COMBINATION VACCINE COMPRISING AN ATTENUATED BOVINE VIRAL DIHARRHEA VIRUS

BACKGROUND OF THE INVENTION

TECHNICAL FIELD

The present invention relates to the field of animal health and in particular to combinantion vaccines which comprise an attenuated bovine viral diarrhea virus (BVDV) and at least one further immunological active component for treating or preventing diseases or disorders in cattle caused by infectious agents.

BACKGROUND INFORMATION

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 Plagemann, 1992; Thiel et al., 1996). The division of BVDV into 2 species is based on significant differences at the level of genomic sequences (summarized in Heinz et al., 2000) which are also obvious from limited cross neutralizing antibody reactions (Ridpath et al. 1994). The viral proteins of BVDV, and any other virus of the pestivirus family, are arranged in the polyprotein in the order NH_2-N^{pro}-C-E^{rns}-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH (Lindenbach and Rice, 2001). Protein C (= core- or capsidprotein) and the glycoproteins E^{rns}, E1 and E2 represent structural components of the BVDV. E^{rns} and E2 were found to be targets for antibody neutralization (Donis et al., 1988; Paton et al., 1992; van Rijn et al., 1993; Weiland et al., 1990,1992). E^{rns} lacks a typical membrane anchor and is secreted in considerable amounts from the infected cells; this protein has been reported to exhibit RNase activity (Hulst et al., 1994; Schneider et al., 1993; Windisch et al., 1996). The function of this enzymatic activity for the viral life cycle is presently unknown. The enzymatic activity depends on the presence of two stretches of amino acids conserved between the pestivirus E^{rns} and different known RNases of plant and fungal origin. Both of these conserved sequences contain a histidine residue (Schneider et al., 1993). Exchange of each of these residues against lysine in the E^{rns} protein of a Classical Swine Fever Virus (CSFV) vaccine strain resulted in the destruction of RNase activity (Hulst et al., 1998). Introduction of these mutations into the genome of the CSFV vaccine strain did not influence viral viability or growth properties but led to a virus exhibiting a cytopathogenic phenotype (Hulst et al., 1998). Similarly, Meyers et al. showed that an RNase negative variant of the virulent CSFV strain Alfort/Tübingen was fully viable. However, the respective virus mutant showed no cytopathogenic phenotype (Meyers et al., 1999).

N^{pro} represents the first protein encoded by the long open reading frame in the pestivirus RNA. N^{pro} represents a nonstructural protein that has protease activity and cleaves itself of the nascent polyprotein (Stark et al., 1993; Wiskerchen et al., 1991) presumably already during translation. N^{pro} is a cysteine protease (Rümenapf et al., 1998) that is not essential for virus replication (Tratschin et al., 1998). Recently, it was shown that N^{pro} somehow interferes with the cellular antiviral defense so that it can be hypothesized to modulate the immune system within an infected host (Rüggli et al., 2003). Mayer and coworkers presented indications for an attenuation of CSFV in consequence of a deletion of the N^{pro} gene (Mayer et al., 2004).

Present BVDV vaccines for the prevention and treatment of BVDV infections still have drawbacks (Oirschot et al. 1999). Vaccines against the classical BVDV-1 provide only partial protection from BVDV-2 infection, and vaccinated dams may produce calves that are persistently infected with virulent BVDV-2 (Bolin et al., 1991, Ridpath et al., 1994). This problem is probably due to the great antigenic diversity between type 1 and type 2 strains which is most pronounced in the glycoprotein E2, the major antigen for virus neutralization (Tijssen et al., 1996). Most monoclonal antibodies against type 1 strains fail to bind to type 2 viruses (Ridpath et al., 1994).

Currently, licensed BVDV MLV vaccines are produced using attenuated viruses obtained via repeated passage in bovine or porcine cells (Coggins et al.,Cornell Vet. 51: 539- , 1961; Philips et al., Am. J. Vet. Res. 36: 135-, 1975), or using chemically modified viruses which exhibit a temperature-sensitive phenotype (Lobmann et al., Am. J. Vet. Res. 45: 2498-, 1984; 47: 557-561,1986). A single dose of MLV vaccine is sufficient for immunization, and duration of the immunity can last for years in vaccinated cattle. However, as these vaccines have been developed using type 1 BVDV virus strains, the protection is against type 1 virus only. Moreover, these vaccines, although attenuated, are most often associated with safety problems. The vaccine viruses may cross the placenta of pregnant animals, e.g. cows and lead to clinical manifestations in the fetus and/or the induction of persistently infected calves. Therefore, they cannot be applied to breeding herds that contain pregnant cows. Pregnant cows have to be kept separate from vaccinated cattle to protect fetuses and must not be vaccinated themselves.

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 usually associated with mild to subclinical infections. The most important role of PI-3 is to serve as an

initiator that can 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 signs worsen with the onset of bacterial pneumonia. Fatalities from uncomplicated PI-3 pneumonia are rare. Lesions include cranioventral lung consolidation, bronchiolitis, and alveolitis with marked congestion and hemorrhage. 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 can also be infected by respiratory syncytial viruses. This virus was named for its characteristic cytopathic effect—the formation of syncytial cells. Antigenic subtypes are known to exist for BRSV, and preliminary evidence suggests that there may be antigenic subtypes of BRSV. 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 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 can be 0-20%. Signs include increased rectal temperature 40-42°C, depression, 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 of interstitial pneumonia. *See also* atypical 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: 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 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 can occur in bulls (infectious pustular balanoposthitis) and cows (IPV) within 1-3 days of mating or close contact with an infected animal. Transmission can 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 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 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 can also occur up to 100 days after infection. Abortions can 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 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 infection can 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 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 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 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 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 can 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 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 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.

Other viruses, including Breda virus, a calici-like virus, ***Adenovirus***, ***Astrovirus*** and ***Parvovirus***,

have been demonstrated in the feces of calves with diarrhea and can produce diarrhea in calves experimentally. However, these agents can 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. ***Manheimia haemolytica*** (formerly *Pasteurella haemolytica*) biotype A, 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 pyogenes*** is frequently isolated. Under normal conditions, *M. haemolytica* remains confined to the upper respiratory tract, in 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 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; 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 pneumonia or fibrinonecrotic pneumonia. The pneumonia has a bronchopneumonic pattern. Grossly, there is extensive reddish black to grayish 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. 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 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 that may be followed by septicemia and infection of multiple organs. Occasionally, *H. somnus* is associated with extensive pleuritis. *H. somnus* can cause an acute, usually fatal, septicemic disease that can 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, 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 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 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 necrosis occur. Suppurative vaginitis, cervicitis, and endometritis have been documented in cows infected experimentally and naturally after 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 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. 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 opisthotonos, followed by coma and death

within several hours. Affected animals may be blind, and retinal hemorrhages with gray foci of retinal necrosis are sometimes seen. Signs such 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 total cell count of the CSF is markedly increased, and neutrophils predominate. During septicemia, the organism can 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 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.

The exact role of mycoplasmas and ureaplasmas in BRD requires better definition. Mycoplasmas can be recovered from the respiratory tract of nonpneumonic 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***, ***Mycoplasma bovis***, and ***Ureaplasma*** spp. Experimental infections usually result in inapparent 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 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 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 as well as on dairy 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. The acute pulmonary lesion is a bronchointerstitial pneumonia. The anteroventral parts of the lungs are affected but, in severe cases, entire lobes can 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 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* can also be a significant cause of the classic infertility syndrome usually attributed to *Campylobacter fetus venerealis*. There are several 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. Individual bulls vary in their susceptibility to infection because some become permanent carriers, while others appear to be resistant to infection. Bulls can 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 can carry *C. fetus* for ≥ 2 yr. IgA antibodies are shed in cervical mucus in significant amounts in ~50% of cows for 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 is a variable degree of mucopurulent endometritis that causes early embryonic death, prolonged luteal phases, irregular estrous cycles, repeat breeding and, as a result, protracted calving periods. 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.

Leptospirosis is a contagious disease of animals, including man, caused by various immunologically distinct leptospiral serovars, most of which are regarded as subgroups of ***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 USA, the disease is primarily due to the serovars ***Leptospira hardjo***, ***Leptospira pomona***, and ***Leptospira grippotyphosa***. However, ***Leptospira canicola*** and ***Leptospira icterohaemorrhagiae*** serovars also have been isolated. Calves may have fever, 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 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, 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 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, 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 mos. 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. 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 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.

Actinomyces (Corynebacterium) pyogenes causes sporadic abortion in the last trimester. Rarely, the incidence in a herd may reach enzootic (64%) levels. The bacteria are present on mucous membranes of many normal cows, as well as in uterine and abscess discharges. They gain entry to the bloodstream and cause an endometritis and placentitis, which is diffuse with a reddish brown to brown color. The fetus is usually autolyzed, with fibrinous pericarditis, pleuritis, or peritonitis possible.

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 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.

Clostridium haemolyticum is a soil-borne organism that may be found naturally in the GI tract of cattle. It can 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 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 β 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 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 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.

Clostridium chauvoei occurs naturally in the intestinal tract of animals. It probably can 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, and after gaining access to the bloodstream, are 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 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 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 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 α toxin) damage hepatic parenchyma, thereby permitting the bacteria to multiply and produce a lethal amount of toxin. Usually, death 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 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 grayish 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 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-debilitant. Wounds caused by accident, castration, docking, insanitary 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 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 susceptible to malignant edema but not to blackleg.

Infectious disease caused by ***Clostridium sordellii*** are 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 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 β 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 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 opisthotonos. 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. 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 can 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 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.

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 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, that provide a suitable anaerobic environment.

Infection with *Salmonella spp* can produce diarrhea in animals of all ages, especially 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. Conic enteritis may develop occasionally in cattle. Pregnant animals may abort. In older animals, the disease is manifested by dysentery and toxemia, and mortality can

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 can contaminate feed and water, 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 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 are ***Cryptosporidium parvum*** and ***Mycobacterium avium paratuberculosis***. Paratuberculosis is a chronic, contagious enteritis characterized by 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 can be infected experimentally. Distribution is worldwide. There are conflicting data on the involvement of the organism in Crohn's disease, a chronic enteritis in people. Animals with paratuberculosis should be considered as potential zoonotic risks until the 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 can 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. Infection is acquired early in life, but clinical signs rarely develop in cattle < 2 yrs 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 can also be present in colostrum and milk of infected cows, and intrauterine infections have also been described. After 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 a

rise in ineffective serum antibody. However, fecal shedding begins before clinical signs are apparent. *Mycobacterium avium paratuberculosis* can 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 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. 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 can be severe in immunocompromised individuals. If severe disease in calves is seen, other disease agents or concurrent infections should be ruled out. Although *C. parvum* can 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 intramembranous and resides within the brush border of the intestinal epithelial cells.

Chlamydia psittaci causes sporadic abortion after the fourth month of gestation but usually in the last trimester. The chlamydia cause placentitis, fetal pneumonia, and hepatitis. Stained smears of cotyledons may reveal the organisms; if not, tissues may be cultured in embryonating chicken eggs. Abortion is usually sporadic in cows, but an ovine chlamydial vaccine has been used in cattle.

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, Klebsiella spp. Pseudomonas aeruginosa, 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. 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 can 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.***

An infection of the lower respiratory tract, usually resulting in bronchitis or pneumonia, can 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 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 coughing and respiratory distress and even failure. Reduced weight-gains, reduced milk yields, and weight loss accompany many infections in cattle. Patent subclinical infections can 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 worldwide. The causative protozoan, ***Trichomonas (Tritrichomonas) foetus***, is pyriform and ordinarily 10-15 × 5-10 µm, 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. Although *T foetus* can 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 can remain 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 can 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 can also occur but are not as common as losses earlier in gestation. *Trichomonas foetus* has been found in vaginal cultures taken as late as 8 months of gestation and, apparently, live calves can 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 can be transmitted transplacentally in dogs, cattle, goats, sheep, and cats, and subsequent offspring may be affected. Tachyzoites are 5-7 × 1-5 µm, 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 µm in diameter are found in neural cells; the cyst wall is amorphous and up to 4 µm thick. Cysts have no septa and enclose slender 7 × 1.5 µm bradyzoites. In dairy cattle, *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 can 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 can 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 can 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 nonspecific 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.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 Serum neutralisation against NY93/C (BVDV type II)

Fig. 2 Serum neutralisation assay against KE9 (BVDV type I)

Fig. 3 Serum neutralisation assay against NY93/C (BVDV type II)

All subsequent sequences show the deleted regions indicated with dashes (-), which are also numbered, whereas the sequences in the sequence listing attached hereto are continuously numbered without the deleted regions or amino acid codons.

SEQ ID NO:1	XIKE-A-cDNA sequence
SEQ ID NO:2	XIKE-A-NdN-cDNA sequence
SEQ ID NO:3	XIKE-B-cDNA sequence
SEQ ID NO:4	XIKE-B-NdN-cDNA
SEQ ID NO:5	XIKE-A amino acid sequence
SEQ ID NO:6	XIKE-A-NdN amino acid sequence
SEQ ID NO:7	XIKE-B amino acid sequence

SEQ ID NO:8	XIKE-B-NdN amino acid sequence
SEQ ID NO:9	XIKE-C-NdN amino acid sequence
SEQ ID NO:10	XIKE-C-NdN-cDNA sequence
SEQ ID NO:11	XIKE-C-cDNA sequence
SEQ ID NO:12	XIKE-C amino acid sequence

SUMMARY

The present disclosure relates to combination vaccines for the treatment and/or prophylaxis of cattle against microbiological infections, wherein one of the infections is caused by BVDV. The combination vaccine as described herein comprises at least one attenuated BVDV, wherein said attenuated BVDV comprises at least one mutation in the coding sequence for glycoprotein E^{rns} and at least another mutation in the coding sequence for N^{pro} which preferably leads to combined inactivation of the RNase activity residing in glycoprotein E^{rns} in addition to the inactivation of the (hypothesized) immunomodulating activity residing in N^{pro}. The invention also relates to methods for producing such combination vaccines.

In one aspect, there is provided a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections, wherein said combination vaccine comprises

a. one or more attenuated BVDV, having at least one mutation in the coding sequence for glycoprotein E^{rns} and/or at least another mutation in the coding sequence for N^{pro}, wherein said mutation in the coding sequence for glycoprotein E^{rns} leads to inactivation of RNase activity residing in E^{rns} and/or said mutation in the coding sequence for N^{pro} leads to inactivation of said N^{pro}; and

b. one or more

antigen of one or more pathogens selected from the group consisting of: 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), Bovie Parvovirus (PPV), Adenovirus, Astrovirus, Mannheimia haemolytica (formerly Pasteurella haemolytica), Pasteurella multocida, Haemophilus somnus (Histophilus ovis and Haemophilus agni), Actinomyces such as Corynebacterium, Actinomyces pyogenes, Chlamydia psittaci, Campylobacter fetus venerealis and Campylobacter fetus fetus (formerly C fetus intestinalis), Leptospira interrogans, Leptospira hardjo, Leptospira pomona, Leptospira grippotyphosa, Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo such as Leptospira hardjoprajitno and 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., preferably Klebsiella pneumoniae, Salmonella typhimurium; Salmonella newport, Mycobacterium avium paratuberculosis, Cryptsporidium parvum, Cryptsporidium hominis, Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus uberus, Mycoplasma spp., preferably 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 wherein such BVDV component is safe for use in pregnant cows.

According to a preferred embodiment, the present disclosure relates to a combination vaccine for the treatment and/or prophylaxis of cattle against infections of the respiratory and reproductive system in cattle, wherein the combination vaccine comprises an attenuated BVDV as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by Bovine Herpes virus (BHV), Bovine Respiratory Syncytial Virus (BRSV), Parainfluenza Virus (PI-3), Campylobacter fetus, Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira prajitno, Leptospira icterohaemmorrhagiae, Leptospira hardjo, Leptospira bovis, Leptospira interrogans and/or Leptospira ponoma. According to a more preferred embodiment, the combination vaccine comprises an attenuated BVDV as described herein and at least one antigen of BHV, BRSV, PI-3, Campylobacter fetus, Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii Leptospira prajitno, Leptospira hardjo (Leptospira hardjo prajitno and Leptospira hardjo-bovis), Leptospira icterohaemmorrhagiae, Leptospira bovis, Leptospira interrogans and/or Leptospira ponoma.

According to a more preferred embodiment, the present disclosure relates to a combination vaccine for the treatment and /or prophylaxis of cattle against viral infections of the respiratory and reproductive system in cattle caused by BVDV, PI-3, BRSV, IBR and/or BHV, wherein said vaccine comprises at least an attenuated BVDV as described herein and at least one further

immunological active component effective for the treatment and/or prophylaxis of infections caused by PI-3, BRSV IBR, and BHV. According to a further more preferred embodiment, the present disclosure relates to a combination vaccine for the treatment and /or prophylaxis of cattle against viral infections of the respiratory and reproductive system in cattle caused by BVDV, PI-3, IBR, BRSV and/or BHV, wherein said vaccine comprises at least an attenuated BVDV as described herein and at least one antigen of PI-3, IBR, BRSV and/or BHV.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS OF TERMS USED IN THE DESCRIPTION:

Before the embodiments of the present invention it must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a BVDV" includes a plurality of such BVDV, reference to the "cell" is a reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by reference in their entireties including for the purpose of describing and disclosing the cell lines, vectors, and methodologies as reported in the publications, which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

The term "BVDV" as used herein refers to all viruses belonging to species bovine viral diarrhea virus (BVDV) type 1 (BVDV-1) and BVDV type 2 (BVDV-2), including any sub-species such as 1a, 1b, 2a, 2,b, and the like in the genus *Pestivirus* within the family *Flaviviridae* (Heinz et al., 2000). The more classical BVDV type 1 strains and the more recently recognized BVDV type 2 strains display some limited but distinctive differences in nucleotide and amino acid sequences.

"Protein C" or "C protein" or "C-protein" as used herein relates to a structural component of the pestivirus virion (Thiel et al., 1991). "Protein C" is the capsid or core protein of pestiviruses. Said term, depending on the context, may also relate to the "Protein C" with one or several amino acids exchanges resulting from mutation of the encoding nucleotide sequence.

The term "N^{pro}" as understood herein relates to the first protein encoded by the viral open reading frame that cleaves itself from the rest of the synthesized polyprotein (Stark, et al., J. Virol. 67:7088-7093 (1993); Wiskerchen, et al., Virol. 65:4508-4514 (1991)). Said term, depending on the context, may also relate to the remaining "N^{pro}" amino acids after mutation of the encoding nucleotide sequence or to the coding nucleotide sequence for said protein itself. "Protease activity residing in N^{pro}" relates to the polypeptide cleavage activity of said "N^{pro}".

Inactivation of N^{pro} as used herein means the prevention or considerable reduction of the probable immunemodulating activity of N^{pro} by mutation. In a preferred embodiment this mutation prevents or considerably reduces the interference of N^{pro} with the induction of an interferon response by the infected cells as described by Rüggli et al., (2003). In this case, the inactivation of N^{pro} would allow the cell to mount a normal interferon response.

"Processing signal" as used herein relates to a substance that ensures the generation of a functional N-terminal of the C protein of the pestivirus, preferably of BVDV, in particular a substance selected from the group of ubiquitin, LC3, SUMO-1, NEDD8, GATE-16 and GABA(A)RAP. Also proteases selected from the group of Intein, picornavirus 3C, caridovirus 2A and p15 of rabbit hemorrhagic disease virus are understood as "processing signals" as used herein. Any other similar processing signal known to the skilled person that ensures the generation of a functional N-terminal of the C protein shall also be comprised in the term "processing signal".

" E^{rns}" as used herein relates to the glycoprotein E^{rns} which represents a structural component of the pestivirus virion (Thiel et al., 1991). E^{rns} lacks a typical membrane anchor and is secreted in considerable amounts from the infected cells; this protein has been reported to exhibit RNase activity (Hulst et al., 1994; Schneider et al., 1993; Windisch et al., 1996). It should be noted that the term glycoprotein E0 is often used synonymously with glycoprotein E^{rns} in publications. Said term, depending on the context, may also relate to the mutated "E^{rns}" protein after mutation of the encoding nucleotide sequence or to the coding nucleotide sequence for said protein itself. "RNase activity residing in glycoprotein E^{rns}" relates to the RNA cleavage activity of said glycoprotein, i.e. the ability of the glycoprotein E^{rns} to hydrolyze RNA. The term "inactivation of the RNase activity residing in said glycoprotein" refers to the inability or reduced capability of a modified glycoprotein E^{rns} to hydrolyze RNA as compared to the unmodified wild type of said glycoprotein E^{rns}.

Inactivation of E^{rns} as used herein means RNase activity not significantly above the level measured for noninfected control cells in an RNase assay as described in Meyers et al., 1999. "Not significantly above the level measured for noninfected control cells in an RNase assay as described in Meyers et al., 1999", means for example, that the RNase activity is less than 150% compared to the noninfected control cells.

Attenuation: "An attenuated pestivirus or BVDV particle" as used herein means that there is a statistically significant difference between the virulence of attenuated pestivirus or BVDV particles of the present invention, wherein said attenuated viral particles being attenuated by a method described herein, and wild-type pestivirus or BVDV isolates from which said attenuated pestivirus or BVDV particles have been derived, for the predominant clinical parameters, in case of BVDV for diarrhea, pyrexia and lethality in animals infected with the same dose, preferably $6x10^6 TCID_{50}$. Thus, said attenuated BVDV particles do not cause diarrhea, pyrexia and lethality and thus may be used in a vaccine.

"Bovine pathogen" as used herein means a microorganism that has an impact on the healthiness of cattle.

"Immunological active component" or "immunologically active component" as used herein means a component that induces or stimulates the immune response in an animal to which said component is administered. According to a preferred embodiment, said immune response is directed to said component or to an microorganism comprising said component. According to a further preferred embodiment, the immunological active component is an attenuated microorganism, including modified live virus (MLV), a killed-microorganism or at least an immunological active part of a microorganism.

"Immunological active part of a microorganism" as used herein means a protein-, sugar-, and or glycoprotein containing fraction of a microorganism that comprises at least one antigen that induces or stimulates the immune response in an animal to which said component is administered. According to a preferred embodiment, said immune response is directed to said immunological active part of a microorganism or to a microorganism comprising said immunological active part.

The term "vaccine" as used herein refers to a pharmaceutical composition comprising at least one immunologically active component that induces an immunological response in an animal and

possibly but not necessarily one or more additional components that enhance the immunological activity of said active component. A vaccine may additionally comprise further components typical to pharmaceutical compostions. The immunologically active component of a vaccine may comprise complete virus particles in either their original form or as attenuated particles in a so-called modified live vaccine (MLV) or particles inactivated by appropriate methods in a so-called killed vaccine (KV). In another form, the immunologically active component of a vaccine may comprise appropriate elements of said organisms (subunit vaccines) whereby these elements are generated either by destroying the whole particle or the growth cultures containing such particles and optionally, subsequent purification steps yielding the desired structure(s), or by synthetic processes including an appropriate manipulation by use of a suitable system based on, for example, bacteria, insects, mammalian or other species, plus optionally subsequent isolation and purification procedures, or by induction of said synthetic processes in the animal needing a vaccine by direct incorporation of genetic material using suitable pharmaceutical compositions (polynucleotide vaccination). A vaccine may comprise one or simultaneously more than one of the elements described above. The term "vaccine" as understood herein is a vaccine for veterinary use comprising antigenic substances and is administered for the purpose of inducing a specific and active immunity against a disease provoked by a microbiological infection, preferably by a BVDV infection. The BVDV as described herein, confer active immunity that may be transferred passively via maternal antibodies against the immunogens it contains and sometimes also against antigenically related organisms. A vaccine of the invention refers to a vaccine as defined above, wherein one immunologically active component is a BVDV or derived from a nucleotide sequence that is more than 70% homologous to any known BVDV sequence (sense or antisense).

The term "live vaccine" refers to a vaccine comprising a replication competent, in particular, a replication compentent viral active component.

"Combination vaccine" as used herein means a vaccine that comprises attenuated BVDV as desribed herein together with a monovalent, bivalent or multivalent combination of immunological active component(s).

"Microbiological infection" as used herein means an infection as caused by a microorganism that is pathogenic for cattle. Such microorganisms inculde but are not limited to bacteria, viruses, yeasts or fungi, mycoplasms, and parasites.

A fragment" according to the invention is any subunit of a polynucleotide molecule according to the invention, i.e. any subset. For DNA, said fragment is characterized in that it is shorter than the DNA covering the full-length viral genome.

A functional variant" of the nucleotide molecule as used herein is a nucleotide molecule which possesses a biological activity (either functional or structural) that is substantially similar to the nucleotide molecule according to the invention. The term "functional variant" also includes "a fragment", "a functional variant", "a variant based on the degenerative nucleic acid code" or "a chemical derivative". Such "a functional variant" e.g. may carry one or several nucleotide exchanges, deletions or insertions. Said functional variant at least partially retains its biological activity, e.g. functions as an infectious clone or a vaccine strain, or even exhibits improved biological activity.

"Possess a biological activity that is substantially similar" means with respect to the pestiviruses provided herewith, for example, that said pestivirus is attenuated in a manner described herein and results in an non-pathogenic virus suitable for the production of live attenuated virus, which loses ability to pass the placenta but mediates an immune response after vaccination.

A "variant based on the degenerative nature of the genetic code" is a variant resulting from the fact that a certain amino acid may be encoded by several different nucleotide triplets. Said variant at least partially retains its biological activity, or even exhibits improved biological activity.

A molecule is "substantially similar" to another molecule if both molecules have substantially similar nucleotide sequences or biological activity. Thus, provided that two molecules possess a similar activity, they are considered variants as that term is used herein if the nucleotide sequence is not identical, and two molecules which have a similar nucleotide sequence are considered variants as that term is used herein even if their biological activity is not identical.

A "mutation" as used herein relates to modifications in the nucleic acid molecules encoding the proteins / amino acids according to the invention. Said mutations relate to, but are not limited to, substitutions (replacement of one or several nucleotides/base pairs), deletions (removal of one or several nucleotides/base pairs), and/or insertions (addition of one or several nucleotides/base pairs). As used herein, mutation may refer to a single mutation or several mutations, therefore, often the term "mutation(s)" is used and relates to both a single mutation and several mutations. Said

mutations include, but are not limited to point mutations (single nucleotide mutations) or larger mutations wherein e.g. parts of the encoding nucleic acid molecules are deleted, substituted and/or additional coding nucleic acids are inserted. Said mutations may result in a modified expressed polypeptide due to the change in the coding sequence. Such modified polypeptides are desired, as set out in the disclosure of the invention as set out below.

Additional components to enhance the immune response are constituents commonly referred to as "adjuvants", like e.g. aluminiumhydroxide, mineral or other oils or ancillary molecules added to the vaccine or generated by the body after the respective induction by such additional components, like but not restricted to interferons, interleukins or growth factors.

A "pharmaceutical composition" essentially consists of one or more ingredients capable of modifying physiological e.g. immunological functions of the organism it is administered to, or of organisms living in or on the organism. The term includes, but is not restricted to, antibiotics or antiparasitics, as well as other constituents commonly used to achieve certain other objectives like, but not limited to, processing traits, sterility, stability, feasibility to administer the composition via enteral or parenteral routes such as oral, intranasal, intravenous, intramuscular, subcutaneous, intradermal or other suitable route, tolerance after administration, and controlled release properties. One non-limiting example of such a pharmaceutical composition, solely given for demonstration purposes, could be prepared as follows: Cell culture supernatant of an infected cell culture is mixed with a stabilizer (e.g. spermidine and/or BSA (bovine serum albumin)) and the mixture is subsequently lyophilized or dehydrated by other methods. Prior to vaccination, said mixture is then rehydrated in aqueous (e.g. saline, PBS (phosphate buffered saline)) or non-aqueous solutions (e.g. oil emulsion, aluminum-based adjuvant).

DISCLOSURE OF THE INVENTION

The solution to the above technical problem is achieved by the description and the embodiments characterized in the claims.

The present invention relates to a combinantion vaccine for the treatment and/or prophylaxis of microbiological infection in cattle, that comprises a live attenuated BVDV as described herein and at least one further immunological active component for treating or preventing diseases or disorders in cattle caused by an infectious agents other than BVDV.

Attenuated BVDV

It was decribed in WO 99/64604 that BVDV can be attenuated by introducing at least one mutation in the coding sequence of glycoporiteion E^{rns}, wherein said mutation(s) result in an incatvation of the RNAse activity residing in the E^{rns} gene region. Moreover, it has also surprisingly been found that BVDV can be more effectively attenuated by introducing at least one mutation in the coding sequence for glycoprotein E^{rns} and at least another mutation in the coding sequence for N^{pro} which preferably leads to the combined inactivation of the RNase activity residing in glycoprotein E^{rns} in addition to the inactivation of the immunomodulating activity residing in N^{pro} (WO2005/111201). An immunomodulating effect in one aspect is indicated but not limited to the indicated function for one pestivirus in an exemplary manner by Rüggli et al. (2003).Thus according to one aspect, the present invention provides a combination vaccine that comprises at least an attenuated BVDV having at least one mutation in the coding sequence for glycoprotein E^{rns} and/or at least another mutation in the coding sequence for N^{pro}. Preferably, in such attenuated BVDV, said mutation in the coding sequence for glycoprotein E^{rns} leads to inactivation of the RNase activity residing in E^{rns} and/or said mutation in the coding sequence for N^{pro} leads to inactivation of said N^{pro}.

The attenuated BVDV as described herein can be advantageously used in combination vaccines for the treatment and/or prophylaxis of microbiological infections in cattle. Surprisingly, the BVDV as described herein, comprising any of the modifications in the N^{pro} and E^{rns} gene region are safe for use in pregnant animals as they do not cross the placenta. This is exemplified in a non-limiting manner for BVDV in example 3. Furthermore, the BVDV with defined mutations within the N^{pro} and E^{rns} as a basis for attenuation will allow to avoid the risk of reversion to a more pathogenic strain. A further advantage of said attenuating mutations lies in their molecular uniqueness, which allows to use them as distinctive labels for an attenuated BVDV and to distinguish them from BVDV from the field. Therefore, in a further aspect the present invention provides a combination vaccine that comprises at least an attenuated BVDV having at least one mutation in the coding sequence for glycoprotein E^{rns} and at least another mutation in the coding sequence for N^{pro}. Preferably, in such attenuated BVDV, said mutation in the coding sequence for glycoprotein E^{rns} leads to inactivation of the RNase activity residing in E^{rns} and/or said mutation in the coding sequence for N^{pro} leads to inactivation of said N^{pro}. Said inactivation may take place by any mutation known to the person skilled in the art of the E^{rns}- and the N^{pro}-coding sequence, wherein the mutations are any mutation as defined in the "definitions" section, such as deletions, insertion

mutations and/or substitution mutations. Most preferably, the mutation(s) are deletions, as the likelihood for reversion to the wild type is the lowest for deletions.

Altogether, the term attenuated BVDV or attenuated pestivirus in general as used herein, means but is not limited to any attenuated BVDV or pestivirus, having at least one modification in the coding sequence for glycoprotein E^{rns} and/or at least one modification in the coding sequence for N^{pro}. In the following, specific embodiments of any of such modification in the E^{rns} and/or N^{pro} are described more in detail. According to a preferred aspect, the term attenuated BVDV or attenuated pestivirus in general as used herein, means, but is not limited to, any attenuated BVDV or pestivirus, having at least one modification in the coding sequence for glycoprotein E^{rns} and at least one modification in the coding sequence for N^{pro} However, it is hereby understood that the present invention shall not be limited to the specific modification described herein. A person skilled in the art with the knowledge of the teaching provided herewith, is able to generate and introduce further modifications within the glycoprotein E^{rns} and/or N^{pro} having the effect of attenuation as described herein.

Modifications of the E^{rns} of BVDV

It has been shown that the glycoprotein E^{rns} forms a disulfide-bonded homodimer of about 97 kD, wherein each monomer consists of 227 amino acids corresponding to the amino acids 268 to 494 of the CSFV polyprotein as described by Rümenapf et al. (1993). The genome sequence of the Alfort/Tübingen strain of CSFV is available in the GenBank/EMBL data library under accession number J04358; alternatively, the amino acid sequence for the BVDV strain CP7 can be accessed in the GenBank/EMBL data library (accession number U63479); in the BVDV CP7 polyprotein, the E^{rns} protein corresponds to residues 271 to 497. Two regions of amino acids are highly conserved in glycoprotein E^{rns} as well as in some plant and fungal RNase-active proteins (Schneider et al., 1993). These two regions are of particular importance to the RNase enzymatic activity. The first region consists of the region at the amino acids at position 295 to 307 (298 to 310 for BVDV strain cp7) and the second region consists of the amino acids at position 338 to 357 (341 to 360 for BVDV strain cp7) of said viral polyprotein as exemplified for the Alfort strain of CSFV in Meyers et al., 1999 (numbering according to the published deduced amino acid sequence of CSFV strain Alfort/Tübingen (Meyers et al., 1989). The amino acids of particular importance to the RNase activity as mentioned above are by no means limited to the exact position as defined for the Alfort/Tübingen strain of CSFV but are simply used in an exemplary manner to point out the preferred amino acids being at that position or corresponding to that position in other strains such as

found in BVDV, BDV and pestiviruses in general since they are highly conserved. For pestiviruses other than the CSFV Alfort/Tübingen strain the numbering of the positions of the preferred amino acids can be different but an expert in the field of the molecular biology of pestiviruses will easily identify these preferred amino acids by the high degree of conservation of this amino acid sequence and the position of these motifs in the sequence context. In one particular non-limiting example, the position of CSFV Alfort/Tübingen 346 is identical to position 349 of BVDV strain cp7.

As a consequence, the present invention preferably relates to a BVDV according to the invention, wherein said mutation(s) in the coding sequence for glycoprotein E^{rns} are located in the encoding nucleotide sequence corresponding to amino acids at position 298 to 310 and/or position 341 to 360. Preferably, such a mutation is (amino acids are given in the one letter symbols; the amino acid before the position number indicates the amino acid to be substituted, the amino acid after the position number the substituting amino acid (del indicates deletion) for example, H300L, which means that histidine at position 300 was substituted by leucine:

Suitable modification of the glycoprotein E^{rns} are for example, the single substitutions/deletions: S298G, H300K, H300L, H300R, H300del, W303G, P304del, E305A, C308G, R343G, E345del, W346G, K348A, H349K, H349L, H349del, H349Q, H349SV (mutation H349S and insertion of V), K348R, W351P, W351G, W351L, W351K, W351H; the double substitutions/deletions: H300L/H349L, K348del/H349del, H349del/G350del, E345del/H349del, W303G/E305A, H300K/H349K, H300K/H349L and the triple deletions: L299del/H300del/G301del, K348del/H349del/G350del. Numbering is according to the published amino acid sequence of BVDV CP7 for all the mutants listed above. All the above-listed mutants were at least tested as BVDV mutants without mutations in the N^{pro}region. Suitable mutants of the pestiviral such BVDV glycoprotein E^{rns} are provided, for example, by WO 99/64604, which is incorporated herein at its whole. It should be noted, however, that according to a further preferred embodiment of the present invention, at least one additional mutation in the N^{pro} region, as disclosed in further detail below, must be present.

It was particularly found that deletion or substitution of the histidine residue at position 349 (BVDV) leads to effective inactivation of E^{rns} and therefore leads to particularly useful BVDV live vaccines. The present invention demonstrates that BVDV are viable and code for an E^{rns} protein without RNase activity when the histidine residue at position at position 349 (numbering according to the published sequence of BVDV CP7 (Meyers et al., 1996b)) is deleted. Thus, preferably, the

BVDV as used in the combination vaccine bears a mutation in the coding sequence for glycoprotein E^{rns} is a deletion or substitution of the histidine residue at position 349. Even more specifically, the putative active site of the RNase is represented by the conserved E^{rns} sequences SLHGIWPEKICTG (SEQ ID NO 13) and/or LQRHEWNKHGWCNWFHIEPW (SEQ ID NO 14) (sequence of the BVDV-2 New York'93 protein is given here in an exemplary manner; minor changes can possibly be found in other BVDV sequences but the identity of the motif will always be obvious for an expert in the field). As an example, the corresponding amino acid sequences of BVDV-1 CP7 would be SLHGIWPEKICTG (SEQ ID NO 13) and/or LQRHEWNKHGWCNWYNIEPW (SEQ ID NO 15). Thus, preferably, the BVDV of the combination vaccine bears mutation(s) in the coding sequence for glycoprotein E^{rns} are located in the nucleotide sequence coding for the conserved E^{rns} sequence SLHGIWPEKICTG (SEQ ID NO 13) and/or LQRHEWNKHGWCNWFHIEPW (SEQ ID NO 14). These sequences are representing the putative active site of the RNase. The sequences SLHGIWPEKIC (SEQ ID NO 16) and RHEWNKHGWCNW (SEQ ID NO 17) of the putative E^{rns} active site are even more conserved across pestiviruses. Thus, preferably, the BVDV used for the preparation of a combination vaccine as described herein has at least one mutation in the coding sequence of the N^{pro} protein and/or the glycoprotein E^{rns}, wherein said mutation(s) in the coding sequence for glycoprotein E^{rns} are located in the nucleotide sequence coding for the conserved E^{rns} sequence SLHGIWPEKIC (SEQ ID NO 16) and/or RHEWNKHGWCNW (SEQ ID NO 15). Preferably, the mutation is located in only one of said sequences. Thus the BVDV of the combination vaccine described herein having at least one mutation in the coding sequence of the N^{pro} protein and/or the glycoprotein E^{rns}, wherein said mutation(s) in the coding sequence for glycoprotein E^{rns} are located in the nucleotide sequence coding for the conserved E^{rns} sequence SLHGIWPEKIC (SEQ ID NO 16) or RHEWNKHGWCNW (SEQ ID NO 17). Preferably, such mutations concern two different amino acids, i.e. are double mutations. Thus, said mutations may be 1 to 3 nucleotide mutations in two different triplets encoding two amino acids. Thus, the invention also relates to a combination vaccine comprising a live attenuated BVDV having at least one mutation in the coding sequence of the N^{pro} protein and/or the glycoprotein E^{rns}, wherein said mutation(s) in the coding sequence for glycoprotein E^{rns} are two mutations located in the nucleotide sequence coding for the conserved E^{rns} sequence SLHGIWPEKIC (SEQ ID NO 16) and/or RHEWNKHGWCNW (SEQ ID NO 17). Preferably, such mutations concern a single amino acid. Thus, said mutation may be 1 to 3 nucleotide mutations in one triplett encoding one amino acid. Thus, the invention also relates to combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections comprising a live attenuated BVDV having at least one mutation in the coding sequence of the N^{pro} protein and/or the glycoprotein E^{rns}, wherein a single

mutation is located in the conserved E^{rns} sequence SLHGIWPEKIC (SEQ ID NO 16) and/or RHEWNKHGWCNW (SEQ ID NO 17).

Modification of the N^{pro} gene of BVDV

As mentioned above, the attenuated BVDV provided herein, having at least one mutation in the coding sequence of the glycoprotein E^{rns} and/or in the coding sequence of the the N^{Pro} protein, wherein said mutation preferably results in inactivation of the RNase activity residing in the glycoprotein E^{RNS} and/or of the immunomodulating activity residing in N^{pro}. Inactivation of N^{pro} is achieved in BVDV of the specified formula described more in detail below, wherein between 0 and all amino acids of N^{pro} are present; ubiquitin or LC3 or another sequence serves as processing signal (e.g. SUMO-1, NEDD8, GATE-16,GABA(A)RAP, or proteases like e.g. Intein, picornavirus 3C, cardovirus 2A, or p15 of rabbit hemorrhagic disease virus) are present or absent. In case a processing signal is present, the coding sequence of the processing signal is inserted at or close to the C-terminal end of the (remaining part of the) N^{pro}-protein. Only in the case that a processing signal is present, any number of amino acids coding for N^{pro} (=N^{pro} amino acids) may be present. In case no processing signal sequence is inserted, a maximum of 12 amino acids, preferably aminoterminal amino acids, of N^{pro} may be present, the remaining amino acids have to be deleted. Furthermore, other than the E^{rns} mutations as disclosed above (at least one of which has to be present in the attenuated BVDV as described herein), the remaining sequences of the attenuated BVDV may remain unchanged, i.e. are not mutated, or may also have mutations close to the N-terminal end of the C-protein. A number of more specific embodiments as disclosed below exemplify this.

Thus, the attenuated BVDV of the combination vaccine is modified in the coding regions of the E^{rns} as described above and/or in the N^{pro}, wherein said mutation(s) in the coding sequence for N^{pro} leads to an encoded polyprotein as characterized by the following formula:

$$[N^{pro}]_x\text{-}[PS]_y\text{-}[C\text{-term}]$$

and wherein:

$[N^{pro}]$ relates to the N^{pro} portion of said polyprotein, wherein "x" represents the number of amino acids of the N^{pro} present in the polyprotein;

[PS] relates to a processing signal selected from: ubiquitin, LC3, SUMO-1, NEDD8, GATE-16 or GABA(A)RAP or proteases like e.g. Intein, picornavirus 3C, cardovirus 2A, or p15 of rabbit hemorrhagic disease virus, or any processing signal known to the skilled person that

ensures the generation of a functional N-terminal of the C-protein. "Y" may be = 0, which means that no processing signal is present (= PS is absent), or "Y" may be = 1, which means that a processing signal is present (= PS present).

[C-term] relates to the complete pestivirus, in particular the complete BVDV polyprotein except for N^{pro}, but including the capsid (C)-protein and any other protein present in the pestivirus polyprotein, in particular in the BVDV polyprotein including the carboxyterminal NS5B. Preferably, the glycoprotein E^{rns} in said [C-term] is mutated, in such that the RNase activity residing in the glycoprotein E^{rns} is inactivated. The term "any other protein present in the pestivirus polyprotein /BVDV polyprotein" relates to E^{rns}, E1, E2, p7, NS2, NS3, NS4A, NS4B and NS5A, wherein glycoprotein E^{rns} is mutated, preferably as disclosed herein (see above), in such that the RNase activity residing in the glycoprotein E^{rns} is inactivated. Preferably, the pestivirus, in particular the BVDV according to the invention has a C-protein which is not mutated except for the amino acid at position 2 which is changed from D to N. Therefore, [C-term*] is the same as [C-term] but with a mutation at position 2 of the C-protein (N instead of D);

if "y" is = 0 (means no [PS] present) then"x" is 0 to 12, (means no N^{pro} specific amino acid or 1 to 12 amino acids of N^{pro}, preferably of the N-terminus of N^{pro}, are present);

if "y" is = 1 (means [PS] is present) then "x" is 0 to 168; (means no N^{pro} specific amino acid or 1 to all 168 amino acids of N^{pro}, preferably of the N-terminus of N^{pro}, are present).

Also more preferably, the mutation(s) in the coding sequence for N^{pro} lead to an encoded polyprotein as characterized by the following formula:

$$[N^{pro}]_1\text{-}[PS]_0\text{-}[C\text{-term}]$$

and wherein the definitions are as defined above.

A specific example thereof is disclosed below, wherein the N-terminal methionine is followed by the C-protein and any other protein present in the polyprotein including the carboxyterminal NS5B Hence, most preferably, the attenunated BVDV as described herein is encoded by a polyprotein as characterized by the following formula:

$$M[C\text{-term}].$$

and wherein the definitions are as defined above.

Also more preferably, the invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections, wherein said vaccine comprises an attenuated BVDV that is modified in the coding regions of the E^{rns} as described above and/or in the N^{pro}, wherein said mutation(s) in the coding sequence for N^{pro} leads to an encoded polyprotein as characterized by the following formula:

$$[N^{pro}]_3\text{-}[PS]_0\text{-}[C\text{-term}]$$

and wherein the definitions are as defined above.

A further specific example of an attenuated BVDV is disclosed below, wherein the N-terminal methionine is followed by the N^{pro} sequence EL and the C-protein and any other protein present in the polyprotein including the carboxyterminal NS5B. Hence, most preferably, the attenuated BVDV as described herein is encoded by a polyprotein as characterized by the following formula:

$$MEL\text{-}[C\text{-term}]$$

and wherein the definitions are as defined above.

Also more preferably, the invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections, wherein said vaccine comprises an attenuated BVDV that is modified in the coding regions of the E^{rns} as described above and/or in the N^{pro}, wherein said mutation(s) in the coding sequence for N^{pro} leads to an encoded polyprotein as characterized by the following formula:

$$[N^{pro}]_4\text{-}[PS]_0\text{-}[C\text{-term}]$$

and wherein the definitions are as defined above.

A further specific example of BVDV is disclosed below, wherein the N-terminal methionine is followed by the N^{pro} sequence ELF (SEQ ID NO 18) and the C-protein and any other protein present in the polyprotein including the carboxyterminal NS5B. Hence, most preferably, the invention refers to a BVDV, wherein said mutation(s) in the coding sequence for N^{pro} leads to an encoded polyprotein as characterized by the following formula:

$$MELF\text{-}[C\text{-term}].$$

and wherein the definitions are as defined above.

Also more preferably, the invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections, wherein said vaccine comprises an attenuated BVDV that is modified in the coding regions of the E^{rns} as described above and/or in the N^{pro}, wherein said mutation(s) in the coding sequence for N^{pro} leads to an encoded polyprotein as characterized by the following formula:

$$[N^{pro}]_6\text{-}[PS]_b\text{-}[C\text{-term}]$$

and wherein the definitions are as defined above.

A further specific example of an attenuated BVDV as described herein is given below, wherein the N-terminal methionine is followed by the N^{pro} sequence ELFSN (SEQ ID NO 19) and the C-protein and any other protein present in the polyprotein including the carboxyterminal NS5B. Hence, most preferably, the invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections, wherein said vaccine comprises an attenuated BVDV that is modified in the coding regions of the E^{rns} as described above and/or in the N^{pro}, wherein said mutation(s) in the coding sequence for N^{pro} leads to an encoded polyprotein as characterized by the following formula:

MELFSN-[C-term].

and wherein the definitions are as defined above.

A further specific example of an attenuated BVDV as described herein is given below, wherein the N-terminal methionine is followed by the N^{pro} sequence ELFSNE (SEQ ID NO 20); ELFSNEL (SEQ ID NO 21); ELFSNELL (SEQ ID NO 22); ELFSNELLY (SEQ ID NO 23); ELFSNELLYK (SEQ ID NO 24); or ELFSNELLYKT (SEQ ID NO 25) and the C-protein and any other protein present in the polyprotein including the carboxyterminal NS5B. Hence, most preferably, the invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections, wherein said vaccine comprises an attenuated BVDV that is modified in the coding regions of the E^{rns} as described above and/or in the N^{pro}, wherein said mutation(s) in the coding sequence for N^{pro} leads to an encoded polyprotein as characterized by the following formula:

MELFSN-[C-term].
MELFSNE-[C-term];
MELFSNEL--[C-term];

MELFSNELL-[C-term];
MELFSNELLY-[C-term];
MELFSNELLYK- [C-TERM]
MELFSNELLYKT- [C-TERM]

and wherein the definitions are as defined above.

Also more preferably, the invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections, wherein said vaccine comprises an attenuated BVDV that is modified in the coding regions of the E^{rns} as described above and/or in the N^{pro}, wherein said mutation(s) in the coding sequence for N^{pro} leads to an encoded polyprotein as characterized by the following formula:

$$[N^{pro}]_4\text{-}[PS]_0\text{-}[C\text{-term}^*]$$

and wherein the definitions are as defined above except for the fact that the aminoterminal part of the C-protein is changed, also as described above.

A further specific example of BVDV is disclosed below, wherein the N-terminal methionine is followed by the N^{pro} sequence ELF and in the C-protein sequence, the amino acid at position 2 is changed from D to N. Therefore, the aminoterminal C-protein sequence is SNEGSK (SEQ ID NO 26), instead of SDEGSK (SEQ ID NO 27). Hence, most preferably, the invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections, wherein said vaccine comprises an attenuated BVDV that is modified in the coding regions of the E^{rns} as described above and/or in the N^{pro}, wherein said mutation(s) in the coding sequence for N^{pro} leads to an encoded polyprotein as characterized by the following formula:

MELF-[C-term*],

wherein in the C-protein the amino acid at position 2 is changed from D to N, and

wherein the definitions are as defined above.

Also more preferably, the invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections, wherein said vaccine comprises an attenuated BVDV that is modified in the coding regions of the E^{rns} as described above and/or in the

N^{pro}, wherein said mutation(s) in the coding sequence for N^{pro} lead to an encoded polyprotein as characterized by the following formula:

$$[N^{pro}]_k\text{-}[PS]_1\text{-}[C\text{-term}],$$

wherein the definitions are as defined as above,

and wherein PS is any of the PS disclosed above, and more preferably selected from the group of ubiquitin or LC3.

A further specific example of BVDV is disclosed below, wherein the N-terminal methionine is followed by any 21 or 28 N^{pro} amino acids, ubiquitin or LC3, and the C-protein. Hence most preferably, the invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections, wherein said vaccine comprises an attenuated BVDV that is modified in the coding regions of the E^{ms} as described above and/or of the N^{pro}, wherein said mutation(s) in the coding sequence for N^{pro} lead to an encoded polyprotein as characterized by the following formula:

$[N^{pro}]_{22}\text{-}[PS]_1\text{-}[C\text{-term}]$, wherein preferably, the PS is ubiquitin or LC3 or

$[N^{pro}]_{29}\text{-}[PS]_1\text{-}[C\text{-term}]$, wherein preferably, the PS is ubiquitin or LC3.

Ubiquitin is a well known highly conserved cellular protein of 76 amino acids. Among other functions, ubiquitin is a key player in protein catabolism since conjugation with ubiquitin can mark a protein for degradation via the proteasome. Ubiquitin conjugated with or fused to other proteins via the carboxyterminal glycine can be cleaved off by cellular ubiquitin-specific proteases. Thus, fusion of a protein to the carboxyterminus of ubiquitin will usually result in defined proteolytic cleavage of the fusion protein into its components when expressed within a cell.

LC3 (light chain 3 of microtubule associated proteins) represents a cellular protein of 125 amino acids that serves a variety of functions (length given for bovine LC3). Recently, a fundamental role of the protein in autophagy has been defined. During this process, LC3 is activated by carboxyterminal cleavage. Thereby, a new carboxyterminus is generated that consists of glycine. LC3 is then conjugated via the carboxyterminal glycine to phosphatidylethanolamine present in the membranes of autophagic vesicles. Because of this process, a protein fused to the carboxyterminus of LC3 will be cleaved off by a cellular protease at a defined position.

Also more preferably the invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections, wherein said vaccine comprises an attenuated BVDV that is modified in the coding regions of the E^{rns} as described above and/or in the N^{pro}, wherein said mutation(s) in the coding sequence for N^{pro} lead to an encoded polyprotein as characterized by the following formula selected from the group of :

$[N^{pro}]_2$-$[PS]_y$-[C-term] and preferably ME-$[PS]_y$-[C-term];

$[N^{pro}]_5$-$[PS]_y$-[C-term] and preferably MELFS-$[PS]_y$-[C-term];

$[N^{pro}]_7$-$[PS]_y$-[C-term] and preferably MELFSNE-$[PS]_y$-[C-term];

$[N^{pro}]_8$-$[PS]_y$-[C-term] and preferably MELFSNEL-$[PS]_y$-[C-term];

$[N^{pro}]_9$-$[PS]_y$-[C-term] and preferably MELFSNELL-$[PS]_y$-[C-term];

$[N^{pro}]_{10}$-$[PS]_y$-[C-term] and preferably MELFSNELLY-$[PS]_y$-[C-term];

$[N^{pro}]_{11}$-$[PS]_y$-[C-term] and preferably MELFSNELLYK-$[PS]_y$-[C-term]; and

$[N^{pro}]_{12}$-$[PS]_y$-[C-term] and preferably MELFSNELLYKT-$[PS]_y$-[C-term]

and wherein the definitions are as defined as above. The preferably disclosed embodiments refer to BVDV.

Most preferably, y is 0 (no PS present).

Suitable E^{RNS} N^{pro} double mutants of BVDV include those listed in the table below:

Table: BVDV E^{Rns} N^{pro} double mutants:

[illegible]	[illegible]
S298G	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
H300K	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
H300L	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-,

	MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
H300del	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
W303G	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
P304del	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
E305A	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
C308G	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
R343G	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
E345del	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
W346G	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
K348A	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-,

	MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
K348R	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
H349K	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
H349L	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
H349Q	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
H349del	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
H349SV (mutation H349S and insertion of V)	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
W351P	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
W351K	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
H300L/H349L	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-,

	MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
H300K/H349K	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- . MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
H300K/H349L	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
W303G/E305A	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
K348del/H349del	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
E345del/H349del	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
H349del/G350del	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
L299del/H300del/G301del	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-
L299del/H300del/G301del	ME-, MEL-, MELF-, MELFSN-, MELFSNEL-, MELFSNELL-, MELFSNELLY-, MELFSNELLYK-,or MELFSNELLYKT-, MESDEGSK- , MELFSSDEGSK- ,MELFSNESDEGSK-, MELFSNELSDEGSK-, MELFSNELLSDEGSK-, MELFSNELLYSDEGSK-, MELFSNELLYKSDEGSK-, MELFSNELLYKTSDEGSK-

The N^{pro} –mutations are defined by their remaining amino terminal sequences. For example; MELF- mutant means that the coding region of N^{pro} is completely deleted except of the amino terminal amino acids MELF.

According to one embodiment of the present invention, the attenuated BVDV of the combination vaccine as provided herein is a BVDV type 1. Preferably, the attenuated BVDV is based on one of the following BVDV type 1 strains: NADL, Osloss, SD-1, CP7 or KE9, wherein each of the strains comprises at least one of the E^{RNS} and/or N^{pro} mutations as described above, and preferably at least one of the double-mutants within the E^{RNS} and N^{pro} region as listed in the table above. According to a further embodiment, said attenuated BVDV of the combination vaccine as provided herein is a BVDV type 2. Preferably, the attenuated BVDV is based on one of the following BVDV type 2 strains: 890, C413, or New York'93C, wherein each of the strains comprises at least one of the E^{RNS} and/or N^{pro} mutations as described above, and preferably at least one of the double-mutants within the E^{RNS} and N^{pro} region as listed in the table above.

BVDV-1 and BVDV-2 are differentiated according to features of their genomic sequences (Heinz et al., 2000 and references therein). BVDV-1 as disclosed herein may be used in the manufacture of a composition for use in the prevention and/or treatment of BVDV type 1 infections in breeding stocks of cattle, in pregnant cows and in the induction of fetal protection against BVDV type 1 infection is pregnant cows. Surprisingly, a BVDV-2 as disclosed herein may be used in the manufacture of a combination vaccine for use in the prevention and/or treatment of BVDV type 1 infections in breeding stocks of cattle. In particular, the invention relates to the use of a BVDV type 2 as described herein in the manufacture of a combination vaccine for use in the prevention and/or treatment of BVDV type 1 infections in pregnant cows. Preferably, the BVDV type 2 as provided herein may be used in the manufacture of a combination vaccine for use in the induction of fetal protection against BVDV type 1 infections in pregnant cows. Surprisingly also, a BVDV-1 as disclosed herein may be used in the manufacture of a combination vaccine for use in the prevention and/or treatment of BVDV type 2 infections in breeding stocks of cattle. In particular, the invention relates to the use of a BVDV type 1 as described herein in the manufacture of a combination vaccine for use in the prevention and/or treatment of BVDV type 2 infections in pregnant cows. Preferably, the BVDV type 1 according to the invention may be used in the manufacture of a combination vaccine for use in the induction of fetal protection against BVDV type 2 infections in pregnant cows.

Most preferred is the use of BVDV type 1 and type 2 in combination for the manufacture of the combination vaccines described herein for use in the prevention and/or treatment of BVDV type 1 and or type 2 infections in breeding stocks of cattle, in pregnant cows and in the induction of fetal

protection against BVDV type 1 and/or type 2 infections in pregnant cows. Thus, preferably the combination vaccines provided herewith comprise one or more attenuated BVDV type 1 and type 2 as desribed above. For example, the combination vaccines provided herewith comprise an attenuated BVDV of type 1 and type 2, based on the strains: NADL/890; NADL/C413; NADL/NewYork'93/C; CP7/890; CP7/C413; CP7/NewYork'93/C; KE9/890; KE9/C413; KE9/NewYork'93/C, wherein each of the strains comprises at least one of the E^{RNS} and/or N^{pro} mutations as described above, and more preferably at least one of the double-mutants within the E^{RNS} and N^{pro} region as listed in the table above. More preferably, any of the combination vaccines provided herewith may include one of more sub-types of attenuated BVDV type 1 and one or more sub-types of attenuated BVDV-2, e.g one or more attenuated BVDV of sub-types 1a, 1b, 1c, 1d, 1e, 1f, 1g, 1h, and the like and one or more attenuated BVDV of sub-types 2a, 2b and the like. Most preferred is a combination vaccine comprising attenuated BVDV of sub-types 1a, 1b, and 2a. Thus, according to a preferred embodiment of the present invention, the phrase "attenuated BVDV (types 1 and/or 2)" includes but is not limited to combinations of BVD viruses comprising one or more attenuated BVDV of type 1, preferably of sub-type 1b and one or more attenuated BVDV of type 2, preferably of subtype 2a. According to further embodiment of the present invention the phrase "attenuated BVDV (types 1 and/or 2)" includes but is not limited to combinations of BVD viruses comprising one or more "attenuated BVDV of sub-type 1a, one or more attenuated BVDV of sub-type 1b, and one or more attenuated BVDV of type 2, preferably of sub-type 2a.

If more than one attenuated BVDV is used in the combination vaccines as described herein, each of the attenuated BVDV should mutated in same genomic site of the E^{RNS} and/or the N^{pro} such that the none of the attenuated BVDV can recombine with any of the others to eliminate the mutations which are essential and responsible for the attenuation of the viruses. For example if BVDV type 1a with one of the following E^{RNS} mution is used: H349K, H349L, H349del, H349Q, H349SV (mutation H349S and insertion of V), a BVDV type 1b and/or type 2 should be used, which are/is mutated in the same site of the E^{RNS}, i.e. at position 349 or at amino acid position which corresponds to position 349 of BVDV type 1 in order to avoid revertation of attenuated BVDV type 1 or type 2. This principle also applies to any mutation within the N^{pro} region. Thus, according to a prefered embodiment of the present invention, the phrase "attenuated BVDV (types 1 and/or 2)" includes but is not limited to combinations of BVD viruses comprising one or more attenuated BVDV of type 1 and one or more attenuated BVDV of type 2, wherein each of the attenuated BVDV is mutated in same genomic site of the E^{RNS} and/or the N^{pro} such that none of the attenuated BVDV can recombine with any of the others to eliminate the mutations within the E^{RNS} and N^{pro}

which are essential and responsible for the attenuation of the viruses. According to another embodiment of the present invention, the phrase "attenuated BVDV (types 1 and/or 2)" includes, but is not limited to, a combination of BVD viruses comprising one or more "attenuated BVDV of sub-type 1a, one or more attenuated BVDV of sub-type 1b, and one or more attenuated BVDV of type 2, preferably of sub-type 2a, wherein each of the attenuated BVDV is mutated in same genomic site of the E^{RNS} and/or the N^{pro} such that none of attenuated BVDV can recombine with any of the others to eliminate the mutations within the E^{RNS} and/or N^{pro} which are essential and responsible for the attenuation of the viruses.

Preparation of the attenuated BVDV

Another important aspect of the invention is a method for attenuating a pestivirus, characterized in that at least one mutation in the coding sequence for glycoprotein E^{rns} and/or at least another mutation in the coding sequence for N^{pro} is generated in the BVDV genome.

According to a more preferred embodiment, said method comprises the steps:

a) reverse transcription of a wild-type pestivirus nucleotide sequence into a cDNA;
b) cloning said cDNA;
c) introducing mutations selected from the group of deletions, insertion mutations and/or substitution mutations into said cDNA, wherein said mutations are located in the coding sequence encoding glycoprotein E^{rns} and/or the protease N^{pro},
d) incorporating the cDNA into a plasmid or into a DNA virus capable of directing the transcription of BVDV cDNA into RNA in vitro or upon infection of suitable cells.

Regarding the method for attenuating a BVDV according to the invention, said preferred method comprises the steps:

a) reverse transcription of a wild-type BVDV nucleotide sequence into a cDNA;
b) cloning said cDNA;
c) introducing mutations selected from the group of deletions, insertion mutations and/or substitution mutations into said cDNA, wherein said mutations are located in the coding sequence encoding glycoprotein E^{rns} and/or the protease N^{pro},
d) incorporating the cDNA into a plasmid or into a DNA virus capable of directing the transcription of BVDV cDNA into RNA in vitro or upon infection of suitable cells.

There are several nucleotide sequences known in the art, which represents the basis for the production of a polynucleotide molecule coding for a BVDV attenuated as decribed herein, having at least one mutation in the coding sequence of N^{pro} and/or at least one in the coding sequence of glycoprotein E^{rns}, wherein said mutations result in a combined inactivation of the RNase activity residing in glycoprotein E^{rns} and in the inactivation of the immunomodulating activity residing in N^{pro}. Examples of nuclecic acid sequences of wild-type sequences of several BVDVstrains are listed below:

Bovine viral diarrhea virus 1	
Strain NADL	NCBI GenBank Accession No. [M31182]
Strain Osloss	NCBI GenBank Accession No. [M96687]
Strain SD-1	NCBI GenBank Accession No. [M96751]
Strain CP7	NCBI GenBank Accession No. [U63479]
Strain KE9	(SEQ ID NO:1)
Bovine viral diarrhea virus 2	
Strain 890	NCBI GenBank Accession No. [U18059]
Strain C413	NCBI GenBank Accession No. [AF002227]
Strain NewYork'93/C	NCBI GenBank Accession No. [AF502399]

The mutations/modifications relating to the coding sequences of N^{pro} and E^{rns} are described above more in detail. Having this information, a person skilled in the art is able to realize the manufacture of any polynucleotide/polynucleic acid coding for an attenuated BVDV as provided herewith. Molecular methods for introducing a mutation into a polynucleotide sequence, as well as the cloning and amplification of said mutated polynucleotide are for example provided by Sambrook et al. 1989 or Ausubel et al. 1994.

Most preferably, the wild type BVDV which is to be mutated as disclosed herein corresponds to amino acid sequence SEQ ID No. 5 (termed XIKE A) or is a functional variant thereof. Most preferably also, the BVDV has a N^{pro} mutation as described herein corresponding to amino acid sequence SEQ ID No. 6 (termed XIKE-A-NdN) or is a functional variant thereof. Preferably, such a functional variant is at least 65% homologous to the amino acid sequence disclosed herein. On the amino acid level, homologies are very roughly: BVDV-1/-BVDV-1: 93%; BVDV-1/-BVDV-2: 84%; BVDV-2/-BVDV-2: 98%. Therefore, more preferably, such a functional variant is at least

70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90% homologous to the amino acid sequence disclosed herein. More preferably also, such functional variant is at least 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous to the amino acid sequence disclosed herein. Most preferably, such functional variant is at least 99% or 99.9% homologous to the amino acid sequence disclosed herein.

Most preferably also, the attenuated BVDV as described herein has a E^{ms} mutation which has a deletion of the codon coding for histidine 349 (termed XIKE-B), or is a functional variant thereof. Most preferably also, the attenuated BVDV has both a E^{ms} mutation and/or a N^{pro} mutation as described herein, wherein the codon coding for histidine 349 of E^{ms} is deleted and also the complete N^{pro} coding region is deleted, except for codons 1 to 4, thus amino acids MELF of N^{pro} remain. Said double mutant corresponds to amino acid sequence SEQ ID No. 8 (termed XIKE-B-NdN) or is a functional variant thereof. Preferably, such a functional variant is at least 65% homologous to the amino acid sequence disclosed herein. More preferably, such a functional variant is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90% homologous to the amino acid sequence disclosed herein. More preferably also, such functional variant is at least 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous to the amino acid sequence disclosed herein. Most preferably, such functional variant is at least 99% or 99.9% homologous to the amino acid sequence disclosed herein.

Most preferably also, the BVDV according to the invention has an E^{ms} mutation according to the invention which has a substitution of the codon coding for histidine 300 by the codon coding for leucine (termed XIKE-C), or is a functional variant thereof. Most preferably also, the BVDV according to the invention has both a E^{ms} mutation and a N^{pro} mutation according to the invention, wherein the codon coding for histidine 300 is substituted by the codon coding for leucine and also the complete N^{pro} coding region is deleted, except for codons 1 to 4, thus amino acids MELF of N^{pro} remain. Said mutant corresponds to amino acid sequence SEQ ID No. 10 (termed XIKE-C NdN) or is a functional variant thereof. Preferably, such a functional variant is at least 65% homologous to the amino acid sequence disclosed herein. More preferably, such a functional variant is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89% or 90% homologous to the amino acid sequence disclosed herein. More preferably also, such functional variant is at least 91%, 92%, 93%, 94%, 95%, 96%, 97% or 98% homologous to the amino acid sequence disclosed herein. Most preferably, such functional variant is at least 99% or 99.9% homologous to the amino acid sequence disclosed herein.

Combination partners:

As described above, the present invention relates to a combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections, wherein said combination vaccine comprises attenuated BVDV (types 1 and/or 2) as decribed above, and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by a bovine pathogen other than BVDV. As mentioned above, the combination vaccine preferably comprises attenuated BVDV type 1 and BVDV type 2, both having at least one mutation in the coding sequence for glycoprotein E^{rns} and/or at least another mutation in the coding sequence for N^{pro}, wherein said mutation in the coding sequence for glycoprotein E^{rns} leads to inactivation of RNase activity residing in E^{rns} and/or said mutation in the coding sequence for N^{pro} leads to inactivation of said N^{pro}. Even more preferably, each of the attenuated BVDV is mutated in same genomic site of the E^{RNS} and/or the N^{pro} such that none of the attenuated BVDV can recombine with any of the others to eliminate the mutations within the E^{RNS} and/or N^{pro}, which are essential and responsible for the attenuation of the viruses. Even more preferably, the combination vaccine comprises attenuated BVDV type 1 and BVDV type 2, both having at least one mutation in the coding sequence for glycoprotein E^{rns} and at least another mutation in the coding sequence for N^{pro}, wherein said mutation in the coding sequence for glycoprotein E^{rns} leads to inactivation of RNase activity residing in E^{rns} and/or said mutation in the coding sequence for N^{pro} leads to inactivation of said N^{pro}. Most preferably, each of the attenuated BVDV is mutated in same genomic site of the E^{RNS} and the N^{pro} such that none of the attenuated BVDV can recombine with any of the others to eliminate the mutations within the E^{RNS} and/or N^{pro}, which are essential and responsible for the attenuation of the viruses.

Relevant bovine pathogens other than BVDV include but are not limited to: i) **pathogens of viral origin** such as 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 (PPV), 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, 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, and Streptococcus uberus **iii) pathogens of other origin,** such as Mycoplasma dispar, Mycoplasma bovis, and Ureaplasma spp., Tritrichomonas foetus, Trichophyton verrucosum, Trichophyton mentagrophytes, Trichophyton sarkisovii, Neospora caninum (formerly Toxoplasma gondii), Cryptsporidium parvum, Cryptsporidium hominis, Babesia bigemina and Babesia bovis, and Dictyocaulus viviparous (Lungworm disease).

Thus, the present invention relates to a combination vaccine for the treatment and /or prophylaxis of cattle against microbiological infections in cattle, wherein said vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and a further immunological active component effective for the treatment and/or prophylaxis of infections caused by 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), Bovie 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, 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, Cryptsporidium parvum, Cryptsporidium hominis, Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus uberus, 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).

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 said vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of 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 (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 hardjo, Leptospira pomona, and Leptospira grippotyphosa, Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo (Leptospira hardjoprajitno and 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, Cryptsporidium parvum, Cryptsporidium hominis, Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus uberus, 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)

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological 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 attenuated BVDV (types 1 and/or 2) 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 BVDV 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological 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 attenuated BVDV (types 1 and/or 2) 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **BRSV [combo 007]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of BRSV **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of BHV **[combo 010]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR and PI-3 [combo 011]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen 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 and reproductive

systems in cattle, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR and BRSV [combo 013]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR and BRSV **[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 and reproductive systems in cattle, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **PI-3 and BRSV [combo 017]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of PI-3 and BRSV **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the

treatment and/or prophylaxis of infections caused by **IBR, PI-3 and BRSV [combo 021]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3 and BRSV **[combo 022]**. Preferably, **all viral antigens are modified live viruses [combo 023]**. According to a further embodiment of said combination vaccine, the **IBR and PI-3 antigens are modified live viruses and BRSV antigen is a killed virus [combo 024]**. According to a further embodiment of said combination vaccine, the **IBR, PI-3 and BRSV antigens are killed viruses [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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, BRSV and BHV [combo 026]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, BRSV and BHV **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **PI-3, BRSV and BHV [combo 028]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of PI-3, BRSV 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3 and BHV **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV** and **BHV [combo 032]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV and BHV **[combo 033]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological 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 attenuated BVDV (types 1 and/or 2) 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 infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of H. somnus **[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 infections of the respiratory and reproductive systems in cattle, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV** and **H. somnus [combo 040]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV and H. somnus **[combo 041]**. Preferably, all viral antigens are modified live viruses. According to a further embodiment of said combination vaccine, the IBR and PI-3 antigens are modified live viruses, whereas the BRSV antigen is a killed virus **[combo 038]**. According to a further embodiment of said combination vaccine, the IBR, PI-3 and BRSV antigens are killed viruses **[combo 042]**. According to a further embodiment, any of said combination vaccines, preferably the combination vaccine that comprises killed IBR, killed PI-3 and killed BRSV as 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, BHV** and **H. somnus [combo 044]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BHV and H. somnus **[combo 045]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by one or more pathogenic **specie(s) of Leptospira**, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemmorrhagiae, Leptospira bovis, Leptospira interrogans and Leptospira pomona. **[combo 046]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigen of at least one or more pathogenic specie(s) of Leptospira, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno

and Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemmorrhagiae, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR,** and one or more pathogenic **specie(s) of Leptospira**, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemmorrhagiae, Leptospira bovis, Leptospira interrogans and Leptospira pomona. **[combo 048].** According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR and one or more pathogenic specie(s) of Leptospira, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemmorrhagiae, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, preferably a live modified virus, and 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3,** and one or more pathogenic **specie(s) of Leptospira**, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-

bovis), Leptospira prajitno, Leptospira icterohaemmorrhagiae, Leptospira bovis, Leptospira interrogans and Leptospira pomona **[combo 052]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3 and one or more pathogenic specie(s) of Leptospira, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemmorrhagiae, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV** and one or more pathogenic **specie(s) of Leptospira**, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemmorrhagiae, Leptospira bovis, Leptospira interrogans and Leptospira pomona **[combo 056]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV and one or more pathogenic species of Leptospira, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira

prajitno, Leptospira icterohaemmorrhagiae, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by IBR, PI-3, BRSV, **Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo (Leptospira hardjoprajitno and/or Leptospira hardjo-bovis), Leptospira icterohaemorrhagiae, and Leptospira pomona [combo 058].** According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, Leptospira canicola, Leptospira grippotyphosa, 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]**.

According to a further embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein, **live modified viruses of IBR, PI-3, BRSV,** 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 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, BHV** and one or more pathogenic **specie(s) of Leptospira**, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemmorrhagiae, Leptospira bovis, Leptospira interrogans and Leptospira pomona. **[combo 064]**. According to a more preferred

embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, BHV and one or more pathogenic specie(s) of Leptospira, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemmorrhagiae, Leptospira bovis, Leptospira interrogans and Leptospira pomona **[combo 065]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by one or more pathogenic **specie(s) of Leptospira**, as mentioned above, and **H. somnus [combo 066]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least antigen of one or more pathogenic species of Leptospira, as mentioned above, and H. somnus **[combo 067]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR and one or more pathogenic species of Leptospira, as mentioned above, and H. somnus **[combo 069]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen

of IBR, PI-3, and one or more pathogenic species of Leptospira, as mentioned above, and 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV**, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, and one or more pathogenic species of Leptospira, as mentioned above, and 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV,

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by IBR, PI-3, BRSV, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, BHV and one or more pathogenic species of Leptospira, as mentioned above, and 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least antigen of one or more pathogenic species of Leptospira, as mentioned above, and Campylobacter fetus **[combo 081]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Leptospira canicola, Leptospira grippotyphosa, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least antigen of Leptospira canicola, 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]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 **Campylobacter fetus [combo 086]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR and one or more pathogenic species of Leptospira, as mentioned above, and Campylobacter fetus**[combo 087]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, and one or more pathogenic species of Leptospira, as mentioned above, and Campylobacter fetus **[combo 089]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV**, and one or more pathogenic **specie(s) of Leptospira**, as mentioned above, and **Campylobacter fetus [combo 090]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, and one or more pathogenic species of Leptospira, as mentioned above, and 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, BHV and one or more pathogenic species of Leptospira, as mentioned above, and 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, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least antigen of one or more pathogenic species of Leptospira, as mentioned above, 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, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, one or more pathogenic species of Leptospira, as mentioned above, H. somnus and Campylobacter fetus **[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, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, and one or more pathogenic species of Leptospira, as mentioned above, H. somnus and Campylobacter fetus **[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, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen 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]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV**, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, and one or more pathogenic species of Leptospira, as mentioned above, H. somnus and Campylobacter fetus **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by IBR, PI-3, BRSV, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo (Leptospira hardjoprajitno and/or Leptospira hardjo-bovis), Leptospira icterohaemorrhagiae, Leptospira pomona, H. somnus and Campylobacter fetus **[combo 104]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, BHV and one or more pathogenic species of Leptospira, as mentioned above, H. somnus and Campylobacter fetus **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by BHV, BRSV, PI-3, IBR, Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii Leptospira hardjo (Leptospira hardjoprajitno and/or Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemmorrhagiae, Leptospira bovis, Leptospira interrogans and Campylobacter fetus **[combo 107]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV as described herein and at least one antigen of BHV, BRSV, IBR, PI-3, Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii Leptospira hardjo (Leptospira hardjoprajitno and/or Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemmorrhagiae, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused

by **Pasteurella haemolytica and Pasteurella multocida [combo 109]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of Pasteurella haemolytica bacterin and Pasteurella multocida bacterin. **[combo 110]** According to a further preferred embodiment, said combination vaccine comprises neomycin and thimerosal as preservatives **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, Pasteurella haemolytica and Pasteurella multocida [combo 112]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, preferably as live modified viruses, and Pasteurella haemolytica bacterin and Pasteurella multocida bacterin **[combo 113]**. According to a further preferred embodiment, said combination vaccine comprises neomycin and thimerosal as preservatives **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, preferably as live modified viruses, and Pasteurella haemolytica 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 combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, Pasteurella haemolytica and Pasteurella multocida [combo 118]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types

1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, preferably as live modified viruses, and 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, BHV, Pasteurella haemolytica and Pasteurella multocida [combo 121]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, BHV, preferably as live modified viruses, and Pasteurella haemolytica 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Mycoplasma bovis [combo 124]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least antigen of Mycoplasma bovis **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, and Mycoplasma bovis [combo 126]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least antigen of IBR, preferably as live modified viruses, and Mycoplasma bovis **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, and Mycoplasma bovis [combo 128]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, preferably as live modified viruses, and Mycoplasma bovis **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, and Mycoplasma bovis [combo 130]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, preferably as live modified viruses, and Mycoplasma bovis **[combo 131]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, BHV, and Mycoplasma bovis [combo 132]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, BHV, preferably as live modified viruses, and Mycoplasma bovis **[combo 133]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Pasteurella haemolytica, Pasteurella multocida and Mycoplasma bovis [combo 134]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of Pasteurella haemolytica, Pasteurella multocida and Mycoplasma bovis **[combo 135]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, Pasteurella haemolytica, Pasteurella multocida and Mycoplasma bovis [combo 136]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, preferably as live modified viruses, Pasteurella haemolytica, Pasteurella multocida and Mycoplasma bovis **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, Pasteurella haemolytica, Pasteurella multocida and Mycoplasma bovis [combo 138]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, preferably as live modified viruses, Pasteurella haemolytica, Pasteurella multocida and Mycoplasma bovis **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, Pasteurella haemolytica, Pasteurella multocida and Mycoplasma bovis [combo 140]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, preferably as live modified viruses, Pasteurella haemolytica, Pasteurella multocida and Mycoplasma bovis **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, BHV, Pasteurella haemolytica, Pasteurella multocida and Mycoplasma**

bovis [combo 140]. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, BHV, preferably as live modified viruses, Pasteurella haemolytica, Pasteurella multocida and Mycoplasma bovis **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, preferably as live modified virus, and 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, preferably as live modified viruses, and 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, Pasteurella haemolytica, Pasteurella multocida and H. somnus [combo 148]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **IBR, PI-3, BRSV, BHV, Pasteurella haemolytica, Pasteurella multocida and H. somnus [combo 150]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one antigen of IBR, PI-3, BRSV, BHV, preferably as live modified viruses, and 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 an immunological active component effective for the treatment and/or prophylaxis of infections caused by one or more pathogenic **species of Leptospira**, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo, Leptospira prajitno, Leptospira icterohaemmorrhagiae, 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 antigen of one or more specie(s) of Leptospira, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-bovis),, Leptospira prajitno, Leptospira icterohaemmorrhagiae, Leptospira bovis, Leptospira interrogans and Leptospira pomona. **[combo 153]**.

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 immunological active component effective for the treatment and/or prophylaxis of infections 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]**.

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 immunological active component effective for the treatment and/or prophylaxis of infections caused by one or more pathogenic **specie(s) of Leptospira**, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemmorrhagiae, 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 antigen of Campylobacter fetus and of one or more **specie(s) of Leptospira**, preferably selected from Leptospira canicola, Leptospira grippotyphosa, Leptospira borgpetersenii, Leptospira hardjo (Leptospira hardjoprajitno and Leptospira hardjo-bovis), Leptospira prajitno, Leptospira icterohaemmorrhagiae, 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, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological 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 attenuated BVDV (types 1 and/or 2) as 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, 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 an immunological 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, 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, 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, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) 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, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156 and 157]**, that further comprises an immunological active component 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, 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 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 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 antigen of Clostridium perfringens Types A, B, C, and/or D, and Clostridium tetani **[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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and antigens, preferably toxins, 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 an immunological active component 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 antigen 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, 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 antigen 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, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 Mycoplasma bovis [combo 174].** According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigens, preferably toxins, 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 vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and antigens, preferably toxins, of Clostridium chauvoei, Clostridium septicum, Clostridium novyi, Clostridium sordellii, and Clostridium perfringens Types C and D and H. somnus. **[combo 177].**

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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, Mycoplasma bovis, and H.somnus [combo 178].** According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigens, preferably toxins, 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].**

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by Salmonella, preferably **Salmonella dublin, Salmonella newport and**

Salmonella typhimurium [combo 180]. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and toxins of Salmonella, preferably Salmonella dublin, Salmonella newport, and Salmonella typhimurium **[combo 181]**.

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 and 179]**, that further comprises an immunological active component 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, 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 and 179], that further comprises antigen, preferably a toxin, of Salmonella, preferably **Salmonella dublin, salmonella newport and Salmonella typhimurium [combo 183]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Pasteurella haemolytica, Pasteurella multocida, Salmonella, preferably Salmonella dublin, Salmonella newport and Salmonella typhimurium [combo 184]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) 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 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Moraxella bovis and/or Klebsiella spp. , preferably Klebsiella pneumoniae [combo 187]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and toxins of **Moraxella bovis and/or Klebsiella spp. 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, 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, and 186]**, that further comprises an immunological active component effective for the treatment and/or prophylaxis of infections caused by infections caused by **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, 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, 174, 175, 176, 177, 178, 179, 180, 181, 184, 185 and 186], that further comprises antigen, preferably a toxin, of **Moraxella bovis and/or Klebsiella spp., preferably, 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Escherichia coli [combo 191]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and toxins of Escherichia coli **[combo 192]**.

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 immunological active component effective for the treatment and/or prophylaxis of infections caused by infections caused by Eschericia 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, 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 antigen, preferably a toxin, of Escherichia coli **[combo 194]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 attenuated BVDV (types 1 and/or 2) as described herein and **Pasteurella haemolytica, Pasteurella multocida, Salmonella dublin,Salmonella typhimurium and Escherichia coli** Bacterin-Toxoid **[combo 196]**. According to more preferred embodiment, 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, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **bovine Rotavirus [combo 198]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigen of bovine Rotavirus **[combo 199]**.

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, 195, 196, and 197]**, that further comprises an immunological 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 [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 **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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **bovine Coronavirus [combo 202]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) 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 immunological 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, 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].**

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **bovine Coronavirus and bovine Rotavirus [combo 206]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigen 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, 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 immunological active component effective for the treatment and/or prophylaxis of infections caused by 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, 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 antigen of bovine Coronavirus and bovine Rotavirus **[combo 209]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Cryptosporidium parvum [combo 210]**. According to a preferred

embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) 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, 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 immunological 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 attenuated BVDV (types 1 and/or 2) as described herein and at least

one further immunological 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 attenuated BVDV (types 1 and/or 2) 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 immunological active component effective for the treatment and/or prophylaxis of infections caused by 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, 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 hominis **[combo 217]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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 vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigen of Cryptosporidium parvum and Cryptosporidium hominis **[combo 219]**.

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 immunological active component 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, 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 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Mycobacterium avium paratuberculosis [combo 222]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) 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, 184 184, 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 immunological 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, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological 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 attenuated BVDV (types 1 and/or 2) as described herein and antigen of Adenovirus **[combo 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, 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, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224 and 225]**, that further comprises an immunological active 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, 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]**.

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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Astrovirus [combo 230]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) 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, 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, 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 an immunological 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological 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 attenuated BVDV (types 1 and/or 2) 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, 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 immunological 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 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, 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 vaccine for the treatment and/or prophylaxis of cattle against microbiological infections in cattle, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Cryptosporidium parvum, Adenovirus, Astrovirus, bovine Parvovirus and Mycobacterim avium paratuberculosis [combo 238]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigen of Cryptosporidium parvum, Adenovirus, Astrovirus, bovine Parvovirus and Mycobacterim 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, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component 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, bovine Parvovirus and Mycobacterim avium paratuberculosis [combo 240]. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigen of Escherichia coli, Salmonella spp., preferably Salmonella dublin, Salmonella typhimurium and Salmonella newport, bovine rotavirus and bovine Coronavirus, Cryptosporidium parvum, Adenovirus, Astrovirus, bovine Parvovirus and Mycobacterim 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Streptococcus spp., preferably Streptococcus uberus and /or Streptococcus dysgalactiae [combo 242]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigen of Streptococcus spp., preferably Streptococcus uberus and/or Streptococcus dysgalactiae, **[combo 243]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigen of Streptococcus spp., preferably Streptococcus uberus 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, wherein the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Streptococcus spp., preferably Streptococcus uberus and /or Streptococcus dysgalactiae and/or Staphylococcus aureus [combo 245]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigen of Streptococcus spp., preferably Streptococcus uberus 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, 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, 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 an immunological active component effective for the treatment and/or prophylaxis of infections caused by infections caused by **Streptococcus spp., preferably Streptococcus uberus and/or Streptococcus dysgalactiae, and/or Staphylococcus aureus [combo 247]**. 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, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240 and 241], that further comprises antigen **of Streptococcus spp., preferably Streptococcus uberus 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, 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, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240 and 241], that further comprises antigen **of Streptococcus spp., preferably Streptococcus uberus 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Streptococcus spp., preferably Streptococcus uberus and/or Streptococcus dysgalactiae, Staphylococcus aureus, Klebsiella spp. and Mycoplasma spp. [combo 250]**. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigen of **Streptococcus spp., preferably Streptococcus uberus, Streptococcus dysgalactiae, and/or Streptococcus aureus, Klebsiella spp. and Mycoplasma spp. [combo 251]**. According to a more preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigen of **Streptococcus spp., preferably Streptococcus uberus 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 attenuated BVDV (types 1 and/or 2) as described herein and at least one further immunological active component effective for the treatment and/or prophylaxis of infections caused by **Trichophyten and Microsporus**, preferably selected from **Trichophyton verrucosum, Trichophyton mentagrophytes, Trichophyton equinum, Trichophyton sarkisovii, Microsporum canis, Microsporum canis var. obesum, Microsporum canis var. distortum, and**

Microsporum gypseum [combo 253]. According to a preferred embodiment, the combination vaccine comprises attenuated BVDV (types 1 and/or 2) as described herein and antigen of Trichophyten, **and Microsporus**, preferably selected from Trichophyton verrucosum, Trichophyton mentagrophytes, Trichophyton equinum, Trichophyton sarkisovii, Microsporum canis, Microsporum canis var. obesum, Microsporum canis var. distortum, and Microsporum gypseum **[combo 254]**.

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, 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 an immunological active component effective for the treatment and/or prophylaxis of infections caused by infections caused by **Trichophyten and Microsporus**, preferably selected from **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 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, 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 antigen of Trichophyten and Microsporus, preferably selected from Trichophyton verrucosum, Trichophyton mentagrophytes, Trichophyton equinum, Trichophyton sarkisovii, Microsporum canis, Microsporum canis var. obesum, Microsporum canis var. distortum, and Microsporum gypseum **[combo 256]**.

According to further embodiment, the source of the combination vaccine is, Alpha 7™, ALPHA-7/MB™, ALPHA-CD™, BAR-VAC® 7, BAR-VAC® 7/SOMNUS, BAR-VAC® 8, BAR-VAC® CD, BAR-VAC® C/DT, BREED-BACK™ FP 10, BREED-BACK™ FP 10 HS, BREED-BACK™ FP 5, BREED-BACK™ FP 5 HS, BREED-BACK-10™, CALIBER® 3, CALIBER® 7, ELITE 4™, ELITE 9™, ELITE 9-HS™, EXPRESS®10, EXPRESS®10-HS, EXPRESS® 3, EXPRESS® 3/Lp, EXPRESS 4®, EXPRESS® 5, EXPRESS® 5-HS, EXPRESS® 5-PHM, EXPRESS® I, EXPRESS® I/LP, OCU-GUARD® MB, PULMO-GUARD™ MpB, PULMO-GUARD™ PH-M, PULMO-GUARD™ PH-M/SDT, PULMO-GUARD™ PHM-1, TETGUARD™, VIBRIO-LEPTO-5™ (all of **Boehringer Ingelheim, St. Joseph, MO**); Cobalt™ 7, I-Site™, Lepto 5, Master Guard® Preg 5, Master Guard® 10, Master Guard® 10 + Vibrio, Master Guard® J5, P.H.M. Bac® 1, Pre-vent 6™, 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™ 4, Scour Vac™ 9, Scour Vac™ E coli + C, Somnumune®, Titanium™ 3, Titanium™ 4, Titanium™ 4 L5, Titanium™ 5, Titanium™ 5 L5, Titanium® 5+P.H.M. Bac®-1, Titanium™ BRSV 3, Titanium™ IBR, Titanium™ IBR-LP (all of **Agri Laboratories Inc., St. Joseph, MO**); Herd-Vac® 3, Herd-Vac® 3 S, Herd-Vac® 8, Herd-Vac® 9, Surround™ 4, Surround™ 4+HS, Surround™ 8, Surround™ 9, Surround™ 9+HS, Surround™ HS, Surround™ L5, Surround™ 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 + 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 (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, 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 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, 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® 7, 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-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®, Virabos™-4 with Immunostim® (all of **Bioniche Animal Health, Athens, GA**), wherein attenuated BVDV, as described herein, is added. Alternatively, when BVDV antigen is present in any of those vaccines, attenuated BVDV, as described herein, is added, or BVDV present any of those vaccines is substituted by attenuated BVDV, as described herein.

Formulations:

An important apect of the present invention is the preparation of the combination vaccine(s). The skilled person knows additional components which may be comprised in said composition (see also Remington's Pharmaceutical Sciences. (1990). 18th ed. Mack Publ., Easton). The expert may use known injectable, physiologically acceptable sterile solutions. 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 pharmaceutical compositions 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 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. Stabilizers include albumin and alkalisalts of ethylendiamintetracetic 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 (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 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, the present invention contemplates compositions comprising from about 50 ug to about 2000 ug of adjuvant and preferably about 250 ug/ ml dose of the vaccine composition. In another preferred embodiment, the present invention contemplates vaccine compositions comprising from about 1ug/ml to about 60 ug/ml of antibiotics, and more preferably less than about 30 ug/ml of antibiotics.

For example, in a composition according to the invention, 10^4 to 10^6 $TCID_{50}$ of attenutated BVDV may be solved in 25% (v/v) SGS (Sucrose 75 mg, Gelatine 20 mg, Potassium hydroxide 0.274 mg, L- glutamic acid 0.72 mg, Potassium dihydrogen phosphate 0.516 mg, Dipotassium phosphate 1.254 mg, and 2 ml with water for injection), and 5 % (v/v) cell culture medium, and 1 ml with water for injection. This is further mixed with at least one further antigen of a bovine pathogen, as listed above.

According to a further embodiment the combination vaccine 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).

Dose and administration:

According to the present invention, an effective amount of a combination vaccine administered to cattle, including pregnant cows and calves nursing pregnant cows, provides effective immunity against microbiological infections caused by BVDV and at least one further pathogen as listed above. Prefered combinations of antigens for the treatment and prophylaxis of microbiological diseases in cattle are listed above.

Acording to one embodiment, the combination vaccine is administered to calves in two doses at an interval of about 3 to 4 weeks. For example, the first administration is performed when the animal is about 1 to about 3 months of age. The second administration is performed about 1 to about 4 weeks after the first administration of the combination vaccine. According to a further embodiment, revaccination is performed in an interval of 6 to 12 month after administration of the second dose. In a preferred embodiment, the first administration is performed about 5 weeks prior to animal breeding. The second administration is performed about 2 weeks prior to animal breeding. Administration of subsequent vaccine doses is preferably done on a 6 month to an annual basis. In another preferred embodiment, animals vaccinated before the age of about 6 months should be revaccinated after 6 months of age. Administration of subsequent vaccine doses is preferably done on an annual basis.

The amount of combination vaccine that is effective depends on the ingredients of the vaccine and the schedule of administration. Typically, when an inactivated virus or a modified live virus preparation is used in the combination vaccine, an amount of the vaccine containing about 10^2 to about 10^9 $TCID_{50}$ per dose, preferably about 10^3 to about 10^8 $TCID_{50}$ per dose, more preferably, about 10^4 to about 10^8 $TCID_{50}$ per dose. For example, about 10^5 to about 10^8 $TCID_{50}$ 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 modivied viruses. Typically, when bacterial antigen is used in the combination vaccine, the vaccine contains an amount of about 10^5 to about 10^9 colony forming units (CFU) per dose, preferably, about 10^4 to about 10^8 (CFU) per dose, more preferably about 10^5 to about 10^6 (CFU) per dose.

In the event, the combination vaccine comprises live modified IBR, the amount of IBR antigen is preferably in a range of about 10^5 to $10^{7.5}$ $TCID_{50}$ 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 $TCID_{50}$ 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 $10^{6.5}$ $TCID_{50}$ per dose. In the event, the combination vaccine comprises killed IBR, the amount of IBR antigen is preferably in a range of about $10^{7.0}$ to $10^{9.0}$ $TCID_{50}$ 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 $10^{9.2}$ $TCID_{50}$ 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 $10^{7.5}$ $TCID_{50}$ 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 the amount of H. somnus antigen is preferably in a range of about $10^{6.0}$ to 10^{9} (CFU) per dose.

The composition according to the invention may be applied intradermally, intratracheally, intravaginally, intramuscularly or intranasally, and preferably intramuscularly or intranasally. In an animal body, it can prove advantageous to apply the pharmaceutical compositions as described above via an intravenous or by direct injection into target tissues. For systemic application, the intravenous, intravascular, intramuscular, intranasal, intraarterial, intraperitoneal, oral, or intrathecal routes are preferred. A more local application can be effected subcutaneously, intradermally, intracutaneously, intracardially, intralobally, intramedullarly, intrapulmonarily or directly in or near the tissue to be treated (connective-, bone-, muscle-, nerve-, epithelial tissue). Depending on the desired duration and effectiveness of the treatment, the compositions according to the invention may be administered once or several times, as well as intermittently, for instance on a daily basis for several days, weeks or months and in different dosages.

Methods for treatment:

Yet another important embodiment of the invention is a method for the prophylaxis or treatment of diseases caused by BVDV, and further bovine pathogenic microorganism(s), wherein an attenuated BVDV as described herein and further immunological active components effective for the treatment and/or prophylaxis of the infection caused by said further bovine pathogenic microorganism is administered to an animal in need thereof at a suitable dose, as known to the skilled person.

EXAMPLES

The following examples serve to further illustrate the present invention; but the same should not be construed as limiting the scope of the invention disclosed herein.

EXAMPLE 1

BVDV XIKE-B: foetopathogenicity assessment in pregnant heifers

BVDV XIKE-B, an RNase negative mutant of the highly pathogenic BVDV type 2 isolate NewYork'93/C was recovered from the infectious cDNA clone pKANE40B and showed wild type-like (wt-like) growth characteristics in tissue culture. In animal experiments, the mutant virus was found to be considerably attenuated so that it represented a promising candidate for development of a live attenuated vaccine virus (Meyer et al., 2002). To test whether this attenuated virus is still able to cross the placenta and infect the fetus, pregnant heifers were infected with XIKE-B. As a control, wild type BVDV recovered from cDNA clone pKANE40A was used. The respective virus named XIKE-A expresses an active E^{rns} RNase in the infected cell. The study aimed to assess the safety of XIKE-A and XIKE-B in pregnant animals.

EXPERIMENTAL DESIGN

Ten pregnant heifers were selected from a BVDV negative herd. The following groups of 5 heifers were included in the trial:

	No.	Inoculation	Virus
Group 1:	5	One i. n. administration, 3 ml in each nostril	XIKE-A
Group 2:	5	One i. n. administration, 3 ml in each nostril	XIKE-B

Heifers were moved to the experimental facilities 8 days before inoculations. Pregnancy status was confirmed after transport into the experimental facility. Heifers were between days 60 and 90 of gestation on the day of inoculation. Inoculation took place for all animals at one point of time.

Heifers were monitored for the presence of clinical signs of BVDV infection including abortions during the observation period. Blood samples were collected from the animals for serology, antigen detection and white blood cells were counted. The experiment was terminated 9 weeks after infection. Non-aborted cows were slaughtered, the uterus examined and collected. Fetal organ samples were collected during routine necropsy and examined for BVDV infection.

The presence of fetal infection was the main evaluation parameter, composed from the number of BVDV-related cow mortalities, the number of BVDV-related abortions and the number of BVDV positive fetuses at termination. In addition to the main parameter, clinical signs characteristic for

BVDV infection, viraemia and white blood cell counts in cows, and rectal temperature after challenge were evaluated.

Animals

Heifers were purchased from a farm free of BVDV. Only animals, which met the following inclusion criteria, were used.

Inclusion criteria

- Free of BVD antibodies; each individual was tested in the serum antibody test prior to transport and at the initiation of the study (at the animal test facility).
- Free of BVDV; plasma and/or buffy-coat preparation from each individual was tested by a suitable test.
- Clinically healthy at the initiation of the study judged upon physical examination. The health examination of the animals was accomplished in accordance with the current, generally accepted veterinary practice.
- Pregnancy confirmed by physical examination before inoculation. Pregnancy was between 60 - 90 days at the time of inoculation, proven by insemination records.

Test Strain A

Description:	XIKE A, live virus BVDV strain
Composition:	Experimental material comprising of cell culture supernatant of low passaged XIKE-A
BVD components:	BVDV type II strain: XIKE-A
Supplied by:	Dr. Gregor Meyers, "Bundesforschungsanstalt für Viruskrankheiten der Tiere" (BFAV), Paul-Ehrlich-Straße 28, 72076 Tübingen, Germany
Applied BVD virus dose:	Type 1 strain:10^5 $TCID_{50}$/6ml (TCID = Tissue Culture Infective Dose)
Applied vaccine volume:	3 ml per nostril
Application route:	Intranasal
Preparation of dosage form:	The inoculum was sent in a pre-diluted frozen form in a 50 ml vial on dry ice and was stored at –70 °C before inoculation. Immediately before inoculation of Group 1 heifers, the material was thawed avoiding local temperatures above 37 °C. After no ice was visible in the fluid, material was gently stirred and immediately used for inoculation of the animals.
Unused inoculum:	The volume of the unused material was measured

	and split on two aliquots before immediate freezing in dry ice or liquid nitrogen and stored for re-titration purposes. Virus and contaminated plastic or glassware were incubated with an appropriate volume of an 8 – 10 % formaldehyde solution for at least 24 hours at room temperature before discarding in order to inactivate viruses.

Test Strain B

Description:	XIKE B, live virus BVDV strain
Composition:	Experimental material comprising of cell culture supernatant of low passaged XIKE-B
BVD components:	BVDV type II strain: XIKE-B
Supplied by:	Dr. Gregor Meyers, "Bundesforschungsanstalt für Viruskrankheiten der Tiere" (BFAV), Paul-Ehrlich-Straße 28, 72076 Tübingen, Germany
Applied BVD virus dose:	Type I strain: 10^5 $TCID_{50}$/6ml (TCID = Tissue Culture Infective Dose)
Applied vaccine volume:	3 ml per nostril
Application route:	Intranasal
Preparation of dosage form:	The inoculum was sent in a pre-diluted frozen form in a 50 ml vial on dry ice and was stored at –70 °C before inoculation. Immediately before inoculation of Group 2 heifers, the material was thawed avoiding local temperatures above 37 °C. After no ice was visible in the fluid, material was gently stirred and immediately used for inoculation of the animals.
Unused vaccine:	The volume of the unused material was measured and split on two aliquots before immediate freezing in dry ice or liquid nitrogen and stored for re-titration purposes. Virus and contaminated plastic or glassware was incubated with an appropriate volume of an 8 – 10 % formaldehyde solution for at least 24 hours at room temperature before discarding in order to inactivate viruses.

Pregnancy Control

Pregnancy was confirmed immediately before inoculation.

Inoculation of Heifers

The date of inoculation is Day 0 of the experiment.

In each nostril, 3 ml of the test material was administered intranasally by syringe without needle.

Each time a new sterile syringe was taken. Administration was performed during the aspiration phase in order to minimize loss of fluid via expiration of material.

Post-Inoculation Observations

Collection and examination of blood samples

Blood was collected following standard, aseptic procedures (disinfecting the bleeding site). A new sterile syringe and needle was used for each animal.

Blood collection to prepare serum

At least 10 ml blood was collected from the heifers immediately before inoculation, then weekly after infection and at the termination of the study. Serum was stored at –20 °C until required.

Blood collection for leukocyte counts and buffy coat preparations

For leukocyte counting, 3 ml blood was transferred immediately after collection to suitable sterile vessels (Venoject, Terumo Europe N.V., Leuven, Belgium), pre-filled with 0.06 ml EDTA (0.235 MOL/L).

For buffy coat preparations, at least 15 ml blood was transferred immediately after collection to suitable sterile vessels, pre-filled with 0.1 ml Heparin solution (Na-heparin for inj., 5 000 IU/ml lot. A7B163A exp. date: 11/2000: Gedeon Richter RT, Budapest, Hungary) yielding at least 20 IU Heparin per ml blood in the blood sample. The content was carefully mixed thereafter.

For preparation of buffy coats and leukocyte counting, blood was collected from the heifers

- on every day, between Day 0 and Day 14 after infection;
- on every second day, between Day 15 and Day 40, or until all animals were negative for virus isolation for three consecutive sampling time points.

Preparation of serum

Blood was allowed to clot at room temperature, and separated by centrifugation. Each serum sample was divided into two aliquots of at least 2 ml each. One set of aliquots was assayed for BVDV specific antibodies by ELISA. The rest of the sera was frozen and stored at –20 °C until required.

Leukocyte counts

Leukocyte counts were determined with a coulter-counter semi-automated electronic device (Diatron Minicell-16, Messtechnik GmbH, Wien, Austria) with a claimed accuracy of 0.1 x 10^9 / l, 100 / µl. The instrument was used (calibration and leukocyte-counts) according to the manufacturer's recommendations.

Preparation of buffy coats

Heparin blood samples were transported to the laboratory as soon as possible. Buffy coat preparation procedure, following a standard laboratory procedure was performed under aseptic conditions (sterile pipettes, handling, clean bench etc.).

The obtained buffy coats were re-suspended in a small volume (2 ml) of RPMI 1640 and frozen at – 70 °C in two aliquots of 0.5 ml. The residual 1ml buffy coats were immediately used for determination of blood cell associated BVDV by co-cultivation in a permissive cell culture.

BVD serum antibody ELISA-test

Each serum sample was tested for the presence of BVDV-antibodies using a suitable and validated ELISA test (Svanovir™ BVDV antibody test Cat# 10-2200-10). Each test was validated and performed according to the manufacturer's recommendations. Positive samples were diluted according to the $\log_2$ scale to determine BVDV antibody titers.

BVD antigen assay(s)

Each buffy coat sample was assayed for the presence of BVDV by co-cultivation of the freshly prepared buffy-coats with susceptible cells or a cell-line. No freezing was allowed before co-cultivation.

Plasma was collected and provided to Man-Gene from each sample.

Clinical observations

Observation of heifers

Animals were examined daily from Day 0 - 42 post inoculation for the presence of clinical symptoms by a sufficiently trained veterinarian.

All clinical signs were recorded and described by their nature, consistence/touch, severity (mild, medium or severe) location, size of the area affected, and they were scored according to agreed and standard definitions. Special attention was paid to respiratory signs (respiration, its rate; nasal or ocular discharge; conjunctivitis, sneezing, coughing, etc.) and diarrhea.

Rectal temperatures

Rectal temperatures were measured daily in each heifer, at the same hour of the day (preferably in the morning) from 5 days prior to the inoculation until 21 days post infection.

Daily measurement of rectal temperature was continued until each animal had rectal temperatures below or equal to 39 °C for at least 3 consecutive days.

Detection of interrupted pregnancy

Pregnancy was confirmed and suspicion for abortion or resorption of the fetus was established by rectal examination. A trained veterinarian examined all animals at inoculation, 1, and 2 months post-inoculation. The examination was carried out according to the generally accepted veterinary practice.

Heifers were examined daily for any sign of abortion until termination of the study (8 - 12 weeks post-challenge).

Termination of the Study

The study was terminated by slaughtering the heifers and extracting the fetuses. Fetuses and fetal material were transferred into closed transport containers marked with the number of the cow and the date/time. Containers were transported to a selected necropsy room.

Necropsy of the heifers was not required. Necropsy was performed on fetuses, findings recorded and a panel of samples collected as described below.

Post-Mortem Examination

A detailed necropsy of the experimental animals was done in each case of death. Post-mortem examinations were carried out by an experienced veterinary surgeon and the data were recorded on appropriate data sheets. Further laboratory tests were performed according to the clinical signs and lesions observed. If the diagnosis of the necropsy referred to a disease caused by microbial agent

the diagnosis was verified by an appropriate test, specific for the agent. Each tissue sample was collected in at least 2 separate, labeled containers and snap-frozen in liquid nitrogen. Samples were stored at –70 °C until required.

Aborted fetuses and study termination

At least the following tissue samples were collected from the fetuses:

- exudate from the peritoneal cavity or thorax, if present,
- mesenteric lymph nodes,
- spleen,
- thymus,
- cerebellum,
- kidney,
- bone marrow from the sternum,
- sample from the placenta, if available.

Dead or sacrificed heifers

At least the following tissue samples were collected:

- blood for buffy coat, if available,
- blood for serum, if available,
- Peyer's patches,
- mesenteric lymph nodes,
- spleen,
- kidney,
- uterus, including a sample from the placenta, if available.

Storage and Transport of Samples

Samples:	Storage:
Serum	–20 °C
Buffy coat	–70 °C
Virus	–70 °C
Tissue from heifers	–70 °C
Tissue from fetuses	–70 °C

Samples were sent for laboratory analysis as required by the sponsor. The choice of samples and the timing of transport were agreed with the study monitor or the project manager. As a matter of

general principle, samples coming from aborted material or from new-born calves were investigated as soon as possible.

RESULTS

Summary of BVD related clinical and laboratory data

Group 1

Animal No.	Conclusion	BVD
526	BVD abortion (uterus with placenta post-mortem)	NT (no sample found)
598	BVD abortion (foetus post-mortem)	+ (foetus)*
615	Clinical BVD abortion	- (foetus)*
618	BVD abortion (foetus post-mortem)	- (foetus)*
626	Died due to BVD	+ (foetus) / + (heifer)

NT: not tested

Group 2

Animal No.	Conclusion	BVD
469	Clinical BVD abortion	- (foetus)*
565	Expected BVD abortion; non-viable foetus	+ (foetus)
588	Normal	- (foetus)
608	Normal	+ (foetus)
619	BVD abortion (foetus post-mortem)	- (foetus)*

* Foetuses were autolysed at the time of sampling

Conclusion:

The study aimed to assess the safety of XIKE-A and XIKE-B in pregnant animals. Ten pregnant heifers were selected from a BVDV negative herd. Two groups of 5 heifers were included in the trial: one was inoculated with XIKE-A the other with XIKE-B virus strain. Heifers were between days 60 and 90 of gestation on the day of inoculation. Heifers were monitored for the presence of clinical signs of BVDV infection including abortions during the observation period. Blood samples were collected from the animals for serology, antigen detection and white blood cells were counted. The experiment was terminated 9 weeks after infection. Non-aborted cows were slaughtered, the uterus examined and collected. Fetal organ samples were collected during routine necropsy and examined for BVDV infection.

The XIKE-B virus proved to be less pathogenic than XIKE-A, nevertheless BVD related abortion and infection of the foetus was observed in the XIKE-B group, too. Therfore it can be concluded that the inactivation of the E^{rns} RNase does not prevent fetal infection.

EXAMPLE 2

BVDV XIKE-A-NdN: foetopathogenicity assessment in pregnant heifers

The N^{pro} gene has been shown to be nonessential for growth of CSFV in tissue culture (Tratschin et al., 1998). Even though a proof for BVDV attenuation in consequence of N^{pro} deletion is still missing, a role of this protein in the interaction between virus and host seemed to be possible and was actually indicated by recent experiments for CSFV (Mayer et al., 2004; Rüggli et al., 2003). We therefore wanted to investigate, whether the deletion of the major part of the N^{pro} coding sequence leads to a virus that no longer infects the fetus in pregnant heifers. The N^{pro} gene, except for the 5' terminal 4 codons, was deleted from the full length cDNA clone pKANE40A according to standard procedures. The resulting mutant full length clone was used as template for in vitro transcription and the resulting cRNA was transfected into MDBK cells as described (Meyer et al., 2002). The recovered virus was amplified in tissue culture and then used in the animal experiment described below. BVDV XIKE-B served as a control since it was shown before that it is able to cross the placenta (EXAMPLE 1).

OBJECTIVE(S)/PURPOSE OF THE STUDY

The study aims to assess the safety of a live attenuated BVDV with a genomic deletion of most of the N^{pro} coding region in pregnant animals.

Material and Methods applied are described in Example 1

STUDY DESIGN

Eight pregnant heifers were assigned at random to two groups. They were treated and observed according to the following schedule:

	Group1	Group 2
N	5	3
Treatments	XIKE-A-NdN	XIKE-B / control
Route	Intramuscular	

Vaccination time	between days 60 and 90 of pregnancy (day 0 of the study)
Observations Post-vaccination (in life)	• Clinical signs • Serum at days 0, 14, 28, 42 and at termination • WBC at day 0 and then daily for 14 days • Buffy coat at day 0 and then daily for 14 days
Post-mortem (day 60)	• Gross-pathology • Organ panel for virus isolation

Type of study:	open controlled clinical study
Experimental unit:	Individual animal
Method of blinding:	Partial blinding. No detailed procedures for blinding and access to treatment schedule were applied. The observing veterinarian at the study location and the pathologist were not be aware of the treatment; they only received a protocol extract relevant to their tasks. Vaccination was performed by the investigator or his assignee. Samples for virus isolation were coded by the investigator until all results are available.

RESULTS

All heifers were healthy and pregnant at study start. All animals proved to be free of BVDV and BVDV antibodies before the initiation of the study.

Preparation and Control of the Virus used for the Infection

Samples were collected throughout the dilution steps and assayed on the day of preparation, i.e. without freezing by co-cultivation on suitable tissue culture. The results of virus titration are shown in the following table.

Sample ID	Virus strain	Dilution / description	Log_{10} titre/ml
VT1a	XIKE-A/NdN (S)	1:2 (at 4 °C)	4.4
VT1b		#2a on ice without opening	4.0
VT1c		Return of #2b	2.8
VT2a	XIKE-B	1:2.2 (at 4 °C)	2.3
VT2b		#3a on ice without opening	2.8
VT2c		Return of #3b	Negative

Clinical Symptoms of BVDV Infection

The table below gives a summary about the animals that had clinical signs during the observation period.

Clinical signs and the days post inoculation (DPI) when they were observed

Group 1 (XIKE-A NdN) Group 2 (XIKE-B)

Clinical sign	Animal ID	Animal ID	
	1583	1438	1585
Loss of appetite	8	-	10
Lachrymation	-	-	-
Conjunctivitis	-	-	-
Nasal discharge	-	-	-
Oral erosion	-	-	-
Oral haemorrhage	-	-	-
Diarrhoea	-	-	-
Coughing	-	12	10-13
Abnormal breathing	-	-	-
Elevated respiratory rate	-	-	-
Hoof erosion	-	-	-

Only mild and transient clinical signs were observed in some of the animals in each group.

In Group 1, one out of the 5 heifers had loss of appetite on day 8 PI. In Group 2, two out of the 3 animals had clinical signs. Both heifers experienced coughing around day 21 PI that was accompanied with loss of appetite in one of the animals.

Rectal Temperatures

No abnormal temperature changes were detected before the inoculation of the animals. The few cases of elevated temperatures measured after the inoculation are summarised in the table below.

Group	Animal ID	Temperature (°C)	PI day
1	1583	39.9	8
	1621	39.0	5
2	1438	39.0	2
	1585	40.8	9

One animal in each group had slightly elevated temperature, and also one animal in each group had fever. Fever was detected on day 8 or 9 PI. Temperature values always returned to normal values on the following day.

Leukocyte Counts

Some leukopenia was observed in all groups between PI days 3 – 8. The number of animals with at least 40 % reduction in white blood cell count is noted below:

Group	Number of animals having leukopenia / total
1	3/5 (60%)
2	1/3 (33%)

Serology (BVDV antibodies)

In compliance with the study protocol, all heifers were free of BVDV antibodies before vaccination. In Group 1 (inoculated with XIKE-A NdN) and Group 2 (inoculated with XIKE-B), complete seroconversion was detected only at study termination (2 months after inoculation).

BVD virus isolation from buffy coats

No viremia was detected

BVD virus isolation from fetal tissue samples

	Group 1	Group 2
N	5	3
Treatments	XIKE-A-NdN	XIKE-B / control
Route	Intramuscular	Intramuscular
Number of fetuses in which fetal transmission was detected:	4 out of 5 foetuses infected	2 out of 3 foetuses infected
Conclusion of the virus used for treatment has the potential to be transmitted over the placenta:	Fetal transmission for XIKE-A-NdN observed	Fetal transmission for XIKE – B observed

Conclusion

The N^{pro} deletion resulted in a considerable attenuation of the BVDV in comparison to the parental virus XIKE-A that was shown to be highly pathogenic (Meyer et al., 2002). However, the N^{pro} deletion alone is not preventing transmission of a NY93 based virus recombinant to the foetus after inoculation of pregnant cows.

EXAMPLE 3

BVDV XIKE-B-NdN: foetopathogenicity assessment in pregnant heifers

To be able to test the potential of a combination of RNase inactivation and N^{pro} deletion with regard

to BVDV attenuation and fetal transmission, different BVDV-2 mutants with deletions within the N^{pro} coding region were established based on the infectious cDNA clone pKANE40B, the RNase negative mutant of pKANE40A with a deletion of codon 349. The recovered viruses were analyzed with regard to presence of the desired mutations, the absence of second site mutations in the regions flanking the introduced changes and their growth characteristics in tissue culture. XIKE-B-NdN (V-pK88C), a variant containing a deletion of the complete N^{pro} coding region except for codons 1 to 4 in addition to the RNase inactivating deletion of codon 349 was chosen for an animal experiment since it combined the desired mutations with acceptable growth characteristics. The aim of the study was to assess the safety of a live attenuated BVDV isolate in pregnant animals.

Five BVDV-negative, pregnant heifers were inoculated intranasally with an infective dose of 10^5 $TCID_{50}$/animal XIKE-B-NdN. Clinical data were recorded daily. Blood samples were collected for white blood cell counting, buffy-coat preparation, and serology. After termination of the study fetal tissues were collected for virus isolation.

MATERIAL AND METHODS:

As detailed for example 1:

RESULTS

No clinical data were observed (data not shown). Leukocyte counts remained virtually unchanged except for a significant decrease by approximately 40% below the baseline value (day0) in heifer no- 1015 on a single day (day 6 p.i.) (data not shown).

a) Analysis of buffy coat preparations:

Approximately 10^6 leukocytes were cultured in duplicates with MDBK-cells in 24-well tissue culture plates for 5 days. Samples were freeze-thawed twice. One hundred microliter aliquots of thawed samples were inoculated onto freshly seeded 24-well tissue culture plates and tested for virus by indirect immuno-fluorescence staining (mAb Code 4, directed against a conserved epitope in nonstructural protein NS3). No BVDV could be isolated from the buffy coat preparations of animals # 921, 1013, 1015, 1055 and 1075, whereas positive controls clearly showed the correct conduction of the test.

b) Post-mortem examination of fetal tissues

After termination of the study the following fetal tissues were collected for virus isolation: spleen, kidney, thymus, sternum, cerebellum, placenta, intestine and abdominal fluid. Briefly, tissue

suspensions were made in a mortar using sterile sea sand and ice-cold PBS without Ca^{2+} and Mg^{2+}. Mortars were rinsed with 1 ml ice-cold PBS without Ca^{2+} and Mg^{2+} and suspensions were centrifuged for 10 minutes at 2000 x g (4°C). The supernatant was first passed through a disposable 0.45 µm filter holder, followed by a second filter passage (0.2 µm pore size). Virus isolation was carried out in duplicate (400 µl fetal tissue suspension or 100 µl fetal abdominal fluid) on a monolayer of MDBK-cells in a 24 wells tissue culture plate (37°C, 7% CO2). Tissue samples were controlled daily for cytopathic effects or bacterial contamination, and after an incubation time of 5 days, plates were frozen and thawed twice. 100 µl of samples were passaged to freshly seeded MDBK-cells. Virus was detected by indirect immuno-fluorescence staining (mAb Code 4). No BVDV could be detected in the tissue samples or fetal abdominal fluid.

c) Serological Findings

Serum neutralization titres were determined before inoculation, 1 month post-inoculation and at termination of the study. Sera from all animals were tested in triplicate for neutralizing antibodies against NY93/C, and the endpoint dilution was read by indirect immunofluorescence staining. Results were expressed as the endpoint dilution, which neutralized approximately 100 $TCID_{50}$ and calculated by the method of Kaerber. No definite data could be obtained for day 0, and 1 and 2 weeks post infection as the sera were toxic for MBDK-cells in dilutions up to 1:16 and no neutralization could be detected at higher dilutions. Starting with the third week post vaccination all animals developed neutralizing antibodies against the homologous BVDV-2 virus NY'93/C lasting till the end of the experiment (Fig. 1).

d) Conclusions

The data obtained during the animal study clearly show that BVDV XIKE-B-NdN represents a highly attenuated virus. In contrast to wild type virus or the single mutants XIKE-B or XIKE-A-NdN that show fetal transmission in pregnant heifers at high rates the double mutant did not cross the placenta. BVDV XIKE-B-NdN as well as similar double mutants are extremely suitable for use in a live attenuated vaccine.

Efficacy and crossprotection study

Two possible problems have to be faced with regard to vaccination with attenuated virus mutants BVDV XIKE-B or BVDV XIKE-B-NdN. First, there is a general problem concerning cross protection between BVDV-1 and BVDV-2. At least vaccination with inactivated BVDV-1 vaccines did not prevent the transmission of BVDV-2 to the foetus in pregnant animals. Since protection

against fetal infection represents the major aim of anti BVDV vaccination, such vaccines cannot be regarded to induce a protective immunity on a broad range. The question therefore was, whether vaccination with live attenuated BVDV-2 can prevent virus transmission to the foetus. Second, the reduced growth rates of BVDV XIKE-B-NdN might result in only a low level of protection not able to prevent transplacental infection of the foetus in pregnant heifers. To address these problems, an animal study was started. The animals (2 groups of 10 animals each) were vaccinated either with BVDV XIKE-B or XIKE-B-NdN (intended dosage: 1ml of supernatant with 10^5 $TCID_{50}$ of virus). None of the animals showed significant clinical signs after the vaccination except for one animal of the nonvaccinated control group with mild coughing for one day. Rectal temperature values were below 39 °C except for one animal of the nonvaccinated control group that was 39.1°C for one day. Buffy coat samples prepared after vaccination were analysed for the presence of virus as described above. The experiments showed that only 5 of the 20 animals contained virus in the blood for 1 or 2 days at 4 to 8 days post infection.

Four weeks after vaccination, insemination of the animals was carried out. Challenge infections were performed 60 to 90 days later using either a BVDV-1 strain (BVDV KE-9, heterologous challenge, animals vaccinated with XIKE-B) or a heterologous BVDV-2 strain (BVDV KE-13, homologous challenge, animals vaccinated with XIKE-B-NdN) (intended dosage: 10^5 TCID50 in 6ml). From each group of vaccinated animals 5 pregnant heifers were randomly selected for the challenge infection. Animals vaccinated with BVDV XIKE-B were challenged with the BVDV-1 strain KE-9, whereas heifers vaccinated with BVDV XIKE-B/NdN were challenged with BVDV-2 KE-13. In addition, 2 nonvaccinated control animals were infected with each of the challenge viruses. The vaccinated animals did not show viremia or clinical symptoms upon challenge infection. The challenge was successful as all non-vaccinated controls were BVDV positive. Only mild signs of disease were observed in the control groups. The white blood cell counts were nearly normal (not shown).

Serum neutralization titers were determined before inoculation, 1 month post-inoculation, before challenge, 1 month after challenge and at termination of the study. Sera from all animals were tested in triplicates for neutralizing antibodies against KE9 and NY93/C (1456Nase), and the endpoint dilution was read by indirect immunofluorescence staining. Results were expressed as the endpoint dilution, which neutralized approximately 100 $TCID_{50}$ and calculated by the method of Kaerber. At some of the higher antibody titres, the used endpoint dilution was not high enough. Against KE9, only animals vaccinated with XIKE-B developed low antibody titres starting about week 4. At

challenge, all animals had antibody titres, which increased considerably starting around week 4 post challenge. XIKE-B vaccinated animals had higher antibody titres then those vaccinated with XIKE-B-NdN vaccinated. All animals developed about the same neutralization titre against NY93/C four weeks post vaccination, with marginally lower titres in XIKE-B-NdN vaccinated animals. After challenge, all animals had high antibody titres. Fig. 2 shows the serum neutralization assay against KE9 (BVDV-1) and Fig. 3 shows the serum neutralization assay against NY93/C (BVDV-2).

Analysis of tissue samples obtained after termination of the study from the foetuses revealed that the material obtained from the vaccinated animals gave negative results whereas transmission had occurred in all 4 control animals. Thus, it is clear that the established BVDV-2 mutants are well suited as efficient cross protective vaccine viruses .

Conclusion

The challenge was successful as all non-vaccinated controls were BVDV viraemic and foetuses of all non-vaccinated controls were BVDV positive. Both isolates gave full protection under the present test and assay conditions. Isolate XIKE-B, with the single genetic marker was shown to cross-protect against type 1 BVDV challenge in terms of BVD viraemia and transmission to the foetus after challenge. Isolate XIKE-B-NdN with the double genetic marker was able to fully protect against a heterologue type 2 BVDV challenge strain in terms of BVD viraemia and transmission to the foetus after challenge:

1. Isolate XIKE-B (type 2 isolate) was shown to cross-protect against type 1 BVDV challenge in terms of BVD viraemia and transmission to the foetus after challenge under the present test and assay conditions (n=4).
2. Isolate XIKE-B-NdN (type 2 isolate) fully protected against a heterologues type 2 BVDV challenge strain in terms of BVD viraemia and transmission to the foetus after challenge under the present test and assay conditions (n=5).

EXAMPLE 4

Establishment of N^{pro} mutants

This Example further analyzes BVDV-2 mutants with N^{pro} deletions. Different mutants with deletions in the N^{pro}-coding region of the genome were established. Initially, only true deletions or a deletion accompanied by a point mutation were introduced.

A: $[N^{pro}]_1$-[C-term];

B: $[N^{pro}]_5$-[C-term];

C: $[N^{pro}]_4$-[C-term];

D: $[N^{pro}]_6$-[C-term];

E: $[N^{pro}]_4$-[C-term*]

In the formulas $[N^{pro}]_x$ represents the number of residues of the aminoterminus of N^{pro} that are left in the mutated polyprotein amino acids, [C-term] is the complete polyprotein except for N^{pro} (starting with the C protein and ending with NS5B), and [C-term*] is the same as [C-term] but with a mutation at position 2 of the C protein (N instead of D). The growth rates of the recovered viruses were considerably lower than those of wild type XIKE-A or the RNase negative mutant XIKE-B. There are two possible explanations for this finding: (i) dependent on the virus strain, sequences of variable length of the N^{pro}-coding region are necessary for efficient translation initiation (Mayers et al., 2001; Tautz et al., 1999) and (ii) the fusion of additional sequences to the aminoterminus of the capsid protein interferes with capsid protein function. To obtain better growing N^{pro} deletion mutants, a second set of mutants was generated with either a bovine ubiquitin gene or a fragment of the bovine LC3-coding sequence replacing the major part of the N^{pro} gene. These constructs allow efficient translation and generate a capsid protein with the correct amino terminus.

$[N^{pro}]_{22}$-[PS]- [C-term]

wherein PS is ubiquitin or LC3, C-term is the complete polyprotein except for N^{pro} (starting with the C protein and ending with NS5B).

The growth rates of these mutants were more similar to what was determined for XIKE-A. It even seemed that the two RNase positive viruses according to the formula $[N^{pro}]_{22}$-[PS]- [C-term] named V-pK87F and V-pK87G showed no significant growth retardation at all, whereas the RNase negative counterpart V-pK88G once again was somewhat hampered in propagation but to a lesser extent than the formerly described mutants.

Further examples of N^{pro} deletion mutants may be:

*ME*SDEGSK... (SEQ ID NO 28)

*MELFS*SDEGSK... (SEQ ID NO 29)

*MELFSNE*SDEGSK... (SEQ ID NO 30)

*MELFSNEL*SDEGSK... (SEQ ID NO 31)

*MELFSNELL*SDEGSK... (SEQ ID NO 32)

*MELFSNELLY*SDEGSK... (SEQ ID NO 33)

*MELFSNELLYK*SDEGSK... (SEQ ID NO 34)

*MELFSNELLYKT*SDEGSK... (SEQ ID NO 35)

MELFSNELLYKT represents the aminoterminal sequence of N^{pro} of the BVDV isolate NewYork93/C.

It may also be possible to use variants of this sequence with one or several mutations. Especially the naturally occurring variations as found in other pestiviruses can be expected to be functional. Therefore, the complete list of the tested or proposed variants with the different parts of the aminoterminal end of N^{pro} can be enlarged by equivalent sets with amino acid exchanges. Below, typical examples of the respective sequences are given for several pestiviruses but the possible variations are not limited to these examples.

BVDV NewYork93/C:	*MELFSNELLYKT*	
BVDV CP13:	*MELISNELLYKT*	(SEQ ID NO 36)
BVDV SD1:	*MELITNELLYKT*	(SEQ ID NO 37)
CSFV Brescia:	*MELNHFELLYKT*	(SEQ ID NO 38)
BDV X818:	*MELNKFELLYKT*	(SEQ ID NO 39)

Thus, these variants for example may include: *MELI*-$[PS]_0$-[C-term];

MELIS-$[PS]_0$-[C-term];

MELISN-$[PS]_0$-[C-term];

MELISNE-$[PS]_0$-[C-term];

MELISNEL-$[PS]_0$-[C-term];

MELISNELL-$[PS]_0$-[C-term];

MELISNELLY-$[PS]_0$-[C-term];

MELISNELLYK-$[PS]_0$-[C-term];

MELISNELLYKT-$[PS]_0$-[C-term];

MELIT-$[PS]_0$-[C-term];

MELITN-$[PS]_0$-[C-term];

MELITNE-$[PS]_0$-[C-term];

MELITNEL-$[PS]_0$-[C-term];

MELITNELL-$[PS]_0$-[C-term];

MELITNELLY-$[PS]_0$-[C-term];

MELITNELLYK-$[PS]_0$-[C-term];

MELITNELLYKT-[PS]$_0$-[C-term];

These formulas may also have [PS]$_1$, i.e. PS may also be one of the PS as described herein.

Sequences belonging to the N^{pro} protein are in italics. Amino acid exchanges with regard to the sequence of BVDV NewYork93/C are in bold.

Further examples can be found e.g. by using the GenBank accession numbers given in Becher et al., 2003, Virology 311, 96-104) or by standard sequence data searches.

A further possibility could be the use of a processing signal (PS) inserted between the (residual) N^{pro} sequence and the aminoterminus of the capsid protein. The PS leads to a cleavage that generates a functional capsid protein. The configuration of such constructs could be as follows:

$[N^{pro}]_{22}$-PS[C-term]

PS: Processing signal. Can either be a target for a protease (e.g. ubiquitin, LC3 as defined herein or a protease or an unstable peptide leading to processing at its own carboxyterminus like e.g. intein (Chong et al. 1998 and references therein) or 3C of picornaviruses, 2A of cardioviruses or aphtoviruses, p15 of rabbit hemorrhagic disease virus or the corresponding protease of other caliciviruses (Proter 1993, and references therein; Meyers et al., 2000 and references therein). When using a PS, a large number of different variants are possible since the PS ensures the generation of the correct amino terminus of the capsid protein C. Thus, when using a PS construct, all kinds of deletions or mutations of the N^{pro} sequence are expected to result in viable mutants as long as the reading frame is not shifted or translation stopped by an in frame stop codon. As an example we established a viable CSFV N^{pro} deletion mutant according to the formula

$[N^{pro}]_{29}$-PS[C-term]

Especially interesting could be N^{pro} mutations blocking the proteolytic activity of the protein. Rümenapf et al. (1998) have published the identification of the active site residues of the protease for CSFV Alfort Tübingen. The respective amino acids (glutamic acid at position 22, histidine at position 49 and cysteine at postion 69) are conserved for other pestiviruses. Thus, exchanges of any amino acid expect for serine or threonine for the cysteine at position 69 will result in destruction of the protease activity. Similarly, changing the glutamic acid at position 22 will most likely result in inactivation of the protease unless the new amino acid is aspartic acid. Similarly, most if not all exchanges at position 49 will lead to an inactive protease.

EXAMPLE 5

Preparation of combination vaccines according to the invention

Vaccine A

IBR, BVDV types 1 and 2, BRSV

Attenuated BVDV type 1 and 2 strains, having at least one mutation in the coding sequence for glycoprotein E^{rns} and/or at least another mutation in the coding sequence for N^{pro}, wherein said mutation in the coding sequence for glycoprotein E^{rns} leads to inactivation of RNase activity residing in E^{rns} and/or said mutation in the coding sequence for N^{pro} leads to inactivation of said N^{pro}, are grown in MDBK-cells until a $TCID_{50}$ of about $10^{5.0}$ to $10^{8.1}$ per ml cell culture fluid. A live attenuated strain of IBR is grown in MDBK cells until a $TCID_{50}$ of about $10^{5.0}$ to $10^{8.6}$ per ml cell culture fluid. A live attenuated strain of BRSV is grown in MDBK cells until a $TCID_{50}$ of about $10^{5.0}$ to $10^{7.2}$ per ml cell culture fluid. Each virus containing culture fluid is collected and lyophilized. Equal amounts of the lyphilized antigens are mixed. For reconstitution, an aqueous solution containing 1 to 3% : 0,8 ml of Alhydrogel is used. One dose of the combinantion vaccine contains 4 ml of the reconstituted antigens. A final dose includes IBR ($10^{5.0}$ to $10^{8.6}$ $TCID_{50}$), BVDV-1 ($10^{5.0}$ to $10^{8.1}$ $TCID_{50}$), BVDV-2 ($10^{5.0}$ to $10^{8.1}$ $TCID_{50}$), and BRSV ($10^{5.0}$ to $10^{7.2}$ $TCID_{50}$).

Vaccine B

IBR, BVDV types 1 and 2, PI3 BRSV

The preparation of the IBR, BVDV 1 and 2 and BRSV antigens is performed as described for vaccine A. In addition, a live attenuated strain of PI3 is grown in MDBK cells until a $TCID_{50}$ of about $10^{4.2}$ to $10^{6.5}$ per ml cell culture fluid. Afterwards, the PI3 containing culture fluid is harvested and lyophilized. An amount of $10^{4.2}$ to $10^{6.5}$ ($TCID_{50}$) of the lyophlized antigen is mixed with the lyophilized IBR, BVDV types 1 and 2, and BRSV antigens. The mixture is then reconstituted in 4 ml as described for Vaccine A. A final dose includes IBR ($10^{5.0}$ to $10^{8.6}$ $TCID_{50}$), BVDV-1 ($10^{5.0}$ to $10^{8.1}$ $TCID_{50}$), BVDV-2 ($10^{5.0}$ to $10^{8.1}$ $TCID_{50}$), BRSV ($10^{5.0}$ to $10^{7.2}$ $TCID_{50}$), and PI3 ($10^{4.2}$ to $10^{6.5}$ $TCID_{50}$).

Vaccine C

BVDV types 1 and 2, PI3, BRSV, Mannheimia (Pasteurella) haemolytica

BVDV 1 and 2, BRSV and PI3 viruses are grown as described for vaccines A and B. After the culture fluids are harvested, the viruses are inactivated and lyophilized. Mannheima (Pasteurella) haemalytica is grown until $10^{8.0}$ to $10^{11.0}$ cells per ml culture. The bacteria are inactivated and the culture fluid is lyophilized or freeze dried. An amount of $10^{8.0}$ to $10^{11.0}$ lyophlized or freeze dried bacteria cells are mixed with the lyophilized BVDV types 1 and 2 antigen (each in an amount of $10^{5.0}$ to $10^{8.1}$ $TCID_{50}$), PI3 antigen ($10^{7.3}$ to $10^{8.3}$ $TCID_{50}$) and BRSV antigen ($10^{5.0}$ to $10^{7.2}$ $TCID_{50}$). The reconstituted suspension (5 ml per dose) further contains 30 to 50 mg Aluminium hydroxide, 0.4 to 0.8 mg Quil A (Saponin), 0.04 to 0.06 mg sodium timerfonate and traces of neomycin. Final antigen amounts per dose are BVDV-1 ($10^{5.0}$ to $10^{8.1}$ $TCID_{50}$), BVDV-2 ($10^{5.0}$ to $10^{8.1}$ $TCID_{50}$), PI3 ($10^{7.3}$ to $10^{8.3}$ $TCID_{50}$) BRSV ($10^{5.0}$ to $10^{7.2}$ $TCID_{50}$) and Mannheima (Pasteurella) haemalytica ($10^{8.0}$ to $10^{11.0}$ cells).

Vaccine D

BVDV types 1 and 2, IBR, BRSV, PI3, Leptospira canicola, Leptospira grippo, Leptospira hardjo, Leptospira ponoma, Leptospora borgpetersenii hardjo-bovis

Modified live viruses of BVDV 1 and 2, BRSV, IBR, and PI3 are grown as described for vaccines A and B. After the culture fluids are harvested, the viruses are lyophilized. Leptospira canicola, Leptospira grippo, Leptospira hardjo, Leptospira ponoma, Leptospora borgpetersenii hardjo-bovis are separately cultivated until reaching $10^{8.0}$ to $10^{11.0}$ cells per ml culture. The bacteria cultures are inactivated and the culture fluids are lyophilized or freeze dried. 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 $10^{7.0}$ $TCID_{50}$), modified live PI3 ($10^{7.2}$ to $10^{8.3}$ $TCID_{50}$), modified live BRSV ($10^{5.0}$ to $10^{7.0}$ $TCID_{50}$) and modified live IBR ($10^{6.1}$ to $10^{7.7}$ $TCID_{50}$). 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 $10^{7.0}$ $TCID_{50}$), BVDV-2 ($10^{5.0}$ to $10^{7.0}$ $TCID_{50}$), PI3 ($10^{7.3}$ to $10^{8.3}$ $TCID_{50}$) BRSV ($10^{5.0}$ to $10^{7.0}$ $TCID_{50}$), PI3 ($10^{7.3}$ to $10^{8.3}$ $TCID_{50}$), and Leptospira canicola, Leptospira grippo, Leptospira hardjo, Leptospira ponoma, and Leptospora borgpetersenii hardjo-bovis (each $10^{8.0}$ to $10^{11.0}$ cells).

Vaccine E

BVDV types 1 and 2, IBR, BRSV, PI3, and H.somnus

Modified live viruses of BVDV 1 and 2, BRSV, IBR, and PI3 are grown as described for vaccines A and B. After the culture fluids are harvested, the viruses 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. $10^{7.1}$ to $10^{9.2}$ of the loyphilized or freeze dried bacteria are reconstituted with the lyophilized modified BVDV types 1 and 2 (each in an amount of $10^{5.0}$ to $10^{7.0}$ $TCID_{50}$), modified live PI3 ($10^{7.3}$ to $10^{8.3}$ $TCID_{50}$), modified live BRSV ($10^{5.0}$ to $10^{7.0}$ $TCID_{50}$) and modified live IBR ($10^{6.1}$ to $10^{7.7}$ $TCID_{50}$). 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 $10^{7.0}$ $TCID_{50}$), BVDV-2 ($10^{5.0}$ to $10^{7.0}$ $TCID_{50}$), PI3 ($10^{7.3}$ to $10^{8.3}$ $TCID_{50}$) BRSV ($10^{5.0}$ to $10^{7.0}$ $TCID_{50}$), PI3 ($10^{7.3}$ to $10^{8.3}$ $TCID_{50}$), and H.somnus ($10^{7.1}$ to $10^{9.2}$ cells).

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The teachings and contents of all references (articles, patents, book portions, presentations, and the like) cited herein, including those listed below, are expressly incorporated by reference herein.

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CLAIMS:

1. A combination vaccine for the treatment and/or prophylaxis of cattle against microbiological infections, wherein said combination vaccine comprises

   a. one or more attenuated BVDV, having at least one mutation in the coding sequence for glycoprotein E^{rns} and/or at least another mutation in the coding sequence for N^{pro}, wherein said mutation in the coding sequence for glycoprotein E^{rns} leads to inactivation of RNase activity residing in E^{rns} and/or said mutation in the coding sequence for N^{pro} leads to inactivation of said N^{pro}; and

   b. one or more

antigen of one or more pathogens selected from the group consisting of: 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), Bovie Parvovirus (PPV), Adenovirus, Astrovirus, Mannheimia haemolytica (formerly Pasteurella haemolytica), Pasteurella multocida, Haemophilus somnus (Histophilus ovis and Haemophilus agni), Actinomyces such as Corynebacterium, Actinomyces pyogenes, Chlamydia psittaci, Campylobacter fetus venerealis and Campylobacter fetus fetus (formerly C fetus intestinalis), Leptospira interrogans, Leptospira hardjo, Leptospira pomona, Leptospira grippotyphosa, Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo such as Leptospira hardjoprajitno and 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., preferably Klebsiella pneumoniae, Salmonella typhimurium; Salmonella newport, Mycobacterium avium paratuberculosis, Cryptsporidium parvum, Cryptsporidium hominis, Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus uberus, Mycoplasma spp., preferably 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 wherein such BVDV component is safe for use in pregnant cows.

2. The combination vaccine according to claim 1, wherein said mutation(s) in the coding sequence for glycoprotein E^{rns} are located in the encoding nucleotide sequence corresponding to amino acids at positions 298 to 310 and/or positions 341 to 360.

3. The combination vaccine according to claim 1, wherein said mutation(s) in the coding sequence for glycoprotein E^{rns} are located in the nucleotide sequence coding for the conserved E^{rns} sequence SLHGIWPEKIC (SEQ ID NO 16) and/or RHEWNKHGWCNW (SEQ ID NO 15).

4. The combination vaccine according to any one of claims 1 to 3, wherein said mutation(s) in the coding sequence for N^{pro} leads to an encoded polyprotein as characterized by the following formula:

$$[N^{pro}]_x\text{-}[\textbf{C-term}],$$

wherein $[N^{pro}]$ relates to the N^{pro} portion of said polypeptide, wherein "**x**" represents the number of amino acids of the N^{pro} present in the polyprotein, wherein **[C-term]** relates to the complete pestivirus, in particular the complete BVDV polyprotein except for N^{pro}, but including the capsid (C)-protein and any other protein present in the pestivirus polyprotein, in particular in the BVDV polyprotein including the carboxyterminal NS5B.

5. The combination vaccine according to any one of claims 1 to 4, wherein the one or more pathogens are selected from the group consisting of: 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 (PPV), and Adenovirus and Astrovirus.

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caatgcacta agcactttag ctgtgtgagg ttaactcgac gtccacggtt ggactaggga 11820
agacctctaa cagcccc                                               11837
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<210> 5
<211> 3913
<212> PRT
<213> Wildtyp BVDV: XIKE-A

<400> 5

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Met Glu Leu Phe Ser Asn Glu Leu Leu Tyr Lys Thr Tyr Lys Gln Lys
1               5                  10                  15

Pro Ala Gly Val Val Glu Pro Val Tyr Asp Val Asn Gly Arg Pro Leu
            20                  25                  30

Phe Gly Glu Ser Ser Asp Leu His Pro Gln Ser Thr Leu Lys Leu Pro
        35                  40                  45
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His Gln Arg Gly Ser Ala Asn Ile Leu Thr Asn Ala Arg Ser Leu Pro
50 55 60

Arg Lys Gly Asp Cys Arg Arg Gly Asn Val Tyr Gly Pro Val Ser Gly
65 70 75 80

Ile Tyr Ile Lys Pro Gly Pro Ile Tyr Tyr Gln Asp Tyr Val Gly Pro
85 90 95

Val Tyr His Arg Ala Pro Leu Glu Leu Cys Arg Glu Ala Ser Met Cys
100 105 110

Glu Thr Thr Arg Arg Val Gly Arg Val Thr Gly Ser Asp Gly Lys Leu
115 120 125

Tyr His Ile Tyr Ile Cys Ile Asp Gly Cys Ile Leu Leu Lys Arg Ala
130 135 140

Thr Arg Asn Gln Pro Glu Val Leu Lys Trp Val Tyr Asn Arg Leu Asn
145 150 155 160

Cys Pro Leu Trp Val Thr Ser Cys Ser Asp Glu Gly Ser Lys Gly Ala
165 170 175

Thr Ser Lys Lys Gln Pro Lys Pro Asp Arg Ile Glu Lys Gly Lys Met
180 185 190

Lys Ile Ala Pro Lys Glu Thr Glu Lys Asp Cys Lys Thr Arg Pro Pro
195 200 205

Asp Ala Thr Ile Val Val Glu Gly Val Lys Tyr Gln Val Lys Lys Lys
210 215 220

Gly Lys Val Arg Gly Lys Asn Thr Gln Asp Gly Leu Tyr His Asn Lys
225 230 235 240

Asn Lys Pro Pro Glu Ser Arg Lys Lys Leu Glu Lys Ala Leu Leu Ala
245 250 255

Trp Ala Ile Leu Ala Ala Val Leu Leu Gln Leu Val Thr Gly Glu Asn
260 265 270

Ile Thr Gln Trp Asn Leu Met Asp Asn Gly Thr Glu Gly Ile Gln Gln
275 280 285

Ala Met Phe Leu Arg Gly Val Asn Arg Ser Leu His Gly Ile Trp Pro
290 295 300

Glu Lys Ile Cys Thr Gly Val Pro Thr His Leu Ala Thr Asp Tyr Glu
305 310 315 320

Leu Lys Glu Ile Val Gly Met Met Asp Ala Ser Glu Lys Thr Asn Tyr
325 330 335

Thr Cys Cys Arg Leu Gln Arg His Glu Trp Asn Lys His Gly Trp Cys
340 345 350

Asn Trp Phe His Ile Glu Pro Trp Ile Trp Leu Met Asn Lys Thr Gln
355 360 365

Asn Asn Leu Thr Glu Gly Gln Pro Leu Arg Glu Cys Ala Val Thr Cys
370 375 380

Arg Tyr Asp Lys Glu Thr Glu Leu Asn Ile Val Thr Gln Ala Arg Asp
385 390 395 400

Arg Pro Thr Thr Leu Thr Gly Cys Lys Lys Gly Lys Asn Phe Ser Phe
405 410 415

Ala Gly Val Ile Leu Asp Gly Pro Cys Asn Phe Lys Val Ser Val Glu
420 425 430

Asp Val Leu Phe Lys Glu His Asp Cys Gly Asn Met Leu Gln Glu Thr
435 440 445

Ala Ile Gln Leu Leu Asp Gly Ala Thr Asn Thr Ile Glu Gly Ala Arg
450 455 460

Val Gly Thr Ala Lys Leu Thr Thr Trp Leu Gly Lys Gln Leu Gly Ile
465 470 475 480

Leu Gly Lys Lys Leu Glu Asn Lys Ser Lys Ala Trp Phe Gly Ala His
485 490 495

Ala Ala Ser Pro Tyr Cys Gly Val Glu Arg Lys Ile Gly Tyr Val Trp
500 505 510

Tyr Thr Lys Asn Cys Thr Pro Ala Cys Leu Pro Arg Asn Thr Arg Ile
515 520 525

Ile Gly Pro Gly Lys Phe Asp Thr Asn Ala Glu Asp Gly Lys Ile Leu
530 535 540

His Glu Met Gly Gly His Leu Ser Glu Phe Val Leu Leu Ser Leu Val
545 550 555 560

Val Leu Ser Asp Phe Ala Pro Glu Thr Ala Ser Val Ile Tyr Leu Val
565 570 575

Leu His Phe Ala Ile Pro Gln Ser His Val Asp Val Asp Thr Cys Asp
580 585 590

Lys Asn Gln Leu Asn Leu Thr Val Ala Thr Thr Val Ala Glu Val Ile
595 600 605

Pro Gly Thr Val Trp Asn Leu Gly Lys Tyr Val Cys Ile Arg Pro Asp
610 615 620

Trp Trp Pro Tyr Glu Thr Thr Thr Val Phe Val Ile Glu Glu Ala Gly
625 630 635 640

Gln Val Ile Lys Leu Met Leu Arg Ala Ile Arg Asp Leu Thr Arg Ile
645 650 655

Trp Asn Ala Ala Thr Thr Thr Ala Phe Leu Ile Phe Leu Val Lys Ala

660 665 670

Leu Arg Gly Gln Leu Ile Gln Gly Leu Leu Trp Leu Met Leu Ile Thr
675 680 685

Gly Ala Gln Gly Phe Pro Glu Cys Lys Glu Gly Phe Gln Tyr Ala Ile
690 695 700

Ser Lys Asp Arg Lys Met Gly Leu Leu Gly Pro Glu Ser Leu Thr Thr
705 710 715 720

Thr Trp His Leu Pro Thr Lys Lys Ile Val Asp Ser Met Val His Val
725 730 735

Trp Cys Glu Gly Lys Asp Leu Lys Ile Leu Lys Met Cys Thr Lys Glu
740 745 750

Glu Arg Tyr Leu Val Ala Val His Glu Arg Ala Leu Ser Thr Ser Ala
755 760 765

Glu Phe Met Gln Ile Ser Asp Gly Thr Ile Gly Pro Asp Val Ile Asp
770 775 780

Met Pro Asp Asp Phe Glu Phe Gly Leu Cys Pro Cys Asp Ser Lys Pro
785 790 795 800

Val Ile Lys Gly Lys Phe Asn Ala Ser Leu Leu Asn Gly Pro Ala Phe
805 810 815

Gln Met Val Cys Pro Gln Gly Trp Thr Gly Thr Ile Glu Cys Thr Leu
820 825 830

Ala Asn Gln Asp Thr Leu Asp Thr Thr Val Ile Arg Thr Tyr Arg Arg
835 840 845

Thr Thr Pro Phe Gln Arg Arg Lys Trp Cys Thr Tyr Glu Lys Ile Ile
850 855 860

Gly Glu Asp Ile Tyr Glu Cys Ile Leu Gly Gly Asn Trp Thr Cys Ile
865 870 875 880

Thr Gly Asp His Ser Arg Leu Lys Asp Gly Pro Ile Lys Lys Cys Lys
885 890 895

Trp Cys Gly His Asp Phe Val Asn Ser Glu Gly Leu Pro His Tyr Pro
900 905 910

Ile Gly Lys Cys Met Leu Ile Asn Glu Ser Gly Tyr Arg Tyr Val Asp
915 920 925

Asp Thr Ser Cys Asp Arg Gly Gly Val Ala Ile Val Pro Ser Gly Thr
930 935 940

Val Lys Cys Arg Ile Gly Asn Val Thr Val Gln Val Ile Ala Thr Asn
945 950 955 960

Asn Asp Leu Gly Pro Met Pro Cys Ser Pro Ala Glu Val Ile Ala Ser
965 970 975

Glu Gly Pro Val Glu Lys Thr Ala Cys Thr Phe Asn Tyr Ser Arg Thr
980 985 990

Leu Pro Asn Lys Tyr Tyr Glu Pro Arg Asp Arg Tyr Phe Gln Gln Tyr
995 1000 1005

Met Leu Lys Gly Glu Trp Gln Tyr Trp Phe Asp Leu Asp Ser Val
1010 1015 1020

Asp His His Lys Asp Tyr Phe Ser Glu Phe Ile Ile Ile Ala Val
1025 1030 1035

Val Ala Leu Leu Gly Gly Lys Tyr Val Leu Trp Leu Leu Ile Thr
1040 1045 1050

Tyr Thr Ile Leu Ser Glu Gln Met Ala Met Gly Ala Gly Val Asn
1055 1060 1065

Thr Glu Glu Ile Val Met Ile Gly Asn Leu Leu Thr Asp Ser Asp
1070 1075 1080

Ile Glu Val Val Val Tyr Phe Leu Leu Leu Tyr Leu Ile Val Lys
1085 1090 1095

Glu Glu Leu Ala Arg Lys Trp Ile Ile Leu Val Tyr His Ile Leu
1100 1105 1110

Val Ala Asn Pro Met Lys Thr Ile Gly Val Val Leu Leu Met Leu
1115 1120 1125

Gly Gly Val Val Lys Ala Ser Arg Ile Asn Ala Asp Asp Gln Ser
1130 1135 1140

Ala Met Asp Pro Cys Phe Leu Leu Val Thr Gly Val Val Ala Val
1145 1150 1155

Leu Met Ile Ala Arg Arg Glu Pro Ala Thr Leu Pro Leu Ile Val
1160 1165 1170

Ala Leu Leu Ala Ile Arg Thr Ser Gly Phe Leu Leu Pro Ala Ser
1175 1180 1185

Ile Asp Val Thr Val Ala Val Val Leu Ile Val Leu Leu Leu Ala
1190 1195 1200

Ser Tyr Ile Thr Asp Tyr Phe Arg Tyr Lys Lys Trp Leu Gln Leu
1205 1210 1215

Leu Phe Ser Leu Ile Ala Gly Ile Phe Ile Ile Arg Ser Leu Lys
1220 1225 1230

His Ile Asn Gln Met Glu Val Pro Glu Ile Ser Met Pro Ser Trp
1235 1240 1245

Arg Pro Leu Ala Leu Val Leu Phe Tyr Ile Thr Ser Thr Ala Ile
1250 1255 1260

Thr Thr Asn Trp Asp Ile Asp Leu Ala Gly Phe Leu Leu Gln Trp
1265 1270 1275

Ala Pro Ala Val Ile Met Met Ala Thr Met Trp Ala Asp Phe Leu
1280 1285 1290

Thr Leu Ile Ile Val Leu Pro Ser Tyr Glu Leu Ser Lys Leu Tyr
1295 1300 1305

Phe Leu Lys Asn Val Arg Thr Asp Val Glu Lys Asn Trp Leu Gly
1310 1315 1320

Lys Val Lys Tyr Arg Gln Ile Ser Ser Val Tyr Asp Ile Cys Asp
1325 1330 1335

Ser Glu Glu Ala Val Tyr Leu Phe Pro Ser Arg His Lys Ser Gly
1340 1345 1350

Ser Arg Pro Asp Phe Ile Leu Pro Phe Leu Lys Ala Val Leu Ile
1355 1360 1365

Ser Cys Ile Ser Ser Gln Trp Gln Val Val Tyr Ile Ser Tyr Leu
1370 1375 1380

Ile Leu Glu Ile Thr Tyr Tyr Met His Arg Lys Ile Ile Asp Glu
1385 1390 1395

Val Ser Gly Gly Ala Asn Phe Leu Ser Arg Leu Ile Ala Ala Ile
1400 1405 1410

Ile Glu Leu Asn Trp Ala Ile Asp Asp Glu Glu Cys Lys Gly Leu
1415 1420 1425

Lys Lys Leu Tyr Leu Leu Ser Gly Arg Ala Lys Asn Leu Ile Val
1430 1435 1440

Lys His Lys Val Arg Asn Glu Ala Val His Arg Trp Phe Gly Glu
1445 1450 1455

Glu Glu Ile Tyr Gly Ala Pro Lys Val Ile Thr Ile Ile Lys Ala
1460 1465 1470

Ser Thr Leu Ser Lys Asn Arg His Cys Ile Ile Cys Thr Ile Cys
1475 1480 1485

Glu Gly Lys Glu Trp Asn Gly Ala Asn Cys Pro Lys Cys Gly Arg
1490 1495 1500

Gln Gly Lys Pro Ile Thr Cys Gly Met Thr Leu Ala Asp Phe Glu
1505 1510 1515

Glu Lys His Tyr Lys Lys Ile Phe Ile Arg Glu Glu Ser Ser Cys
1520 1525 1530

Pro Val Pro Phe Asp Pro Ser Cys His Cys Asn Tyr Phe Arg His
1535 1540 1545

Asp Gly Pro Phe Arg Lys Glu Tyr Lys Gly Tyr Val Gln Tyr Thr

1550 1555 1560

Ala Arg Gly Gln Leu Phe Leu Arg Asn Leu Pro Ile Leu Ala Thr
1565 1570 1575

Lys Met Lys Leu Leu Met Val Gly Asn Leu Gly Ala Glu Ile Gly
1580 1585 1590

Asp Leu Glu His Leu Gly Trp Val Leu Arg Gly Pro Ala Val Cys
1595 1600 1605

Lys Lys Ile Thr Asn His Glu Lys Cys His Val Asn Ile Met Asp
1610 1615 1620

Lys Leu Thr Ala Phe Phe Gly Ile Met Pro Arg Gly Thr Thr Pro
1625 1630 1635

Arg Ala Pro Val Arg Phe Pro Thr Ala Leu Leu Lys Val Arg Arg
1640 1645 1650

Gly Leu Glu Thr Gly Trp Ala Tyr Thr His Gln Gly Gly Ile Ser
1655 1660 1665

Ser Val Asp His Val Thr Ala Gly Lys Asp Leu Leu Val Cys Asp
1670 1675 1680

Ser Met Gly Arg Thr Arg Val Val Cys His Ser Asn Asn Lys Met
1685 1690 1695

Thr Asp Glu Thr Glu Tyr Gly Ile Lys Thr Asp Ser Gly Cys Pro
1700 1705 1710

Glu Gly Ala Arg Cys Tyr Val Leu Asn Pro Glu Ala Val Asn Ile
1715 1720 1725

Ser Gly Thr Lys Gly Ala Met Val His Leu Gln Lys Thr Gly Gly
1730 1735 1740

Glu Phe Thr Cys Val Thr Ala Ser Gly Thr Pro Ala Phe Phe Asp
1745 1750 1755

Leu Lys Asn Leu Lys Gly Trp Ser Gly Leu Pro Ile Phe Glu Ala
1760 1765 1770

Ser Ser Gly Arg Val Val Gly Arg Val Lys Val Gly Lys Asn Glu
1775 1780 1785

Asp Ser Lys Pro Thr Lys Leu Met Ser Gly Ile Gln Thr Val Ser
1790 1795 1800

Lys Asn Gln Thr Asp Leu Ala Asp Ile Val Lys Lys Leu Thr Ser
1805 1810 1815

Met Asn Arg Gly Glu Phe Lys Gln Ile Thr Leu Ala Thr Gly Ala
1820 1825 1830

Gly Lys Thr Thr Glu Leu Pro Arg Ser Val Ile Glu Glu Ile Gly
1835 1840 1845

```
Arg His  Lys Arg Val Leu Val  Leu Ile Pro Leu Arg  Ala Ala Ala
     1850                1855                1860

Glu Ser  Val Tyr Gln Tyr Met  Arg Val Lys Tyr Pro  Ser Ile Ser
     1865                1870                1975

Phe Asn  Leu Arg Ile Gly Asp  Met Lys Glu Gly Asp  Met Ala Thr
     1980                1885                1890

Gly Ile  Thr Tyr Ala Ser Tyr  Gly Tyr Phe Cys Gln  Leu Pro Gln
     1895                1900                1905

Pro Lys  Leu Arg Ala Ala Met  Val Glu Tyr Ser Tyr  Ile Phe Leu
     1910                1915                1920

Asp Glu  Tyr His Cys Ala Thr  Pro Glu Gln Leu Ala  Ile Ile Gly
     1925                1930                1935

Lys Ile  His Arg Phe Ala Glu  Asn Leu Arg Val Val  Ala Met Thr
     1940                1945                1950

Ala Thr  Pro Ala Gly Thr Val  Thr Thr Thr Gly Gln  Lys His Pro
     1955                1960                1965

Ile Glu  Glu Phe Ile Ala Pro  Glu Val Met Lys Gly  Glu Asp Leu
     1970                1975                1980

Gly Ser  Glu Tyr Leu Asp Ile  Ala Gly Leu Lys Ile  Pro Thr Glu
     1985                1990                1995

Glu Met  Lys Gly Asn Met Leu  Val Phe Ala Pro Thr  Arg Asn Met
     2000                2005                2010

Ala Val  Glu Thr Ala Lys Lys  Leu Lys Ala Lys Gly  Tyr Asn Ser
     2015                2020                2025

Gly Tyr  Tyr Tyr Ser Gly Glu  Asn Pro Glu Asn Leu  Arg Val Val
     2030                2035                2040

Thr Ser  Gln Ser Pro Tyr Val  Val Val Ala Thr Asn  Ala Ile Glu
     2045                2050                2055

Ser Gly  Val Thr Leu Pro Asp  Leu Asp Thr Val Val  Asp Thr Gly
     2060                2065                2070

Leu Lys  Cys Glu Lys Arg Val  Arg Ile Ser Ser Lys  Met Pro Phe
     2075                2080                2085

Ile Val  Thr Gly Leu Lys Arg  Met Ala Val Thr Ile  Gly Glu Gln
     2090                2095                2100

Ala Gln  Arg Arg Gly Arg Val  Gly Arg Val Lys Pro  Gly Arg Tyr
     2105                2110                2115

Tyr Arg  Ser Gln Glu Thr Ala  Ser Gly Ser Lys Asp  Tyr His Tyr
     2120                2125                2130
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Asp Leu Leu Gln Ala Gln Arg Tyr Gly Ile Glu Asp Gly Ile Asn
2135 2140 2145

Val Thr Lys Ser Phe Arg Glu Met Asn Tyr Asp Trp Ser Leu Tyr
2150 2155 2160

Glu Glu Asp Ser Leu Met Ile Thr Gln Leu Glu Val Leu Asn Asn
2165 2170 2175

Leu Leu Ile Ser Glu Asp Leu Pro Ala Ala Val Lys Asn Ile Met
2180 2185 2190

Ala Arg Thr Asp His Pro Glu Pro Ile Gln Leu Ala Tyr Asn Ser
2195 2200 2205

Tyr Glu Asn Gln Ile Pro Val Leu Phe Pro Lys Ile Lys Asn Gly
2210 2215 2220

Glu Val Thr Asp Ser Tyr Glu Asn Tyr Thr Tyr Leu Asn Ala Arg
2225 2230 2235

Lys Leu Gly Glu Asp Val Pro Ala Tyr Val Tyr Ala Thr Glu Asp
2240 2245 2250

Glu Asp Leu Ala Val Asp Leu Leu Gly Met Asp Trp Pro Asp Pro
2255 2260 2265

Gly Asn Gln Gln Val Val Glu Thr Gly Arg Ala Leu Lys Gln Val
2270 2275 2280

Thr Gly Leu Ser Thr Ala Glu Asn Ala Leu Leu Ile Ala Leu Phe
2285 2290 2295

Gly Tyr Val Gly Tyr Gln Thr Leu Ser Lys Arg His Ile Pro Met
2300 2305 2310

Ile Thr Asp Ile Tyr Thr Leu Glu Asp His Arg Leu Glu Asp Thr
2315 2320 2325

Thr His Leu Gln Phe Ala Pro Asn Ala Ile Arg Thr Asp Gly Lys
2330 2335 2340

Asp Ser Glu Leu Lys Glu Leu Ala Val Gly Asp Leu Asp Lys Tyr
2345 2350 2355

Val Asp Ala Leu Val Asp Tyr Ser Lys Gln Gly Met Lys Phe Ile
2360 2365 2370

Lys Val Gln Ala Glu Lys Val Arg Asp Ser Gln Ser Thr Lys Glu
2375 2380 2385

Gly Leu Gln Thr Ile Lys Glu Tyr Val Asp Lys Phe Ile Gln Ser
2390 2395 2400

Leu Thr Glu Asn Lys Glu Glu Ile Ile Arg Tyr Gly Leu Trp Gly
2405 2410 2415

Val His Thr Ala Leu Tyr Lys Ser Leu Ala Ala Arg Leu Gly His

2420 2425 2430

Glu Thr Ala Phe Ala Thr Leu Val Val Lys Trp Leu Ala Phe Gly
2435 2440 2445

Gly Glu Thr Val Ser Ala His Ile Lys Gln Val Ala Val Asp Leu
2450 2455 2460

Val Val Tyr Tyr Ile Ile Asn Lys Pro Ser Phe Pro Gly Asp Thr
2465 2470 2475

Glu Thr Gln Gln Glu Gly Arg Arg Phe Val Ala Ser Leu Phe Ile
2480 2485 2490

Ser Ala Leu Ala Thr Tyr Thr Tyr Lys Thr Trp Asn Tyr Asn Asn
2495 2500 2505

Leu Gln Arg Val Val Glu Pro Ala Leu Ala Tyr Leu Pro Tyr Ala
2510 2515 2520

Thr Ser Ala Leu Lys Leu Phe Thr Pro Thr Arg Leu Glu Ser Val
2525 2530 2535

Val Ile Leu Ser Ser Thr Ile Tyr Lys Thr Tyr Leu Ser Ile Arg
2540 2545 2550

Lys Gly Lys Ser Asp Gly Leu Leu Gly Thr Gly Ile Ser Ala Ala
2555 2560 2565

Met Glu Ile Leu Asn Gln Asn Pro Ile Ser Val Gly Ile Ser Val
2570 2575 2580

Met Leu Gly Val Gly Ala Ile Ala Ala His Asn Ala Ile Glu Ser
2585 2590 2595

Ser Glu Gln Lys Arg Thr Leu Leu Met Lys Val Phe Val Lys Asn
2600 2605 2610

Phe Leu Asp Gln Ala Ala Thr Asp Glu Leu Val Lys Glu Asn Pro
2615 2620 2625

Glu Lys Ile Ile Met Ala Leu Phe Glu Ala Val Gln Thr Ile Gly
2630 2635 2640

Asn Pro Leu Arg Leu Ile Tyr His Leu Tyr Gly Val Tyr Tyr Lys
2645 2650 2655

Gly Trp Glu Ala Lys Glu Leu Ala Glu Lys Thr Ala Gly Arg Asn
2660 2665 2670

Leu Phe Thr Leu Ile Met Phe Glu Ala Phe Glu Leu Leu Gly Met
2675 2680 2685

Asp Ser Glu Gly Lys Ile Arg Asn Leu Ser Gly Asn Tyr Ile Leu
2690 2695 2700

Asp Leu Ile Phe Asn Leu His Asn Lys Leu Asn Lys Gly Leu Lys
2705 2710 2715

Lys Leu Val Leu Gly Trp Ala Pro Ala Pro Leu Ser Cys Asp Trp
2720 2725 2730

Thr Pro Ser Asp Glu Arg Ile Ser Leu Pro His Asn Asn Tyr Leu
2735 2740 2745

Arg Val Glu Thr Arg Cys Pro Cys Gly Tyr Glu Met Lys Ala Ile
2750 2755 2760

Lys Asn Val Ala Gly Lys Leu Thr Lys Val Glu Glu Lys Gly Ser
2765 2770 2775

Phe Leu Cys Arg Asn Arg Leu Gly Arg Gly Pro Pro Asn Phe Lys
2780 2785 2790

Val Thr Lys Phe Tyr Asp Asp Asn Leu Ile Glu Val Lys Pro Val
2795 2800 2805

Ala Arg Leu Glu Gly Gln Val Asp Leu Tyr Tyr Lys Gly Val Thr
2810 2815 2820

Ala Lys Leu Asp Tyr Asn Asn Gly Lys Val Leu Leu Ala Thr Asn
2825 2830 2835

Lys Trp Glu Val Asp His Ala Phe Leu Thr Arg Leu Val Lys Lys
2840 2845 2850

His Thr Gly Ile Gly Phe Lys Gly Ala Tyr Leu Gly Asp Arg Pro
2855 2860 2865

Asp His Gln Asp Leu Val Asp Arg Asp Cys Ala Thr Ile Thr Lys
2870 2875 2880

Asn Ser Val Gln Phe Leu Lys Met Lys Lys Gly Cys Ala Phe Thr
2885 2890 2895

Tyr Asp Leu Thr Ile Ser Asn Leu Val Arg Leu Ile Glu Leu Val
2900 2905 2910

His Lys Asn Asn Leu Gln Glu Arg Glu Ile Pro Thr Val Thr Val
2915 2920 2925

Thr Thr Trp Leu Ala Tyr Ser Phe Val Asn Glu Asp Leu Gly Thr
2930 2935 2940

Ile Lys Pro Val Leu Gly Glu Lys Val Ile Pro Glu Pro Pro Glu
2945 2950 2955

Glu Leu Ser Leu Gln Pro Thr Val Arg Leu Val Thr Thr Glu Thr
2960 2965 2970

Ala Ile Thr Ile Thr Gly Glu Ala Glu Val Met Thr Thr Gly Ile
2975 2980 2985

Thr Pro Val Val Glu Met Lys Glu Glu Pro Gln Leu Asp His Gln
2990 2995 3000

Ser Thr Thr Leu Lys Val Gly Leu Lys Glu Gly Glu Tyr Pro Gly
3005 3010 3015

Pro Gly Val Asn Pro Asn His Leu Ala Glu Val Ile Asp Glu Lys
3020 3025 3030

Asp Asp Arg Pro Phe Val Leu Ile Ile Gly Asn Lys Gly Ser Thr
3035 3040 3045

Ser Asn Arg Ala Arg Thr Ala Lys Asn Ile Arg Leu Tyr Lys Gly
3050 3055 3060

Asn Asn Pro Arg Glu Ile Arg Asp Leu Met Ser Gln Gly Arg Ile
3065 3070 3075

Leu Thr Val Ala Leu Lys Glu Leu Asp Pro Glu Leu Lys Glu Leu
3080 3085 3090

Val Asp Tyr Lys Gly Thr Phe Leu Asn Arg Glu Ala Leu Glu Ala
3095 3100 3105

Leu Ser Leu Gly Lys Pro Ile Lys Arg Lys Thr Thr Thr Ala Met
3110 3115 3120

Ile Arg Arg Leu Ile Glu Pro Glu Val Glu Glu Glu Leu Pro Asp
3125 3130 3135

Trp Phe Gln Ala Glu Glu Pro Leu Phe Leu Glu Ala Lys Ile Gln
3140 3145 3150

Asn Asp Leu Tyr His Leu Ile Gly Ser Val Asp Ser Ile Lys Ser
3155 3160 3165

Lys Ala Lys Glu Leu Gly Ala Thr Asp Asn Thr Lys Ile Val Lys
3170 3175 3180

Glu Val Gly Ala Arg Thr Tyr Thr Met Lys Leu Ser Ser Trp Ser
3185 3190 3195

Thr Gln Val Thr Lys Lys Gln Met Ser Leu Ala Pro Leu Phe Glu
3200 3205 3210

Glu Leu Leu Leu Lys Cys Pro Pro Cys Ser Lys Ile Ser Lys Gly
3215 3220 3225

His Met Val Ser Ala Tyr Gln Leu Ala Gln Gly Asn Trp Glu Pro
3230 3235 3240

Leu Gly Cys Gly Val Tyr Met Gly Thr Ile Pro Ala Arg Arg Leu
3245 3250 3255

Lys Ile His Pro Tyr Glu Ala Tyr Leu Lys Leu Lys Glu Leu Val
3260 3265 3270

Glu Val Glu Ser Ser Arg Ala Thr Ala Lys Glu Ser Ile Ile Arg
3275 3280 3285

Glu His Asn Thr Trp Ile Leu Arg Lys Val Arg His Glu Gly Asn

3290 3295 3300

Leu Arg Thr Lys Ser Met Ile Asn Pro Gly Lys Ile Ser Asp Gln
3305 3310 3315

Leu Cys Arg Asp Gly His Lys Arg Asn Ile Tyr Asn Lys Ile Ile
3320 3325 3330

Gly Ser Thr Met Ala Ser Ala Gly Ile Arg Leu Glu Lys Leu Pro
3335 3340 3345

Val Val Arg Ala Gln Thr Asp Thr Thr Ser Phe His Gln Ala Ile
3350 3355 3360

Arg Glu Lys Ile Asp Lys Thr Glu Asn Lys Gln Thr Pro Glu Leu
3365 3370 3375

His Glu Glu Leu Met Lys Val Phe Asp Cys Leu Lys Ile Pro Glu
3380 3385 3390

Leu Lys Glu Ser Tyr Asp Glu Val Ser Trp Glu Gln Leu Glu Ala
3395 3400 3405

Gly Ile Asn Arg Lys Gly Ala Ala Gly Tyr Leu Glu Ser Lys Asn
3410 3415 3420

Ile Gly Glu Val Leu Asp Thr Glu Lys His Ile Val Glu Gln Leu
3425 3430 3435

Ile Lys Asp Leu Arg Lys Gly Lys Lys Ile Arg Tyr Tyr Glu Thr
3440 3445 3450

Ala Ile Pro Lys Asn Glu Lys Arg Asp Val Ser Asp Asp Trp Glu
3455 3460 3465

Ala Gly Glu Phe Val Asp Glu Lys Lys Pro Arg Val Ile Gln Tyr
3470 3475 3480

Pro Asp Ala Lys Val Arg Leu Ala Ile Thr Lys Val Met Tyr Lys
3485 3490 3495

Trp Val Lys Gln Lys Pro Val Val Ile Pro Gly Tyr Glu Gly Lys
3500 3505 3510

Thr Pro Leu Phe Asp Ile Phe Asn Lys Val Lys Lys Glu Trp Asp
3515 3520 3525

Ser Phe Gln Asp Pro Val Ala Val Ser Phe Asp Thr Lys Ala Trp
3530 3535 3540

Asp Thr Gln Val Thr Ser Arg Asp Leu Met Leu Ile Lys Asp Ile
3545 3550 3555

Gln Lys Tyr Tyr Phe Lys Arg Ser Ile His Lys Phe Leu Asp Thr
3560 3565 3570

Ile Thr Glu His Met Val Glu Val Pro Val Ile Thr Ala Asp Gly
3575 3580 3585

Glu Val Tyr Ile Arg Asn Gly Gln Arg Gly Ser Gly Gln Pro Asp
3590 3595 3600

Thr Ser Ala Gly Asn Ser Met Leu Asn Val Leu Thr Met Ile Tyr
3605 3610 3615

Ala Phe Cys Lys Ser Thr Gly Ile Pro Tyr Arg Gly Phe Ser Arg
3620 3625 3630

Val Ala Arg Ile His Val Cys Gly Asp Asp Gly Phe Leu Ile Thr
3635 3640 3645

Glu Arg Gly Leu Gly Leu Lys Phe Ser Glu Lys Gly Met Gln Ile
3650 3655 3660

Leu His Glu Ala Gly Lys Pro Gln Lys Ile Thr Glu Gly Asp Lys
3665 3670 3675

Met Lys Val Ala Tyr Arg Phe Glu Asp Ile Glu Phe Cys Ser His
3680 3685 3690

Thr Pro Val Pro Val Arg Trp Ala Asp Asn Thr Ser Ser Tyr Met
3695 3700 3705

Ala Gly Arg Ser Thr Ala Thr Ile Leu Ala Lys Met Ala Thr Arg
3710 3715 3720

Leu Asp Ser Ser Gly Glu Arg Gly Ser Thr Ala Tyr Glu Lys Ala
3725 3730 3735

Val Ala Phe Ser Phe Leu Leu Met Tyr Ser Trp Asn Pro Val Val
3740 3745 3750

Arg Arg Ile Cys Leu Leu Val Leu Ser Gln Phe Pro Glu Ile Ser
3755 3760 3765

Pro Ser Lys Asn Thr Ile Tyr Tyr Tyr Gln Gly Asp Pro Ile Ala
3770 3775 3780

Ala Tyr Arg Glu Val Ile Gly Lys Gln Leu Cys Glu Leu Lys Arg
3785 3790 3795

Thr Gly Phe Glu Lys Leu Ala Gly Leu Asn Leu Ser Met Thr Thr
3800 3805 3810

Leu Gly Ile Trp Thr Lys His Thr Ser Lys Arg Leu Ile Gln Ala
3815 3820 3825

Cys Val Glu Ile Gly Lys Arg Glu Gly Thr Trp Leu Val Asn Ala
3830 3835 3840

Asp Arg Leu Ile Ala Gly Lys Thr Gly Lys Phe Tyr Ile Pro Ser
3845 3850 3855

Thr Gly Val Thr Leu Leu Gly Lys His Tyr Glu Glu Ile Asn Leu
3860 3865 3870

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Lys Gln  Lys Ala Ala Gln Pro  Pro Ile Glu Gly Val  Asp Arg Tyr
    3875                 3880                 3885

Lys Leu  Gly Pro Ile Val Asn  Val Ile Leu Arg Arg  Leu Arg Val
    3890                 3895                 3900

Met Leu  Met Thr Val Ala Ser  Gly Ser Trp
    3905                 3910
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<210> 6
<211> 3749
<212> PRT
<213> Artificial Sequence: Mutated SVUV: XIKE-A-NdN

<400> 6

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Met Glu Leu Phe Ser Asp Glu Gly Ser Lys Gly Ala Thr Ser Lys Lys
1               5                  10                  15

Gln Pro Lys Pro Asp Arg Ile Glu Lys Gly Lys Met Lys Ile Ala Pro
            20                  25                  30

Lys Glu Thr Glu Lys Asp Cys Lys Thr Arg Pro Pro Asp Ala Thr Ile
        35                  40                  45

Val Val Glu Gly Val Lys Tyr Gln Val Lys Lys Lys Gly Lys Val Arg
    50                  55                  60

Gly Lys Asn Thr Gln Asp Gly Leu Tyr His Asn Lys Asn Lys Pro Pro
65                  70                  75                  80

Glu Ser Arg Lys Lys Leu Glu Lys Ala Leu Leu Ala Trp Ala Ile Leu
                85                  90                  95

Ala Ala Val Leu Leu Gln Leu Val Thr Gly Glu Asn Ile Thr Gln Trp
            100                 105                 110

Asn Leu Met Asp Asn Gly Thr Glu Gly Ile Gln Gln Ala Met Phe Leu
        115                 120                 125

Arg Gly Val Asn Arg Ser Leu His Gly Ile Trp Pro Glu Lys Ile Cys
    130                 135                 140

Thr Gly Val Pro Thr His Leu Ala Thr Asp Tyr Glu Leu Lys Glu Ile
145                 150                 155                 160

Val Gly Met Met Asp Ala Ser Glu Lys Thr Asn Tyr Thr Cys Cys Arg
                165                 170                 175

Leu Gln Arg His Glu Trp Asn Lys His Gly Trp Cys Asn Trp Phe His
            180                 185                 190

Ile Glu Pro Trp Ile Trp Leu Met Asn Lys Thr Gln Asn Asn Leu Thr
        195                 200                 205

Glu Gly Gln Pro Leu Arg Glu Cys Ala Val Thr Cys Arg Tyr Asp Lys
    210                 215                 220
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Glu Thr Glu Leu Asn Ile Val Thr Gln Ala Arg Asp Arg Pro Thr Thr
225 230 235 240

Leu Thr Gly Cys Lys Lys Gly Lys Asn Phe Ser Phe Ala Gly Val Ile
245 250 255

Leu Asp Gly Pro Cys Asn Phe Lys Val Ser Val Glu Asp Val Leu Phe
260 265 270

Lys Glu His Asp Cys Gly Asn Met Leu Gln Glu Thr Ala Ile Gln Leu
275 280 285

Leu Asp Gly Ala Thr Asn Thr Ile Glu Gly Ala Arg Val Gly Thr Ala
290 295 300

Lys Leu Thr Thr Trp Leu Gly Lys Gln Leu Gly Ile Leu Gly Lys Lys
305 310 315 320

Leu Glu Asn Lys Ser Lys Ala Trp Phe Gly Ala His Ala Ala Ser Pro
325 330 335

Tyr Cys Gly Val Glu Arg Lys Ile Gly Tyr Val Trp Tyr Thr Lys Asn
340 345 350

Cys Thr Pro Ala Cys Leu Pro Arg Asn Thr Arg Ile Ile Gly Pro Gly
355 360 365

Lys Phe Asp Thr Asn Ala Glu Asp Gly Lys Ile Leu His Glu Met Gly
370 375 380

Gly His Leu Ser Glu Phe Val Leu Leu Ser Leu Val Val Leu Ser Asp
385 390 395 400

Phe Ala Pro Glu Thr Ala Ser Val Ile Tyr Leu Val Leu His Phe Ala
405 410 415

Ile Pro Gln Ser His Val Asp Val Asp Thr Cys Asp Lys Asn Gln Leu
420 425 430

Asn Leu Thr Val Ala Thr Thr Val Ala Glu Val Ile Pro Gly Thr Val
435 440 445

Trp Asn Leu Gly Lys Tyr Val Cys Ile Arg Pro Asp Trp Trp Pro Tyr
450 455 460

Glu Thr Thr Thr Val Phe Val Ile Glu Glu Ala Gly Gln Val Ile Lys
465 470 475 480

Leu Met Leu Arg Ala Ile Arg Asp Leu Thr Arg Ile Trp Asn Ala Ala
485 490 495

Thr Thr Thr Ala Phe Leu Ile Phe Leu Val Lys Ala Leu Arg Gly Gln
500 505 510

Leu Ile Gln Gly Leu Leu Trp Leu Met Leu Ile Thr Gly Ala Gln Gly
515 520 525

Phe Pro Glu Cys Lys Glu Gly Phe Gln Tyr Ala Ile Ser Lys Asp Arg
530 535 540

Lys Met Gly Leu Leu Gly Pro Glu Ser Leu Thr Thr Thr Trp His Leu
545 550 555 560

Pro Thr Lys Lys Ile Val Asp Ser Met Val His Val Trp Cys Glu Gly
565 570 575

Lys Asp Leu Lys Ile Leu Lys Met Cys Thr Lys Glu Glu Arg Tyr Leu
580 585 590

Val Ala Val His Glu Arg Ala Leu Ser Thr Ser Ala Glu Phe Met Gln
595 600 605

Ile Ser Asp Gly Thr Ile Gly Pro Asp Val Ile Asp Met Pro Asp Asp
610 615 620

Phe Glu Phe Gly Leu Cys Pro Cys Asp Ser Lys Pro Val Ile Lys Gly
625 630 635 640

Lys Phe Asn Ala Ser Leu Leu Asn Gly Pro Ala Phe Gln Met Val Cys
645 650 655

Pro Gln Gly Trp Thr Gly Thr Ile Glu Cys Thr Leu Ala Asn Gln Asp
660 665 670

Thr Leu Asp Thr Thr Val Ile Arg Thr Tyr Arg Arg Thr Thr Pro Phe
675 680 685

Gln Arg Arg Lys Trp Cys Thr Tyr Glu Lys Ile Ile Gly Glu Asp Ile
690 695 700

Tyr Glu Cys Ile Leu Gly Gly Asn Trp Thr Cys Ile Thr Gly Asp His
705 710 715 720

Ser Arg Leu Lys Asp Gly Pro Ile Lys Lys Cys Lys Trp Cys Gly His
725 730 735

Asp Phe Val Asn Ser Glu Gly Leu Pro His Tyr Pro Ile Gly Lys Cys
740 745 750

Met Leu Ile Asn Glu Ser Gly Tyr Arg Tyr Val Asp Asp Thr Ser Cys
755 760 765

Asp Arg Gly Gly Val Ala Ile Val Pro Ser Gly Thr Val Lys Cys Arg
770 775 780

Ile Gly Asn Val Thr Val Gln Val Ile Ala Thr Asn Asn Asp Leu Gly
785 790 795 800

Pro Met Pro Cys Ser Pro Ala Glu Val Ile Ala Ser Glu Gly Pro Val
805 810 815

Glu Lys Thr Ala Cys Thr Phe Asn Tyr Ser Arg Thr Leu Pro Asn Lys
820 825 830

Tyr Tyr Glu Pro Arg Asp Arg Tyr Phe Gln Gln Tyr Met Leu Lys Gly

835 840 845

Glu Trp Gln Tyr Trp Phe Asp Leu Asp Ser Val Asp His His Lys Asp
850 855 860

Tyr Phe Ser Glu Phe Ile Ile Ile Ala Val Val Ala Leu Leu Gly Gly
865 870 875 880

Lys Tyr Val Leu Trp Leu Leu Ile Thr Tyr Thr Ile Leu Ser Glu Gln
885 890 895

Met Ala Met Gly Ala Gly Val Asn Thr Glu Glu Ile Val Met Ile Gly
900 905 910

Asn Leu Leu Thr Asp Ser Asp Ile Glu Val Val Val Tyr Phe Leu Leu
915 920 925

Leu Tyr Leu Ile Val Lys Glu Glu Leu Ala Arg Lys Trp Ile Ile Leu
930 935 940

Val Tyr His Ile Leu Val Ala Asn Pro Met Lys Thr Ile Gly Val Val
945 950 955 960

Leu Leu Met Leu Gly Gly Val Val Lys Ala Ser Arg Ile Asn Ala Asp
965 970 975

Asp Gln Ser Ala Met Asp Pro Cys Phe Leu Leu Val Thr Gly Val Val
980 985 990

Ala Val Leu Met Ile Ala Arg Arg Glu Pro Ala Thr Leu Pro Leu Ile
995 1000 1005

Val Ala Leu Leu Ala Ile Arg Thr Ser Gly Phe Leu Leu Pro Ala
1010 1015 1020

Ser Ile Asp Val Thr Val Ala Val Val Leu Ile Val Leu Leu Leu
1025 1030 1035

Ala Ser Tyr Ile Thr Asp Tyr Phe Arg Tyr Lys Lys Trp Leu Gln
1040 1045 1050

Leu Leu Phe Ser Leu Ile Ala Gly Ile Phe Ile Ile Arg Ser Leu
1055 1060 1065

Lys His Ile Asn Gln Met Glu Val Pro Glu Ile Ser Met Pro Ser
1070 1075 1080

Trp Arg Pro Leu Ala Leu Val Leu Phe Tyr Ile Thr Ser Thr Ala
1085 1090 1095

Ile Thr Thr Asn Trp Asp Ile Asp Leu Ala Gly Phe Leu Leu Gln
1100 1105 1110

Trp Ala Pro Ala Val Ile Met Met Ala Thr Met Trp Ala Asp Phe
1115 1120 1125

Leu Thr Leu Ile Ile Val Leu Pro Ser Tyr Glu Leu Ser Lys Leu
1130 1135 1140

Tyr Phe Leu Lys Asn Val Arg Thr Asp Val Glu Lys Asn Trp Leu
1145 1150 1155

Gly Lys Val Lys Tyr Arg Gln Ile Ser Ser Val Tyr Asp Ile Cys
1160 1165 1170

Asp Ser Glu Glu Ala Val Tyr Leu Phe Pro Ser Arg His Lys Ser
1175 1180 1185

Gly Ser Arg Pro Asp Phe Ile Leu Pro Phe Leu Lys Ala Val Leu
1190 1195 1200

Ile Ser Cys Ile Ser Ser Gln Trp Gln Val Val Tyr Ile Ser Tyr
1205 1210 1215

Leu Ile Leu Glu Ile Thr Tyr Tyr Met His Arg Lys Ile Ile Asp
1220 1225 1230

Glu Val Ser Gly Gly Ala Asn Phe Leu Ser Arg Leu Ile Ala Ala
1235 1240 1245

Ile Ile Glu Leu Asn Trp Ala Ile Asp Asp Glu Glu Cys Lys Gly
1250 1255 1260

Leu Lys Lys Leu Tyr Leu Leu Ser Gly Arg Ala Lys Asn Leu Ile
1265 1270 1275

Val Lys His Lys Val Arg Asn Glu Ala Val His Arg Trp Phe Gly
1280 1285 1290

Glu Glu Glu Ile Tyr Gly Ala Pro Lys Val Ile Thr Ile Ile Lys
1295 1300 1305

Ala Ser Thr Leu Ser Lys Asn Arg His Cys Ile Ile Cys Thr Ile
1310 1315 1320

Cys Glu Gly Lys Glu Trp Asn Gly Ala Asn Cys Pro Lys Cys Gly
1325 1330 1335

Arg Gln Gly Lys Pro Ile Thr Cys Gly Met Thr Leu Ala Asp Phe
1340 1345 1350

Glu Glu Lys His Tyr Lys Lys Ile Phe Ile Arg Glu Glu Ser Ser
1355 1360 1365

Cys Pro Val Pro Phe Asp Pro Ser Cys His Cys Asn Tyr Phe Arg
1370 1375 1380

His Asp Gly Pro Phe Arg Lys Glu Tyr Lys Gly Tyr Val Gln Tyr
1385 1390 1395

Thr Ala Arg Gly Gln Leu Phe Leu Arg Asn Leu Pro Ile Leu Ala
1400 1405 1410

Thr Lys Met Lys Leu Leu Met Val Gly Asn Leu Gly Ala Glu Ile
1415 1420 1425

Gly Asp Leu Glu His Leu Gly Trp Val Leu Arg Gly Pro Ala Val
1430 1435 1440

Cys Lys Lys Ile Thr Asn His Glu Lys Cys His Val Asn Ile Met
1445 1450 1455

Asp Lys Leu Thr Ala Phe Phe Gly Ile Met Pro Arg Gly Thr Thr
1460 1465 1470

Pro Arg Ala Pro Val Arg Phe Pro Thr Ala Leu Leu Lys Val Arg
1475 1480 1485

Arg Gly Leu Glu Thr Gly Trp Ala Tyr Thr His Gln Gly Gly Ile
1490 1495 1500

Ser Ser Val Asp His Val Thr Ala Gly Lys Asp Leu Leu Val Cys
1505 1510 1515

Asp Ser Met Gly Arg Thr Arg Val Val Cys His Ser Asn Asn Lys
1520 1525 1530

Met Thr Asp Glu Thr Glu Tyr Gly Ile Lys Thr Asp Ser Gly Cys
1535 1540 1545

Pro Glu Gly Ala Arg Cys Tyr Val Leu Asn Pro Glu Ala Val Asn
1550 1555 1560

Ile Ser Gly Thr Lys Gly Ala Met Val His Leu Gln Lys Thr Gly
1565 1570 1575

Gly Glu Phe Thr Cys Val Thr Ala Ser Gly Thr Pro Ala Phe Phe
1580 1585 1590

Asp Leu Lys Asn Leu Lys Gly Trp Ser Gly Leu Pro Ile Phe Glu
1595 1600 1605

Ala Ser Ser Gly Arg Val Val Gly Arg Val Lys Val Gly Lys Asn
1610 1615 1620

Glu Asp Ser Lys Pro Thr Lys Leu Met Ser Gly Ile Gln Thr Val
1625 1630 1635

Ser Lys Asn Gln Thr Asp Leu Ala Asp Ile Val Lys Lys Leu Thr
1640 1645 1650

Ser Met Asn Arg Gly Glu Phe Lys Gln Ile Thr Leu Ala Thr Gly
1655 1660 1665

Ala Gly Lys Thr Thr Glu Leu Pro Arg Ser Val Ile Glu Glu Ile
1670 1675 1680

Gly Arg His Lys Arg Val Leu Val Leu Ile Pro Leu Arg Ala Ala
1685 1690 1695

Ala Glu Ser Val Tyr Gln Tyr Met Arg Val Lys Tyr Pro Ser Ile
1700 1705 1710

Ser Phe Asn Leu Arg Ile Gly Asp Met Lys Glu Gly Asp Met Ala

1715 1720 1725

Thr Gly Ile Thr Tyr Ala Ser Tyr Gly Tyr Phe Cys Gln Leu Pro
1730 1735 1740

Gln Pro Lys Leu Arg Ala Ala Met Val Glu Tyr Ser Tyr Ile Phe
1745 1750 1755

Leu Asp Glu Tyr His Cys Ala Thr Pro Glu Gln Leu Ala Ile Ile
1760 1765 1770

Gly Lys Ile His Arg Phe Ala Glu Asn Leu Arg Val Val Ala Met
1775 1780 1785

Thr Ala Thr Pro Ala Gly Thr Val Thr Thr Thr Gly Gln Lys His
1790 1795 1800

Pro Ile Glu Glu Phe Ile Ala Pro Glu Val Met Lys Gly Glu Asp
1805 1810 1815

Leu Gly Ser Glu Tyr Leu Asp Ile Ala Gly Leu Lys Ile Pro Thr
1820 1825 1830

Glu Glu Met Lys Gly Asn Met Leu Val Phe Ala Pro Thr Arg Asn
1835 1840 1845

Met Ala Val Glu Thr Ala Lys Lys Leu Lys Ala Lys Gly Tyr Asn
1850 1855 1860

Ser Gly Tyr Tyr Tyr Ser Gly Glu Asn Pro Glu Asn Leu Arg Val
1865 1870 1875

Val Thr Ser Gln Ser Pro Tyr Val Val Val Ala Thr Asn Ala Ile
1880 1885 1890

Glu Ser Gly Val Thr Leu Pro Asp Leu Asp Thr Val Val Asp Thr
1895 1900 1905

Gly Leu Lys Cys Glu Lys Arg Val Arg Ile Ser Ser Lys Met Pro
1910 1915 1920

Phe Ile Val Thr Gly Leu Lys Arg Met Ala Val Thr Ile Gly Glu
1925 1930 1935

Gln Ala Gln Arg Arg Gly Arg Val Gly Arg Val Lys Pro Gly Arg
1940 1945 1950

Tyr Tyr Arg Ser Gln Glu Thr Ala Ser Gly Ser Lys Asp Tyr His
1955 1960 1965

Tyr Asp Leu Leu Gln Ala Gln Arg Tyr Gly Ile Glu Asp Gly Ile
1970 1975 1980

Asn Val Thr Lys Ser Phe Arg Glu Met Asn Tyr Asp Trp Ser Leu
1985 1990 1995

Tyr Glu Glu Asp Ser Leu Met Ile Thr Gln Leu Glu Val Leu Asn
2000 2005 2010

Asn Leu Leu Ile Ser Glu Asp Leu Pro Ala Ala Val Lys Asn Ile
2015 2020 2025

Met Ala Arg Thr Asp His Pro Glu Pro Ile Gln Leu Ala Tyr Asn
2030 2035 2040

Ser Tyr Glu Asn Gln Ile Pro Val Leu Phe Pro Lys Ile Lys Asn
2045 2050 2055

Gly Glu Val Thr Asp Ser Tyr Glu Asn Tyr Thr Tyr Leu Asn Ala
2060 2065 2070

Arg Lys Leu Gly Glu Asp Val Pro Ala Tyr Val Tyr Ala Thr Glu
2075 2080 2085

Asp Glu Asp Leu Ala Val Asp Leu Leu Gly Met Asp Trp Pro Asp
2090 2095 2100

Pro Gly Asn Gln Gln Val Val Glu Thr Gly Arg Ala Leu Lys Gln
2105 2110 2115

Val Thr Gly Leu Ser Thr Ala Glu Asn Ala Leu Leu Ile Ala Leu
2120 2125 2130

Phe Gly Tyr Val Gly Tyr Gln Thr Leu Ser Lys Arg His Ile Pro
2135 2140 2145

Met Ile Thr Asp Ile Tyr Thr Leu Glu Asp His Arg Leu Glu Asp
2150 2155 2160

Thr Thr His Leu Gln Phe Ala Pro Asn Ala Ile Arg Thr Asp Gly
2165 2170 2175

Lys Asp Ser Glu Leu Lys Glu Leu Ala Val Gly Asp Leu Asp Lys
2180 2185 2190

Tyr Val Asp Ala Leu Val Asp Tyr Ser Lys Gln Gly Met Lys Phe
2195 2200 2205

Ile Lys Val Gln Ala Glu Lys Val Arg Asp Ser Gln Ser Thr Lys
2210 2215 2220

Glu Gly Leu Gln Thr Ile Lys Glu Tyr Val Asp Lys Phe Ile Gln
2225 2230 2235

Ser Leu Thr Glu Asn Lys Glu Glu Ile Ile Arg Tyr Gly Leu Trp
2240 2245 2250

Gly Val His Thr Ala Leu Tyr Lys Ser Leu Ala Ala Arg Leu Gly
2255 2260 2265

His Glu Thr Ala Phe Ala Thr Leu Val Val Lys Trp Leu Ala Phe
2270 2275 2280

Gly Gly Glu Thr Val Ser Ala His Ile Lys Gln Val Ala Val Asp
2285 2290 2295

Leu Val Val Tyr Tyr Ile Ile Asn Lys Pro Ser Phe Pro Gly Asp
2300 2305 2310

Thr Glu Thr Gln Gln Glu Gly Arg Arg Phe Val Ala Ser Leu Phe
2315 2320 2325

Ile Ser Ala Leu Ala Thr Tyr Thr Tyr Lys Thr Trp Asn Tyr Asn
2330 2335 2340

Asn Leu Gln Arg Val Val Glu Pro Ala Leu Ala Tyr Leu Pro Tyr
2345 2350 2355

Ala Thr Ser Ala Leu Lys Leu Phe Thr Pro Thr Arg Leu Glu Ser
2360 2365 2370

Val Val Ile Leu Ser Ser Thr Ile Tyr Lys Thr Tyr Leu Ser Ile
2375 2380 2385

Arg Lys Gly Lys Ser Asp Gly Leu Leu Gly Thr Gly Ile Ser Ala
2390 2395 2400

Ala Met Glu Ile Leu Asn Gln Asn Pro Ile Ser Val Gly Ile Ser
2405 2410 2415

Val Met Leu Gly Val Gly Ala Ile Ala Ala His Asn Ala Ile Glu
2420 2425 2430

Ser Ser Glu Gln Lys Arg Thr Leu Leu Met Lys Val Phe Val Lys
2435 2440 2445

Asn Phe Leu Asp Gln Ala Ala Thr Asp Glu Leu Val Lys Glu Asn
2450 2455 2460

Pro Glu Lys Ile Ile Met Ala Leu Phe Glu Ala Val Gln Thr Ile
2465 2470 2475

Gly Asn Pro Leu Arg Leu Ile Tyr His Leu Tyr Gly Val Tyr Tyr
2480 2485 2490

Lys Gly Trp Glu Ala Lys Glu Leu Ala Glu Lys Thr Ala Gly Arg
2495 2500 2505

Asn Leu Phe Thr Leu Ile Met Phe Glu Ala Phe Glu Leu Leu Gly
2510 2515 2520

Met Asp Ser Glu Gly Lys Ile Arg Asn Leu Ser Gly Asn Tyr Ile
2525 2530 2535

Leu Asp Leu Ile Phe Asn Leu His Asn Lys Leu Asn Lys Gly Leu
2540 2545 2550

Lys Lys Leu Val Leu Gly Trp Ala Pro Ala Pro Leu Ser Cys Asp
2555 2560 2565

Trp Thr Pro Ser Asp Glu Arg Ile Ser Leu Pro His Asn Asn Tyr
2570 2575 2580

Leu Arg Val Glu Thr Arg Cys Pro Cys Gly Tyr Glu Met Lys Ala

2585 2590 2595

Ile Lys Asn Val Ala Gly Lys Leu Thr Lys Val Glu Glu Lys Gly
2600 2605 2610

Ser Phe Leu Cys Arg Asn Arg Leu Gly Arg Gly Pro Pro Asn Phe
2615 2620 2625

Lys Val Thr Lys Phe Tyr Asp Asp Asn Leu Ile Glu Val Lys Pro
2630 2635 2640

Val Ala Arg Leu Glu Gly Gln Val Asp Leu Tyr Tyr Lys Gly Val
2645 2650 2655

Thr Ala Lys Leu Asp Tyr Asn Asn Gly Lys Val Leu Leu Ala Thr
2660 2665 2670

Asn Lys Trp Glu Val Asp His Ala Phe Leu Thr Arg Leu Val Lys
2675 2680 2685

Lys His Thr Gly Ile Gly Phe Lys Gly Ala Tyr Leu Gly Asp Arg
2690 2695 2700

Pro Asp His Gln Asp Leu Val Asp Arg Asp Cys Ala Thr Ile Thr
2705 2710 2715

Lys Asn Ser Val Gln Phe Leu Lys Met Lys Lys Gly Cys Ala Phe
2720 2725 2730

Thr Tyr Asp Leu Thr Ile Ser Asn Leu Val Arg Leu Ile Glu Leu
2735 2740 2745

Val His Lys Asn Asn Leu Gln Glu Arg Glu Ile Pro Thr Val Thr
2750 2755 2760

Val Thr Thr Trp Leu Ala Tyr Ser Phe Val Asn Glu Asp Leu Gly
2765 2770 2775

Thr Ile Lys Pro Val Leu Gly Glu Lys Val Ile Pro Glu Pro Pro
2780 2785 2790

Glu Glu Leu Ser Leu Gln Pro Thr Val Arg Leu Val Thr Thr Glu
2795 2800 2805

Thr Ala Ile Thr Ile Thr Gly Glu Ala Glu Val Met Thr Thr Gly
2810 2815 2820

Ile Thr Pro Val Val Glu Met Lys Glu Glu Pro Gln Leu Asp His
2825 2830 2835

Gln Ser Thr Thr Leu Lys Val Gly Leu Lys Glu Gly Glu Tyr Pro
2840 2845 2850

Gly Pro Gly Val Asn Pro Asn His Leu Ala Glu Val Ile Asp Glu
2855 2860 2865

Lys Asp Asp Arg Pro Phe Val Leu Ile Ile Gly Asn Lys Gly Ser
2870 2875 2880

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Thr Ser  Asn Arg Ala Arg Thr  Ala Lys Asn Ile Arg  Leu Tyr Lys
     2885                 2890                 2895

Gly Asn  Asn Pro Arg Glu Ile  Arg Asp Leu Met Ser  Gln Gly Arg
     2900                 2905                 2910

Ile Leu  Thr Val Ala Leu Lys  Glu Leu Asp Pro Glu  Leu Lys Glu
     2915                 2920                 2925

Leu Val  Asp Tyr Lys Gly Thr  Phe Leu Asn Arg Glu  Ala Leu Glu
     2930                 2935                 2940

Ala Leu  Ser Leu Gly Lys Pro  Ile Lys Arg Lys Thr  Thr Thr Ala
     2945                 2950                 2955

Met Ile  Arg Arg Leu Ile Glu  Pro Glu Val Glu Glu  Glu Leu Pro
     2960                 2965                 2970

Asp Trp  Phe Gln Ala Glu Glu  Pro Leu Phe Leu Glu  Ala Lys Ile
     2975                 2980                 2985

Gln Asn  Asp Leu Tyr His Leu  Ile Gly Ser Val Asp  Ser Ile Lys
     2990                 2995                 3000

Ser Lys  Ala Lys Glu Leu Gly  Ala Thr Asp Asn Thr  Lys Ile Val
     3005                 3010                 3015

Lys Glu  Val Gly Ala Arg Thr  Tyr Thr Met Lys Leu  Ser Ser Trp
     3020                 3025                 3030

Ser Thr  Gln Val Thr Lys Lys  Gln Met Ser Leu Ala  Pro Leu Phe
     3035                 3040                 3045

Glu Glu  Leu Leu Leu Lys Cys  Pro Pro Cys Ser Lys  Ile Ser Lys
     3050                 3055                 3060

Gly His  Met Val Ser Ala Tyr  Gln Leu Ala Gln Gly  Asn Trp Glu
     3065                 3070                 3075

Pro Leu  Gly Cys Gly Val Tyr  Met Gly Thr Ile Pro  Ala Arg Arg
     3080                 3085                 3090

Leu Lys  Ile His Pro Tyr Glu  Ala Tyr Leu Lys Leu  Lys Glu Leu
     3095                 3100                 3105

Val Glu  Val Glu Ser Ser Arg  Ala Thr Ala Lys Glu  Ser Ile Ile
     3110                 3115                 3120

Arg Glu  His Asn Thr Trp Ile  Leu Arg Lys Val Arg  His Glu Gly
     3125                 3130                 3135

Asn Leu  Arg Thr Lys Ser Met  Ile Asn Pro Gly Lys  Ile Ser Asp
     3140                 3145                 3150

Gln Leu  Cys Arg Asp Gly His  Lys Arg Asn Ile Tyr  Asn Lys Ile
     3155                 3160                 3165
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Ile Gly Ser Thr Met Ala Ser Ala Gly Ile Arg Leu Glu Lys Leu
3170 3175 3180

Pro Val Val Arg Ala Gln Thr Asp Thr Thr Ser Phe His Gln Ala
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Ile Arg Glu Lys Ile Asp Lys Thr Glu Asn Lys Gln Thr Pro Glu
3200 3205 3210

Leu His Glu Glu Leu Met Lys Val Phe Asp Cys Leu Lys Ile Pro
3215 3220 3225

Glu Leu Lys Glu Ser Tyr Asp Glu Val Ser Trp Glu Gln Leu Glu
3230 3235 3240

Ala Gly Ile Asn Arg Lys Gly Ala Ala Gly Tyr Leu Glu Ser Lys
3245 3250 3255

Asn Ile Gly Glu Val Leu Asp Thr Glu Lys His Ile Val Glu Gln
3260 3265 3270

Leu Ile Lys Asp Leu Arg Lys Gly Lys Lys Ile Arg Tyr Tyr Glu
3275 3280 3285

Thr Ala Ile Pro Lys Asn Glu Lys Arg Asp Val Ser Asp Asp Trp
3290 3295 3300

Glu Ala Gly Glu Phe Val Asp Glu Lys Lys Pro Arg Val Ile Gln
3305 3310 3315

Tyr Pro Asp Ala Lys Val Arg Leu Ala Ile Thr Lys Val Met Tyr
3320 3325 3330

Lys Trp Val Lys Gln Lys Pro Val Val Ile Pro Gly Tyr Glu Gly
3335 3340 3345

Lys Thr Pro Leu Phe Asp Ile Phe Asn Lys Val Lys Lys Glu Trp
3350 3355 3360

Asp Ser Phe Gln Asp Pro Val Ala Val Ser Phe Asp Thr Lys Ala
3365 3370 3375

Trp Asp Thr Gln Val Thr Ser Arg Asp Leu Met Leu Ile Lys Asp
3380 3385 3390

Ile Gln Lys Tyr Tyr Phe Lys Arg Ser Ile His Lys Phe Leu Asp
3395 3400 3405

Thr Ile Thr Glu His Met Val Glu Val Pro Val Ile Thr Ala Asp
3410 3415 3420

Gly Glu Val Tyr Ile Arg Asn Gly Gln Arg Gly Ser Gly Gln Pro
3425 3430 3435

Asp Thr Ser Ala Gly Asn Ser Met Leu Asn Val Leu Thr Met Ile
3440 3445 3450

Tyr Ala Phe Cys Lys Ser Thr Gly Ile Pro Tyr Arg Gly Phe Ser

3455 3460 3465

Arg Val Ala Arg Ile His Val Cys Gly Asp Asp Gly Phe Leu Ile
3470 3475 3480

Thr Glu Arg Gly Leu Gly Leu Lys Phe Ser Glu Lys Gly Met Gln
3485 3490 3495

Ile Leu His Glu Ala Gly Lys Pro Gln Lys Ile Thr Glu Gly Asp
3500 3505 3510

Lys Met Lys Val Ala Tyr Arg Phe Glu Asp Ile Glu Phe Cys Ser
3515 3520 3525

His Thr Pro Val Pro Val Arg Trp Ala Asp Asn Thr Ser Ser Tyr
3530 3535 3540

Met Ala Gly Arg Ser Thr Ala Thr Ile Leu Ala Lys Met Ala Thr
3545 3550 3555

Arg Leu Asp Ser Ser Gly Glu Arg Gly Ser Thr Ala Tyr Glu Lys
3560 3565 3570

Ala Val Ala Phe Ser Phe Leu Leu Met Tyr Ser Trp Asn Pro Val
3575 3580 3585

Val Arg Arg Ile Cys Leu Leu Val Leu Ser Gln Phe Pro Glu Ile
3590 3595 3600

Ser Pro Ser Lys Asn Thr Ile Tyr Tyr Tyr Gln Gly Asp Pro Ile
3605 3610 3615

Ala Ala Tyr Arg Glu Val Ile Gly Lys Gln Leu Cys Glu Leu Lys
3620 3625 3630

Arg Thr Gly Phe Glu Lys Leu Ala Gly Leu Asn Leu Ser Met Thr
3635 3640 3645

Thr Leu Gly Ile Trp Thr Lys His Thr Ser Lys Arg Leu Ile Gln
3650 3655 3660

Ala Cys Val Glu Ile Gly Lys Arg Glu Gly Thr Trp Leu Val Asn
3665 3670 3675

Ala Asp Arg Leu Ile Ala Gly Lys Thr Gly Lys Phe Tyr Ile Pro
3680 3685 3690

Ser Thr Gly Val Thr Leu Leu Gly Lys His Tyr Glu Glu Ile Asn
3695 3700 3705

Leu Lys Gln Lys Ala Ala Gln Pro Pro Ile Glu Gly Val Asp Arg
3710 3715 3720

Tyr Lys Leu Gly Pro Ile Val Asn Val Ile Leu Arg Arg Leu Arg
3725 3730 3735

Val Met Leu Met Thr Val Ala Ser Gly Ser Trp
3740 3745

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Pro Ala Gly Val Val Glu Pro Val Tyr Asp Val Asn Gly Arg Pro Leu
20 25 30

Phe Gly Glu Ser Ser Asp Leu His Pro Gln Ser Thr Leu Lys Leu Pro
35 40 45

His Gln Arg Gly Ser Ala Asn Ile Leu Thr Asn Ala Arg Ser Leu Pro
50 55 60

Arg Lys Gly Asp Cys Arg Arg Gly Asn Val Tyr Gly Pro Val Ser Gly
65 70 75 80

Ile Tyr Ile Lys Pro Gly Pro Ile Tyr Tyr Gln Asp Tyr Val Gly Pro
85 90 95

Val Tyr His Arg Ala Pro Leu Glu Leu Cys Arg Glu Ala Ser Met Cys
100 105 110

Glu Thr Thr Arg Arg Val Gly Arg Val Thr Gly Ser Asp Gly Lys Leu
115 120 125

Tyr His Ile Tyr Ile Cys Ile Asp Gly Cys Ile Leu Leu Lys Arg Ala
130 135 140

Thr Arg Asn Gln Pro Glu Val Leu Lys Trp Val Tyr Asn Arg Leu Asn
145 150 155 160

Cys Pro Leu Trp Val Thr Ser Cys Ser Asp Glu Gly Ser Lys Gly Ala
165 170 175

Thr Ser Lys Lys Gln Pro Lys Pro Asp Arg Ile Glu Lys Gly Lys Met
180 185 190

Lys Ile Ala Pro Lys Glu Thr Glu Lys Asp Cys Lys Thr Arg Pro Pro
195 200 205

Asp Ala Thr Ile Val Val Glu Gly Val Lys Tyr Gln Val Lys Lys Lys
210 215 220

Gly Lys Val Arg Gly Lys Asn Thr Gln Asp Gly Leu Tyr His Asn Lys
225 230 235 240

Asn Lys Pro Pro Glu Ser Arg Lys Lys Leu Glu Lys Ala Leu Leu Ala
245 250 255

Trp Ala Ile Leu Ala Ala Val Leu Leu Gln Leu Val Thr Gly Glu Asn
260 265 270

Ile Thr Gln Trp Asn Leu Met Asp Asn Gly Thr Glu Gly Ile Gln Gln
275 280 285

Ala Met Phe Leu Arg Gly Val Asn Arg Ser Leu His Gly Ile Trp Pro
290 295 300

Glu Lys Ile Cys Thr Gly Val Pro Thr His Leu Ala Thr Asp Tyr Glu
305 310 315 320

Leu Lys Glu Ile Val Gly Met Met Asp Ala Ser Glu Lys Thr Asn Tyr
325 330 335

Thr Cys Cys Arg Leu Gln Arg His Glu Trp Asn Lys Gly Trp Cys Asn
340 345 350

Trp Phe His Ile Glu Pro Trp Ile Trp Leu Met Asn Lys Thr Gln Asn
355 360 365

Asn Leu Thr Glu Gly Gln Pro Leu Arg Glu Cys Ala Val Thr Cys Arg
370 375 380

Tyr Asp Lys Glu Thr Glu Leu Asn Ile Val Thr Gln Ala Arg Asp Arg
385 390 395 400

Pro Thr Thr Leu Thr Gly Cys Lys Lys Gly Lys Asn Phe Ser Phe Ala
405 410 415

Gly Val Ile Leu Asp Gly Pro Cys Asn Phe Lys Val Ser Val Glu Asp
420 425 430

Val Leu Phe Lys Glu His Asp Cys Gly Asn Met Leu Gln Glu Thr Ala
435 440 445

Ile Gln Leu Leu Asp Gly Ala Thr Asn Thr Ile Glu Gly Ala Arg Val
450 455 460

Gly Thr Ala Lys Leu Thr Thr Trp Leu Gly Lys Gln Leu Gly Ile Leu
465 470 475 480

Gly Lys Lys Leu Glu Asn Lys Ser Lys Ala Trp Phe Gly Ala His Ala
485 490 495

Ala Ser Pro Tyr Cys Gly Val Glu Arg Lys Ile Gly Tyr Val Trp Tyr
500 505 510

Thr Lys Asn Cys Thr Pro Ala Cys Leu Pro Arg Asn Thr Arg Ile Ile
515 520 525

Gly Pro Gly Lys Phe Asp Thr Asn Ala Glu Asp Gly Lys Ile Leu His
530 535 540

Glu Met Gly Gly His Leu Ser Glu Phe Val Leu Leu Ser Leu Val Val
545 550 555 560

Leu Ser Asp Phe Ala Pro Glu Thr Ala Ser Val Ile Tyr Leu Val Leu
565 570 575

His Phe Ala Ile Pro Gln Ser His Val Asp Val Asp Thr Cys Asp Lys
580 585 590

Asn Gln Leu Asn Leu Thr Val Ala Thr Thr Val Ala Glu Val Ile Pro
595 600 605

Gly Thr Val Trp Asn Leu Gly Lys Tyr Val Cys Ile Arg Pro Asp Trp
610 615 620

Trp Pro Tyr Glu Thr Thr Thr Val Phe Val Ile Glu Glu Ala Gly Gln
625 630 635 640

Val Ile Lys Leu Met Leu Arg Ala Ile Arg Asp Leu Thr Arg Ile Trp
645 650 655

Asn Ala Ala Thr Thr Thr Ala Phe Leu Ile Phe Leu Val Lys Ala Leu
660 665 670

Arg Gly Gln Leu Ile Gln Gly Leu Leu Trp Leu Met Leu Ile Thr Gly
675 680 685

Ala Gln Gly Phe Pro Glu Cys Lys Glu Gly Phe Gln Tyr Ala Ile Ser
690 695 700

Lys Asp Arg Lys Met Gly Leu Leu Gly Pro Glu Ser Leu Thr Thr Thr
705 710 715 720

Trp His Leu Pro Thr Lys Lys Ile Val Asp Ser Met Val His Val Trp
725 730 735

Cys Glu Gly Lys Asp Leu Lys Ile Leu Lys Met Cys Thr Lys Glu Glu
740 745 750

Arg Tyr Leu Val Ala Val His Glu Arg Ala Leu Ser Thr Ser Ala Glu
755 760 765

Phe Met Gln Ile Ser Asp Gly Thr Ile Gly Pro Asp Val Ile Asp Met
770 775 780

Pro Asp Asp Phe Glu Phe Gly Leu Cys Pro Cys Asp Ser Lys Pro Val
785 790 795 800

Ile Lys Gly Lys Phe Asn Ala Ser Leu Leu Asn Gly Pro Ala Phe Gln
805 810 815

Met Val Cys Pro Gln Gly Trp Thr Gly Thr Ile Glu Cys Thr Leu Ala
820 825 830

Asn Gln Asp Thr Leu Asp Thr Thr Val Ile Arg Thr Tyr Arg Arg Thr
835 840 845

Thr Pro Phe Gln Arg Arg Lys Trp Cys Thr Tyr Glu Lys Ile Ile Gly
850 855 860

Glu Asp Ile Tyr Glu Cys Ile Leu Gly Gly Asn Trp Thr Cys Ile Thr
865 870 875 880

Gly Asp His Ser Arg Leu Lys Asp Gly Pro Ile Lys Lys Cys Lys Trp

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                885                 890                 895

Cys Gly His Asp Phe Val Asn Ser Glu Gly Leu Pro His Tyr Pro Ile
            900                 905                 910

Gly Lys Cys Met Leu Ile Asn Glu Ser Gly Tyr Arg Tyr Val Asp Asp
        915                 920                 925

Thr Ser Cys Asp Arg Gly Gly Val Ala Ile Val Pro Ser Gly Thr Val
    930                 935                 940

Lys Cys Arg Ile Gly Asn Val Thr Val Gln Val Ile Ala Thr Asn Asn
945                 950                 955                 960

Asp Leu Gly Pro Met Pro Cys Ser Pro Ala Glu Val Ile Ala Ser Glu
                965                 970                 975

Gly Pro Val Glu Lys Thr Ala Cys Thr Phe Asn Tyr Ser Arg Thr Leu
            980                 985                 990

Pro Asn Lys Tyr Tyr Glu Pro Arg  Asp Arg Tyr Phe Gln  Gln Tyr Met
        995                1000                1005

Leu Lys  Gly Glu Trp Gln Tyr  Trp Phe Asp Leu Asp  Ser Val Asp
    1010                1015                1020

His His  Lys Asp Tyr Phe Ser  Glu Phe Ile Ile Ile  Ala Val Val
    1025                1030                1035

Ala Leu  Leu Gly Gly Lys Tyr  Val Leu Trp Leu Leu  Ile Thr Tyr
    1040                1045                1050

Thr Ile  Leu Ser Glu Gln Met  Ala Met Gly Ala Gly  Val Asn Thr
    1055                1060                1065

Glu Glu  Ile Val Met Ile Gly  Asn Leu Leu Thr Asp  Ser Asp Ile
    1070                1075                1080

Glu Val  Val Val Tyr Phe Leu  Leu Leu Tyr Leu Ile  Val Lys Glu
    1085                1090                1095

Glu Leu  Ala Arg Lys Trp Ile  Ile Leu Val Tyr His  Ile Leu Val
    1100                1105                1110

Ala Asn  Pro Met Lys Thr Ile  Gly Val Val Leu Leu  Met Leu Gly
    1115                1120                1125

Gly Val  Val Lys Ala Ser Arg  Ile Asn Ala Asp Asp  Gln Ser Ala
    1130                1135                1140

Met Asp  Pro Cys Phe Leu Leu  Val Thr Gly Val Val  Ala Val Leu
    1145                1150                1155

Met Ile  Ala Arg Arg Glu Pro  Ala Thr Leu Pro Leu  Ile Val Ala
    1160                1165                1170

Leu Leu  Ala Ile Arg Thr Ser  Gly Phe Leu Leu Pro  Ala Ser Ile
    1175                1180                1185
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Asp Val Thr Val Ala Val Val Leu Ile Val Leu Leu Leu Ala Ser
1190 1195 1200

Tyr Ile Thr Asp Tyr Phe Arg Tyr Lys Lys Trp Leu Gln Leu Leu
1205 1210 1215

Phe Ser Leu Ile Ala Gly Ile Phe Ile Ile Arg Ser Leu Lys His
1220 1225 1230

Ile Asn Gln Met Glu Val Pro Glu Ile Ser Met Pro Ser Trp Arg
1235 1240 1245

Pro Leu Ala Leu Val Leu Phe Tyr Ile Thr Ser Thr Ala Ile Thr
1250 1255 1260

Thr Asn Trp Asp Ile Asp Leu Ala Gly Phe Leu Leu Gln Trp Ala
1265 1270 1275

Pro Ala Val Ile Met Met Ala Thr Met Trp Ala Asp Phe Leu Thr
1280 1285 1290

Leu Ile Ile Val Leu Pro Ser Tyr Glu Leu Ser Lys Leu Tyr Phe
1295 1300 1305

Leu Lys Asn Val Arg Thr Asp Val Glu Lys Asn Trp Leu Gly Lys
1310 1315 1320

Val Lys Tyr Arg Gln Ile Ser Ser Val Tyr Asp Ile Cys Asp Ser
1325 1330 1335

Glu Glu Ala Val Tyr Leu Phe Pro Ser Arg His Lys Ser Gly Ser
1340 1345 1350

Arg Pro Asp Phe Ile Leu Pro Phe Leu Lys Ala Val Leu Ile Ser
1355 1360 1365

Cys Ile Ser Ser Gln Trp Gln Val Val Tyr Ile Ser Tyr Leu Ile
1370 1375 1380

Leu Glu Ile Thr Tyr Tyr Met His Arg Lys Ile Ile Asp Glu Val
1385 1390 1395

Ser Gly Gly Ala Asn Phe Leu Ser Arg Leu Ile Ala Ala Ile Ile
1400 1405 1410

Glu Leu Asn Trp Ala Ile Asp Asp Glu Glu Cys Lys Gly Leu Lys
1415 1420 1425

Lys Leu Tyr Leu Leu Ser Gly Arg Ala Lys Asn Leu Ile Val Lys
1430 1435 1440

His Lys Val Arg Asn Glu Ala Val His Arg Trp Phe Gly Glu Glu
1445 1450 1455

Glu Ile Tyr Gly Ala Pro Lys Val Ile Thr Ile Ile Lys Ala Ser
1460 1465 1470

Thr Leu Ser Lys Asn Arg His Cys Ile Ile Cys Thr Ile Cys Glu
1475 1480 1485

Gly Lys Glu Trp Asn Gly Ala Asn Cys Pro Lys Cys Gly Arg Gln
1490 1495 1500

Gly Lys Pro Ile Thr Cys Gly Met Thr Leu Ala Asp Phe Glu Glu
1505 1510 1515

Lys His Tyr Lys Lys Ile Phe Ile Arg Glu Glu Ser Ser Cys Pro
1520 1525 1530

Val Pro Phe Asp Pro Ser Cys His Cys Asn Tyr Phe Arg His Asp
1535 1540 1545

Gly Pro Phe Arg Lys Glu Tyr Lys Gly Tyr Val Gln Tyr Thr Ala
1550 1555 1560

Arg Gly Gln Leu Phe Leu Arg Asn Leu Pro Ile Leu Ala Thr Lys
1565 1570 1575

Met Lys Leu Leu Met Val Gly Asn Leu Gly Ala Glu Ile Gly Asp
1580 1585 1590

Leu Glu His Leu Gly Trp Val Leu Arg Gly Pro Ala Val Cys Lys
1595 1600 1605

Lys Ile Thr Asn His Glu Lys Cys His Val Asn Ile Met Asp Lys
1610 1615 1620

Leu Thr Ala Phe Phe Gly Ile Met Pro Arg Gly Thr Thr Pro Arg
1625 1630 1635

Ala Pro Val Arg Phe Pro Thr Ala Leu Leu Lys Val Arg Arg Gly
1640 1645 1650

Leu Glu Thr Gly Trp Ala Tyr Thr His Gln Gly Gly Ile Ser Ser
1655 1660 1665

Val Asp His Val Thr Ala Gly Lys Asp Leu Leu Val Cys Asp Ser
1670 1675 1680

Met Gly Arg Thr Arg Val Val Cys His Ser Asn Asn Lys Met Thr
1685 1690 1695

Asp Glu Thr Glu Tyr Gly Ile Lys Thr Asp Ser Gly Cys Pro Glu
1700 1705 1710

Gly Ala Arg Cys Tyr Val Leu Asn Pro Glu Ala Val Asn Ile Ser
1715 1720 1725

Gly Thr Lys Gly Ala Met Val His Leu Gln Lys Thr Gly Gly Glu
1730 1735 1740

Phe Thr Cys Val Thr Ala Ser Gly Thr Pro Ala Phe Phe Asp Leu
1745 1750 1755

Lys Asn Leu Lys Gly Trp Ser Gly Leu Pro Ile Phe Glu Ala Ser

1760 1765 1770

Ser Gly Arg Val Val Gly Arg Val Lys Val Gly Lys Asn Glu Asp
1775 1780 1785

Ser Lys Pro Thr Lys Leu Met Ser Gly Ile Gln Thr Val Ser Lys
1790 1795 1800

Asn Gln Thr Asp Leu Ala Asp Ile Val Lys Lys Leu Thr Ser Met
1805 1810 1815

Asn Arg Gly Glu Phe Lys Gln Ile Thr Leu Ala Thr Gly Ala Gly
1820 1825 1830

Lys Thr Thr Glu Leu Pro Arg Ser Val Ile Glu Glu Ile Gly Arg
1835 1840 1845

His Lys Arg Val Leu Val Leu Ile Pro Leu Arg Ala Ala Ala Glu
1850 1855 1860

Ser Val Tyr Gln Tyr Met Arg Val Lys Tyr Pro Ser Ile Ser Phe
1865 1870 1875

Asn Leu Arg Ile Gly Asp Met Lys Glu Gly Asp Met Ala Thr Gly
1880 1885 1890

Ile Thr Tyr Ala Ser Tyr Gly Tyr Phe Cys Gln Leu Pro Gln Pro
1895 1900 1905

Lys Leu Arg Ala Ala Met Val Glu Tyr Ser Tyr Ile Phe Leu Asp
1910 1915 1920

Glu Tyr His Cys Ala Thr Pro Glu Gln Leu Ala Ile Ile Gly Lys
1925 1930 1935

Ile His Arg Phe Ala Glu Asn Leu Arg Val Val Ala Met Thr Ala
1940 1945 1950

Thr Pro Ala Gly Thr Val Thr Thr Thr Gly Gln Lys His Pro Ile
1955 1960 1965

Glu Glu Phe Ile Ala Pro Glu Val Met Lys Gly Glu Asp Leu Gly
1970 1975 1980

Ser Glu Tyr Leu Asp Ile Ala Gly Leu Lys Ile Pro Thr Glu Glu
1985 1990 1995

Met Lys Gly Asn Met Leu Val Phe Ala Pro Thr Arg Asn Met Ala
2000 2005 2010

Val Glu Thr Ala Lys Lys Leu Lys Ala Lys Gly Tyr Asn Ser Gly
2015 2020 2025

Tyr Tyr Tyr Ser Gly Glu Asn Pro Glu Asn Leu Arg Val Val Thr
2030 2035 2040

Ser Gln Ser Pro Tyr Val Val Val Ala Thr Asn Ala Ile Glu Ser
2045 2050 2055

Gly Val Thr Leu Pro Asp Leu Asp Thr Val Val Asp Thr Gly Leu
2060 2065 2070

Lys Cys Glu Lys Arg Val Arg Ile Ser Ser Lys Met Pro Phe Ile
2075 2080 2085

Val Thr Gly Leu Lys Arg Met Ala Val Thr Ile Gly Glu Gln Ala
2090 2095 2100

Gln Arg Arg Gly Arg Val Gly Arg Val Lys Pro Gly Arg Tyr Tyr
2105 2110 2115

Arg Ser Gln Glu Thr Ala Ser Gly Ser Lys Asp Tyr His Tyr Asp
2120 2125 2130

Leu Leu Gln Ala Gln Arg Tyr Gly Ile Glu Asp Gly Ile Asn Val
2135 2140 2145

Thr Lys Ser Phe Arg Glu Met Asn Tyr Asp Trp Ser Leu Tyr Glu
2150 2155 2160

Glu Asp Ser Leu Met Ile Thr Gln Leu Glu Val Leu Asn Asn Leu
2165 2170 2175

Leu Ile Ser Glu Asp Leu Pro Ala Ala Val Lys Asn Ile Met Ala
2180 2185 2190

Arg Thr Asp His Pro Glu Pro Ile Gln Leu Ala Tyr Asn Ser Tyr
2195 2200 2205

Glu Asn Gln Ile Pro Val Leu Phe Pro Lys Ile Lys Asn Gly Glu
2210 2215 2220

Val Thr Asp Ser Tyr Glu Asn Tyr Thr Tyr Leu Asn Ala Arg Lys
2225 2230 2235

Leu Gly Glu Asp Val Pro Ala Tyr Val Tyr Ala Thr Glu Asp Glu
2240 2245 2250

Asp Leu Ala Val Asp Leu Leu Gly Met Asp Trp Pro Asp Pro Gly
2255 2260 2265

Asn Gln Gln Val Val Glu Thr Gly Arg Ala Leu Lys Gln Val Thr
2270 2275 2280

Gly Leu Ser Thr Ala Glu Asn Ala Leu Leu Ile Ala Leu Phe Gly
2285 2290 2295

Tyr Val Gly Tyr Gln Thr Leu Ser Lys Arg His Ile Pro Met Ile
2300 2305 2310

Thr Asp Ile Tyr Thr Leu Glu Asp His Arg Leu Glu Asp Thr Thr
2315 2320 2325

His Leu Gln Phe Ala Pro Asn Ala Ile Arg Thr Asp Gly Lys Asp
2330 2335 2340

Ser Glu Leu Lys Glu Leu Ala Val Gly Asp Leu Asp Lys Tyr Val
2345 2350 2355

Asp Ala Leu Val Asp Tyr Ser Lys Gln Gly Met Lys Phe Ile Lys
2360 2365 2370

Val Gln Ala Glu Lys Val Arg Asp Ser Gln Ser Thr Lys Glu Gly
2375 2380 2385

Leu Gln Thr Ile Lys Glu Tyr Val Asp Lys Phe Ile Gln Ser Leu
2390 2395 2400

Thr Glu Asn Lys Glu Glu Ile Ile Arg Tyr Gly Leu Trp Gly Val
2405 2410 2415

His Thr Ala Leu Tyr Lys Ser Leu Ala Ala Arg Leu Gly His Glu
2420 2425 2430

Thr Ala Phe Ala Thr Leu Val Val Lys Trp Leu Ala Phe Gly Gly
2435 2440 2445

Glu Thr Val Ser Ala His Ile Lys Gln Val Ala Val Asp Leu Val
2450 2455 2460

Val Tyr Tyr Ile Ile Asn Lys Pro Ser Phe Pro Gly Asp Thr Glu
2465 2470 2475

Thr Gln Gln Glu Gly Arg Arg Phe Val Ala Ser Leu Phe Ile Ser
2480 2485 2490

Ala Leu Ala Thr Tyr Thr Tyr Lys Thr Trp Asn Tyr Asn Asn Leu
2495 2500 2505

Gln Arg Val Val Glu Pro Ala Leu Ala Tyr Leu Pro Tyr Ala Thr
2510 2515 2520

Ser Ala Leu Lys Leu Phe Thr Pro Thr Arg Leu Glu Ser Val Val
2525 2530 2535

Ile Leu Ser Ser Thr Ile Tyr Lys Thr Tyr Leu Ser Ile Arg Lys
2540 2545 2550

Gly Lys Ser Asp Gly Leu Leu Gly Thr Gly Ile Ser Ala Ala Met
2555 2560 2565

Glu Ile Leu Asn Gln Asn Pro Ile Ser Val Gly Ile Ser Val Met
2570 2575 2580

Leu Gly Val Gly Ala Ile Ala Ala His Asn Ala Ile Glu Ser Ser
2585 2590 2595

Glu Gln Lys Arg Thr Leu Leu Met Lys Val Phe Val Lys Asn Phe
2600 2605 2610

Leu Asp Gln Ala Ala Thr Asp Glu Leu Val Lys Glu Asn Pro Glu
2615 2620 2625

Lys Ile Ile Met Ala Leu Phe Glu Ala Val Gln Thr Ile Gly Asn

2630 2635 2640

Pro Leu Arg Leu Ile Tyr His Leu Tyr Gly Val Tyr Tyr Lys Gly
2645 2650 2655

Trp Glu Ala Lys Glu Leu Ala Glu Lys Thr Ala Gly Arg Asn Leu
2660 2665 2670

Phe Thr Leu Ile Met Phe Glu Ala Phe Glu Leu Leu Gly Met Asp
2675 2680 2685

Ser Glu Gly Lys Ile Arg Asn Leu Ser Gly Asn Tyr Ile Leu Asp
2690 2695 2700

Leu Ile Phe Asn Leu His Asn Lys Leu Asn Lys Gly Leu Lys Lys
2705 2710 2715

Leu Val Leu Gly Trp Ala Pro Ala Pro Leu Ser Cys Asp Trp Thr
2720 2725 2730

Pro Ser Asp Glu Arg Ile Ser Leu Pro His Asn Asn Tyr Leu Arg
2735 2740 2745

Val Glu Thr Arg Cys Pro Cys Gly Tyr Glu Met Lys Ala Ile Lys
2750 2755 2760

Asn Val Ala Gly Lys Leu Thr Lys Val Glu Glu Lys Gly Ser Phe
2765 2770 2775

Leu Cys Arg Asn Arg Leu Gly Arg Gly Pro Pro Asn Phe Lys Val
2790 2785 2790

Thr Lys Phe Tyr Asp Asp Asn Leu Ile Glu Val Lys Pro Val Ala
2795 2800 2805

Arg Leu Glu Gly Gln Val Asp Leu Tyr Tyr Lys Gly Val Thr Ala
2810 2815 2820

Lys Leu Asp Tyr Asn Asn Gly Lys Val Leu Leu Ala Thr Asn Lys
2825 2830 2835

Trp Glu Val Asp His Ala Phe Leu Thr Arg Leu Val Lys Lys His
2840 2845 2850

Thr Gly Ile Gly Phe Lys Gly Ala Tyr Leu Gly Asp Arg Pro Asp
2855 2860 2865

His Gln Asp Leu Val Asp Arg Asp Cys Ala Thr Ile Thr Lys Asn
2870 2875 2880

Ser Val Gln Phe Leu Lys Met Lys Lys Gly Cys Ala Phe Thr Tyr
2885 2890 2895

Asp Leu Thr Ile Ser Asn Leu Val Arg Leu Ile Glu Leu Val His
2900 2905 2910

Lys Asn Asn Leu Gln Glu Arg Glu Ile Pro Thr Val Thr Val Thr
2915 2920 2925

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Thr Trp  Leu Ala Tyr Ser Phe  Val Asn Glu Asp Leu  Gly Thr Ile
     2930                2935                2940

Lys Pro  Val Leu Gly Glu Lys  Val Ile Pro Glu Pro  Pro Glu Glu
     2945                2950                2955

Leu Ser  Leu Gln Pro Thr Val  Arg Leu Val Thr Thr  Glu Thr Ala
     2960                2965                2970

Ile Thr  Ile Thr Gly Glu Ala  Glu Val Met Thr Thr  Gly Ile Thr
     2975                2980                2985

Pro Val  Val Glu Met Lys Glu  Glu Pro Gln Leu Asp  His Gln Ser
     2990                2995                3000

Thr Thr  Leu Lys Val Gly Leu  Lys Glu Gly Glu Tyr  Pro Gly Pro
     3005                3010                3015

Gly Val  Asn Pro Asn His Leu  Ala Glu Val Ile Asp  Glu Lys Asp
     3020                3025                3030

Asp Arg  Pro Phe Val Leu Ile  Ile Gly Asn Lys Gly  Ser Thr Ser
     3035                3040                3045

Asn Arg  Ala Arg Thr Ala Lys  Asn Ile Arg Leu Tyr  Lys Gly Asn
     3050                3055                3060

Asn Pro  Arg Glu Ile Arg Asp  Leu Met Ser Gln Gly  Arg Ile Leu
     3065                3070                3075

Thr Val  Ala Leu Lys Glu Leu  Asp Pro Glu Leu Lys  Glu Leu Val
     3080                3085                3090

Asp Tyr  Lys Gly Thr Phe Leu  Asn Arg Glu Ala Leu  Glu Ala Leu
     3095                3100                3105

Ser Leu  Gly Lys Pro Ile Lys  Arg Lys Thr Thr Thr  Ala Met Ile
     3110                3115                3120

Arg Arg  Leu Ile Glu Pro Glu  Val Glu Glu Glu Leu  Pro Asp Trp
     3125                3130                3135

Phe Gln  Ala Glu Glu Pro Leu  Phe Leu Glu Ala Lys  Ile Gln Asn
     3140                3145                3150

Asp Leu  Tyr His Leu Ile Gly  Ser Val Asp Ser Ile  Lys Ser Lys
     3155                3160                3165

Ala Lys  Glu Leu Gly Ala Thr  Asp Asn Thr Lys Ile  Val Lys Glu
     3170                3175                3180

Val Gly  Ala Arg Thr Tyr Thr  Met Lys Leu Ser Ser  Trp Ser Thr
     3185                3190                3195

Gln Val  Thr Lys Lys Gln Met  Ser Leu Ala Pro Leu  Phe Glu Glu
     3200                3205                3210
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Leu Leu Leu Lys Cys Pro Pro Cys Ser Lys Ile Ser Lys Gly His
3215 3220 3225

Met Val Ser Ala Tyr Gln Leu Ala Gln Gly Asn Trp Glu Pro Leu
3230 3235 3240

Gly Cys Gly Val Tyr Met Gly Thr Ile Pro Ala Arg Arg Leu Lys
3245 3250 3255

Ile His Pro Tyr Glu Ala Tyr Leu Lys Leu Lys Glu Leu Val Glu
3260 3265 3270

Val Glu Ser Ser Arg Ala Thr Ala Lys Glu Ser Ile Ile Arg Glu
3275 3280 3285

His Asn Thr Trp Ile Leu Arg Lys Val Arg His Glu Gly Asn Leu
3290 3295 3300

Arg Thr Lys Ser Met Ile Asn Pro Gly Lys Ile Ser Asp Gln Leu
3305 3310 3315

Cys Arg Asp Gly His Lys Arg Asn Ile Tyr Asn Lys Ile Ile Gly
3320 3325 3330

Ser Thr Met Ala Ser Ala Gly Ile Arg Leu Glu Lys Leu Pro Val
3335 3340 3345

Val Arg Ala Gln Thr Asp Thr Thr Ser Phe His Gln Ala Ile Arg
3350 3355 3360

Glu Lys Ile Asp Lys Thr Glu Asn Lys Gln Thr Pro Glu Leu His
3365 3370 3375

Glu Glu Leu Met Lys Val Phe Asp Cys Leu Lys Ile Pro Glu Leu
3380 3385 3390

Lys Glu Ser Tyr Asp Glu Val Ser Trp Glu Gln Leu Glu Ala Gly
3395 3400 3405

Ile Asn Arg Lys Gly Ala Ala Gly Tyr Leu Glu Ser Lys Asn Ile
3410 3415 3420

Gly Glu Val Leu Asp Thr Glu Lys His Ile Val Glu Gln Leu Ile
3425 3430 3435

Lys Asp Leu Arg Lys Gly Lys Lys Ile Arg Tyr Tyr Glu Thr Ala
3440 3445 3450

Ile Pro Lys Asn Glu Lys Arg Asp Val Ser Asp Asp Trp Glu Ala
3455 3460 3465

Gly Glu Phe Val Asp Glu Lys Lys Pro Arg Val Ile Gln Tyr Pro
3470 3475 3480

Asp Ala Lys Val Arg Leu Ala Ile Thr Lys Val Met Tyr Lys Trp
3485 3490 3495

Val Lys Gln Lys Pro Val Val Ile Pro Gly Tyr Glu Gly Lys Thr

3500 3505 3510

Pro Leu Phe Asp Ile Phe Asn Lys Val Lys Lys Glu Trp Asp Ser
3515 3520 3525

Phe Gln Asp Pro Val Ala Val Ser Phe Asp Thr Lys Ala Trp Asp
3530 3535 3540

Thr Gln Val Thr Ser Arg Asp Leu Met Leu Ile Lys Asp Ile Gln
3545 3550 3555

Lys Tyr Tyr Phe Lys Arg Ser Ile His Lys Phe Leu Asp Thr Ile
3560 3565 3570

Thr Glu His Met Val Glu Val Pro Val Ile Thr Ala Asp Gly Glu
3575 3580 3585

Val Tyr Ile Arg Asn Gly Gln Arg Gly Ser Gly Gln Pro Asp Thr
3590 3595 3600

Ser Ala Gly Asn Ser Met Leu Asn Val Leu Thr Met Ile Tyr Ala
3605 3610 3615

Phe Cys Lys Ser Thr Gly Ile Pro Tyr Arg Gly Phe Ser Arg Val
3620 3625 3630

Ala Arg Ile His Val Cys Gly Asp Asp Gly Phe Leu Ile Thr Glu
3635 3640 3645

Arg Gly Leu Gly Leu Lys Phe Ser Glu Lys Gly Met Gln Ile Leu
3650 3655 3660

His Glu Ala Gly Lys Pro Gln Lys Ile Thr Glu Gly Asp Lys Met
3665 3670 3675

Lys Val Ala Tyr Arg Phe Glu Asp Ile Glu Phe Cys Ser His Thr
3690 3685 3690

Pro Val Pro Val Arg Trp Ala Asp Asn Thr Ser Ser Tyr Met Ala
3695 3700 3705

Gly Arg Ser Thr Ala Thr Ile Leu Ala Lys Met Ala Thr Arg Leu
3710 3715 3720

Asp Ser Ser Gly Glu Arg Gly Ser Thr Ala Tyr Glu Lys Ala Val
3725 3730 3735

Ala Phe Ser Phe Leu Leu Met Tyr Ser Trp Asn Pro Val Val Arg
3740 3745 3750

Arg Ile Cys Leu Leu Val Leu Ser Gln Phe Pro Glu Ile Ser Pro
3755 3760 3765

Ser Lys Asn Thr Ile Tyr Tyr Tyr Gln Gly Asp Pro Ile Ala Ala
3770 3775 3780

Tyr Arg Glu Val Ile Gly Lys Gln Leu Cys Glu Leu Lys Arg Thr
3785 3790 3795

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Gly Phe  Glu Lys Leu Ala Gly  Leu Asn Leu Ser Met  Thr Thr Leu
    3800                 3805                 3810

Gly Ile  Trp Thr Lys His Thr  Ser Lys Arg Leu Ile  Gln Ala Cys
    3815                 3820                 3825

Val Glu  Ile Gly Lys Arg Glu  Gly Thr Trp Leu Val  Asn Ala Asp
    3830                 3835                 3840

Arg Leu  Ile Ala Gly Lys Thr  Gly Lys Phe Tyr Ile  Pro Ser Thr
    3845                 3850                 3855

Gly Val  Thr Leu Leu Gly Lys  His Tyr Glu Glu Ile  Asn Leu Lys
    3860                 3865                 3870

Gln Lys  Ala Ala Gln Pro Pro  Ile Glu Gly Val Asp  Arg Tyr Lys
    3875                 3880                 3885

Leu Gly  Pro Ile Val Asn Val  Ile Leu Arg Arg Leu  Arg Val Met
    3890                 3895                 3900

Leu Met  Thr Val Ala Ser Gly  Ser Trp
    3905                 3910
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<212> PRT
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<400> 8

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Gln Pro Lys Pro Asp Arg Ile Glu Lys Gly Lys Met Lys Ile Ala Pro
            20                  25                  30

Lys Glu Thr Glu Lys Asp Cys Lys Thr Arg Pro Pro Asp Ala Thr Ile
        35                  40                  45

Val Val Glu Gly Val Lys Tyr Gln Val Lys Lys Lys Gly Lys Val Arg
    50                  55                  60

Gly Lys Asn Thr Gln Asp Gly Leu Tyr His Asn Lys Asn Lys Pro Pro
65                  70                  75                  80

Glu Ser Arg Lys Lys Leu Glu Lys Ala Leu Leu Ala Trp Ala Ile Leu
                85                  90                  95

Ala Ala Val Leu Leu Gln Leu Val Thr Gly Glu Asn Ile Thr Gln Trp
            100                 105                 110

Asn Leu Met Asp Asn Gly Thr Glu Gly Ile Gln Gln Ala Met Phe Leu
        115                 120                 125

Arg Gly Val Asn Arg Ser Leu His Gly Ile Trp Pro Glu Lys Ile Cys
```

130 135 140

Thr Gly Val Pro Thr His Leu Ala Thr Asp Tyr Glu Leu Lys Glu Ile
145 150 155 160

Val Gly Met Met Asp Ala Ser Glu Lys Thr Asn Tyr Thr Cys Cys Arg
165 170 175

Leu Gln Arg His Glu Trp Asn Lys Gly Trp Cys Asn Trp Phe His Ile
180 185 190

Glu Pro Trp Ile Trp Leu Met Asn Lys Thr Gln Asn Asn Leu Thr Glu
195 200 205

Gly Gln Pro Leu Arg Glu Cys Ala Val Thr Cys Arg Tyr Asp Lys Glu
210 215 220

Thr Glu Leu Asn Ile Val Thr Gln Ala Arg Asp Arg Pro Thr Thr Leu
225 230 235 240

Thr Gly Cys Lys Lys Gly Lys Asn Phe Ser Phe Ala Gly Val Ile Leu
245 250 255

Asp Gly Pro Cys Asn Phe Lys Val Ser Val Glu Asp Val Leu Phe Lys
260 265 270

Glu His Asp Cys Gly Asn Met Leu Gln Glu Thr Ala Ile Gln Leu Leu
275 280 285

Asp Gly Ala Thr Asn Thr Ile Glu Gly Ala Arg Val Gly Thr Ala Lys
290 295 300

Leu Thr Thr Trp Leu Gly Lys Gln Leu Gly Ile Leu Gly Lys Lys Leu
305 310 315 320

Glu Asn Lys Ser Lys Ala Trp Phe Gly Ala His Ala Ala Ser Pro Tyr
325 330 335

Cys Gly Val Glu Arg Lys Ile Gly Tyr Val Trp Tyr Thr Lys Asn Cys
340 345 350

Thr Pro Ala Cys Leu Pro Arg Asn Thr Arg Ile Ile Gly Pro Gly Lys
355 360 365

Phe Asp Thr Asn Ala Glu Asp Gly Lys Ile Leu His Glu Met Gly Gly
370 375 380

His Leu Ser Glu Phe Val Leu Leu Ser Leu Val Val Leu Ser Asp Phe
385 390 395 400

Ala Pro Glu Thr Ala Ser Val Ile Tyr Leu Val Leu His Phe Ala Ile
405 410 415

Pro Gln Ser His Val Asp Val Asp Thr Cys Asp Lys Asn Gln Leu Asn
420 425 430

Leu Thr Val Ala Thr Thr Val Ala Glu Val Ile Pro Gly Thr Val Trp
435 440 445

Asn Leu Gly Lys Tyr Val Cys Ile Arg Pro Asp Trp Trp Pro Tyr Glu
450 455 460

Thr Thr Thr Val Phe Val Ile Glu Glu Ala Gly Gln Val Ile Lys Leu
465 470 475 480

Met Leu Arg Ala Ile Arg Asp Leu Thr Arg Ile Trp Asn Ala Ala Thr
485 490 495

Thr Thr Ala Phe Leu Ile Phe Leu Val Lys Ala Leu Arg Gly Gln Leu
500 505 510

Ile Gln Gly Leu Leu Trp Leu Met Leu Ile Thr Gly Ala Gln Gly Phe
515 520 525

Pro Glu Cys Lys Glu Gly Phe Gln Tyr Ala Ile Ser Lys Asp Arg Lys
530 535 540

Met Gly Leu Leu Gly Pro Glu Ser Leu Thr Thr Thr Trp His Leu Pro
545 550 555 560

Thr Lys Lys Ile Val Asp Ser Met Val His Val Trp Cys Glu Gly Lys
565 570 575

Asp Leu Lys Ile Leu Lys Met Cys Thr Lys Glu Glu Arg Tyr Leu Val
580 585 590

Ala Val His Glu Arg Ala Leu Ser Thr Ser Ala Glu Phe Met Gln Ile
595 600 605

Ser Asp Gly Thr Ile Gly Pro Asp Val Ile Asp Met Pro Asp Asp Phe
610 615 620

Glu Phe Gly Leu Cys Pro Cys Asp Ser Lys Pro Val Ile Lys Gly Lys
625 630 635 640

Phe Asn Ala Ser Leu Leu Asn Gly Pro Ala Phe Gln Met Val Cys Pro
645 650 655

Gln Gly Trp Thr Gly Thr Ile Glu Cys Thr Leu Ala Asn Gln Asp Thr
660 665 670

Leu Asp Thr Thr Val Ile Arg Thr Tyr Arg Arg Thr Thr Pro Phe Gln
675 680 685

Arg Arg Lys Trp Cys Thr Tyr Glu Lys Ile Ile Gly Glu Asp Ile Tyr
690 695 700

Glu Cys Ile Leu Gly Gly Asn Trp Thr Cys Ile Thr Gly Asp His Ser
705 710 715 720

Arg Leu Lys Asp Gly Pro Ile Lys Lys Cys Lys Trp Cys Gly His Asp
725 730 735

Phe Val Asn Ser Glu Gly Leu Pro His Tyr Pro Ile Gly Lys Cys Met
740 745 750

Leu Ile Asn Glu Ser Gly Tyr Arg Tyr Val Asp Asp Thr Ser Cys Asp
755 760 765

Arg Gly Gly Val Ala Ile Val Pro Ser Gly Thr Val Lys Cys Arg Ile
770 775 780

Gly Asn Val Thr Val Gln Val Ile Ala Thr Asn Asn Asp Leu Gly Pro
785 790 795 800

Met Pro Cys Ser Pro Ala Glu Val Ile Ala Ser Glu Gly Pro Val Glu
805 810 815

Lys Thr Ala Cys Thr Phe Asn Tyr Ser Arg Thr Leu Pro Asn Lys Tyr
820 825 830

Tyr Glu Pro Arg Asp Arg Tyr Phe Gln Gln Tyr Met Leu Lys Gly Glu
835 840 845

Trp Gln Tyr Trp Phe Asp Leu Asp Ser Val Asp His His Lys Asp Tyr
850 855 860

Phe Ser Glu Phe Ile Ile Ile Ala Val Val Ala Leu Leu Gly Gly Lys
865 870 875 880

Tyr Val Leu Trp Leu Leu Ile Thr Tyr Thr Ile Leu Ser Glu Gln Met
885 890 895

Ala Met Gly Ala Gly Val Asn Thr Glu Glu Ile Val Met Ile Gly Asn
900 905 910

Leu Leu Thr Asp Ser Asp Ile Glu Val Val Val Tyr Phe Leu Leu Leu
915 920 925

Tyr Leu Ile Val Lys Glu Glu Leu Ala Arg Lys Trp Ile Ile Leu Val
930 935 940

Tyr His Ile Leu Val Ala Asn Pro Met Lys Thr Ile Gly Val Val Leu
945 950 955 960

Leu Met Leu Gly Gly Val Val Lys Ala Ser Arg Ile Asn Ala Asp Asp
965 970 975

Gln Ser Ala Met Asp Pro Cys Phe Leu Leu Val Thr Gly Val Val Ala
980 985 990

Val Leu Met Ile Ala Arg Arg Glu Pro Ala Thr Leu Pro Leu Ile Val
995 1000 1005

Ala Leu Leu Ala Ile Arg Thr Ser Gly Phe Leu Leu Pro Ala Ser
1010 1015 1020

Ile Asp Val Thr Val Ala Val Val Leu Ile Val Leu Leu Leu Ala
1025 1030 1035

Ser Tyr Ile Thr Asp Tyr Phe Arg Tyr Lys Lys Trp Leu Gln Leu
1040 1045 1050

Leu Phe Ser Leu Ile Ala Gly Ile Phe Ile Ile Arg Ser Leu Lys

1055 1060 1065

His Ile Asn Gln Met Glu Val Pro Glu Ile Ser Met Pro Ser Trp
1070 1075 1080

Arg Pro Leu Ala Leu Val Leu Phe Tyr Ile Thr Ser Thr Ala Ile
1085 1090 1095

Thr Thr Asn Trp Asp Ile Asp Leu Ala Gly Phe Leu Leu Gln Trp
1100 1105 1110

Ala Pro Ala Val Ile Met Met Ala Thr Met Trp Ala Asp Phe Leu
1115 1120 1125

Thr Leu Ile Ile Val Leu Pro Ser Tyr Glu Leu Ser Lys Leu Tyr
1130 1135 1140

Phe Leu Lys Asn Val Arg Thr Asp Val Glu Lys Asn Trp Leu Gly
1145 1150 1155

Lys Val Lys Tyr Arg Gln Ile Ser Ser Val Tyr Asp Ile Cys Asp
1160 1165 1170

Ser Glu Glu Ala Val Tyr Leu Phe Pro Ser Arg His Lys Ser Gly
1175 1180 1185

Ser Arg Pro Asp Phe Ile Leu Pro Phe Leu Lys Ala Val Leu Ile
1190 1195 1200

Ser Cys Ile Ser Ser Gln Trp Gln Val Val Tyr Ile Ser Tyr Leu
1205 1210 1215

Ile Leu Glu Ile Thr Tyr Tyr Met His Arg Lys Ile Ile Asp Glu
1220 1225 1230

Val Ser Gly Gly Ala Asn Phe Leu Ser Arg Leu Ile Ala Ala Ile
1235 1240 1245

Ile Glu Leu Asn Trp Ala Ile Asp Asp Glu Glu Cys Lys Gly Leu
1250 1255 1260

Lys Lys Leu Tyr Leu Leu Ser Gly Arg Ala Lys Asn Leu Ile Val
1265 1270 1275

Lys His Lys Val Arg Asn Glu Ala Val His Arg Trp Phe Gly Glu
1280 1285 1290

Glu Glu Ile Tyr Gly Ala Pro Lys Val Ile Thr Ile Ile Lys Ala
1295 1300 1305

Ser Thr Leu Ser Lys Asn Arg His Cys Ile Ile Cys Thr Ile Cys
1310 1315 1320

Glu Gly Lys Glu Trp Asn Gly Ala Asn Cys Pro Lys Cys Gly Arg
1325 1330 1335

Gln Gly Lys Pro Ile Thr Cys Gly Met Thr Leu Ala Asp Phe Glu
1340 1345 1350

Glu Lys His Tyr Lys Lys Ile Phe Ile Arg Glu Glu Ser Ser Cys
1355 1360 1365

Pro Val Pro Phe Asp Pro Ser Cys His Cys Asn Tyr Phe Arg His
1370 1375 1380

Asp Gly Pro Phe Arg Lys Glu Tyr Lys Gly Tyr Val Gln Tyr Thr
1385 1390 1395

Ala Arg Gly Gln Leu Phe Leu Arg Asn Leu Pro Ile Leu Ala Thr
1400 1405 1410

Lys Met Lys Leu Leu Met Val Gly Asn Leu Gly Ala Glu Ile Gly
1415 1420 1425

Asp Leu Glu His Leu Gly Trp Val Leu Arg Gly Pro Ala Val Cys
1430 1435 1440

Lys Lys Ile Thr Asn His Glu Lys Cys His Val Asn Ile Met Asp
1445 1450 1455

Lys Leu Thr Ala Phe Phe Gly Ile Met Pro Arg Gly Thr Thr Pro
1460 1465 1470

Arg Ala Pro Val Arg Phe Pro Thr Ala Leu Leu Lys Val Arg Arg
1475 1480 1485

Gly Leu Glu Thr Gly Trp Ala Tyr Thr His Gln Gly Gly Ile Ser
1490 1495 1500

Ser Val Asp His Val Thr Ala Gly Lys Asp Leu Leu Val Cys Asp
1505 1510 1515

Ser Met Gly Arg Thr Arg Val Val Cys His Ser Asn Asn Lys Met
1520 1525 1530

Thr Asp Glu Thr Glu Tyr Gly Ile Lys Thr Asp Ser Gly Cys Pro
1535 1540 1545

Glu Gly Ala Arg Cys Tyr Val Leu Asn Pro Glu Ala Val Asn Ile
1550 1555 1560

Ser Gly Thr Lys Gly Ala Met Val His Leu Gln Lys Thr Gly Gly
1565 1570 1575

Glu Phe Thr Cys Val Thr Ala Ser Gly Thr Pro Ala Phe Phe Asp
1580 1585 1590

Leu Lys Asn Leu Lys Gly Trp Ser Gly Leu Pro Ile Phe Glu Ala
1595 1600 1605

Ser Ser Gly Arg Val Val Gly Arg Val Lys Val Gly Lys Asn Glu
1610 1615 1620

Asp Ser Lys Pro Thr Lys Leu Met Ser Gly Ile Gln Thr Val Ser
1625 1630 1635

Lys Asn Gln Thr Asp Leu Ala Asp Ile Val Lys Lys Leu Thr Ser
1640 1645 1650

Met Asn Arg Gly Glu Phe Lys Gln Ile Thr Leu Ala Thr Gly Ala
1655 1660 1665

Gly Lys Thr Thr Glu Leu Pro Arg Ser Val Ile Glu Glu Ile Gly
1670 1675 1680

Arg His Lys Arg Val Leu Val Leu Ile Pro Leu Arg Ala Ala Ala
1685 1690 1695

Glu Ser Val Tyr Gln Tyr Met Arg Val Lys Tyr Pro Ser Ile Ser
1700 1705 1710

Phe Asn Leu Arg Ile Gly Asp Met Lys Glu Gly Asp Met Ala Thr
1715 1720 1725

Gly Ile Thr Tyr Ala Ser Tyr Gly Tyr Phe Cys Gln Leu Pro Gln
1730 1735 1740

Pro Lys Leu Arg Ala Ala Met Val Glu Tyr Ser Tyr Ile Phe Leu
1745 1750 1755

Asp Glu Tyr His Cys Ala Thr Pro Glu Gln Leu Ala Ile Ile Gly
1760 1765 1770

Lys Ile His Arg Phe Ala Glu Asn Leu Arg Val Val Ala Met Thr
1775 1780 1785

Ala Thr Pro Ala Gly Thr Val Thr Thr Thr Gly Gln Lys His Pro
1790 1795 1800

Ile Glu Glu Phe Ile Ala Pro Glu Val Met Lys Gly Glu Asp Leu
1805 1810 1815

Gly Ser Glu Tyr Leu Asp Ile Ala Gly Leu Lys Ile Pro Thr Glu
1820 1825 1830

Glu Met Lys Gly Asn Met Leu Val Phe Ala Pro Thr Arg Asn Met
1835 1840 1845

Ala Val Glu Thr Ala Lys Lys Leu Lys Ala Lys Gly Tyr Asn Ser
1850 1855 1860

Gly Tyr Tyr Tyr Ser Gly Glu Asn Pro Glu Asn Leu Arg Val Val
1865 1870 1875

Thr Ser Gln Ser Pro Tyr Val Val Val Ala Thr Asn Ala Ile Glu
1880 1885 1890

Ser Gly Val Thr Leu Pro Asp Leu Asp Thr Val Val Asp Thr Gly
1895 1900 1905

Leu Lys Cys Glu Lys Arg Val Arg Ile Ser Ser Lys Met Pro Phe
1910 1915 1920

Ile Val Thr Gly Leu Lys Arg Met Ala Val Thr Ile Gly Glu Gln

1925 1930 1935

Ala Gln Arg Arg Gly Arg Val Gly Arg Val Lys Pro Gly Arg Tyr
1940 1945 1950

Tyr Arg Ser Gln Glu Thr Ala Ser Gly Ser Lys Asp Tyr His Tyr
1955 1960 1965

Asp Leu Leu Gln Ala Gln Arg Tyr Gly Ile Glu Asp Gly Ile Asn
1970 1975 1980

Val Thr Lys Ser Phe Arg Glu Met Asn Tyr Asp Trp Ser Leu Tyr
1985 1990 1995

Glu Glu Asp Ser Leu Met Ile Thr Gln Leu Glu Val Leu Asn Asn
2000 2005 2010

Leu Leu Ile Ser Glu Asp Leu Pro Ala Ala Val Lys Asn Ile Met
2015 2020 2025

Ala Arg Thr Asp His Pro Glu Pro Ile Gln Leu Ala Tyr Asn Ser
2030 2035 2040

Tyr Glu Asn Gln Ile Pro Val Leu Phe Pro Lys Ile Lys Asn Gly
2045 2050 2055

Glu Val Thr Asp Ser Tyr Glu Asn Tyr Thr Tyr Leu Asn Ala Arg
2060 2065 2070

Lys Leu Gly Glu Asp Val Pro Ala Tyr Val Tyr Ala Thr Glu Asp
2075 2080 2085

Glu Asp Leu Ala Val Asp Leu Leu Gly Met Asp Trp Pro Asp Pro
2090 2095 2100

Gly Asn Gln Gln Val Val Glu Thr Gly Arg Ala Leu Lys Gln Val
2105 2110 2115

Thr Gly Leu Ser Thr Ala Glu Asn Ala Leu Leu Ile Ala Leu Phe
2120 2125 2130

Gly Tyr Val Gly Tyr Gln Thr Leu Ser Lys Arg His Ile Pro Met
2135 2140 2145

Ile Thr Asp Ile Tyr Thr Leu Glu Asp His Arg Leu Glu Asp Thr
2150 2155 2160

Thr His Leu Gln Phe Ala Pro Asn Ala Ile Arg Thr Asp Gly Lys
2165 2170 2175

Asp Ser Glu Leu Lys Glu Leu Ala Val Gly Asp Leu Asp Lys Tyr
2180 2185 2190

Val Asp Ala Leu Val Asp Tyr Ser Lys Gln Gly Met Lys Phe Ile
2195 2200 2205

Lys Val Gln Ala Glu Lys Val Arg Asp Ser Gln Ser Thr Lys Glu
2210 2215 2220

Gly Leu Gln Thr Ile Lys Glu Tyr Val Asp Lys Phe Ile Gln Ser
2225 2230 2235

Leu Thr Glu Asn Lys Glu Glu Ile Ile Arg Tyr Gly Leu Trp Gly
2240 2245 2250

Val His Thr Ala Leu Tyr Lys Ser Leu Ala Ala Arg Leu Gly His
2255 2260 2265

Glu Thr Ala Phe Ala Thr Leu Val Val Lys Trp Leu Ala Phe Gly
2270 2275 2280

Gly Glu Thr Val Ser Ala His Ile Lys Gln Val Ala Val Asp Leu
2285 2290 2295

Val Val Tyr Tyr Ile Ile Asn Lys Pro Ser Phe Pro Gly Asp Thr
2300 2305 2310

Glu Thr Gln Gln Glu Gly Arg Arg Phe Val Ala Ser Leu Phe Ile
2315 2320 2325

Ser Ala Leu Ala Thr Tyr Thr Tyr Lys Thr Trp Asn Tyr Asn Asn
2330 2335 2340

Leu Gln Arg Val Val Glu Pro Ala Leu Ala Tyr Leu Pro Tyr Ala
2345 2350 2355

Thr Ser Ala Leu Lys Leu Phe Thr Pro Thr Arg Leu Glu Ser Val
2360 2365 2370

Val Ile Leu Ser Ser Thr Ile Tyr Lys Thr Tyr Leu Ser Ile Arg
2375 2380 2385

Lys Gly Lys Ser Asp Gly Leu Leu Gly Thr Gly Ile Ser Ala Ala
2390 2395 2400

Met Glu Ile Leu Asn Gln Asn Pro Ile Ser Val Gly Ile Ser Val
2405 2410 2415

Met Leu Gly Val Gly Ala Ile Ala Ala His Asn Ala Ile Glu Ser
2420 2425 2430

Ser Glu Gln Lys Arg Thr Leu Leu Met Lys Val Phe Val Lys Asn
2435 2440 2445

Phe Leu Asp Gln Ala Ala Thr Asp Glu Leu Val Lys Glu Asn Pro
2450 2455 2460

Glu Lys Ile Ile Met Ala Leu Phe Glu Ala Val Gln Thr Ile Gly
2465 2470 2475

Asn Pro Leu Arg Leu Ile Tyr His Leu Tyr Gly Val Tyr Tyr Lys
2480 2485 2490

Gly Trp Glu Ala Lys Glu Leu Ala Glu Lys Thr Ala Gly Arg Asn
2495 2500 2505

Leu Phe Thr Leu Ile Met Phe Glu Ala Phe Glu Leu Leu Gly Met
2510 2515 2520

Asp Ser Glu Gly Lys Ile Arg Asn Leu Ser Gly Asn Tyr Ile Leu
2525 2530 2535

Asp Leu Ile Phe Asn Leu His Asn Lys Leu Asn Lys Gly Leu Lys
2540 2545 2550

Lys Leu Val Leu Gly Trp Ala Pro Ala Pro Leu Ser Cys Asp Trp
2555 2560 2565

Thr Pro Ser Asp Glu Arg Ile Ser Leu Pro His Asn Asn Tyr Leu
2570 2575 2580

Arg Val Glu Thr Arg Cys Pro Cys Gly Tyr Glu Met Lys Ala Ile
2585 2590 2595

Lys Asn Val Ala Gly Lys Leu Thr Lys Val Glu Glu Lys Gly Ser
2600 2605 2610

Phe Leu Cys Arg Asn Arg Leu Gly Arg Gly Pro Pro Asn Phe Lys
2615 2620 2625

Val Thr Lys Phe Tyr Asp Asp Asn Leu Ile Glu Val Lys Pro Val
2630 2635 2640

Ala Arg Leu Glu Gly Gln Val Asp Leu Tyr Tyr Lys Gly Val Thr
2645 2650 2655

Ala Lys Leu Asp Tyr Asn Asn Gly Lys Val Leu Leu Ala Thr Asn
2660 2665 2670

Lys Trp Glu Val Asp His Ala Phe Leu Thr Arg Leu Val Lys Lys
2675 2680 2685

His Thr Gly Ile Gly Phe Lys Gly Ala Tyr Leu Gly Asp Arg Pro
2690 2695 2700

Asp His Gln Asp Leu Val Asp Arg Asp Cys Ala Thr Ile Thr Lys
2705 2710 2715

Asn Ser Val Gln Phe Leu Lys Met Lys Lys Gly Cys Ala Phe Thr
2720 2725 2730

Tyr Asp Leu Thr Ile Ser Asn Leu Val Arg Leu Ile Glu Leu Val
2735 2740 2745

His Lys Asn Asn Leu Gln Glu Arg Glu Ile Pro Thr Val Thr Val
2750 2755 2760

Thr Thr Trp Leu Ala Tyr Ser Phe Val Asn Glu Asp Leu Gly Thr
2765 2770 2775

Ile Lys Pro Val Leu Gly Glu Lys Val Ile Pro Glu Pro Pro Glu
2780 2785 2790

Glu Leu Ser Leu Gln Pro Thr Val Arg Leu Val Thr Thr Glu Thr

2795 2800 2805

Ala Ile Thr Ile Thr Gly Glu Ala Glu Val Met Thr Thr Gly Ile
2810 2815 2820

Thr Pro Val Val Glu Met Lys Glu Glu Pro Gln Leu Asp His Gln
2825 2830 2835

Ser Thr Thr Leu Lys Val Gly Leu Lys Glu Gly Glu Tyr Pro Gly
2840 2845 2850

Pro Gly Val Asn Pro Asn His Leu Ala Glu Val Ile Asp Glu Lys
2855 2860 2865

Asp Asp Arg Pro Phe Val Leu Ile Ile Gly Asn Lys Gly Ser Thr
2870 2875 2880

Ser Asn Arg Ala Arg Thr Ala Lys Asn Ile Arg Leu Tyr Lys Gly
2885 2890 2895

Asn Asn Pro Arg Glu Ile Arg Asp Leu Met Ser Gln Gly Arg Ile
2900 2905 2910

Leu Thr Val Ala Leu Lys Glu Leu Asp Pro Glu Leu Lys Glu Leu
2915 2920 2925

Val Asp Tyr Lys Gly Thr Phe Leu Asn Arg Glu Ala Leu Glu Ala
2930 2935 2940

Leu Ser Leu Gly Lys Pro Ile Lys Arg Lys Thr Thr Thr Ala Met
2945 2950 2955

Ile Arg Arg Leu Ile Glu Pro Glu Val Glu Glu Glu Leu Pro Asp
2960 2965 2970

Trp Phe Gln Ala Glu Glu Pro Leu Phe Leu Glu Ala Lys Ile Gln
2975 2980 2985

Asn Asp Leu Tyr His Leu Ile Gly Ser Val Asp Ser Ile Lys Ser
2990 2995 3000

Lys Ala Lys Glu Leu Gly Ala Thr Asp Asn Thr Lys Ile Val Lys
3005 3010 3015

Glu Val Gly Ala Arg Thr Tyr Thr Met Lys Leu Ser Ser Trp Ser
3020 3025 3030

Thr Gln Val Thr Lys Lys Gln Met Ser Leu Ala Pro Leu Phe Glu
3035 3040 3045

Glu Leu Leu Leu Lys Cys Pro Pro Cys Ser Lys Ile Ser Lys Gly
3050 3055 3060

His Met Val Ser Ala Tyr Gln Leu Ala Gln Gly Asn Trp Glu Pro
3065 3070 3075

Leu Gly Cys Gly Val Tyr Met Gly Thr Ile Pro Ala Arg Arg Leu
3080 3085 3090

Lys Ile His Pro Tyr Glu Ala Tyr Leu Lys Leu Lys Glu Leu Val
3095 3100 3105

Glu Val Glu Ser Ser Arg Ala Thr Ala Lys Glu Ser Ile Ile Arg
3110 3115 3120

Glu His Asn Thr Trp Ile Leu Arg Lys Val Arg His Glu Gly Asn
3125 3130 3135

Leu Arg Thr Lys Ser Met Ile Asn Pro Gly Lys Ile Ser Asp Gln
3140 3145 3150

Leu Cys Arg Asp Gly His Lys Arg Asn Ile Tyr Asn Lys Ile Ile
3155 3160 3165

Gly Ser Thr Met Ala Ser Ala Gly Ile Arg Leu Glu Lys Leu Pro
3170 3175 3180

Val Val Arg Ala Gln Thr Asp Thr Thr Ser Phe His Gln Ala Ile
3185 3190 3195

Arg Glu Lys Ile Asp Lys Thr Glu Asn Lys Gln Thr Pro Glu Leu
3200 3205 3210

His Glu Glu Leu Met Lys Val Phe Asp Cys Leu Lys Ile Pro Glu
3215 3220 3225

Leu Lys Glu Ser Tyr Asp Glu Val Ser Trp Glu Gln Leu Glu Ala
3230 3235 3240

Gly Ile Asn Arg Lys Gly Ala Ala Gly Tyr Leu Glu Ser Lys Asn
3245 3250 3255

Ile Gly Glu Val Leu Asp Thr Glu Lys His Ile Val Glu Gln Leu
3260 3265 3270

Ile Lys Asp Leu Arg Lys Gly Lys Lys Ile Arg Tyr Tyr Glu Thr
3275 3280 3285

Ala Ile Pro Lys Asn Glu Lys Arg Asp Val Ser Asp Asp Trp Glu
3290 3295 3300

Ala Gly Glu Phe Val Asp Glu Lys Lys Pro Arg Val Ile Gln Tyr
3305 3310 3315

Pro Asp Ala Lys Val Arg Leu Ala Ile Thr Lys Val Met Tyr Lys
3320 3325 3330

Trp Val Lys Gln Lys Pro Val Val Ile Pro Gly Tyr Glu Gly Lys
3335 3340 3345

Thr Pro Leu Phe Asp Ile Phe Asn Lys Val Lys Lys Glu Trp Asp
3350 3355 3360

Ser Phe Gln Asp Pro Val Ala Val Ser Phe Asp Thr Lys Ala Trp
3365 3370 3375

Asp Thr Gln Val Thr Ser Arg Asp Leu Met Leu Ile Lys Asp Ile
3380 3385 3390

Gln Lys Tyr Tyr Phe Lys Arg Ser Ile His Lys Phe Leu Asp Thr
3395 3400 3405

Ile Thr Glu His Met Val Glu Val Pro Val Ile Thr Ala Asp Gly
3410 3415 3420

Glu Val Tyr Ile Arg Asn Gly Gln Arg Gly Ser Gly Gln Pro Asp
3425 3430 3435

Thr Ser Ala Gly Asn Ser Met Leu Asn Val Leu Thr Met Ile Tyr
3440 3445 3450

Ala Phe Cys Lys Ser Thr Gly Ile Pro Tyr Arg Gly Phe Ser Arg
3455 3460 3465

Val Ala Arg Ile His Val Cys Gly Asp Asp Gly Phe Leu Ile Thr
3470 3475 3480

Glu Arg Gly Leu Gly Leu Lys Phe Ser Glu Lys Gly Met Gln Ile
3485 3490 3495

Leu His Glu Ala Gly Lys Pro Gln Lys Ile Thr Glu Gly Asp Lys
3500 3505 3510

Met Lys Val Ala Tyr Arg Phe Glu Asp Ile Glu Phe Cys Ser His
3515 3520 3525

Thr Pro Val Pro Val Arg Trp Ala Asp Asn Thr Ser Ser Tyr Met
3530 3535 3540

Ala Gly Arg Ser Thr Ala Thr Ile Leu Ala Lys Met Ala Thr Arg
3545 3550 3555

Leu Asp Ser Ser Gly Glu Arg Gly Ser Thr Ala Tyr Glu Lys Ala
3560 3565 3570

Val Ala Phe Ser Phe Leu Leu Met Tyr Ser Trp Asn Pro Val Val
3575 3580 3585

Arg Arg Ile Cys Leu Leu Val Leu Ser Gln Phe Pro Glu Ile Ser
3590 3595 3600

Pro Ser Lys Asn Thr Ile Tyr Tyr Tyr Gln Gly Asp Pro Ile Ala
3605 3610 3615

Ala Tyr Arg Glu Val Ile Gly Lys Gln Leu Cys Glu Leu Lys Arg
3620 3625 3630

Thr Gly Phe Glu Lys Leu Ala Gly Leu Asn Leu Ser Met Thr Thr
3635 3640 3645

Leu Gly Ile Trp Thr Lys His Thr Ser Lys Arg Leu Ile Gln Ala
3650 3655 3660

Cys Val Glu Ile Gly Lys Arg Glu Gly Thr Trp Leu Val Asn Ala

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    3665                3670                3675

Asp Arg  Leu Ile Ala Gly Lys  Thr Gly Lys Phe Tyr  Ile Pro Ser
    3680                3685                3690

Thr Gly  Val Thr Leu Leu Gly  Lys His Tyr Glu Glu  Ile Asn Leu
    3695                3700                3705

Lys Gln  Lys Ala Ala Gln Pro  Pro Ile Glu Gly Val  Asp Arg Tyr
    3710                3715                3720

Lys Leu  Gly Pro Ile Val Asn  Val Ile Leu Arg Arg  Leu Arg Val
    3725                3730                3735

Met Leu  Met Thr Val Ala Ser  Gly Ser Trp
    3740                3745
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<212> PRT
<213> Artificial sequence:XIKE-C EVDV-Sequence

<400> 9

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Pro Ala Gly Val Val Glu Pro Val Tyr Asp Val Asn Gly Arg Pro Leu
            20                  25                  30

Phe Gly Glu Ser Ser Asp Leu His Pro Gln Ser Thr Leu Lys Leu Pro
        35                  40                  45

His Gln Arg Gly Ser Ala Asn Ile Leu Thr Asn Ala Arg Ser Leu Pro
    50                  55                  60

Arg Lys Gly Asp Cys Arg Arg Gly Asn Val Tyr Gly Pro Val Ser Gly
65                  70                  75                  80

Ile Tyr Ile Lys Pro Gly Pro Ile Tyr Tyr Gln Asp Tyr Val Gly Pro
                85                  90                  95

Val Tyr His Arg Ala Pro Leu Glu Leu Cys Arg Glu Ala Ser Met Cys
            100                 105                 110

Glu Thr Thr Arg Arg Val Gly Arg Val Thr Gly Ser Asp Gly Lys Leu
        115                 120                 125

Tyr His Ile Tyr Ile Cys Ile Asp Gly Cys Ile Leu Leu Lys Arg Ala
    130                 135                 140

Thr Arg Asn Gln Pro Glu Val Leu Lys Trp Val Tyr Asn Arg Leu Asn
145                 150                 155                 160

Cys Pro Leu Trp Val Thr Ser Cys Ser Asp Glu Gly Ser Lys Gly Ala
                165                 170                 175
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Thr Ser Lys Lys Gln Pro Lys Pro Asp Arg Ile Glu Lys Gly Lys Met
180 185 190

Lys Ile Ala Pro Lys Glu Thr Glu Lys Asp Cys Lys Thr Arg Pro Pro
195 200 205

Asp Ala Thr Ile Val Val Glu Gly Val Lys Tyr Gln Val Lys Lys Lys
210 215 220

Gly Lys Val Arg Gly Lys Asn Thr Gln Asp Gly Leu Tyr His Asn Lys
225 230 235 240

Asn Lys Pro Pro Glu Ser Arg Lys Lys Leu Glu Lys Ala Leu Leu Ala
245 250 255

Trp Ala Ile Leu Ala Ala Val Leu Leu Gln Leu Val Thr Gly Glu Asn
260 265 270

Ile Thr Gln Trp Asn Leu Met Asp Asn Gly Thr Glu Gly Ile Gln Gln
275 280 285

Ala Met Phe Leu Arg Gly Val Asn Arg Ser Leu Leu Gly Ile Trp Pro
290 295 300

Glu Lys Ile Cys Thr Gly Val Pro Thr His Leu Ala Thr Asp Tyr Glu
305 310 315 320

Leu Lys Glu Ile Val Gly Met Met Asp Ala Ser Glu Lys Thr Asn Tyr
325 330 335

Thr Cys Cys Arg Leu Gln Arg His Glu Trp Asn Lys His Gly Trp Cys
340 345 350

Asn Trp Phe His Ile Glu Pro Trp Ile Trp Leu Met Asn Lys Thr Gln
355 360 365

Asn Asn Leu Thr Glu Gly Gln Pro Leu Arg Glu Cys Ala Val Thr Cys
370 375 380

Arg Tyr Asp Lys Glu Thr Glu Leu Asn Ile Val Thr Gln Ala Arg Asp
385 390 395 400

Arg Pro Thr Thr Leu Thr Gly Cys Lys Lys Gly Lys Asn Phe Ser Phe
405 410 415

Ala Gly Val Ile Leu Asp Gly Pro Cys Asn Phe Lys Val Ser Val Glu
420 425 430

Asp Val Leu Phe Lys Glu His Asp Cys Gly Asn Met Leu Gln Glu Thr
435 440 445

Ala Ile Gln Leu Leu Asp Gly Ala Thr Asn Thr Ile Glu Gly Ala Arg
450 455 460

Val Gly Thr Ala Lys Leu Thr Thr Trp Leu Gly Lys Gln Leu Gly Ile
465 470 475 480

Leu Gly Lys Lys Leu Glu Asn Lys Ser Lys Ala Trp Phe Gly Ala His

485 490 495

Ala Ala Ser Pro Tyr Cys Gly Val Glu Arg Lys Ile Gly Tyr Val Trp
500 505 510

Tyr Thr Lys Asn Cys Thr Pro Ala Cys Leu Pro Arg Asn Thr Arg Ile
515 520 525

Ile Gly Pro Gly Lys Phe Asp Thr Asn Ala Glu Asp Gly Lys Ile Leu
530 535 540

His Glu Met Gly Gly His Leu Ser Glu Phe Val Leu Leu Ser Leu Val
545 550 555 560

Val Leu Ser Asp Phe Ala Pro Glu Thr Ala Ser Val Ile Tyr Leu Val
565 570 575

Leu His Phe Ala Ile Pro Gln Ser His Val Asp Val Asp Thr Cys Asp
580 585 590

Lys Asn Gln Leu Asn Leu Thr Val Ala Thr Thr Val Ala Glu Val Ile
595 600 605

Pro Gly Thr Val Trp Asn Leu Gly Lys Tyr Val Cys Ile Arg Pro Asp
610 615 620

Trp Trp Pro Tyr Glu Thr Thr Thr Val Phe Val Ile Glu Glu Ala Gly
625 630 635 640

Gln Val Ile Lys Leu Met Leu Arg Ala Ile Arg Asp Leu Thr Arg Ile
645 650 655

Trp Asn Ala Ala Thr Thr Thr Ala Phe Leu Ile Phe Leu Val Lys Ala
660 665 670

Leu Arg Gly Gln Leu Ile Gln Gly Leu Leu Trp Leu Met Leu Ile Thr
675 680 685

Gly Ala Gln Gly Phe Pro Glu Cys Lys Glu Gly Phe Gln Tyr Ala Ile
690 695 700

Ser Lys Asp Arg Lys Met Gly Leu Leu Gly Pro Glu Ser Leu Thr Thr
705 710 715 720

Thr Trp His Leu Pro Thr Lys Lys Ile Val Asp Ser Met Val His Val
725 730 735

Trp Cys Glu Gly Lys Asp Leu Lys Ile Leu Lys Met Cys Thr Lys Glu
740 745 750

Glu Arg Tyr Leu Val Ala Val His Glu Arg Ala Leu Ser Thr Ser Ala
755 760 765

Glu Phe Met Gln Ile Ser Asp Gly Thr Ile Gly Pro Asp Val Ile Asp
770 775 780

Met Pro Asp Asp Phe Glu Phe Gly Leu Cys Pro Cys Asp Ser Lys Pro
785 790 795 800

Val Ile Lys Gly Lys Phe Asn Ala Ser Leu Leu Asn Gly Pro Ala Phe
805 810 815

Gln Met Val Cys Pro Gln Gly Trp Thr Gly Thr Ile Glu Cys Thr Leu
820 825 830

Ala Asn Gln Asp Thr Leu Asp Thr Thr Val Ile Arg Thr Tyr Arg Arg
835 840 845

Thr Thr Pro Phe Gln Arg Arg Lys Trp Cys Thr Tyr Glu Lys Ile Ile
850 855 860

Gly Glu Asp Ile Tyr Glu Cys Ile Leu Gly Gly Asn Trp Thr Cys Ile
865 870 875 880

Thr Gly Asp His Ser Arg Leu Lys Asp Gly Pro Ile Lys Lys Cys Lys
885 890 895

Trp Cys Gly His Asp Phe Val Asn Ser Glu Gly Leu Pro His Tyr Pro
900 905 910

Ile Gly Lys Cys Met Leu Ile Asn Glu Ser Gly Tyr Arg Tyr Val Asp
915 920 925

Asp Thr Ser Cys Asp Arg Gly Gly Val Ala Ile Val Pro Ser Gly Thr
930 935 940

Val Lys Cys Arg Ile Gly Asn Val Thr Val Gln Val Ile Ala Thr Asn
945 950 955 960

Asn Asp Leu Gly Pro Met Pro Cys Ser Pro Ala Glu Val Ile Ala Ser
965 970 975

Glu Gly Pro Val Glu Lys Thr Ala Cys Thr Phe Asn Tyr Ser Arg Thr
980 985 990

Leu Pro Asn Lys Tyr Tyr Glu Pro Arg Asp Arg Tyr Phe Gln Gln Tyr
995 1000 1005

Met Leu Lys Gly Glu Trp Gln Tyr Trp Phe Asp Leu Asp Ser Val
1010 1015 1020

Asp His His Lys Asp Tyr Phe Ser Glu Phe Ile Ile Ile Ala Val
1025 1030 1035

Val Ala Leu Leu Gly Gly Lys Tyr Val Leu Trp Leu Leu Ile Thr
1040 1045 1050

Tyr Thr Ile Leu Ser Glu Gln Met Ala Met Gly Ala Gly Val Asn
1055 1060 1065

Thr Glu Glu Ile Val Met Ile Gly Asn Leu Leu Thr Asp Ser Asp
1070 1075 1080

Ile Glu Val Val Val Tyr Phe Leu Leu Leu Tyr Leu Ile Val Lys
1085 1090 1095

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Glu Glu  Leu Ala Arg Lys Trp  Ile Ile Leu Val Tyr  His Ile Leu
1100                 1105                 1110

Val Ala  Asn Pro Met Lys Thr  Ile Gly Val Val Leu  Leu Met Leu
1115                 1120                 1125

Gly Gly  Val Val Lys Ala Ser  Arg Ile Asn Ala Asp  Asp Gln Ser
1130                 1135                 1140

Ala Met  Asp Pro Cys Phe Leu  Leu Val Thr Gly Val  Val Ala Val
1145                 1150                 1155

Leu Met  Ile Ala Arg Arg Glu  Pro Ala Thr Leu Pro  Leu Ile Val
1160                 1165                 1170

Ala Leu  Leu Ala Ile Arg Thr  Ser Gly Phe Leu Leu  Pro Ala Ser
1175                 1180                 1185

Ile Asp  Val Thr Val Ala Val  Val Leu Ile Val Leu  Leu Leu Ala
1190                 1195                 1200

Ser Tyr  Ile Thr Asp Tyr Phe  Arg Tyr Lys Lys Trp  Leu Gln Leu
1205                 1210                 1215

Leu Phe  Ser Leu Ile Ala Gly  Ile Phe Ile Ile Arg  Ser Leu Lys
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His Ile  Asn Gln Met Glu Val  Pro Glu Ile Ser Met  Pro Ser Trp
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Lys Val  Lys Tyr Arg Gln Ile  Ser Ser Val Tyr Asp  Ile Cys Asp
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Ser Arg  Pro Asp Phe Ile Leu  Pro Phe Leu Lys Ala  Val Leu Ile
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Ser Thr Leu Ser Lys Asn Arg His Cys Ile Ile Cys Thr Ile Cys
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Gly Lys Asn Thr Gln Asp Gly Leu Tyr His Asn Lys Asn Lys Pro Pro
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Glu Ser Arg Lys Lys Leu Glu Lys Ala Leu Leu Ala Trp Ala Ile Leu
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Ala Ala Val Leu Leu Gln Leu Val Thr Gly Glu Asn Ile Thr Gln Trp
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Leu Tyr Leu Ile Val Lys Glu Glu Leu Ala Arg Lys Trp Ile Ile Leu
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Val Met Leu Gly Val Gly Ala Ile Ala Ala His Asn Ala Ile Glu
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Ser Ser Glu Gln Lys Arg Thr Leu Leu Met Lys Val Phe Val Lys
2435 2440 2445

Asn Phe Leu Asp Gln Ala Ala Thr Asp Glu Leu Val Lys Glu Asn
2450 2455 2460

Pro Glu Lys Ile Ile Met Ala Leu Phe Glu Ala Val Gln Thr Ile
2465 2470 2475

Gly Asn Pro Leu Arg Leu Ile Tyr His Leu Tyr Gly Val Tyr Tyr
2480 2485 2490

Lys Gly Trp Glu Ala Lys Glu Leu Ala Glu Lys Thr Ala Gly Arg
2495 2500 2505

Asn Leu Phe Thr Leu Ile Met Phe Glu Ala Phe Glu Leu Leu Gly
2510 2515 2520

Met Asp Ser Glu Gly Lys Ile Arg Asn Leu Ser Gly Asn Tyr Ile
2525 2530 2535

Leu Asp Leu Ile Phe Asn Leu His Asn Lys Leu Asn Lys Gly Leu
2540 2545 2550

Lys Lys Leu Val Leu Gly Trp Ala Pro Ala Pro Leu Ser Cys Asp
2555 2560 2565

Trp Thr Pro Ser Asp Glu Arg Ile Ser Leu Pro His Asn Asn Tyr
2570 2575 2580

Leu Arg Val Glu Thr Arg Cys Pro Cys Gly Tyr Glu Met Lys Ala
2585 2590 2595

Ile Lys Asn Val Ala Gly Lys Leu Thr Lys Val Glu Glu Lys Gly
2600 2605 2610

Ser Phe Leu Cys Arg Asn Arg Leu Gly Arg Gly Pro Pro Asn Phe
2615 2620 2625

Lys Val Thr Lys Phe Tyr Asp Asp Asn Leu Ile Glu Val Lys Pro
2630 2635 2640

Val Ala Arg Leu Glu Gly Gln Val Asp Leu Tyr Tyr Lys Gly Val
2645 2650 2655

Thr Ala Lys Leu Asp Tyr Asn Asn Gly Lys Val Leu Leu Ala Thr
2660 2665 2670

Asn Lys Trp Glu Val Asp His Ala Phe Leu Thr Arg Leu Val Lys
2675 2680 2685

Lys His Thr Gly Ile Gly Phe Lys Gly Ala Tyr Leu Gly Asp Arg
2690 2695 2700

Pro Asp His Gln Asp Leu Val Asp Arg Asp Cys Ala Thr Ile Thr
2705 2710 2715

Lys Asn Ser Val Gln Phe Leu Lys Met Lys Lys Gly Cys Ala Phe
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Thr Tyr Asp Leu Thr Ile Ser Asn Leu Val Arg Leu Ile Glu Leu
2735 2740 2745

Val His Lys Asn Asn Leu Gln Glu Arg Glu Ile Pro Thr Val Thr
2750 2755 2760

Val Thr Thr Trp Leu Ala Tyr Ser Phe Val Asn Glu Asp Leu Gly
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Thr Ile Lys Pro Val Leu Gly Glu Lys Val Ile Pro Glu Pro Pro
2780 2785 2790

Glu Glu Leu Ser Leu Gln Pro Thr Val Arg Leu Val Thr Thr Glu
2795 2800 2805

Thr Ala Ile Thr Ile Thr Gly Glu Ala Glu Val Met Thr Thr Gly
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Ile Thr Pro Val Val Glu Met Lys Glu Glu Pro Gln Leu Asp His
2825 2830 2835

Gln Ser Thr Thr Leu Lys Val Gly Leu Lys Glu Gly Glu Tyr Pro
2840 2845 2850

Gly Pro Gly Val Asn Pro Asn His Leu Ala Glu Val Ile Asp Glu
2855 2860 2865

Lys Asp Asp Arg Pro Phe Val Leu Ile Ile Gly Asn Lys Gly Ser
2870 2875 2880

Thr Ser Asn Arg Ala Arg Thr Ala Lys Asn Ile Arg Leu Tyr Lys
2885 2890 2895

Gly Asn Asn Pro Arg Glu Ile Arg Asp Leu Met Ser Gln Gly Arg
2900 2905 2910

Ile Leu Thr Val Ala Leu Lys Glu Leu Asp Pro Glu Leu Lys Glu
2915 2920 2925

Leu Val Asp Tyr Lys Gly Thr Phe Leu Asn Arg Glu Ala Leu Glu
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Ala Leu Ser Leu Gly Lys Pro Ile Lys Arg Lys Thr Thr Thr Ala
2945 2950 2955

Met Ile Arg Arg Leu Ile Glu Pro Glu Val Glu Glu Glu Leu Pro
2960 2965 2970

Asp Trp Phe Gln Ala Glu Glu Pro Leu Phe Leu Glu Ala Lys Ile
2975 2980 2985

Gln Asn Asp Leu Tyr His Leu Ile Gly Ser Val Asp Ser Ile Lys
2990 2995 3000

Ser Lys Ala Lys Glu Leu Gly Ala Thr Asp Asn Thr Lys Ile Val
3005 3010 3015

Lys Glu Val Gly Ala Arg Thr Tyr Thr Met Lys Leu Ser Ser Trp
3020 3025 3030

Ser Thr Gln Val Thr Lys Lys Gln Met Ser Leu Ala Pro Leu Phe
3035 3040 3045

Glu Glu Leu Leu Leu Lys Cys Pro Pro Cys Ser Lys Ile Ser Lys
3050 3055 3060

Gly His Met Val Ser Ala Tyr Gln Leu Ala Gln Gly Asn Trp Glu
3065 3070 3075

Pro Leu Gly Cys Gly Val Tyr Met Gly Thr Ile Pro Ala Arg Arg
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Leu Lys Ile His Pro Tyr Glu Ala Tyr Leu Lys Leu Lys Glu Leu
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Val Glu Val Glu Ser Ser Arg Ala Thr Ala Lys Glu Ser Ile Ile
3110 3115 3120

Arg Glu His Asn Thr Trp Ile Leu Arg Lys Val Arg His Glu Gly
3125 3130 3135

Asn Leu Arg Thr Lys Ser Met Ile Asn Pro Gly Lys Ile Ser Asp
3140 3145 3150

Gln Leu Cys Arg Asp Gly His Lys Arg Asn Ile Tyr Asn Lys Ile
3155 3160 3165

Ile Gly Ser Thr Met Ala Ser Ala Gly Ile Arg Leu Glu Lys Leu
3170 3175 3180

Pro Val Val Arg Ala Gln Thr Asp Thr Thr Ser Phe His Gln Ala
3185 3190 3195

Ile Arg Glu Lys Ile Asp Lys Thr Glu Asn Lys Gln Thr Pro Glu
3200 3205 3210

Leu His Glu Glu Leu Met Lys Val Phe Asp Cys Leu Lys Ile Pro

3215 3220 3225

Glu Leu Lys Glu Ser Tyr Asp Glu Val Ser Trp Glu Gln Leu Glu
3230 3235 3240

Ala Gly Ile Asn Arg Lys Gly Ala Ala Gly Tyr Leu Glu Ser Lys
3245 3250 3255

Asn Ile Gly Glu Val Leu Asp Thr Glu Lys His Ile Val Glu Gln
3260 3265 3270

Leu Ile Lys Asp Leu Arg Lys Gly Lys Lys Ile Arg Tyr Tyr Glu
3275 3280 3285

Thr Ala Ile Pro Lys Asn Glu Lys Arg Asp Val Ser Asp Asp Trp
3290 3295 3300

Glu Ala Gly Glu Phe Val Asp Glu Lys Lys Pro Arg Val Ile Gln
3305 3310 3315

Tyr Pro Asp Ala Lys Val Arg Leu Ala Ile Thr Lys Val Met Tyr
3320 3325 3330

Lys Trp Val Lys Gln Lys Pro Val Val Ile Pro Gly Tyr Glu Gly
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Lys Thr Pro Leu Phe Asp Ile Phe Asn Lys Val Lys Lys Glu Trp
3350 3355 3360

Asp Ser Phe Gln Asp Pro Val Ala Val Ser Phe Asp Thr Lys Ala
3365 3370 3375

Trp Asp Thr Gln Val Thr Ser Arg Asp Leu Met Leu Ile Lys Asp
3380 3385 3390

Ile Gln Lys Tyr Tyr Phe Lys Arg Ser Ile His Lys Phe Leu Asp
3395 3400 3405

Thr Ile Thr Glu His Met Val Glu Val Pro Val Ile Thr Ala Asp
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Gly Glu Val Tyr Ile Arg Asn Gly Gln Arg Gly Ser Gly Gln Pro
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Asp Thr Ser Ala Gly Asn Ser Met Leu Asn Val Leu Thr Met Ile
3440 3445 3450

Tyr Ala Phe Cys Lys Ser Thr Gly Ile Pro Tyr Arg Gly Phe Ser
3455 3460 3465

Arg Val Ala Arg Ile His Val Cys Gly Asp Asp Gly Phe Leu Ile
3470 3475 3480

Thr Glu Arg Gly Leu Gly Leu Lys Phe Ser Glu Lys Gly Met Gln
3485 3490 3495

Ile Leu His Glu Ala Gly Lys Pro Gln Lys Ile Thr Glu Gly Asp
3500 3505 3510

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Lys Met  Lys Val Ala Tyr Arg  Phe Glu Asp Ile Glu  Phe Cys Ser
     3515                 3520                 3525

His Thr  Pro Val Pro Val Arg  Trp Ala Asp Asn Thr  Ser Ser Tyr
     3530                 3535                 3540

Met Ala  Gly Arg Ser Thr Ala  Thr Ile Leu Ala Lys  Met Ala Thr
     3545                 3550                 3555

Arg Leu  Asp Ser Ser Gly Glu  Arg Gly Ser Thr Ala  Tyr Glu Lys
     3560                 3565                 3570

Ala Val  Ala Phe Ser Phe Leu  Leu Met Tyr Ser Trp  Asn Pro Val
     3575                 3580                 3585

Val Arg  Arg Ile Cys Leu Leu  Val Leu Ser Gln Phe  Pro Glu Ile
     3590                 3595                 3600

Ser Pro  Ser Lys Asn Thr Ile  Tyr Tyr Tyr Gln Gly  Asp Pro Ile
     3605                 3610                 3615

Ala Ala  Tyr Arg Glu Val Ile  Gly Lys Gln Leu Cys  Glu Leu Lys
     3620                 3625                 3630

Arg Thr  Gly Phe Glu Lys Leu  Ala Gly Leu Asn Leu  Ser Met Thr
     3635                 3640                 3645

Thr Leu  Gly Ile Trp Thr Lys  His Thr Ser Lys Arg  Leu Ile Gln
     3650                 3655                 3660

Ala Cys  Val Glu Ile Gly Lys  Arg Glu Gly Thr Trp  Leu Val Asn
     3665                 3670                 3675

Ala Asp  Arg Leu Ile Ala Gly  Lys Thr Gly Lys Phe  Tyr Ile Pro
     3690                 3685                 3690

Ser Thr  Gly Val Thr Leu Leu  Gly Lys His Tyr Glu  Glu Ile Asn
     3695                 3700                 3705

Leu Lys  Gln Lys Ala Ala Gln  Pro Pro Ile Glu Gly  Val Asp Arg
     3710                 3715                 3720

Tyr Lys  Leu Gly Pro Ile Val  Asn Val Ile Leu Arg  Arg Leu Arg
     3725                 3730                 3735

Val Met  Leu Met Thr Val Ala  Ser Gly Ser Trp
     3740                 3745
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Ile Glu Pro Trp
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Ile Glu Pro Trp
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Arg His Glu Trp Asn Lys His Gly Trp Cys Asn Trp
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Met Glu Leu Phe Ser Asn Glu Leu Leu Tyr
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Ser Asn Glu Gly Ser Lys
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Ser Asp Glu Gly Ser Lys
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Met Glu Ser Asp Gln Gly Ser Lys
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Met Glu Leu Phe Ser Ser Asp Gln Gly Ser Lys
1 5 10

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Lys

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Ser Lys

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Met Glu Leu Asn Lys Phe Glu Leu Leu Tyr Lys Thr
1 5 10