa and  
30 later form diphtheritic plaques. Conjunctivitis with corneal opacity may develop as the only manifestation of BHV-1 infection. In the absence of bacterial pneumonia, recovery generally

occurs 4-5 days after the onset of clinical signs. Abortions may occur concurrently with respiratory disease but may also occur up to 100 days after infection. Abortions may occur regardless of the severity of disease in the dam. Abortions generally occur during the second half of pregnancy, but early embryonic death may also occur. The first signs of genital infections in 5 cows are frequent urination, elevation of the tailhead, and a mild vaginal discharge. The vulva is swollen, and small papules, then erosions and ulcers, are present on the mucosal surface. If secondary bacterial infections do not occur, animals recover in 10-14 days. If bacterial infection occurs, there may be inflammation of the uterus and transient infertility, with purulent vaginal discharge for several weeks. In bulls, similar lesions occur on the penis and prepuce. BHV-1 10 infection may be severe in young calves and cause a generalized disease. Pyrexia, ocular and nasal discharges, respiratory distress, diarrhea, incoordination, and eventually convulsions and death may occur in a short period after generalized viral infection. IBR is rarely fatal in cattle unless complicated by bacterial pneumonia. In uncomplicated IBR infections, most lesions are restricted to the upper respiratory tract and trachea. Petechial to ecchymotic hemorrhages may be 15 found in the mucous membranes of the nasal cavity and the paranasal sinuses. Focal areas of necrosis develop in the nose, pharynx, larynx, and trachea. The lesions may coalesce to form plaques. The sinuses are often filled with a serous or serofibrinous exudate. As the disease progresses, the pharynx becomes covered with a serofibrinous exudate, and blood-tinged fluid may be found in the trachea. The pharyngeal and pulmonary lymph nodes may be acutely 20 swollen and hemorrhagic. The tracheitis may extend into the bronchi and bronchioles; when this occurs, epithelium is sloughed in the airways. The viral lesions are often masked by secondary bacterial infections. In young animals with generalized BHV-1 infection, erosions and ulcers overlaid with debris may be found in the nose, esophagus, and forestomachs. In addition, white 25 foci may be found in the liver, kidney, spleen, and lymph nodes. Aborted fetuses may have pale, focal, necrotic lesions in all tissues, but which are especially visible in the liver.

A number of other Bovine Respiratory Viruses have been identified as being involved in BRD. *Bovine herpesvirus-4* has been implicated in several diseases, including BRD. Bovine adenovirus has been associated with a wide spectrum of diseases, with bovine adenovirus type 3 being the serotype most often associated with BRD. Two serotypes of bovine rhinovirus have 30 been recognized to cause respiratory tract infections in cattle. Other viruses reported to be associated with BRD include *bovine reovirus*, *enterovirus*, and *coronavirus*. These viruses have

a role similar to the other viruses previously discussed in that, in combination with other stressors, they may serve as initiators of bacterial pneumonia. *Bovine coronavirus* is also commonly associated with diarrhea in calves. It replicates in the epithelium of the upper respiratory tract and in the enterocytes of the intestine, where it produces similar lesions to 5 rotavirus but also infects the epithelial cells of the large intestine to produce atrophy of the colonic ridges. Vaccines are not available for prevention of these viral respiratory diseases.

*Bovine rotavirus* is the most common viral cause of diarrhea in calves. Group A and B rotavirus are involved, but group A is the most prevalent and clinically important and contains 10 several serotypes of differing virulence. Rotavirus replicates in the mature absorptive and enzyme-producing enterocytes on the villi of the small intestine, leading to rupture and sloughing of the enterocytes with release of virus to infect adjacent cells. Rotavirus does not infect the immature cells of the crypts. With virulent strains of rotavirus, the loss of enterocytes exceeds the ability of the intestinal crypts to replace them; hence, villous height is reduced, with a consequent decrease in intestinal absorptive surface area and intestinal digestive enzyme activity.

15 Other viruses, including Breda virus, a calici-like virus, *Adenovirus*, *Astrovirus* and *Parvovirus*, have been demonstrated in the feces of calves with diarrhea and may produce diarrhea in calves experimentally. However, these agents may also be demonstrated in the feces of healthy calves. The importance of these agents in the syndrome of neonatal diarrhea has yet to be determined. *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*) biotype A, 20 serotype 1 is the bacterium most frequently isolated from the lungs of cattle with BRD. Although less frequently cultured than *M. haemolytica*, *Pasteurella multocida* is also an important cause of bacterial pneumonia. When pulmonary abscessation occurs, generally in association with chronic pneumonia, *Actinomyces (Arcanobacterium) pyogenes* is frequently isolated. Under normal conditions, *M. haemolytica* generally remains confined to the upper respiratory tract, in 25 particular the tonsillar crypts, and is difficult to culture from healthy cattle. After stress or viral infection, the replication rate of *M. haemolytica* in the upper respiratory tract increases rapidly, as does the likelihood of culturing the bacterium. The increased bacterial growth rate and colonization of the lungs may be due to suppression of the host's defense mechanism related to environmental stressors or viral infections. It is during this log phase of growth that virulence 30 factors are elaborated by *M. haemolytica*, such as an exotoxin that has been referred to as leukotoxin. The interaction between the virulence factors of the bacteria and host defenses results

in tissue damage and development of pneumonia. Clinical signs of bacterial pneumonia are often preceded by signs of viral infection of the respiratory tract. With the onset of bacterial pneumonia, the severity of clinical signs increases and are characterized by depression and toxemia. There will be pyrexia (40-41°C); serous to mucopurulent nasal discharge; moist cough; 5 and a rapid, shallow respiratory rate. Auscultation of the cranioventral lung field reveals increased bronchial sounds, crackles, and wheezes. In severe cases, pleurisy may develop, which is characterized by an irregular breathing pattern and grunting on expiration. The animal will become unthrifty in appearance if the pneumonia becomes chronic, which is usually associated with the formation of pulmonary abscesses. *M. haemolytica* causes a severe, acute fibrinous 10 pneumonia or fibrinonecrotic pneumonia. The pneumonia has a bronchopneumonic pattern. Grossly, there is extensive reddish black to greyish brown cranioventral regions of consolidation with gelatinous thickening of interlobular septa and fibrinous pleuritis. There are extensive thromboses, foci of lung necrosis, and limited evidence of bronchitis and bronchiolitis. *P. multocida* is associated with a less fulminating fibrinous to fibrinopurulent bronchopneumonia. 15 In contrast to *M. haemolytica*, *P. multocida* is associated with only small amounts of fibrin exudation, some thromboses, limited lung necrosis, and suppurative bronchitis and bronchiolitis.

*Haemophilus somnus* (recently reclassified as *Histophilus somni*) is being increasingly recognized as an important pathogen in BRD; these bacteria are normal inhabitants of the nasopharynx of cattle. *H. somnus* infection of the lungs results in purulent bronchopneumonia 20 that may be followed by septicemia and infection of multiple organs. Occasionally, *H. somnus* is associated with extensive pleuritis. *H. somnus* may cause an acute, usually fatal, septicemic disease that may involve the nervous, musculoskeletal, circulatory, and respiratory systems, either singly or together. The reproductive system is often affected but usually without the other systems being clinically involved. The disease may be characterized by fever, severe depression, 25 ataxia, weakness, blindness, coma, and death within several hours to several days. It occurs sporadically in individual beef and dairy cattle and is found nearly worldwide. *H. somnus* is a gram-negative, nonmotile, nonsporeforming, pleomorphic coccobacillus that requires an enriched medium and a microaerophilic atmosphere for culture. It appears to be identical to *Histophilus ovis* and *Haemophilus agni*, etiologic agents of ovine septicemia, mastitis, and 30 epididymitis; however, transmission of *H. somnus* between sheep and cattle has not been demonstrated. Pathogenic and nonpathogenic strains have been differentiated by intracisternal

inoculation of young calves with organisms from various sources. Pathogenic and nonpathogenic strains of *H. somnus* are carried in the sheath and prepuce of males, the vagina of female cattle, and in the nasal passages of both sexes. The organism may colonize the respiratory tract, presumably after inhalation, and is frequently found in urine. Prevalence of the organism in cattle 5 is probably high because high titers of specific antibodies are found in a large proportion of tested cattle. Several disease syndromes caused by *H. somnus* have been recognized, including thrombomeningoencephalitis, fibrinopurulent bronchopneumonia, fibrinous pleuritis, and polyarthritis. Myocardial and skeletal muscle necroses occur. Suppurative vaginitis, cervicitis, and endometritis have been documented in cows infected experimentally and naturally after 10 breeding, and the organism is a cause of sporadic abortion. Strains of *H. somnus* that cause disease adhere to the endothelium of vessels, resulting in contraction, exposure of collagen, platelet adhesion, and thrombosis. TME results when this occurs in the brain and associated membranes, after invasion of the organism into the bloodstream of susceptible cattle. Strains 15 may adhere to endothelium in vessels of the pleura, myocardium, synovium, or a variety of other tissues and produce inflammation in those sites (e.g., infections of the larynx and middle ear have been recorded). The susceptibility of individual animals and variations in the preference of strains of the organism for vessels in different tissues may be important in the development of the form of disease, but the mechanisms involved are incompletely understood. Reproductive problems may not necessarily be preceded by bacteremia, but the pathogenesis is poorly defined. 20 A fever as high as 42°C is often the first sign of disease; however, this usually falls to normal or subnormal within hours. Other findings are determined by the system(s) involved and may include rapid respiration, stiffness, knuckling at the fetlocks, severe depression, ataxia, paralysis, and opisthotonus, followed by coma and death within several hours. Affected animals may be blind, and retinal hemorrhages with grey foci of retinal necrosis are sometimes seen. Signs such 25 as hypersensitivity, convulsions, excitement, nystagmus, and circling occur inconsistently and may be related to the regions of the CNS affected in the course of disease development. Occasionally, animals are found dead, indicating a rapidly fatal course. A marked change in the total and differential WBC count is common; leukopenia and neutropenia occur in severe, usually acute, fatal disease, while neutrophilia may be present in less severe disease. In TME, the 30 total cell count of the CSF is markedly increased, and neutrophils predominate. During septicemia, the organism may be recovered from blood, synovial fluid, CSF, brain, kidneys,

urine, and a variety of other organs. The lesions are characterized by vascular thrombosis and infarction of the surrounding tissue. Randomly distributed red to brown foci of necrosis with hemorrhage on the surface and cut sections of the brain and spinal cord, retina, skeletal muscle, myocardium, kidney, intestine, and spleen are characteristic. A fibrinopurulent meningitis with 5 cloudy CSF may sometimes be seen on the surface of the brain and spinal cord, and a polyserositis, especially of joints and pleura, may occur. An acute fibrinous bronchopneumonia with tissue necrosis may develop after airborne infections.

Except for *M. bovis*, the exact role of mycoplasmas and ureaplasmas in BRD requires better definition. Mycoplasmas may be recovered from the respiratory tract of nonpneumonic 10 calves, but the frequency of isolation is greater in those with respiratory tract disease. The mycoplasmas commonly recovered from the lungs of pneumonic calves include *Mycoplasma dispar*, *Ureaplasma* spp. Experimental infections usually result in unapparent to mild signs of respiratory disease. This does not preclude a synergistic role for mycoplasmas in conjunction with viruses and bacteria in BRD. Lesions described include peribronchial and peribronchiolar 15 lymphoid cuffing and alveolitis. Culture of these organisms requires special media and conditions and may take up to a week for growth of the organisms.

Chlamydiae have been identified in various parts of the world as a cause of enzootic pneumonia in calves. The causative agent is *Chlamydia psittaci*. Some respiratory isolates from 20 calves have properties of immunotypes 1 and 6 and are similar to strains recovered from intestinal infections and abortions of cattle and sheep. Immunotype 6 has been recovered from pneumonic lungs of calves and pigs. Thus, the GI tract must be considered as an important site in the pathogenesis of chlamydial infections and as a natural reservoir and source of the organisms. Chlamydial pneumonia has affected calves under a whole range of conditions, including dairy 25 farms. A synergism between *Chlamydia* and *P. haemolytica* has been demonstrated experimentally. Calves with chlamydial pneumonia are usually febrile, lethargic, and dyspneic, and have a serous and later mucopurulent nasal discharge and a dry hacking cough. Calves of weanling age are affected most frequently, but older cattle may also show signs of infection. The acute pulmonary lesion is a bronchointerstitial pneumonia. The anteroventral parts of the lungs 30 are affected but, in severe cases, entire lobes may be involved. The dry cough is attributed to tracheitis. Microscopic changes in the lungs include suppurative bronchitis and alveolitis progressing to type II pneumocyte hyperplasia and interstitial thickening.

Bovine genital campylobacteriosis is a venereal disease of cattle characterized primarily by early embryonic death, infertility, a protracted calving season, and occasionally, abortion. Distribution is probably worldwide. The cause is the motile, gram-negative, curved or spiral, polar flagellated bacterium *Campylobacter fetus venerealis* or *Campylobacter fetus fetus*. For 5 many years, it was thought that *C. fetus fetus* (formerly *C. fetus intestinalis*) was generally an intestinal organism, only occasionally caused abortion in cattle, and was not a cause of infertility. However, it has been shown that *C. fetus fetus* may also be a significant cause of the classic infertility syndrome usually attributed to *Campylobacter fetus venerealis*. There are several 10 strains of *C. fetus fetus*, and the only way to determine if a strain is a cause of infertility is to test that possibility in a group of heifers. *Campylobacter* spp are very labile and are destroyed quickly by heating, drying, and exposure to the atmosphere. Unless cultured quickly after collection from the animal and grown under microaerophilic or anaerobic conditions, campylobacters will not grow. *Campylobacter fetus* is transmitted venereally and also by contaminated instruments, bedding, or by artificial insemination using contaminated semen. 15 Individual bulls vary in their susceptibility to infection because some become permanent carriers, while others appear to be resistant to infection. Bulls may also transmit the infection mechanically for several hours after copulating with an infected cow. In cows, the duration of the carrier state is also variable; some clear the infection rapidly, while others may carry *C. fetus* for  $\geq 2$  yr. IgA antibodies are shed in cervical mucus in significant amounts in  $\sim 50\%$  of cows for 20 several months after infection and are useful diagnostically. Although most of the genital tract may be free of infection when a cow eventually conceives, the vagina may remain chronically infected, even through pregnancy. Cows are systemically normal, but there are variable degrees of mucopurulent endometritis that causes early embryonic death, prolonged luteal phases, irregular estrous cycles, repeat breeding and, as a result, protracted calving periods. 25 Observed abortions are not common. In herds not managed intensively, disease may be noticed only when pregnancy examinations reveal low or marginally low pregnancy rates but, more importantly, great variations in gestation lengths, especially when the disease has recently been introduced to the herd. In subsequent years, infertility is usually confined to replacement heifers and a few susceptible cows. Bulls are asymptomatic and produce normal semen.

30 Leptospirosis is a contagious disease of animals, including man, caused by various immunologically distinct leptospiral serovars, most of which are regarded as subgroups of

5 *Leptospira interrogans*. Infections may be asymptomatic or cause various signs, including fever, icterus, hemoglobinuria, renal failure, infertility, abortion, and death. After acute infection, leptospires frequently localize in the kidneys or reproductive organs and are shed in the urine, sometimes in large numbers for months or years. Because the organisms survive in surface waters for extended periods, the disease is often waterborne.

In the U.S. the disease is primarily due to the serovars *Leptospira hardjo*, *Leptospira interrogans* serovar *hardjo* (*hardjo Prajitno*), *L. borgpetersenii* serovar *hardjo* (*hardjo Bovis*), *Leptospira pomona*, and *Leptospira grippotyphosa*. However, *Leptospira canicola* and *Leptospira icterohaemorrhagiae* serovars also have been isolated. Calves may have fever, 10 anorexia, and dyspnea, and in *Leptospira pomona* infections, icterus, hemoglobinuria, and anemia. Body temperature may rise suddenly to 40.5-41°C. Hemoglobinuria rarely lasts longer than 48-72 hrs. Icterus clears rapidly and is followed by anemia. The RBC's begin to increase in 15 number by 4-5 days and return to normal 7-10 days later. However, *Leptospira hardjo* infections usually do not cause hemolytic anemia, which makes diagnosis more difficult. Morbidity and mortality are higher in calves than in adult cattle. In older cattle, signs vary greatly and diagnosis is more difficult. Enzootic *Leptospira hardjo* infections, which usually result in abnormal milk, 20 are more obvious in dairy than in beef cattle. Signs usually are restricted to lowered milk and calf production; a hemolytic crisis does not occur. The milk is thick, yellow, and blood-tinged; it may contain clots, although there is little evidence of mammary inflammation. Milk production returns to normal in 10-14 days, even in the absence of treatment. Abortion and stillbirths, which 25 are common in *Leptospira pomona* infections and sporadic in *Leptospira hardjo* infections, generally occur 3-10 weeks after initial infection. The abortions are more common during the third trimester. An abortion storm in a breeding herd is often the first indication that leptospirosis exists, because the mild initial signs often pass unnoticed. In endemically infected herds, 30 abortions occur mostly in younger animals and are sporadic, rather than being manifested as abortion storms. Calves reared by previously infected cows are protected by colostral antibodies for up to 6 months. The calves generally have an antibody titer similar to that of their dams. In the acute form, anemia, icterus, hemoglobinuria, and submucosal hemorrhages are prominent. The kidneys are swollen, with multifocal petechial and ecchymotic hemorrhages that become pale with time. The liver may be swollen, with minute areas of focal necrosis. Petechiae in other

organs are seen in fulminating cases; however, in the more prevalent *Leptospira hardjo* infections, the lesions are primarily restricted to the kidneys.

Brucellosis is caused by bacteria of the genus *Brucella* and is characterized by abortion, retained placenta, and to a lesser extent, orchitis and infection of the accessory sex glands in males. The disease in cattle, water buffalo, and bison is caused almost exclusively by *Brucella abortus*; however, *Brucella suis* or *Brucella melitensis* is occasionally implicated in some cattle herds. *Brucella suis* does not appear to be contagious from cow to cow. *Brucella abortus* Infection spreads rapidly and causes many abortions in unvaccinated herds. Typically, in a herd in which disease is endemic, an infected cow aborts only once after exposure; subsequent gestations and lactations appear normal. After exposure, many cattle become bacteremic for a short period and develop agglutinins and other antibodies; others resist infection, and a small percentage of infected cows recover. A positive serum agglutination test usually precedes abortion or a normal parturition but may be delayed in ~15% of animals. The incubation period may be variable and is related to the stage of gestation at the time of exposure. Organisms are shed in milk and uterine discharges, and the cow may become temporarily sterile. Bacteria may be found in the uterus during pregnancy, uterine involution, and infrequently, for a prolonged time in the nongravid uterus. Shedding from the vagina largely disappears with reduction of the fluids after parturition. Some infected cows that aborted previously shed brucellae from the uterus at subsequent normal parturitions. Organisms are shed in milk for a variable length of time—in most cattle for life. Natural transmission occurs by ingestion of organisms, which are present in large numbers in aborted fetuses, fetal membranes, and uterine discharges. Cattle may ingest contaminated feed and water, or lick contaminated genitals of other animals. Venereal transmission by infected bulls to susceptible cows appears to be rare. Transmission may occur by artificial insemination when *Brucella*-contaminated semen is deposited in the uterus but, reportedly, not when deposited in the midcervix. Brucellae may enter the body through mucous membranes, conjunctivae, wounds, or even intact skin. Mechanical vectors (eg, other animals, including man) may spread infection. Brucellae have been recovered from fetuses and from manure that has remained in a cool environment for > 2 mo. Exposure to direct sunlight kills the organisms within a few hours. Abortion is the most obvious manifestation. Infections may also cause stillborn or weak calves, retained placentas, and reduced milk yield. Usually, general health is not impaired in uncomplicated abortions. Seminal vesicles, ampullae, testicles, and

epididymides may be infected in bulls; therefore, organisms are in the semen. Agglutinins may be demonstrated in seminal plasma from infected bulls. Testicular abscesses may occur. Long-standing infections may result in arthritic joints in some cattle.

5 Clostridia are relatively large, anaerobic, spore-forming, rod-shaped organisms. The spores are oval, sometimes spherical, and are central, subterminal, or terminal in position. The vegetative forms of clostridia in tissue fluids of infected animals occur singly, in pairs, or rarely in chains. Differentiation of the various pathogenic and related species is based on cultural characteristics, spore shape and position, biochemical reactions, and the antigenic specificity of toxins or surface antigens. The natural habitats of the organisms are the soil and intestinal tract of 10 animals, including man. Pathogenic strains may be acquired by susceptible animals either by wound contamination or by ingestion. Diseases thus produced are a constant threat to successful livestock production in many parts of the world.

15 *Clostridium haemolyticum* is a soil-borne organism that may be found naturally in the GI tract of cattle. It may survive for long periods in contaminated soil or in bones from carcasses of animals that had been infected. After ingestion, latent spores ultimately become lodged in the liver. The incubation period is extremely variable, and the onset depends on the presence of a locus of anaerobiosis in the liver. Such a nidus for germination is most often caused by fluke 20 infection, much less often by high nitrate content of the diet, accidental liver puncture, liver biopsy, or any other cause of localized necrosis. When conditions for anaerobiosis are favorable, the spores germinate, and the resulting vegetative cells multiply and produce  $\beta$  toxin (phospholipase C), which causes intravascular hemolysis and its sequelae, including hemolytic anemia and hemoglobinuria. Cattle may be found dead without premonitory signs. Usually, there 25 is a sudden onset of severe depression, fever, abdominal pain, dyspnea, dysentery, and hemoglobinuria. Anemia and jaundice are present in varying degrees. Edema of the brisket may occur. Hgb and RBC levels are quite low. The duration of clinical signs varies from ~12 hr in pregnant cows to ~3-4 days in other cattle. The mortality in untreated animals is ~95%. Some cattle suffer from subclinical attacks of the disease and thereafter act as immune carriers. Dehydration, anemia, and sometimes subcutaneous edema are present. There is bloody fluid in 30 the abdominal and thoracic cavities. The lungs are not grossly affected, and the trachea contains bloody froth with hemorrhages in the mucosa. The small intestine and occasionally the large intestine are hemorrhagic; their contents often contain free or clotted blood. An anemic infarct in

the liver is virtually pathognomonic; it is slightly elevated, lighter in color than the surrounding tissue, and outlined by a bluish red zone of congestion. The kidneys are dark, friable, and usually studded with petechiae. The bladder contains purplish red urine. After death, *rigor mortis* sets in more rapidly than usual.

5        *Clostridium chauvoei* occurs naturally in the intestinal tract of animals. It probably may remain viable in the soil for many years, although it does not actively grow there. Contaminated pasture appears to be a source of organisms. Outbreaks of blackleg have occurred in cattle on farms in which recent excavations have occurred, which suggests that disturbance of soil may activate latent spores. The organisms probably are ingested, pass through the wall of the GI tract,  
10      and after gaining access to the bloodstream, deposited in muscle and other tissues. In cattle, blackleg infection is endogenous, in contrast to malignant edema. Lesions develop without any history of wounds, although bruising or excessive exercise may precipitate some cases. Commonly, the animals that contract blackleg are of the beef breeds, in excellent health, gaining weight, and usually the best animals of their group. Outbreaks occur in which a few new cases  
15      are found each day for several days. Most cases occur in cattle from 6 months to 2 years old, but thrifty calves as young as 6 weeks and cattle as old as 10-12 years may be affected. The disease usually occurs in summer and fall and is uncommon during the winter. In sheep, the disease is not restricted to the young, and most cases follow some form of injury such as shearing cuts, docking, crutching, or castration. Usually, onset is sudden and a few cattle may be found dead  
20      without premonitory signs. Acute lameness and marked depression are common. Initially, there is a fever but, by the time clinical signs are obvious, the temperature may be normal or subnormal. Characteristic edematous and crepitant swellings develop in the hip, shoulder, chest, back, neck, or elsewhere. At first, the swelling is small, hot, and painful. As the disease rapidly progresses, the swelling enlarges, there is crepitation on palpation, and the skin becomes cold  
25      and insensitive as the blood supply to the area diminishes. General signs include prostration and tremors. Death occurs in 12-48 hrs. In some cattle, the lesions are restricted to the myocardium and the diaphragm, with no reliable ante mortem evidence of the localized lesion.

*Clostridium novyi* has been suspected but not yet confirmed as a cause of sudden death in cattle and pigs fed high-level grain diets, and in which pre-existing lesions of the liver were not detectable. The lethal and necrotizing toxins (primarily  $\alpha$  toxin) damage hepatic parenchyma, thereby permitting the bacteria to multiply and produce a lethal amount of toxin. Usually, death  
30

is sudden with no well-defined signs. Affected animals tend to lag behind the flock, assume sternal recumbency, and die within a few hours. Most cases occur in the summer and early fall when liver fluke infection is at its height. The disease is most prevalent in 1- to 4-year-old sheep and is limited to animals infected with liver flukes. Differentiation from acute fascioliasis may be 5 difficult, but peracute deaths of animals that show typical lesions on necropsy should arouse suspicion of infectious necrotic hepatitis. The most characteristic lesions are the greyish yellow necrotic foci in the liver that often follow the migratory tracks of the young flukes. Other common findings are an enlarged pericardial sac filled with straw-colored fluid, and excess fluid in the peritoneal and thoracic cavities. Usually, there is extensive rupture of the capillaries in the 10 subcutaneous tissue, which causes the adjacent skin to turn black (hence the common name, black disease).

*Clostridium septicum* is found in soil and intestinal contents of animals (including man) throughout the world. Infection ordinarily occurs through contamination of wounds containing devitalized tissue, soil, or some other tissue-debilitating. Wounds caused by accident, castration, 15 docking, unsanitary vaccination, and parturition may become infected. General signs, such as anorexia, intoxication, and high fever, as well as local lesions, develop within a few hours to a few days after predisposing injury. The local lesions are soft swellings that pit on pressure and extend rapidly because of the formation of large quantities of exudate that infiltrates the subcutaneous and intramuscular connective tissue of the affected areas. The muscle in such areas 20 is dark brown to black. Accumulations of gas are uncommon. Severe edema of the head of rams occurs after infection of wounds inflicted by fighting. Malignant edema associated with lacerations of the vulva at parturition is characterized by marked edema of the vulva, severe toxemia, and death in 24-48 hours. Similarity to blackleg is marked, and differentiation made on necropsy is unreliable; laboratory confirmation is the only certain procedure. Horses and pigs are 25 susceptible to malignant edema but not to blackleg.

Infectious disease caused by *Clostridium sordellii* is also manifested as malignant edema in cattle, and also characterized by a nongaseous, nonhemorrhagic, edematous swelling of the head, face, and neck of young rams. This infection is initiated in young rams by their continual butting of one another. The bruised and battered subcutaneous tissues provide conditions suitable 30 for growth of pathogenic clostridia, and the breaks in the skin offer an opportunity for their entrance

Infection with *C. perfringens* types **A, B and C** causes severe enteritis, dysentery, toxemia, and high mortality in young calves. Types B and C both produce the highly necrotizing and lethal  $\beta$  toxin that is responsible for the severe intestinal damage. This toxin is sensitive to proteolytic enzymes, and disease is associated with inhibition of proteolysis in the intestine. Sow 5 colostrum, which contains a trypsin inhibitor, has been suggested as a factor in the susceptibility of young piglets. Type C also causes enterotoxemia in adult cattle. In calves, there is acute diarrhea, dysentery, abdominal pain, convulsions, and opisthotonus. Death may occur in a few hours, but less severe cases survive for a few days, and recovery over a period of several days is possible. Hemorrhagic enteritis with ulceration of the mucosa is the major lesion in all species. 10 Grossly, the affected portion of the intestine is deep blue-purple and appears at first glance to be an infarction associated with mesenteric torsion. Smears of intestinal contents may be examined for large numbers of gram-positive, rod-shaped bacteria, and filtrates made for detection of toxin and subsequent identification by neutralization with specific antiserum.

This classic enterotoxemia caused by *C. perfringens* type **D** rarely occurs in cattle. It is 15 worldwide in distribution and may occur in animals of any age. The disease has been suspected in well-nourished beef calves nursing high-producing cows grazing lush pasture and in sudden death syndrome in feedlot cattle; however, supportive laboratory evidence in the latter is lacking. Acutely affected calves not found dead show mania, convulsions, blindness, and death in a few hours. Subacutely affected calves are stuporous for a few days and may recover.

20 Tetanus toxemia is caused by a specific neurotoxin produced by *Clostridium tetani* in necrotic tissue. Almost all mammals are susceptible to this disease. Although tetanus is worldwide in distribution, there are some areas, such as the northern Rocky Mountain section of the USA, where the organism is rarely found in the soil and where tetanus is almost unknown. In general, the occurrence of *C. tetani* in the soil and the incidence of tetanus in man and horses is 25 higher in the warmer parts of the various continents. *Clostridium tetani*, an anaerobe with terminal, spherical spores, is found in soil and intestinal tracts. In most cases, it is introduced into the tissues through wounds, particularly deep puncture wounds, which provide a suitable anaerobic environment.

Infection with *Salmonella* spp may produce diarrhea in animals of all ages, especially 30 those that are stressed, closely stocked, or exposed to a heavily contaminated feed or water supply. Salmonellosis is caused by many species of salmonellae and characterized clinically by

one or more of three major syndromes—septicemia, acute enteritis, and chronic enteritis. The incidence has increased with the intensification of livestock production. Young calves usually develop the septicemic form. Adult cattle develop acute enteritis. Chronic enteritis may develop occasionally in cattle. Pregnant animals may abort. In older animals, the disease is manifested by 5 dysentery and toxemia, and mortality may be significant. While many other *Salmonella* spp may cause disease, the more relevant in cattle are *S. typhimurium*, *S. dublin*, and *S. newport*. Although their resulting clinical patterns are not distinct, different species of salmonellae tend to differ in their epidemiology. Plasmid profile and drug-resistance patterns are sometimes useful markers for epidemiologic studies. Feces of infected animals may contaminate feed and water, 10 milk, fresh and processed meats from abattoirs, plant and animal products used as fertilizers or feedstuffs, pasture and rangeland, and many inert materials. The organisms may survive for months in wet, warm areas such as in feeder pig barns or in water dugouts but survive less than 1 week in composted cattle manure. Rodents and wild birds also are sources of infection. The prevalence of infection varies among species and countries and is much higher than the incidence 15 of clinical disease, which is commonly precipitated by stressful situations such as sudden deprivation of feed, transportation, drought, crowding, parturition, and the administration of some drugs.

Further relevant gastro-intestinal pathogens that may be used in the present invention include *Escherichia coli*, *Cryptosporidium parvum* and *Mycobacterium avium paratuberculosis*. *Escherichia coli* infection causes severe intestinal disease in young animals characterized as neonatal diarrhea, post weaning diarrhea, edema disease, and/or septicemia depending upon the virulence factors present in the strain causing the infection. Calves infected with pathogenic *E. coli* may develop severe diarrhea causing fatal dehydration, or fatal septicemic infections. Paratuberculosis is a chronic contagious enteritis characterized by 20 persistent and progressive diarrhea, weight loss, debilitation, and eventually death. It affects cattle, sheep, goats, llamas, camels, farmed deer, and other domestic, exotic, and wild ruminants. It has also been recognized in wild rabbits; horses and pigs may be infected experimentally. Distribution is worldwide.

Animals with paratuberculosis should be considered as potential zoonotic risks until the 30 situation is clarified. The causative organism is *Mycobacterium avium paratuberculosis*, formerly known as *M. paratuberculosis* or *M. johnei*. Occasionally, other *M. avium* subspecies

are isolated from cases. The organism is quite resistant and may survive on pasture for more than 1 year, but sunlight, alkaline soils, and drying reduce its survival rate. It is shed in large numbers in feces of infected animals, and infection is acquired by ingestion of contaminated feed and water. Introduction of the disease into a clean herd is usually by subclinically infected carriers.

5 Infection is acquired early in life, but clinical signs rarely develop in cattle < 2 years old. Resistance increases with age, and cattle first exposed as adults are unlikely to become infected. Most calves are infected soon after birth either by nursing udders contaminated with feces from infected animals or by being housed in contaminated pens. The organism may also be present in colostrum and milk of infected cows, and intrauterine infections have also been described. After 10 ingestion, the bacteria infect macrophages in the mucosa of the lower small intestine and in associated lymph nodes. Most animals will eliminate infection by an early cell-mediated immune response that encourages microbicidal activity in macrophages. In susceptible animals, the organisms multiply and provoke a chronic enteritis that leads to clinical disease. This may take months to years to develop and is usually paralleled by a decline in cell-mediated immunity and 15 a rise in ineffective serum antibody. However, fecal shedding begins before clinical signs are apparent. *Mycobacterium avium paratuberculosis* may be isolated from feces, mesenteric and ileocecal lymph nodes, thickened intestinal walls, and less frequently the udder and the reproductive tracts of both sexes.

Cryptosporidiosis is an enterocolitis of cosmopolitan distribution caused by the coccidian 20 parasite *Cryptosporidium parvum*. It is not host-specific and is common in young ruminants, particularly calves; it is also found in man and pigs and is rare in dogs, cats, and horses. Other cryptosporidia cause disease in reptiles and birds. The disease in calves, characterized by weight loss and watery diarrhea, is clinically indistinguishable from many other causes of calf diarrhea. *Cryptosporidium parvum* is a minute protozoan that is transmitted by the fecal-oral route. 25 Oocysts are sporulated (four sporozoites) when shed in the feces and, therefore, are immediately infective. The mean incubation period is ~4 days. Calves 1-3 weeks old seem to be most susceptible. Signs such as anorexia, weight loss, diarrhea, and tenesmus, resemble those caused by several other intestinal pathogens; however, infections without signs do occur. Uncomplicated cryptosporidiosis is seldom fatal. Disease may be severe in immuno-compromised individuals. If 30 severe disease in calves is seen, other disease agents or concurrent infections should be ruled out. Although *C. parvum* may infect virtually the entire intestinal tract, the distal small intestine

usually is affected most severely. Infection in horses is limited to the small intestine. Gross lesions may consist of hyperemic intestinal mucosa and yellowish intestinal contents. Microscopically, mild to severe villous atrophy with spherical organisms in the brush border is evident. Unlike *Eimeria* and *Isospora* spp, which are intracellular parasites, *C. parvum* is 5 intramembranous and resides within the brush border of the intestinal epithelial cells.

Inflammation of the mammary gland (mastitis) is almost always due to the effects of infection by bacterial or mycotic pathogens. Mastitis may be associated with infection by many other organisms, including *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp, *Pseudomonas aeruginosa*, 10 *Actinomyces pyogenes*, *Mycoplasma* spp, *Nocardia asteroides*, *Serratia*, *Mycobacterium* spp, *Clostridium perfringens*, *Pasteurella* spp, yeasts, and *Prototheca* spp.

Dermatomycoses (Dermatophytosis) in animals are anthropozoonotic diseases of the skin and related tissue. Clinical symptoms are characterized by loss of hair in the affected area, hyperemia, scaling and asbestos-like scabs. Inflammation is often accompanied by suppuration. 15 Dermatomycoses are often also characterized by localized infection of the skin. Dermatomycoses in animals carry a substantial socioeconomic impact. Diseased animals required prolonged treatment and may spread infection to both animals and humans. Dermatophytosis are caused by mycosis infections of *Trichophyton* spp. or *Microsporum* spp. Most relevant causes for cattle are *Trichophyton verrucosum*, *Trichophyton mentagrophytes* or *Trichophyton sarkisovii*.

20 An infection of the lower respiratory tract, usually resulting in bronchitis or pneumonia, may be caused by any of several parasitic nematodes, including *Dictyocaulus viviparus* in cattle. This lungworm belongs to the superfamily Trichostrongyloidea and has direct life cycles. The cattle lungworm is common in northwest Europe and is the cause of severe outbreaks of "husk" or "hoose" in young grazing cattle. Because *D. viviparus* infection in cattle is the most 25 economically important, it has been most investigated and many of the observations from it are applicable to the other species. Clinical disease usually develops on first exposure to sufficient infective larvae. In cattle, this usually occurs during their first season at pasture; however, an increase in the number of older cattle affected has been reported. Signs of lungworm infection range from moderate coughing with slightly increased respiratory rates to severe persistent 30 coughing and respiratory distress and even failure. Reduced weight-gains, reduced milk yields,

and weight loss accompany many infections in cattle. Patent subclinical infections may occur in all species. The most consistent signs in cattle are tachypnea and coughing.

Trichomoniasis is a venereal protozoal disease of cattle characterized primarily by early fetal death and infertility, resulting in extended calving intervals. Distribution is probably 5 worldwide. The causative protozoan, *Trichomonas (Tritrichomonas) foetus*, is pyriform and ordinarily  $10-15 \times 5-10 \mu\text{m}$ , but there is considerable pleomorphism. It may become spherical when cultured in artificial media. At its anterior end, there are three flagella about the same length as the body of the parasite. An undulating membrane extends the length of the body and is bordered by a marginal filament that continues beyond the membrane as a posterior flagellum. 10 Although *T. foetus* may survive the process used for freezing semen, it is killed by drying or high temperatures. *Trichomonas foetus* is found in the genital tracts of cattle. When cows are bred naturally by an infected bull, 30-90% become infected, suggesting that strain differences exist. Variation in breed susceptibility to trichomoniasis may also exist. Bulls of all ages may remain 15 infected indefinitely but this is less likely in younger males. By contrast, most cows are free of infection within 3 months after breeding. However, immunity is not long lasting and reinfection does occur. Transmission may also occur when the semen from infected bulls is used for artificial insemination. The most common sign is infertility caused by embryonic death. This results in repeat breeding and a prolonged calving season. Fetal death and abortions may also occur but are not as common as losses earlier in gestation. *Trichomonas foetus* has been found in 20 vaginal cultures taken as late as 8 months of gestation and, apparently, live calves may be born to infected dams. Pyometra occasionally develops after breeding.

*Neospora caninum* is an obligate intracellular protozoan parasite that has been confused previously with *Toxoplasma gondii*. Only asexual stages are known, and they resemble *T. gondii*. The complete life cycle of *N. caninum* is unknown, but it may be transmitted 25 transplacentally in dogs, cattle, goats, sheep, and cats, and subsequent offspring may be affected. Tachyzoites are  $5-7 \times 1-5 \mu\text{m}$ , depending on the stage of division. They divide by endodyogeny. Tachyzoites are found in myocytes, neural cells, dermal cells, macrophages, and other cells. Tissue cysts up to  $100 \mu\text{m}$  in diameter are found in neural cells; the cyst wall is amorphous and up to  $4 \mu\text{m}$  thick. Cysts have no septa and enclose slender  $7 \times 1.5 \mu\text{m}$  bradyzoites. In dairy cattle, 30 *N. caninum* is a major cause of abortion in many countries, particularly in the USA. Calves may be aborted, stillborn, born underweight, weak, or paralyzed, or they may become paralyzed

within 4 weeks of birth. Non-suppurative encephalitis is the main lesion in aborted fetal tissues. Abortion may occur throughout gestation, and some cows may abort again; dams of these calves are clinically normal.

Babesiosis is caused by intraerythrocytic protozoan parasites of the genus *Babesia*. A wide range of domestic and wild animals and occasionally man is affected by the disease, which is transmitted by ticks and has a worldwide distribution. Two important species in cattle—*Babesia bigemina* and *Babesia bovis*—are widespread in tropical and subtropical areas and are the focus of this discussion. In endemic areas, two features are important in determining the risk of clinical disease: 1) calves have a degree of immunity (related both to colostral-derived antibodies and to age) that persists for ~6 months, and 2) animals that recover from *Babesia* infections are immune for life. Thus, at high levels of tick transmission, all newborn calves will become infected with *Babesia* by 6 mos. of age, show few if any clinical signs, and subsequently be immune. This situation of endemic stability may be upset by either a natural (eg, climatic) or artificial (eg, acaricide treatment) reduction in tick numbers to levels where tick transmission of *Babesia* to calves is insufficient to ensure all are infected during this critical early period. Other circumstances that may lead to clinical outbreaks include the introduction of susceptible cattle to endemic areas and the incursion of *Babesia*-infected ticks into previously tick-free areas. Strain variation in immunity has been demonstrated but is probably not of significance in the field. The acute disease generally runs a course of ~1 week. The first sign is fever (frequently 41°C or higher), which persists throughout, and is accompanied later by inappetence, increased respiratory rate, muscle tremors, anemia, jaundice, and loss of weight with hemoglobinemia and hemoglobinuria in the final stages. CNS involvement due to sludging of parasitized erythrocytes in brain capillaries occurs frequently with *B. bovis* infection. Either constipation or diarrhea may be present. Pregnant cows often abort. With virulent strains of *B. bovis*, a hypotensive shock syndrome, combined with generalized non-specific inflammation, coagulation disturbances, and erythrocytic stasis in capillaries, contribute to the pathogenesis. With most strains of *B. bigemina*, the pathogenic effects relate more directly to erythrocyte destruction. Animals that recover from the acute disease remain infected for a number of years with *B. bovis* and for a few months in the case of *B. bigemina*. No signs are apparent during this carrier state. Lesions include an enlarged and friable spleen; a swollen liver with an enlarged gallbladder containing thick granular bile; congested, dark-colored kidneys; and generalized anemia and jaundice. The

urine is often, but not invariably, red. Other organs, including the brain and heart, may show congestion or petechial hemorrhages. The susceptibility of cattle breeds to *Babesia* infections varies; for example, Brahman cattle are more resistant to *B. bovis* infection than are British breeds.

5 As described above, the present invention also relates to combination vaccines and/or the combined use of immunogenic compositions for the treatment and/or prophylaxis of cattle against microbiological infections, wherein the infections are caused by *M. bovis* and at least one further relevant cattle pathogen. The combination vaccine as described herein comprises at least one *M. bovis* antigen, preferably the attenuated, avirulent *M. bovis* as provided herewith and one  
10 or more further immunologically active components effective for the treatment and/or prophylaxis of infections caused by one or more further relevant pathogen of cattle. The combined use or the method of co-administration of two or more antigens of pathogens affecting cattle comprises administering a first immunogenic composition comprising *M. bovis* antigen, preferably the attenuated, avirulent *M. bovis* as provided herewith and at least one further  
15 immunologically active component effective for the treatment and/or prophylaxis of infections caused by a further relevant pathogen of cattle.

Relevant cattle pathogens other than *M. bovis* include those listed in the background section above but are not limited to: **i) pathogens of viral origin** such as Bovine viral diarrhea virus (BVDV) type 1 (BVDV-1) and type 2 (BVDV-2), Parainfluenza-3 Virus (PI-3), Infectious  
20 Bovine Rhinotracheitis virus (IBR), Bovine Respiratory Syncytial Virus (BRSV), Bovine Herpesvirus (BHV), Bovine Rotavirus (BRV), Bovine Enterovirus (BEV), Bovine Coronavirus (BCV), Bovine Rabies (BR), Bovine Parvovirus (BPV), and Adenovirus and Astrovirus; **ii) pathogens of bacterial origin**, such as *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*), *Pasteurella multocida*, *Haemophilus somnus* (*Histophilus ovis* and *Haemophilus agni*), *Actinomyces* (*Corynebacterium*), *Actinomyces pyogenes*, *Chlamydia psittaci*, *Campylobacter fetus venerealis* and *Campylobacter fetus fetus* (formerly *C. fetus intestinalis*), *Leptospira interrogans*, *Leptospira pomona*, and *Leptospira grippotyphosa*, *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), *Brucella abortus*, *Brucella suis* and *Brucella melitensis*, *Escherichia coli*, *Listeria monocytogenes*, *Chlamydia psittaci*, *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium haemolyticum*, *Clostridium novyi*, *Clostridium sordellii*, *Clostridium perfringens*, *Clostridium*

*tetani, Moraxella bovis, Klebsiella spp, Klebsiella pneumoniae, Salmonella typhimurium; Salmonella newport, Mycobacterium avium paratuberculosis, Staphylococcus aureus, Streptococcus dysgalactiae, Mycoplasma dispar, and Ureaplasma spp., and Streptococcus uberis*

**iii) pathogens of other origin**, such as *Tritrichomonas foetus, Trichophyton verrucosum,*

5 *Trichophyton mentagrophytes, Trichophyton sarkisovii, Neospora caninum* (formerly *Toxoplasma gondii*), *Cryptosporidium parvum, Cryptosporidium hominis, Babesia bigemina* and *Babesia bovis*, and *Dictyocaulus viviparous* (Lungworm disease).

The combined use or the method of co-administration of two or more antigens of pathogens affecting cattle comprises administering a first immunogenic composition comprising

10 *M. bovis* antigen, preferably the attenuated, avirulent *M. bovis* as provided herewith and at least one further immunologically active component effective for the treatment and/or prophylaxis of infections caused by a further pathogen of cattle, wherein said further pathogen of cattle is selected from the group consisting of: **i) pathogens of viral origin** such as Bovine viral diarrhea

virus (BVDV) type 1 (BVDV-1) and type 2 (BVDV-2), Parainfluenza-3 Virus (PI-3), Infectious

15 Bovine Rhinotracheitis virus (IBR), Bovine Respiratory Syncytial Virus (BRSV), Bovine Herpesvirus (BHV), Bovine Rotavirus (BRV), Bovine Enterovirus (BEV), Bovine Coronavirus (BCV), Bovine Rabies (BR), Bovine Parvovirus (BPV), and Adenovirus and Astrovirus; **ii) pathogens of bacterial origin**, such as *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*), *Pasteurella multocida, Haemophilus somnus* (*Histophilus ovis* and *Haemophilus agni*), *Actinomyces* (*Corynebacterium*), *Actinomyces pyogenes, Chlamydia psittaci, Campylobacter fetus venerealis* and *Campylobacter fetus fetus* (formerly *C. fetus intestinalis*), *Leptospira interrogans, Leptospira pomona, and Leptospira grippotyphosa, Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo* (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), *Brucella abortus, Brucella suis* and *Brucella melitensis, Escherichia coli, Listeria monocytogenes, Chlamydia psittaci, Clostridium chauvoei, Clostridium septicum, Clostridium haemolyticum, Clostridium novyi, Clostridium sordellii, Clostridium perfringens, Clostridium tetani, Moraxella bovis, Klebsiella spp, Klebsiella pneumoniae, Salmonella typhimurium; Salmonella newport, Mycobacterium avium paratuberculosis, Staphylococcus aureus, Streptococcus dysgalactiae, Mycoplasma dispar, and Ureaplasma spp., and Streptococcus uberis*

30 and **iii) pathogens of other origin**, such as *Tritrichomonas foetus, Trichophyton verrucosum, Trichophyton mentagrophytes, Trichophyton sarkisovii, Neospora caninum* (formerly

*Toxoplasma gondii*), *Cryptosporidium parvum*, *Cryptosporidium hominis*, *Babesia bigemina* and *Babesia bovis*, and *Dictyocaulus viviparous* (Lungworm disease) or any other pathogen listed in the background section or known to be pathogenic in cattle.

The present invention relates to combination vaccines and/or the combined use of 5 immunogenic compositions for the treatment and/or prophylaxis of cattle against microbiological infections, wherein the infections are caused by *M. bovis* and at least one further cattle relevant pathogen, wherein said vaccine or combined use comprises or makes use of an *M bovis* antigen, preferably the avirulent, attenuated *M. bovis*, as described herein, and a further immunologically active component effective for the treatment and/or prophylaxis of infections caused by Bovine 10 viral diarrhea virus (BVDV) type 1 (BVDV-1) and type 2 (BVDV-2), Parainfluenza-3 Virus (PI-3), Infectious Bovine Rhinotracheitis virus (IBR), Bovine Respiratory Syncytial Virus (BRSV), Bovine Herpesvirus (BHV), Bovine Rotavirus (BRV), Bovine Enterovirus (BEV), Bovine Coronavirus (BCV), Bovine Rabies (BR), Bovine Parvovirus (BPV), Adenovirus Astrovirus, *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*), *Pasteurella multocida*, 15 *Haemophilus somnus* (*Histophilus ovis* and *Haemophilus agni*), Actinomyces (Corynebacterium), *Actinomyces pyogenes*, *Chlamydia psittaci*, *Campylobacter fetus venerealis* and *Campylobacter fetus fetus* (formerly *C. fetus intestinalis*), *Leptospira interrogans*, *Leptospira pomona*, and *Leptospira grippotyphosa*, *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), 20 *Brucella abortus*, *Brucella suis* and *Brucella melitensis*, *Escherichia coli*, *Listeria monocytogenes*, *Chlamydia psittaci*, *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium haemolyticum*, *Clostridium novyi*, *Clostridium sordellii*, *Clostridium perfringens*, *Clostridium tetani*, *Moraxella bovis*, *Klebsiella* spp, *Klebsiella pneumoniae*, *Salmonella typhimurium*; *Salmonella newport*, *Mycobacterium avium paratuberculosis*, *Cryptosporidium parvum*, 25 *Cryptosporidium hominis*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Mycoplasma* spp, *Mycoplasma dispar*, and *Ureaplasma* spp., *Tritrichomonas foetus*, *Trichophyton verrucosum*, *Trichophyton mentagrophytes*, *Trichophyton sarkisovii*, *Neospora caninum* (formerly *Toxoplasma gondii*), *Babesia bigemina* and *Babesia bovis*, and *Dictyocaulus viviparous* (Lungworm disease) and/or any other pathogen known to be pathogenic in cattle, 30 including the pathogens discussed in the background.

The combined use or the method of co-administration of two or more antigens of pathogens affecting cattle comprises administering a first immunogenic composition comprising *M. bovis* antigen, preferably the attenuated, avirulent *M. bovis*, as provided herewith, and at least one further immunogenic composition comprising one or more further immunologically active components effective for the treatment and/or prophylaxis of infections caused by a further pathogen of cattle, wherein said further pathogen of cattle is selected from the group consisting of: Bovine viral diarrhea virus (BVDV) type 1 (BVDV-1) and type 2 (BVDV-2), Parainfluenza-3 Virus (PI-3), Infectious Bovine Rhinotracheitis virus (IBR), Bovine Respiratory Syncytial Virus (BRSV), Bovine Herpesvirus (BHV), Bovine Rotavirus (BRV), Bovine Enterovirus (BEV), Bovine Coronavirus (BCV), Bovine Rabies (BR), Bovine Parvovirus (PPV), Adenovirus Astrovirus, *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*), *Pasteurella multocida*, *Haemophilus somnus* (*Histophilus ovis* and *Haemophilus agni*), Actinomyces (Corynebacterium), *Actinomyces pyogenes*, *Chlamydia psittaci*, *Campylobacter fetus venerealis* and *Campylobacter fetus fetus* (formerly *C. fetus intestinalis*), *Leptospira interrogans*, *Leptospira pomona*, and *Leptospira grippotyphosa*, *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), *Brucella abortus*, *Brucella suis* and *Brucella melitensis*, *Escherichia coli*, *Listeria monocytogenes*, *Chlamydia psittaci*, *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium haemolyticum*, *Clostridium novyi*, *Clostridium sordellii*, *Clostridium perfringens*, *Clostridium tetani*, *Moraxella bovis*, *Klebsiella* spp, *Klebsiella pneumoniae*, *Salmonella typhimurium*; *Salmonella newport*, *Mycobacterium avium paratuberculosis*, *Cryptosporidium parvum*, *Cryptosporidium hominis*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Mycoplasma* spp, *Mycoplasma dispar*, *Mycoplasma bovis*, and *Ureaplasma* spp., *Tritrichomonas foetus*, *Trichophyton verrucosum*, *Trichophyton mentagrophytes*, *Trichophyton sarkisovii*, *Neospora caninum* (formerly *Toxoplasma gondii*), *Babesia bigemina* and *Babesia bovis*, and *Dictyocaulus viviparous* (Lungworm disease) and/or any other pathogen listed in the background section or known to be pathogenic in cattle. Preferably, the further immunogenic composition comprises an antigen of one or more of any of the cattle relevant pathogens as listed above.

According to a further embodiment, the present invention relates to a combination vaccine or the combined use of immunogenic compositions for the treatment and/or prophylaxis

of cattle against infections of the respiratory and/or reproductive systems in cattle, wherein the combination vaccine or combined use comprises a *M. bovis* antigen, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one further immunologically active component effective for the treatment and/or prophylaxis of infections caused by **IBR [combo 001]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis*, as described herein, and at least one antigen of **IBR [combo 002]**. According to a preferred embodiment, the IBR antigen is a **live modified virus [combo 003]**. According to a further embodiment, the combination vaccine of attenuated *M. bovis* and **IBR contains an antibiotic, e.g. neomycin, for preservation [combo 004]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one further immunologically active component effective for the treatment and/or prophylaxis of infections caused by **PI-3 [combo 005]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen of **PI-3 [combo 006]**

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one further immunologically active component effective for the treatment and/or prophylaxis of infections caused by **BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2) **[combo 007]**. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein and at least one antigen of BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2) **[combo 008]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one further

immunologically active component effective for the treatment and/or prophylaxis of infections caused by **BHV [combo 009]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen of **BHV [combo 010]**.

5 According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections 10 caused by **IBR and PI-3 [combo 011]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR and PI-3 **[combo 012]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory 15 and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR and BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2) **[combo 013]**. According to a preferred embodiment, the combination vaccine comprises 20 *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR and BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2) **[combo 014]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory 25 and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR and BHV [combo 015]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, 30 and at least one antigen each of IBR and BHV **[combo 016]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **PI-3 and BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2) [combo 017]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of PI-3 and BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2) [combo 018].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **PI-3 and BHV [combo 019]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of PI-3 and BHV [combo 020].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3 and BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2) [combo 021]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein and at least one antigen each of IBR, PI-3 and BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2) [combo 022]. Preferably, all viral antigens are modified live viruses [combo 023].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory

and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **BRSV and BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2) [combo 024]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen of each BRSV and BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2 [combo 025]).

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2 and **BHV [combo 026]**). According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR BHV, and BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2 [combo 027]).

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2 and **BHV [combo 028]**). According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of PI-3 and BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2 and BHV [combo 029]).

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*,

preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3 and BHV [combo 030]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, BHV and PI-3 **[combo 031]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2 and **BHV [combo 032]**). According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR BHV PI-3, and BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2) **[combo 033]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against bacterial infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one further immunologically active component effective for the treatment and/or prophylaxis of infections caused by *H. somnus* **[combo 034]**. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen of *H. somnus* **[combo 035]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral and bacterial infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR and *H. somnus* [combo 036]**. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of *H. somnus* and IBR **[combo 037]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral and bacterial infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, and *H. somnus* [combo 038]**. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, and *H. somnus* [combo 039].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2) and *H. somnus* [combo 040]. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3 and BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2) and one antigen of *H. somnus* [combo 041]. According to a further embodiment of said combination vaccine, the IBR, PI-3 antigens are killed viruses [combo 042]. According to a further embodiment, any of said combination vaccines, preferably the combination vaccine that comprises killed IBR and killed PI-3 antigens, contains neomycin and thimerosal as preservatives [combo 043].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against viral infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), **BHV** and *H. somnus* [combo 044]. According to a more preferred embodiment, the

combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, BHV, PI-3, and BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2) and one antigen of *H. somnus* [combo 045].

5 According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one further immunologically active component effective for the treatment and/or prophylaxis of infections  
10 caused by one or more pathogenic species of **Leptospira**, preferably selected from the group consisting of *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira borgpetersenii*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), *Leptospira prajitno*, *Leptospira icterohaemorrhagiae*, *Leptospira bovis*, *Leptospira interrogans* and *Leptospira pomona*. [combo 046]. According to a more preferred embodiment, the combination vaccine  
15 comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein and one or more antigens of at least one or more pathogenic species of **Leptospira**, preferably selected from the group consisting of *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira borgpetersenii*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), *Leptospira prajitno*, *Leptospira icterohaemorrhagiae*, *Leptospira bovis*, *Leptospira interrogans* and *Leptospira pomona* [combo 047].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active  
25 components effective for the treatment and/or prophylaxis of infections caused by **IBR** and one or more pathogenic species of **Leptospira**, preferably selected from the group consisting of *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira borgpetersenii*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), *Leptospira prajitno*, *Leptospira icterohaemorrhagiae*, *Leptospira bovis*, *Leptospira interrogans* and *Leptospira pomona*.  
30 [combo 048]. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein and at least one

antigen each of IBR and one or more pathogenic specie(s) of *Leptospira*, preferably selected from the group consisting of *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira borgpetersenii*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), *Leptospira prajitno*, *Leptospira icterohaemorrhagiae*, *Leptospira bovis*, *Leptospira interrogans* and *Leptospira pomona* [combo 049].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR and Leptospira pomona** [combo 050]. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen of IBR, preferably a live modified virus, and at least one *Leptospira pomona* bacterin [combo 51].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, and one or more pathogenic species of Leptospira**, preferably selected from the group consisting of *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira borgpetersenii*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), *Leptospira prajitno*, *Leptospira icterohaemorrhagiae*, *Leptospira bovis*, *Leptospira interrogans* and *Leptospira pomona* [combo 052]. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, and PI-3, and one or more antigens each of one or more pathogenic specie(s) of *Leptospira*, preferably selected from the group consisting of *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira borgpetersenii*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), *Leptospira prajitno*, *Leptospira icterohaemorrhagiae*, *Leptospira bovis*, *Leptospira interrogans* and *Leptospira pomona* [combo 053].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and/or *Leptospira hardjo-bovis*), *Leptospira icterohaemorrhagiae*, and *Leptospira pomona* [combo 054]**. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of **IBR, PI-3, *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo*, *Leptospira icterohaemorrhagiae* and *Leptospira pomona* [combo 055]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2) and one or more pathogenic species of **Leptospira**, preferably selected from the group consisting of *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira borgpetersenii*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), *Leptospira prajitno*, *Leptospira icterohaemorrhagiae*, *Leptospira bovis*, *Leptospira interrogans* and *Leptospira pomona* **[combo 056]**. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2) and one or more antigens each of one or more pathogenic species of **Leptospira**, preferably selected from the group consisting of *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira borgpetersenii*, *Leptospira prajitno*, *Leptospira icterohaemorrhagiae*, *Leptospira bovis*, *Leptospira interrogans* and *Leptospira pomona* **[combo 057]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably

the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by IBR, PI-3, BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and/or 5 *Leptospira hardjo-bovis*), *Leptospira icterohaemorrhagiae*, and *Leptospira pomona* [combo 058]. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), and one or more antigens each of *Leptospira canicola*, *Leptospira grippotyphosa*, 10 *Leptospira hardjo* (*Leptospira hardjoprajitno* and/or *Leptospira hardjo-bovis*), *Leptospira icterohaemorrhagiae*, and *Leptospira pomona* [combo 059]. According to a preferred embodiment, **the viral antigens are killed viruses** and the bacterial antigens are bacterins [combo 060]. Preferably, said combination vaccines as described in this paragraph further contain neomycin and thimerosal as preservatives [combo 061].

15 According to a further embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, **live modified viruses of IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), and bacterin of *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and/or *Leptospira hardjo-bovis*), *Leptospira icterohaemorrhagiae* and *Leptospira pomona* [combo 062]. According to a further preferred embodiment, the combination vaccine 20 described in this paragraph comprises neomycin as a preservative [combo 063].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably 25 the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type2), **BHV** and one or more pathogenic **species of Leptospira**, preferably selected from the group consisting of *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira borgpetersenii*, *Leptospira hardjo* 30 (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), *Leptospira prajitno*, *Leptospira icterohaemorrhagiae*, *Leptospira bovis*, *Leptospira interrogans* and *Leptospira pomona*.

[**combo 064**]. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR BHV, PI-3, and BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), and one or more antigens each of one or more pathogenic species of 5 Leptospira, preferably selected from the group consisting of *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira borgpetersenii*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), *Leptospira prajitno*, *Leptospira icterohaemorrhagiae*, *Leptospira bovis*, *Leptospira interrogans* and *Leptospira pomona* [**combo 065**].

According to a further embodiment, the present invention relates to a combination 10 vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by one or more pathogenic **species of Leptospira**, as mentioned above, and *H. somnus* [**combo 066**]. According to a more preferred 15 embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen of one or more pathogenic species of Leptospira, as mentioned above, and at least one antigen of *H. somnus* [**combo 067**].

According to a further embodiment, the present invention relates to a combination 20 vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR** and one or more pathogenic **specie(s) of Leptospira**, as mentioned above, and *H. somnus* [**combo 068**]. According to a more preferred 25 embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen of IBR and at least one antigen each of one or more pathogenic species of Leptospira, as mentioned above, and at least one antigen of *H. somnus* [**combo 069**].

According to a further embodiment, the present invention relates to a combination 30 vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the

treatment and/or prophylaxis of infections caused by **IBR**, **PI-3** and one or more pathogenic specie(s) of **Leptospira**, as mentioned above, and ***H. somnus* [combo 070]**. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of **IBR**, **PI-3**, and at least one antigen each of one or more pathogenic species of **Leptospira**, as mentioned above, and at least one antigen of ***H. somnus* [combo 071]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR**, **PI-3**, ***Leptospira canicola***, ***Leptospira grippotyphosa***, ***Leptospira hardjo* (*Leptospira hardjoprajitno* and/or *Leptospira hardjo-bovis*)**, ***Leptospira icterohaemorrhagiae***, ***Leptospira pomona*** and ***H. somnus* [combo 072]**. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of **IBR**, **PI-3**, ***Leptospira canicola***, ***Leptospira grippotyphosa***, ***Leptospira hardjo* (*Leptospira hardjoprajitno* and/or *Leptospira hardjo-bovis*)**, ***Leptospira icterohaemorrhagiae***, ***Leptospira pomona***, and ***H. somnus* [combo 073]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR**, **PI-3**, **BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), and one or more pathogenic specie(s) of **Leptospira**, as mentioned above, and ***H. somnus* [combo 074]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of **IBR**, **PI-3**, **BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), and one or more antigens each of one or more pathogenic species of **Leptospira**, as mentioned above, and at least one antigen of ***H. somnus* [combo 075]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV** (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), ***Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo (Leptospirahardjo prajitno and/or Leptospira hardjo-bovis), Leptospira icterohaemorrhagiae, Leptospira pomona and H. somnus [combo 076]***. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), ***Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo, Leptospira icterohaemorrhagiae, Leptospira pomona and H. somnus [combo 077]***.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by IBR, PI-3, BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), **BHV** and one or more pathogenic **specie(s) of *Leptospira***, as mentioned above, and ***H. somnus [combo 078]***. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), BHV and one or more antigens each of one or more pathogenic species of *Leptospira*, as mentioned above, and at least one antigen of *H. somnus [combo 079]*.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by one or more pathogenic **specie(s) of *Leptospira***, as mentioned above, and ***Campylobacter fetus [combo 080]***. According to a more

preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one or more antigens each of one or more pathogenic species of *Leptospira*, as mentioned above, and at least one antigen of *Campylobacter fetus* [combo 081].

5 According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by *Leptospira canicola*, *Leptospira grippotyphosa*,  
10 *Leptospira hardjo* (*Leptospira hardjoprajitno* and/or *Leptospira hardjo-bovis*), *Leptospira Icterohaemorrhagiae*, *Leptospira pomona* and *Campylobacter fetus* [combo 082]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least antigen each of *Leptospira canicola*,  
15 *Leptospira grippotyphosa*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and *Leptospira hardjo-bovis*), *Leptospira Icterohaemorrhagiae*, *Leptospira pomona* and *Campylobacter fetus* [combo 083]. According to a more preferred embodiment, the bacterial antigens are chemically inactivated, aluminum hydroxide adsorbed, whole cultures of said bacteria [combo 084]. According to a further preferred embodiment, said combination vaccine comprises gentamicin and Amphotericin B as preservatives [combo 085].

20 According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR** and one or more pathogenic **specie(s)**  
25 **of Leptospira**, as mentioned above, and *Campylobacter fetus* [combo 086]. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen of **IBR** and one or more antigens each of one or more pathogenic species of *Leptospira*, as mentioned above, and at least one antigen of *Campylobacter fetus* [combo 087].

30 According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle,

wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3** and one or more pathogenic **specie(s) of Leptospira**, as mentioned above, and **Campylobacter fetus [combo 088]**. According  
5 to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of **IBR, PI-3**, and one or more antigens each of one or more pathogenic species of **Leptospira**, as mentioned above, and at least one antigen of **Campylobacter fetus [combo 089]**.

According to a further embodiment, the present invention relates to a combination  
10 vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), and one or more pathogenic **specie(s) of Leptospira**, as mentioned above, and **Campylobacter fetus [combo 090]**. According to a more  
15 preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), and one or more antigens each of one or more pathogenic species of **Leptospira**, as mentioned above, and at least  
20 one antigen of **Campylobacter fetus [combo 091]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), **BHV**, one or more pathogenic **specie(s) of Leptospira**, as mentioned above, and **Campylobacter fetus [combo 092]**. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of **IBR, PI-3, BVDV (type 1 and/or type 2)**, **BHV** and one or  
30

more antigens each of one or more pathogenic species of *Leptospira*, as mentioned above, and at least one antigen of *Campylobacter fetus* [combo 093].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, 5 wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by one or more pathogenic specie(s) of **Leptospira**, as mentioned above, *H. somnus* and *Campylobacter fetus* [combo 094]. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the 10 attenuated and avirulent *M. bovis* as described herein, and one or more antigens each of one or more pathogenic species of *Leptospira*, as mentioned above, and at least one antigen each of *H. somnus* and *Campylobacter fetus* [combo 095].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, 15 wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR**, one or more pathogenic specie(s) of **Leptospira**, as mentioned above, *H. somnus* and *Campylobacter fetus* [combo 096]. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the 20 attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of **IBR**, *H. somnus* and *Campylobacter fetus*, and one or more antigens each of one or more pathogenic species of *Leptospira*, as mentioned above, [combo 097].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, 25 wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR**, **PI-3**, and one or more pathogenic specie(s) of **Leptospira**, as mentioned above, *H. somnus* and *Campylobacter fetus* [combo 098]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, 30 preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen

each of IBR, PI-3, *H. somnus* and *Campylobacter fetus* and one or more atingens each of one or more pathogenic species of *Leptospira*, as mentioned above, **[combo 099]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, 5 wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by IBR, PI-3, *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo*, *Leptospira icterohaemorrhagiae*, *Leptospira pomona*, *H. somnus* and *Campylobacter fetus* **[combo 100]**. According to a further embodiment, the 10 combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and/or *Leptospira hardjo-bovis*), *Leptospira icterohaemorrhagiae*, *Leptospira pomona*, *H. somnus* and *Campylobacter fetus* **[combo 101]**.

15 According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), and one or more pathogenic **specie(s) of Leptospira**, as mentioned above, *H. somnus* and *Campylobacter fetus* **[combo 102]**. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, BVDV (type 1 and/or type 2), *H. somnus* 20 and *Campylobacter fetus* and one or more antigens each of one or more pathogenic species of *Leptospira*, as mentioned above **[combo 100]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the 30 treatment and/or prophylaxis of infections caused by IBR, PI-3, BVDV (type 1 and/or type 2),

preferably attenuated BVDV (type 1 and/or type 2), *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and/or *Leptospira hardjo-bovis*), *Leptospira icterohaemorrhagiae*, *Leptospira Pomona*, *H. somnus* and *Campylobacter fetus* [combo 103]. According to a further embodiment, the combination vaccine comprises *M. bovis*, 5 preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo* (*Leptospira hardjoprajitno* and/or *Leptospira hardjo-bovis*), *Leptospira icterohaemorrhagiae*, *Leptospira pomona*, *H. somnus* and *Campylobacter fetus* [combo 104].

10 According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, 15 preferably attenuated BVDV (type 1 and/or type 2), **BHV** and one or more pathogenic **specie(s) of Leptospira**, as mentioned above, ***H. somnus*** and ***Campylobacter fetus*** [combo 105]. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), **BHV**, ***H. somnus*** and ***Campylobacter fetus***, and one or more antigens each of one or 20 more pathogenic species of *Leptospira*, as mentioned above, [combo 106].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises *M. bovis*, preferably 25 the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **BHV, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), **PI-3, IBR, *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira borgpetersenii* *Leptospira hardjo* (*Leptospira hardjoprajitno* and/or *Leptospira hardjo-bovis*), *Leptospira prajitno*, *Leptospira icterohaemorrhagiae*, *Leptospira bovis*, *Leptospira interrogans* and *Campylobacter fetus*** [combo 107]. According to a more preferred embodiment, the combination vaccine comprises 30

attenuated *M. bovis*, as described herein, and at least one antigen each of BHV, BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), IBR, PI-3, *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira borgpetersenii* *Leptospira hardjo* (*Leptospira hardjoprajitno* and/or *Leptospira hardjo-bovis*), *Leptospira prajitno*, *Leptospira icterohaemorrhagiae*, *Leptospira borgpetersenii*, *Leptospira bovis*, *Leptospira interrogans* and *Campylobacter fetus* [**combo 108**].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **Pasteurella haemolytica** and **Pasteurella multocida** [b**combo 109**]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of *Pasteurella haemolytica* bacterin and *Pasteurella multocida* bacterin. [b**combo 110**] According to a further preferred embodiment, said combination vaccine comprises neomycin and thimerosal as preservatives [b**combo 111**].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR**, **Pasteurella haemolytica** and **Pasteurella multocida** [b**combo 112**]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen of each of IBR, preferably as live modified viruses, *Pasteurella haemolytica* bacterin and *Pasteurella multocida* bacterin [b**combo 113**]. According to a further preferred embodiment, said combination vaccine comprises neomycin and thimerosal as preservatives [b**combo 114**].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment

and/or prophylaxis of infections caused by **IBR, PI-3, *Pasteurella haemolytica* and *Pasteurella multocida* [combo 115]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, preferably as live modified viruses, *Pasteurella haemolytica* 5 bacterin and *Pasteurella multocida* bacterin **[combo 116]**. According to a further preferred embodiment, said combination vaccine comprises neomycin and thimerosal as preservatives **[combo 117]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the 10 combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), ***Pasteurella haemolytica* and *Pasteurella multocida* [combo 118]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, 15 preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, preferably as live modified viruses, BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), *Pasteurella haemolytica* bacterin and *Pasteurella multocida* bacterin **[combo 119]**. According to a further preferred embodiment, said combination vaccine comprises neomycin and thimerosal as preservatives **[combo 120]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), **BHV, *Pasteurella haemolytica* and *Pasteurella multocida* [combo 121]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis*, as described herein, and at least one antigen each of IBR, PI-3, BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), BHV, preferably as live modified viruses, *Pasteurella haemolytica* 30 bacterin and *Pasteurella multocida* bacterin **[combo 122]**. According to a further preferred

embodiment, said combination vaccine comprises neomycin and thimerosal as preservatives [combo 123].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one further immunologically active component effective for the treatment and/or prophylaxis of infections caused by **BRSV** [combo 124]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least antigen of BRSV [combo 125].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, and BRSV** [combo 126]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least antigen of each IBR, preferably as live modified viruses, and BRSV [combo 127].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, and BRSV** [combo 128]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, preferably as live modified viruses, and BRSV [combo 129].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, and BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2) [combo 130]. According to a preferred

embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, BRSV, preferably as live modified viruses, and BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2) [combo 131].

5 According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, BHV, and BVDV (type 1 and/or 10 type 2)**, preferably attenuated BVDV (type 1 and/or type 2) [combo 132]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, BRSV, BHV, preferably as live modified viruses, and BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2) [combo 133].

15 According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **Pasteurella haemolytica, Pasteurella multocida and BRSV [combo 134]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of *Pasteurella haemolytica, Pasteurella multocida* and BRSV [combo 135].

20 According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, Pasteurella haemolytica, Pasteurella multocida and BRSV [combo 136]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, preferably as live modified viruses, *Pasteurella haemolytica, Pasteurella multocida* and BRSV [combo 137].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, *Pasteurella haemolytica*, *Pasteurella multocida* and BRSV [combo 138]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, preferably as live modified viruses, *Pasteurella haemolytica*, *Pasteurella multocida* and BRSV [combo 139].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), ***Pasteurella haemolytica*, *Pasteurella multocida* and BRSV [combo 140]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, BVDV (type 1 and/or type 2), preferably as live modified viruses, *Pasteurella haemolytica*, *Pasteurella multocida* and BRSV [combo 141].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), **BHV, *Pasteurella haemolytica*, *Pasteurella multocida* and BRSV [combo 140]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), BHV, preferably as live modified viruses, *Pasteurella haemolytica*, *Pasteurella multocida* and BRSV [combo 141].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by ***Pasteurella haemolytica, Pasteurella multocida and H. somnus [combo 142]***. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of *Pasteurella haemolytica, Pasteurella multocida* and *H. somnus [combo 143]*.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, *Pasteurella haemolytica, Pasteurella multocida and H. somnus [combo 144]***. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, preferably as live modified virus, *Pasteurella haemolytica, Pasteurella multocida* and *H. somnus [combo 145]*.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, *Pasteurella haemolytica, Pasteurella multocida and H. somnus [combo 146]***. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, preferably as live modified viruses, *Pasteurella haemolytica, Pasteurella multocida* and *H. somnus [combo 147]*.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment

and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), **Pasteurella haemolytica, Pasteurella multocida and H. somnus [combo 148]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, BVDV (type 1 and/or type 2), preferably attenuated BVDV (type 1 and/or type 2), preferably as live modified viruses, and *Pasteurella haemolytica, Pasteurella multocida and H. somnus [combo 149]*.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BVDV (type 1 and/or type 2)**, preferably attenuated BVDV (type 1 and/or type 2), **BHV, Pasteurella haemolytica, Pasteurella multocida and H. somnus [combo 150]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one antigen each of IBR, PI-3, BVDV (type 1 and/or type 2), BHV, preferably as live modified viruses, *Pasteurella haemolytica, Pasteurella multocida and H. somnus [combo 151]*.

According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150 and 151], that further comprises immunologically active components effective for the treatment and/or prophylaxis of infections caused by one or more pathogenic **species of Leptospira**, preferably selected from the group consisting of *Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo, Leptospira prajitno, Leptospira icterohaemorrhagiae, Leptospira bovis, Leptospira interrogans and Leptospira pomona [combo 152]*. According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150 and 151], that further comprises one or more antigens each of one or more specie(s) of *Leptospira*, preferably selected from the group consisting of *Leptospira*

*canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemorrhagiae, Leptospira bovis, Leptospira interrogans and Leptospira pomona.* [combo 153].

5 According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150 and 151], that further comprises an immunologically active component effective for the treatment and/or prophylaxis of infections 10 caused by **Campylobacter fetus** [combo 154]. According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150 and 151], that further comprises antigen of **Campylobacter fetus** [combo 155].

15 According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150 and 151], that further comprises an immunologically active component effective for the treatment and/or prophylaxis of infections 20 caused by one or more pathogenic **specie(s) of Leptospira**, preferably selected from the group consisting of *Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemorrhagiae, Leptospira bovis, Leptospira interrogans and Leptospira pomona*, and **Campylobacter fetus** [combo 156]. According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150 and 151], that further comprises one or more antigen each of *Campylobacter fetus* and of one or more 25 **specie(s) of Leptospira**, preferably selected from the group consisting of *Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-bovis), Leptospira prajitno, Leptospira*

*icterohaemorrhagiae*, *Leptospira bovis*, *Leptospira interrogans* and *Leptospira pomona*, and. [combo 157].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, 5 wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one further immunologically active component effective for the treatment and/or prophylaxis of infections caused by ***Clostridium perfringens*, preferably Types A, C and/or D** [combo 158]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as 10 described herein, and toxins of *Clostridium perfringens* Types C and D [combo 254]. According to a more preferred embodiment, said vaccine comprises antigens, preferably toxins, of *Clostridium perfringens*, preferably Types A, B, C, and/or D [combo 159].

According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 15 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 20 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156 and 157], that further comprises an immunologically active component effective for the treatment and/or prophylaxis of infections caused by infections caused by ***Clostridium perfringens*, preferably Types A, C and/or D** [combo 160]. According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 25 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156 and 157], that further comprises an immunologically active component effective for the treatment and/or prophylaxis of infections caused by infections caused by ***Clostridium perfringens*, preferably Types A, C and/or D** [combo 160]. According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 30 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156 and 157].

107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125,  
126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144,  
145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156 and 157], that further comprises  
5 antigen of *Clostridium perfringens*, preferably, Types A, C, and/or D [**combo 161**]. According to  
a further embodiment, the present invention relates to a combination vaccine according to any  
one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016,  
017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035,  
036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054,  
055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073,  
10 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092,  
093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111,  
112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130,  
131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149,  
150, 151, 152, 153, 154, 155, 156 and 157], that further comprises antigen of *Clostridium  
perfringens* Types, B, C, and/or D [**combo 162**].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by ***Clostridium perfringens* Types A, C and/or D, and *Clostridium tetani* [combo 163]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and toxins of *Clostridium perfringens* Types A, C and/or D, and *Clostridium tetani* [**combo 164**]. According to a more preferred embodiment, said vaccine comprises antigens, preferably toxins, of *Clostridium perfringens* Types A, B, C, and/or D, and *Clostridium tetani* [**combo 165**].

According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068,

069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087,  
088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106,  
107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125,  
126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144,  
5 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156 and 157], that further comprises  
immunologically active components effective for the treatment and/or prophylaxis of infections  
caused by infections caused by ***Clostridium perfringens* Types A, C and/or D, and *Clostridium***  
***tetani* [combo 166].** According to a further embodiment, the present invention relates to a  
combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008,  
10 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027,  
028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046,  
047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065,  
066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084,  
085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103,  
15 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122,  
123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141,  
142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156 and 157], that further  
comprises antigen of ***Clostridium perfringens* Types A, C, and/or D, and *Clostridium tetani***  
**[combo 167].** According to a further embodiment, the present invention relates to a combination  
20 vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011,  
012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030,  
031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049,  
050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068,  
069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087,  
25 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106,  
107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125,  
126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144,  
145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156 and 157], that further comprises one  
or more antigens of ***Clostridium perfringens* Types A, B, C, and/or D, and *Clostridium tetani***  
30 **[combo 168].**

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by ***Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium sordellii*, and *Clostridium perfringens* Types A, C and/or D [combo 169]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and one or more antigens, preferably toxins, each of *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium sordellii*, and *Clostridium perfringens* Types A, C and/or D [combo 170].

According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156 and 157], that further comprises immunologically active components effective for the treatment and/or prophylaxis of infections caused by infections caused by ***Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium sordellii*, and *Clostridium perfringens* Types A, C and/or D [combo 171]**.  
According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108,

109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127,  
128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146,  
147, 148, 149, 150, 151, 152, 153, 154, 155, 156 and 157], that further comprises one or more  
5 antigens each of of *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium*  
*sordellii*, and *Clostridium perfringens* Types A, C and/or D [**combo 172**]. According to a further  
embodiment, the present invention relates to a combination vaccine according to any one of  
[combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017,  
018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036,  
037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055,  
10 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074,  
075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093,  
094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112,  
113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131,  
132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150,  
15 151, 152, 153, 154, 155, 156 and 157], that further comprises one or more antigens each of  
*Clostridium perfringens* Types, A, B, C, and/or D, *Clostridium chauvoei*, *Clostridium septicum*,  
*Clostridium novyi*, *Clostridium sordellii* and *Clostridium tetani* [**combo 173**].

According to more preferred embodiment, the present invention relates to a combination  
vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle,  
20 wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M.*  
*bovis* as described herein, and further immunologically active components effective for the  
treatment and/or prophylaxis of infections caused by *Clostridium chauvoei*, *Clostridium*  
*septicum*, *Clostridium novyi*, *Clostridium sordellii*, *Clostridium perfringens* Types A, C  
and/or D and BRSV [**combo 174**]. According to a preferred embodiment, the combination  
25 vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein,  
and one or more antigens, preferably toxins, each of *Clostridium chauvoei*, *Clostridium*  
*septicum*, *Clostridium novyi*, *Clostridium sordellii*, and *Clostridium perfringens* Types A, C  
and/or D and *Mycoplasma bovis* [**combo 175**].

According to more preferred embodiment, the present invention relates to a combination  
30 vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle,  
wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M.*

5 *bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium sordellii*, *Clostridium perfringens* Types A, C and/or D, and *H. somnus*. [combo 176]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and one or more antigens, preferably toxins, each of *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium sordellii*, and *Clostridium perfringens* Types C and D and *H. somnus*. [combo 177].

10 According to more preferred embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium sordellii*, *Clostridium perfringens* Types A, C 15 and/or D, **BRSV**, and **H.somnus** [combo 178]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and one or more antigens, preferably toxins, each of *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium sordellii*, and *Clostridium perfringens* Types A, C and/or D, *Mycoplasma bovis*, and *H.somnus* [combo 179].

20 According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by *Salmonella*, preferably *Salmonella dublin*, 25 *Salmonella newport* and *Salmonella typhimurium* [combo 180]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and one or more toxins of *Salmonella*, preferably each of *Salmonella dublin*, *Salmonella newport*, and/or *Salmonella typhimurium* [combo 181].

30 According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030,

031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049,  
050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068,  
069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087,  
088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106,  
5 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125,  
126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144,  
145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162,  
163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178 and 179], that  
10 further comprises immunologically active components effective for the treatment and/or  
prophylaxis of infections caused by infections caused by *Salmonella*, preferably ***Salmonella dublin, Salmonella newport and Salmonella typhimurium [combo 182]***. According to a further  
embodiment, the present invention relates to a combination vaccine according to any one of  
[combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017,  
018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036,  
15 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055,  
056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074,  
075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093,  
094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112,  
113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131,  
20 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150,  
151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168,  
169, 170, 171, 172, 173, 174, 175, 176, 177, 178 and 179], that further comprises one or more  
antigens, preferably toxins, of *Salmonella*, preferably each of ***Salmonella dublin, salmonella newport and/or Salmonella typhimurium [combo 183]***.

25 According to a preferred embodiment, the present invention relates to a combination  
vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle,  
wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M.*  
*bovis* as described herein, and further immunologically active components effective for the  
treatment and/or prophylaxis of infections caused by ***Pasteurella haemolytica, Pasteurella***  
30 ***multocida, Salmonella, preferably Salmonella dublin, Salmonella newport and Salmonella typhimurium [combo 184]***. According to a preferred embodiment, the combination vaccine

comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and *Pasteurella haemolytica*, *Pasteurella multocida*, *Salmonella*, preferably *Salmonella dublin*, *Salmonella newport*, and *Salmonella typhimurium* Bacterin-Toxoid [combo 185]. According to more preferred embodiment, said combination vaccine comprises multiple isolates of 5 *Pasteurella haemolytica* Type A1 and an associated toxoid fraction, and single isolates of *P. multocida*, *S. dublin*, and *S. typhimurium* [combo 186].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by *Moraxella bovis* and/or *Klebsiella spp.*, 10 preferably *Klebsiella pneumoniae* [combo 187]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and one or more toxins each of *Moraxella bovis* and/or *Klebsiella spp.* 15 preferably *Klebsiella pneumoniae* [combo 188].

According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 20 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 25 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, and 186], that further comprises immunologically active components effective for the treatment and/or prophylaxis of infections caused by infections caused by 30 *Moraxella bovis* and/or *Klebsiella spp.*, preferably *Klebsiella pneumoniae* [combo 189]. According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013,

014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032,  
033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051,  
052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070,  
071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089,  
5 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108,  
109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127,  
128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146,  
147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 163, 164, 165, 169, 170,  
10 174, 175, 176, 177, 178, 179, 180, 181, 184, 185 and 186], that further comprises one or more  
antigens, preferably toxins, each of ***Moraxella bovis* and/or *Klebsiella* spp., preferably,**

15 ***Klebsiella pneumoniae* [combo 190].**

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by ***Escherichia coli* [combo 191]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and one or more toxins of ***Escherichia coli* [combo 192]**.

20 According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189 and 190], that further comprises an immunologically

active component effective for the treatment and/or prophylaxis of infections caused by infections caused by *Escherichia coli* [combo 193]. According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 5 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 10 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189 and 190], that further comprises antigen, preferably a toxin, of *Escherichia coli* [combo 194].

15 According to a preferred embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by *Pasteurella haemolytica*, *Pasteurella multocida*, *Salmonella dublin*, *Salmonella typhimurium* and *Escherichia coli* [combo 195]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and one or more antigens each of *Pasteurella haemolytica*, *Pasteurella multocida*, *Salmonella dublin*, *Salmonella typhimurium* and *Escherichia coli* Bacterin-Toxoid [combo 196]. According to more preferred embodiment, 20 said combination vaccine comprises multiple isolates of *Pasteurella haemolytica* Type A1 and an associated toxoid fraction, and single isolates of *P. multocida*, *S. dublin*, and *S. typhimurium* [combo 197].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, 30 wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the

treatment and/or prophylaxis of infections caused by **bovine Rotavirus [combo 198]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein and antigen of bovine Rotavirus **[combo 199]**.

5 According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 10 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 15 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 195, 196, and 197], that further comprises an immunologically active component effective for the treatment and/or prophylaxis of infections caused by infections caused by **bovine Rotavirus [200]**. According to a further embodiment, the present invention relates to a combination vaccine according to any one of 20 [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 25 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 30 188, 189, 190, 191, 192, 193, 194, 195, 196 and 197], that further comprises **antigen of bovine Rotavirus [combo 201]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one further immunologically active component effective for the treatment and/or prophylaxis of infections caused by **bovine Coronavirus [combo 202]**.  
5 According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and antigen of bovine Coronavirus **[combo 203]**.

According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196 and 197], that further comprises an immunologically active component effective for the treatment and/or prophylaxis of infections caused by infections caused by **bovine Coronavirus [combo 204]**. According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196 and 197].

151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, and 197], that further comprises antigen of bovine Coronavirus **[combo 205]**.

5 According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **bovine Coronavirus and bovine** 10 **Rotavirus [combo 206]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein and one or more antigens each of bovine Coronavirus and bovine Rotavirus **[combo 207]**.

According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 15 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 20 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196 and 197], that further 25 comprises immunologically active components effective for the treatment and/or prophylaxis of infections caused by **bovine Coronavirus and bovine Rotavirus [combo 208]**. According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070,

071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089,  
090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108,  
109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127,  
128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146,  
5 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164,  
165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183,  
184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, and 197 ], that further  
comprises one or more antigens each of bovine Coronavirus and bovine Rotavirus [**combo 209**].

According to a further embodiment, the present invention relates to a combination  
10 vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle,  
wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M.*  
*bovis* as described herein, and at least one further immunologically active component effective  
for the treatment and/or prophylaxis of infections caused by *Cryptosporidium parvum* [**combo**  
15 **210**]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*,  
preferably the attenuated and avirulent *M. bovis* as described herein, and antigen of  
*Cryptosporidium parvum* [**combo 211**].

According to a further embodiment, the present invention relates to a combination  
vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011,  
012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030,  
20 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049,  
050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068,  
069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087,  
088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106,  
107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125,  
25 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144,  
145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162,  
163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181,  
182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199,  
30 200, 201, 202, 203, 204, 205, 206, 207, 208 and 209], that further comprises an immunologically  
active component effective for the treatment and/or prophylaxis of infections caused by  
infections caused by *Cryptosporidium parvum* [**combo 212**]. According to a further

embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208 and 209], that further comprises antigen of *Cryptosporidium parvum* [combo 213].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one further immunologically active component effective for the treatment and/or prophylaxis of infections caused by *Cryptosporidium hominis* [combo 214]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and antigen of *Cryptosporidium hominis* [combo 215].

According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162,

163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181,  
182, 183, 184 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199,  
200, 201, 202, 203, 204, 205, 206, 207, 208 and 209], that further comprises an immunologically  
active component effective for the treatment and/or prophylaxis of infections caused by  
5 infections caused by *Cryptosporidium hominis* [combo 216]. According to a further  
embodiment, the present invention relates to a combination vaccine according to any one of  
[combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017,  
018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036,  
037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055,  
10 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074,  
075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093,  
094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112,  
113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131,  
132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150,  
15 151, 152, 153, 154, 155, 156, 157 ,158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168,  
169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187,  
188, 189, 190, 191, 192, 193, 194, 195, 196, 197 198, 199, 200, 201, 202, 203, 204 205, 206,  
207, 208 and 209], that further comprises antigen of *Cryptosporidium hominis* [combo 217].

According to a further embodiment, the present invention relates to a combination  
20 vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle,  
wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M.  
bovis* as described herein, and further immunologically active components effective for the  
treatment and/or prophylaxis of infections caused by *Cryptosporidium parvum* and  
*Cryptosporidium hominis* [combo 218]. According to a preferred embodiment, the combination  
25 vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein,  
and one or more antigens each of *Cryptosporidium parvum* and *Cryptosporidium hominis*  
[combo 219].

According to a further embodiment, the present invention relates to a combination  
30 vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011,  
012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030,  
031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049,

050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068,  
069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087,  
088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106,  
107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125,  
5 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144,  
145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162,  
163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181,  
182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199,  
10 200, 201, 202, 203, 204, 205, 206, 207, 208 and 209], that further comprises immunologically  
active components effective for the treatment and/or prophylaxis of infections caused by  
infections caused by *Cryptosporidium parvum* and *Cryptosporidium hominis* [combo 220].  
According to a further embodiment, the present invention relates to a combination vaccine  
according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013,  
014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032,  
15 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051,  
052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070,  
071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089,  
090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108,  
109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127,  
20 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146,  
147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164,  
165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183,  
184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202,  
203, 204, 205, 206, 207, 208 and 209], that further comprises one or more antigens each of  
25 *Cryptosporidium parvum* and *Cryptosporidium hominis* [combo 221].

According to a further embodiment, the present invention relates to a combination  
vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle,  
wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M.*  
*bovis* as described herein, and at least one further immunologically active component effective  
30 for the treatment and/or prophylaxis of infections caused by *Mycobacterium avium*  
*paratuberculosis* [combo 222]. According to a preferred embodiment, the combination vaccine

comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and antigen of *Mycobacterium avium paratuberculosis* [combo 223].

According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220 and 221], that further comprises an immunologically active component effective for the treatment and/or prophylaxis of infections caused by infections caused by *Mycobacterium avium paratuberculosis* [combo 224]. According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215,

216, 217, 218, 219, 220 and 221], that further comprises antigen of *Mycobacterium avium paratuberculosis* [combo 225].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, 5 wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one further immunologically active component effective for the treatment and/or prophylaxis of infections caused by **Adenovirus** [combo 226]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and antigen of Adenovirus [combo 10 227].

According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 15 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 20 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224 and 225], that further comprises an immunologically active 25 component effective for the treatment and/or prophylaxis of infections caused by infections caused by **Adenovirus** [combo 228]. According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082,

083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101,  
102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120,  
121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139,  
140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158,  
5 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176,  
177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195,  
196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214,  
215, 216, 217, 218, 219, 220, 221, 222, 223, 224, and 225], that further comprises antigen of  
Adenovirus **[combo 229]**.

10 According to a further embodiment, the present invention relates to a combination  
vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle,  
wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M.*  
*bovis* as described herein, and at least one further immunologically active component effective  
for the treatment and/or prophylaxis of infections caused by **Astrovirus [combo 230]**. According  
15 to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the  
attenuated and avirulent *M. bovis* as described herein, and antigen of **Astrovirus [combo 231]**.

According to a further embodiment, the present invention relates to a combination  
vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011,  
012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030,  
20 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049,  
050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068,  
069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087,  
088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106,  
107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125,  
25 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144,  
145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162,  
163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181,  
182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199,  
200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218,  
30 219, 220, 221, 222, 223, 224, 225, 226, 227, 228 and 229], that further comprises an  
immunologically active component effective for the treatment and/or prophylaxis of infections

caused by infections caused by **Astrovirus [combo 232]**. According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228 and 229], that further comprises antigen of **Astrovirus [combo 233]**.

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and at least one further immunologically active component effective for the treatment and/or prophylaxis of infections caused by **bovine Parvovirus [combo 234]**. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and antigen of bovine Parvovirus **[combo 235]**.

According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125,

126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144,  
145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162,  
163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181,  
182, 183, 184, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199,  
5 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218,  
219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232 and 233], that further  
comprises an immunologically active component effective for the treatment and/or prophylaxis  
of infections caused by infections caused by **bovine Parvovirus [combo 236]**. According to a  
further embodiment, the present invention relates to a combination vaccine according to any one  
10 of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017,  
018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036,  
037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055,  
056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074,  
075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093,  
15 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112,  
113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131,  
132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150,  
151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168,  
169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187,  
20 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206,  
207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225,  
226, 227, 228, 229, 230, 231, 232, and 233], that further comprises antigen of bovine Parvovirus  
[combo 237].

According to a further embodiment, the present invention relates to a combination  
25 vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle,  
wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M.*  
*bovis* as described herein, and further immunologically active components effective for the  
treatment and/or prophylaxis of infections caused by ***Cryptosporidium parvum*, Adenovirus,**  
**Astrovirus, bovine Parvovirus and *Mycobacterium avium paratuberculosis* [combo 238]**.  
30 According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably  
the attenuated and avirulent *M. bovis* as described herein, and one or more antigens each of

*Cryptosporidium parvum*, Adenovirus, Astrovirus, bovine Parvovirus and *Mycobacterium avium paratuberculosis* [combo 239].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, 5 wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by *Escherichia coli*, *Salmonella* spp., preferably *Salmonella dublin*, *Salmonella typhimurium* and *Salmonella newport*, bovine Rotavirus and bovine Coronavirus, *Cryptosporidium parvum*, Adenovirus, Astrovirus, 10 bovine Parvovirus and *Mycobacterium avium paratuberculosis* [combo 240]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and one or more antigens each of *Escherichia coli*, *Salmonella* spp., preferably *Salmonella dublin*, *Salmonella typhimurium* and *Salmonella newport*, bovine rotavirus and bovine Coronavirus, *Cryptosporidium parvum*, Adenovirus, 15 Astrovirus, bovine Parvovirus and *Mycobacterium avium paratuberculosis* [combo 241].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by *Streptococcus* spp., preferably *Streptococcus uberis* and /or *Streptococcus dysgalactiae* [combo 242]. According to a preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and antigen of *Streptococcus* spp., preferably each of *Streptococcus uberis* and/or *Streptococcus dysgalactiae*, [combo 243]. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and one or more antigens of *Streptococcus* spp., preferably each of *Streptococcus uberis* and/or *Streptococcus dysgalactiae* [combo 244].

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, 30 wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the

treatment and/or prophylaxis of infections caused by **Streptococcus spp., preferably Streptococcus uberis and /or Streptococcus dysgalactiae and/or Staphylococcus aureus [combo 245]**. According to a more preferred embodiment, the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and one or more 5 antigens of *Streptococcus* spp., preferably each of *Streptococcus uberis* and/or *Streptococcus dysgalactiae*, and/or ***Staphylococcus aureus [combo 246]***.

According to a further embodiment, the present invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 10 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 15 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 20 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, and 241], that further comprises immunologically active components effective for the treatment and/or prophylaxis of infections caused by infections caused by **Streptococcus spp., preferably Streptococcus uberis and/or Streptococcus dysgalactiae, and/or Staphylococcus aureus [combo 247]**. According to a further embodiment, the present invention 25 relates to a combination vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120,

121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139,  
140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158,  
254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176,  
177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195,  
5 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214,  
215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233,  
234, 235, 236, 237, 238, 239, 240 and 241], that further comprises one or more antigens each of  
10 **Streptococcus spp., preferably *Streptococcus uberis* and/or *Streptococcus dysgalactiae*, and/or *Staphylococcus aureus* [combo 248].** According to a further embodiment, the present  
invention relates to a combination vaccine according to any one of [combo 001, 002, 003, 004,  
005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023,  
024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042,  
043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061,  
062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080,  
15 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099,  
100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118,  
119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137,  
138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156,  
157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174,  
20 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193,  
194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212,  
213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231,  
232, 233, 234, 235, 236, 237, 238, 239, 240 and 241], that further comprises one or more antigens of  
25 **several serotypes of *Streptococcus spp.*, preferably of *several serotypes* each of *Streptococcus uberis* and/or *Streptococcus dysgalactiae*, and/or *Staphylococcus aureus* [combo 249].**

According to a further embodiment, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of infections caused by **Streptococcus spp., preferably**

5 *Streptococcus uberis*, *Streptococcus dysgalactiae* and/or *Staphylococcus aureus*, **Klebsiella**  
spp. and **Mycoplasma** spp. [combo 250]. According to a preferred embodiment, the  
combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as  
described herein, and one or more antigens of **Streptococcus** spp., preferably each of  
10 *Streptococcus uberis*, *Streptococcus dysgalactiae*, and/or *Streptococcus aureus*, **Klebsiella**  
spp. and **Mycoplasma** spp. [combo 251]. According to a more preferred embodiment, the  
combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as  
described herein, and one or more antigens of **Streptococcus** spp., preferably each of  
15 *Streptococcus uberis* and/or *Streptococcus dysgalactiae*, *Staphylococcus aureus*, **Klebsiella**  
spp., **Mycoplasma** spp. and endotoxin [combo 252].

According to a further embodiment, the present invention relates to a combination  
vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle,  
wherein the combination vaccine comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as  
described herein, and further immunologically active components effective for the  
15 treatment and/or prophylaxis of infections caused by **Trichophyton** and **Microsporum**,  
preferably selected from the group consisting of *Trichophyton verrucosum*, *Trichophyton*  
*mentagrophytes*, *Trichophyton equinum*, *Trichophyton sarkisovii*, *Microsporum canis*,  
*Microsporum canis* var. *obesum*, *Microsporum canis* var. *distortum*, and *Microsporum*  
20 *gypseum* [combo 253]. According to a preferred embodiment, the combination vaccine  
comprises *M. bovis*, preferably the attenuated and avirulent *M. bovis* as described herein, and one  
or more antigens each of **Trichophyton**, and **Microsporum**, preferably selected from the group  
consisting of *Trichophyton verrucosum*, *Trichophyton mentagrophytes*, *Trichophyton equinum*,  
*Trichophyton sarkisovii*, *Microsporum canis*, *Microsporum canis* var. *obesum*, *Microsporum*  
25 *canis* var. *distortum*, and *Microsporum gypseum* [combo 254].

25 According to a further embodiment, the present invention relates to a combination  
vaccine according to any one of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011,  
012, 013, 014, 015, 016, 017, 018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030,  
031, 032, 033, 034, 035, 036, 037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049,  
050, 051, 052, 053, 054, 055, 056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068,  
30 069, 070, 071, 072, 073, 074, 075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087,  
088, 089, 090, 091, 092, 093, 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106,

107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125,  
126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144,  
145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162,  
163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181,  
5 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200,  
201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219,  
220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238,  
239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, and 251], that further comprises  
10 immunologically active components effective for the treatment and/or prophylaxis of infections  
caused by infections caused by **Trichophyton and Microsporum**, preferably selected from the  
group consisting of *Trichophyton verrucosum*, *Trichophyton mentagrophytes*, *Trichophyton equinum*,  
*Trichophyton sarkisovii*, *Microsporum canis*, *Microsporum canis* var. *obesum*,  
*Microsporum canis* var. *distortum*, and *Microsporum gypseum* [combo 255]. According to a  
further embodiment, the present invention relates to a combination vaccine according to any one  
15 of [combo 001, 002, 003, 004, 005, 006, 007, 008, 009, 010, 011, 012, 013, 014, 015, 016, 017,  
018, 019, 020, 021, 022, 023, 024, 025, 026, 027, 028, 029, 030, 031, 032, 033, 034, 035, 036,  
037, 038, 039, 040, 041, 042, 043, 044, 045, 046, 047, 048, 049, 050, 051, 052, 053, 054, 055,  
056, 057, 058, 059, 060, 061, 062, 063, 064, 065, 066, 067, 068, 069, 070, 071, 072, 073, 074,  
075, 076, 077, 078, 079, 080, 081, 082, 083, 084, 085, 086, 087, 088, 089, 090, 091, 092, 093,  
20 094, 095, 096, 097, 098, 099, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112,  
113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131,  
132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150,  
151, 152, 153, 154, 155, 156, 157, 158, 254, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168,  
169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187,  
25 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206,  
207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225,  
226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244,  
245, 246, 247, 248, 249, 250, 251, and 252], that further comprises one or more antigens each of  
Trichophyton and Microsporum, preferably selected from the group consisting of *Trichophyton*  
30 *verrucosum*, *Trichophyton mentagrophytes*, *Trichophyton equinum*, *Trichophyton sarkisovii*,

*Microsporum canis*, *Microsporum canis* var. *obesum*, *Microsporum canis* var. *distortum*, and *Microsporum gypseum* [combo 256].

According to a further aspect, the present invention also relates to the combined use or co-administration of any of the antigens provided in the combination vaccines [combo 1 to 5 combo 256]. Preferably, the *M. bovis* antigen is provided in a first immunogenic composition, and any other antigen/antigens are provided in one or more further immunogenic compositions to be administered.

According to further embodiment, the immunologically active component(s) effective for the treatment and/or prophylaxis of infections caused by one or more cattle relevant pathogen(s) 10 as source of the combination vaccine provided herewith is any one included in the vaccines selected from the group consisting of: Alpha 7<sup>TM</sup>, ALPHA-7/MB<sup>TM</sup>, ALPHA-CD<sup>TM</sup>, BAR-VAC® 7, BAR-VAC® 7/SOMNUS, BAR-VAC® 8, BAR-VAC® CD, BAR-VAC® C/DT, BREED-BACK<sup>TM</sup> FP 10, BREED-BACK<sup>TM</sup> FP 10 HS, BREED-BACK<sup>TM</sup> FP 5, BREED-BACK<sup>TM</sup> FP 5 HS, BREED-BACK-10<sup>TM</sup>, CALIBER® 3, CALIBER® 7, ELITE 4<sup>TM</sup>, ELITE 15 9<sup>TM</sup>, ELITE 9-HS<sup>TM</sup>, EXPRESS® 10, EXPRESS® 10-HS, EXPRESS® 3, EXPRESS® 3/Lp, EXPRESS 4<sup>®</sup>, EXPRESS® 5, EXPRESS® 5-HS, EXPRESS® 5-PHM, EXPRESS® I, EXPRESS® I/LP, OCU-GUARD® MB, PULMO-GUARD<sup>TM</sup> MpB, PULMO-GUARD<sup>TM</sup> PH-M, PULMO-GUARD<sup>TM</sup> PH-M/SDT, PULMO-GUARD<sup>TM</sup> PHM-1, TETGUARD<sup>TM</sup>, VIBRIO-LEPTO-5<sup>TM</sup> (all of **Boehringer Ingelheim, St. Joseph, MO**); Cobalt<sup>TM</sup> 7, I-Site<sup>TM</sup>, Lepto 5, Master Guard® 20 Preg 5, Master Guard® 10, Master Guard® 10 + Vibrio, Master Guard® J5, P.H.M. Bac® 1, Pre-vent 6<sup>TM</sup>, Respromune® 4, Respromune® 4 + Somnumune® (IM, SC), Respromune® 5 I-B-P+BRSV, Respromune® 5+L5, Respromune® 5+L5 Somnus, Respromune® 5+Somnumune, Respromune® 5+VL5, Respromune® 8, Respromune® 9, Respromune® 10, Scour Vac<sup>TM</sup> 4, Scour Vac<sup>TM</sup> 9, Scour Vac<sup>TM</sup> E coli + C, Somnumune®, Titanium<sup>TM</sup> 3, Titanium<sup>TM</sup> 4, 25 Titanium<sup>TM</sup> 4 L5, Titanium<sup>TM</sup> 5, Titanium<sup>TM</sup> 5 L5, Titanium® 5+P.H.M. Bac®-1, Titanium<sup>TM</sup> BRSV 3, Titanium<sup>TM</sup> IBR, Titanium<sup>TM</sup> IBR-LP (all of **Agri Laboratories Inc., St. Joseph, MO**); Herd-Vac® 3, Herd-Vac® 3 S, Herd-Vac® 8, Herd-Vac® 9, Surround<sup>TM</sup> 4, Surround<sup>TM</sup> 4+HS, Surround<sup>TM</sup> 8, Surround<sup>TM</sup> 9, Surround<sup>TM</sup> 9+HS, Surround<sup>TM</sup> HS, Surround<sup>TM</sup> L5, Surround<sup>TM</sup> V-L5 (all of **BioCor, Omaha, NE (Pfizer)**); Mycomune® (**Biomune Co., Lenexa, KS**); Bluetongue vaccine, Bovine Virus Diarrhea Vaccine, *Campylobacter fetus* bacterin-bovine, Essential 1, Essential 2, Essential 2+P, Essential 3, Essential 3+T, Essential 4, Lepto-5,

*Mannheimia haemolytica-Pasteurella multocida* bacterin, Pre-breed 6, Pre-breed 8, Respira-1, Respira-3, Wart Vaccine (all of **Colorado Serum Company, Denver, CO**); Pyramid® 3, Pyramid® 4, Pyramid® 4 + Presponse® SQ, Pyramid® 5, Pyramid® 8, Pyramid® 9, Pyramid® IBR, Pyramid® IBR+Lepto, Triangle® 1 + Type II BVD, Triangle® 3 + VL5, Triangle® 4 + 5 HS, Triangle® 4 + PH/HS, Triangle® 4 + PH-K, Triangle® 4 + Type II BVD, Triangle® 9 + HS, Triangle® 9 + PH-K, Triangle® + Type II BVD, Trichguard®, Trichguard® + V5L, TriVib 5L® (all of **Fort Dodge Animal Health, Overland Park, KS (Wyeth)**); J-5 *Escherichia coli* Bacterin, Serpens Species Bacterin; *Staphylococcus aureus* bacterin-toxoid (all of **Hygieia Biological Laboratories, Woodland, CA**); Endovac-Bovi® with Immuneplus® (**Immvac, Inc., Columbia, MO**); 20/20 Vision® with Spur®, L5 SQ, Neoguard™, MasterGuard® Preg 5, Once PMH®, Once PMH® SQ, Vibralone™-L5, Vision® 7 Somnus with Spur®, Vision® 7 with Spur®, Vision® 8 Somnus with Spur®, Vision® 8 with Spur®, Vision® CD-T with Spur®, Vision® CD with Spur®, Vista™ IBR SQ, Vista™ 3 SQ, Vista™ 5 SQ, Vista™ 5 L5 SQ, Vista™ Once SQ, VL5 SQ, Volar®, (all of **Intervet Inc., Millsboro, DE**); Vac®, Reliant® 3, Reliant® 4, Reliant® IBR, Reliant® IBR/BVD, Reliant® IBR/Lepto, Reliant® Plus BVD-K 15 (Dual IBR™), Reliant® Plus (Dual IBR™), Respishield™ 4, Respishield™ 4 L5, Respishield™ HM (all of **Merial LTD, Duluth, GA**); Arsenal® 4.1, Arsenal® IBR, Arsenal® IBR BVD, Bovine Pili Shield™, Bovine Pili Shield™+C, Clostri Shield® 7, Clostri Shield® BCD, Fusogard®, Lepto Shield™ 5, Pinkeye Shield™ XT4, Salmo Shield® T, Salmo Shield® TD, 20 Scour Bos™ 4, Scour Bos™ 9, Somnu Shield™, Trep Shield™ HW, Vib Shield® L5, Vib Shield® Plus, Vib Shield® Plus L5, Vira Shield® 2, Vira Shield® 2+BRSV, Vira Shield® 3, Vira Shield® 3+VL5, Vira Shield® 4, Vira Shield® 4+L5, Vira Shield® 5, Vira Shield® 5+L5, Vira Shield® 5+L5 Somnus, Vira Shield® 5+Somnus, Vira Shield® 5+VL5, Vira Shield® 5+VL5 Somnus, Vira Shield® 6, Vira Shield® 6+Somnus, Wart Shield™ (all of **Novartis 25 Animal Health, Basel, Switzerland**); Bovi-K® 4, Bovi-Shield™ 3, Bovi-Shield™ 4, Bovi-Shield™ BRSV, Bovi-Shield® FP™ 4+L5, Bovi-Shield® GOLD 3, Bovi-Shield® GOLD 5, Bovi-Shield® GOLD FP™ 5 L5, Bovi-Shield® GOLD FP™ 5 VL5, Bovi-Shield® Gold IBR-BVD, Bovi-Shield® Gold IBR-BVD-BRSV-LP, Bovi-Shield™ IBR, Bovi-Shield™ IBR-BRSV-LP, Bovi-Shield™ IBR-BVD, Bovi-Shield™ IBR-BVD-BRSV-LP, Bovi-Shield™ IBR-PI3-BRSV, Calf-Guard®, CattleMaster® 4, CattleMaster® 4+L5, CattleMaster® 4+VL5, 30 CattleMaster® BVD-K, CattleMaster® Gold FP™ 5, CattleMaster® Gold FP™ 5 L5,

Defensor® 3, Fortress® 7, Fortress® 8, Fortress® CD, Leptoferm®-5, One Shot®, One Shot Ultra™ 7, One Shot Ultra™ 8, PregGuard™ FP 9, PregGuard® Gold FP™ 10, Resvac® BRSV/Somubac®, Resvac® 4/Somubac®, ScourGuard 3® (K), ScourGuard 3® (K)/C, Somubac®, Spirovac®, Spirovac® L5, Spirovac® VL5, StayBred™ VL5, TSV-2™, Ultrabac® 5, Ultrabac® 7/Somubac®, Ultrabac® 8, Ultrabac® CD, UltraChoice™ 7, UltraChoice™ 8, UltraChoice™ CD, Upjohn J-5 Bacterin™, Vibrin® (all of **Pfizer Inc., New York, NY**); Covexin® 8 Vaccine, Electroid® 7 Vaccine, Electroid® D, Guardian™, Jencine® 2, Jencine® 3, Jencine® 4, Nasalgen® IP Vaccine, Piliguard® Pinkeye-1 Trivalent, Piliguard® Pinkeye + 7, Piliguard® Pinkeye Triview®, Siteguard® G, Siteguard® MLG Vaccine (all of **Schering-10 Plough Animal Health Corporation, Kenilworth, NJ**); Myco-Bac™ B, Poly-Bac B® 3, Poly-Bac B® Somnus, Super Poly-Bac B® Somnus (all of **Texas Vet Lab, Inc., San Angelo, TX**), Virabos™-3 with Immunostim®, Virabos™-4 + *H. somnus* with Immunostim®, and Virabos™-4 with Immunostim® (all of **Bioniche Animal Health, Athens, GA**), wherein the *M. bovis* antigen, preferably the attenuated, avirulent *M. bovis* as described herein, is added. Alternatively, 15 when *M. bovis* antigen is present in any of those vaccines, attenuated avirulent *M. bovis*, as described herein, is added, or the *M. bovis* antigen present any of those vaccines is substituted by the attenuated, avirulent *M. bovis* as described herein.

#### Dose and administration

According to the present invention, an effective amount of a combination vaccine 20 administered to cattle provides effective immunity against microbiological infections caused by *M. bovis* and at least one further pathogen as listed above. Preferred combinations of antigens for the treatment and prophylaxis of microbiological diseases in cattle are listed above.

In preferred forms, the dose volume of the combination vaccine as well as of each immunogenic composition when separately co-administered is no more than 5ml, more 25 preferably no more than 3 ml, and more preferably no more than 2 ml. In a most preferred embodiment, the dose is 2 ml, preferably administered intranasally, with 1 ml being administered in each nostril, more preferably administered intramuscularly, even more preferably administered subcutaneously, and most preferably administered both intranasally and subcutaneously on one occasion as a single dose. In some preferred forms, a second or subsequent administration of the 30 immunogenic composition would be administered after the first administration. Such a subsequent administration would preferably occur at least 10 days after the initial administration,

more preferably between at least 10 –32 days, more preferably between at least 12-30 days, still more preferably at least 14 days, and most preferably between at least 14-28 days. In most preferred forms, the vaccine would be administered either as a single dose, preferably on Day 0 or, in alternative forms, 14-28 days thereafter, preferably on Day 0 and 14-28 days thereafter with exposure to pathogenic forms of *M. bovis* not occurring until after the completion of the immunizing regimen. In a most preferred form, no booster is necessary and the vaccine is administered only one time. The vaccine is administered to animals from 1 day of age through adulthood, preferably to calves from 1 day of age through young adult cattle 2 years of age, more preferably to calves from 1 day of age through 16 weeks of age, and most preferably to calves from 6 weeks to 12 weeks of age. Such administration lessened or reduced signs of *M. bovis* infection as described below. Preferably, signs of *M. bovis* infection in the group vaccinated as described above are reduced by at least 50%, more preferably at least 60%, even more preferably at least 70%, and even more preferably at least 75% in comparison to the non-vaccinated group. Lung pathology assessment, specifically the percentage of lung consolidation attributed to lesions due to *M. bovis*, as customarily scored for various species, was made post-necropsy. Preferably lung lesions are reduced by at least 33%, more preferably at least 50%, even more preferably at least 70%, even more preferably at least 80%, even more preferably at least 90%, and most preferably by at least 95% in comparison to a non-vaccinated group.

The amount of combination vaccine that is effective depends on the ingredients of the vaccine and the schedule of administration. Typically, when bacterial antigen is used in the combination vaccine or in a combined use, the vaccine or immunogenic composition contains an amount of about  $10^3$  to about  $10^{10}$  colony forming units (CFU) of the bacterial antigen per dose, preferably, about  $10^4$  to about  $10^9$  CFU of the bacterial antigen per dose, more preferably about  $10^5$  to about  $10^6$  CFU of the bacterial antigen per dose. For instance, the attenuated, avirulent *M. bovis* is preferably used in amounts of about  $10^2$  to about  $10^{10}$  CFU per dose, preferably about  $10^3$  to about  $10^9$  CFU per dose, even more preferably in an amount of about  $10^8$  to about  $10^{10}$  CFU per dose, most preferably in an amount of about  $2.1 \times 10^9$  CFU per dose.

Typically, when an inactivated virus or a modified live virus preparation is used in the combination vaccine or in a combined use, the vaccine or immunogenic composition containing about  $10^2$  to about  $10^9$  TCID<sub>50</sub> viral antigen per dose, preferably about  $10^3$  to about  $10^8$  TCID<sub>50</sub> viral antigen per dose, more preferably, about  $10^4$  to about  $10^8$  TCID<sub>50</sub> viral antigen per dose. For

example, about  $10^5$  to about  $10^8$  TCID<sub>50</sub> per dose of attenuated BVDV (types 1 and 2) is effective when administered twice to the animal during a period of about 3 to 4 weeks. In general, inactivated antigen is normally used in higher amounts than live modified viruses.

In the event the combination vaccine comprises live modified IBR, the amount of IBR 5 antigen is preferably in a range of about  $10^5$  to  $10^{7.5}$  TCID<sub>50</sub> per dose. In the event the combination vaccine comprises live modified PI3, the amount of PI3 antigen is preferably in a range of about  $10^7$  to  $10^9$  TCID<sub>50</sub> per dose. In the event the combination vaccine comprises live modified BRSV, the amount of BRSV antigen is preferably in a range of about  $10^{4.5}$  to  $10^{6.5}$  TCID<sub>50</sub> per dose. In the event the combination vaccine comprises killed antigens the TCID<sub>50</sub> or 10 CFU indicates the amount of antigen per dose in the live culture before inactivation, and for IBR, the amount of IBR antigen is preferably in a range of about  $10^{7.0}$  to  $10^{9.0}$  TCID<sub>50</sub> per dose. In the event the combination vaccine comprises killed PI3, the amount of PI3 antigen is preferably in a range of about  $10^{7.2}$  to  $10^{9.2}$  TCID<sub>50</sub> per dose. In the event the combination vaccine comprises killed BRSV, the amount of BRSV antigen is preferably in a range of about  $10^{5.0}$  to  $10^{7.5}$  TCID<sub>50</sub> 15 per dose. In the event the combination vaccine comprises killed Leptospira spp. the amount of each Leptospira spp. antigen is preferably in a range of about  $10^{7.0}$  to  $10^{10}$  (CFU) per dose. In the event the combination vaccine comprises killed *H. somnus*, and/or killed *Pasteurella multocida*, and/or killed *Mannheimia haemolytica* the amount of *H. somnus* antigen and/or *Pasteurella multocida* antigen, and/or *Mannheimia haemolytica* antigen is preferably in a range of about  $10^{6.0}$  20 to  $10^{10}$  colony forming unit (CFU) per dose.

#### Combined use/ method of treatment

A further aspect of the present invention relates to the combined use of immunogenic 25 compositions for the treatment and/or prophylaxis of cattle against microbiological infections, wherein the infections are caused by *M. bovis* and at least one further cattle relevant pathogen.

Yet another important embodiment of the invention is a method for the prophylaxis or treatment of diseases caused by *M. bovis*, and further cattle pathogenic microorganism(s), wherein a *M. bovis* antigen, preferably the attenuated, avirulent *M. bovis* as described herein, and further immunologically active components effective for the treatment and/or prophylaxis of 30 the infection caused by said further cattle pathogenic microorganism, preferably as described

herein, are administered to an animal in need thereof at a suitable dose, as known to the skilled person.

The combined use or the method of co-administration of two or more antigens of pathogens affecting cattle comprises administering a first immunogenic composition comprising 5 a *M. bovis* antigen, preferably the attenuated, avirulent *M. bovis* as provided herewith, and at least one further immunogenic composition comprising an immunologically active component effective for the treatment and/or prophylaxis of infections caused by a further pathogen of cattle. Preferably, the further pathogen is one of the pathogens as listed herein. Preferably, the first and the further immunogenic compositions are administered separately. Preferably, the 10 further immunogenic composition comprises one or more immunologically active component(s) effective for the treatment and/or prophylaxis of infections caused by a pathogen of cattle other than *M. bovis*. More preferably, the first and the further immunogenic compositions are administered together by means such as mixing before administration and/or by formulating the first and the further immunogenic compositions in a single container.

15 The co-administration of each of the immunogenic compositions occurs simultaneously, which means at least within 48 hours, preferably within 24 hours, even more preferably within 12 hours, even more preferably within 6 hours, even more preferably within 3 hours, even more preferably within 2 hours, even more preferably within 1 hour. The route of administration of each of the immunogenic compositions depends on the mode-of-action and may be the same, but 20 also could be different.

According to a further embodiment the *M. bovis* antigen as provided herewith and one or more further immunologically active component(s) effective for the treatment and/or prophylaxis of infections caused by a further cattle relevant pathogen other than *M. bovis* can be used a medicament. Preferably, that medicament is a vaccine and can be used for lessening or reducing 25 the signs of a *M. bovis* infection. Most preferably, that medicament or vaccine can be used for lessening or reducing the signs of a *M. bovis* infection and associated with or caused by an infection of the further cattle relevant antigen.

#### Formulations

30 A further aspect of the present invention is the preparation of the combination vaccine(s). One of skill in the art can determine additional components which are present in the composition

of the invention. (see also Remington's Pharmaceutical Sciences, (1990) 18th ed. Mack Publ., Easton). Known injectable, physiologically acceptable sterile solutions may be used. For preparing a ready-to-use solution for parenteral injection or infusion, aqueous isotonic solutions, such as e.g. saline or corresponding plasma protein solutions, are readily available. The 5 pharmaceutical compositions of the present invention may be present as lyophylisates or dry preparations, which can be reconstituted with a known injectable solution directly before use under sterile conditions, e.g. as a kit of parts.

In addition, the immunogenic and vaccine compositions of the present invention can include one or more veterinary-acceptable carriers. As used herein, "a veterinary-acceptable 10 carrier" includes any and all solvents, dispersion media, coatings, adjuvants, stabilizing agents, diluents, preservatives, antibacterial and antifungal agents, isotonic agents, adsorption delaying agents, and the like.

Diluents can include water, saline, dextrose, ethanol, glycerol, and the like. Isotonic agents can include sodium chloride, dextrose, mannitol, sorbitol, and lactose, among others. 15 Stabilizers include albumin and alkali salts of ethylenediamintetraacetic acid, among others.

Adjuvants include, but are not limited to, the RIBI adjuvant system (Ribi Inc.), alum, aluminum hydroxide gel, Cholesterol, oil-in water emulsions, water-in-oil emulsions such as, e. g., Freund's complete and incomplete adjuvants, Block co-polymer (CytRx, Atlanta GA), SAF-M (Chiron, Emeryville CA), CARBOPOL®, AMPHIGENO adjuvant, saponin, Quil A, QS-21 20 (Cambridge Biotech Inc., Cambridge MA), GPI-0100 (Galenica Pharmaceuticals, Inc., Birmingham, AL) or other saponin fractions, monophosphoryl lipid A, Avridine lipid-amine adjuvant, heat-labile enterotoxin from *E. coli* (recombinant or otherwise), cholera toxin, or muramyl dipeptide, among many others.

The immunogenic compositions can further include one or more other 25 immunomodulatory agents such as, e. g., interleukins, interferons, or other cytokines. The immunogenic compositions can also include Gentamicin and Merthiolate. While the amounts and concentrations of adjuvants and additives useful in the context of the present invention can readily be determined by the skilled artisan, preferably the composition comprises from about 50 ug to about 2000 ug of adjuvant and preferably about 250 ug/ ml dose of the vaccine 30 composition. In another preferred embodiment, the present invention an antibiotic is present in an amount of from about 1ug/ml to about 60 ug/ml of and preferably less than about 30 ug/ml.

According to a further embodiment the combination vaccine (or immunogenic composition) is first dehydrated. If the composition is first lyophilized or dehydrated by other methods, then, prior to vaccination, said composition is rehydrated in aqueous (e.g. saline, PBS (phosphate buffered saline)) or non-aqueous solutions (e.g. oil emulsion (mineral oil, or vegetable/metabolizable oil based/single or double emulsion based), aluminum-based, carbomer based adjuvant).

According to a further embodiment, the immunogenic composition or combination vaccine as provided herewith, which comprises at least one *M. bovis* antigen and one or more further immunologically active component(s) effective for the treatment and/or prophylaxis of infections caused by a further cattle relevant pathogen other than *M. bovis* are formulated as fix-dose combination vaccine. Preferably, the immunogenic composition or combination vaccine provided herewith, in particular the fix-dose combination vaccine is formulated for use as a single-dose or multi-dose vaccine, whereas the formulation for use as a single-dose vaccine is most preferred. In other words the immunogenic compositions or vaccine, preferably the fix-dose combination vaccine provided herewith is formulated for the administration as a multi-dose or single-dose, whereas the formulation for the administration as single-dose is most preferred. As shown in the example section, such a single dose administration of the *M. bovis* antigen is effective in lessening or reducing the signs of an *M. bovis* infection. Thus, according to a further embodiment, the immunogenic compositions provided herewith, in particular the fix-dose combination vaccine is formulated for use as a single-dose vaccine, wherein the administration of such single-dose is effective in lessening or reducing the signs of an *M. bovis* infection.

#### Modes of Administration and Dosing

Compositions of the present invention may be administered in any conventional manner. Examples of administration methods include any that afford access by cells of the immune system to the immunogenic composition, including but not limited to, oral, transdermal, intradermal, intravenous, subcutaneous, intramuscular, intraocular, intraperitoneal, intrarectal, intravaginal, intranasal, intragastrical, intratracheal, intrapulmonary, or any combination thereof. Preferred modes of administration are intramuscular, subcutaneous and intranasal, with subcutaneous and intranasal being especially preferred. If desired or necessary, booster

immunizations may be given once or several times at various intervals. However, it is a preferred embodiment of the present invention that the vaccination be administered as a single-dose. After administration of such a vaccine, an immune response is elicited in the animal and signs of *M. bovis* infection are reduced in incidence and/or severity, as well as a reduction in rate of 5 mortality, in comparison to animals exposed to wild-type bacteria or isolates after challenge with a virulent form of *M. bovis*.

In preferred forms, the dose volume of the vaccine is no more than 5ml, more preferably no more than 3 ml, and more preferably no more than 2ml. In a most preferred embodiment, the dose would be 2 ml, preferably administered intranasally, with 1 ml being administered in each 10 nostril, even more preferably administered subcutaneously, and most preferably administered both intranasally and subcutaneously on one occasion as a single dose. In some preferred forms, a second or subsequent administration of the immunogenic composition would be administered after the first administration.

Such a subsequent administration would preferably occur at least 10 days after the initial 15 administration, more preferably between at least 10 –32 days, more preferably between at least 12-30 days, still more preferably at least 14 days, and most preferably between at least 14-28 days. In most preferred forms, the vaccine would be administered either on Day 0 as a single dose, or, in alternative forms, on Day 0 and 14-28 days thereafter with exposure to pathogenic forms of *M. bovis* not occurring until after the completion of the immunizing regimen. In a most 20 preferred form, no booster is necessary and the vaccine is administered only one time. The vaccine is administered to animals from 1 day of age through adulthood, preferably to calves from 1 day of age through young adult cattle 2 years of age, more preferably to calves from 1 day of age through 16 weeks of age, and most preferably to calves from 6 weeks to 12 weeks of age. Such administration reduced signs of *M. bovis* infection as described below. In fact, the 25 studies herein show that signs of *M. bovis* infection in the group vaccinated as described above were reduced by at least 50%, more preferably, at least 60%, even more preferably, at least 70%, even more preferably, at least 75%, more preferably, at least 80%, still more preferably at least 83%, more preferably, at least 85%, and, most preferably, at least 90% in comparison to the non-vaccinated group.

30 In a preferred embodiment, the immunogenic compositions of the present invention are effective in stimulating an onset of immunity within 14 days following a single dose

administration. In an additionally preferred embodiment the immunogenic composition of the present invention is effective in stimulating duration of immunity of at least 42 days following a single dose administration of the immunogenic composition.

In another preferred embodiment, the immunogenic compositions of the present invention may be co-administered to an animal (preferably cattle). Specifically two or more antigens may be administered to an animal of the *M. bovis* antigen of the present invention and at least one other immunologically active component(s) effective for the treatment and/or prophylaxis of infections caused by a further cattle relevant pathogen other than *M. bovis* (discussed in more detail *infra*). The *M. bovis* antigen of the present invention and the immunologically active component(s) may be co-administered or administered separately. An example of separate co-administration includes the *M. bovis* antigen and the immunologically active component(s) occurring within 2 days. Alternatively, the *M. bovis* antigen and one or more immunologically active component(s) may be formulated as fix-dose combination vaccine. Another preferred embodiment would be administering the two or more antigens which comprise *M. bovis* antigen and one or more immunologically active component(s) effective for the treatment and/or prophylaxis of infections caused by a further cattle relevant pathogen other than *M. bovis* are in one only dose. Alternatively two, three, or four doses may be administered that is, co-administered or administered separately.

In a preferred embodiment, the immunogenic composition of the present invention contains an adjuvant. Adjuvants may include aluminum hydroxide and aluminum phosphate, saponins e.g., Quil A, QS-21 (Cambridge Biotech Inc., Cambridge MA), GPI-0100 (Galenica Pharmaceuticals, Inc., Birmingham, AL), water-in-oil emulsion, oil-in-water emulsion, water-in-oil-in-water emulsion. The emulsion may be based in particular on light liquid paraffin oil (European Pharmacopea type); isoprenoid oil such as squalane or squalene; oil resulting from the oligomerization of alkenes, in particular of isobutene or decene; esters of acids or of alcohols containing a linear alkyl group, more particularly plant oils, ethyl oleate, propylene glycol di-caprylate/caprate), glyceryl tri-(caprylate/caprate) or propylene glycol dioleate; esters of branched fatty acids or alcohols, in particular isostearic acid esters. The oil is used in combination with emulsifiers to form the emulsion. The emulsifiers are preferably nonionic surfactants, in particular esters of sorbitan, of mannide (e.g. anhydromannitol oleate), of glycol, of polyglycerol, of propylene glycol and of oleic, isostearic, ricinoleic or hydroxystearic acid,

which are optionally ethoxylated, and polyoxypropylene-polyoxyethylene copolymer blocks, in particular the Pluronic products, especially L121. See Hunter et al., The Theory and Practical Application of Adjuvants (Ed. Stewart-Tull, D. E. S.). John Wiley and Sons, NY, pp51-94 (1995) and Todd et al., Vaccine 15:564-570 (1997), hereby entirely incorporated by reference.

5 For example, it is possible to use the SPT emulsion described on page 147 of "Vaccine Design, The Subunit and Adjuvant Approach" edited by M. Powell and M. Newman, Plenum Press, 1995, and the emulsion MF59 described on page 183 of this same book.

A further instance of an adjuvant is a compound chosen from the polymers of acrylic or methacrylic acid and the copolymers of maleic anhydride and alkenyl derivative. Advantageous 10 adjuvant compounds are the polymers of acrylic or methacrylic acid which are cross-linked, especially with polyalkenyl ethers of sugars or polyalcohols. These compounds are known by the term carbomer (Phameuropa Vol. 8, No. 2, June 1996, hereby entirely incorporated by reference). Persons skilled in the art may also refer to U. S. Patent No. 2,909,462 (hereby entirely incorporated by reference) which describes such acrylic polymers cross-linked with a 15 polyhydroxylated compound having at least 3 hydroxyl groups, preferably not more than 8, the hydrogen atoms of at least three hydroxyls being replaced by unsaturated aliphatic radicals having at least 2 carbon atoms. The preferred radicals are those containing from 2 to 4 carbon atoms, e.g. vinyls, allyls and other ethylenically unsaturated groups. The unsaturated radicals may themselves contain other substituents, such as methyl. The products sold under the name 20 Carbopol (BF Goodrich, Ohio, USA) are particularly appropriate. They are cross-linked with an allyl sucrose or with allyl pentaerythritol. Among them, there may be mentioned Carbopol 974P, 934P and 971P. Most preferred is the use of Cabopol 971P. Among the copolymers of maleic anhydride and alkenyl derivative, are the copolymers EMA (Monsanto), which are copolymers 25 of maleic anhydride and ethylene. The dissolution of these polymers in water leads to an acid solution that will be neutralized, preferably to physiological pH, in order to give the adjuvant solution into which the immunogenic, immunological or vaccine composition itself will be incorporated.

Further suitable adjuvants include, but are not limited to, the RIBI adjuvant system (Ribi 30 Inc.), Block co-polymer (CytRx, Atlanta GA), SAF-M (Chiron, Emeryville CA), monophosphoryl lipid A, Avridine lipid-amine adjuvant, heat-labile enterotoxin from *E. coli* (recombinant or otherwise), cholera toxin, IMS 1314 or muramyl dipeptide, or naturally

occurring or recombinant cytokines or analogs thereof or stimulants of endogenous cytokine release, among many others.

Preferably, the adjuvant is added in an amount of about 100 µg to about 1 g per dose. Even more preferably the adjuvant is added in an amount of about 100 µg to about 500 mg per dose. Even more preferably the adjuvant is added in an amount of about 500 µg to about 250 mg per dose. Even more preferably the adjuvant is added in an amount of about 750 µg to about 100 mg per dose. Even more preferably the adjuvant is added in an amount of about 1mg to about 50 mg per dose. Even more preferably the adjuvant is added in an amount of about 1 mg to about 10 mg per dose. Most preferably the adjuvant is added in an amount of about 1 mg per dose.

10

#### Carriers

In addition, the immunogenic and vaccine compositions of the present invention may include one or more veterinary-acceptable carriers. Thus, the present invention relates to the use of an *M. bovis* strain, attenuated through multiple passage or serial attenuation as described above, as a medicine, preferably as a veterinary medicine. *M. bovis* strains attenuated as described above may be used for the preparation of a pharmaceutical composition, as described herein, for the prophylaxis or treatment of infections caused by *M. bovis*. As noted above, those pharmaceutical compositions/vaccine compositions may be used for the treatment and/or prophylaxis of animals susceptible to infection by *M. bovis*.

20

#### Methods of treatment or prophylaxis

In another aspect of the present invention, the invention is a method for the treatment or prophylaxis including a lessening of the incidence of wild type infection in a herd or reduction in the severity of signs of *M. bovis* infection associated with wild type *M. bovis* infected animals. 25 administered immunogenic compositions in accordance with the present invention in comparison to animals that are either not vaccinated or vaccinated with vaccines available prior to the present invention is provided. Additionally, administration of the vaccine in accordance with the present invention reduces the number of animals in a herd that become infected with *M. bovis*. Such a method generally involves the administration of a therapeutically effective amount of an *M. bovis* strain attenuated through the methods disclosed above, to a subject or herd of subjects in 30 need of such a treatment. Preferably, clinical symptoms are lessened in incidence or severity by

at least 10%, more preferably by at least 20%, still more preferably by at least 30%, even more preferably by at least 40%, still more preferably by at least 50%, even more preferably by at least 60%, still more preferably by at least 70%, even more preferably by at least 80%, still more preferably by at least 90%, and most preferably by at least 95% in comparison to animals that are 5 either not vaccinated or vaccinated with an *M. bovis* immunogenic composition that was available prior to the present invention but subsequently infected by wild-type *M. bovis*.

## EXAMPLES

The following examples are provided for the purposes of illustration and are not intended 10 to limit the scope of the present invention

### **Example 1: Preparation and testing of a high-passage *M. bovis* isolate vaccine**

#### **Materials and Methods**

Forty-four (44) calves were reared. Post-weaning, the calves were randomly assigned to 1 15 of 5 groups. Calves were  $6 \pm 2$  weeks of age at initiation of the study and received vaccination according to group assignment on Day 0 and Day 14. Approximately four (4) weeks later (Day 27), calves were challenged with virulent *M. bovis* or media only according to group assignment. Sixteen (16) days after challenge, (Day 43) the calves were necropsied. The group treatments are summarized in Table 1.

20

**Table 1: Group Treatments**

Groups	Animals / group	Test Substance			Challenge		
		Article	Dose / Route	Admin Schedule	Material	Dose / Route	Admin Schedule
Group 1	8	<i>M. bovis</i> MLV	~ $10^9$ CFU in 2 ml/ SQ and ~ $10^9$ CFU 2mL IN	Day 0 and 14	<i>M. bovis</i> (Single Isolate Fresh)	30 ml of challenge material and 10 ml of PBS	Day 27 (approx 4 weeks after vaccination)
Group 2	9	<i>M. bovis</i> MLV	~ $10^9$ CFU in 2 ml SQ	Day 0	<i>M. bovis</i> (Single Isolate Fresh)	30 ml of challenge material and 10 ml of PBS	Day 27 (approx 4 weeks after vaccination)
Group	9	<i>M. bovis</i>	~ $10^8$ CFU	Day 0	<i>M. bovis</i>	30 ml of	Day 27

3		<i>bovis</i> MLV	in 2 ml SQ		(Single Isolate Fresh)	challenge material and 10 ml of PBS	(approx 4 weeks after vaccination)
Group 4	9	<i>M. bovis</i> MLV	$\sim 10^7$ CFU in 2 ml SQ	Day 0	<i>M. bovis</i> (Single Isolate Fresh)	30 ml of challenge material and 10 ml of PBS	Day 27 (approx 4 weeks after vaccination)
Group 5	9	Media Only	2 ml SQ	Day 0	<i>M. bovis</i> (Single Isolate Fresh)	30 ml of challenge material and 10 ml of PBS	Day 27 (approx 4 weeks after vaccination)

High passage live *Mycoplasma bovis* strain, ATCC PTA-9666 (vaccine candidate 052823A131 MS+4), which was passaged 135 times in raw culture media, was used for vaccination. This strain was obtained from naturally occurring disease outbreak then serially 5 passaged in a modified (i.e. modified to remove CNS components therefrom) Friis Media to prepare the material. More specifically, the isolate was grown in Friis media supplemented with 10% yeast extract and 20% horse serum. The culture was grown  $24 \pm 2$  hours at  $37^\circ\text{C}$  after inoculation with an appropriate volume of seed culture determined before the study. Material was then frozen at  $<-60^\circ\text{C}$ . Prior to vaccination, the material was rapidly thawed and three dose 10 levels were prepared using Friis media as the diluent.

1e9 CFU Preparation (Groups 1 and 2):

2 ml dose administered subcutaneously and (group 1 only) 2 ml dose administered intranasally (1 ml in each nostril).

SQ Total: 8.4E8 CFU / Animal

15 IN Total: 8.4E8 CFU / Animal

1e8 CFU Preparation (Group 3):

2 ml dose administered subcutaneously.

SQ Total: 9.4E7 CFU / Animal

20

1e8 CFU Preparation (Group 4):

2 ml dose administered subcutaneously.

SQ Total: 1.0E7 CFU / Animal

Modified Live Vaccine *M. bovis* PTA-9666 (2<sup>nd</sup> Vaccination)

High passage live *Mycoplasma bovis* PTA-9666 in raw culture media.

The isolate used for vaccination was obtained from naturally occurring disease outbreak then serially passaged in the modified Friis Media. Material was prepared at BIVI. The isolate was grown in Friis media supplemented with 10% yeast extract and 20% horse serum. The culture was grown  $24 \pm 2$  hours at  $37^\circ\text{C}$  after inoculation with an appropriate volume of seed culture determined before the study. Material was frozen ( $<-60^\circ\text{C}$ ). Prior to vaccination, material was rapidly thawed and a single dose level was prepared using Friis media as the diluent.

10 1e9 CFU Preparation (Group 1):

2 ml dose administered subcutaneously and 2 ml dose administered intranasally (1 ml in each nostril).

SQ Total: 7.2E8 CFU / Animal

IN Total: 7.2E8 CFU / Animal

15 Media Only (1<sup>st</sup> Vaccination) Friis Media supplemented with 10% yeast extract and 20% horse serum dose administered subcutaneously.

SQ Total: 0 CFU / Animal serum

A summary of the study timeline is below in Table 2.

**Table 2: Study Timeline**

Day	Event	Samples	Testing
-42	Acquire Animals	--	--
-42 to 0	General Observations (Daily)	--	--
-35	Collect samples	Nasal swab (Wet/Dry)	<i>M. bovis</i> (Culture/PCR)
		Blood (SST)	<i>M. bovis</i> (ELISA)
		Ear-notch	BVDV (IHC)
-3 to -1	Transfer animals	--	--
0 to 28	Clinical assessment	--	--
0	Collect samples	Nasal swab (Wet/Dry)	<i>M. bovis</i> (PCR)
		Blood (SST)	<i>M. bovis</i> (ELISA)
	<b>1<sup>st</sup> Vaccination (All Groups)</b>	--	--
	Injection site evaluation	--	--

14	Injection site evaluation.	--	--
27	Collect samples	Nasal swab (Wet/Dry)	<i>M. bovis</i> (Culture/PCR)
		Blood (SST)	<i>M. bovis</i> (ELISA)
	<b>2<sup>nd</sup> Vaccination (Group 1 only)</b>	--	--
27 to 43	Collect samples	Nasal swab (Wet/Dry)	<i>M. bovis</i> (Culture/PCR)
		Blood (SST)	<i>M. bovis</i> (ELISA)
	Injection site evaluation	--	--
	<b>Challenge</b>	--	--
27	Clinical observation (Daily)	--	--
34	Collect samples	Nasal swab (Wet/Dry)	<i>M. bovis</i> (Culture/PCR)
		Blood (SST)	<i>M. bovis</i> (ELISA)
43	Collect samples (Pre)	Nasal swab (Wet/Dry)	<i>M. bovis</i> (Culture/PCR)
		Blood (SST)	<i>M. bovis</i> (ELISA)
	<b>Necropsy and Gross Pathology (% Lung path)</b>	--	--
	Collect samples (Post)	Tonsil swab (Wet/Dry)	<i>M. bovis</i> (Culture/PCR)
		Lung Tissue (Preserved)	<i>M. bovis</i> (IHC)
		Lung Tissue (Fresh)	<i>M. bovis</i> (Culture/PCR)

## SAMPLING

### Swab

Nasal swabs were collected from all calves on Days -22, 0, 14, 27, 34 and 43 (or day of post). At necropsy, tonsil swabs were collected from all calves. Joint swabs were taken from animals with clinical abnormalities. In addition, samples were taken from other locations in certain animals showing area involvement. In all cases, three sterile swabs were rubbed around the regions, as aseptically as possible, for a few seconds and then removed. The swabs were placed back into the transport containers. Two swabs were placed into individual transport containers with media and one was placed into a transport container without media. The swabs were appropriately labeled with the Study number, calf ID number and date. Sample collections were recorded on the Sample Collection Record. The swabs were stored on ice and transported the same day to BIVI – St. Joseph. The deliveries of the swabs were documented on a Specimen

Delivery Form.

Sera

Blood was collected from all calves on Days -42, 0, 14, 27, 34 and 43 (or day of post).

5 Blood was collected aseptically from a jugular vein from each calf into one 12.5 mL Serum Separator Tube (SST). Sample collections were recorded on the Sample Collection Record. The blood was delivered to BIVI – St. Joseph with the delivery being documented on a Specimen Delivery Form. Serum was then harvested from each tube by centrifugation and transferred to a sterile cryovial. The samples were stored at 4° C until testing followed by long term storage at -10 20° C.

Tissue

From all animals, samples of lung were collected at the discretion of the Study Investigator. Areas in which gross lesions were observed were targeted for sampling. Samples were placed in whirl pack bags and labeled with the animal number. The tissue samples were 15 stored on ice and transported the same day to BIVI – St. Joseph. An additional set of lung tissues were collected and placed into 10% formalin solution and appropriately labeled. Fixed tissue samples were shipped to ISU - Veterinary Diagnostic Laboratory. The sample sites selected for each calf are listed on the Sample Collection Record.

20 TESTING

Microbiology

Swabs placed in the transport media and tissue samples were shipped to BIVI for *Mycoplasma bovis* isolation (day of posting samples only). Briefly, swabs were swirled in 5mL Mycoplasma selective broth. A small sample (approx. 5mm) was cut from lung tissue and 25 homogenized in 5 mL of complete Friis media. 100ul of homogenate were added to Mycoplasma selective broth. Cultures were incubated at 37C / 5% CO<sub>2</sub>. After 4-14 days, the broth was examined for growth and subcultured to plates for isolation. All positive subculture samples were stored at -70° C.

30 PCR

Swabs from each calf not placed in transport media and tissue samples were shipped to BIVI where DNA was extracted and tested by PCR using primers and probe specific for the *uvrC* gene of *M. bovis* developed at BIVI. Results of PCR were expressed as positive or negative for *M. bovis* DNA detection.

5

### Serology

Serum samples were tested using an ELISA commercially available by Biovet (Canada) using the protocol provided with the test kit. ELISA results are expressed as Optical Density (OD) readings. Sample OD's were compared to the Positivity level (Mean OD<sub>p</sub> x 0.3) established by the positive control included in the test kit. Positive results were then interpreted according to the manufacturer's classification scheme. 0 being no seroconversion to 4 being very strong seroconversion (see seroconversion below in Table 3).

**Table 3: Seroconversion**

Interval	Interpretation
OD sample < Positivity Level	Negative (0)
Positivity Level < OD Sample < 1.75 * Positivity Level	+1
1.75 * Positivity Level < OD Sample < 2.3 * Positivity Level	+2
2.3 * Positivity Level < OD Sample < 3 * Positivity Level	+3
OD Sample > 3 * Positivity Level	+4

15

### Histopathology/IHC

Formalin-fixed tissues were sent to ISU Veterinary Diagnostic laboratory and tested by hematoxylin/eosin stained slide and immunohistochemistry using monoclonal antibodies specific for *M. bovis*

20

### Clinicals

Daily general observations were carried out from Day 0 to Day 27 and then daily clinical observations made from Day 28 to euthanasia and necropsy. Clinical and general observations noted deviation from the norm and were documented.

25

### Necropsy

Following euthanasia, each animal was necropsied. The thoracic cavity and trachea were examined for each calf and gross observations recorded on the Necropsy Report Record. The

lungs and about 6 inches of trachea from each calf were removed intact for further examination and sample collection.

#### Lung Pathology

5 For each set of lungs, each lung lobe was examined by visualization and by palpation. The quality and amount of pathology present (as a percent) per each lung lobe due to *M. bovis* was determined. Each lung lobe percent was then weighted and summed to determine the percentage of total lung with pathology.

#### 10 Joint Pathology

Affected joints were examined and gross observations recorded.

**Table 4: Statistical Methods of Analysis between groups for each parameter**

Parameter	Scoring System	Evaluation Of Each Parameter	Statistical Analysis between Groups
Total % Lung Pathology	Gross Pathology  Sum of % lung lobe pathology multiplied by lobular fraction of total lung per animal	Total % Lung Pathology Scores between groups	Wilcoxon Test
Clinical Signs of Coughing Post-Challenge	Clinical Observation (per day)  0 = No Coughing 1 = Coughing	Number of animals with coughing scores > 0 from Day 27 (DPC 0) to Day 43 (DPC 16) / total number of animals in the group.	Fisher's Exact Test
		Number of days with coughing scores > 0 from Day 27 (DPC 0) to Day 43 (DPC 16) / total number of days.	Fisher's Exact Test
Clinical Signs of Lameness Post-Challenge	Clinical Observation (per day)  Lameness Severity Score (per limb)	Number of animals with total lameness scores > 0 from Day 27 (DPC 0) to Day 43 (DPC 16) / total number of animals in the group.	Fisher's Exact Test

	<p>0 = No Lameness 1 = Mild 2 = Moderate 3 = Severe Note: Score of 5 if removed early from the study for humane reasons</p> <p>Sum of lameness score per animal</p>	Number of days with total lameness scores > 0 from Day 27 (DPC 0) to Day 43 (DPC 16) / total number of days.	Fisher's Exact Test
		Total lameness Scores between groups	Wilcoxon Test
Clinical Signs of Joint Swelling Post-Challenge	Clinical Observation (per day)	Number of animals with total joint swelling scores > 0 from Day 27 (DPC 0) to Day 43 (DPC 16) / total number of animals in the group.	Fisher's Exact Test
	Joint Swelling Score (per animal) 0 = No Swelling 1-12 = Number of joints with swelling Note: Last score for the remainder of the study if removed early from the study for humane reasons	Number of days with total joint swelling scores > 0 from Day 27 (DPC 0) to Day 43 (DPC 16) / total number of days.	Fisher's Exact Test
		Total Joint Swelling Scores between groups	Wilcoxon Test
Early Removal Rates (Mortality or Culling Rate)	Clinical Observation (per day) 0 = Alive 1 = Death or early removal for humane reasons	Number of animals removed from study prior to Day 43 (DPC 16) / total number of animals in the group	Fisher's Exact Test
Clinical Signs of Arthritis Score Post-Challenge	Clinical Observation (per day)	Number of animals with arthritis score > 0 from Day 27 (DPC 0) to Day 43 (DPC 16) / total number of animals in the group.	Fisher's Exact Test
	Sum of total lameness and joint swelling scores	Number of days with arthritis score > 0 from Day 27 (DPC 0) to Day 43 (DPC 16) / total number of days.	Fisher's Exact Test
		Total Arthritis Scores between groups	Wilcoxon Test
		Total Arthritis Scores between groups by day	Wilcoxon Test

Percent Reduction for All Parameters

Groups compared using percent reduction [1-(vaccinate/challenge)] and statistical significance for each parameter.

Table 5. Percent Reduction and Significance for all Parameters as Compared to the Challenge Control Group – Summary

Group	Total % Lung Pathology Scores	Clinical Signs of Coughing Post-Challenge		Clinical Signs of Lameness Post Challenge		
		Number of Animals with coughing scores >0	Days with coughing scores >0	Number of animals with total lameness scores >0	Days with total lameness >0	Total Lameness scores
1	68%	0%	-57%	33%	-8%	70%
2	30%	-19%	-129%	11%	-8%	31%
3	57%	-14%	-86%	5%	-8%	10%
4	71%	77%	85%	-11%	-17%	6%

Table 5. (con't)

Group	Clinical Signs of Joint Swelling Post-Challenge			Early Removal Rates	Clinical Signs of Arthritis Score Post-Challenge		
	Number of animals with total joint swelling scores >0	Days with total joint swelling scores >0	Total joint swelling scores		Number of animals removed early from study	Number of animals with arthritis score >0	Total Arthritis scores
1	33%	0%	63%	83%	17%	-8%	67%
2	26%	-8%	58%	55%	11%	-8%	39%
3	-33%	-8%	22%	43%	-33%	-8%	12%
4	-14%	-17%	32%	33%	-33%	-17%	10%

Group 1 = 2 Hi Dose (dual IN/SQ Day 0 and Day 14)

Group 2= 1 Hi Dose (SQ Day 0)

Group 3=1 Mid Dose (SQ Day 0)

Group 4= 1 Lo Dose (SQ Day 0)

Group 5= Challenge Only Control (all values are in comparison to challenge controls)

## DISCUSSION

The objective of this study was to evaluate the efficacy of a live high passage *M. bovis* vaccine candidate at 3 different dosage levels (1E9, 1E8 and 1E7 CFU per dose) administered 5 subcutaneously (SQ) with a single dose (Day 0). A vaccine control group was also included and given two doses (days 0 and 14) SQ and intranasally (IN) with the high dose level of vaccine.

The challenge and vaccine candidate *Mycoplasma bovis* isolates used in this study originated from different naturally infected farms. The challenge isolate was previously shown to cause both lung and joint disease during experimental challenge and predominated in mixed 10 isolate challenge studies. The live vaccine candidates are high passage isolates originally derived from diagnostic samples. High passage of the vaccine candidates was performed by serial limiting dilution in Friis base media supplemented with horse serum and yeast extract. Although no appreciable difference has been observed in the growth rate in the Friis complete media, at high passage, vaccine candidate PTA-9666 has demonstrated restricted growth on some 15 *Mycoplasma* selective agar formulations while the low passage parent isolate shows no apparent growth restriction. Also, genotypes of the challenge and vaccine isolates are dissimilar (as determined by insertion sequence PCR fingerprinting).

The challenge procedure using a total volume of 30mL of the challenge isolate and resulted in the challenge control group showing lung pathology in all animals and joint 20 involvement in 6 of 8 animals.

Multiple parameters were investigated during this study to access vaccine benefits and identify possible primary parameters for future studies. Of those parameters, animal removal 25 rates and joint clinical signs (lameness and joint swelling) were proposed as indicators of joint protection. Lung pathology (percent gross lung lesions) and clinical signs of coughing were proposed as indicators of lung protection. Lung lesion scores are provided in Figure 1. Total lameness scores were also taken and are presented in Figure 2.

Post-challenge, the SQ/IN two high dose vaccine group (group 1) had a lower incidence of joint swelling and removal rates, compared with the challenge control group (group 5). In addition, group 1 showed a trend in a reduced incidence of arthritis as shown in Figure 4. In the 30 SQ/IN single high dose vaccine group (group 2), a trend was observed in a lower incidence of

joint swelling as shown in Figure 3. Other groups did show a reduction in the incidence of joint clinical signs and removal rates.

5 Five animals tested positive for *M. bovis* antibodies prior to or on Day 0 and 5 of 9 within the challenge control group tested positive during the vaccination phase prior to challenge (see Protocol Deviation 3 & 4). Reasons for the serum titers prior to exposure may include ELISA reagent cross-reactivity, maternal antibodies and/or residual material from prior studies.

10 The data for this isolate passaged 135 times was compared to the data for this isolate when passaged at 106 times. Specifically, the data was compared where both passages of the isolate were administered two times subcutaneously and intranasally. In the 135 passage study, only 1 animal out of 8 were removed from the study due to death and culling. In the 106 passage study, 4 out of 9 animals were removed from the study due to death and culling. Death and culling was reduced in the isolate passaged 135 times by 31.9%. The results are summarized in the Table 6.

<b>Table 6: Comparison of 135 passage to 106 passage for death and culling</b>			
	Administration Method	Number of Animals Removed due to Death and culling	Percentage
135 passage	Two times subcutaneously and intranasaly	1/8	12.5%
106 passage (Live VacI)	Two times subcutaneously and intranasaly	4/9	44.4%
Difference			31.9%

15 CONCLUSIONS

20 *Mycoplasma bovis* vaccine candidate PTA-9666 via a simultaneous intranasal and subcutaneous route administered 2 times with a 2 week interval at a high dose level (1E9 CFU) showed a reduction of total joint swelling scores and early removal rates (joint protection) in colostrum deprived calves when challenged.

20

**Example 2: Efficacy of live *Mycoplasma bovis* vaccine (05-2823 P106) using various administration routes**

**Materials and Methods**

42 weaned calves negative for *Mycoplasma bovis* were reared. At post weaning (6 weeks ±2 weeks), the calves were randomly assigned to 1 of 6 groups. Calves were allowed to adjust for six (6) days and received vaccination according to group assignment on Day 0. Approximately four (4) weeks later (Day 28) all calves were challenged with virulent *M. bovis* according to group assignment. Fourteen (14) days after challenge (Day 42), the calves were necropsied. Three live vaccine candidates were used to immunize the calves. They are referred to as *M. bovis* Live Vaccine I, *M. bovis* Live Vaccine II, and *M. bovis* Live Vaccine III. These three representative strains include 052823A106, deposited with the ATCC in Manassas, VA on October 16, 2007 under the terms of the Budapest Treaty and designated as PTA-8694 (Live VacI); 05249A102, also deposited with the ATCC in Manassas, VA on October 16, 2007 under the terms of the Budapest Treaty and designated as PTA 8696 (Live Vac II); and 0519021B106, also deposited with the ATCC in Manassas, VA on October 16, 2007 and designated as PTA 8695 (Live Vac III).

Groups 1, 2, and 3 were given Live Vaccine I on Day 0 and Day 14. Group 1 was administered 2 mL subcutaneously and 2 mL intranasally for each administration. Group II was administered 2 mL subcutaneously for each administration. Group III was administered 2 mL intranasally for each administration. Group IV was a control group and was administered 2 mL of media subcutaneously and 2 mL of media intranasally for each administration. Group V was administered Live Vaccine II on Day 0 and Day 14 at a dose of 2 mL subcutaneously and 2 mL intranasally for each administration. Group VI was given Live Vaccine III on Day 0 and Day 14 at a dose of 2 mL subcutaneously and 2mL intranasally for each administration. All groups were challenged with virulent *M. bovis* on Day 28. A summary of the Study Design is illustrated in Table 7.

**Table 7: Group Treatments**

Groups	Animals / group	Test Substance			Challenge		
		Article	Dose / Route	Admin Schedule	Material	Dose / Route	Admin Schedule
Group 1	10	<i>M. bovis</i> Live I	2 ml SQ and 2mL	Day 0 and 14	<i>M. bovis</i> (24466-192)	120 ml of challenge material with 15 ml PBS	Day 28 (approx 4 weeks after vaccination)

			IN				
Group 2	10	<i>M. bovis</i> Live I	2 mL SQ	Day 0 and 14	<i>M. bovis</i> (24466-192)	120 ml of challenge material with 15 ml PBS	Day 28 (approx 4 weeks after vaccination)
Group 3	9	<i>M. bovis</i> Live I	2mL IN	Day 0 and 14	<i>M. bovis</i> (24466-192)	120 ml of challenge material with 15 ml PBS	Day 28 (approx 4 weeks after vaccination)
Group 4	9	Media Only	2 mL SQ and 2mL IN	Day 0 and 14	<i>M. bovis</i> (24466-192)	120 ml of challenge material with 15 ml PBS	Day 28 (approx 4 weeks after vaccination)
Group 5	2	<i>M. bovis</i> Live II	2 mL SQ and 2mL IN	Day 0 and 14	<i>M. bovis</i> (24466-192)	120 ml of challenge material with 15 ml PBS	Day 28 (approx 4 weeks after vaccination)
Group 6	2	<i>M. bovis</i> Live III	2 mL SQ and 2mL IN	Day 0 and 14	<i>M. bovis</i> (24466-192)	120 ml of challenge material with 15 ml PBS	Day 28 (approx 4 weeks after vaccination)

The *M. bovis* Live Vaccine I isolate used for vaccination was obtained from naturally occurring disease outbreak then serially passaged (106 times) in modified Friis Media. The isolate was grown in Friis media supplemented with 10% yeast extract and 20% horse serum.

5 The culture was grown 24 ± 2 hours at 37° C after inoculation with an appropriate volume of seed culture determined before the study. The isolate was used without dilution. The average pre and post vaccination concentration was found to be 3.0E9 CFU / ml.

The *M. bovis* Live Vaccine II isolate used for vaccination was obtained from naturally occurring disease outbreak then serially passaged (102 times) in modified Friis Media. The 10 isolate was grown in Friis media supplemented with 10% yeast extract and 20% horse serum. The culture was grown 24 ± 2 hours at 37° C after inoculation with an appropriate volume of seed culture determined before the study. The isolate was used without dilution. The average pre-vaccination concentration was found to be 7.8E8 CFU / ml.

The *M. bovis* Live Vaccine III isolate used for vaccination was obtained from naturally 15 occurring disease outbreak then serially passaged (106 times) in modified Friis Media. The

isolate was grown in Friis media supplemented with 10% yeast extract and 20% horse serum. The culture was grown 24 ± 2 hours at 37° C after inoculation with an appropriate volume of seed culture determined before the study. The isolate was used without dilution. The average pre vaccination concentration was found to be 1.7E8 CFU / ml.

5 The challenge material, a virulent *M. bovis* isolate was obtained from naturally occurring disease outbreak. The average pre and post challenge concentration was found to be 1.8E9 CFU/ml.

Samples were taken from the animals, such as nasal swabs and blood tests. The Sample Schedule is summarized in Table 8.

10 **Table 8: Study Timeline**

Day	Event	Samples	Testing
approx. -42	Acquire Animals	--	--
-42 to 0	General Observations (Daily)	--	--
Approx -35	Collect samples	Nasal swab (Wet/Dry) Blood (SST) Ear-notch	<i>M. bovis</i> (Culture/PCR) <i>M. bovis</i> (ELISA) BVDV (IHC)
-6	Transfer animals	--	--
0 to 28	Clinical assessment	--	--
0	Collect samples	Nasal swab (Wet/Dry) Blood (SST)	<i>M. bovis</i> (Culture/PCR) <i>M. bovis</i> (ELISA)
	<b>1<sup>st</sup> Vaccination</b>	--	--
14	Injection site evaluation. Collect samples	-- Nasal swab (Wet/Dry) Blood (SST)	-- <i>M. bovis</i> (Culture/PCR) <i>M. bovis</i> (ELISA)
	<b>2<sup>nd</sup> Vaccination</b>	--	--
27	Collect samples	Nasal swab (Wet/Dry) Blood (SST)	<i>M. bovis</i> (Culture/PCR) <i>M. bovis</i> (ELISA)
28	<b>Challenge</b>	--	--
29 to 42	Clinical observation (Daily)	--	--
35	Collect samples	Nasal swab (Wet/Dry) Blood (SST)	<i>M. bovis</i> (Culture/PCR) <i>M. bovis</i> (ELISA)
41	Collect samples	Nasal swab (Wet/Dry) Blood (SST)	<i>M. bovis</i> (Culture/PCR) <i>M. bovis</i> (ELISA)
42	<b>Necropsy and Gross Pathology</b> Collect samples (Post)	-- Tonsil swab (Wet/Dry) Lung Tissue (Preserved) Lung Tissue (Fresh) Joint swabs (Wet/Dry)	-- <i>M. bovis</i> (Culture/PCR) <i>M. bovis</i> (IHC) <i>M. bovis</i> (Culture/PCR) <i>M. bovis</i> (Culture/PCR)

## SAMPLING

Nasal swabs were collected from all calves on Days 0, 14, 27, 35 and 41. At necropsy, tonsil swabs were collected from all calves. Joint swabs were taken from animals with clinical abnormalities. In addition, samples were taken from other locations in certain animals showing 5 area involvement. In all cases, three sterile swabs were rubbed around the regions, as aseptically as possible, for a few seconds and then removed.

### Tissue

From all animals, samples of lung were collected. Areas in which gross lesions were 10 observed were targeted for sampling. An additional set of lung tissues were collected and placed into 10% formalin solution.

### Sera

Blood was collected from all calves on Days 0, 14, 27, 35 and 41. Blood was collected 15 aseptically from a jugular vein from each calf into one 12.5 mL Serum Separator Tube (SST).

## TESTING

### Microbiology

Briefly, swabs were swirled in 5mL Mycoplasma selective broth. A small sample 20 (approx. 5mm) was cut from lung tissue and homogenized in 2mL of complete Friis media. 100ul of homogenate were added to Mycoplasma selective broth. Cultures were incubated at 37C/ 5% CO<sub>2</sub>. After 4-14 days, the broth was examined for growth and subcultured to plates for isolation. All positive subculture samples were stored at -70° C.

### 25 PCR

DNA was extracted and tested by PCR using primers and probe specific for the *uvrC* gene of *M. bovis*. Results of PCR were expressed as positive or negative for *M. bovis* DNA detection.

### 30 Serology

Serum samples were tested using an ELISA commercially available by Biovet (Canada) using the protocol provided with the test kit. ELISA results are expressed as Optical Density (OD) readings. Sample OD's were compared to the Positivity level (Mean OD<sub>p</sub> x 0.3) established by the positive control included in the test kit. Positive results were then interpreted 5 according to the manufacturer's classification scheme, with 0 being no seroconversion to 4 being very strong seroconversion.

#### Histopathology/IHC

Formalin-fixed tissues were sent to ISU Veterinary Diagnostic laboratory and tested by 10 hematoxylin/eosin stained slide and immunohistochemistry using monoclonal antibodies specific for *M. bovis*.

#### Clinical Signs

Daily general observations were carried out from Day 0 to Day 28 and then daily clinical 15 observations made from Day 29 to euthanasia and necropsy. Clinical and general observations noted deviation from the norm and were documented.

#### Necropsy

Following euthanasia, each animal was necropsied. The thoracic cavity and trachea were 20 examined for each calf and gross observations recorded on the Necropsy Report Record. The lungs and about 6 inches of trachea from each calf were removed intact for further examination and sample collection.

For each set of lungs, the dorsal and ventral lung surfaces were photographed with an appropriate ear tag alongside each view.

25

#### Lung Pathology

For each set of lungs, each lung lobe was examined by visualization and by palpation. An approximation of how much pathology was present (as a percent) per each lung lobe due to *M. bovis* was determined. Each lung lobe percent was then weighted and summed to determine the 30 percentage of total lung with pathology.

#### Joint Pathology

Affected joints were examined and gross observations recorded.

#### Changes to the Study Protocol

Six animals were excluded from analysis. One animal each was excluded from Groups I, 5 II, and III. Three animals were excluded from Group IV. The one animal in Group II was found dead with lung pathology inconsistent with *M. bovis*, and the rest were excluded from analysis for testing positive for *M. bovis*. Twenty-two animals were removed prior to study termination from Day 30-38 for humane reasons. One animal in Group 5 was removed on Day 40, prior to 10 study termination, because the animal died by asphyxiation after becoming trapped in the feed bunk.

### Results and Discussion

#### Post-Challenge Clinical Signs

Clinical observations were made from Day 28 through Day 42. Coughing, labored respiration, 15 depression, swollen joints, lameness and droopy ear were clinical observations noted during this phase of the study. Clinical signs were divided into three types (respiratory, joint and other) typical of *Mycoplasma bovis* infection. Respiratory signs included coughing, rapid/labored respiration and nasal discharge. Joint signs included swollen joints and lameness.

#### 20 Lung Pathology

At necropsy, lungs were collected and observed for lesions associated with *M. bovis*. Animals exhibited variability in pathological features such as consolidation and nodular lesion formation. Results of lung involvement were expressed as a percent using a scoring system, which reflects the percentage of the total lung with gross pathology associated with *M. bovis* 25 infection. In some cases, determination of lung percent involvement was hampered by adhesions or the atypical nature of lesions.

#### Joint Pathology

At necropsy, joints from animals that previously exhibited clinical symptoms (swelling 30 and/or lameness) were examined for gross pathology. Areas affected varied by animal and may involve the carpus, hock, stifle, fetlock and/or elbow. Animals presented with gross swelling,

increased synovial fluid, abnormal fluid appearance, or thickening of the joint capsule. In more severely affected calves, fibrin was present as was erosion of the articular surface. Samples of joint fluid and/or surface swabs were tested by culture and PCR for the presence of *Mycoplasma bovis*.

5 PCR Detection of *M. bovis* from Nasal, Tonsil and Lung Samples

The nasal passages were sampled from each animal by swab on Day 0, 14, 27, 35 and 41 or Day of Necropsy. In addition, during the post-mortem, samples of tonsils were taken by swab and representative lung tissue was recovered. The frequency of detection using real-time PCR targeting a general *M. bovis* marker (uvrC) was analyzed. In addition, tonsil and lung tissue were 10 analyzed using a recently developed end-point PCR assay targeting markers not found in the *M. bovis* challenge isolate but found in all vaccine candidates.

*M. bovis* Serology

All samples were tested in the Biovet *M. bovis* ELISA to monitor the serological 15 response to *M. bovis*. Seroconversion was scored according to grouped multipliers of positivity ODs. The mean serological scores detected from each group on Day 0, 14, 27, 35 and Post (post represents a range of study days from 37 to 41 due to early removal of certain animals) were analyzed. Figure 5 illustrates the serology results for Live Vac I, II, III, and controls and Figure 6 20 illustrates the comparison of serology for Live Vac I using various routes of administration.

DISCUSSION

The objective of this study was to assess the efficacy of an experimental live *Mycoplasma bovis* vaccine (05-2823 P106) using various 2mL administration routes (SQ, IN, SQ+IN) 25 fourteen days apart and a dual challenge model in the target species. The challenge model used a high volume administered to two locations of the lung with the addition of an IV administration. In addition, two other live vaccine candidates (05-249 P102 and 05-1902-1 P106) were evaluated for efficacy using only the SQ+IN route.

The challenge and vaccine candidate *Mycoplasma bovis* isolates originated from different naturally infected farms. The challenge isolate was previously shown to cause both lung and 30 joint disease during experimental challenge and predominated in mixed isolate challenge studies. The live vaccine candidates are high passage isolates originally derived from diagnostic samples.

High passage of the vaccine candidates was performed by serial limiting dilution in Friis base media supplemented with horse serum and yeast extract. Although no appreciable difference has been observed in the growth rate in the Friis complete media, at high passage, vaccine candidate 05-2823 P106 has demonstrated restricted growth on some *Mycoplasma* selective agar 5 formulations while the low passage parent isolate shows no apparent growth restriction. Also, genotypes of the challenge and vaccine isolates are dissimilar (as determined by insertion sequence PCR fingerprinting).

The challenge procedure using a total volume of 120mL of the challenge isolate administered to each animal resulted in the challenge only group showing lung pathology and 10 joint involvement in all animals.

Multiple parameters were investigated during this study to access vaccine benefits. Of those parameters, animal removal rates and joint clinical symptoms were used as primary indicators of joint protection. Lung pathology (percent gross lung lesions) was used as the primary indicator of lung protection. Other data such as detection of organism from tissue, joint 15 distribution, and serology provided additional data for conformation.

All groups showed some lung and joint protective benefit after receiving the vaccine candidate *Mycoplasma bovis* Live Vaccine I (05-2823 P106) regardless of route or route combination as demonstrated by a reduction in lung lesions, joint clinical symptoms and animal removal rates. The combined SQ and IN route (Group 1) resulted in the greatest reduction of 20 lung lesions (86%) compared to the groups using only a single route. Additionally, results of lung lesions, joint clinical symptoms and removal rate reductions suggest benefit from receiving the two other vaccine candidates Live Vaccine II (05-249 P102) and Live Vaccine III (05-1902-1 P106) by a combined SQ and IN route. ELISA results demonstrated a strong humoral response to vaccination with all vaccine candidates.

25 All vaccine candidates demonstrated safety. No animals from any group receiving a vaccine presented with clinical symptoms during the vaccination period and only one animal that had received Live Vaccine III (05-1902-1 P106) showed reactivity at an injection site. Additionally, results of PCR showed non-challenge *M. bovis* detection from the tonsil tissue of only groups receiving a vaccine candidate via the IN route and detection of non-challenge from 30 lung tissue in only a single animal that had received Live Vaccine I (05-2823 P106) by both IN and SQ routes. A comparison of serology for Live Vac I, II, II, and no vaccine where the vaccine

was administered subcutaneously and intramuscularly, is provided in Figure 5. Figure 5 illustrates that Live Vac III consistently had the highest level of serology, with Live Vac II being equal in serology with Live Vac III for days 27, 35, and post necropsy. All groups had higher serology than the group given no vaccine. Figure 6 illustrates a comparison of the serology for 5 Live Vac I, using various routes of administration. Subcutaneous and intramuscular administration consistently had the highest serology over the period of the study, with intramuscular administration only being in second. All groups had higher serology values than those animals not administered vaccine.

## 10 CONCLUSIONS

Protective benefits (respiratory and joint) were observed for *M. bovis* vaccine candidates Live Vaccine I (05-2823 P106), Live Vaccine II (05-249 P102) and Live Vaccine III (05-1902-1 P106) via a simultaneous intranasal and subcutaneous route administered 2 times with a 2 week interval in colostrum deprived calves using a dual lung/joint challenge.

15 Protective benefits (respiratory and joint) were observed for *M. bovis* vaccine candidate Live Vaccine I (05-2823 P106) with either intranasal or subcutaneous route administered 2 times with a 2 week interval in colostrum deprived calves using a dual lung/joint challenge.

### Example 3: DNA fingerprinting

20 The DNA fingerprinting process was used to differentiate *M. bovis* strains by isolating, amplifying and detecting DNA using the methods and primers as disclosed in WO 2008-030619.

#### **Materials and Methods**

25 *Mycoplasma sp.* isolates were used in the studies. Isolates were obtained from in-house sources or field isolates obtained from infected animals. Isolates were grown using a combination of Mycoplasma-selective agar and broth for 1-7 days. To isolate DNA, broth cultures were spun and pelleted. DNA from the pellet was then extracted (using the Qiagen DNeasy Tissue Kit and resuspended in molecular grade water). Genomic DNA was quantitated using Picogreen (Invitrogen). Primers were designed based on the known insertion sequences (transposable elements) present in the bacterial genome (*Mycoplasma bovis*) and are disclosed in 30 WO 2008-030619. Outwardly facing primers were manually selected from the element ends (excluding the terminal repeat regions) at a Tm of 55-58C. PCR reactions were then carried out

using a multiplex PCR master mix (Qiagen Multiplex PCR Kit). The reactions contained 1x Master mix, 300nM of each primer and 1ng of template DNA. Thermal cycling conditions were 95°C for 15 minutes, 35 cycles of 94°C for 30 seconds, 56.1°C for 90 seconds, 72°C for 2 minutes, with a final extension of 72°C for 4 minutes and a 4°C hold. The amplified products 5 were separated on a 4% agarose gel with ethidium bromide (Invitrogen E-gel), run for 50 minutes at room temperature and imaged under UV light.

### Results and Discussion

The results showed that each of the isolates used in this application had a unique fingerprint. However, as shown in Example 2, each isolate was also an effective attenuated live 10 culture vaccine that was effective at providing cross protection against a challenge isolate having a different fingerprint than any of the vaccine candidates. Three field isolates, 05-2823 P106 (PTA-8694), 05-249 P102 (PTA-8696), and 05-1902-1-P106 (PTA-8695), were grown and DNA isolated according to the above protocol. 2-5ng of DNA from each isolate was amplified according to the above protocol using a multiplex of 4 sets of IS primers identified as SEQ ID 15 Nos. 1-8 as disclosed in WO 2008-030619. The amplified products were separated on a Invitrogen E-gel 4% agarose gel containing ethidium bromide (according to manufacturer) for 50 minutes and visualized under UV light. All isolates produced unique patterns. The patterns were reproducible using independent aliquots under the sample PCR reaction conditions.

20 **Example 4: Comparative analysis of efficacy of *Mycoplasma bovis* vaccine at passage 135 as compared to vaccine at the lower passage level of 106.**

The study in Example 2 used the *Mycoplasma bovis* vaccine at the lower passage 106, and measured the effectiveness in protecting vaccinated calves from 3 key manifestations of *Mycoplasma bovis* disease: clinical signs of respiratory disease, lameness, and “early removal” 25 or euthanasia and death resulting from a severe myriad of clinical signs caused by *Mycoplasma bovis* disease. The latter manifestation of disease is obviously most consequential, and hence, was considered the most important feature of vaccine efficacy. This most important parameter was used to measure any differences in efficacy between vaccine at passage 106 and passage 135.

30 In the study described in Example 2 with passage 106 vaccine, Group 1(Live Vac I SQ+IN) received vaccine twice at 14 day intervals and via two routes of administration,

intranasal (IN) and subcutaneously (SQ), at each vaccination event. Separate groups of calves were also vaccinated with passage 106 vaccine. Most pertinent was Group 2 (Live Vac I SQ) which was given vaccine twice at 14 day intervals but via one route of administration, only subcutaneously (SQ), at each vaccination event.

5 Following vaccination, both the vaccinated Group 1 and Group 2 and a separate assembly (Group 4) of non-vaccinated (No Vac) calves were purposely exposed to virulent *Mycoplasma bovis* delivered directly into each calf. Disease resulting from the challenge exposure was measured and compared among the groups, and specifically, comparing the most significant disease “early removal” (death) in vaccinated Group 1 and Group 2 to the non-vaccinated (No 10 Vac) calves of Group 4 to measure the effectiveness of the passage 106 vaccine. As presented in Table 8, passage 106 vaccinated Group 2 given vaccine twice at 14 day intervals but via one route of administration, subcutaneously, provided a notable 56% reduction in death/euthanasia. Group 1(Live Vac I SQ+IN) given passage 106 vaccine twice at 14 day intervals and via two 15 routes of administration, intranasal (IN) and subcutaneously (SQ), at each vaccination event, provided the same notable 56% reduction in death/euthanasia of vaccinated calves.

TABLE 9

Group		Respiratory			Joint			Early Removal		
		Affected	Frequency	% Reduction	Affected	Frequency	% Reduction	Affected	Frequency	% Reduction
1	Live Vac I (SQ+IN)	3 / 9	33%	0%	6 / 9	67%	33%	4 / 9	44%	56%
2	Live Vac I (SQ)	0 / 9	0%	100%	7 / 9	78%	22%	4 / 9	44%	56%
4	No Vac	2 / 6	33%		6 / 6	100%		6 / 6	100%	

20 In this study the *Mycoplasma bovis* vaccine at the higher passage 135 was also evaluated for its effectiveness in protecting vaccinated calves from the most important manifestation of *Mycoplasma bovis* disease, “early removal rates” or euthanasia and death resulting from a severe myriad of clinical signs caused by *Mycoplasma bovis* disease. In the study described in Example 1, Group 1 also received vaccine twice at 14-day intervals via two routes of administration, intranasal (IN) and subcutaneously (SQ), at each vaccination event as was done in the study

described in Example 2, with passage 106 vaccine. However, the critical difference between the vaccines in Group 1 of each of these two studies was passage level. In the study of Example 2 Group 1 was given vaccine at passage level 106, and in this second Study of Example 1 Group 1 received vaccine at the higher passage 135.

5 Separate groups of calves were also vaccinated with passage 135 vaccine in the study described in Example 1. Most pertinent again was Group 2 which was given vaccine only once via one route of administration, subcutaneously (SQ). Following vaccination, both the vaccinated Group 1 and Group 2 and a separate assembly (Group 5) of non-vaccinated calves were 10 purposely exposed to virulent *Mycoplasma bovis* delivered directly into each calf. Disease resulting from the challenge exposure was measured and compared among the groups. Specifically, efficacy was determined by comparing the most significant disease "early removal 15 rates" (death) in vaccinated Group 1 and Group 2 to the non-vaccinated calves of Group 5 to measure the effectiveness of the passage 135 vaccine. As presented in Table 10, Group 1 given passage 135 vaccine twice at 14 day intervals via two routes of administration, intranasal (IN) and subcutaneously (SQ), at each vaccination event provided a quite unanticipated and very high effectiveness by reducing calf early removal rates (death) by 83%, or much higher efficacy than was achieved with passage 106 vaccine that provided a notable but lower 56% reduction of early removal rates. The comparative results between the two studies appear in Table 11.

20 Also unanticipated was the efficacy achieved in Group 2 the study of Example 1, with a single dose of vaccine. Group 2 received just a single dose of passage 135 vaccine via one route of administration, subcutaneously. This higher passage 135 vaccine provided a 55.6% (56%) reduction in calf death/euthanasia. By contrast, the 106 passage vaccine required two doses of 25 vaccine, a priming dose followed by a booster 14 days later in the study of Example 2 to achieve comparable efficacy delivered in just one dose (no booster needed) to calves receiving passage 135 vaccine in the study of Example 1.

5 **TABLE 10: Percent Reduction and Significance for all Parameters in comparison to the Challenge Control**

Group	Total % Lung Pathology Scores	Clinical Signs of Coughing Post-Challenge		Clinical Signs of Lameness Post Challenge		
		Number of Animals with coughing scores >0	Days with coughing scores >0	Number of animals with total lameness scores >0	Days with total lameness scores >0	Total Lameness scores
1	68% *	0%	-57%	33%	-8%	70% *
2	30%	-19%	-129%	11%	-8%	31%

10 Table 10: (con't)

Group	Clinical Signs of Joint Swelling Post-Challenge			Early Removal Rates	Clinical Signs of Arthritis Score Post-Challenge		
	Number of animals with total joint swelling scores >0	Days with total joint swelling scores >0	Total joint swelling scores		Number of animals removed early from study	Number of animals with arthritis score >0	Total Arthritis scores
1	33%	0%	63% **	83% **	17%	-8%	67% *
2	26%	-8%	58% *	55%	11%	-8%	39%

Group 1 = 2 Hi Dose (dual IN/SQ Day 0 and Day 14)

Group 2 = 1 Hi Dose (SQ Day 0)

15

**Table 11: Comparative Results of Passage 135 and Passage 106 Vaccine**

Study Number	Group	Vaccine Passage	Doses	Booster	Route of Vaccination	Reduction: Death due to <i>M. bovis</i>
Example 2	1	106	2	Yes	IN & SQ	56%
Example 1	1	135	2	Yes	IN & SQ	83% <sup>a</sup>
Example 2	2	106	2	Yes	SQ	56%
Example 1	2	135	1 <sup>b</sup>	No	SQ	56%

20 (a) passage 135 vaccine provides unanticipated reduction in death due to *M. bovis* compared to passage 106  
 (b) passage 135 vaccine is effective as single dose without need for booster, whereas passage 106 requires a booster for comparable reduction in death due to *M. bovis*

Hence, the passage 135 vaccine yielded an improved and unexpectedly higher level of efficacy by significantly reducing death due to *Mycoplasma bovis* infection using a more convenient, less costly single dose of vaccine, whereas the lower passage 106 vaccine required two doses of vaccine to provide comparable efficacy. In addition, substantially better efficacy of 5 83% reduction in death was achieved with high passage Group 1 of the study of Example 1 using an identical vaccination regimen as used with low passage Group 1 of the study of Example 2, the only difference being the passage 135 vaccine in the former and passage 106 vaccine in the latter study. The passage 135 vaccine reduced death due to *Mycoplasma bovis* by 83% as compared to a lower but notable efficacy with passage 106 vaccine of 56%. Typically the 10 efficacy of a vaccine is reduced on progressive passages *in vitro* as the microorganism further adapts to cell culture and loses expression of potentially immunogenic virulence proteins. Unexpectedly, the avirulent *M. bovis* vaccine of this invention demonstrated improved effectiveness in response to higher *in vitro* passages, and specifically in progressing from passage 106 to passage 135 vaccine.

15

#### **Example 5: Minimum immunizing doses**

##### **Materials and Methods**

An *M. bovis*, avirulent live bacterial culture in lyophilized presentation that was rehydrated with sterile water diluent was used at 2 x 2 mL doses administered at 2 to 3 week intervals via 20 subcutaneous injection in cattle 6 weeks of age or older.

The study design utilized 3 vaccine treated groups and 1 placebo treated each containing 15 to 17 calves. Test animals all received two subcutaneous doses of experimental vaccines formulated at 3 different antigen levels or a placebo vaccine composed of the media used to produce the *Mycoplasma Bovis* culture. Calves were 6-weeks of age at the time of administration 25 of the first dose of vaccine or placebo. Calves were challenged on day 42 and post-challenge observations were made for 28 days. Primary parameters of mortality/culling, and lameness and joint swelling on any day were observed to assess the efficacy of the vaccine.

Table 12 summarizes the conclusions made from the analysis of the data.

30

##### **Table 12: Minimum Immunizing Doses**

Variable	Treatment	No. of calves	Prevented Fraction	Lower 95% CL	Upper 95% CL	Conclusion
Mortality/ Culling	Low Dose = $10^{6.8}$ / dose	16	0.04	-0.859	0.5113	Incomplete Efficacy
	Middle Dose = $10^{7.9}$ / dose	15	0.1467	-0.631	0.6163	Incomplete Efficacy
	High Dose = $10^{8.9}$ / dose	17	<b>0.6235</b>	0.0855	0.9268	<b>Efficacy Established</b>
	Placebo	15				
Presence of Lameness	Low Dose = $10^{6.8}$ / dose	16	0.2857	-0.1	0.5994	Incomplete Efficacy
	Middle Dose = $10^{7.9}$ / dose	15	0.0857	-0.324	0.4046	Incomplete Efficacy
	High Dose = $10^{8.9}$ / dose	17	<b>0.6667</b>	0.3653	0.8513	<b>Efficacy Established</b>
	Placebo	15				
Presence of Swelling	Low Dose = $10^{6.8}$ / dose	16	0.1818	-0.468	0.5908	Incomplete Efficacy
	Middle Dose = $10^{7.9}$ / dose	15	0.0303	-0.693	0.4606	Incomplete Efficacy
	High Dose = $10^{8.9}$ / dose	17	<b>0.5722</b>	0.0498	0.866	<b>Efficacy Established</b>
	Placebo	15				

## CONCLUSIONS

A preferred dose of  $10^{8.9}$  CCU<sub>50</sub> was efficacious as assessed.

### 5 Example 6: Safety - Dissemination and Transmission

#### Materials and Methods

An *M. bovis*, avirulent live bacterial culture in lyophilized presentation that was rehydrated with sterile water diluent was used at 2 x 2 mL doses administered at 2 to 3 week intervals via subcutaneous injection in cattle 6 weeks of age or older.

The dissemination and transmission of the vaccine strain was also evaluated in colostrum deprived calves. In this study >10 logs of low passage, Master seed-derived (MS +3) was administered via the subcutaneous route to 10 calves. Samples including 15 different tissues and swabs were collected from treated calves at weekly intervals for 5 weeks after administration. To 5 assess transmission of the vaccine, 10 sentinel calves were commingled with the vaccinated calves throughout the study. Table 13 shows the results of this study.

**Table 13: Summary of experimental design and results of dissemination and transmission**

Treatment	Administration	No. of calves	Dose	Outcome
Mycoplasma bovis working seed (MS + 3)	Subcutaneous injection	10	$10^{10.5}$ /dose	No <i>M. bovis</i> isolated beyond 14 days post vaccination. No lesions or clinical signs
Commingled w/ treated calves	Animal to animal contact	10	Sentinels	No <i>M. bovis</i> detected. No lesions or clinical signs

## 10 CONCLUSIONS

The 14 day sampling was the only recovery of *Mycoplasma bovis* vaccine, avirulent live culture, and only in one tonsil tissue sample from a single calf in the vaccinated group. The remaining 129 samples collected from dosed calves from day 7 through day 35 after vaccination were all negative for *M. bovis*.

15 Within this study, an equal number of sentinel calves were commingled with the vaccinated calves. No *M. bovis* was isolated from any of the swab samples or tissues from the sentinel calves.

The data from Examples 5 and 6 demonstrated the safety and efficacy of the vaccine of the present invention. The vaccines of the present invention were highly effective in preventing 20 disease due to *M. bovis* following two subcutaneous injections at the minimum immunizing dose ( $10^{8.9}$ /dose). The safety evaluations in young, highly susceptible colostrum deprived (CD) calves confirmed the safety of a 20-fold overdose injected subcutaneously. The safety studies also demonstrated the vaccine is safe when injected subcutaneously.

## 25 Example 7: Preparation of combination vaccines

### *Vaccine A*

*M. bovis, IBR, and BVDV types 1 and 2*

Attenuated live BVDV type 1 and 2 strains, having at least one mutation in the coding sequence for glycoprotein E<sup>rns</sup> and/or at least another mutation in the coding sequence for N<sup>pro</sup>, wherein said mutation in the coding sequence for glycoprotein E<sup>rns</sup> leads to inactivation of RNase activity residing in E<sup>rns</sup> and/or said mutation in the coding sequence for N<sup>pro</sup> leads to inactivation of said N<sup>pro</sup> (as described in WO2005/111201, hereby entirely incorporated by reference), are grown in MDBK-cells until a TCID<sub>50</sub> of about 10<sup>5.0</sup> to 10<sup>8.1</sup> per ml cell culture fluid. A live attenuated strain of IBR is grown in MDBK cells until a TCID<sub>50</sub> of about 10<sup>5.0</sup> to 10<sup>8.6</sup> per ml cell culture fluid. A live attenuated strain of *M. bovis* as described above is grown in MDBK cells until a CFU of about 10<sup>10</sup> per ml cell culture fluid. Each culture fluids are collected. Equal amounts of the antigens are mixed and lyophilized by standard techniques. For reconstitution, an aqueous solution is used. One dose of the combination vaccine contains 2 ml of the reconstituted antigens. A final dose includes IBR (10<sup>5.0</sup> to 10<sup>8.6</sup> TCID<sub>50</sub>), BVDV-1 (10<sup>5.0</sup> to 10<sup>8.1</sup> TCID<sub>50</sub>), BVDV-2 (10<sup>5.0</sup> to 10<sup>8.1</sup> TCID<sub>50</sub>), and *M. bovis* (2.1 x 10<sup>9</sup>CFU).

15

**Vaccine B***M. bovis, IBR, BVDV types 1 and 2, and PI3*

The preparation of the IBR, BVDV 1 and 2 and *M. bovis* antigens are grown as described for vaccine A. In addition, a live attenuated strain of PI3 is grown in MDBK cells until a TCID<sub>50</sub> of about 10<sup>4.2</sup> to 10<sup>6.5</sup> per ml cell culture fluid. Afterwards, the PI3 containing culture fluid is harvested. An amount of 10<sup>4.2</sup> to 10<sup>6.5</sup> (TCID<sub>50</sub>) of the PI3 antigen is mixed with the IBR, and BVDV types 1 and 2. The mixture is then lyophilized by standard techniques, so that one dose of the reconstituted combination vaccine contains 2 ml as described for Vaccine A. A final dose includes IBR (10<sup>5.0</sup> to 10<sup>8.6</sup> TCID<sub>50</sub>), BVDV-1 (10<sup>5.0</sup> to 10<sup>8.1</sup> TCID<sub>50</sub>), BVDV-2 (10<sup>5.0</sup> to 10<sup>8.1</sup> TCID<sub>50</sub>), *M. bovis* (2.1 x 10<sup>9</sup>CFU), and PI3 (10<sup>4.2</sup> to 10<sup>6.5</sup> TCID<sub>50</sub>).

25

**Vaccine C***M. bovis, BVDV types 1 and 2, PI3, Mannheimia (Pasteurella) haemolytica*

BVDV 1 and 2, *M. bovis* bacterium according to the present invention, and PI3 viruses are grown as described for vaccines A and B. After the culture fluids are harvested, the antigens are lyophilized. Mannheimia (Pasteurella) haemolytica is grown until the titer reaches 10<sup>8.0</sup> to 10<sup>11</sup> cells per ml of culture. The bacteria are inactivated and the culture fluid is lyophilized or

freeze dried, or formulated as a liquid that will not inactivate attenuated cultures of BVD, *M. bovis*, and PI3. An amount of  $10^{8.0}$  to  $10^{11.0}$  lyophilized or freeze dried or formulated liquid bacteria cells are mixed with the lyophilized BVDV types 1 and 2 antigen (each in an amount of  $10^{5.0}$  to  $10^{8.1}$  TCID<sub>50</sub>), PI3 antigen ( $10^{7.3}$  to  $10^{8.3}$  TCID<sub>50</sub>) and *M. bovis* antigen ( $2.1 \times 10^9$  CFU).  
5 Final antigen amounts per dose are BVDV-1 ( $10^{5.0}$  to  $10^{8.1}$  TCID<sub>50</sub>), BVDV-2 ( $10^{5.0}$  to  $10^{8.1}$  TCID<sub>50</sub>), PI3 ( $10^{7.3}$  to  $10^{8.3}$  TCID<sub>50</sub>) *M. bovis* ( $2.1 \times 10^9$  CFU), and *Mannheimia* (Pasteurella) haemolytica ( $10^{8.0}$  to  $10^{11.0}$  cells).

#### **Vaccine D**

10 *M. bovis* BVDV types 1 and 2, IBR, PI3, *Leptospira canicola*, *Leptospira grippotyphosa*,  
*Leptospira hardjo*, *Leptospira pomoma*, *Leptospira borgpetersenii hardjo-bovis*  
BVDV 1 and 2, *M. bovis*, IBR, and PI3 are grown as described for vaccines A and B.  
After the culture fluids are harvested, the viruses and *M. bovis* are lyophilized. *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo*, *Leptospira pomoma*, *Leptospira 15 borgpetersenii hardjo-bovis* are separately cultivated until reaching  $10^{8.0}$  to  $10^{11.0}$  cells per ml of culture. The *Leptospira* cultures are inactivated and the culture fluids are lyophilized or freeze dried, or formulated as a liquid that is non-virucidal for the live antigens of the vaccine. Each of the  $10^{8.0}$  to  $10^{11.0}$  of the lyophilised or freeze dried bacteria cells are reconstituted with the lyophilized modified BVDV types 1 and 2 (each in an amount of  $10^{5.0}$  to  $10^{7.0}$  TCID<sub>50</sub>), modified 20 live PI3 ( $10^{7.3}$  to  $10^{8.3}$  TCID<sub>50</sub>), modified live *M. bovis* ( $2.1 \times 10^9$  CFU) and modified live IBR ( $10^{6.1}$  to  $10^{7.7}$  TCID<sub>50</sub>) using sterile water for injection, or the lyophilized components are reconstituted using the liquid non-virucidal formulation of the *Leptospira* cultures. The 25 reconstituted suspension (2 ml per dose) contains traces of neomycin as preservative. Final antigen amounts per dose are BVDV-1 ( $10^{5.0}$  to  $10^{7.0}$  TCID<sub>50</sub>), BVDV-2 ( $10^{5.0}$  to  $10^{7.0}$  TCID<sub>50</sub>), PI3 ( $10^{7.3}$  to  $10^{8.3}$  TCID<sub>50</sub>) *M. bovis* ( $2.1 \times 10^9$  CFU), PI3 ( $10^{7.3}$  to  $10^{8.3}$  TCID<sub>50</sub>), and *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo*, *Leptospira pomoma*, and *Leptospira 25 borgpetersenii hardjo-bovis* (each  $10^{8.0}$  to  $10^{11.0}$  cells).

#### **Vaccine E**

30 *M. bovis*, BVDV types 1 and 2, IBR, PI3, and *H.somnus*

BVDV 1 and 2, *M. bovis*, IBR, and PI3 are grown as described for vaccines A and B. After the culture fluids are harvested, the viruses and *M. bovis* are lyophilized. *H. somnus* is cultivated until achieving  $10^{7.1}$  to  $10^{9.2}$  cells per ml culture. The bacteria culture is inactivated and the culture fluid is lyophilized or freeze dried, or formulated as a liquid that is non-virucidal for the live antigens of the vaccine.  $10^{7.1}$  to  $10^{9.2}$  of the lyophilized or freeze dried bacteria are reconstituted with the lyophilized modified BVDV types 1 and 2 (each in an amount of  $10^{5.0}$  to  $10^{7.0}$  TCID<sub>50</sub>), modified live PI3 ( $10^{7.3}$  to  $10^{8.3}$  TCID<sub>50</sub>), modified live *M. bovis* ( $2.1 \times 10^9$  CFU), and modified live IBR ( $10^{6.1}$  to  $10^{7.7}$  TCID<sub>50</sub>) using sterile water for injection, or the lyophilized components are reconstituted using the liquid non-virucidal formulation of the bacterial 5 *H.somnus* culture. The reconstituted suspension (2 ml per dose) contains traces of neomycin as preservative. Final antigen amounts per dose are BVDV-1 ( $10^{5.0}$  to  $10^{7.0}$  TCID<sub>50</sub>), BVDV-2 ( $10^{5.0}$  to  $10^{7.0}$  TCID<sub>50</sub>), PI3 ( $10^{7.3}$  to  $10^{8.3}$  TCID<sub>50</sub>) *M. bovis* ( $2.1 \times 10^9$  CFU), PI3 ( $10^{7.3}$  to  $10^{8.3}$  TCID<sub>50</sub>), and *H.somnus* ( $10^{7.1}$  to  $10^{9.2}$  cells).  
10

15

### ***Vaccine F***

#### *M. bovis, IBR, BVDV types 1 and 2, PI3 and BRSV*

The preparation of the IBR, PI3, BVDV 1 and 2 and *M. bovis* antigens are grown as described for vaccine A and B. In addition, a live attenuated strain of BRSV is grown in MDBK cells until a TCID<sub>50</sub> of about  $10^{5.0}$  to  $10^{7.2}$  per ml cell culture fluid. Afterwards, the BRSV 20 containing culture fluid is harvested. After the culture fluids are harvested, the antigens are mixed and lyophilized as described for vaccine A and B. An amount of  $10^{5.0}$  to  $10^{7.2}$  of the BRSV antigen is mixed with the IBR, BVDV types 1 and 2, and *M. bovis* antigens. The mixture is then reconstituted in 2 ml dose volume as described for Vaccine A. For reconstitution, an aqueous solution is used. One dose of the combination vaccine contains 2 ml of the reconstituted 25 antigens. A final dose includes IBR ( $10^{5.0}$  to  $10^{8.6}$  TCID<sub>50</sub>), BVDV-1 ( $10^{5.0}$  to  $10^{8.1}$  TCID<sub>50</sub>), BVDV-2 ( $10^{5.0}$  to  $10^{8.1}$  TCID<sub>50</sub>), *M. bovis* ( $2.1 \times 10^9$  CFU), PI3 ( $10^{4.2}$  to  $10^{6.5}$  TCID<sub>50</sub>) and BRSV ( $10^{5.0}$  to  $10^{7.2}$  TCID<sub>50</sub>).

## CLAIMS

1. An immunogenic composition comprising an attenuated, avirulent *Mycoplasma bovis* bacterium strain, wherein the bacterium is passaged more than 110 times, wherein the attenuated, avirulent *Mycoplasma bovis* strain is selected from the group consisting of: the attenuated 5 *Mycoplasma bovis* bacteria strain deposited with the ATCC under accession number PTA-9666 and PTA-9667, any attenuated descendant *Mycoplasma bovis* bacteria strains thereof when used as live vaccine, reduces the mortality rate in a group of animals of at least 56% as compared to a non-vaccinated control group of animals after the administration of a single dose of said vaccine.
2. The immunogenic composition according to claim 1, wherein said attenuated, avirulent 10 *Mycoplasma bovis* bacterium strain when used as live vaccine, reduces the mortality rate in a group of animals of at least 83% as compared to a non-vaccinated control group of animals.
3. The immunogenic composition according to claim 1 or 2, characterized in that the 15 immunogenic composition comprises at least 1.0E7 CFU of the live bacteria of the attenuated, avirulent *Mycoplasma bovis* bacteria per dose.
4. The immunogenic composition according to any one of claims 1 to 3, wherein one dose of the immunogenic composition is formulated in 1 or 2 ml. 20
5. The immunogenic composition according to any one of claims 1 to 3, wherein said immunogenic composition is formulated for a single-dose administration.
6. The immunogenic composition according to any one of claims 1 to 5, wherein the 25 immunogenic composition is effective in stimulating an onset of immunity within 14 days following a single dose administration.
7. The immunogenic composition according to any one of claims 1 to 6, wherein the immunogenic composition is effective in stimulating duration of immunity of at least 42 days following a single dose administration of the immunogenic composition.
8. The immunogenic composition according to any one of claims 1 to 7, wherein said immunogenic composition further comprises one or more further immunologically active 25

component(s) effective for the treatment and/or prophylaxis of microbiological infection in cattle caused by a cattle pathogen other than *Mycoplasma bovis*.

9. The immunogenic composition according to claim 8, wherein said microbiological infection in cattle caused by a cattle pathogen other than *Mycoplasma bovis* is caused by one or

5 more immunogenic components selected from the group consisting of: Bovine viral diarrhea

virus (BVDV), Parainfluenza-3 Virus (PI-3), Infectious Bovine Rhinotracheitis virus (IBR),

Bovine Respiratory Syncytial Virus (BRSV), Bovine Herpesvirus (BHV), Bovine Rotavirus

(BRV), Bovine Enterovirus (BEV), Bovine Coronovirus (BCV), Bovine Rabies (BR), Bovine

Parvovirus (BPV), Adenovirus Astrovirus, Mannheimia haemolytica (formerly Pasteurella

10 haemolytica), Pasteurella multocida, Haemophilus somnus (*Histophilus ovis* and *Haemophilus*

agni), *Actinomyces* (*Corynebacterium*), *Actinomyces pyogenes*, *Chlamydia psittaci*,

Campylobacter fetus venerealis and Campylobacter fetus fetus (formerly *C* fetus intestinalis),

Leptospira interrogans, Leptospira hardjo, Leptospira pomona, and Leptospira grippotyphosa,

Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo (Leptospira hardjoprajitno and

15 Leptospira hardjo-bovis), Brucella abortus, Brucella suis and Brucella melitensis, Listeria

monocytogenes, *Chlamydia psittaci*, Clostridium chauvoei, Clostridium septicum, Clostridium

haemolyticum, Clostridium novyi, Clostridium sordellii, Clostridium perfringens, Clostridium

tetani, Moraxella bovis, Klebsiella spp, Klebsiella pneumoniae, *Salmonella typhimurium*;

Salmonella newport, *Mycobacterium avium* paratuberculosis, Cryptosporidium parvum,

20 Cryptosporidium hominis, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus*

uberis, *Streptococcus agalactiae*, *Escherichia coli*, *Mycoplasma* spp, *Mycoplasma* dispar, and

Ureaplasma spp., *Tritrichomonas foetus*, *Trichophyton verrucosum*, *Trichophyton*

mentagrophytes, *Trichophyton sarkisovii*, *Neospora caninum* (formerly *Toxoplasma gondii*),

Babesia bigemina and Babesia bovis, *Dictyocaulus viviparous* (Lungworm disease), and

25 combinations thereof.

10. The immunogenic composition according to any one of claims 1 to 9, wherein the immunogenic composition is a vaccine.

11. A method of producing an immunogenic composition according to any one of claims 1 to 10, comprising admixing said bacteria of an attenuated, avirulent *Mycoplasma bovis* strain with a

30 pharmaceutical acceptable carrier.

12. A method for the treatment or prophylaxis of infections caused by *Mycoplasma bovis*, comprising, administering an effective amount of the immunogenic composition according to any one of claims 1 to 10 to an animal, wherein said treatment or prophylaxis is selected from the group consisting of reducing signs of *Mycoplasma bovis* infection, reducing the severity of or incidence of clinical signs of *Mycoplasma bovis* infection, reducing the mortality of animals from *Mycoplasma bovis* infection, and combinations thereof.

13. A method for reducing the incidence of mortality of animals resulting from infection by *Mycoplasma bovis* comprising administering the immunogenic composition according to any one of claims 1 to 10 to an animal.

14. The method of claim 13, wherein said mortality is reduced by at least 56%.

15. The method according to any one of claims 12 to 14, wherein two doses are administered to said animal.

16. The method according to claim 15, wherein the second dose is administered at least 10 days after the first administration.

17. The method according to any one of claims 12 to 14, wherein only a single dose is administered to said animal.

18. The method according to any one of claims 12 to 17, wherein the immunogenic composition is administered to animals from day 1 of age.

19. A method of producing an immunogenic composition comprising attenuating *Mycoplasma bovis*, comprising,

a. passaging *Mycoplasma bovis* bacteria more than 135 times to produce a cultured *Mycoplasma bovis* bacteria;

b. obtaining the cultured *Mycoplasma bovis* bacteria;

c. testing the cultured *Mycoplasma bovis* bacteria obtained under step b) for their pathogenicity and immunogenicity; and

d. propagating the non-pathogenic, but immunogenic *Mycoplasma bovis* bacteria to obtain the attenuated *Mycoplasma bovis* bacteria.

20. The method according to claim 19, wherein the *Mycoplasma bovis* bacteria are passaged in vitro.

21. The method according to claim 19 or 20, wherein the pathogenicity testing comprises:

- a. infecting cattle with the passaged *Mycoplasma bovis* bacteria; and
- b. monitoring the infected cattle for developing clinical symptoms of a *Mycoplasma bovis* infection.

22. The method according to any one claims 19 to 21, wherein the immunogenic testing comprises:

- a. infecting cattle with the passaged *Mycoplasma bovis* bacteria; and
- b. monitoring the development of the humoral antibody response against *Mycoplasma bovis* in the infected cattle.

**Boehringer Ingelheim Vetmedica, Inc.**

**Patent Attorneys for the Applicant/Nominated Person**

**SPRUSON & FERGUSON**

Fig. 1

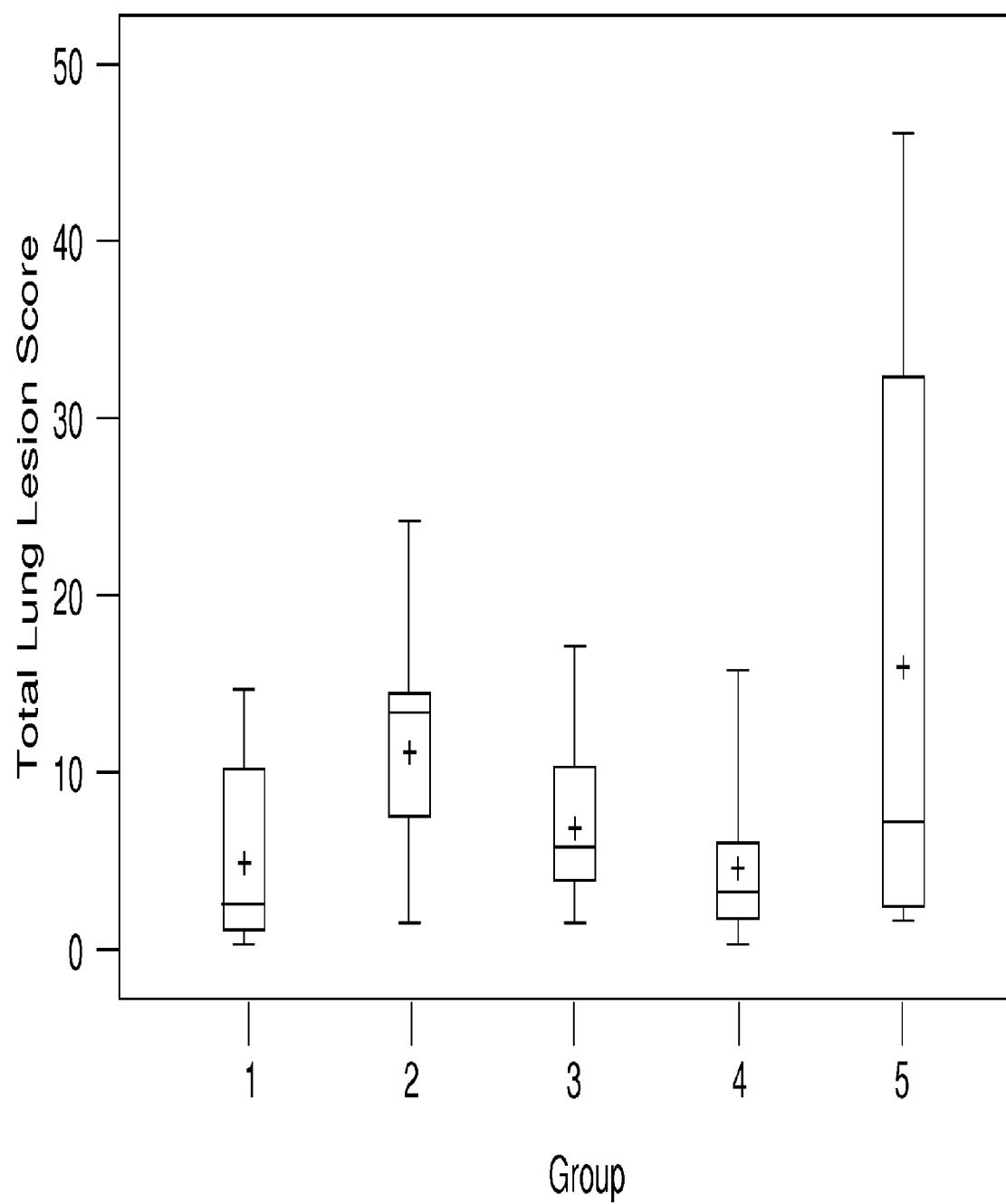


Fig. 2

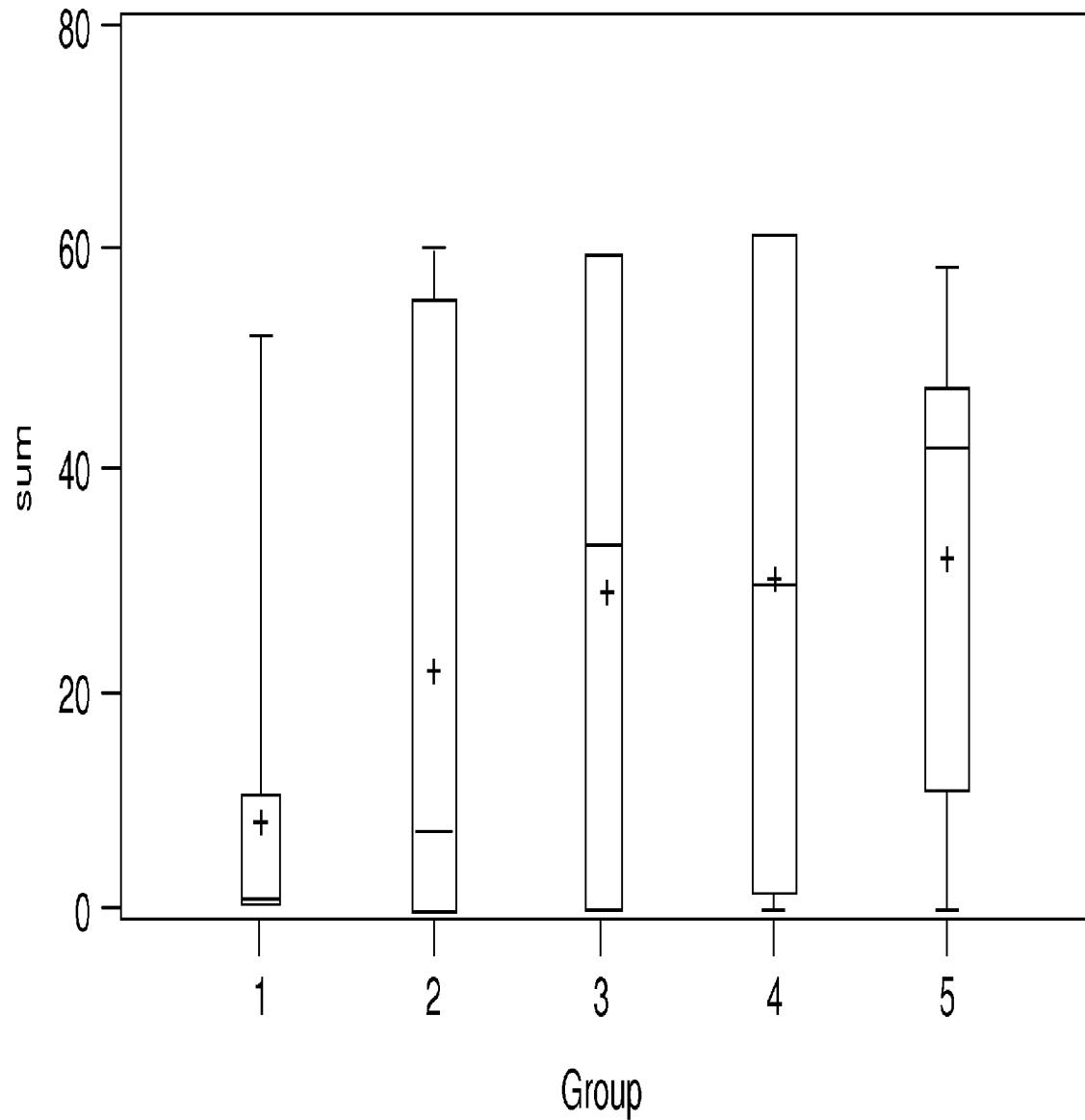


Fig. 3

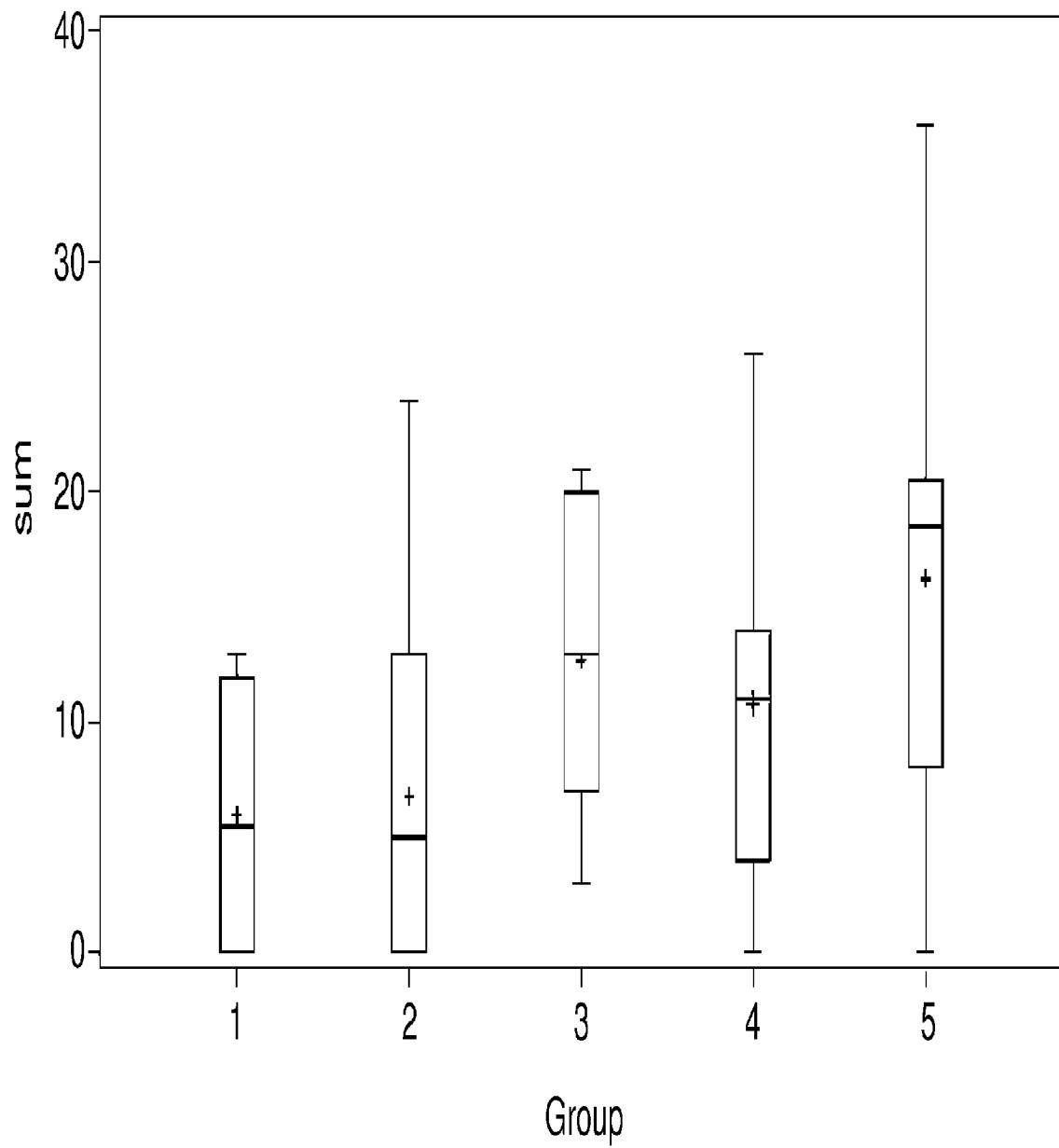


Fig. 4

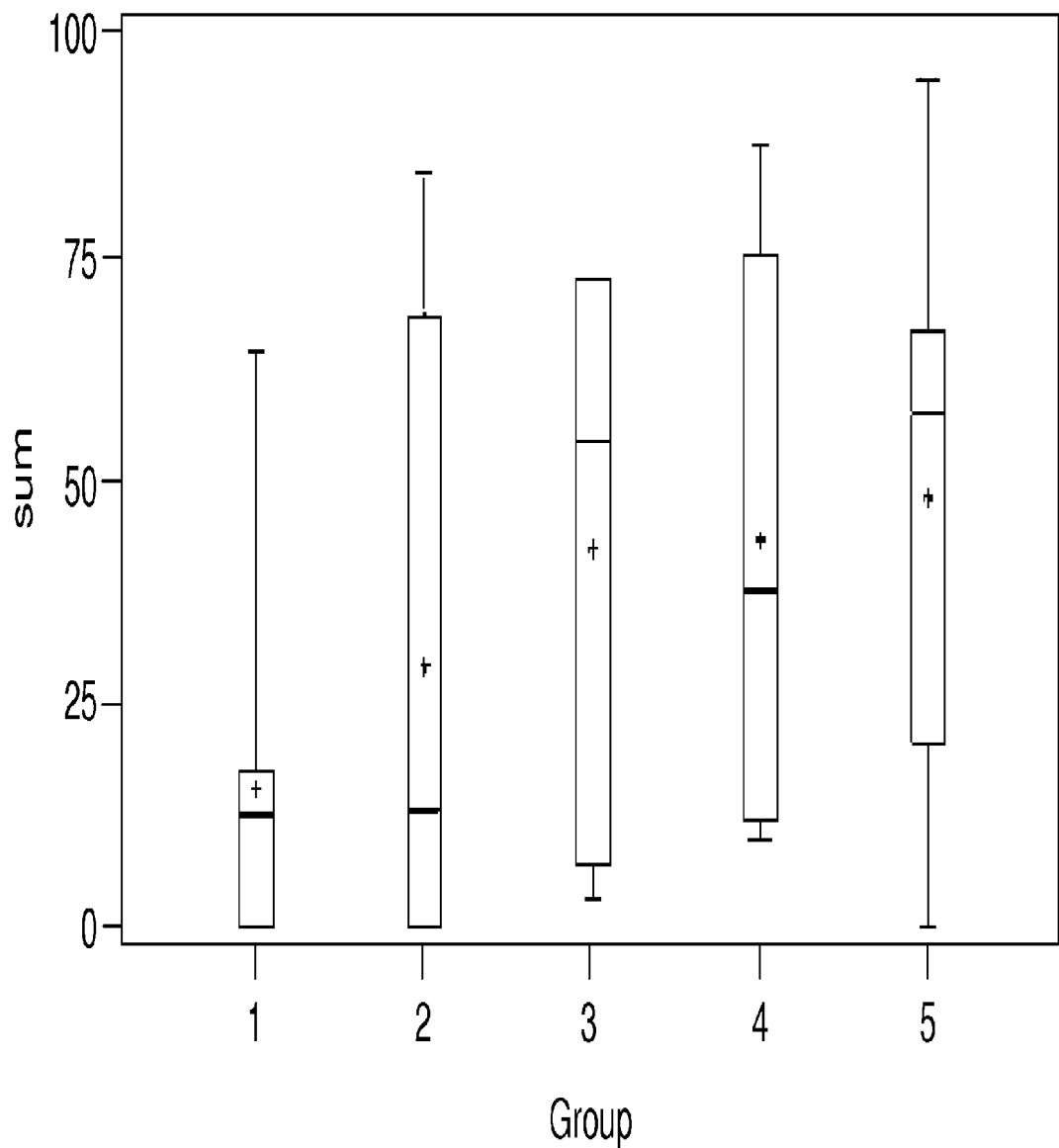


Fig. 5

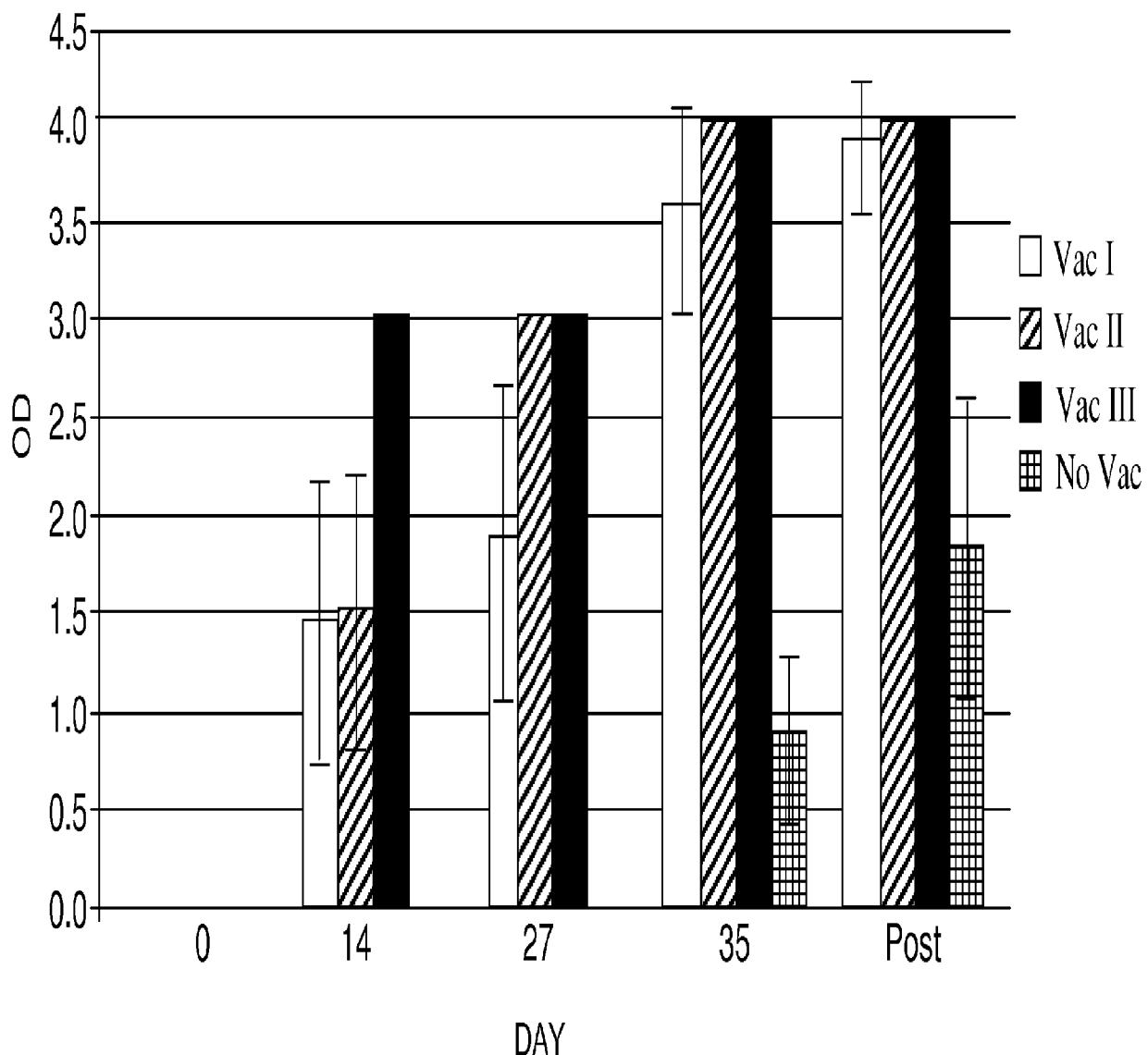


Fig. 6

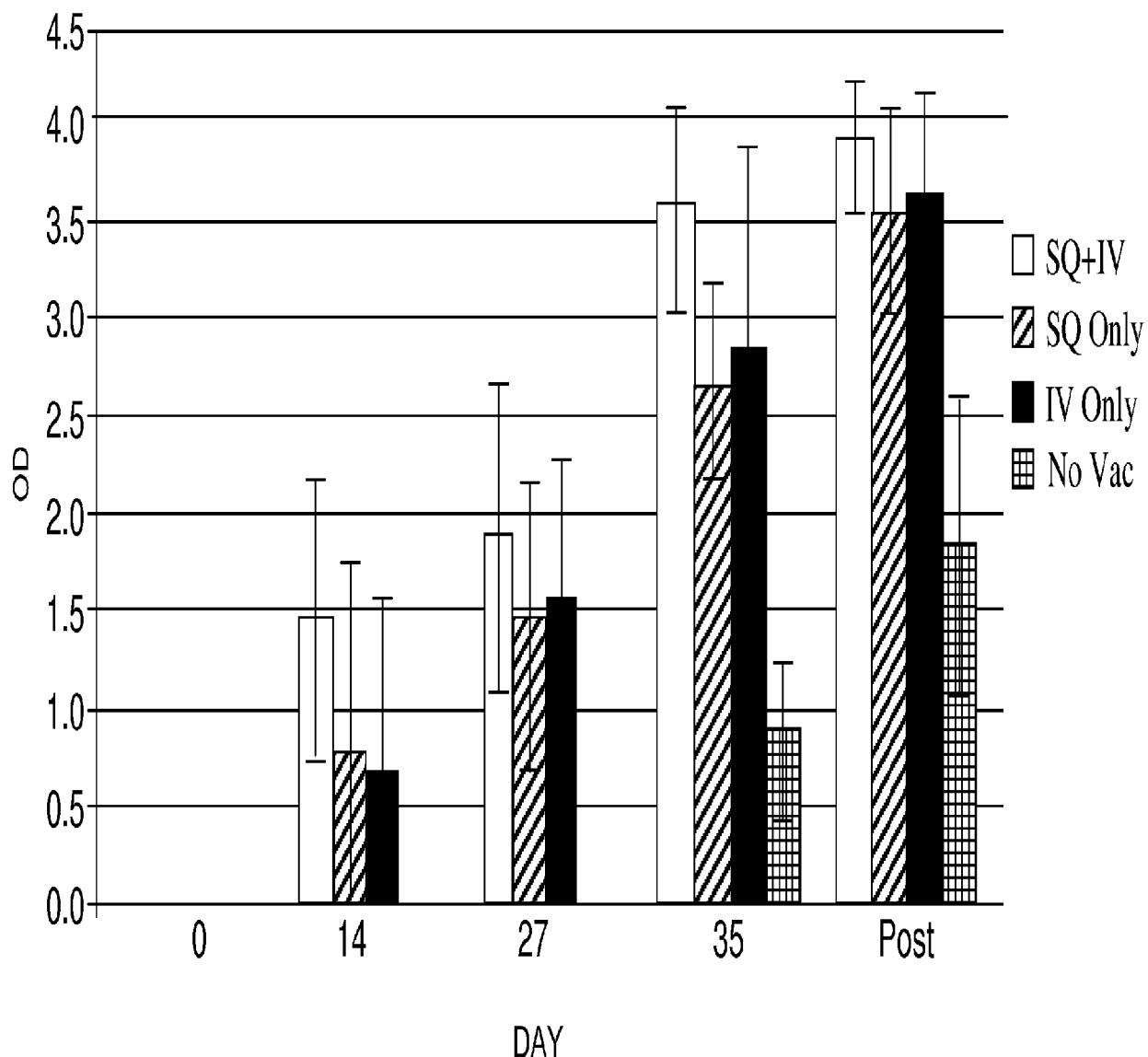


Fig. 7.

Nucleotide substitution in lipoate protein ligase gene and protein

**a. Nucleotide substitution (identified by BLAST alignment)**

Isolates 1 (24/24 reads) and 2 (11/11 reads) have G at this position

BI-1 : 792

SEQ ID. NO. 9 cgcccgttagctattggtacaattaacttctcgcttgcagttgaaggccaaagaattga

BI-3 : 792

SEQ ID. NO. 10 cgcccgttagctattggtacaattaacttctcgcttgcagttgaaggccaaataattga

G  
↓

T  
↑

Isolates 3 (4/4 reads) and 4 (15/15 reads) have T at this position

**b. Amino acid substitution (identified by translation)**

In BI-1 and BI-2, Arginine (R) codon (AGA)

In BI-3 and BI-4, Isoleucine (I) codon (ATA)

BI-1 : 241

SEQ ID. NO. 11 LVNTKYKNWDWNYGLSPRYEYNRDLAIGTINFSLAVEGQRIEKIKISGDFFAKKDITE 300

SEQ ID. NO. 12 LVNTKYKNWDWNYGLSPRYEYNRDLAIGTINFSLAVEGQ IEKIKISGDFFAKKDITE

BI-3 : 241

SEQ ID. NO. 13 LVNTKYKNWDWNYGLSPRYEYNRDLAIGTINFSLAVEGQIEKIKISGDFFAKKDITE 300

▲

Fig. 7 (CONT.)

## Nucleotide substitution in Era (GTPase) gene and protein

#### a. Nucleotide substitution (identified by BLAST alignment)

Isolates 1 (10/10 reads) and 2 (13/13 reads) have G at this position

Isolates 3 (20/20 reads) and 4 (7/7 reads) have A at this position

**b. No Amino acid substitution (identified by translation)**

In BI-1 and BI-2, Leucine (L) codon (TTG)

In BI-3 and BI-4, Leucine (L) codon (TTA)

BI-1: 61  
SEQ ID. NO. 16 IHKPLNLLGESLNKEAFSSINDIDCILFLTPVNEEIKSGDKLILERIANSKNKIAVISKI

SEQID NO. 17 THKPLNLGESLNKEAFSSINDDCILELTPVNEEJKSGDKLILERTJANSKNKTAVTSKT

BI-3: 61  
SEQ ID. NO. 18 IHKPLNLLGESLNKEAFSSINDIDCILFLTPVNEEIKSGDKLILERIANSKNKIAVISKI

Fig. 7 (CONT.)

Nucleotide substitution in P81 lipoprotein gene and protein

**a. Nucleotide substitution (identified by BLAST alignment)**

Isolates 1 (6/6 reads) and 2 (8/8 reads) have G at this position

Arrow highlights nt 3 of gene

BI-1 : 61

SEQ ID. NO. 19 cacataaaatatttaaggacatattatgagtaagaaaaataaattatgattgggcttc

G  
↓

BI-3 : 61

SEQ ID. NO. 20 cacataaaatatttaaggacatattattagtaagaaaaataaattatgattgggcttc

T  
↑

Isolates 3 (14/14 reads) and 4 (24/25 reads) have T at this position.

One BI-4 read was called "G" but was within a 5 nt-region of poor quality

**b. Amino acid substitution (identified by translation)**

In BI-1 and BI-2, Methionine start codon (M) codon (AT~~G~~)

In BI-3 and BI-4, Isoleucine (I) codon (AT~~T~~)

BI-1: 1

SEQ ID. NO. 21 MSKKNKLIGLSSSTAIPLLA AVSAKCGGTVN YEDLGDAKKISLGVSFSSGQPQ

SEQ ID. NO. 22 +SKKNKLIGLSSSTAIPLLA AVSAKCGGTVN YEDLGDAKKISLGVSFSSGQPQ

BI-3 1

SEQ ID. NO. 23 ISKKNKLIGLSSSTAIPLLA AVSAKCGGTVN YEDLGDAKKISLGVSFSSGQPQ

▲

Fig. 7 (CONT.)

Nucleotide substitution in Membrane protein gene and product

**a. Nucleotide substitution (identified by BLAST alignment)**

Isolates 1 (4/4 reads) and 2 (4/4 reads) have A at this position

BI-1 961  
SEQ ID. NO. 24 tttaacacagtaaacatctagaatatctagagacaaccgtgaaagaagagtgttcggctat

BI-3 961            ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
SEQ ID. NO. 25 tttaacacagtaaacatctagaatatctagagacgaccgtgaaagaagagtgttcggctat

A ↓  
G ↑

Isolates 3 (21/21 reads) and 4 (12/12 reads) have G at this position

**b. Amino acid substitution (identified by translation)**

In BI-1 and BI-2, Asparagine (N) codon (AAC)

In BI-3 and BI-4, Aspartic acid (D) codon (GAC)

BI-1 : 301  
SEQ ID. NO. 26 AAIINEDGTISSHGFAPDDQFNTVTSRISRDNRERRVFGYDSPYGRSPDNV

SEQ ID. NO. 27 AAIINEDGTISSHGFAPDDQFNTVTSRISRD+RERRVFGYDSPYGRSPDNV

BI-3: 301  
SEQ ID. NO. 28 AAIINEDGTISSHGFAPDDQFNTVTSRISRDRERRVFGYDSPYGRSPDNV



Fig. 7 (CONT.)

## Nucleotide substitution in ribosomal protein RpsB gene and product

#### a. Nucleotide substitution (identified by BLAST alignment)

Isolates 1 (19/19 reads) and 2 (36/36 reads) have G at this position

BI-1 : 781  
SEQ ID. NO. 29 ggaaaagctaaattgcttaccaaggagatgacaaagttatattgcctgagttcaaaaca  
BI-3 : 781 ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||  
SEQ ID. NO. 30 ggaaaagctaaattgcttaccaaggagatgacaaagttatattacctgagttcaaaaca  
A ↑

Isolates 3 (14/14 reads) and 4 (14/14 reads) have A at this position

**b. No amino acid substitution (identified by translation)**

In BI-1 and BI-2, Leucine (L) codon (TTG)

In BI-3 and BI-4, Leucine (L) codon (TTA)

BI-1:241  
SEQ ID. NO. 31 AKSITLIMITILADAIATRGGKAKFAYOGDDKVLPEFKDRVONPREFVNORRSFEOTGA

SEQ ID. NO. 32 AKSITLIMTILADAIATRGGKAKFAYOGDDKVILPEFKDRVONPRFVNORRSFEOTGA

BI-3 : 241  
SEQ ID. NO. 33 AKSITLIMTILADAIATRGGKAKFAYOGDDKVILPEFKDRVONPRFVNORRSFEOTGA

Fig. 7. (CONT.)

**Nucleotide substitution in conserved hypothetical protein and gene**

**a. Nucleotide substitution (identified by BLAST alignment)**

Isolates 1 (16/16 reads) and 2 (9/9 reads) have G at this position

BI-1 : 1  
SEQ ID. NO. 33 atgattgccgatcagaatagtggcaagagaattcgttccctgttgaaggatctattata  
BI-3 : 1  
SEQ ID. NO. 34 atgattgccgatcagaatagtggcaagagaattcgttccctgttaaggatctattata

The alignment shows two sequences side-by-side. The top sequence (BI-1) has a 'G' at the 10th position, indicated by a vertical arrow pointing down from the text 'Isolates 1 (16/16 reads) and 2 (9/9 reads) have G at this position'. The bottom sequence (BI-3) has a 'T' at the 10th position, indicated by a vertical arrow pointing up from the text 'Isolates 3 (22/22 reads) and 4 (11/11 reads) have T at this position'. Both sequences have a 'T' at the 11th position, indicated by a vertical arrow pointing up from the text 'Isolates 3 (22/22 reads) and 4 (11/11 reads) have T at this position'.

Isolates 3 (22/22 reads) and 4 (11/11 reads) have T at this position

**b. Amino acid substitution (identified by translation)**

In BI-1 and BI-2, Glutamate (E) codon (GAA)

In BI-3 and BI-4, STOP (\*) codon (TAA)

BI-1 : 1  
SEQ ID. NO. 35 MIADQNSGKRISFPVEGSIIDVQAYKYDGTLYRQWNGVKVLRNTNKHYVLLMYKTRVSEQ

SEQ ID. NO. 36 MIADQNSGKRISFPV GSIIIDVQAYKYDGTLYRQWNGVKVLRNTNKHYVLLMYKTRVSEQ

BI-3 : 1  
SEQ ID. NO. 37 MIADQNSGKRISFPV\*GSIIIDVQAYKYDGTLYRQWNGVKVLRNTNKHYVLLMYKTRVSEQ



Fig. 7 (CONT.)

Nucleotide substitution in conserved hypothetical protein and gene

**a. Nucleotide substitution (identified by BLAST alignment)**

Isolates 1 (10/10 reads) and 2 (28/28 reads) have G at this position

BI-1 : 7561      G ↓  
SEQ ID. NO. 38 aatgaatttagtaagt cattatatgaaagcgcaaaaagctctgccaagttgcata  
BI-3 : 39786      ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||  
SEQ ID. NO. 39 aataaatttagtaagt cattatatgaaagcgcaaaaagctctgccaagttgcata  
A ↑

Isolates 3 (12/12 reads) and 4 (18/18 reads) have A at this position

**b. Amino acid substitution (identified by translation)**

In BI-1 and BI-2, Glutamate (E) codon (GAA)

In BI-3 and BI-4, Lysine (K) codon (AAA)

BI-1: 2465  
SEQ ID. NO. 40 SGYWAGLDGFDVSSLNGTVEKMFDEIFGSPDKKGVL ETQHGLTKNEIAKFLDSSANEFS

SEQ ID. NO. 41 SGYWAGLDGFDVSSLNGTVEKMFDEIFGSPDKKGVL ETQHGLTKNEIAKFLDSSAN+F S

BI-3: 2465  
SEQ ID. NO. 42 SGYWAGLDGFDVSSLNGTVEKMFDEIFGSPDKKGVL ETQHGLTKNEIAKFLDSSANEFS

Fig. 8

## #1. Lipoate protein ligase protein; Query is BI-1, Sbjct is BI-4

Output from Blastp is formatted for dots representing identical amino acid residues.  
Length=345

Identities = 344/345 (99%), Positives = 344/345 (99%), Gaps = 0/345 (0%)

Query 1 MILVEPIRNGKYVKDGAYWLAIQIWAMNHRLNEKIVFPGIAAPHIQLGYFQNPEVEVNF 60  
Sbjct 1 ..... 60

Query 61 KYLKEHNLEVRRNTGGGAIYIDDNSVNVCYLIPYDEKDNILGNYDKFYEPTIKMLKELG 120  
Sbjct 61 ..... 120

Query 121 AKNVVQSGKNDLTIDGKKVSGAAMMLNGDVIYGGNSLLYKVDYDAMVDSLNPNRKKIEAK 180  
Sbjct 121 ..... 180

Query 181 GVKSIRQRVAPLSNYFDEQYRNLDIFEFKDLVIKKLFGVDDLSKVKRYELTEQDWAQVDE 240  
Sbjct 181 ..... 240

Query 241 LVNTKYKNWDWNYGLSPRYEYNRDARLAIGTINFSLAVEGQRIEKIKISGdffAKKDITE 300  
Sbjct 241 ..... I ..... 300

Query 301 LEKALVGTKMTFDDLVKAFKDADIQSYYFNEISPEEVSKIILDEE 345  
Sbjct 301 ..... 345

Fig. 8 (CONT.)

Length=345

Identities = 344/345 (99%), Positives = 344/345 (99%), Gaps = 0/345 (0%)

Query 1 MILVEPIRNGKYVKDGAYWLAIQIWAMNHLRLNEKIVFPGIAAPHIQLGYFQNPEVEVNF 60  
 MILVEPIRNGKYVKDGAYWLAIQIWAMNHLRLNEKIVFPGIAAPHIQLGYFQNPEVEVNF

Sbjct 1 MILVEPIRNGKYVKDGAYWLAIQIWAMNHLRLNEKIVFPGIAAPHIQLGYFQNPEVEVNF 60

Query 61 KYLKEHNLEVRRNTGGGAIYIDDNSVNVCYLIPYDEKDNILGNYDKFYEPTIKMLKELG 120  
 KYLKEHNLEVRRNTGGGAIYIDDNSVNVCYLIPYDEKDNILGNYDKFYEPTIKMLKELG

Sbjct 61 KYLKEHNLEVRRNTGGGAIYIDDNSVNVCYLIPYDEKDNILGNYDKFYEPTIKMLKELG 120

Query 121 AKNVVQSGKNDLTIDGKKVSGAAMMLNGDVIYGGNSLLYKVDYDAMVDSLNPNRKKIEAK 180  
 AKNVVQSGKNDLTIDGKKVSGAAMMLNGDVIYGGNSLLYKVDYDAMVDSLNPNRKKIEAK

Sbjct 121 AKNVVQSGKNDLTIDGKKVSGAAMMLNGDVIYGGNSLLYKVDYDAMVDSLNPNRKKIEAK 180

Query 181 GVKSIRQRVAPLSNYFDEQYRNLDIFEKFDLVIKKLFGVDDLSKVKRYELTEQDWAQVDE 240  
 GVKSIRQRVAPLSNYFDEQYRNLDIFEKFDLVIKKLFGVDDLSKVKRYELTEQDWAQVDE

Sbjct 181 GVKSIRQRVAPLSNYFDEQYRNLDIFEKFDLVIKKLFGVDDLSKVKRYELTEQDWAQVDE 240

Query 241 LVNTKYKNWDWNYGLSPRYEYNRDLAIGTINFSLAVEGQRIEKIKISGDFFAKKDITE 300  
 LVNTKYKNWDWNYGLSPRYEYNRDLAIGTINFSLAVEGQ IEKIKISGDFFAKKDITE

Sbjct 241 LVNTKYKNWDWNYGLSPRYEYNRDLAIGTINFSLAVEGQIEKIKISGDFFAKKDITE 300

Query 301 LEKALVGTKMTFDDLVKAFKDADIQSYFFNEISPEEVSKIILDEE 345

LEKALVGTKMTFDDLVKAFKDADIQSYFFNEISPEEVSKIILDEE

Sbjct 301 LEKALVGTKMTFDDLVKAFKDADIQSYFFNEISPEEVSKIILDEE 345

Comments: Single amino acid substitution. BI-1 and BI-2 have AGA Arginine (R) codon; BI-3 and BI-4 have ATA isoleucine (I) codon.

The sequence corresponding to this gene in BI-3 is contained within two different scaffolds, without extraneous nucleotides.

Fig. 8 (CONT.)

## #2. Era (GTPase) gene and protein; Query is BI-1, Sbjct is BI-3

Output from Blastp is formatted for dots representing identical amino acid residues.  
Length=290

Identities = 290/290 (100%), Positives = 290/290 (100%), Gaps = 0/290 (0%)

Query 1 MKVCIISILGRPNVGKSSLNKKIIKYDLAIVSNVPQTTRDQIMGVYTENDYQFVFVDTPG 60  
Sbjct 1 ..... 60

Query 61 IHKPLNLLGESLNKEAFSSINDIDCILFLTPVNEEIKSGDKLILERIANSKNKIAVISKI 120  
Sbjct 61 ..... 120

Query 121 DIAKSPDDISKKIKSLEEFNFQKIIISVSNKNDKSIDSIEILKEYSYEAPPFYDEDYITD 180  
Sbjct 121 ..... 180

Query 181 KSMRFMAKEYIRESAINLLTDELPHSIAVEVQDFIEEDRITINAVIYVKKDSQKGILIG 240  
Sbjct 181 ..... 240

Query 241 KGASMIKKIGTNARMKMGHQFNSKVTLNKVKVSNKWINDKSALKKFGYN 290  
Sbjct 241 ..... 290

Fig. 8 (CONT.)

Pairwise alignment from Blastp

Length=290

Identities = 290/290 (100%), Positives = 290/290 (100%), Gaps = 0/290 (0%)

Query 1 MKVCIISILGRPNVGKSSLNKIIKYDLAIVSNVPQTTRDQIMGVYTENDYQFVFVDTG 60

MKVCIISILGRPNVGKSSLNKIIKYDLAIVSNVPQTTRDQIMGVYTENDYQFVFVDTG

Sbjct 1 MKVCIISILGRPNVGKSSLNKIIKYDLAIVSNVPQTTRDQIMGVYTENDYQFVFVDTG 60

Query 61 IHKPLNLLGESLNKEAFSSINDIDCILFLTPVNEEIKSGDKLILERIANSKNKIAVISKI 120

IHKPLNLLGESLNKEAFSSINDIDCILFLTPVNEEIKSGDKLILERIANSKNKIAVISKI

Sbjct 61 IHKPLNLLGESLNKEAFSSINDIDCILFLTPVNEEIKSGDKLILERIANSKNKIAVISKI 120

Query 121 DLAKSPDDISKKIKSLEEFNFQKIISVSNKNDKSIDSLEIELKEYSYEAPPFYDEDYITD 180

DLAKSPDDISKKIKSLEEFNFQKIISVSNKNDKSIDSLEIELKEYSYEAPPFYDEDYITD

Sbjct 121 DLAKSPDDISKKIKSLEEFNFQKIISVSNKNDKSIDSLEIELKEYSYEAPPFYDEDYITD 180

Query 181 KSMRFMAKEYIRESAINLLTDELPHSIAVEVQDFIEEDRITINAVIYVKKDSQKGILIG 240

KSMRFMAKEYIRESAINLLTDELPHSIAVEVQDFIEEDRITINAVIYVKKDSQKGILIG

Sbjct 181 KSMRFMAKEYIRESAINLLTDELPHSIAVEVQDFIEEDRITINAVIYVKKDSQKGILIG 240

Query 241 KGASMIKKIGTNARMKMGHQFNSKVTLNKVKVSNKWINDKSALKKFGYN 290

KGASMIKKIGTNARMKMGHQFNSKVTLNKVKVSNKWINDKSALKKFGYN

Sbjct 241 KGASMIKKIGTNARMKMGHQFNSKVTLNKVKVSNKWINDKSALKKFGYN 290

Comments: Single synonymous nucleotide substitution. BI-1 and BI-2 have TTG Leucine codon; BI-3 and BI-4 have TTA leucine codon. There is no amino acid change.

2010238689 26 Oct 2016

Fig. 8 (CONT.)

Output from Blastn is formatted for dots representing identical nucleotides  
Length=873

Identities = 872/873 (99%), Gaps = 0/873 (0%)

Query 1	ATGAAGGTATGTATTATTAGTATTAGTATTAGGGCGTCCAAATGTTGGCAAATCCTCATTATTA	60
Sbjct 1	.....	60
Query 61	AACAAAATTATTAAATATGATCTTGCTATTGTATCTAACGTTCCGCAAACAACTAGAGAT	120
Sbjct 61	.....	120
Query 121	CAAATAATGGGAGTGTACACTGAAAATGATTATCAGTTGTTTTGTTGACACGCCCGGA	180
Sbjct 121	.....	180
Query 181	ATTCATAAACCATGAAATTGCTAGGGAGTCATTAAACAAAGAAGCATTAGCTCAATA	240
Sbjct 181	.....A.....	240
Query 241	AACGACATTGATTGCATTTATTTAACCCCGTAAATGAAGAGATTAAGTCAGGGGAT	300
Sbjct 241	.....	300
Query 301	AAATTAAATTCTAGAAAGAATTGCTAATTCAAAAAATAAAATTGCTGTAATATCAAAATT	360
Sbjct 301	.....	360
Query 361	GATTTAGCTAAATCACCAGACGATATTCTAAGAAAATTAAGAGCTTAGAAGAATTCAAT	420
Sbjct 361	.....	420
Query 421	TTTCAAAAAATTATTCCTGTTCTAATAAAATGACAAATCAATTGACTCACTAATTGAA	480
Sbjct 421	.....	480

Fig. 8 (CONT.)

Query	481	ATATTAAAGGAGTATTACAGAAGCTCCTCCATTTACGACGAAGACTATATTACAGAT	540
Sbjct	481	.....	540
Query	541	AAATCGATGAGATTTATGGCTAAAGAATACATTGAAAGCGCTATAAATCTTTAACG	600
Sbjct	541	.....	600
Query	601	GACGAATTACCGCATTCAATTGCTGTCGAAGTGCAGGATTATTGAAGAAGAACAGA	660
Sbjct	601	.....	660
Query	661	ATAACAATTAAATGCCGTAAATTATGTTAAAAAGATTGCAAAAGGAATTAAATTGGC	720
Sbjct	661	.....	720
Query	721	AAAGGGGCATCAATGATTAAAAAAATTGGCACCAATGCTAGAATGAAAATGGGCCATCAG	780
Sbjct	721	.....	780
Query	781	TTCAATAGTAAAGTTACGCTTAATTAAAGGTAAAGTTCTAATAATGGATCAATGAC	840
Sbjct	781	.....	840
Query	841	AAAAGTGCCTAAAAAAATTGGCTATAATTAA	873
Sbjct	841	.....	873

## FIG. 8 (CONT.)

Pairwise alignment Blastn

Length=873

Identities = 872/873 (99%), Gaps = 0/873 (0%)

Query 1 ATGAAGGTATGTATTATTAGTATTAGTATTAGCCTCAAATGTTGGCAAATCCTCATTATTA 60  
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct 1 ATGAAGGTATGTATTATTAGTATTAGCCTCAAATGTTGGCAAATCCTCATTATTA 60

Query 61 AACAAAATTATTAAATATGATCTTGCTATTGTATCTAACGTTCCGCAAACAACAGAGAT 120  
||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct 61 AACAAAATTATTAAATATGATCTTGCTATTGTATCTAACGTTCCGCAAACAACAGAGAT 120

Query 121 CAAATAATGGGAGTGTACACTGAAAATGATTATCAGTTGTTTTGTTGACACGCCCGA 180  
||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct 121 CAAATAATGGGAGTGTACACTGAAAATGATTATCAGTTGTTTTGTTGACACGCCCGA 180

Query 181 ATTCTAAACCATTGAATTGCTAGGGAGTCATTAAACAAAGAAGCATTAGCTCAATA 240  
||||||||||||||||||||||||||||||||||||||||||||

Sbjct 181 ATTCTAAACCATTAAATTGCTAGGGAGTCATTAAACAAAGAAGCATTAGCTCAATA 240

Query 241 AACGACATTGATTGCATTTATTTAACCCCGTAAATGAAGAGAGTTAAGTCAGGGAT 300  
||||||||||||||||||||||||||||||||||||||||

Sbjct 241 AACGACATTGATTGCATTTATTTAACCCCGTAAATGAAGAGAGTTAAGTCAGGGAT 300

Query 301 AAATTAATTCTAGAAAGAATTGCTAATTCAAAAAATAAAATTGCTGTAATATCAAAATT 360  
||||||||||||||||||||||||||||||||||||||||

Sbjct 301 AAATTAATTCTAGAAAGAATTGCTAATTCAAAAAATAAAATTGCTGTAATATCAAAATT 360

Fig. 8 (CONT.)

Query	361	GATTTAGCTAAATCACCAGACGATATTCTAAGAAAATTAAGAGCTTAGAAGAATTCAAT	420
Sbjct	361	GATTTAGCTAAATCACCAGACGATATTCTAAGAAAATTAAGAGCTTAGAAGAATTCAAT	420
Query	421	TTTCAAAAAATTATTCTGTTCTAATAAAAATGACAAATCAATTGACTCACTAATTGAA	480
Sbjct	421	TTTCAAAAAATTATTCTGTTCTAATAAAAATGACAAATCAATTGACTCACTAATTGAA	480
Query	481	ATATTAAAGGAGTATTACGAAAGCTCCTCCATTACGACGAAGACTATATTACAGAT	540
Sbjct	481	ATATTAAAGGAGTATTACGAAAGCTCCTCCATTACGACGAAGACTATATTACAGAT	540
Query	541	AAATCGATGAGATTATGGCTAAAGAATAACATTCTGAAAGCGCTATAAATCTTTAACG	600
Sbjct	541	AAATCGATGAGATTATGGCTAAAGAATAACATTCTGAAAGCGCTATAAATCTTTAACG	600
Query	601	GACGAATTACCGCATTCAATTGCTGTCGAAGTGCAGGATTATTGAAGAAGAACAGA	660
Sbjct	601	GACGAATTACCGCATTCAATTGCTGTCGAAGTGCAGGATTATTGAAGAAGAACAGA	660
Query	661	ATAACAATTAAATGCCGTAAATTATGTTAAAAAGATTGCAAAAGGAATTAAATTGGC	720
Sbjct	661	ATAACAATTAAATGCCGTAAATTATGTTAAAAAGATTGCAAAAGGAATTAAATTGGC	720
Query	721	AAAGGGGCATCAATGATTAATGGCACCAATGCTAGAATGAAAATGGCCATCAG	780
Sbjct	721	AAAGGGGCATCAATGATTAATGGCACCAATGCTAGAATGAAAATGGCCATCAG	780

## FIG. 8 (CONT.)

Query 781 TTCAATAGTAAAGTTACGCTTAATTAAAGGTAAAGTTCTAATAATGGATCAATGAC 840  
||||||||||||||||||||||||||||||||||||

Sbjct 781 TTCAATAGTAAAGTTACGCTTAATTAAAGGTAAAGTTCTAATAATGGATCAATGAC 840

Query 841 AAAAGTGCCTAAAAAAATTGGCTATAATTAA 873  
||||||||||||||||||||||||||||

Sbjct 841 AAAAGTGCCTAAAAAAATTGGCTATAATTAA 873

Comments: Single synonymous nucleotide substitution. BI-1 and BI-2 have TTG Leucine codon; BI-3 and BI-4 have TTA leucine codon. There is no amino acid change.

## FIG. 8 (CONT.)

## #3. P81 lipoprotein; Query is BI-1, Sbjct is BI-3

Output from Blastp is formatted for dots representing identical amino acid residues.

Length=728

Identities = 727/728 (99%), Positives = 728/728 (100%), Gaps = 0/728 (0%)

Query 1	MSKKNKLMIGLSSTAIPLLA AVSAKCGGTVN YEDLGDAKKISLGVSFSSGQPQWNTMAS	60
Sbjct 1	I.....	60
Query 61	LIKYYNEAHKNDKHFLPVELKHLGSGYPEGENTVITELKAKRNEVVNLAFNYGSLASRLA	120
Sbjct 61	.....	120
Query 121	SSEM RDLYKMDKVLNFEDNDKDISV D LKNINEKFARANSNTENLPNN GTFMIPMLKSIQV	180
Sbjct 121	.....	180
Query 181	MSANAPV LQYIFKTFENKGAKFDDSFKKSARYQDIMTNGKGDESEVQKLWGEFESSQQDA	240
Sbjct 181	.....	240
Query 241	VKKLTISSTFENLEELLNFANIAQKSFKNSAAKNSRLHILGVDDVSGLIQSLPYAMINA	300
Sbjct 241	.....	300
Query 301	DANDFFIQTGLVKNKTTVNYKKIKDKNNKSVKALSEIYNKFKE SLATKS LTLLAGGEYTS	360
Sbjct 301	.....	360
Query 361	SYQT KHEYAFGIGSTAGYRHFISDKTKVIFTLKGTDVSGEKDKEFKNVIKKTEKGVDQ	420
Sbjct 361	.....	420
Query 421	LFVTFKENANKVYKSTVDTDKLDDEEKDSL RYSYKSLDSATDSKMDEILKKITNTDPEAS	480
Sbjct 421	.....	480

## FIG. 8 (CONT.)

Query	481	DNKQWLLELRDNQSDIKTVKEKGAQEVGTVIETKISGASKYKIFFLNDLVRKELSS	540
Sbjct	481	.....	540
Query	541	KGTLQEKEKELIVFAVPGKWNKSNEKRVVIYSQGPSLIGVSRGAKPDRAAKNFAKFLTSLEKI	600
Sbjct	541	.....	600
Query	601	DITLSKYDKDMKKTDSRDKPYKQVTPAQFISDAASYVFPVKGFEKTDTISKIKNKYIVHT	660
Sbjct	601	.....	660
Query	661	YNELKEAVTNKNVVIYEEPAGFHSSSFRESLGSAFKSAYLKAKNDQPLEDFDKEIIGSII	720
Sbjct	661	.....	720
Query	721	ASSSQLK	728
Sbjct	721	.....	728

## FIG. 8 (CONT.)

Pairwise alignment from Blastp  
Length=728

Identities = 727/728 (99%), Positives = 728/728 (100%), Gaps = 0/728 (0%)

Query 1	MSKKNKLMIGLSSTAIPLLA AVSAKCGGTVN YEDLGDAKKISLGVS FSSGQPQWN TMAS	60
	+SKKKNKLMIGLSSTAIPLLA AVSAKCGGTVN YEDLGDAKKISLGVS FSSGQPQWN TMAS	
Sbjct 1	ISKKNKLMIGLSSTAIPLLA AVSAKCGGTVN YEDLGDAKKISLGVS FSSGQPQWN TMAS	60
Query 61	LIKYYNEAHKNDKHFLPVELKHLGSGYPEGENTVITELKAKRNEVVNLAFNYGSLASRLA	120
	LIKYYNEAHKNDKHFLPVELKHLGSGYPEGENTVITELKAKRNEVVNLAFNYGSLASRLA	
Sbjct 61	LIKYYNEAHKNDKHFLPVELKHLGSGYPEGENTVITELKAKRNEVVNLAFNYGSLASRLA	120
Query 121	SSEM RDLYKMDKVLNFEDNDKDISV D LKNINEKFARANSNTENLPNN GTFMIPMLKSIQV	180
	SSEM RDLYKMDKVLNFEDNDKDISV D LKNINEKFARANSNTENLPNN GTFMIPMLKSIQV	
Sbjct 121	SSEM RDLYKMDKVLNFEDNDKDISV D LKNINEKFARANSNTENLPNN GTFMIPMLKSIQV	180
Query 181	MSANAPVLQYIFKTFENKGAKFDDSFKKSARYQDIMTNGKGDESEVQKLWGEFESSQQDA	240
	MSANAPVLQYIFKTFENKGAKFDDSFKKSARYQDIMTNGKGDESEVQKLWGEFESSQQDA	
Sbjct 181	MSANAPVLQYIFKTFENKGAKFDDSFKKSARYQDIMTNGKGDESEVQKLWGEFESSQQDA	240
Query 241	VKKLTISSTFENLEELLNFANIAQKSFKN SAAKNSRLHILGVDDVSGLIQSLPYAMINA	300
	VKKLTISSTFENLEELLNFANIAQKSFKN SAAKNSRLHILGVDDVSGLIQSLPYAMINA	
Sbjct 241	VKKLTISSTFENLEELLNFANIAQKSFKN SAAKNSRLHILGVDDVSGLIQSLPYAMINA	300
Query 301	DANDFFIQTGLVKNKTTVNYKKIKDKNNKSVKALSEIYNKFKESLATKS LTLLAGGEYTS	360
	DANDFFIQTGLVKNKTTVNYKKIKDKNNKSVKALSEIYNKFKESLATKS LTLLAGGEYTS	
Sbjct 301	DANDFFIQTGLVKNKTTVNYKKIKDKNNKSVKALSEIYNKFKESLATKS LTLLAGGEYTS	360

## FIG. 8 (CONT.)

Query	361	SYQTKHEYAFGIGSTAGYRHNFISDKTKVIFTLKGTDSGEKDKEFKNVIKKTEGV DQ	420
		SYQTKHEYAFGIGSTAGYRHNFISDKTKVIFTLKGTDSGEKDKEFKNVIKKTEGV DQ	
Sbjct	361	SYQTKHEYAFGIGSTAGYRHNFISDKTKVIFTLKGTDSGEKDKEFKNVIKKTEGV DQ	420
Query	421	LFVTFKENANKVYKSTVDTDKLDDEEKDSLRYSYKSLDSATDSKMDEILKKITNTDPEAS	480
		LFVTFKENANKVYKSTVDTDKLDDEEKDSLRYSYKSLDSATDSKMDEILKKITNTDPEAS	
Sbjct	421	LFVTFKENANKVYKSTVDTDKLDDEEKDSLRYSYKSLDSATDSKMDEILKKITNTDPEAS	480
Query	481	DNKQWLLFLREDNQSDIKTVKEKGAQEVGTVIETKISGASKYKIFFLND ELLVRKELSS	540
		DNKQWLLFLREDNQSDIKTVKEKGAQEVGTVIETKISGASKYKIFFLND ELLVRKELSS	
Sbjct	481	DNKQWLLFLREDNQSDIKTVKEKGAQEVGTVIETKISGASKYKIFFLND ELLVRKELSS	540
Query	541	KGTIQEKEKELIVFAVPGKWNKSNEKRV IYSQGPSLIGVSRGAKPDRAAKNFAKFLTSLEKI	600
		KGTIQEKEKELIVFAVPGKWNKSNEKRV IYSQGPSLIGVSRGAKPDRAAKNFAKFLTSLEKI	
Sbjct	541	KGTIQEKEKELIVFAVPGKWNKSNEKRV IYSQGPSLIGVSRGAKPDRAAKNFAKFLTSLEKI	600
Query	601	DITLSKYDKDMKKT KDSRDKPYKQVTPAQFISDAASYVFPVGF EKTDTSKIKNKYIVHT	660
		DITLSKYDKDMKKT KDSRDKPYKQVTPAQFISDAASYVFPVGF EKTDTSKIKNKYIVHT	
Sbjct	601	DITLSKYDKDMKKT KDSRDKPYKQVTPAQFISDAASYVFPVGF EKTDTSKIKNKYIVHT	660
Query	661	YNELKEAVTNKVVIIYEEPAGFHSSSFRESLGSAFKSAYLKAKNDQPLEDFDEIIGSII	720
		YNELKEAVTNKVVIIYEEPAGFHSSSFRESLGSAFKSAYLKAKNDQPLEDFDEIIGSII	
Sbjct	661	YNELKEAVTNKVVIIYEEPAGFHSSSFRESLGSAFKSAYLKAKNDQPLEDFDEIIGSII	720
Query	721	ASSSQLK 728	
		ASSSQLK	
Sbjct	721	ASSSQLK 728	

Comments: Single amino acid substitution. BI-1 and BI-2 have ATG Methionine (M) start codon; BI-3 and BI-4 have ATT isoleucine (I) codon and lack the P81 start codon.

## FIG. 8 (CONT.)

## #4. Membrane protein; Query is BI-1, Sbjct is BI-3

Output from Blastp is formatted for dots representing identical amino acid residues.  
Length=735

Identities = 734/735 (99%), Positives = 735/735 (100%), Gaps = 0/735 (0%)

Query 1	MSIFKKKKNRVIFATLAFGAIISSTSGVLIYRSSSDIDL	SRVIFSSSTNSELANNKNNIN	60
Sbjct 1	.....	.....	60
Query 61	KAIDAIKDNNVVENEKPVVIKPAEVPKIKIPDVAKE	TNPSPEVRNSIRPEKTEPLI	120
Sbjct 61	.....	.....	120
Query 121	NWVTKEIFIHGKVNVATIEVTPDRVISDYDKDRKISNVNPYQNI	IIVSKVLNVEVTQELRD	180
Sbjct 121	.....	.....	180
Query 181	KSVKNALNGNDGTGLFAGTFFFLNNIITSSKDLKTAEDAVMNNP	WIYRDNIHRYERLLN	240
Sbjct 181	.....	.....	240
Query 241	NPNVVNFLKEDAKKEYPNKNFDSIVQRQIWLIHNL	DQTKFTKLAKDAESFLSQGLVISPR	300
Sbjct 241	.....	.....	300
Query 301	AAIINEDGTISSHGFAPDDQFNTVTSRISRDNRERRVFGYD	SPYGRSPDNWEGSYPGWK	360
Sbjct 301	.....	D.....	360
Query 361	KEDVTSDFQKYNVSSADGIKLTKLTREKPEKGSGALNEG	LVVEIDASNTSGYDKTLKL	420
Sbjct 361	.....	.....	420
Query 421	INELKKDKVQVTSYRIKNMGNNDPSQFRDILNALPDNIPQ	LELFFSAEATNTSSLIALE	480
Sbjct 421	.....	.....	480

## FIG. 8 (CONT.)

Query	481	NKRIKELSLYTLGNSLLHKWSFNPLALRNTIEWINTVDYNVS RD FRPNT SIPTRITFDTIA	540
Sbjct	481	.....	540
Query	541	FDSDDFKNKSFERINDGLRMVYFARNNEPFFQAGLGPGLNP DHNEGNNSY PMGLDFSRVE	600
Sbjct	541	.....	600
Query	601	GIKSLRNLVFNDVVKSNNTPRKIRR LTFNNSEAF EISSDELSNASFEHFATDSMDPYSK	660
Sbjct	601	.....	660
Query	661	PKIMFSNGDTT NVIKISDSNELDQKAVWNLSKFFEYNEKLKASKRINVPKDALKLKEQLE	720
Sbjct	661	.....	720
Query	721	RLGYKVNQDGNIY	735
Sbjct	721	.....	735

## FIG. 8 (CONT.)

Pairwise alignment from Blastp

Length=735

Identities = 734/735 (99%), Positives = 735/735 (100%), Gaps = 0/735 (0%)

Query 1 MSIFKKKKNRVIFATLAFGAIISSTSGVLIYRSSSDIDLRSVIFSSSTNSELANNKNNIN 60  
MSIFKKKKNRVIFATLAFGAIISSTSGVLIYRSSSDIDLRSVIFSSSTNSELANNKNNIN

Sbjct 1 MSIFKKKKNRVIFATLAFGAIISSTSGVLIYRSSSDIDLRSVIFSSSTNSELANNKNNIN 60

Query 61 KAIDAIKDNNVENEKPVVIKPAEVPKIKIPDVAKETNPSPEVRNSIRPEKTEPLIACP 120  
KAIDAIKDNNVENEKPVVIKPAEVPKIKIPDVAKETNPSPEVRNSIRPEKTEPLIACP  
Sbjct 61 KAIDAIKDNNVENEKPVVIKPAEVPKIKIPDVAKETNPSPEVRNSIRPEKTEPLIACP 120

Query 121 NVVTKEIFIHGKVNVNATIEVTPDRVISDYDKDRKISNVNPYQNIIVSKVLNVEVTQELRD 180  
NVVTKEIFIHGKVNVNATIEVTPDRVISDYDKDRKISNVNPYQNIIVSKVLNVEVTQELRD  
Sbjct 121 NVVTKEIFIHGKVNVNATIEVTPDRVISDYDKDRKISNVNPYQNIIVSKVLNVEVTQELRD 180

Query 181 KSVKNALNGNDGTGLFAGTFFVFLNNIITSSKDLKTAEDAVMNNPWIYRDNIHRYERLLN 240  
KSVKNALNGNDGTGLFAGTFFVFLNNIITSSKDLKTAEDAVMNNPWIYRDNIHRYERLLN  
Sbjct 181 KSVKNALNGNDGTGLFAGTFFVFLNNIITSSKDLKTAEDAVMNNPWIYRDNIHRYERLLN 240

Query 241 NPNVVNFLKEDAKKEYPNKNFDSIVQRQIWLHNLQDKFTKLAKDAESFLSQGLVISPR 300  
NPNVVNFLKEDAKKEYPNKNFDSIVQRQIWLHNLQDKFTKLAKDAESFLSQGLVISPR  
Sbjct 241 NPNVVNFLKEDAKKEYPNKNFDSIVQRQIWLHNLQDKFTKLAKDAESFLSQGLVISPR 300

Query 301 AAIINEDGTISSHGFAPDDQFNTVTSRISRDNRERRVFGYDSPYGRSPDNWEGSYPGWK 360  
AAIINEDGTISSHGFAPDDQFNTVTSRISRD+RERRVFGYDSPYGRSPDNWEGSYPGWK  
Sbjct 301 AAIINEDGTISSHGFAPDDQFNTVTSRISRDERRVFGYDSPYGRSPDNWEGSYPGWK 360

FIG. 8 (CONT.)

Query	361	KEDVTSDFQKYNVSSADGIKLTREKPEKGSGALNEGLVIEDASNTSGYDKTLKL	420
		KEDVTSDFQKYNVSSADGIKLTREKPEKGSGALNEGLVIEDASNTSGYDKTLKL	
Sbjct	361	KEDVTSDFQKYNVSSADGIKLTREKPEKGSGALNEGLVIEDASNTSGYDKTLKL	420
Query	421	INELKKDKVQVTSYRIKNMGNNDPSQKFRDILNALPDNIPQLELFFSAATNTSSLIALE	480
		INELKKDKVQVTSYRIKNMGNNDPSQKFRDILNALPDNIPQLELFFSAATNTSSLIALE	
Sbjct	421	INELKKDKVQVTSYRIKNMGNNDPSQKFRDILNALPDNIPQLELFFSAATNTSSLIALE	480
Query	481	NKRIKELSLYTLGNSSLHKWSFNPLALRNTEWINTVDYNVS RD F R P N T S I P T R I T F D T I A	540
		NKRIKELSLYTLGNSSLHKWSFNPLALRNTEWINTVDYNVS RD F R P N T S I P T R I T F D T I A	
Sbjct	481	NKRIKELSLYTLGNSSLHKWSFNPLALRNTEWINTVDYNVS RD F R P N T S I P T R I T F D T I A	540
Query	541	FDSDDFKNKSFERINDGLRMVYFARNNEPFFQAGLGPGLNP DHNEGNNNSYPMGLDFSRVE	600
		FDSDDFKNKSFERINDGLRMVYFARNNEPFFQAGLGPGLNP DHNEGNNNSYPMGLDFSRVE	
Sbjct	541	FDSDDFKNKSFERINDGLRMVYFARNNEPFFQAGLGPGLNP DHNEGNNNSYPMGLDFSRVE	600
Query	601	GIKSLRNLVFNDVVKSNNTPRKIRRLTLFNNSEAFEISSDELSNASFEHFATDSMDPYSK	660
		GIKSLRNLVFNDVVKSNNTPRKIRRLTLFNNSEAFEISSDELSNASFEHFATDSMDPYSK	
Sbjct	601	GIKSLRNLVFNDVVKSNNTPRKIRRLTLFNNSEAFEISSDELSNASFEHFATDSMDPYSK	660
Query	661	PKIMFSNGDTTNVIKISDSNELDQKAVWNLSKFFEYNEKLKASKRINVPKDALKLKEQLE	720
		PKIMFSNGDTTNVIKISDSNELDQKAVWNLSKFFEYNEKLKASKRINVPKDALKLKEQLE	
Sbjct	661	PKIMFSNGDTTNVIKISDSNELDQKAVWNLSKFFEYNEKLKASKRINVPKDALKLKEQLE	720

2010238689

26 Oct 2016

FIG. 8 (CONT.)

Query 721 RLGYKVVNQDGNIY 735

RLGYKVVNQDGNIY

Sbjct 721 RLGYKVVNQDGNIY 735

Comments: Single amino acid substitution. BI-1 and BI-2 have AAC Asparagine (N) codon; BI-3 and BI-4 have GAC aspartic acid (D) codon.

FIG. 8 (CONT.)

**#5. Ribosomal protein RpsB and gene; Query is BI-1, Sbjct is BI-3**

Output from Blastp is formatted for dots representing identical amino acid residues.

Length=317

Identities = 317/317 (100%), Positives = 317/317 (100%), Gaps = 0/317 (0%)

Query 1	MENENLKVEQATTAENNIAEKADDSKASKEVKPTIVSREKLLEAGTYFGHKKSMWNPKMK	60
Sbjct 1	.....	60
Query 61	EFLYPQSQRGMHMINTNVTIQRLEFAYNILNKFVAKNPRTTFIFVGTKQAKDTIKENAL	120
Sbjct 61	.....	120
Query 121	RTGSFYVSERWLGGTLTNASTIFKRVKVMEELEAQAAKKFQGYTKKEGLIKQKELDKLHK	180
Sbjct 121	.....	180
Query 181	NLDGIRKMQLPSFMIVADPNVDAIAVKEARSKGVKVGILDSNSNPDAVDFGIPANDDS	240
Sbjct 181	.....	240
Query 241	AKSITLIMTILADAIATARGGKAKFAYQGDDKVLPEFKTDKVQNPRFVNQRRSFEQTGA	300
Sbjct 241	.....	300
Query 301	QTVRNVEKTTSAEVTE	317
Sbjct 301	.....	317

## FIG. 8 (CONT.)

Pairwise alignment from Blastp  
Length=317

Identities = 317/317 (100%), Positives = 317/317 (100%), Gaps = 0/317 (0%)

Query 1	MENENLKV <b>E</b> QATTAENNIAEKADD <b>S</b> KASKEV <b>K</b> P <b>T</b> IVS <b>R</b> E <b>K</b> L <b>E</b> AGTY <b>F</b> GH <b>K</b> K <b>S</b> M <b>W</b> N <b>P</b> K <b>M</b> K	60
	MENENLKV <b>E</b> QATTAENNIAEKADD <b>S</b> KASKEV <b>K</b> P <b>T</b> IVS <b>R</b> E <b>K</b> L <b>E</b> AGTY <b>F</b> GH <b>K</b> K <b>S</b> M <b>W</b> N <b>P</b> K <b>M</b> K	
Sbjct 1	MENENLKV <b>E</b> QATTAENNIAEKADD <b>S</b> KASKEV <b>K</b> P <b>T</b> IVS <b>R</b> E <b>K</b> L <b>E</b> AGTY <b>F</b> GH <b>K</b> K <b>S</b> M <b>W</b> N <b>P</b> K <b>M</b> K	60
Query 61	EFLYPQS <b>K</b> RM <b>M</b> H <b>M</b> INT <b>N</b> VL <b>Q</b> R <b>L</b> E <b>F</b> A <b>Y</b> N <b>I</b> L <b>N</b> K <b>F</b> V <b>A</b> K <b>N</b> P <b>R</b> T <b>F</b> I <b>F</b> V <b>G</b> T <b>K</b> Q <b>A</b> K <b>D</b> T <b>I</b> K <b>E</b> N <b>A</b> L	120
	EFLYPQS <b>K</b> RM <b>M</b> H <b>M</b> INT <b>N</b> VL <b>Q</b> R <b>L</b> E <b>F</b> A <b>Y</b> N <b>I</b> L <b>N</b> K <b>F</b> V <b>A</b> K <b>N</b> P <b>R</b> T <b>F</b> I <b>F</b> V <b>G</b> T <b>K</b> Q <b>A</b> K <b>D</b> T <b>I</b> K <b>E</b> N <b>A</b> L	
Sbjct 61	EFLYPQS <b>K</b> RM <b>M</b> H <b>M</b> INT <b>N</b> VL <b>Q</b> R <b>L</b> E <b>F</b> A <b>Y</b> N <b>I</b> L <b>N</b> K <b>F</b> V <b>A</b> K <b>N</b> P <b>R</b> T <b>F</b> I <b>F</b> V <b>G</b> T <b>K</b> Q <b>A</b> K <b>D</b> T <b>I</b> K <b>E</b> N <b>A</b> L	120
Query 121	RTGSF <b>Y</b> V <b>S</b> ER <b>W</b> L <b>G</b> GT <b>L</b> T <b>N</b> AST <b>I</b> F <b>K</b> R <b>V</b> K <b>V</b> M <b>E</b> E <b>L</b> A <b>Q</b> A <b>A</b> K <b>F</b> Q <b>G</b> Y <b>T</b> K <b>E</b> GL <b>I</b> K <b>Q</b> K <b>E</b> LD <b>K</b> L <b>H</b> K	180
	RTGSF <b>Y</b> V <b>S</b> ER <b>W</b> L <b>G</b> GT <b>L</b> T <b>N</b> AST <b>I</b> F <b>K</b> R <b>V</b> K <b>V</b> M <b>E</b> E <b>L</b> A <b>Q</b> A <b>A</b> K <b>F</b> Q <b>G</b> Y <b>T</b> K <b>E</b> GL <b>I</b> K <b>Q</b> K <b>E</b> LD <b>K</b> L <b>H</b> K	
Sbjct 121	RTGSF <b>Y</b> V <b>S</b> ER <b>W</b> L <b>G</b> GT <b>L</b> T <b>N</b> AST <b>I</b> F <b>K</b> R <b>V</b> K <b>V</b> M <b>E</b> E <b>L</b> A <b>Q</b> A <b>A</b> K <b>F</b> Q <b>G</b> Y <b>T</b> K <b>E</b> GL <b>I</b> K <b>Q</b> K <b>E</b> LD <b>K</b> L <b>H</b> K	180
Query 181	NLD <b>G</b> IR <b>K</b> M <b>Q</b> SL <b>P</b> SM <b>I</b> V <b>A</b> DP <b>N</b> V <b>D</b> AI <b>A</b> V <b>K</b> EAR <b>S</b> KG <b>V</b> K <b>V</b> I <b>G</b> I <b>L</b> D <b>S</b> N <b>S</b> N <b>P</b> DA <b>V</b> DF <b>G</b> I <b>P</b> AN <b>D</b> S	240
	NLD <b>G</b> IR <b>K</b> M <b>Q</b> SL <b>P</b> SM <b>I</b> V <b>A</b> DP <b>N</b> V <b>D</b> AI <b>A</b> V <b>K</b> EAR <b>S</b> KG <b>V</b> K <b>V</b> I <b>G</b> I <b>L</b> D <b>S</b> N <b>S</b> N <b>P</b> DA <b>V</b> DF <b>G</b> I <b>P</b> AN <b>D</b> S	
Sbjct 181	NLD <b>G</b> IR <b>K</b> M <b>Q</b> SL <b>P</b> SM <b>I</b> V <b>A</b> DP <b>N</b> V <b>D</b> AI <b>A</b> V <b>K</b> EAR <b>S</b> KG <b>V</b> K <b>V</b> I <b>G</b> I <b>L</b> D <b>S</b> N <b>S</b> N <b>P</b> DA <b>V</b> DF <b>G</b> I <b>P</b> AN <b>D</b> S	240
Query 241	AKS <b>I</b> T <b>L</b> IM <b>T</b> I <b>L</b> AD <b>A</b> I <b>A</b> T <b>A</b> R <b>G</b> K <b>A</b> K <b>F</b> A <b>Y</b> Q <b>G</b> DD <b>K</b> V <b>I</b> L <b>P</b> E <b>F</b> K <b>T</b> DR <b>V</b> Q <b>N</b> P <b>R</b> F <b>V</b> N <b>Q</b> R <b>R</b> S <b>F</b> E <b>Q</b> T <b>G</b> A	300
	AKS <b>I</b> T <b>L</b> IM <b>T</b> I <b>L</b> AD <b>A</b> I <b>A</b> T <b>A</b> R <b>G</b> K <b>A</b> K <b>F</b> A <b>Y</b> Q <b>G</b> DD <b>K</b> V <b>I</b> L <b>P</b> E <b>F</b> K <b>T</b> DR <b>V</b> Q <b>N</b> P <b>R</b> F <b>V</b> N <b>Q</b> R <b>R</b> S <b>F</b> E <b>Q</b> T <b>G</b> A	
Sbjct 241	AKS <b>I</b> T <b>L</b> IM <b>T</b> I <b>L</b> AD <b>A</b> I <b>A</b> T <b>A</b> R <b>G</b> K <b>A</b> K <b>F</b> A <b>Y</b> Q <b>G</b> DD <b>K</b> V <b>I</b> L <b>P</b> E <b>F</b> K <b>T</b> DR <b>V</b> Q <b>N</b> P <b>R</b> F <b>V</b> N <b>Q</b> R <b>R</b> S <b>F</b> E <b>Q</b> T <b>G</b> A	300
Query 301	QT <b>V</b> R <b>N</b> VE <b>K</b> TT <b>T</b> SA <b>E</b> V <b>T</b> E	317
	QT <b>V</b> R <b>N</b> VE <b>K</b> TT <b>T</b> SA <b>E</b> V <b>T</b> E	
Sbjct 301	QT <b>V</b> R <b>N</b> VE <b>K</b> TT <b>T</b> SA <b>E</b> V <b>T</b> E	317

Comments: Single synonymous nucleotide substitution. BI-1 and BI-2 have TTG Leucine codon; BI-3 and BI-4 have TTA leucine codon. There is no amino acid change.

## FIG. 8 (CONT.)

Output from Blastn is formatted for dots representing identical nucleotides  
Length=954

Identities = 953/954 (99%), Gaps = 0/954 (0%)

Strand=Plus/Plus

Query 1	ATGGAAAACGAAACTTAAAGTTGAACAAGCTACTACACGCTGAAAATAATAGCTGAA	60
Sbjct 1	.....	60
Query 61	AAAGCTGATGATTCTAAAGCTTCAAAAGAAGTAAACCTACTATTGTTCTAGAGAAAAA	120
Sbjct 61	.....	120
Query 121	TTATTAGAAGCTGGAACATATTTGGTCATAAAAAAGTATGTGAAATCCTAAAATGAAG	180
Sbjct 121	.....	180
Query 181	GAATTTCTATACCCACAATCAAACGTGGAATGCATATGATCAACACAAATGTTACATTG	240
Sbjct 181	.....	240
Query 241	CAACGTTAGAATTGCATACAACATTGAAACAAATTGTTGCTAAAAATCCTAGAACAA	300
Sbjct 241	.....	300
Query 301	ACATTTATTTGTTGTTACTAAGAAGCAAGCTAAAGACACAATTAAAGAAAATGCGTTA	360
Sbjct 301	.....	360
Query 361	AGAACTGGCAGTTCTATGTATCTGAAAGATGATTAGGTGGAACATTAACATAATGCTTCT	420
Sbjct 361	.....	420
Query 421	ACAATTTCAAAAGAGTTAAAGTAATGGAAGAATTAGAAGCTCAAGCTGCTAAGAAATTC	480
Sbjct 421	.....	480

## FIG. 8 (CONT.)

Query	481	CAAGGATATACCAAAAAAGAAGGTCTAATCAAACAAAAGAATTAGACAAATTACACAAA	540
Sbjct	481	.....	540
Query	541	AATCTTGATGGTATAAGAAAGATGCAAAGCCTCCATCTTATGATTGTTGCTGACCCCT	600
Sbjct	541	.....	600
Query	601	AACGTTGATGCTATAGCAGTTAAAGAAGCAAGAAGCAAGGGTGTAAAGTTATAGGTATC	660
Sbjct	601	.....	660
Query	661	TTAGACTCAAACCTCTAATCCTGATGCTGTTGACTTGGTATTCTGCAAATGATGATTCA	720
Sbjct	661	.....	720
Query	721	GCTAAAAGTATTACTTAATTATGACAATTTAGCTGACGCAATCGCTACTGCTCGTGGT	780
Sbjct	721	.....	780
Query	781	GGAAAAGCTAAATTGCTTACCAAGGAGATGACAAAGTTATATTGCCTGAGTTCAAAACA	840
Sbjct	781	..... <b>A</b> .....	840
Query	841	GACAGAGTTCAAAATCCAAGATTGTTAACCAAAGAAGAAGCTTGAACAAACTGGTGCC	900
Sbjct	841	.....	900
Query	901	CAAACAGTTAGAAATGTAGAAAAACTACAACAAGCGCTGAAGTTACAGAATAA	954
Sbjct	901	.....	954

## FIG. 8. (CONT.)

Pairwise alignment Blastn

Length=954

Identities = 953/954 (99%), Gaps = 0/954 (0%)

Strand=Plus/Plus

Query 1	ATGGAAAACGAAAACCTAAAGTTGAACAAGCTACTACAGCTGAAAATAATATAGCTGAA	60
Sbjct 1	ATGGAAAACGAAAACCTAAAGTTGAACAAGCTACTACAGCTGAAAATAATATAGCTGAA	60
Query 61	AAAGCTGATGATTCTAAAGCTTCAAAAGAAGTAAACCTACTATTGTTCTAGAGAAAAA	120
Sbjct 61	AAAGCTGATGATTCTAAAGCTTCAAAAGAAGTAAACCTACTATTGTTCTAGAGAAAAA	120
Query 121	TTATTAGAAGCTGGAACATATTTGGTCATAAAAAAGTATGTGAAATCCTAAAATGAAG	180
Sbjct 121	TTATTAGAAGCTGGAACATATTTGGTCATAAAAAAGTATGTGAAATCCTAAAATGAAG	180
Query 181	GAATTCTATACCCACAATCAAAACGTGGAATGCATATGATCAACACAAATGTTACATTG	240
Sbjct 181	GAATTCTATACCCACAATCAAAACGTGGAATGCATATGATCAACACAAATGTTACATTG	240
Query 241	CAACGTTAGAATTGCATACAACATTTGAACAAATTGTTGCTAAAATCCTAGAAC	300
Sbjct 241	CAACGTTAGAATTGCATACAACATTTGAACAAATTGTTGCTAAAATCCTAGAAC	300
Query 301	ACATTTATTTGTTGGTACTAAGAAGCAAGCTAAAGACACAATTAAAGAAAATGCGTTA	360
Sbjct 301	ACATTTATTTGTTGGTACTAAGAAGCAAGCTAAAGACACAATTAAAGAAAATGCGTTA	360

## FIG. 8 (CONT.)

Query	361	AGAACTGGCAGTTCTATGTATCTGAAAGATGATTAGGTGGAACATTAACATAATGCTTCT	420
Sbjct	361	AGAACTGGCAGTTCTATGTATCTGAAAGATGATTAGGTGGAACATTAACATAATGCTTCT	420
Query	421	ACAATTTCAAAAGAGTTAAAGTAATGGAAGAATTAGAAGCTCAAGCTGCTAAGAAATTTC	480
Sbjct	421	ACAATTTCAAAAGAGTTAAAGTAATGGAAGAATTAGAAGCTCAAGCTGCTAAGAAATTTC	480
Query	481	CAAGGATATACCAAAAAAGAAGGTCTAATCAAACAAAAGAATTAGACAAATTACACAAA	540
Sbjct	481	CAAGGATATACCAAAAAAGAAGGTCTAATCAAACAAAAGAATTAGACAAATTACACAAA	540
Query	541	AATCTTGATGGTATAAGAAAGATGCAAAGCCTCCATCATTTATGATTGTTGCTGACCCT	600
Sbjct	541	AATCTTGATGGTATAAGAAAGATGCAAAGCCTCCATCATTTATGATTGTTGCTGACCCT	600
Query	601	AACGTTGATGCTATAGCAGTTAAAGAAGCAAGAAGCAAGGGTGTAAAGTTATAGGTATC	660
Sbjct	601	AACGTTGATGCTATAGCAGTTAAAGAAGCAAGAAGCAAGGGTGTAAAGTTATAGGTATC	660

## FIG. 8 (CONT.)

Query	661	TTAGACTCAAACCTAAATCCTGATGCTGTTGACTTGGTATTCCCTGCAAATGATGATTCA	720
Sbjct	661	TTAGACTCAAACCTAAATCCTGATGCTGTTGACTTGGTATTCCCTGCAAATGATGATTCA	720
Query	721	GCTAAAAGTATTACTTAATTATGACAATTTAGCTGACGCAATCGCTACTGCTCGTGGT	780
Sbjct	721	GCTAAAAGTATTACTTAATTATGACAATTTAGCTGACGCAATCGCTACTGCTCGTGGT	780
Query	781	GGAAAAGCTAAATTGCTTACCAAGGAGATGACAAAGTTATTCGCTGAGTTCAAAACA	840
Sbjct	781	GGAAAAGCTAAATTGCTTACCAAGGAGATGACAAAGTTATTCGCTGAGTTCAAAACA	840
Query	841	GACAGAGTTCAAATCCAAGATTGTTAACCAAGAAGAAGCTTGAACAAACTGGTGCC	900
Sbjct	841	GACAGAGTTCAAATCCAAGATTGTTAACCAAGAAGAAGCTTGAACAAACTGGTGCC	900
Query	901	CAAACAGTTAGAAATGTAGAAAAAACTACAACAAGCGCTGAAGTTACAGAATAA	954
Sbjct	901	CAAACAGTTAGAAATGTAGAAAAAACTACAACAAGCGCTGAAGTTACAGAATAA	954

Comments: Single synonymous nucleotide substitution. BI-1 and BI-2 have TTG Leucine codon; BI-3 and BI-4 have TTA leucine codon. There is no amino acid change.

## FIG. 8 (CONT.)

## #6. Conserved hypothetical protein; Query is BI-1, Sbjct is BI-3

Output from Blastp is formatted for dots representing identical amino acid residues. X in BI-3 (Sbjct) is a stop codon

Length=203

Identities = 202/203 (99%), Positives = 202/203 (99%), Gaps = 0/203 (0%)

Query 1	MIADQNSGKRISFPVEGSIIDVQAYKYDGTLYRQWNGVKVLRNTNKHYVLLMYKTRVSEQ	60
Sbjct 1	.....X.....	60
Query 61	NNHNWVYRDYVLWFLPKHSMYNALILLKPSKKQNSYINVASYPIYEDNTIKFIDLDLDI	120
Sbjct 61	.....	120
Query 121	KAYPSNTVSIVDSEEFKENSKIYNYPDKLKQLWEGTQEVMQHYERQGYFFNEELINYI	180
Sbjct 121	.....	180
Query 181	DLGKKDCSIAKKFRASKYKKKNK	203
Sbjct 181	.....	203

2010238689 26 Oct 2016

FIG. 8 (CONT.)

Pairwise alignment from Blastp. X in BI-3 (Sbjct) is a stop codon  
Length=203

Identities = 202/203 (99%), Positives = 202/203 (99%), Gaps = 0/203 (0%)

Query 1 MIADQNSGKRISFPVEGSIIDVQAYKYDGTLYRQWNGVKVLRNTNKHYVLLMYKTRVSEQ 60

MIADQNSGKRISFPV GSIIDVQAYKYDGTLYRQWNGVKVLRNTNKHYVLLMYKTRVSEQ

Sbjct 1 MIADQNSGKRISFPVXGSIIDVQAYKYDGTLYRQWNGVKVLRNTNKHYVLLMYKTRVSEQ 60

Query 61 NNHNWVYRDYVLWFLPKHSMYNALILLKPSKKQNSYINVASYPIYEDNTIKFIDLDI 120

NNHNWVYRDYVLWFLPKHSMYNALILLKPSKKQNSYINVASYPIYEDNTIKFIDLDI

Sbjct 61 NNHNWVYRDYVLWFLPKHSMYNALILLKPSKKQNSYINVASYPIYEDNTIKFIDLDI 120

Query 121 KAYPSNTVSIVDSEEFKENS KIYNYPDKLKQLVWEGTQEVMQHYERQGYFFNEEIIINYYI 180

KAYPSNTVSIVDSEEFKENS KIYNYPDKLKQLVWEGTQEVMQHYERQGYFFNEEIIINYYI

Sbjct 121 KAYPSNTVSIVDSEEFKENS KIYNYPDKLKQLVWEGTQEVMQHYERQGYFFNEEIIINYYI 180

Query 181 DLGKKDCSIAKKFRASKYKKKNK 203

DLGKKDCSIAKKFRASKYKKKNK

Sbjct 181 DLGKKDCSIAKKFRASKYKKKNK 203

Comments: Single non-synonymous nucleotide substitution. BI-1 and BI-2 have GAA Glutamate (E) codon; BI-3 and BI-4 have TAA Stop (X) codon.

## FIG. 8 (CONT.)

## #7. Membrane protein; Query is BI-1, Sbjct is BI-4

Output from Blastp is formatted for dots representing identical amino acid residues.

Length=2707

Identities = 2706/2707 (99%), Positives = 2707/2707 (100%), Gaps = 0/2707 (0%)

Query 1	MWRLSKEVFKSLSK	NKIMVIGLSILIFITS	AVFTLLSSLRSSIVSG	FENYKKLSVKHDLS	60	
Sbjct 1	.....	.....	.....	.....	60	
Query 61	VDLNLPSQGSAYNQ	GYFVNGEVLGQNGV	KEYKPIKYI	LANETGEYRDSVENVLY	120	
Sbjct 61	.....	.....	.....	.....	120	
Query 121	IKLSNFTGIDGN	NSNSNYYIRRDDL	DILYSIYSSNKNNS	IIVEFKLGNDENDANKA	180	
Sbjct 121	.....	.....	.....	.....	180	
Query 181	QADRTFNIYEKKND	KFEIVTDTKSLNS	SEKVNF	EKKDLTLS	DLMILKKGTDSSNP	240
Sbjct 181	.....	.....	.....	.....	.....	240
Query 241	EQVQPLFINVLDK	KITNEY	SVGNNWVAEKK	IIIIPANEWIEKFGF	KKHGNDNFV	300
Sbjct 241	.....	.....	.....	.....	.....	300
Query 301	NKEILSKFLDP	VEPNNS	DLLDEKS	KVKSEWTISDFY	GKSTIEVKPAK	360
Sbjct 301	.....	.....	.....	.....	.....	360
Query 361	KNLIAKKSRNV	SFERWNYHTTY	VGDKKNQWTGAF	KTFVDELEKS	KDDHNSDNYRKWK	420
Sbjct 361	.....	.....	.....	.....	.....	420
Query 421	EFSNWT	KNIKTVFEPYDSS	KFPKEVKISTL	INELDV	KQKLYNSDD	480
Sbjct 421	.....	.....	.....	.....	.....	480

FIG. 8 (CONT.)

Query	481	GSAYKIGKSGAKNIAEIEFIDRKDPNYERKLLDAINDKDLKNKNFNFIFKQEAYEVTKNI	540
Sbjct	481	.....	540
Query	541	IIDKIAKEVGGRKNIGLRKTITVDAIDEKTKQNVHFINTGDENGEVDGIKLNVGKLF	600
Sbjct	541	.....	600
Query	601	EQKNKSALIPTNRFDESVRQNQLPPYIASLLIQTIGKNLFPDPKYVEPIYFAEVTDMN	660
Sbjct	601	.....	660
Query	661	PVTKAIRKENSKIVLLNKYLVNENNRSIDQNTLDSEYKKLNGLITFRGNRYKLVTLVSN	720
Sbjct	661	.....	720
Query	721	NQLFWKTVYSEGIDQLGFDKGLLTKWMEQNKVTLATKFIKTDDEGWVKKDQSLANISYVP	780
Sbjct	721	.....	780
Query	781	TQFLSPKAELINDILATGKVDFLSNAIEKELLDSALVKEEFISQENVFLIANALKVLNN	840
Sbjct	781	.....	840
Query	841	NNFASAFSSKINRELLPKIGLDLLYELSHSDSGNIFKSVLFNIFEKVKAKISEKGSLEN	900
Sbjct	841	.....	900
Query	901	QKKYVIDEVNNIYSLINGLAGIDISKYLSAEDIVNFSKEPKVIDAFQNIITSIDAYKFS	960
Sbjct	901	.....	960
Query	961	QYANDWYKNEWRKQVDHNKDKYTNRLLSSGLLINWLFKSVDQKTLKTGLKILINNLDFEKI	1020
Sbjct	961	.....	1020
Query	1021	VNLDDKNSFLYKKLNSSVPSLIDGINVLLKKISKDGKFDNIKEGLNKILQNIDFNVLSKY	1080
Sbjct	1021	.....	1080

## FIG. 8 (CONT.)

Query	1081	LDDHLETNYFEYKKSTFDYELNKEKVLKEKVALKTIRPKDGMALIYGLFKNP GTNREFK	1140
Sbjct	1081	.....	1140
Query	1141	DNLIKMFNLSSKVNESNVENGTTIITPDSDPKLSFSDFLAFFLALLSADQSKAIFKNQ	1200
Sbjct	1141	.....	1200
Query	1201	QIFNEIDKAKQHII SVLLHKRNSANIFDLDIKVETLKRFNVI SNETIINQQVIDKLTNI	1260
Sbjct	1201	.....	1260
Query	1261	ENFLKQTTTSINEKIKVVDEKNKTLADLIYDFNNFSDG DATWRTWKS LIGAYGQASITNK	1320
Sbjct	1261	.....	1320
Query	1321	FSLGAQAFDLLL PWINMLAFNKEANQKEALKFINDFLKLSIDPDILKDINKLAEDENLPS	1380
Sbjct	1321	.....	1380
Query	1381	SADNKFGLSIALHRPEQVTLFNQNNDKFTNAKVEKLASENPKFRKYLISQKRS LIE LLGL	1440
Sbjct	1381	.....	1440
Query	1441	IGASQQYSKYETK PTEEGK IYAPYGIYYETIKKSV DKYFSTKEFW DIRDIALSITRSMQI	1500
Sbjct	1441	.....	1500
Query	1501	NFPIELFDLSRIIINPVLRSMY PQLMTSFVSTQKKNLGSINGNLAYIVLSRIGNFEEIIR	1560
Sbjct	1501	.....	1560
Query	1561	DESKKSELEAYFEQIWSNNDTSLVPLDYNEEITLSLDGARINKLFNENNKKTTVFGIDFM	1620
Sbjct	1561	.....	1620
Query	1621	NLAGK VINGIVEPKELDIVFNDINSYYAKVNYAYLAKNNKAIYNGTLPKNNVEMESLIN	1680
Sbjct	1621	.....	1680

FIG. 8 (CONT.)

Query	1681	TIDDKYILDVNGIKFLIVGEDTTIDYIYPVIDENHLQVNTQNQALVLYNNYGFSRVVAAY	1740
Sbjct	1681	.....	1740
Query	1741	QGNVIKKNLLVVNGSKNSNEAKRNIINIVDSSISDANKLKRFLYNEELDPINPERALRI	1800
Sbjct	1741	.....	1800
Query	1801	TTIERMIGVISSSIALMTLFIIMVSVAIIFIIRRYIANKAKVFGILLAQGYKPIEIAIS	1860
Sbjct	1801	.....	1860
Query	1861	LLSFAAVTSLIGGILGYSIGFRTQILLQNVFSNYWLPKSAIPFDFFALFFNVFIPFIGM	1920
Sbjct	1861	.....	1920
Query	1921	SLLIIVVALISLRKSSIDLITGVDEAPKGKLFTFMKKKFINKKNVKRFRFTLAYSGFWK	1980
Sbjct	1921	.....	1980
Query	1981	LASFEGGSVLLTSIATMFLANFKSFNKTINDTYKNRDYKFVDSLPTVEGGDYSYNPK	2040
Sbjct	1981	.....	2040
Query	2041	ELNNLIYTPIGSLNEGNRETADYFKPGKSSIINPNNDNGMPSDKSPHILSQFSVNVTVD	2100
Sbjct	2041	.....	2100
Query	2101	AGVSADPWLIAYNGMPDSQKAKIDKIRDVLGHQLEWTQSLDDNGELITDPNKPIIKVDSN	2160
Sbjct	2101	.....	2160
Query	2161	GLMSYEDGTGKYDFFKYYKSPNDKQGSFRLAHDEVNKEYVMKIIKTGNSGRNEYRDF	2220
Sbjct	2161	.....	2220
Query	2221	LVRAYKNDVIRKQHEKMIASGKSITNPISNWTKSNNSSDFWLIDKSDLNRQWVNDYFIG	2280
Sbjct	2221	.....	2280

## FIG. 8 (CONT.)

Query	2281	FGGVLFDSYDETYTYLSGTNNVSAKIYGYRKPVDFKNAVKLIDKAGNNLYEVLDKYE	2340
Sbjct	2281	.....	2340
Query	2341	VKNNVYPLVVNDVFAKKHKLGINDLIDFKVWNDRVDRYKQKIIIEKIYANDPVKQADLKNEY	2400
Sbjct	2341	.....	2400
Query	2401	NKKTNAKFQIVGINPTYINDELITTHKAANLLIGMTIDNGFNGVLTQNANPVQVTESAG	2460
Sbjct	2401	.....	2460
Query	2461	LYSVSGYWAGLDGFDVSSLNGTVEKMFDEIFGSPDKKGVLETQHGLTKNEIAKFLDSSA	2520
Sbjct	2461	.....	2520
Query	2521	NEFSKSLYESAKSSAKLHIDEFSKIYNNKLYIALSSSIDSKDIEVGFLQVGSTIEQISI	2580
Sbjct	2521	.K.....	2580
Query	2581	FIIVINFVISLILIMSSIIVSENERNIAIWSILGYSQKEKLMFFGAFIPFLVSAIVI	2640
Sbjct	2581	.....	2640
Query	2641	SIPIVIALISVFGFLSSSIALLSLKWWHVLITSGLMLTIFAITSISVWITINKMKP	2700
Sbjct	2641	.....	2700
Query	2701	VDLLKGK	2707
Sbjct	2701	.....	2707

## FIG. 8 (CONT.)

Pairwise alignment from Blastp.

Length=2707

Identities = 2706/2707 (99%), Positives = 2707/2707 (100%), Gaps = 0/2707 (0%)

Query 1	MWRLSKEVFKSLSKNKMIVGLSILIFITSAVFTLLSSLRSSIVSGFENYKKLSVKHDLS	60
	MWRLSKEVFKSLSKNKMIVGLSILIFITSAVFTLLSSLRSSIVSGFENYKKLSVKHDLS	
Sbjct 1	MWRLSKEVFKSLSKNKMIVGLSILIFITSAVFTLLSSLRSSIVSGFENYKKLSVKHDLS	60
Query 61	VDLNLPSQGSAYNQGYFVNGEVLGQNGVKEYKPIKYLANETGEYRDSVENVLYLQNTEF	120
	VDLNLPSQGSAYNQGYFVNGEVLGQNGVKEYKPIKYLANETGEYRDSVENVLYLQNTEF	
Sbjct 61	VDLNLPSQGSAYNQGYFVNGEVLGQNGVKEYKPIKYLANETGEYRDSVENVLYLQNTEF	120
Query 121	IKLSNFTGIDGNNSNSNYYIRRDDLILYSIYSSNKNNSIVEFKLGNDENDANKASFKLK	180
	IKLSNFTGIDGNNSNSNYYIRRDDLILYSIYSSNKNNSIVEFKLGNDENDANKASFKLK	
Sbjct 121	IKLSNFTGIDGNNSNSNYYIRRDDLILYSIYSSNKNNSIVEFKLGNDENDANKASFKLK	180
Query 181	QADRTFNIYEKKNDKFEIVTDTKSLNSSEKVNFEKKDLTLSMLKKGTDSSNPNIVYA	240
	QADRTFNIYEKKNDKFEIVTDTKSLNSSEKVNFEKKDLTLSMLKKGTDSSNPNIVYA	
Sbjct 181	QADRTFNIYEKKNDKFEIVTDTKSLNSSEKVNFEKKDLTLSMLKKGTDSSNPNIVYA	240
Query 241	EQVQPLFINVLDKKITNEYSGNNWVAEKKGIIIPANEWIEKFGFKKHGNDVFVINEGN	300
	EQVQPLFINVLDKKITNEYSGNNWVAEKKGIIIPANEWIEKFGFKKHGNDVFVINEGN	
Sbjct 241	EQVQPLFINVLDKKITNEYSGNNWVAEKKGIIIPANEWIEKFGFKKHGNDVFVINEGN	300
Query 301	NKEILSKFLDPVEPNNSDLLDEKSKVKSEWTISDFYKGSTIEVKPAKTITIKKDTEITIN	360
	NKEILSKFLDPVEPNNSDLLDEKSKVKSEWTISDFYKGSTIEVKPAKTITIKKDTEITIN	
Sbjct 301	NKEILSKFLDPVEPNNSDLLDEKSKVKSEWTISDFYKGSTIEVKPAKTITIKKDTEITIN	360

FIG. 8 (CONT.)

		KNLIAKKSRNVSFERWNYHTTYVGDKKNQWTGAFKTFVDELEKSDDHNSDNYRKWKNLK	420
		KNLIAKKSRNVSFERWNYHTTYVGDKKNQWTGAFKTFVDELEKSDDHNSDNYRKWKNLK	
		KNLIAKKSRNVSFERWNYHTTYVGDKKNQWTGAFKTFVDELEKSDDHNSDNYRKWKNLK	420
		EFSNWTKNIKTVFEPYDSSKFPKKEVKISTLINEVDVKQKLYNSDDTNNNISERSNSNFPG	480
		EFSNWTKNIKTVFEPYDSSKFPKKEVKISTLINEVDVKQKLYNSDDTNNNISERSNSNFPG	
		EFSNWTKNIKTVFEPYDSSKFPKKEVKISTLINEVDVKQKLYNSDDTNNNISERSNSNFPG	480
		GSAYKIGKSGAKNIAEIEFIDRKDPNYERKLLDAINDKDLKNKNFNFIKQEAYEVTKNI	540
		GSAYKIGKSGAKNIAEIEFIDRKDPNYERKLLDAINDKDLKNKNFNFIKQEAYEVTKNI	
		GSAYKIGKSGAKNIAEIEFIDRKDPNYERKLLDAINDKDLKNKNFNFIKQEAYEVTKNI	540
		IIDKIAKEVGGRKNIGLRKTITVDAIDEKTKQNVHFINTGDENGEVDGIKLNVGKLFG	600
		IIDKIAKEVGGRKNIGLRKTITVDAIDEKTKQNVHFINTGDENGEVDGIKLNVGKLFG	
		IIDKIAKEVGGRKNIGLRKTITVDAIDEKTKQNVHFINTGDENGEVDGIKLNVGKLFG	600
		EQKNKSALIPTNRFDESVRQNQLPPYIASLLIQTIGKNLFPDPKYVEPIYEFAEVTDMN	660
		EQKNKSALIPTNRFDESVRQNQLPPYIASLLIQTIGKNLFPDPKYVEPIYEFAEVTDMN	
		EQKNKSALIPTNRFDESVRQNQLPPYIASLLIQTIGKNLFPDPKYVEPIYEFAEVTDMN	660
		PVTKAIRKENSIVLLNKLYLVNENNRSIDQNTLDSEYKKLNGLITFRGNRYKLVTLVNSN	720
		PVTKAIRKENSIVLLNKLYLVNENNRSIDQNTLDSEYKKLNGLITFRGNRYKLVTLVNSN	
		PVTKAIRKENSIVLLNKLYLVNENNRSIDQNTLDSEYKKLNGLITFRGNRYKLVTLVNSN	720
		NQLFWKTVYSEGIDQLGFDKGLLTKWMEQNVTLATKFIKTDDEGVVKKDQSLANISYVP	780
		NQLFWKTVYSEGIDQLGFDKGLLTKWMEQNVTLATKFIKTDDEGVVKKDQSLANISYVP	
		NQLFWKTVYSEGIDQLGFDKGLLTKWMEQNVTLATKFIKTDDEGVVKKDQSLANISYVP	780
		TQFLSPKAELINDILATGKVDLNSNAIEKELLDSDLVKEEFISQENVFLIANALKVLNN	840
		TQFLSPKAELINDILATGKVDLNSNAIEKELLDSDLVKEEFISQENVFLIANALKVLNN	
		TQFLSPKAELINDILATGKVDLNSNAIEKELLDSDLVKEEFISQENVFLIANALKVLNN	840

FIG. 8 (CONT.)

Query	841	NNFASAFTSSKINRELLPKIGLDLLYELSHSDSGNIFKSVLFNIFEKVKAKISEKGSLEN	900
		NNFASAFTSSKINRELLPKIGLDLLYELSHSDSGNIFKSVLFNIFEKVKAKISEKGSLEN	
Sbjct	841	NNFASAFTSSKINRELLPKIGLDLLYELSHSDSGNIFKSVLFNIFEKVKAKISEKGSLEN	900
Query	901	QKKYVIDEVNNIYSLINGLAGIDISKYLSAEDIVNFSKEPKVIDAFQNIITSIDAYKFS	960
		QKKYVIDEVNNIYSLINGLAGIDISKYLSAEDIVNFSKEPKVIDAFQNIITSIDAYKFS	
Sbjct	901	QKKYVIDEVNNIYSLINGLAGIDISKYLSAEDIVNFSKEPKVIDAFQNIITSIDAYKFS	960
Query	961	QYANDWYKNEWRKQVDHNKDKYTNRLLSSGLLINWLFKSDQKTLKTGLKILINNLDFEKI	1020
		QYANDWYKNEWRKQVDHNKDKYTNRLLSSGLLINWLFKSDQKTLKTGLKILINNLDFEKI	
Sbjct	961	QYANDWYKNEWRKQVDHNKDKYTNRLLSSGLLINWLFKSDQKTLKTGLKILINNLDFEKI	1020
Query	1021	VNLDDKNSFLYKKLNSSVPSLIDGINVLLKKISKDGKFDNIKEGLNKILQNIDFNVLSKY	1080
		VNLDDKNSFLYKKLNSSVPSLIDGINVLLKKISKDGKFDNIKEGLNKILQNIDFNVLSKY	
Sbjct	1021	VNLDDKNSFLYKKLNSSVPSLIDGINVLLKKISKDGKFDNIKEGLNKILQNIDFNVLSKY	1080
Query	1081	LDDHLETNYFEYKKSTFDYELNKEKVLKEKVALKTIRPKDGMMALIYGLFKNP GTNREFK	1140
		LDDHLETNYFEYKKSTFDYELNKEKVLKEKVALKTIRPKDGMMALIYGLFKNP GTNREFK	
Sbjct	1081	LDDHLETNYFEYKKSTFDYELNKEKVLKEKVALKTIRPKDGMMALIYGLFKNP GTNREFK	1140
Query	1141	DNLIKMFNLSSKVNESNVENGTTIITPDSDPDKLSFSDFLAFFLALLSADQSKAIFKNQ	1200
		DNLIKMFNLSSKVNESNVENGTTIITPDSDPDKLSFSDFLAFFLALLSADQSKAIFKNQ	
Sbjct	1141	DNLIKMFNLSSKVNESNVENGTTIITPDSDPDKLSFSDFLAFFLALLSADQSKAIFKNQ	1200
Query	1201	QIFNEIDKAKQHIISVLLHKRNSANIFLDLIDKVVETLKRNFNVISNETIINQQVIDKLTNI	1260
		QIFNEIDKAKQHIISVLLHKRNSANIFLDLIDKVVETLKRNFNVISNETIINQQVIDKLTNI	
Sbjct	1201	QIFNEIDKAKQHIISVLLHKRNSANIFLDLIDKVVETLKRNFNVISNETIINQQVIDKLTNI	1260
Query	1261	ENFLKQTTTSINEKIKVVDEKNKTLADLIYDFNNFSDG DATWRTWKS LIGAYGQASITNK	1320
		ENFLKQTTTSINEKIKVVDEKNKTLADLIYDFNNFSDG DATWRTWKS LIGAYGQASITNK	
Sbjct	1261	ENFLKQTTTSINEKIKVVDEKNKTLADLIYDFNNFSDG DATWRTWKS LIGAYGQASITNK	1320

FIG. 8 (CONT.)

Query	1321	FSLGAQAFDLLLWINMLAFNKEANQKEALKFINDFLKLSIDPDILKDINKLAEDENLPS	1380
		FSLGAQAFDLLLWINMLAFNKEANQKEALKFINDFLKLSIDPDILKDINKLAEDENLPS	
Sbjct	1321	FSLGAQAFDLLLWINMLAFNKEANQKEALKFINDFLKLSIDPDILKDINKLAEDENLPS	1380
Query	1381	SADNKFGLSIALHRPEQVTLFNQNNDKFTNAKVEKLASENPKFRKYLISQKRSLIELLGL	1440
		SADNKFGLSIALHRPEQVTLFNQNNDKFTNAKVEKLASENPKFRKYLISQKRSLIELLGL	
Sbjct	1381	SADNKFGLSIALHRPEQVTLFNQNNDKFTNAKVEKLASENPKFRKYLISQKRSLIELLGL	1440
Query	1441	IGASQQYSKYETKPTEEGKIVAPYGIYYETIKKSVDKYFSTKEFDIRDIALSITRSMQI	1500
		IGASQQYSKYETKPTEEGKIVAPYGIYYETIKKSVDKYFSTKEFDIRDIALSITRSMQI	
Sbjct	1441	IGASQQYSKYETKPTEEGKIVAPYGIYYETIKKSVDKYFSTKEFDIRDIALSITRSMQI	1500
Query	1501	NFPIELFDLSRIIINPVLRSMYPOQMTSFVSTQKKNLGSINGNLAYIVLSRIGNFEEIIR	1560
		NFPIELFDLSRIIINPVLRSMYPOQMTSFVSTQKKNLGSINGNLAYIVLSRIGNFEEIIR	
Sbjct	1501	NFPIELFDLSRIIINPVLRSMYPOQMTSFVSTQKKNLGSINGNLAYIVLSRIGNFEEIIR	1560
Query	1561	DESKKSELEAYFEQIWSNNDSLVPDYNEEITSLDGARINKLFNENNKKTTVFGIDFM	1620
		DESKKSELEAYFEQIWSNNDSLVPDYNEEITSLDGARINKLFNENNKKTTVFGIDFM	
Sbjct	1561	DESKKSELEAYFEQIWSNNDSLVPDYNEEITSLDGARINKLFNENNKKTTVFGIDFM	1620
Query	1621	NLAGKVINGIVEPKELDIVFNDINSYYAKVNYAYLAKNNKAIYNGTLPKNNVEMESLIN	1680
		NLAGKVINGIVEPKELDIVFNDINSYYAKVNYAYLAKNNKAIYNGTLPKNNVEMESLIN	
Sbjct	1621	NLAGKVINGIVEPKELDIVFNDINSYYAKVNYAYLAKNNKAIYNGTLPKNNVEMESLIN	1680
Query	1681	TIDDKYILDVNGIKFLIVGEDTTIDYIYPVIDENHLQVNTQALVYLNNGFSRVAAY	1740
		TIDDKYILDVNGIKFLIVGEDTTIDYIYPVIDENHLQVNTQALVYLNNGFSRVAAY	
Sbjct	1681	TIDDKYILDVNGIKFLIVGEDTTIDYIYPVIDENHLQVNTQALVYLNNGFSRVAAY	1740
Query	1741	QGNVIKKNLLVVNGSKNSNEAKRNIINIVDSSISDANKLKRFLYNELDPINPERALRI	1800
		QGNVIKKNLLVVNGSKNSNEAKRNIINIVDSSISDANKLKRFLYNELDPINPERALRI	
Sbjct	1741	QGNVIKKNLLVVNGSKNSNEAKRNIINIVDSSISDANKLKRFLYNELDPINPERALRI	1800

FIG. 8 (CONT.)

Query	1801	TTIERMIGVISSSIIALMTLFIIMVSVAIIFIIRRYIANKAKVFGILLAQGYKPIEIAIS	1860
		TTIERMIGVISSSIIALMTLFIIMVSVAIIFIIRRYIANKAKVFGILLAQGYKPIEIAIS	
Sbjct	1801	TTIERMIGVISSSIIALMTLFIIMVSVAIIFIIRRYIANKAKVFGILLAQGYKPIEIAIS	1860
Query	1861	LLSFAAVTSLIGGILGYSIGFRTOILLQNVFSNYWLPKSAIPFDFFALFFNVFIPFIGM	1920
		LLSFAAVTSLIGGILGYSIGFRTOILLQNVFSNYWLPKSAIPFDFFALFFNVFIPFIGM	
Sbjct	1861	LLSFAAVTSLIGGILGYSIGFRTOILLQNVFSNYWLPKSAIPFDFFALFFNVFIPFIGM	1920
Query	1921	SLLIIWVALISLRKSSIDLITGVDEAPKGKLFTFMKKKFINKKNVKRFRFTLAYSGFWK	1980
		SLLIIWVALISLRKSSIDLITGVDEAPKGKLFTFMKKKFINKKNVKRFRFTLAYSGFWK	
Sbjct	1921	SLLIIWVALISLRKSSIDLITGVDEAPKGKLFTFMKKKFINKKNVKRFRFTLAYSGFWK	1980
Query	1981	LASFGGSVLLTSIATMGLANFKSFNKTINDTYKNRDYKFVDSLQFVNL	2040
		LASFGGSVLLTSIATMGLANFKSFNKTINDTYKNRDYKFVDSLQFVNL	
Sbjct	1981	LASFGGSVLLTSIATMGLANFKSFNKTINDTYKNRDYKFVDSLQFVNL	2040
Query	2041	ELNNLIYTPIGSLNEGNRETADYFKPGKSSIINPNNDNGMPSDKSPHILSQFSVNTVD	2100
		ELNNLIYTPIGSLNEGNRETADYFKPGKSSIINPNNDNGMPSDKSPHILSQFSVNTVD	
Sbjct	2041	ELNNLIYTPIGSLNEGNRETADYFKPGKSSIINPNNDNGMPSDKSPHILSQFSVNTVD	2100
Query	2101	AGVSADPWLIAYNGMPDSQKAKIDKIRDLVGHQLEWTQSLDDNGELITDPNKPIIKVDSN	2160
		AGVSADPWLIAYNGMPDSQKAKIDKIRDLVGHQLEWTQSLDDNGELITDPNKPIIKVDSN	
Sbjct	2101	AGVSADPWLIAYNGMPDSQKAKIDKIRDLVGHQLEWTQSLDDNGELITDPNKPIIKVDSN	2160
Query	2161	GLMSYEDGTGKKYDFFKYYKSPNDQGSFRLAHWDEVNKEYVMKIIKTGNSGRNEYRDF	2220
		GLMSYEDGTGKKYDFFKYYKSPNDQGSFRLAHWDEVNKEYVMKIIKTGNSGRNEYRDF	
Sbjct	2161	GLMSYEDGTGKKYDFFKYYKSPNDQGSFRLAHWDEVNKEYVMKIIKTGNSGRNEYRDF	2220

FIG. 8 (CONT.)

Query	2221	LVRAYKNDVIRKQHEKMIASGKSITNPISNWTSSNNSSDFWLIDKSDLNRQWVNDYFIG	2280
		LVRAYKNDVIRKQHEKMIASGKSITNPISNWTSSNNSSDFWLIDKSDLNRQWVNDYFIG	
Sbjct	2221	LVRAYKNDVIRKQHEKMIASGKSITNPISNWTSSNNSSDFWLIDKSDLNRQWVNDYFIG	2280
Query	2281	FGGVLFDSYDETYTYLSGTNNVSAKIYGYRKPVDFKNAVKLIDKAGNNLYEVLDKYE	2340
		FGGVLFDSYDETYTYLSGTNNVSAKIYGYRKPVDFKNAVKLIDKAGNNLYEVLDKYE	
Sbjct	2281	FGGVLFDSYDETYTYLSGTNNVSAKIYGYRKPVDFKNAVKLIDKAGNNLYEVLDKYE	2340
Query	2341	VKNNVYPLVVNDVFAKKHKLGINDLIDFKVWNRVDYKQKIIIEKIYANDPVKQADLKNEY	2400
		VKNNVYPLVVNDVFAKKHKLGINDLIDFKVWNRVDYKQKIIIEKIYANDPVKQADLKNEY	
Sbjct	2341	VKNNVYPLVVNDVFAKKHKLGINDLIDFKVWNRVDYKQKIIIEKIYANDPVKQADLKNEY	2400
Query	2401	NKKTKQFQIVGINPTYINDELITTHKAANLLIGMTIDNGFNGVLTQANPQVQTESAG	2460
		NKKTKQFQIVGINPTYINDELITTHKAANLLIGMTIDNGFNGVLTQANPQVQTESAG	
Sbjct	2401	NKKTKQFQIVGINPTYINDELITTHKAANLLIGMTIDNGFNGVLTQANPQVQTESAG	2460
Query	2461	LYSVSGYWAGLDGFVSSLDNGTVEKMFDEIFGSPDKKGVLETQHGLTKNEIAKFLDSSA	2520
		LYSVSGYWAGLDGFVSSLDNGTVEKMFDEIFGSPDKKGVLETQHGLTKNEIAKFLDSSA	
Sbjct	2461	LYSVSGYWAGLDGFVSSLDNGTVEKMFDEIFGSPDKKGVLETQHGLTKNEIAKFLDSSA	2520
Query	2521	NEFSKSLYESAKSSAKLHIDEFSKIKYNNKLYIALSSSIDSKDIEVGFLQVGSTIEQISI	2580
		N+FSKSLYESAKSSAKLHIDEFSKIKYNNKLYIALSSSIDSKDIEVGFLQVGSTIEQISI	
Sbjct	2521	NKFSKSLYESAKSSAKLHIDEFSKIKYNNKLYIALSSSIDSKDIEVGFLQVGSTIEQISI	2580
Query	2581	FIIVINFVISLILIMSSIIVSENERNIAIWSILGYSQKEKLMFFGAFIPFLVSAIVI	2640
		FIIVINFVISLILIMSSIIVSENERNIAIWSILGYSQKEKLMFFGAFIPFLVSAIVI	
Sbjct	2581	FIIVINFVISLILIMSSIIVSENERNIAIWSILGYSQKEKLMFFGAFIPFLVSAIVI	2640

FIG. 8 (CONT.)

Query 2641 SIPIVIALISVFGFLLSSSSIALLSSLKWWHLITSGMLTIFAITSISVWITINKMKP 2700

SIPIVIALISVFGFLLSSSSIALLSSLKWWHLITSGMLTIFAITSISVWITINKMKP

Sbjct 2641 SIPIVIALISVFGFLLSSSSIALLSSLKWWHLITSGMLTIFAITSISVWITINKMKP 2700

Query 2701 VDLLKGK 2707

VDLLKGK

Sbjct 2701 VDLLKGK 2707

Comments: Single non-synonymous nucleotide substitution. BI-1 and BI-2 have GAA Glutamate (E) codon; BI-3 and BI-4 have AAA lysine (K) codon.

In addition to the above difference between pairs of BI isolates, there are two short polyT tracts which are a different length in BI-2 to those present in BI-1, BI-3 and BI-4 (which are identical in these tracts). There are two additional sequence differences called in the BI-3 sequence, that are not found in BI-1, BI-2 or BI-4.

P013001Seq. TXT  
SEQUENCE LISTING

<110> Boehringer Ingelheim Vetmedica, Inc.  
Beck, Michael  
Kittel, Jeff

<120> IMPROVED MODIFIED LIVE VACCINE OF MYCOPLASMA BOVIS

<130> 40106

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<170> PatentIn version 3.5

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P013001Seq. TXT

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