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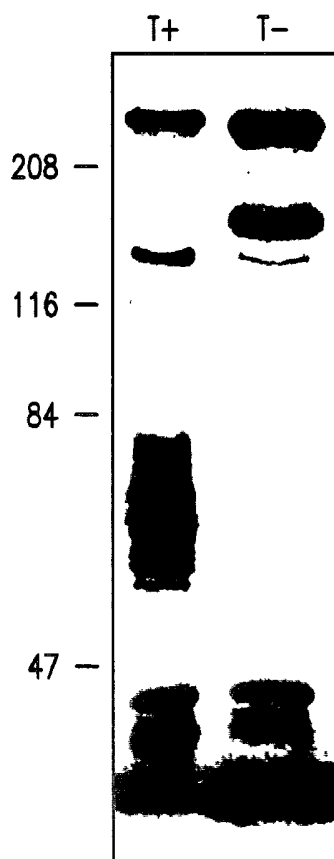
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(54) Title: **MORAXELLA BOVIS** CYTOTOXIN, CYTOTOXIN GENE, ANTIBODIES AND VACCINES FOR PREVENTION
AND TREATMENT OF **MORAXELLA BOVIS** INFECTIONS



(57) Abstract: *Moraxella bovis* cytotoxin and a gene encoding *Moraxella bovis*. Identification, isolation, cloning and identification of nucleotide sequence of the *Moraxella bovis* genes mbxA, mbxB, mbxC and mbxD, partial purification of the native cytotoxin, preparation of partially purified native and a recombinant *Moraxella bovis* cytotoxin, identification of an amino acid sequence of the cytotoxin, preparation of antibodies against the *Moraxella bovis* cytotoxin, preparation of vaccines against *Moraxella bovis*. Method for prevention and treatment of infectious bovine keratoconjunctivitis caused by *Moraxella bovis*.

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MORAXELLA BOVIS CYTOTOXIN, CYTOTOXIN GENE,
ANTIBODIES AND VACCINES FOR PREVENTION
AND TREATMENT OF MORAXELLA BOVIS INFECTIONS

5

BACKGROUND OF THE INVENTION

Field of the Invention

The current invention concerns *Moraxella bovis* cytotoxin and a gene encoding *Moraxella bovis* cytotoxin. In particular,
10 the invention concerns identification, isolation, cloning and identification of nucleotide sequence of the *Moraxella bovis* gene *mbxA* and adjacent genes *mbxB*, *mbxC* and *mbxD*, partial purification of the native cytotoxin, preparation of a purified native and recombinant *Moraxella bovis* cytotoxin, an
15 amino acid sequence of the cytotoxins, preparation of antibodies against the *Moraxella bovis* cytotoxin, preparation of vaccines against *Moraxella bovis* and a method for prevention and treatment of infectious bovine keratoconjunctivitis caused by *Moraxella bovis*.

20 Background and Related Disclosures

Infectious bovine keratoconjunctivitis (IBK) also called pinkeye, caused by *Moraxella bovis* (*M. bovis*) infection ranks as one of the most important cattle diseases in the United States and abroad. Annual estimated incidence of the disease
25 is 5% of all beef cattle with greater than 50% of all herds affected. Epizootics also occur with case attack rates approaching 90-100% of yearling cattle. Affected cattle do not eat and fail to gain weight. Economic losses due to lower market weights of affected cattle and calves and ocular
30 scarring and treatment associated expenses are estimated to

exceed \$150,000,000 annually. Effective control measures that could substantially reduce this expense are not available as the commercially available vaccines have low efficacy.

Animals affected with IBK exhibit corneal ulceration, edema, ocular pain, photophobia and lacrimation. Control of IBK using antimicrobial treatments has been only partially successful. Antimicrobials have been used to eliminate the carrier state in experimental and field trials, but these treatments have drawbacks that include emergence of resistant bacteria in cattle and the general environment, potential for adulteration of the nation's food supply, high cost and marginal economic benefit.

Topical and oral administration of antibiotics has proven somewhat effective for treatment of pinkeye, but the high cost and losses associated with the ongoing disease often outweigh the benefits of such treatment. Topical therapy alone does not eliminate *M. bovis* from non-ocular sites such as nasal choanae and vagina where it is known to reside.

Consequently, an effective vaccine or antibody for prevention and treatment of IBK would be an important component of a successful IBK control program.

So far, vaccines prepared from killed or live bacteria, isolated pili, or ribosomes have had limited benefit for immunization of cattle in the field (Proc. Am. Assoc. Bov. Pract., 20:26-32 (1987)). Nevertheless, a large body of field and experimental data exist to show that acquired resistance to pinkeye does develop after the natural occurrence of the infection.

At least two attributes, namely pili and cytotoxin (leukotoxin/hemolysin/cytolysin), of the *M. bovis* organism are important in the pathogenesis of IBK. *M. bovis* pili

facilitate adhesion of the bacterium to the corneal epithelium and are required for colonization. Pili are highly immunogenic, but have antigenic diversity due to the presence of at least two structural pilin genes and variability in the amino acid composition of the pilin molecule. Variability in pilus gene expression is regulated by a site-specific DNA inversion system. Limited antigenic cross-reactivity was found in vaccines prepared from heterologous pili. *M. bovis* pili in monovalent or multivalent formulations have demonstrated variable efficacy, attributed to pilus gene inversion that occurs during infection (Vet. Microbiol., 45:129-138 (1995)). The emergence of novel pili serotypes during epizootics of IBK has been documented (Am. J. Vet. Res., 50:1437-1441 (1989)). These studies suggest that because of the pili heterogeneity pilus based vaccines will not be 100% effective at preventing IBK.

The *Moraxella bovis* cytotoxin (hemolysin) is an important component of immunity to IBK (FEMS Microbiol. Lett., 124:69-74 (1994)).

Moraxella bovis produces a heat labile, approximately 100 kD cytotoxin (hemolysin) that causes cell lysis by forming pores in cell membranes via a calcium-dependent process (Infect. Immunol., 59:1148-1152 (1991)). *In vitro*, culture filtrates from broth cultures of hemolytic but not nonhemolytic strains of *M. bovis* cause lysis of bovine neutrophils (Am. J. Vet. Res., 51:191-196 (1990)). *In vivo*, the ocular damage caused by a purified hemolytic and cytotoxic fraction of *M. bovis* mimics the lesions seen in naturally occurring IBK (Vet. Microbiol., 42:15-33 (1994)). Unlike the pilin molecule, the *M. bovis* cytotoxin appears to be more

conserved between different *M. bovis* isolates. Serologic studies show that antihemolytic antibodies recognize hemolysin from different strains of *M. bovis* (Am. J. Vet. Res., 46:1011-1014 (1985)) and, thus, form an important component of immunity to IBK (FEMS Microbiol. Lett., 124:69-74 (1994)).

In view of the severity of consequences of infectious bovine keratoconjunctivitis, it would be important to have available an effective vaccine and/or anti-*Moraxella bovis* antibodies that could prevent this condition.

This invention, therefore, concerns an effective IBK vaccine, anti-*Moraxella bovis* antibodies and to that end an isolation and partial purification of the native *Moraxella bovis* cytotoxin, a characterization of the *M. bovis* cytotoxin gene, identification of *M. bovis* cytotoxin amino acid sequence, preparation of recombinant *M. bovis* cytotoxin and determination of its efficacy in a vaccine for prophylaxis and treatment of IBK.

All patents, patent applications and publications cited herein are hereby incorporated by reference in their entirety.

SUMMARY

One aspect of the current invention is a vaccine for prevention of infectious bovine keratoconjunctivitis comprising a purified native or recombinant *Moraxella bovis* cytotoxin.

Still another aspect of the current invention is a DNA sequence of the *Moraxella bovis* gene depicted by SEQ ID NO: 1.

Still another aspect of the current invention is a DNA sequence of the *Moraxella bovis* gene B depicted by SEQ ID NO: 30.

Still another aspect of the current invention is a DNA sequence of the *Moraxella bovis* gene C depicted by SEQ ID NO: 31.

5 Still another aspect of the current invention is a DNA sequence of the *Moraxella bovis* gene D depicted by SEQ ID NO: 36.

Yet another aspect of the current invention is an amino acid sequence of *Moraxella bovis* cytotoxin A depicted by SEQ ID NO: 2 or a partially purified native or recombinantly prepared cytotoxin comprising SEQ ID NO: 2.

Yet another aspect of the current invention is an amino acid sequence of *Moraxella bovis* cytotoxin B depicted by SEQ ID NO: 30 or a partially purified native or recombinantly prepared cytotoxin comprising SEQ ID NO: 18.

15 Yet another aspect of the current invention is an amino acid sequence of *Moraxella bovis* cytotoxin C depicted by SEQ ID NO: 31 or a partially purified native or recombinantly prepared cytotoxin comprising SEQ ID NO: 32.

20 Yet another aspect of the current invention is an amino acid sequence of *Moraxella bovis* cytotoxin D depicted by SEQ ID NO: 36 or a partially purified native or recombinantly prepared cytotoxin comprising SEQ ID NO: 37.

25 Still another aspect of the current invention is a method for purification or partial purification of the *Moraxella bovis* cytotoxin and its stabilization.

Still another aspect of the current invention is a method for prevention of IBK in cattle and calves by vaccinating said cattle or calves with a vaccine comprising a partially purified native or recombinant *Moraxella bovis* cytotoxin comprising SEQ ID NO: 2.

30

BRIEF DESCRIPTION OF FIGURES

Figure 1 is a graph illustrating neutralization of *M. bovis* leukotoxin in bacterial filter permeates by anti T+ serum.

5 Figure 2 is an autoradiogram of the pooled and concentrated void volume fraction of diafiltered retentate from T+ and T- cultures chromatographed on a Superose 6HR column.

10 Figure 3 shows nucleotide (SEQ ID NO: 1) and deduced amino acid (SEQ ID NO: 2) sequences of *M. bovis* RTXA (MbxA) gene.

15 Figure 4 shows the alignment of the deduced amino acid sequence of *M. bovis* RTXA (MbxA) gene (SEQ ID NO: 2) and RTX toxins of *M. (Pasteurella) haemolytica* (LktA) (SEQ ID NO: 3), *A. pleuropneumoniae* (ApxIA) (SEQ ID NO: 4) and *E. coli* (HlyA) (SEQ ID NO: 5).

Figure 5 is an autoradiogram demonstrating MbxA presence in culture supernatants of T+, and Tifton I and absence in T- culture supernatants.

20 Figure 6 is an autoradiogram of culture supernatants from a Tifton I broth culture at 3, 4, 4.5, 5 and 6 hours time points.

25 Figure 7 shows the percent neutralization of cytolysis and hemolysis by non-immune and immune sera alone or preabsorbed with a recombinant expressed carboxy terminus of MbxA.

Figure 8 shows nucleotide (SEQ ID NO: 30) and deduced amino acid (SEQ ID NO: 18) sequences of *M. bovis* RTXB (Mbx3) gene.

Figure 9 shows the alignment of the deduced amino acid sequence of *M. bovis* RTXB (MbxB) gene (SEQ ID NO: 18) and RTX toxins from *M. (Pasteurella) haemolytica* (LktB) (SEQ ID NO: 19), *A. pleuropneumoniae* (ApxIIB) (SEQ ID NO:20) and *E. coli* (HlyB) (SEQ ID NO: 21).

Figure 10 shows nucleotide (SEQ ID NO: 31) and deduced amino acid (SEQ ID Nos: 32) sequences of *M. bovis* RTXC (MbxC) gene.

Figure 11 shows the alignment of the deduced amino acid sequence (SEQ ID NO: 32) of *M. bovis* RTXC (MbxC) gene and RTX toxins of *M. (Pasteurella) haemolytica* (LktC) (SEQ ID NO: 33), *A. pleuropneumoniae* (ApxIIC) (SEQ ID NO:34) and *E. coli* (HlyC) (SEQ ID NO: 35).

Figure 12 shows nucleotide (SEQ ID NO: 36) and deduced amino acid (SEQ ID NO: 37) sequences of *M. bovis* RTXD (MbxD) gene.

Figure 13 shows the alignment of the deduced amino acid sequence (SEQ ID NO: 37) of *M. bovis* RTXD (MbxD) gene and RTX toxins of *M. (Pasteurella) haemolytica* (LktD) gene (SEQ ID NO: 38), *A. pleuropneumoniae* (ApxIID) gene (SEQ ID NO: 39) and *E. coli* (HlyD) gene (SEQ ID NO: 40).

Figure 14 is a graph showing the cumulative number of calves with ulcers in one eye vaccinated with *M. bovis* cytotoxin, adjuvant alone, or saline (control) over time.

Figure 15 is a graph showing a number of calves affected weekly in one group of vaccinated calves and in controls.

Figure 16 is a graph showing cumulative proportion of ulcerated calves during the trial with recombinant cytotoxin vaccine, saline alone or adjuvant alone.

DEFINITIONS

As used herein:

"Cytotoxin," "cytolysin" or "hemolysin" means a protein produced by *Moraxella bovis* that is lytic for cells. The
5 cytotoxin gene encodes 927 amino acids depicted by SEQ ID NO: 2 and is homologous to the internal regions of the published repeats in the structural toxin (RTX) toxins of *E. coli*, *M. (Pasteurella) haemolytica* and *Actinobacillus* species.

"Antigen" or "*M. bovis* antigen" means *M. bovis* cytotoxin
10 as defined above.

"Functionality" or "functional characteristics" means the interaction of antibodies against *M. bovis* cytotoxin, such that the antibody inhibits the activity of *M. bovis* cytotoxin and retards/prevents development of IBK.

15 "ISCOM" means a partially purified cytotoxin formulated in an immunostimulating complex comprising *Quillaja saponins (Quil A)*, cholesterol and phospholipids.

"IBK" means infectious bovine keratoconjunctivitis (pinkeye) caused by *Moraxella bovis*.

20 "*M. bovis*" or "*Moraxella bovis*" means the etiologic agent of IBK which expresses a cytotoxin that lyses corneal epithelial cells and neutrophils and results in corneal ulceration in cattle and calves. Pathogenic isolates of *M. bovis* are hemolytic; non-pathogenic isolates are nonhemolytic.

25 "*mbxA*" means a gene encoding *M. bovis* cytotoxin, said gene comprising a nucleotide sequence of 2784 base pairs depicted as SEQ ID NO: 1.

"*MbxA*" means a protein encoded by *mbxA* gene described as SEQ ID NO: 2.

"Leukotoxic" activity means biological activity which causes lysis of bovine neutrophils and lymphoma cells.

"Hemolytic" activity means biological activity which causes lysis of red blood cells.

5 "Corneotoxic" activity means biological activity which causes lysis of corneal epithelial cells.

"KDO" means 2-keto-3-deoxyoctonate.

"Antibodies" means proteins which structurally interact with the target antigen, i.e., *M. bovis* cytotoxin, and are
10 produced when the antigen is introduced into an animal, such that they stimulate the immune system. The term also includes antibodies produced *in vitro*, such as recombinant antibodies, antibodies produced by hybridoma cell cultures and chimeric proteins, as well as hybridoma cells and chimeric constructs
15 introduced into the host to provide an *in vivo* antibody.

"Antibodies to *M. bovis* antigen" means proteins which structurally interact with the target antigen and inhibit or detect infectious bovine keratoconjunctivitis.

"Monoclonal antibodies" means the monovalent antibodies
20 produced by B cells fused to immortalized cells producing specific antibody to *M. bovis* antigen.

"Polyclonal antibodies" means antibodies directed at *M. bovis* antigen which are not monovalent and are the products of multiple B cells in character.

25 "*M. bovis* antigen DNA" means the sequence of about 2784 nucleotides identified as SEQ ID NO: 1 which encodes a protein comprising an amino acid sequence depicted by SEQ ID NO: 2.

"Vaccine" means a protein, recombinant protein, DNA, RNA, or a fragment thereof preserved from *M. bovis* cytotoxin which,
30 upon administration to a host, is able to provoke an immune

response including but not limited to the production of antibodies, cytokines and other cellular responses.

"Prevention or prophylaxis" means the passive or active immunization with antibodies or vaccines of the invention such that disease or infection does not occur.

"Treatment" means therapeutic use of any protein or antibody to inhibit existing infection in a cattle or calf host.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates generally to infectious bovine keratoconjunctivitis, to vaccines, antibodies, purified or partially purified native or recombinant proteins, DNAs, RNAs and methods for prophylaxis and treatment of infectious bovine keratoconjunctivitis (IBK).

More specifically, the invention concerns identification, isolation, and purification or partial purification of native *M. bovis* antigen (cytotoxin) which causes IBK in cattle and calves, determination of its biological activity, identification and isolation of an *M. bovis* genes *mbxA*, *mbxB*, *mbxC* and *mbxD*, and determination of their nucleotide sequences, deducing their amino acid sequences based on the DNA sequences, partial sequencing of native partially purified cytotoxin, cloning of *M. bovis* genes *mbxA*, *mbxB*, *mbxC* and *MbxD*, expression of recombinant proteins, identification of the amino acid sequences of the expressed proteins and their purification, and preparation of *M. bovis* cytotoxin containing vaccines or polyclonal or monoclonal antibodies against such cytotoxin.

I. Native *Moraxella bovis* Cytotoxin

Moraxella bovis is a bacteria isolated from a cattle infected with *Moraxella bovis* and suffering from infectious bovine keratoconjunctivitis. *M. bovis* strains are pathogenic or nonpathogenic and different isolates may be found in different regions.

A. *M. bovis* Strains and Isolates

A cytolytic strain *M. bovis* (T+) and its noncytolytic subculture (T-), as well as cytolytic strains EPP63 and GLN63 were obtained from Dr. G. W. Pugh, Ames, Iowa. Additional isolates Tifton 1, Tifton 2, GA3, LS-2, LS-3, LS-4, LS-5, LS-6, LS-7 and LS-8 were obtained from field specimens submitted for determination of IBK infections from various states. The strains were isolated according to Example 1.

B. Characteristics of Native *M. bovis* Cytotoxin

Moraxella bovis produces a toxin, herein called cytotoxin, which causes cell lysis.

The cytotoxin of *Moraxella bovis* (*M. bovis*) is a transmembrane pore forming molecule that shares antigenic homology to the *E. coli* α -hemolysin, and is excreted in particulate complexes with the proteins during log phase growth. The cytotoxin has leukotoxic, corneotoxic, and hemolytic activities. Once released into the media, the liberated cytotoxin and protein aggregates can be separated from the whole bacteria by filtration through polycarbonate membranes of a mean pore diameter 0.22 μ M, and can be retained by filters with molecular weight cut-offs that are >100 kDa.

Cytotoxin is an unstable molecule with activity dissipating rapidly after removal from the bacterial cell. Its pathogenic activity is irreversibly inactivated by a number of chemical, enzymatic, and physical treatments,

including trypsin, sodium dodecyl sulfate, high incubation temperature ($>37^{\circ}\text{C}$), and calcium chelation.

The molecular complexity and the lability of the cell free cytotoxin has precluded purification to a monomolecular state.

C. Immunogenicity of *M. bovis* Strains

To investigate the immunogenic potential of cytotoxin, characterization of cytotoxin of *M. bovis* strains from diverse regions of the United States were undertaken.

For this purpose, leukotoxins were obtained from nine field strains and three isolates. These leukotoxins were submitted to neutralization assay as described in Example 21 with immune serum or pre-immune serum. Results are seen in Figure 1.

Figure 1 illustrates neutralization of *M. bovis* leukotoxin in bacterial filter permeates from isolates Tifton 1, Tifton 2, LS-2, LS-3, LS-4, LS-5, LS-6, LS-7, LS-8 and in three cytolytic strains T+, EPP63 and GLN63 with immune rabbit anti-T+ serum compared to neutralization of these permeates with pre-immune serum from the same rabbit. The pre-immune serum was used as the negative control. In this study, the same bacterial filter permeates were incubated with either the immune serum or pre-immune serum under the same conditions. The leukotoxic activity was measured by LDH assay.

As seen in Figure 1, the undiluted anti-T+ diafiltered retentate serum inhibited the cytotoxic activity of all strains and isolates. No such inhibition was observed in samples incubated with pre-immune serum.

These results clearly confirm that the leukotoxic activity of cytotoxin is shared by *M. bovis* strains and

isolates and suggest that a vaccine containing such cytotoxin would immunize the cattle against IBK.

II. Cytotoxin Gene, Protein, Recombinant Protein and DNA

Following the discovery and confirmation that the
5 cytotoxin protein is an antigen which could be used as a
vaccine for cross-strain protection against IBK, cloning,
characterization and sequencing of the *M. bovis* cytotoxin
genes, expression of the proteins encoded by these genes and
the amino acid sequencing of HPLC purified peptides derived
10 from native cytotoxin was undertaken.

A. Isolation of *M. bovis* Cytotoxin for Amino Acid Sequence Analysis

For amino acid sequence analysis, the *M. bovis*
cytotoxin/cytotoxin peptides were partially purified by the
15 procedure described in Example 14. To identify proteins that
were unique to the hemolytic strain T+, purified culture
extracts from hemolytic T+ and nonhemolytic T- *M. bovis*
strains were compared by Western blotting using the procedure
described in Example 15.

20 Several candidate proteins for the *M. bovis* cytotoxin
with molecular masses ranging from 55 to 75 kDa were
identified as shown in Figure 2.

Figure 2 is the autoradiogram of the pooled and
concentrated void volume fraction of diafiltered retentate
25 from T+ and T- cultures chromatographed on a Superose 6
column. The rabbit antiserum to column purified cytotoxin, in
1:400 dilution, was used as the primary antibody. Molecular
mass markers 47, 84, 116 and 208 kDa are indicated. Protein
bands in T+ lane ranging from 55 to 75 kDa were found to
30 represent *M. bovis* cytotoxin protein/peptides that were unique

to T+ diafiltered retentate. A 70 kDa protein in Superose 6 chromatographed T+ diafiltered retentate was subsequently identified on a Coomassie blue stained SDS polyacrylamide gel and was the source for two peptides designated peptides #23 and #26. These peptides were sequenced by N-terminal Edman degradation chemistry.

The respective amino acid sequences for two tryptic peptides #23 and #26 that were derived from the 70 kDa band were FLSELNKELEAE (SEQ ID NO: 6) and FNDIFHSGEGLDLDLSDGA (SEQ ID NO: 13).

BLAST database searches identified relatedness between the two peptides and the deduced amino acid sequences from RTX A genes of *E. coli*, *M. haemolytica*, *A. suis*, *A. pleuropneumoniae*, and *A. actinomycetemcomitans*. These alignments also predicted that peptide #23 was amino to peptide #26. Sequence and orientation-specific degenerate PCR primers 23A and 26A designed from reverse translating the amino acid sequences for peptides #23 and #26, respectively, amplified an approximately 850 bp fragment of genomic DNA from *M. bovis* Tifton I strain.

The deduced amino acid encoded by this region exhibited homology to the RTX family of bacterial exoproteins. Complete cloning and sequencing of *mbxA* was subsequently performed using a PCR based approach as described herein.

B. Homology with RTX Toxins

Amino acid sequencing of HPLC purified peptides derived from the purified cytotoxin protein has yielded amino acid sequences which show homology to internal regions of the published RTX toxins of *E. coli*, *M. haemolytica* and

Actinobacillus spp. Regions of homology are seen in Table 4 and Table 5.

Table 4
Amino Acid Alignment

5	<i>M. bovis</i> cytotoxin	F	L	S	E	L	N	K	E	L	E	A	E	SEQ ID NO: 6
	<i>M. haemolytica</i> leukotoxin	F	L	L	N	L	N	K	E	L	Q	A	E	SEQ ID NO: 7
	<i>E. coli</i> alpha hemolysin	I	L	S	Q	Y	N	K	E	Y	S	V	E	SEQ ID NO: 8
10	<i>A. pleuropneumoniae</i> hemolysin	F	L	I	N	L	N	K	E	L	Q	A	E	SEQ ID NO: 9
	<i>A. pleuropneumoniae</i> hemolysin	L	L	S	Q	Y	N	K	E	Y	S	V	E	SEQ ID NO: 10
	<i>A. suis</i> cytotoxin	F	L	I	N	L	N	K	E	L	Q	A	E	SEQ ID NO: 11
	Consensus sequence	F	L	.	.	L/Y	N	K	E	L/Y	.	A/V	E	SEQ ID NO. 12

15

Table 4 shows amino acid sequence of *M. bovis* cytotoxin peptide #23 aligned with known published RTX toxin amino acid sequences.

Table 5
Alignment of Fragment #26

20

25

<i>M. bovis</i> cytotoxin	F	N	D	I	F	H	S	G	E	G	D	D	L	L	SEQ ID NO: 13
<i>A. pleuropneumoniae</i> serotype 8 cytotoxin	F	R	D	I	F	H	G	A	D	G	D	D	L	L	SEQ ID NO: 14
<i>A. actinomycetemcomitans</i> leukotoxin	F	N	D	V	F	H	G	H	D	G	D	D	L	I	SEQ ID NO: 15
<i>A. pleuropneumoniae</i> serotype 2 cytotoxin	F	R	D	I	F	H	G	A	D	G	D	D	L	L	SEQ ID NO: 16
Consensus sequence	F	.	D	.	F	H	G	A	D	G	D	D	L/i		SEQ ID NO: 17

Table 5 shows amino acid sequence of *M. bovis* cytotoxin peptide peak #26 aligned with known published amino acid sequences of RTX toxins.

5 Obtained results show that a relationship exists between the *M. bovis* cytotoxin and the RTX family of toxins. These results are further supported by the earlier findings of other investigators that a monoclonal antibody to the RTX toxin of *E. coli*, namely alpha-hemolysin, recognized a protein in
10 hemolytic and cytolytic extracts from hemolytic but not nonhemolytic *M. bovis* (Vet. Microbiol., 43:183-196 (1995)).

C. Deduced Amino Acid Sequences

Amino acid sequences of products MbxA, MbxB, MbxC and MbxD of the four genes *mbxA*, *mbxB*, *mbxC* and *mbxD* were deduced.
15 The peptide MbxA is the structural cytotoxin. Peptides MbxB, MbxC and MbxD are involved in *M. bovis* cytotoxin activity.

Specifically, MbxA peptide is encoded by the *mbxA* gene. DNA sequence of the *mbxA* gene was determined according to the Example 17. Plasmid DNA was isolated with Qiagen plasmid
20 minipreps (Qiagen, Valencia, CA). The DNA sequence of the complete cytotoxin gene was obtained by sequencing PCR products directly or PCR products cloned into pCR2.1-TOPO (Invitrogen) with a TOPO TA cloning kit (Invitrogen). PCR
25 primers C-down and B-up, corresponding to conserved regions within respective RTX C and B genes of other RTX toxin producing bacteria, were used for PCR amplification. These
30 primers amplified a 4 kb region that contained an open reading frame (ORF) of 2784 nucleotide bases including stop codon depicted as sequence SEQ ID NO: 1, seen in Figure 3. This gene was designated *mbxA*.

The MbxA protein encoded by *mbxA* gene was deduced to be 927 amino acids with a predicted molecular mass of 98.8 kDa (SEQ ID NO: 2).

There was approximately 40-50% identity between deduced amino acid sequences of *mbxA* and other RTX genes. Specifically, there was 50.4% identity with *M. haemolytica* LktA, 49.1% with *A. pleuropneumoniae* ApxIIA, 48.7% with *A. suis* ClyIIA, 43.5% with *E. coli* HlyA, and 40.9% with *A. actinomycetemcomitans* LtA. A sequence alignment of MbxA with LktA, ApxIIA, and HlyA is shown in Figure 4.

Figure 4 shows alignment of the deduced amino acid sequences of MbxA and RTX toxins of *M. (Pasteurella) haemolytica* (LktA), *A. pleuropneumoniae* (ApxIIA) and *E. coli* (HlyA). Black boxes indicate identical amino acids. Gray boxes indicate highly similar amino acids.

The location of peptides #23 and #26 derived from trypsin digestion of the 70 kDa protein are indicated except that the residue at position 720 was designated serine by N-terminal Edman degradation chemistry. The sequences corresponding to 6 glycine rich repeats are indicated on the top MbxA sequence by double lines. The expressed recombinant internal peptide corresponded to amino acid 438 through 713. The expressed recombinant carboxy peptide corresponded to amino acids 643 through 927. Lysines at positions 536 and 660 (marked by *) are hypothetical acylation modification sites as determined by relatedness of the neighboring amino acids to published consensus sequences (Infect. Immun., 64:3081-7 (1996) and Microbiol. Mol. Biol. Rev., 62:309-33 (1998)) surrounding modified lysine residues of HlyA.

MbxA protein has been found to have as much as 50% identity with other RTX toxins and this homology extends over the length of the protein. The carboxy terminus of MbxA features glycine rich repeats that are characteristic of RTX proteins. Specifically, there are six glycine-rich repeats in the carboxy terminal 325 amino acids of MbxA. Four of these exactly match the predicted L/V-X-G-G-X-G-N/D-D-X (SEQ ID NO: 29) consensus for glycine repeats in RTX toxins.

Identification of MbxA protein in *M. bovis* in culture supernatants is illustrated in Figure 5.

Figure 5 is an autoradiogram demonstrating cytotoxin MbxA in culture supernatants of T+ and Tifton I. Three hour culture supernatants from T+, T- and Tifton I (Tif I) were electrophoresed on a 3.9%/7.5% SDS-polyacrylamide gel, and transblotted to Immobilon-P (Millipore) and probed with rabbit antisera against the expressed recombinant carboxy peptide (1:250 dilution). Molecular mass markers 45 kDa, 66 kDa, 97 kDa, 116 kDa and 200 kDa are indicated. Two proteins with molecular masses of 102 and 105 kDa were present in culture supernatants from both T+ and Tifton I. No immunoreactive proteins were present in the T- culture supernatant. These proteins were concluded to represent full length MbxA protein.

During longer incubations (4-6 hours), smaller immunoreactive proteins with molecular masses ranging from 52 to 91 kDa were identified in the culture supernatant as shown in Figure 6.

Figure 6 is an autoradiogram of culture supernatants from a Tifton I broth culture at 3, 4, 4.5, 5 and 6 hour time points. The blot was probed with rabbit antiserum against the expressed recombinant internal peptide encoded by *MbxA* gene

(amino acids 438 through 713). Molecular mass markers 45, 66, 97, 116 and 200 kDa are indicated.

The appearance of proteins that were smaller than the 102 and 105 kDa predicted full length cytotoxin, following 4-6 hours of incubation, as seen in Figure 6, has been attributed to proteolysis. Hemolytic *M. bovis* produces numerous hydrolytic enzymes including C4 esterase, C8 esterase-lipase, C14 lipase, phosphoamidase, phosphatase, leucine and valine aminopeptidases and gelatinase. Proteolysis is believed to account for the difference in mass between the 70 kDa protein selected for amino acid sequencing and the 98.8 kDa molecular mass predicted for full length MbxA. The data indicate that *M. bovis* could also express smaller molecular mass proteins that share epitopes with the internal region MbxA.

To confirm that MbxA encoded the *M. bovis* cytotoxin, neutralization assays were performed with antisera that was preabsorbed with the recombinant expressed carboxy terminus of MbxA (carboxy peptide). Results are seen in Figure 7.

Figure 7 shows percent neutralization (± 1 standard deviation) of cytolysis (top) and hemolysis (bottom) by non-immune sera alone or preabsorbed with the carboxy peptide. Cytotoxin was tested for neutralization with nonimmune fetal bovine serum (FBS); with preimmune sera A and B obtained from two rabbits A and B; with immune rabbit anti T+ sera obtained from T+ rabbit polyclonal antisera to culture filtrates from *M. bovis* strain T+; and with immune sera A and B obtained from rabbits A and B vaccinated with the carboxy peptide.

As seen in Figure 7, rabbit anti T+ sera lost over 50% of its hemolytic and cytolytic neutralizing capacity following preabsorption with the carboxy peptide. Rabbit antisera

Peptide #26 of MbxA cytotoxin is depicted by the amino acid sequence identified as SEQ ID NO: 13. The peptide #26 aligns with *A. pleuropneumoniae* serotype 8 cytotoxin (SEQ ID NO: 14), *A. actinomycetemcomitans* leukotoxin (SEQ ID NO: 15) and *A. pleuropneumoniae* serotype 2 cytotoxin (SEQ ID NO: 16), as seen in Table 5 (supra). Table 5 also lists a consensus sequence (SEQ ID NO: 17).

Additionally, the deduced amino acid sequences of products of *M. bovis* RTX B (Mbx B), RTX C (Mbx C) and RTX D (Mbx D) genes were determined.

The deduced amino acid sequence of product of *M. bovis* RTX B gene (Mbx B) is depicted by SEQ ID NO: 18. The Mbx B protein encoded by *mbxB* gene was deduced to be 739 amino acids. Amino acid sequence of the Mbx B protein is seen in Figure 8.

Deduced amino acid sequence Mbx B aligned with other products of the RTX B genes, as seen in Figure 9. Figure 9 shows an alignment of *M. bovis* (Mbx B) with the deduced amino acid sequences of RTX B genes from *M. (Pasteurella) haemolytica* (Lkt B) (SEQ ID NO: 19), *A. pleuropneumoniae* (Apx B) (SEQ ID NO: 20) and *E. coli* (Hly B) (SEQ ID NO: 21). Black boxes in Figure 9 indicate identical amino acids. Gray boxes indicate highly similar amino acids.

The deduced amino acid sequence of product of *M. bovis* RTX C gene (Mbx C) is depicted by SEQ ID NO: 32. The Mbx C protein encoded by *mbxC* gene was deduced to be 169 amino acids. Amino acid sequence of the Mbx C protein is seen in Figure 10.

Deduced amino acid sequence Mbx C aligned with other products of the RTX C genes, as seen in Figure 10. Figure 10 shows an alignment of *M. bovis* (Mbx C) with the deduced amino

acid sequences of RTX genes from *M. (Pasteurella) haemolytica* (LktC) (SEQ ID NO: 33), *A. pleuropneumoniae* (ApxIC) (SEQ ID NO: 34) and *E. coli* (HlyC) (SEQ ID NO: 35), as seen in Figure 11. Black boxes in Figure 11 indicate identical amino acids. Gray boxes indicate highly similar amino acids.

The deduced amino acid sequence of MbxD protein encoded by the *M. bovis* RTX gene (*MbxD*) is depicted by SEQ ID NO: 37. The MbxD protein encoded by *mbxD* gene was deduced to be 476 amino acids. Amino acid sequence of the MbxD protein is seen in Figure 12.

Deduced amino acid sequence MbxD aligned with other products of the RTX genes is seen in Figure 13. Figure 13 shows an alignment of *M. bovis* (MbxD) with the deduced amino acid sequences of RTX genes from *M. (Pasteurella) haemolytica* (LktB) (SEQ ID NO: 38), *A. pleuropneumoniae* (ApxIB) (SEQ ID NO: 39) and *E. coli* (HlyB) (SEQ ID NO: 40). Black boxes in Figure 13 indicate identical amino acids. Gray boxes indicate highly similar amino acids.

The above findings conclusively documents the presence of RTX gene (*mbxA*) and other RTX genes in *M. bovis*. The *mbxA* gene encodes a protein MbxA which has been found to be the etiologic agent of infectious bovine keratoconjunctivitis. The data also show that the protein expressed from this gene is responsible for the hemolytic and cytolytic activity present in culture extracts from pathogenic *M. bovis*, and thus proves that the *M. bovis* cytotoxin is an RTX toxin.

D. E. Cytotoxin Gene *mbxA* and *mbxB*, *mbxC* and *mbxD* Genes

Cytotoxin gene *mbxA* and other RTX genes, namely *mbxB*, *mbxC* and *mbxD* belong to a family of RTX genes encoding bacterial pore-forming toxins. RTX genes are composed of four genes

organized 5'-C-A-B-D-3'. The *mbxC* gene is located upstream of *mbxA*. The *mbxB* and *mbxD* are located downstream of *mbxA*.

The product of the RTXA gene is the structural toxin which is activated by the RTXC gene product to become
5 hemolytic. Such activation is mediated by fatty acylation of conserved lysines. The activated toxin is secreted by membrane transport proteins encoded by RTXB and RTXD genes and a third protein TolC.

The cytotoxin gene *mbxA*, disclosed herein, has similar
10 properties to other RTX genes and, as described above, its encoded protein MbxA has 50% identity with the deduced amino acid sequence of *M. haemolytica* leukotoxin. MbxA protein has 6 glycine-rich repeats in the carboxy terminus and two putative lysine acylation sites which are necessary for toxin
15 activation.

RTX toxin gene *mbxA* was now shown to encode the *M. bovis* cytotoxin.

1. Structures of the *mbxA*, *mbxB*, *mbxC* and *mbxD* Genes

20 DNA sequence identified as SEQ ID NO: 1 represents the *M. bovis* RTXA gene *mtxA*. The nucleotide sequence of the *mtxA* gene is shown in Figure 3. The gene *mtxA* comprises an ORF of 2784 nucleotides including stop codon.

The sequence of the cytotoxin gene was determined
25 according to the procedure described in Example 18, using primers identified by the nucleotide sequences SEQ ID Nos: 23-26.

DNA sequence identified as SEQ ID NO: 30 represents the *M. bovis* RTXB gene *mtxB*. The nucleotide sequence of the *mtxB*

gene is shown in Figure 8. The gene *mtxB* comprises an ORF of 2215 nucleotides including stop codon.

DNA sequence identified as SEQ ID NO: 31 represents the *M. bovis* RTX C gene *mtxC*. The nucleotide sequence of the *mtxC* gene is shown in Figure 10. The gene *mtxC* comprises an ORF of 507 nucleotides including stop codon.

DNA sequence identified as SEQ ID NO: 36 represents the *M. bovis* RTX D gene *mtxD*. The nucleotide sequence of the *mtxD* gene is shown in Figure 12. The gene *mtxD* comprises an ORF of 1428 nucleotides including stop codon.

Corresponding protein products encoded by these genes are described above.

2. Cloning of *mbxA*, *mbxB*, *mbxC* and *mbxD* Genes and Expression of Recombinant Proteins

In order to produce highly purified recombinant cytotoxin peptides, portions of the cytotoxin gene were cloned into expression vectors according to procedures described in Example 18. An internal peptide and carboxy peptide were expressed corresponding to amino acids 438 through 713 and 643 through 927, respectively.

3. Confirmation of Recombinant Protein Identity

Identity of produced recombinant protein was confirmed by neutralization studies, described in Example 21 and in Figure 7.

Preparation of recombinant protein was done according to Example 18.

III. Biologically Derived or Recombinant Anti-Moraxella
bovis Vaccines

A. Cytotoxin Vaccines

Vaccine is a biologically derived or recombinantly
5 prepared agent useful for artificially acquired immunization
in a host. The current invention describes the production of
biologically derived and recombinant vaccines for active
immunization of cattle and calves against IBK and includes the
preparation of passive immune products for treatment of
10 established infections.

The scope of the invention is, therefore, intended to
include biologically derived or recombinantly prepared
vaccines based on the antigens (a native or recombinant
cytotoxin or a fragment thereof) of the invention.

15 The native vaccine is produced from isolated and
partially purified native cytotoxin. The recombinant vaccine
is produced by identifying the relevant cytotoxin or a
fragment thereof, cloning them and expressing them using
suitable vectors. This approach yields immunogens which are
20 pure and reproducible in sufficiently large quantities to
allow preparation of a vaccine for active immunization.

Recombinant vaccines are useful for immunization of the
cattle or calves to produce the host's own antibodies against
an *M. bovis* infection. Additionally, the recombinant vaccines
25 may be used for production of passive immunotherapeutic
agents.

These vaccines are also useful for primary protection
against *M. bovis* infection in calves. Providing the herd with
anti-*M. bovis* immunity decreases the risk for outbreaks of IBK
30 in areas where the infection is prevalent.

In addition, *mbxA* DNA or RNA may be introduced into cattle so that propagation and/or expression of the encoded protein occurs in the host using a foreign expression system according to the methods known in the art.

5 The vaccine of the invention contains a cytotoxin identified by the invention, modified in such a way that it is incapable of producing the IBK symptoms but is capable of eliciting the production of specific protective antibodies against the disease when introduced in the body.

10 A DNA or RNA vaccine for prevention and treatment of infections caused by *M. bovis* is developed by utilizing newly identified and isolated DNA (SEQ ID NO: 1) and amino acid (SEQ ID NO: 2) sequences of the cytotoxin made by the *M. bovis* pathogen.

15 The antigen proteins used for preparation of vaccines correspond to cytotoxin or a cytotoxin fragment, which are identified by being a target of the polyclonal or monoclonal antibodies of the invention capable of preventing or ameliorating the disease.

20 A hybrid vector comprising a DNA segment that encodes the protein antigen able to bind selectively and specifically to anti-*M. bovis* antibodies operatively coupled to the vector can also be prepared and expressed. This includes preparation of recombinant vaccines using the viral expression vector outside
25 of the host body and the preparation of DNA vaccines in procaryotic or eukaryotic hosts carrying the hybrid vector which may be introduced into the cattle or calf or a direct introduction of DNA or RNA into cattle or calf cells generating the host's own expression or translation of DNA or

RNA, respectively, and production of proteins eliciting appropriate antibodies.

B. DNA and RNA Vaccines

DNA or RNA vaccines for immunization against *M. bovis* are prepared as described in Science, 259:1745 (1993), hereby
5 incorporated by reference. DNA or RNA vaccines for development of immunity for cattle and calves against IBK are produced according to the methods described ibid.

Briefly, animals are injected, preferably
10 intramuscularly, with DNA vectors encoding the deactivated anti-*M. bovis* antigen DNA or RNA and the antigen is produced. The produced antigen elicits its own immune responses in the form of a formation of a specific antibody against anti-*M. bovis* antigen, thereby providing its own (native) immunity
15 and/or cell mediated responses.

C. Field Trials with Vaccines

Both *in vitro* and *in vivo* studies were performed in order to determine whether the native or recombinantly produced cytotoxin can be used as agent for provoking active immunity
20 against *M. bovis* and for preparation of vaccines.

As described above, antibodies to both native partially purified and recombinantly produced cytotoxins were tested for their cytolytic and/or hemolytic inhibitory activity in neutralization studies described in Examples 7-13, and both
25 were shown to be inhibited with antibodies raised against native or recombinant protein. Both proteins also induced production of antibodies that recognize cytotoxin/cytotoxin peptides.

Field trials were performed for testing both the partially purified native and recombinantly produced cytotoxin.

1. Native Cytotoxin Vaccines

5 *M. bovis* cytotoxin is a labile protein of which activity rapidly disappears when removed from the bacterial cell. This property previously prevented its complete characterization. To overcome this, a method for partial purification and stabilization of cytotoxin was developed and such partially
10 purified and stabilized cytotoxin was used for preparation of a vaccine for field trials.

 In order to determine suitability of such partially purified native *M. bovis* antigen (cytotoxin) for vaccination against *M. bovis*, two field trials were performed where the
15 cytotoxin was administered as a vaccine formulated according to Example 23.

 The first field trial was performed on 82 cattle to determine the suitability of the cytotoxin in a vaccine to prevent IBK. In this trial the vaccine was a partially
20 purified cytotoxin formulated either in Quil A adjuvant or in an immunostimulating complex (ISCOM), a matrix that is composed of Quillaja saponins (Quil A), cholesterol, phospholipids and the antigen of interest. ISCOM based vaccines utilizing antigens of Bovine Herpes Virus-1, Bovine
25 Virus Diarrhea Virus and Bovine Leukemia Virus are known to be successful immunogens in cattle.

 Prior to the field trial testing the *M. bovis* cytotoxin vaccine, a preliminary study had shown that calves vaccinated with a cytotoxin vaccine developed higher tear IgA
30 concentrations than calves vaccinated with a cytotoxin in oil

adjuvant. Results of the first field trial showed that calves receiving the cytotoxin vaccine had a significantly reduced requirement for treatment of IBK and had significantly reduced the severity of clinical IBK as compared to control calves.

5 To further evaluate the efficacy of an ISCOM vaccine using partially purified *M. bovis* cytotoxin, a second field trial was conducted according to Example 23.

Briefly, a group of 104 calves on a farm with a historically high (> 50%) incidence of IBK were randomly
10 assigned to receive one of three different vaccine compositions: A) saline (controls); B) ISCOM-cytotoxin; and C) ISCOM alone (adjuvant).

Calves were vaccinated on days 0 and 21. The individual responsible for all subsequent examinations of the calves was
15 blinded as to treatment group assignments. The calves were then examined once weekly throughout the summer.

In the saline control group A, total of 18 of 34 calves developed IBK, while only 7 of 35 calves in the ISCOM-cytotoxin group developed IBK. Fifteen of 35 calves that
20 received adjuvant alone developed IBK. The cumulative number of calves with ulcers in one eye over time is shown in Figure 14. The cumulative number of calves that develop IBK was significantly less in the vaccinated group by the 10th week and remained so until the end of the study. The number of calves
25 with corneal ulcers during the study is presented in Figure 15.

The cumulative number of vaccinated calves with severe corneal ulcers (ulcer score > 1) was significantly lower in the antigen vaccinated calves than in the controls or in the
30 adjuvant only group on observations. There were no

differences in cumulative ulcers in calves of the control or the adjuvant only groups.

The surface area measurements and the clinical scores of the corneal ulcers are shown in Table 6.

5

Table 6

Surface Area Measurements of the Corneal Ulcers
and Clinical Scores of Vaccinated Calves and Controls

10	Group	Peak Surface area of First corneal ulcer	Surface area of corneal ulcer when first observed	Clinical score of corneal ulcer when first observed
15	Saline (n=34)	1.59±4.0(21.6) ^v	0.714±1.8(11.5) ^v	0.58±0.7 ^v
	Vaccine (n=35)	1.00±5.5(31.2) ^{a,s}	0.082±0.2(9.2) ^{s,c}	0.71±0.8 ^a
20	Adjuvant (n=35)	1.27±2.9(11.5) ^v	1.23±2.91(1.1) ^v	0.171±0.4

The numbers reflect the mean value ± the standard deviation. Numbers in parenthesis reflect the range of values.

Table 7

25 Descriptive Data Regarding Recurrences, Treatment
and Peak Corneal Ulcer Scores

30	Group	No. Recurrences ^a	No. Calves treated	Peak CUS [*]
	Saline (n=34)	5/18 [*]	10 [*]	
	Vaccine (n=35)	3/7	4	
	Adjuvant (n=35)	6/15	9	

* Differences were not significant (P>0.05)

^a Numbers reflect number affected divided by the number of calves with corneal ulcers.

The number of calves in the saline, vaccine and adjuvant groups that received antibiotic treatment, and the number of recurrences was not significantly different (Table 7).

Intact iscoms were present in low concentrations in indirectly stained ultracentrifuged sediments that were examined by electron microscopy.

Beginning on the 8th observation week, and continuing thereafter until the 15th observation week, there were significantly fewer cumulative infections in calves in the vaccine group than in either of the control groups (Figure 14). There were no significant differences in numbers of cumulative infections between the calves that received adjuvant only and those of the controls.

Table 8

Cumulative Numbers of Calves with Corneal Ulcers
at Each Observation

Observation Week	Group					
	Saline (n=34)		Vaccine (n=35)		Adjuvant (n=35)	
	All	≥2 CUS	All	≥2CUS	All	≥2CUS
0	0 (0)	0 (0)	0 (0)	0	0 (0)	0
1	0 (0)	0 (0)	0 (0)	0	0 (0)	0
2	0 (0)	0 (0)	1 (2.9)	0	0 (0)	0
3	1 (2.9)	0 (0)	1 (2.9)	0	1 (2.9)	1
4	1 (2.9)	0 (0)	1 (2.9)	0	3 (8.6)	1
5	2 (5.9)	0 (0)	4 (11.4)	0	3 (8.6)	1
6	3 (8.8)	0 (0)	4 (11.4)	0	5 (14.3)	1
7	5 (14.7)	3 (8.8)	4 (11.4)	0	5 (14.3)	1
8	7 (20.6)	3 (8.8)	4 (11.4)	1	7 (20.0)	3
9	9 (26.5)	4 (11.7)	4 (11.4)	1	8 (22.9)	3

10	12 (35.3)	6 (17.6)	4 (11.4)	1	11 (31.4)	5
11	13 (38.2)	7 (20.6)	4 (11.4)	1	11 (31.4)	6
12	13 (38.2)	7 (20.6)	4 (11.4)	1	12 (31.4)	7
13	14 (41.1)	8 (23.5)	5 (14.3)	1	14 (34.3)	7
5 14	15 (44.1)	8 (23.5)	5 (14.3)	3	15 (40.0)	7
15	16 (47.1)	10 (29.4)	5 (14.3)	3	15 (42.9)	9
16	18 (52.9)	10 (29.4)	7 (20.0)	4	15 (42.9)	9

10 The native vaccine was found to be effective at preventing new cases of IBK and the vaccine afforded protection against natural exposure. The antigenic mass of this experimental vaccine was concentrated from 5 liters of supernatant from hemolytic, toxigenic *M. bovis*. The retentate
 15 of that process was diafiltered extensively, effectively removing any solubilized protein with a molecular mass <100,000 daltons. A companion study showed that the leukotoxin was concentrated and stabilized in the diafiltered retentate. This proved to be antigenic and immunogenic in the
 20 field study described herein.

As measured by decreased cumulative infection, and decreased numbers of affected calves, vaccination with the vaccine derived from cytotoxin induced a resistance to infection under field conditions.

25 Results from this and previous studies provided a rational basis for pursuing the development of the vaccine comprising the native cytotoxin. Unfortunately, the lability of the cytotoxin and the difficulty associated with purifying it in milligram quantities limited its usefulness for
 30 practical use. To overcome these obstacles, techniques of molecular cloning and biotechnology to study the *M. bovis* cytotoxin were undertaken as described above and in Examples

and the recombinant protein was used for preparation of recombinant vaccines.

2. Recombinant Vaccines

Recombinantly prepared cytotoxin was used for preparation of recombinant anti-*M. bovis* vaccine using procedure as described in Example 24.

For the preparation of vaccine, the *M. bovis* cytotoxin was expressed as described in Example 24, and expressed purified protein was mixed with the ISCOM following ISCOM matrix formation. The vaccine was prepared according to Example 24.

For vaccine production, ISCOM matrix preparation was mixed 1:1 (v/v) with the recombinant protein solution. Vaccine (2 ml) was used for primary vaccination and for follow-up booster inoculations. ISCOM matrix adjuvant was used for control.

To determine if recombinantly derived *M. bovis* RTX toxin vaccine could prevent IBK, a vaccine trial was conducted during summer. In this trial, beef calves were randomly divided to receive saline, adjuvant alone (ISCOM matrix), or a recombinant *M. bovis* RTX toxin subunit vaccine. Vaccines were administered subcutaneously and calves were boosted 21 days later. The recombinant toxin vaccine contained the carboxy terminal 338 amino acids of MbxA. The calves were examined once per week for IBK through mid-September. Results indicated that fewer cases of IBK occurred in the recombinant vaccine group.

A total of 93 calves were enrolled in the study. Respective calf numbers in each vaccine group were: 29 CTRL (saline control); 31 ADJ (ISCOM MATRIX contact); and 33 VAC

(ISCOM matrix plus recombinant protein). At the end of the 20 week trial, a total of 47 calves developed corneal ulcers (50.5%). Respective numbers of ulcerated calves were 17 (54.8%), 17 (58.6%), and 13 (39.4%) for the CTRL, ADJ, and VAC groups. Figure 16 shows the cumulative proportion of calves with ulcers at each weekly interval. During weeks 4 and weeks 11 through 15, there were significantly fewer ulcerated calves in the VAC group relative to the CTRL group. At week 4, there were significantly fewer ulcerated calves in the VAC group relative to the ADJ group.

Results of this field trial clearly show that the vaccine comprising recombinantly derived cytotoxin provided immunity against *M. bovis* in vaccinated animals.

(V. Prophylaxis of IBK with Antigen Vaccine

Using vaccines described above comprising either the purified native or recombinantly prepared cytotoxin antigen, the cattle and/or calves are vaccinated using a regimen typically utilized for immunization by vaccinating the animals at least twice in about a 3 week interval and with a booster vaccine at least once a year.

A method of prophylaxis of *M. bovis* infection comprises administering to the animal in need of such treatment a vaccine comprising the cytotoxin or recombinant cytotoxin of this invention capable of endogenous development of an inhibitory amount of anti-*M. bovis* antibodies.

V. Diagnostic Utilities

Cytotoxin of the invention and/or antibodies against *M. bovis* may also be used for diagnostic purposes, such as for detection of *M. bovis* carrier cattle.

In the simplest form, serum obtained from the cattle is reacted with monoclonal or polyclonal antibody raised against native or recombinant *M. bovis* cytotoxin. The antibody binding or forming complex with the antigen indicates the presence of antigen. Following techniques may be advantageously used for *M. bovis* antigen identification.

Antigen capture ELISA is a technique for identifying an antigen in a sample. In the case of *M. bovis* detection, it can be used to identify cytotoxin in tear samples from cattle or respiratory or vaginal secretions to identify carrier cattle. The technique utilizes an antibody directed against the antigen (cytotoxin) which is fixed on a solid support. It then uses traditional ELISA techniques to develop a color change indicative of cytotoxin presence.

Diagnostic PCR utilizes primers, in this case, primers suitable for cytotoxin gene sequence, to amplify DNA from tissue samples, such as ocular, respiratory, or vaginal secretions, for example.

Fluorescence Resonance Energy Transfer (FRET) technology is also suitable to diagnose *M. bovis* carriers. This technology utilizes PCR primers linked to chemicals that fluoresce under certain conditions. If these primers detect the *mbxA* gene in sample DNA or bacteria, they emit certain detectable fluorescent spectra.

Method of diagnosing cattle that are carriers of *M. bovis* by use of antibodies (monoclonal or polyclonal) using an antigen capture ELISA.

Method to diagnose infection with hemolytic *M. bovis* utilizes diagnostic PCR of using DNA sequence data.

UTILITY

The current invention provides native or recombinant vaccines for immunoprotection against *M. bovis* infections and development of IBK in cattle and calves and methods for
5 detection of latent *Moraxella bovis* infections.

EXAMPLE 1Isolation of *Moraxella bovis* Bacteria

This example describes isolation of a cytolytic strain of *Moraxella bovis* T+ and a noncytolytic subculture T-.

10 A hemolytic, pathogenic strain of *Moraxella bovis* (T+) was isolated from a beef cow with infectious bovine keratoconjunctivitis. Cytolytic EPP and GLN 63 strains were furnished by Dr. G. W. Pugh (Ames, Iowa), and additional isolates were obtained from field specimens that were
15 submitted to the laboratory for confirmation of bacterial identification. These additional isolates were recovered from clinically affected cattle located in regions of the United States that included southern Georgia (Tifton 1, Tifton 2), northern Georgia (GA 3), northern California (LS-2, LS-4, LS-
20 6), southern California (LS-3, LS-8), Washington (LS-5), and North Carolina (LS-7). Isolate were identified and propagated as described in Acta Path. Microbiol. Scand., 80B, 629-640 (1972) and in Identification of Non-Enteric Gram-Negative Bacteria, US Dept. Health Education and Welfare Technical
25 Report, Center for Disease Control, Atlanta, GA (1979).

EXAMPLE 2Purification and Concentration of the Cytotoxin

This example describes the procedure used for purification and concentration of the cytotoxin when produced
30 in *M. bovis* shaker cultures.

The cytotoxin was produced in broth shaker cultures that were inoculated with lawn cultures of *M. bovis*. The inoculum was harvested from the surface of 10, 20 hour sheep blood agar plate lawns of *M. bovis* with sterile cotton tipped applicators. The bacteria on the swab were suspended into 10 ml of heart infusion broth (Difco Laboratories, Detroit, MI) spiked with 1.5 mM CaCl_2 .

Flasks were inoculated with the suspension, and were incubated at $35.5 \pm 0.5^\circ\text{C}$ on a rotary shaker set at 200 oscillations per minute. The cultures were removed from the incubator when the optical densities (420 nm) reached 1.85. For purification, the cultures (living whole cells) were centrifuged for one hour at $13,000 \times g$ (4°C) and the supernatants (centrifuged supernatants) were filtered through a sterile $0.2 \mu\text{m}$ polyethersulfone membrane (Gelman Sciences, Ann Arbor, MI).

Permeates from these filtrations (bacterial filter permeate) were concentrated approximately 100 fold by ultrafiltration, and further purified by diafiltration (diafiltered retentate) against 45 volumes of chilled (4°C) buffer consisting of 50 mM Tris (pH 8.2), 500 mM sodium acetate, 1.5 mM CaCl_2 and 20% glycerol. Diafiltration was discontinued after the optical density (280 nm) of the diafiltered permeate reached ≤ 0.01 . Specimens collected at each stage of preparation were examined for hemolytic and leukotoxic activities.

Filters that were used for concentration and diafiltration, included cartridges of spiral wrapped, regenerated cellulose (100 kDa molecular weight cut-off, CH2PRS® system S1Y100 membrane, Amicon, Beverly, Ma), and flat

polyethersulfone membranes (300, or 500 kDa molecular weight cut-off, stirred cell, XM 300 or YM 500 membranes, Amicon).

Results indicated that there were no differences in the retention of the cytotoxin by the filters, but because of ease of use, and capacity, most studies were performed using the regenerated cellulose filter. Each filtration step was conducted according to the manufacturer's recommendations, which included 30 PSI operating pressure, 300 to 500 ml/minute flow rate, and a 5 to 10 PSI pressure differential.

Due to the length of time required for diafiltration, initial measurements of cytolytic activity did not commence until 16 to 20 hours after the broth cultures were first centrifuged. For stability studies, cytolytic activities of the refrigerated retentates and permeates were centrifuged. For stability studies, cytolytic activities of the refrigerated retentates and permeates were re-examined 7, 14, and 57 days later. Cytolytic activities of frozen (-70°C) samples were remeasured after 120 days. All measurements were made from at least duplicate experiments.

EXAMPLE 3

Cytotoxin Preparation for Neutralization Studies

This example describes the procedure used for preparation of cytotoxin for neutralization studies.

Moraxella bovis lawns were grown on trypticase soy agar plates supplemented with 5% sheep blood. After 24 hours of incubation, the bacterial cells were harvested by flooding the plate with 10 ml of Tris buffered saline solution (TBS CaCl₂ 50 mM Tris, 150 mM NaCl, 1.5 mM CaCl₂, pH 8.0), and suspending the bacterial growth with a sterile inoculating loop. The suspensions were collected from the surface of the agar, and then were centrifuged at 27,000 x G for one hour. After

centrifugation, the supernatants were harvested, filtered through 0.22 μ M polyethersulfone membrane (Gelman Biosciences, Ann Arbor, Michigan), and tested for cytolytic activity.

EXAMPLE 4

5 Cytotoxicity Assay Using ^{51}Cr -Labeled Cells

This example describes the assay for determination of cytotoxicity, using cells labeled with $^{51}\text{chromium}$.

A stable, tissue culture adapted cell line of bovine lymphocytes BL-3 cells, supplied by Dr. Gordon Thielen,
10 University of California, Davis, were used as targets in the cytotoxicity assays. The cells were grown from frozen seed cultures at 37°C in 5% CO₂ in Liebovitz L-15 and Minimal Essential Media (1:1; v/v) with penicillin (100 IU/ml), streptomycin (100 μ g/ml), and 15% fetal bovine serum until
15 sufficient density was achieved (3 to 4 days). The cells were harvested by centrifugation, and the pellets were washed 3 times in Dulbecco's phosphate buffered saline solution (DPBS). After the wash, the cells were resuspended in DPBS to a final concentration of 2 X 10⁷/ml, labeled with 200 μ Ci of ^{51}Cr (1
20 hr, 37°C), and then washed three times in McCoy's 5A buffer (Gibco/BRL, Grand Island, NY). After the final wash, the cells were diluted to a final concentration of 1 X 10⁶/ml in McCoy's 5A buffer.

For measurement of cytotoxicity, 500 μ l of labeled BL-3
25 cells were added to 500 μ l of sample and incubated for one hour at 37°C. After incubation, the cells were centrifuged and the radioactivity was measured in 500 μ l of supernatant. Labeled cells (500 μ l) in 500 μ l of labeled cells lysed with Triton X-100 (Sigma, St. Louis, Mo.) served as respective
30 negative and positive controls. The % lysis was calculated using the formula:

$$\% \text{ lysis} = \left[\frac{\text{counts per minute (CPM) of specimen-media CPM}}{\text{Triton X CPT-media CPM}} \right] \times 100$$

5 For calculations relating to specific activity, a unit of cytotoxic activity was defined as the amount of leukotoxin required to release 1% of the total amount of ^{51}Cr from the labeled BL-3 cells.

EXAMPLE 5

10 Cytolytic Assay Using Non-Labeled Target Cells

This example describes the assay for determination of cytotoxicity using non-labeled target cells.

Cytolytic activity for bovine lymphocytes was also measured by assaying the release of lactate dehydrogenase
15 (LDH). These tests were performed by washing BL-3 cells three times a diluting them to a final concentration of 5×10^6 cells/ml in DPBS, adding 100 μl of BL-3 cells to 100 μl of sample in 96-well polypropylene plates (Becton Dickinson, Franklin Lakes, NJ) and incubating the mix for 60 minutes
20 (37°C) while being shaken for 30 seconds every 15 minutes. Duplicate measurements were made on each specimen.

After the incubation was completed, a 50 μl aliquot of supernatant from each well was transferred to a corresponding well in a new polystyrene plate (Costar, Cambridge, Ma), and
25 75 μl of 0.75 mM β -NADH in 100 mM potassium phosphate pH 7.2 was added. The change in absorbance (340 μm) was measured every 15 seconds using an automated reader.

After 50 measurements, lactate dehydrogenase activity was recorded in units/ml with units representing the reciprocal
30 slope of a line plate of $\Delta A/\Delta T$. For validation, lytic activities of 0.22 μM filter sterilized culture supernatants were measured concurrently using ^{51}Cr release and LDH assays.

EXAMPLE 6Neutralization Assay Using ^{51}Cr Labeled Cells

This example describes the assay used for neutralization with $^{51}\text{chromium}$ -labeled cells.

- 5 Prior to neutralization, sera were diluted 1:5 or 1:10 in TBS CaCl_2 or McCoy's 5A buffer and 200 μl was mixed with 500 μl of specimen, refrigerated (4°C) for one hour, and then mixed with 500 μl of labeled BL-3 cells. The cells and sera were incubated (37°C) and rotated at 15 revolutions per minute for
10 one hour before centrifuging each specimen and measuring the radioactivity in 500 μl of supernatant. For controls, dilutions of irrelevant rabbit sera, or fetal bovine serum was substituted.

EXAMPLE 7

15 Neutralization Assay Using Non-Labeled Target Cells

This example describes the assay used for neutralization with non-labeled target cells.

- Cytolytic and hemolytic bacterial filter permeates were prepared by flooding agar lawn cultures of *M. bovis* with 10 ml of TBS CaCl_2 , centrifuging the suspension and filtering the
20 supernatants through sterile polyethersulfone membranes (0.2 μm average pore diameter). Heat inactivated sera were diluted in TBS CaCl_2 . Aliquots (50 μl) of the bacterial filter permeates from T+ and T- were mixed with 50 μl of each serum
25 dilution, and the mixture was refrigerated (4°C) for 60 minutes before the addition of 100 μl of diluted BL-3 cells. The cell-serum mixture was further incubated (37°C) for 60 minutes with gentle agitation for 30 seconds every 15 minutes. After incubation, 50 μl of the mixture was transferred to a
30 new tissue culture plate to measure the cell free LDH activity.

EXAMPLE 8Hemolysin Assay

This example describes conditions used for the hemolysin assay.

5 Bovine erythrocytes, harvested from 10 ml of heparinized blood of a normal cow, were packed by centrifugation and washed three times with TBS CaCl_2 buffer. For the hemolysin assay, 10 μl of packed erythrocytes were added to 500 μl of sera of 10-fold dilutions of sample. The mixtures were
10 incubated for 6 hours (37°C) before final evaluation of hemolytic activity. Controls included washed erythrocytes in TBS CaCl_2 incubated with, or without 10% Triton X as respective positive and negative controls. For measurement of hemolytic activity of column fractions, a mixture of 10 μl of packed,
15 washed bovine erythrocytes and 500 μl of sample, was incubated for 6 hours (37°C). After incubation, the cells were centrifuged, and the absorbance (455 μM) of the supernatant was measured. Units of hemolytic activity were calculated using the formula:

20
$$\text{Hemolytic units} = \frac{\text{Sample O.D.} - \text{Neg. Control O.D.}}{100\% \text{ Lysis O.D.} - \text{Neg. Control O.D.}} \times 100\%$$

Percent hemolysis was calculated from hemolytic units of 100% lysis controls, and the specimen.

25 EXAMPLE 9

Immunoassay

This example describes conditions used for immunoassay.

The 96-well polystyrene plates (Nunc Maxisorp, Naperville, Ill.) were sensitized with 100 μl of solution
30 containing 2 $\mu\text{g}/\text{ml}$ diafiltered cytotoxin, refrigerated for 24 hour, and then washed with TBS CaCl_2 containing 0.05% Tween 20 (TBST). After washing, two-fold doubling dilutions of serum were added to the wells. The plate was then agitated gently

for 60 minutes at 37°C, and washed three times with TBST. After the final wash, either goat anti rabbit or rabbit anti bovine IgG labeled with 100 μ l of alkaline phosphatase (Jackson Immunobiological, West Grove, Pa), diluted 1:25,000 in TBS CaCl₂, was added. Plates containing secondary antibodies were shaken continuously for 60 minutes (37°C), and then washed one more time with TBST. The color reaction was developed by the addition of 100 μ l of 3.8 mM p-nitro phenyl phosphate (disodium salt) dissolved in a buffer consisting of 0.5 mM MgCl₂ and 50 mM Na₂CO₃ (pH 9.5). The plate was agitated gently for 60 minutes at 37°C and then absorbance (400 μ m) was measured using an automated plate reader (Dynatech MR700, Chantilly, Va).

EXAMPLE 10

Chromatography of Diafiltered Retentates

This example describes conditions used for chromatography diafiltered retentates.

Diafiltered retentates of T+ and T- were chromatographed through a 10 x 300 mm Superose 6HR gel filtration column (FPLC, Pharmacia Biotech, Uppsala, Sweden) that was equilibrated with 50 mM Tris, 0.5 M sodium acetate, 1.5 mM CaCl₂ pH 8.0. The column was loaded with 200 μ l of diafiltered retentate and was eluted with buffer at a maximum flow rate of 0.5 ml/min⁻¹. Absorbance (280 nm) of column effluent was measured. Fractions (3.5 ml) were collected, and examined for cytotoxic and hemolytic activity, and for endotoxin content.

EXAMPLE 11

Production of Antibodies

This example describes procedure used for the production of antibodies against *M. bovis* cytotoxin.

New Zealand White rabbits were given 0.5 ml of either diafiltered retentate (50 μ g protein), gel filtration purified cytotoxin (10 μ g protein) or permeates from the 0.22 μ M filtration of T+ or T- broth cultures.

5 Immunizing preparations were mixed 1:1 (v/v) in Freund's complete adjuvant for the primary injection and with incomplete Freund's adjuvant for the booster, 21 days later. The rabbits were exsanguinated three weeks after the booster was given. Convalescent serum from a Holstein calf with
10 clinically apparent infectious bovine keratoconjunctivitis and fetal bovine serum (Hyclone Laboratories, Logan, Utah) were used as respective positive and negative controls.

EXAMPLE 12

General Methods

15 This example describes or gives reference to general methods used in these studies.

Measurement of 2-Keto-3-Deoxyoctonate (KDO)

The concentration of KDO was measured in diafiltrates, and cytolytic Superose 6HR column fractions using a
20 hydroperiodate oxidation assay as described in Handbook of Micromethods for the Biological Sciences, Van Nostrand Reinhold, New York (1976).

Measurement of Endotoxin

The concentration of endotoxin in cytolytic Superose 6HR
25 column fractions was measured semiquantitatively using a commercially available timed gel formation limulin assay (Sigma, St. Louis, Mo.).

Electrophoresis

Proteins were examined using the discontinuous
30 polyacrylamide gel electrophoresis method described in Nature, 227:680-685 (1970).

To reduce non-specific binding, blots were blocked in a buffer containing 0.1% SDS, 50 mM Tris and 150 mM NaCl, pH 7.5, containing either 1.5% goat serum, or 0.5% teleost gelatin (Sigma Inc., St. Louis, Mo). The blocked, washed
5 membranes were incubated with antiserum for 12 hours, and then were washed four times in TBS. After the final wash, blots were incubated with either 1:2500 dilution of goat anti-rabbit immunoglobulin conjugated with alkaline phosphatase, or with 0.5 μ Ci of I^{125} labeled protein A in TBS. The alkaline
10 phosphatase labeled blots were incubated for 12 hours, after which they were washed four times (10 minutes per wash) with a buffer containing 100 mM Tris, 5 mM Tris, 5 mM $MgCl_2$ and 100 mM NaCl (pH 9.5). After the final wash, the blots were developed using nitro blue tetrazolium and bromo-chloro-
15 indonyl-phosphate as described in Antibodies: A Laboratory Manual, 1st Ed., Cold Spring Harbor, Cold Spring Harbor Laboratories, New York (1988). The A- I^{125} protein A- I^{125} labeled blots were incubated for 2 hours, and then washed 3 times, 4 hours per wash, in TBS containing either 1.5% goat serum or
20 0.5% teleost gelatin. After washing, the blots were air dried and autoradiographed.

Protein Quantitation

Protein concentrations were measured using the Lowry protein precipitation method (Protein Assay Kit, Sigma Inc.,
25 St. Louis, Mo.).

EXAMPLE 13

Corneal Epithelial Cell Cultures

This example describes procedures used for culturing corneal epithelial cells.

30 Bovine eyes were collected at slaughter, and the corneal epithelium was harvested by keratectomy. An ophthalmic

solution containing neomycin, polymyxin B and gramicidin (Schein Pharmaceuticals, Port Washing, NY) was applied to the corneas prior to keratectomy in order to reduce the bacterial contamination.

5 The corneal epithelium was dissected from the stroma, and immersed in 50 ml of Minimal Essential Medium (MEM) containing D-valine, penicillin G (100 IV/ml), streptomycin (100 μ g/ml), gentamicin sulfate (100 μ g/ml), and amphotericin B, (250 μ g/ml). Neutral protease, (Dispase II, Boehringer Mannheim, Indianapolis, Inc.) was added in order to initiate separation
10 of the epithelial cells from the remaining stroma.

After 2 hours of incubation, the epithelial sheets were washed three times in fresh media, and were placed epithelial side down, in wells of sterile polystyrene plate. The
15 cultures were then immersed in 2 ml of media containing MEM-D-valine and 10% fetal bovine serum, and incubated in an atmosphere of 5% CO₂ (37°C) until plaques of adherent corneal cells were observed on the bottom of the plate. The epithelial sheets were removed from the plates at that time.
20 These cell cultures were incubated for at least 48 additional hours until they formed an adherent monolayer.

In order to verify the epithelial origin of the adherent cells, sample monolayers were stained with an anti-keratin, epithelium specific monoclonal antibody furnished by Dr. Rheen
25 Wu, University of California, Davis. After 1 hour, the cultures were washed with PBS containing 0.05% Tween 20, and a fluorescein isothiocyanate labeled goat anti-mouse antibody (Organon Teknika, Durham, NC) was applied for 1 hour. The unbound secondary antibody was then washed from the cells
30 using PBS with 0.05% Tween and the monolayer was examined using a fluorescence microscope.

Diafiltered retentate was buffer exchanged into FPLC buffer (50 mM Tris; 1.5 mM CaCl_2 ; 0.9% NaCl; 5% glycerol; pH 8.0) using PD-10 columns (Amersham Pharmacia Biotech, Inc., Piscataway, NJ), concentrated 10-15 fold (Centriprep-10; 5 Millipore, Beverly, MA), filtered through a 0.2 μm filter (Sterile Acrodisc 13; Gelman Sciences), and chromatographed on a Superose 6 gel filtration column (Amersham Pharmacia) in FPLC buffer at room temperature. Column void volume fractions from multiple chromatography runs were pooled and concentrated 10 5-10 fold (Centriprep 10; Millipore).

For analysis, samples were mixed with an equal volume of 2X loading buffer containing 62.5 mM Tris, pH 6.8, 0.7 M β -mercaptoethanol (Bio-Rad Laboratories, Hercules, CA), 20% glycerol, 4.1% sodium dodecyl sulfate (SDS), and 0.2 mg/ml 15 bromophenol blue, heated to 95°-100°C for 5 minutes, electrophoresed using SDS-PAGE (3.9% stacking/7.5% running), transferred to Immobilon-P (0.45 μm pore size; Millipore), and incubated overnight at room temperature on a rotating shaker in blocking buffer (TSN buffer; 20 mM Tris, 0.9% NaCl, 0.1% 20 Tween 20 (pH 7.4) with 5.5% teleostean gelatin (Sigma, St. Louis, MO)). Following incubation, fresh blocking buffer containing rabbit anti-column fraction serum (1:400 dilution) was added and blots were incubated overnight at room temperature on a rotating shaker. The blots were washed 3 25 times in TSN buffer, then immersed in a solution containing 0.5 μCi of ^{125}I -labeled Protein A (NEN Life Science Products, Inc., Boston, MA) per 20 ml TSN buffer, incubated overnight at room temperature on a rotating shaker, washed and autoradiographed (Hyperfilm MP; Amersham).

EXAMPLE 16Amino Acid Sequencing

This example describes conditions used for amino acid sequencing.

5 The concentrated void volume fractions from gel filtration chromatographed diafiltered retentates from T+ were electrophoresed on a SDS-PAGE gel and stained with Coomassie blue. A 70 kDa band that had previously been identified on Western blots as being unique to T+ was excised, digested with
10 trypsin, and tryptic peptides were fractionated by HPLC. Two peptides designated #23 and #26 were selected for N-terminal amino acid sequencing using Edman degradation chemistry. Amino acid sequencing was performed at the Protein Chemistry Laboratory, University of California, Davis (ABI 470A amino
15 acid sequencer; PE Biosystems, Foster City, CA).

EXAMPLE 17Determination of DNA Sequence of Cytotoxin Gene

This example describes conditions used for determination of DNA sequences of *mbxA* gene.

20 All DNA manipulations were performed as recommended by the manufacturers of the enzymes or by using standard published methods. Genomic DNA from *M. bovis* Tifton 1 was isolated as described in Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring
25 Harbor, N.Y. (1989).

Plasmid DNA was isolated with Qiagen plasmid minipreps (Qiagen, Valencia, CA). The DNA sequence of the complete cytotoxin gene was obtained by sequencing PCR products directly or PCR products cloned into pCR2.1-TOPO (Invitrogen)
30 with a TOPO TA cloning kit (Invitrogen). Degenerate PCR primers designated primer 23A, depicted by a sequence 5'-AAY

AAA GAR TTR GAR GCN GAR-3' (SEQ ID NO: 23) and 26A, depicted by a sequence 5'CCY TCN CCR CTR TGR AAD ATR TCR TTR AAT TT-3' (SEQ ID NO: 24) were designed by reverse translating the amino acid sequences of peptides #23 and #26, respectively, primers
5 C-down, 5'ATH GAY TGG ATH GCN CCN TTY GGN GAY-3' (SEQ ID NO: 25) and B-up, 5'-ACT TTA TCC ATC ACR ACT TGR AAR AA-3' (SEQ ID NO: 26) were designed by reverse translating conserved regions of RTXC and B genes of other bacteria.

PCR amplifications were performed with Taq polymerase
10 (Life Technologies) using 30 cycles of 30 seconds each at 95°C, 1 minute at 55°C, and 45 seconds (primers 23A/26A) or 2 minutes (primers C-down/B-up) at 72°C. PCR products were analyzed by agarose gel electrophoresis. Automated DNA sequencing was performed by the DNA sequencing facility,
15 University of California, Davis. Additional primers were designed as needed for sequencing of overlapping fragments on both strands; the DNA sequences of overlapping fragments were assembled with MacDNASIS (Hitachi Software Engineering Co., Ltd., Yokohama, Japan) to yield the final complete sequence.

20

EXAMPLE 18

Expression of Recombinant Peptides

This example describes expression of recombinant peptides.

Internal peptide

25 The PCR product amplified by primers 23A/26A was cloned into pCR2.1-TOPO (Invitrogen) and the resulting recombinant plasmid was digested with EcoRI. The appropriate fragment was gel purified (QIAquick gel extraction kit, Qiagen) and ligated into pProEXHTa (Life Technologies) digested with EcoRI to
30 yield an expression construct that would direct synthesis of the cytotoxin gene from amino acids 438 through 713. This

expression plasmid was introduced into *E. coli* DH5 α , cells were grown to an OD₆₀₀ of 0.6, and expression was induced by adding isopropylthio- β -galactoside (IPTG; Life Technologies) to 1 mM. Induction was continued for 4-6 hours and cells were
5 harvested by centrifugation.

The expressed proteins formed inclusion bodies that were purified as described in Methods Enzymol., 153:461 (1987). Purified inclusion bodies were then solubilized in buffer containing 20 mM Tris (pH 8.0), 1 mM EDTA, and 6 M urea and
10 chromatographed on a SuperDex 200 gel filtration column (Amersham Pharmacia). Peak fractions were pooled, dialyzed against phosphate buffered saline (PBS) and stored at -20°C. Following removal of urea, the purified protein formed a precipitate. This protein was designated internal peptide.

15 Carboxy peptide

The complete cytotoxin gene was amplified from genomic DNA of *M. bovis* Tifton I with primers designated NdeI-start (5'-GAT CAT ATG TCC AAT ATA AAT GTA ATT AAA TCT AA-3') SEQ ID NO: 27 and B-up #2 (5'ATC ACT AGT TCC ATA ATC TAT AAC CAA TGA-
20 3') SEQ ID NO: 28. This primer pair amplified *MbxA* from 6 bases before the ATG start codon to 64 bases past the termination codon and introduced an NdeI restriction site overlapping the ATG start codon. The PCR conditions were as described above except that the elongation step was for 1
25 minute at 72°C.

The PCR product was cloned into pCR2.1-TOPO (Invitrogen). The resulting recombinant plasmid was digested with XhoI-EcoRI and blunt ended with Klenow polymerase (Life Technologies). The appropriate fragment was gel purified, then cloned into
30 blunted ended, BamHI digested and Klenow blunt ended pT7-7 to

yield an expression construct that would direct synthesis of the cytotoxin gene from amino acids 643 through 927.

Recombinant plasmids were transformed into *E. coli* DH5 α , and subsequently purified (Qiagen miniprep kit; Qiagen) prior to transformation into *E. coli* strain BL21 (DE3) (Novagen, Madison, WI) for expression. The cells were grown and induced as described above to produce the recombinant protein, except that for protein purification, inclusion bodies were solubilized in buffer containing 10 mM Tris (pH 8.0), 1 mM EDTA, and 8 M urea, and chromatographed on a Mono Q column (Amersham Pharmacia). Peak fractions were identified by SDS-PAGE, pooled and then chromatographed on a SuperDex 200 gel filtration column. Peak fractions from the SuperDex 200 column were pooled, dialyzed against PBS, and stored at -20°C. This protein, which remained soluble following dialysis, was designated carboxy peptide.

EXAMPLE 19

Preparation of Rabbit Antisera

This example describes preparation of rabbit antisera for recombinant cytotoxin identification and confirmation.

Antisera was prepared by immunizing New Zealand White rabbits with either the pooled void volume fractions or Superose 6 gel-filtration chromatographed diafiltered retentates of *M. bovis* T+ mixed 1:1 (v/v) with Freund's complete adjuvant. Booster vaccinations were performed 21 days later with either the pooled column fractions or culture filtrates mixed 1:1 with Freund's incomplete adjuvant, and serum was harvested 21 days post booster. Sera against the pooled column fractions was designated rabbit anti-column fraction. The sera against the culture filtrates was designated rabbit anti T+. This antisera neutralized

hemolytic and cytolytic activity of partially purified *M. bovis* cytotoxin.

Polyclonal rabbit antisera to the internal (amino acids 438 through 713) and carboxy (amino acids 643 through 927) peptides expressed from *MbxA* were prepared commercially (Antibodies Incorporated, Davis, CA). Before use in immunoblots, this antisera was preabsorbed with an *E. coli* DH5 α lysate. Sera were heat inactivated at 56°C for 1 hour prior to lysis neutralization assays.

10

EXAMPLE 20

Demonstration of Cytotoxin in Culture Supernatants

This example describes conditions used for demonstration of the presence of cytotoxin in culture supernatant.

Moraxella bovis T+, T-, or Tifton I was inoculated into LB broth containing 1.5 mM CaCl₂ and incubated for 3-6 hours with shaking at 250 rpm at 35°C. Following incubation, cultures were centrifuged and the supernatants were harvested and analyzed by SDS-PAGE/transblotting, using rabbit antiserum against the internal or carboxy peptide as the primary antibody.

20

EXAMPLE 21

Lysis Neutralization Assays

This example describes assays performed to study cytotoxicity neutralization and hemolysis neutralization.

Serum test samples were diluted 1:64 in TBS CaCl₂ buffer (50 mM Tris, 150 mM NaCl, 1.5 mM CaCl₂, pH 7.4) and an equal volume of either TBS CaCl₂ buffer or carboxy peptide diluted in TBS CaCl₂ buffer (0.1 mg/ml) was added. The samples were incubated at room temperature for 30 minutes, then at 37°C for 30 minutes, and then centrifuged at maximum speed in a microcentrifuge for 10 minutes. The supernatants were then

30

harvested and mixed with an equal volume of Tifton I diafiltered retentate diluted 1:32 in TBS CaCl₂ buffer. The test samples were incubated for 1 hour at 4°C after which hemolysis or cytolysis neutralization assays were performed as described below.

For hemolysis neutralization assays, 500 µl of 1% (v/v) suspension of washed and pelleted bovine erythrocytes in TBS CaCl₂ buffer was added to 500 µl of each test sample, incubated for 6 hours at 37°C, the samples were inverted once, centrifuged for 1 minute at maximum speed in a microcentrifuge, and 350 µl of supernatant was transferred to a 96 well microtiter plate for determination of the OD₄₅₅ (Spectra Max 250, Molecular Devices Corporation, Sunnyvale, CA). Respective negative and positive controls were TBS CaCl₂ buffer or Tifton I diafiltered retentate diluted 1:32 in TBS CaCl₂ buffer. A control for the carboxy peptide in TBS CaCl₂ buffer was also included. The percent hemolysis was determined using the formula:

$$\% \text{ hemolysis} = \frac{[\text{sample OD} - \text{lysis negative control OD}]}{[\text{positive control OD} - \text{lysis negative control OD}]} \times 100$$

The percent neutralization was calculated by subtracting the percent hemolysis from 100. The results reflect the means from three experiments.

The target cells for cytolysis neutralization assays were labeled bovine lymphocytes (BL-3 cells); supplied by Dr. Gordon Thielen, University of California, Davis. These were prepared by growing cells from frozen stock cultures at 37°C in 6% CO₂ in Liebovitz L-15 medium (Life Technologies) and Minimal Essential Media (Life Technologies) (1:1 v/v) supplemented with penicillin (50 U/ml), streptomycin (50 ug/ml) (Life Technologies), and 15% heat inactivated fetal bovine serum (Hyclone, Logan UT). Cells were harvested by

centrifugation, washed 3 times in PBS, resuspended in McCoys 5A media (Life Technologies) to a final concentration of 2×10^7 /ml, labeled for 1 hour at 37°C with 200 μ Ci of ^{51}Cr (NEN Life Science Products) while rotating at 20 revolutions per minute, and then washed 3 times in McCoys 5A media. Following the final wash, cells were diluted in McCoys 5A media to a final concentration of 4×10^5 /ml.

For cytolysis neutralization assays, 500 μ l of labeled BL-3 cells were added to 500 μ l of each test sample, incubated for 1 hour at 37°C while rotating at 20 revolutions per minute, pelleted in a microcentrifuge, and the radioactivity in 500 μ l of supernatant was determined. Negative and positive controls were as described above for hemolysis neutralization assays. The percent cytolysis was determined using the formula:

$$\% \text{ cytolysis} = \left[\frac{\text{specimen counts per minute (cpm)} - \text{negative control cpm}}{\text{positive control cpm} - \text{negative control cpm}} \right] \times 100$$

The percent neutralization was calculated by subtracting the percent cytolysis from 100. The results reflect means obtained from three experiments.

EXAMPLE 22

Amino Acid Sequence Analysis

This example describes methods used in amino acid sequence analysis.

The relationships between the deduced amino acid sequences and published protein sequences were determined with BLAST database searches. Multiple sequence analyses, alignments, and homology calculations were obtained using the blosum 62 amino acid substitution matrix (SEQWEB software (version 1.1); Genetics Computer Group, Madison, WI). The nucleotide sequence for the *M. bovis* cytotoxin gene has been submitted to the GENBANK database under accession number AF205359.

EXAMPLE 23Field Trials With Native Cytotoxin Vaccine

This example describes conditions used in field trials with native cytotoxin based vaccine.

5 Experimental Subjects

Cattle for the study were part of the herd at the University's Sierra Foothills Field station. The ranch was located in the foothills of the northern Sierra Nevadas at an altitude ranging between 800 and 2400 feet. The subjects
10 included 104 Hereford Angus crossbred yearling calves. The calves, which were born during November and December of the previous year, weighed between 90 and 300 kg, and were composed of equal numbers of females and castrated males. Calves were commingled into a single group and were pastured on either of
15 2 irrigated fields. Other than the pasture forage and trace mineralized salt licks, cattle were not supplemented during the summer. The calves were housed out of doors on irrigated pastures on approximately 100 acres. Pasture forages were composed of Bermuda, fescue, oats, and rye grasses, and smaller
20 amounts of trefoil and clovers, and other plants that were indigenous to the area. For the previous decade, yearling calves at the ranch had an annual prevalence of IBK that exceeded 50%.

Study Design

25 The study was conducted between the dates of May and September. In the May examination, calves were prospectively and randomly allocated to one of 3 groups designated A, B or C. For examination, the calves were restrained in a squeeze chute, and fluorescein dye was instilled into each eye prior to
30 illumination with a light source. Eyes were assigned a clinical score ranging between 0 and 3 with 3 representing an eye that had ruptured, and had expelled the contents. Criteria for lower clinical ocular scores included: 0 = no conjunctivitis or

corneal ulcer; 1 = conjunctivitis with a corneal ulcer having a maximum diameter that was ≤ 0.5 cm, and 2 = a corneal ulcer having a maximum diameter that was > 0.5 cm. Eyes with corneal ulcers were photographed during each examination. Each
5 photographic image included a ruler held on the lower eyelid.

After application of the fluorescein dye, eyes with corneal ulcer scores ≥ 1 were photographed at a constant magnification, and the image was projected onto paper and traced. The surface area of each traced image was measured planimetrically as
10 described. During the first examination in April (Day 1), calves with normal eyes were randomly assigned to one of 3 groups. Calves of the first group (n=34) were given 2 ml of sterile saline solution. Calves of the second group (n=35) were given 2 ml of a cytotoxin enriched, sterile Quil A adjuvanted
15 vaccine. Calves of the third group (n=35) were given 2 ml of a mixture of sterile adjuvant and saline (1:1, v/v). All injections were made subcutaneously in the neck. Calves were boosted with the corresponding product in June. Thereafter, clinical examinations were performed weekly for 15 weeks.

20 Personnel responsible for administering the vaccine, evaluating the clinical scores and for measuring the corneal ulcer diameters had no knowledge of the group assignment of the calves. Each week, calves with corneal ulcer scores ranging between 0 and 1 in either eye were returned to the common
25 pasture. Calves with ulcer scores of 2 or 3 were treated with florfenicol (Nuflor®, 20 mg/kg of body weight intramuscularly) and were housed in a corral until they were retreated 48 hours later. After the second florfenicol treatment, the calves were moved back to their original pasture and were observed weekly
30 for the remainder of the study.

Vaccines

Calves of group A were given 2.0 ml of a vaccine composed of Quil A adjuvanted, diafiltered concentrated cell free

cytotoxin from a toxigenic, hemolytic, non piliated strain of *Moraxella bovis*. Calves of the second group were sham vaccinated with Quil A adjuvant in phosphate buffered saline solution. The third group of calves received 2.0 ml of sterile saline solution. The original piliated strain of *M. bovis* was derived from the eye of an infected cow located in northern California and was identified using biochemical tests described previously. Non piliated isolates were identified during repeated subcultures on blood agar plates. These isolates, devoid of pili, were smooth, did not pit the surface of the agar, and remained homogeneously suspended in broth cultures. Lack of piliation was confirmed using electron microscopy.

Stocks of the pilin negative isolate were stored frozen (-80°C). For vaccine production, stocks were thawed at room temperature and inoculated onto a bovine blood agar plate lawns. After 18 hours of incubation (37°C), the lawns were harvested and were suspended in flasks containing brain heart broth (1 lawn per 500 ml) supplemented 1.5 mM of CaCl₂. The flasks were shaken (200 rpm) for 5 hours (37°C). After incubation, the broth cultures were centrifuged for one hour at 13,000 x g (4°C) and supernatants were harvested and were filter sterilized (0.2 µm average pore diameter; Gelman Sciences, Ann Arbor, Mi). The sterile supernatant was concentrated approximately 40-fold to a final volume of 200 ml by ultrafiltration over a spiral wrapped membrane (molecular weight cut off of 100,000 daltons) of regenerated cellulose (S1Y100 CH2PRS membrane, Amicon Inc, Beverly, MA). The concentrated retentate was then diafiltered using 50 volumes 50 mM Tris, pH 8.2, 50 mM sodium acetate, 1.5 mM CaCl₂, and 20% glycerol (v/v).

Diafiltration was discontinued when the optical density of the permeate was < 0.01 optical density units. Approximately 200 ml of diafiltered retentate was collected, and the protein content was measured using the Lowry method. The specific

cytotoxic and hemolytic activities of the diafiltered retentates were greater than those of the concentrated permeates.

The experimental vaccine preparation was made by diluting the diafiltered retentate to achieve a final concentration of 0.8 mg/ml, and then adding 0.25% (w/v) MEGA-10 (Cal Biochem, San Diego, CA). For the adjuvant control vaccine, an equivalent volume of Tris acetate calcium chloride, glycerol buffer was used in place of the diafiltered retentate. To both solutions, Quil A (Isotec, Sweden) was added to a final concentration of 0.1 %. The mixtures were then placed in dialysis membranes with 2000 kDa molecular weight cut off, and were dialyzed against repeated changes of sterile Dulbecco's phosphate buffered saline solution (GIBCO BRL, Grand Island, New York) for 48 hours at 4°C. After 48 hours, the contents of the dialysis tubing were filter sterilized, and kept refrigerated (4°C) until administered to the subjects 48 hours later.

Statistical evaluation of data

Data including number of calves in each group that were infected, on each date, cumulative numbers of affected calves, and number of corneal ulcers in calves of each group during the weekly evaluation, were compared using the Chi squared analysis. In order to avoid statistical errors caused by repeated measurements in eyes that had more chronic ulcers, data from each week were evaluated independently. Corneal ulcer surface measurements of the calves were compared among groups using the analysis of variance test. Clinical score data were compared across the 3 groups each week using the Kruskal-Wallis test, with inter-group comparisons being made with the Mann-Whitney rank sum test. The data were considered to be significantly different when the p value was ≤ 0.05 . Eyes with recurrences of corneal ulcers were not included in the statistical analyses, but the numbers of recurrences were noted and compared between the 3 groups using the Chi squared analysis.

EXAMPLE 24Field Trials With Recombinant Vaccine

This example describes conditions used in field trials with recombinant anti-M. bovis vaccine.

5 Experimental Subjects

The study population consisted of calves at the University's Sierra Foothills Field Station (SFS), located in Brown's Valley, CA. The terrain consisted of Sierra foothill range pasture. The animals were predominantly Angus or Angus-
10 Hereford crossbred calves. Calves weighed between 98 and 292.7 kg. The study population consisted of 20 intact males, 71 steers, and 2 heifers.

Study DesignPre-enrollment Examination

15 Each calf was restrained in a head catch and both eyes were examined for the presence of corneal opacities indicative of prior ocular disease. Only calves with normal corneas were selected for the study. On day 0, calves were administered a 2 ml subcutaneous injection of either saline (CTRL group), ISCOM
20 matrix adjuvant alone (ADJ group), or recombinant M. bovis cytotoxin-ISCOM matrix adjuvant (VAC group). Calves were randomly assigned to one of the three groups by use of a blocked randomization scheme. Individuals administering vaccine were unaware of the vaccine contents administered. Booster
25 vaccinations were repeated on day 21.

Post-enrollment Examination

Each calf was examined weekly for 20 weeks following the primary vaccination. During these examinations, eyes were examined for the presence of corneal opacities. All eyes with
30 corneal opacification were stained with fluorescein dye, irrigated with sterile isotonic saline solution, and assigned a corneal ulcer score (CUS) of 0, 1, 2, or 3 based on the widest diameter of the ulcer as determined by holding a ruler

next to the eye. The scoring criteria were: 0 = no ulcer; 1 = a corneal ulcer with the widest diameter $\leq$ 5 mm; 2 = a corneal ulcer with the widest diameter $>$ 5 mm; and 3 = a perforating corneal ulcer. Ulcerated eyes were photographed with a ruler held next to the eye for determination of the corneal ulcer surface area measurement (SAM) as described below. Any calves with a CUS of 2 or greater were administered florfenicol (40 mg/kg subcutaneously). To minimize iatrogenic infections, garments including plastic aprons, obstetrical sleeves, and rubber gloves were worn and were rinsed in 1% chlorhexidine solution after each animal was examined.

Bacterial Strains and Culture Conditions

Cloning competent *E. coli* strains TOP10, DH5 α , and BL21 (DE3) were propagated in LB broth or on LB agar (1.5% agar). Antibiotic selection of *E. coli* was made using ampicillin (100 ug/ml).

Expression of recombinant *M. bovis* cytotoxin

The carboxy terminus of the *M. bovis* cytotoxin gene was amplified with primers SNP down (5'-AAT GAC GAT ATC TTT GTT GGT CAA GGT AAA-3') and LNP2 (5'-TAG TAA ATT AAA TNA CTW AAC ACT-3'). PCR amplifications were performed with Taq polymerase using 30 cycles of 30 seconds each at 95°C, 1 minute at 55°C, and 45 seconds at 72°C, followed by a 10 mm incubation at 72°C. The PCR product was cloned into pCR2.1-TOPO with a TOPO TA cloning kit and the resulting recombinant plasmid was digested with EcoRI. The appropriate fragment was gel purified (QIAquick gel extraction kit) and cloned into blunted ended, SmaI digested pT7-7 to yield an expression construct that would direct synthesis of the cytotoxin gene from amino acids 590 through 927. Recombinant plasmids were transformed into *E. coli* DH5 α , and subsequently purified prior to transformation into *E. coli* strain BL21 (DE3) for expression. Cells were grown to an OD₆₀₀ of 0.8, and expression was induced by adding isopropylthio- β -

galactoside to 1 mM. Induction was continued for approximately 9 hours and cells were harvested by centrifugation. The expressed proteins formed inclusion bodies that were purified. Purified inclusion bodies were then solubilized in a buffer containing 4 M urea, 0.25% Triton X-100, 5 mM Tris-HCl (HCl pH 7.5) and 1 mM EDTA and chromatographed (Mono Q column; Amersham Pharmacia). Peak fractions were identified by SDS-PAGE, pooled and protein was quantitated with a BCA kit using a bovine serum albumin standard. The protein solution was sterile filtered and dialyzed against PBS to remove urea and stored at -20°C. Following dialysis the purified protein precipitated. PBS was added to the dialysate to make the final protein concentration 0.5 mg/ml.

Vaccine Preparation

ISCOM matrices were prepared as described in Current Protocols in Immunology, Caligan, John E., Ed. New York, Greene Publication Associates and Wiley-InterScience, except that no protein was incorporated into the ISCOMs. Instead, the purified recombinant protein was mixed separately with ISCOMs following ISCOM matrix formation. A lipid mixture containing 50 mg/ml each of phosphatidylcholine and cholesterol was made by dissolving 100 mg of each in 1 ml of chloroform and combining both solutions. This lipid mixture was then added to a 20% solution (w/v) of decanoyl-N-methylglucamide (Mega 10) made by dissolving 2 g Mega 10 in 10 ml distilled water. The resulting solution was added to phosphate buffered saline (PBS; pH 7.4) (0.1 cc lipid mixture per 1 ml PBS) and the chloroform was removed by heating the solution to 45°C under vacuum until the solution cleared. Quil A was then added to a final concentration of 1 mg/ml. The solution was transferred to dialysis tubing (2000 MW cut-off) and dialyzed extensively for 3 days in 50 mM Tris-HCl, 0.001% thimerosal, pH 7.5 at room temperature. Thimerosal was removed by dialysis against PBS (pH 7.4) for 24-36 hours at 4°C.

Following dialysis the solution was sterile filtered (0.45 or 0.2 μ M pore size) and stored at -20°C until use. The success of ISCOM matrix formation was confirmed by electron microscopy performed at the Department of Medical Pathology, School of 5 Medicine, UC Davis.

For vaccine production, 145 ml of ISCOM dialysate was made to 180 ml with PBS (pH 7.4). For the adjuvant control vaccine (ADJ), the ISCOM matrix preparation was mixed with PBS (pH 7.4) 1:1. For the recombinant protein vaccine (VAC), the ISCOM 10 matrix preparation was mixed 1:1 with the recombinant protein solution. The doses were 2 ml of adjuvant (ADJ) and vaccine (VAC) for both primary and booster vaccinations. The control (CTRL) vaccine was a 2 ml dose of 0.9% saline.

SAM Determination

15 Color slides were projected onto a standardized ruler template to equalize magnifications for subsequent tracing of corneal ulcers. Three free-hand drawings of each ulcer were made on paper. Tracing images were digitized and corneal ulcer surface areas were determined with NIH-image (version 1.62). The 20 mean ulcer size was determined from the three tracings. The limit of detection was 0.008 cm², the area that corresponded to a 1 mm diameter circle. Surface areas less than this were excluded from analysis. Corneal ulcers resulting from scratches were excluded from the analysis. Ulcers resulting from foxtails 25 or suspect foxtails were not included in the analysis on the first week the ulcer was first noted. If eyes remained ulcerated on subsequent weeks, SAM were entered into the analysis.

Data Analysis

The cumulative number of calves with corneal ulcer SAM > 30 0.008 was determined each week for the 20 week trial period. For this analysis, calves were only counted one time. Chi-square analysis was used to compare differences in the cumulative numbers of calves with corneal ulcers during each week.

Commercial software (Statview and SPSS were used for statistical analyses). Differences were considered significant at $P < 0.05$.

WHAT IS CLAIMED

1. A purified or partially purified *Moraxella bovis* cytotoxin, produced by a gene comprising a DNA sequence SEQ ID NO: 1 deposited at GENBANK database under Accession number AF205359.

2. The cytotoxin of claim 1 obtained from culture supernatants of an isolated cytolytic strain of *Moraxella bovis*.

3. The cytotoxin of claim 2 wherein said culture supernatants were purified by centrifugation, filtration, concentration and diafiltration.

4. The cytotoxin of claim 3 wherein said cytotoxin is present in the diafiltered retentate.

5. The cytotoxin of claim 4 wherein said diafiltered retentate comprises a cytotoxin-enriched fraction.

6. The cytotoxin of claim 5, biologically active as hemolysin, leukotoxin or corneotoxin.

7. The cytotoxin of claim 6 of molecular weight of about 95 and 98 kDa.

8. A DNA sequence depicted by SEQ ID NO: 1 or a fragment thereof, encoding an amino acid sequence depicted by SEQ ID NO: 2 or a fragment thereof.

9. The DNA sequence of claim 8 wherein a fragment encodes a protein depicted by SEQ ID NO: 6 or a protein depicted by SEQ ID NO: 13.

10. The DNA sequence of claim 9 encoding the protein depicted by SEQ ID NO: 6.

11. The DNA sequence of claim 9 encoding the protein depicted by SEQ ID NO: 13.

12. An amino acid sequence depicted by SEQ ID NO: 2 or a fragment thereof.

13. The sequence of claim 12 wherein the fragment is depicted by SEQ ID NO: 6.

14. The sequence of claim 12 wherein the fragment is depicted by SEQ ID NO: 13.

15. A recombinant protein comprising an amino acid sequence SEQ ID NO: 2 or a fragment thereof encoded by the DNA sequence depicted by SEQ ID NO: 1, or a fragment thereof.

16. A method for prophylaxis of bovine keratoconjunctivitis comprising a step of vaccinating cattle or calves with a vaccine comprising *M. bovis* cytotoxin.

17. The method of claim 16 wherein the vaccine is based on a native cytotoxin.

18. The method of claim 16 wherein the vaccine is based on a recombinantly derived cytotoxin.

19. A method of diagnosing *M. bovis* in a carrier cattle by reacting the cattle serum with antibodies raised against *M. bovis* cytotoxin comprising an amino acid sequence depicted by SEQ ID NO: 2.

20. The method of claim 19 wherein the *M. bovis* cytotoxin is captured by antigen capture ELISA and identified by reaction with antibodies.

5 21. The method of claim 20 wherein the antibodies are monoclonal.

22. The method of claim 20 wherein the antibodies are polyclonal.

10

23. A vaccine comprising a recombinant *Moraxella bovis* cytotoxin comprising an amino acid sequence depicted by SEQ ID NO: 2 or a fragment thereof.

15 24. The vaccine of claim 23 wherein the fragment is depicted by SEQ ID NO: 6.

25. The vaccine of claim 23 wherein the fragment is depicted by SEQ ID NO: 13.

20

26. A nucleotide sequence depicted by SEQ ID NO: 30, SEQ ID NO: 31 or SEQ ID NO: 36, encoding an amino acid sequence depicted by SEQ ID NO: 18, SEQ ID NO: 32 or SEQ ID NO: 37.

25 27. The sequence of claim 26 depicted by SEQ ID NO: 30.

28. The sequence of claim 26 depicted by SEQ ID NO: 31.

29. The sequence of claim 26 depicted by SEQ ID NO: 36.

30

30. A peptide comprising an amino acid sequence depicted by SEQ ID NO: 18, SEQ ID NO: 32 and SEQ ID NO: 37.

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31. The peptide of claim 30 depicted by SEQ ID NO: 18.
32. The peptide of claim 30 depicted by SEQ ID NO: 32.
- 5 33. The peptide of claim 30 depicted by SEQ ID NO: 38.

FIG. 1

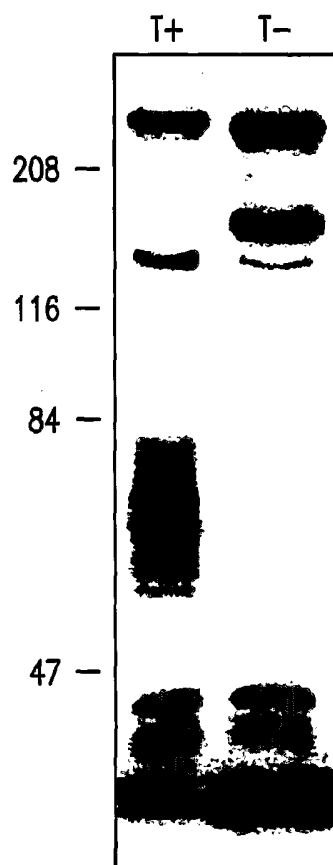
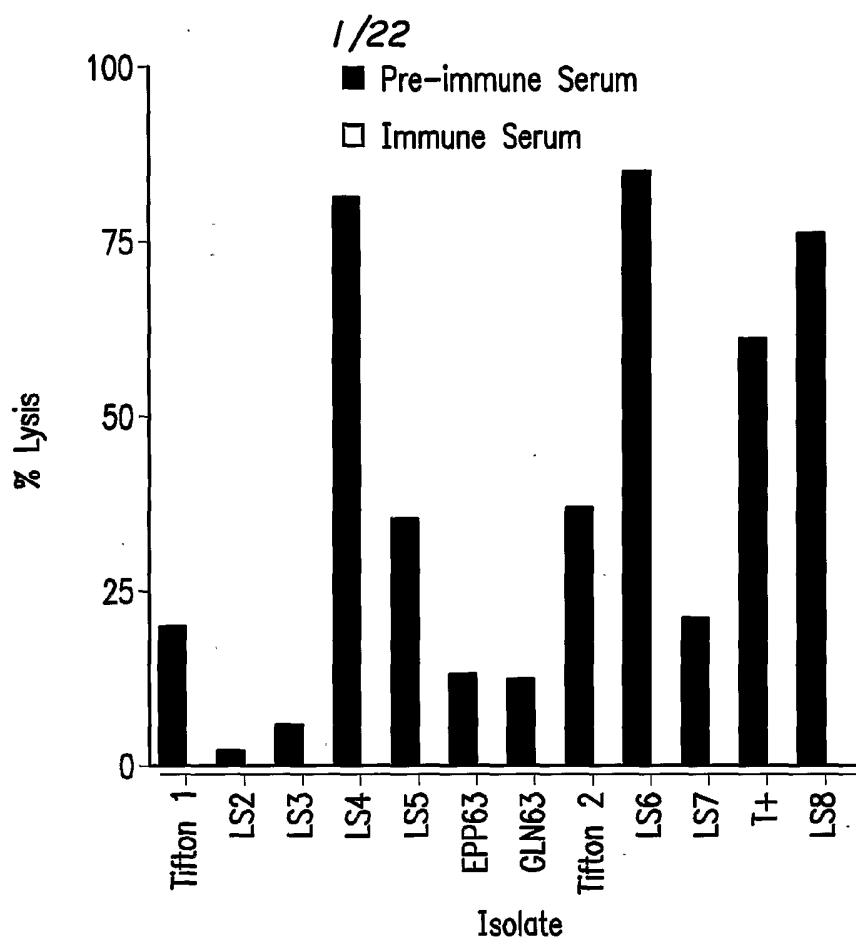


FIG. 2

FIG. 3-1

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1	ATGTCCAATATAAATGTAATTAATCTAATATTCAAGCAGGCTTGAATTCAACAAAGTCT	60
1	M S N I N V I K S N I Q A G L N S T K S	20
61	GGATTAAAAAATCTTTACTTGGCTATTCCCAAAGATTATGATCCGCAAAAAGGTGGGACT	120
21	G L K N L Y L A I P K D Y D P Q K G G T	40
121	TTAAATGATTTTATTAAAGCTGCTGATGAATTAGGTATTGCTCGTTTAGCAGAAGAGCCT	180
41	L N D F E K A A D E L G I A R L A E E P	60
181	AATCACACTGAAACAGCAAAAAAATCTGTTGACACAGTAAATCAGTTTCTCTCTCACA	240
61	N H T E T A K K S V D T V N Q F L S L T	80
241	CAAACTGGTATTGCTATTTCTGCAACAAAATTAGAAAAGTTCTTACAAAAACATTCTACC	300
81	Q T G I A I S A T K L E K F L Q K H S T	100
301	AATAAGTTAGCCAAAGGGTTAGACAGTGTAGAAAATATTGATCGTAAATTAGGTAAAGCA	360
101	N K L A K G L D S V E N I D R K L G K A	120
361	AGTAATGTATTATCAACATTAAGCTCTTTTTTGGGCACTGCATTAGCGGGTATAGAACTT	420
121	S N V L S T L S S F L G T A L A G I E L	140
421	GATTCTTTAATCAAAAAAGGTGATGCTGCACCTGATGCTTTGGCTAAAGCTAGTATTGAC	480
141	D S L I K K G D A A P D A L A K A S I D	160
481	TTGATTAATGAGATAATTGGTAATCTATCTCAGAGTACTCAAACGATTGAAGCATTTTCT	540
161	L I N E I I G N L S Q S T Q T I E A F S	180
541	TCACAGTTAGCAAAGTTAGGTTCTACTATATCGCAGGCTAAAGGCTTCTCTAATATAGGA	600
181	S Q L A K L G S T I S Q A K G F S N I G	200
601	AACAAGTTGCAAACTTAAATTTTTCTAAAACAAATCTTGGTTTGAAATAATTACTGGT	660
201	N K L Q N L N S S K T N L G L E I I T G	220
661	TTGCTATCAGGCATTTCTGCAGGCTTTGCTTTAGCGGATAAAAAATGCATCGACTGGCAAA	720
221	L L S G I S A G F A L A D K N A S T G K	240
721	AAAGTTGCTGCAGGTTTTGAATTAAGCAATCAAGTTATTGGTAATGTAACAAAAGCAATT	780
241	K V A A G F E L S N Q V I G N V T K A I	260
781	TCTTCATATGTTTTAGCACAACGTGTTGCTGCTGGTCTATCAACTACTGGTGCTGTTGCT	840
261	S S Y V L A Q R V A A G L S T T G A V A	280
841	GCTTTAATTACTTCATCGATTATGTTGGCAATTAGTCCTTTGGCATTATGAATGCAGCA	900
281	A L I T S S I M L A I S P L A F M N A A	300
901	GATAAATTCAATCATGCTAATGCTCTTGATGAGTTTGCAAAACAATTCCGAAAATTTGGC	960
301	D K F N H A N A L D E F A K Q F R K F G	320

FIG. 3-2

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961 TATGATGGGGATCATTTATTGGCTGAATATCAGCGTGGTGTGGGTACTATTGAAGCTTCA 1020
321 Y D G D H L L A E Y Q R G V G T I E A S 340

1021 TTA ACTACAATTAGTACGGCATTAGGTGCAGTTTCTGCTGGTGTTCGCTGCTGCTGTA 1080
341 L T T I S T A L G A V S A G V S A A A V 360

1081 GGATCTGCTGTTGGTGCACCGATTGCACTATTAGTTGCAGGTGTTACAGGATTGATCTCT 1140
361 G S A V G A P I A L L V A G V T G L I S 380

1141 GGAATTTTAGAAGCGTCTAAACAGGCAATGTTTGAAAGTGTGCTAACCGTTTACAAGGT 1200
381 G I L E A S K Q A M F E S V A N R L Q G 400

1201 AAAATTTTAGAGTGGGAAAAGCAAAATGGCGGTGAGAACTATTTTGATAAAGGCTATGAT 1260
401 K I L E W E K Q N G G Q N Y F D K G Y D 420

1261 TCTCGTTATGCTGCTTATTTAGCTAATAACTTAAAAATTTTGTCTGAGCTAAATAAAGAG 1320
421 S R Y A A Y L A N N L K F L S E L N K E 440

1321 TTGGAAGCTGAACGTGTTATTGCAATCACCCAACAACGTTGGGATAATAATATTGGTGAG 1380
441 L E A E R V I A I T Q Q R W D N N I G E 460

1381 TTAGCAGGTATTACCAAATTGGGTGAACGCATTAAGAGCGGAAAAGCTTATGCAGATGCT 1440
461 L A G I T K L G E R I K S G K A Y A D A 480

1441 TTTGAAGATGGCAAGAAAGTTGAAGCTGGTTCCAATATTACTTTGGATGCTAAAAGTGGT 1500
481 F E D G K K V E A G S N I T L D A K T G 500

1501 ATCATAGACATTAGTAATTCAAATGGGAAAAAACGCAAGCGTTGCATTTCACTTCGCCT 1560
501 I I D I S N S N G K K T Q A L H F T S P 520

1561 TTGTTAACAGCAGGAAGTGAATCACGTGAACGTTTAACTAATGGTAAATACTCTTATATT 1620
521 L L T A G T E S R E R L T N G K Y S Y I 540

1621 AATAAGTTAAATTCGGACGTGTAAAAAACTGGCAAGTTACAGATGGAGAGGCTAGTTCT 1680
541 N K L K F G R V K N W Q V T D G E A S S 560

1681 AAATTAGATTTCTCTAAAGTTATTACGCGTGTAGCCGAGACAGAAGGCACAGACGAGATT 1740
561 K L D F S K V I Q R V A E T E G T D E I 580

1741 GGTCTAATAGTAAATGCAAAAGCTGGCAATGACGATATCTTTGTTGGTCAAGGTAAAATG 1800
581 G L I V N A K A G N D D I F V G Q G K M 600

1801 AATATTGATGGTGGAGATGGACACGATCGTGTCTTCTATAGTAAAGACGGAGGATTTGGT 1860
601 N I D G G D G H D R V F Y S K D G G F G 620

1861 AATATTACTGTAGATGGTACGAGTGCAACAGAAGCAGGCAGTTATACAGTTAATCGTAAG 1920
621 N I T V D G T S A T E A G S Y T V N R K 640

FIG. 3-3

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1921 GTTGCTCGAGGTGATATCTACCATGAAGTTGTGAAGCGTCAAGAAACCAAGGTGGGTAAA 1980
641 V A R G D I Y H E V V K R Q E T K V G K 660

1981 CGTACTGAAACTATCCAGTATCGTGATTATGAATTAAGAAAAGTTGGGTATGGTTATCAG 2040
661 R T E T I Q Y R D Y E L R K V G Y G Y Q 680

2041 TCTACCGATAATTTGAAATCAGTAGAAGAAGTAATTGGTTCTCAATTTAATGATGTATTC 2100
681 S T D N L K S V E E V I G S Q F N D V F 700

2101 AAAGGTTCTAAATTCAACGACATATTCATAGTGGTGAAGGTGATGATTTACTCGATGGT 2160
701 K G S K F N D I F H S G E G D D L L D G 720

2161 GGTGCTGGTGACGACCGCTTGTTTGGTGGTAAAGGCAACGATCGACTTTCTGGAGATGAA 2220
721 G A G D D R L F G G K G N D R L S G D E 740

2221 GGCGATGATTTACTCGATGGCGGTTCTGGTGATGATGTATTAATGGTGGTGCTGGTAAT 2280
741 G D D L L D G G S G D D V L N G G A G N 760

2281 GATGTCTATATCTTTTCGAAAGGTGATGGTAATGATACTTTGTACGATGGCACGGGCAAT 2340
761 D V Y I F R K G D G N D T L Y D G T G N 780

2341 GATAAATTAGCATTTGCAGATGCAAATATATCTGATATTATGATTGAACGTACCAAAGAG 2400
781 D K L A F A D A N I S D I M I E R T K E 800

2401 GGTATTATAGTTAAACGAAATGATCATTCAAGGTAGTATTAACATACCAAGATGGTACATA 2460
801 G I I V K R N D H S G S I N I P R W Y I 820

2461 ACATCAAATTTACAAAATTATCAAAGTAATAAAACAGATCATAAAATTGAGCAACTAATT 2520
821 T S N L Q N Y Q S N K T D H K I E Q L I 840

2521 GGTAAAGATGGTAGTTATATCACTTCCGATCAAATTGATAAAATTTTGCAAGATAAGAAA 2580
841 G K D G S Y I T S D Q I D K I L Q D K K 860

2581 GATGGTACAGTAATTACATCTCAAGAATTGAAAAAGCTTGCTGATGAGAATAAGAGCCAA 2640
861 D G T V I T S Q E L K K L A D E N K S Q 880

2641 AAATTATCTGCTTCGGACATTGCAAGTAGCTTAAATAAGCTAGTTGGGTCAATGGCACTA 2700
881 K L S A S D I A S S L N K L V G S M A L 900

2701 TTTGGTACAGCAAATAGTGTGAGTTCTAACGCCTTACAGCCAATTACACAACCAACTCAA 2760
901 F G T A N S V S S N A L Q P I T Q P T Q 920

2761 GGAATTTTGGCTCCAAGTGTTTAG SEQ ID NO. 1 2784
921 G I L A P S V * SEQ ID NO. 2 928

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MbxA	~MGTRITLIS	~MSNIN	VIKSNIQAGL	NSTKSLXNL	YELATK..DY	DPQKG	38
Lkta	~MSKRTLSSEK	NGEKNLTAT	RSQLEKAG..	SSEKTIGAKKI	FLYTFQNIQY	QTEGG	59
ApxIIA	~MPTLAAQIK	SSSQGKNG	KNKINQAGTT	LKNGIQTG.	FLYTFQG..	DSGGG	62
H1YA		STLQSAKQSA	ANKESAGQS	TADAKKAE	TULFKDK.	..GGG	61
MbxA	GTNDFFIKAA	DEEGTARLAE	EPNLTETARK	SVDTVNQFLS	TKERFQKH	SINKL	103
Lkta	NGJODLVKVA	EEEGTEVORE	ERNNTATAQT	SLGTITQTAIG	POIDKLOK.	..LKA	121
ApxIIA	NGVODLVKAA	NDLGTEVMRE	ERSNLDIAKI	SFDITQKILG	POIDNEKK.	..NPKI	125
H1YA	SSNDIVRTA	DEEGTEVOYD	EKNGLATKO	VEGTAEKLLIG	POIDKLEOKY	Q..KA	124
MbxA	AKGLDSVEN	IDRKLEKASN	VLSTLSSFLG	TALAGIELDS	LIKK....GD	AAPDAEKAS	163
Lkta	GOALLGSAES	IVGNANKAKT	VLSGIOSIIG	SVAGMDLDE	ALQN.....N	SNCHAEAKAG	180
ApxIIA	GNTELGSASS	TSQNIQKANT	VEGGGSIIG	SVSAVNINE	LQN.....KD	PNQLEAKAG	185
H1YA	GNKLGSAEN	IGDNEKAGS	VLSTFONFLG	TALSSMKIDE	LIKKQKSGN	VSSSEAKAS	189
MbxA	ELITGNLSQIT	QTHLEAFSSQL	AKLGSTISQA	KGFSNIENKL	QNLNFSKTN	LGLEETISL	227
Lkta	SELTENTANSY	KRLDEHGEQI	SQFGSKIQNT	KGIGTIGDK	KNIGGEDKAG	LGIDVTSGLL	245
ApxIIA	ELVGNFASSV	QTVDAFAEQI	SKLGSHLQNV	KGIGGHSNKL	QNLPLIGKAS	LGIDVTSGLL	250
H1YA	QEV.DTAAAL	NNVNSFSQOL	NKEGSLVLSNT	KHENGIVENKL	QNLPNLDNIG	AGLDTVSGIL	253
MbxA	GFALADKNAS	TGKKVAAGFE	LSNOYVGNVT	KALISSVLEAQ	RVAGLSITG	AMAAETISSI	292
Lkta	ALVLADKNAS	TAKKVGAGFE	CANQVGNIT	KAVSSYLEAQ	RVAGLSSIG	PVAAETASTV	310
ApxIIA	GLTLADKEAS	TEKKAAGVE	FANGTIGNVT	KAVSSYLEAQ	RVASGLSSIG	PVAAETASTV	315
H1YA	SFLSNADAD	TGTKAAAGVE	LTTKVLGNVG	KGISQVHIAQ	RVAGGLSTSA	AAAGLEASVV	318
MbxA	PLAFMAADK	ENHARALDEF	AKOERKFGYD	GDHLEAEYQR	GVGTIFASL	TTSTALEGAMS	357
Lkta	PLAFAGIADK	ENFAKSLESY	AERPKLGYD	GDHLEAEYQR	GVGTIFASV	ATNTALEAIA	375
ApxIIA	PSEENVADK	EKOADLIKSY	SERFQKLGVD	GDRELADFHR	ETGTTDASV	TTNTALEAII	380
H1YA	PSEISTADK	EKPAKITEY	SOREKLGVD	GDSELAAFHK	ETGALDASL	RISTVLEASV	383

FIG. 4-1

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FIG. 4-2

MbxA	AAVGSANVAP	IAELVAGVTG	ELSGLEASK	QAMFESVANR	LOGKILEWEK	QNGGQNYFDK	GYDSR	422
Lkta	AAAGSVIASP	IAELVSGITG	VESITLQYSK	QAMFEHVANK	ITHNKIVWEK	NNHGKNYFEN	GYDAR	440
ApXIIA	ASAGSLVAP	VALLVAGVTG	ELITLLEYSK	QAMFEHVANK	VHDIRIVWEK	.KHINKNYFEQ	GYDSR	444
H1YA	AAITTSVIGAP	VSALVGAIVG	ELSGLEASK	QAMFEHVASK	MADVLAEWEK	.KHGKNYFEN	GYDAR	447
peak 23								
MbxA	YAAYLANNLK	ELSELNKELE	AERVIALTQQ	RWDNNIGELA	GITIKIGERIK	SGKAYADAFE	DGRKV	487
Lkta	YLANLQDNMK	ELLNENKELO	AERVIALTQQ	QWNNNIGDLA	GLSRLEGEKVL	SGKAYADAFE	EGKHI	505
ApXIIA	HLADLQDNMK	ELLNENKELO	AERVIALTQQ	RWDNQIGDLA	ALSRRTDKIS	SGKAYADAFE	EGQHQ	509
H1YA	HAALFEDNFK	ILSQYNKEYS	VERSVLETTQQ	HMDITLIGELA	GVTRNGDKIT	SGKSMIDBYME	EGKRL	512
*								
MbxA	EAG...SNL	TEDAKIGITD	TSNSNGKKTQ	ALHETSPLT	AGTESRRLT	NGKYSYTNKL	KFGRV	548
Lkta	KAD...KLV	QLDSANGITD	VSNSGKAKTQ	HLIFRTPLT	PGTHRRRVQ	TGKLEYATKL	NINRV	566
ApXIIA	SYD...SSV	QLDNKNGTIN	LSNTR.KTQ	SVLEERTPLT	PGEEFNRRRIQ	EGKNSYLTKE	HIQRV	569
H1YA	EKKPDEFQKQ	VFDPLKGNID	LSDS..KSST	LKRVITPLLT	PGEEIRRRRQ	SGKYEYKTELE	LVKGV	575
*								
MbxA	KNMQVTD..GE	ASSKLDDESKM	IQRVA....ET	EQ....TDEI	GLIIVNAKAGN	DDIEFVGQCKM	NTDGG	605
Lkta	BSMKITD..GA	ASSTFDLTNV	VORIGTELDN	AGNVTKKET	KETIAKLEGED	DNVAVGSGTIT	EIDEG	630
ApXIIA	DSWTVTD..GD	ASSSVDETNM	VORIAVKFDD	AGNIIESKDT	KILDANIAGN	DNVAVGSSIT	VIDGG	633
H1YA	EKMVTKGVQD	KGSVVDYSNL	IQHASV....	.GN.NQYREI	RUESHLEDGD	DKMFLSAGSA	NIIYAG	634
*								
MbxA	DGHDRIYISK	DGGFENITVD	GTSATEAGSY	FWNRKV..ARG	DIYHEVVKRQ	ELKMGKRTIEI	TOYRD	669
Lkta	EGYDRVHTSR	.GNVGATCTD	ATKETEQGSY	FWNRKV..ETG	KALHEVITSH	TALVGNREK	DEYR.	692
ApXIIA	DGHDRIYHSR	.GEYCALVID	ATAIEFKGSY	SMKRYV..GDS	KALHETIAH	QTNVGNREK	LEYR.	695
H1YA	KGHDVVMYDK	T.DTCYLTCTD	GTATEAGNY	TVTRVVLGCV	KVLOEVKKEQ	EVSAGKRTIEK	TOYRS	698

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MbxA Lkta ApxIIA H1YA	YELRKV.GYG	YQSTONL.KSV	PEVIGSQEND	VEFGSKKENDI	FHSGEQDILL	DGGAGDDEL	GGKGN	733
	HSNNQH.HAG	YVTKDTL.KAV	EEETIGTSHND	TEFGSKKENDI	FNGGQGVDTI	DGNDGNDRLE	GGKGD	756
	REDDRF.HTG	YVTDLSL.KSV	EEETIGSQEND	TEFGSKQDDEV	FHGGNGVDIT	DGNDGDDHLE	GGAGD	759
	VEFTHINGKN	LEETDNLYSV	EEETIGITRAD	KEFGSKHADI	FHGADGDDHI	EGNDGNDRLY	GGKGN	763
peak 26								
MbxA Lkta ApxIIA H1YA	DRESDEGD							742
	DILDEGND							765
	DVIDGGNGN							768
	DILSGNGGD	QLYGGDGNDK	LIGGAGNNYL	NGGDGDELDQ	VQNSLAKNV	LSGGKGNDKL	YGSEG	828
MbxA Lkta ApxIIA H1YA	PLEGGSGD	DMVNGGAGND	VYIFRKGDN	DTILYDGTG.N	DKAFADANL	SDIMIERKE	GITEVK	805
	BFTGGKGN	DLLHGGKGD	TEVERKGBEN	DTITDSG.N	DKLSFSDSNL	KDLTEKVKH	NEVI	827
	NFVGGTGN	DTISGGKDND	LYVHKTGDN	DSITDSG.Q	DKAFSDVNL	KDLTEKKVDS	SEET	830
	ADLDGEGN	DLLKGGYGND	LYRYLSGYGH	HLDDDDGGKD	DKESLADIDF	RDAERREGN	DLLMY	893
MbxA Lkta ApxIIA H1YA	RND.....	HSGSINTPRW	Y.....ITSNL	QNYOSNKTIDH	KTEQLIGKDG	SYTTSDDIDK	FLQDK	859
	TNS.....	KKERVITQNN	FERADFAKEV	PNYKATK.DE	KTEELIGQNG	ERTSKQVDD	LI..A	882
	INQ.....	KGEKVRFGNW	FLDEDLASTV	ANYKATN.DR	KTEELIGRGG	ERTTSEQVDK	LI..K	885
	KAEGNVLSIG	HKNGITTFKNW	FEKE...SGDI	SNFQ.....	LEQIFDKDG	RVLTPDSLKK	ALEYQ	949
MbxA Lkta ApxIIA H1YA	RDGTVITSOE	LEKKLADENKS	QKLSASDITAS	SENKLVGSM	LEGTANSVSS	NALQPIITQPT	QGIEA	924
	KNGKLEQDE	ESKVVNDVIEL	LK.HSKNVITN	SIDKLSSVS	AR.FSSNDSR	NV...VA.PT	SMED	941
	ERNQISAE	ESKVVNDYNT	SK.DRONVSN	SEAKLSSVG	SE.FSSSDFR	NNGTIV.PS	S.ID	945
	QSNK.ASYV	YGNDALAVGS	.QGNLNPLIN	EYSKILISAAE	NFDVKEERAA	ASLQLSG.N	ASDFS	1011
MbxA Lkta ApxIIA H1YA	PSV.....							927
	QSLSLQFAR	AA	AA	AA	AA	AA	AA	953
	VS.NNQLAR	AA	AA	AA	AA	AA	AA	956
	YGRNSITLTA	SA	SA	SA	SA	SA	SA	1023

FIG. 4-3

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FIG. 5

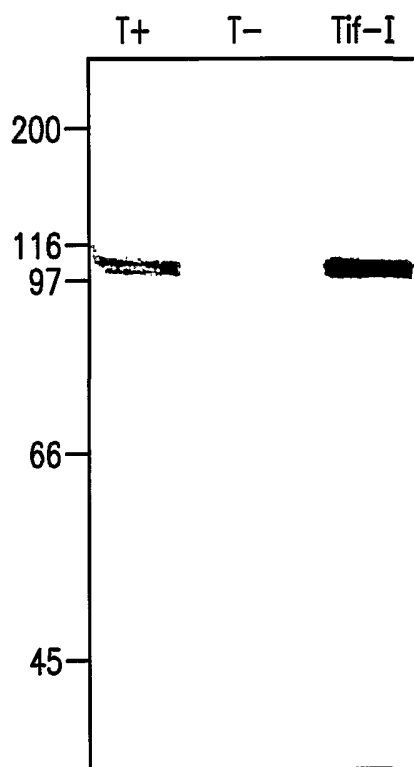
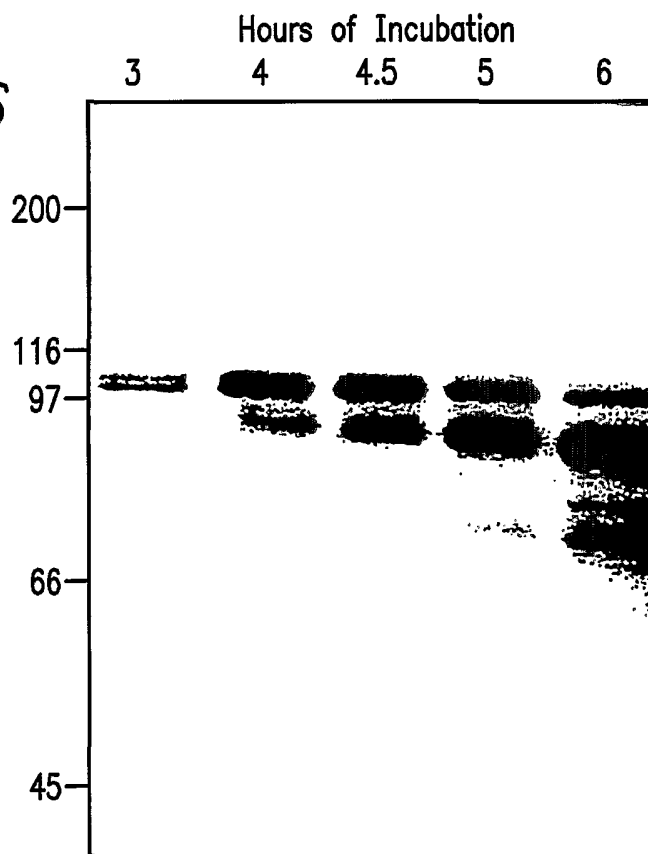


FIG. 6



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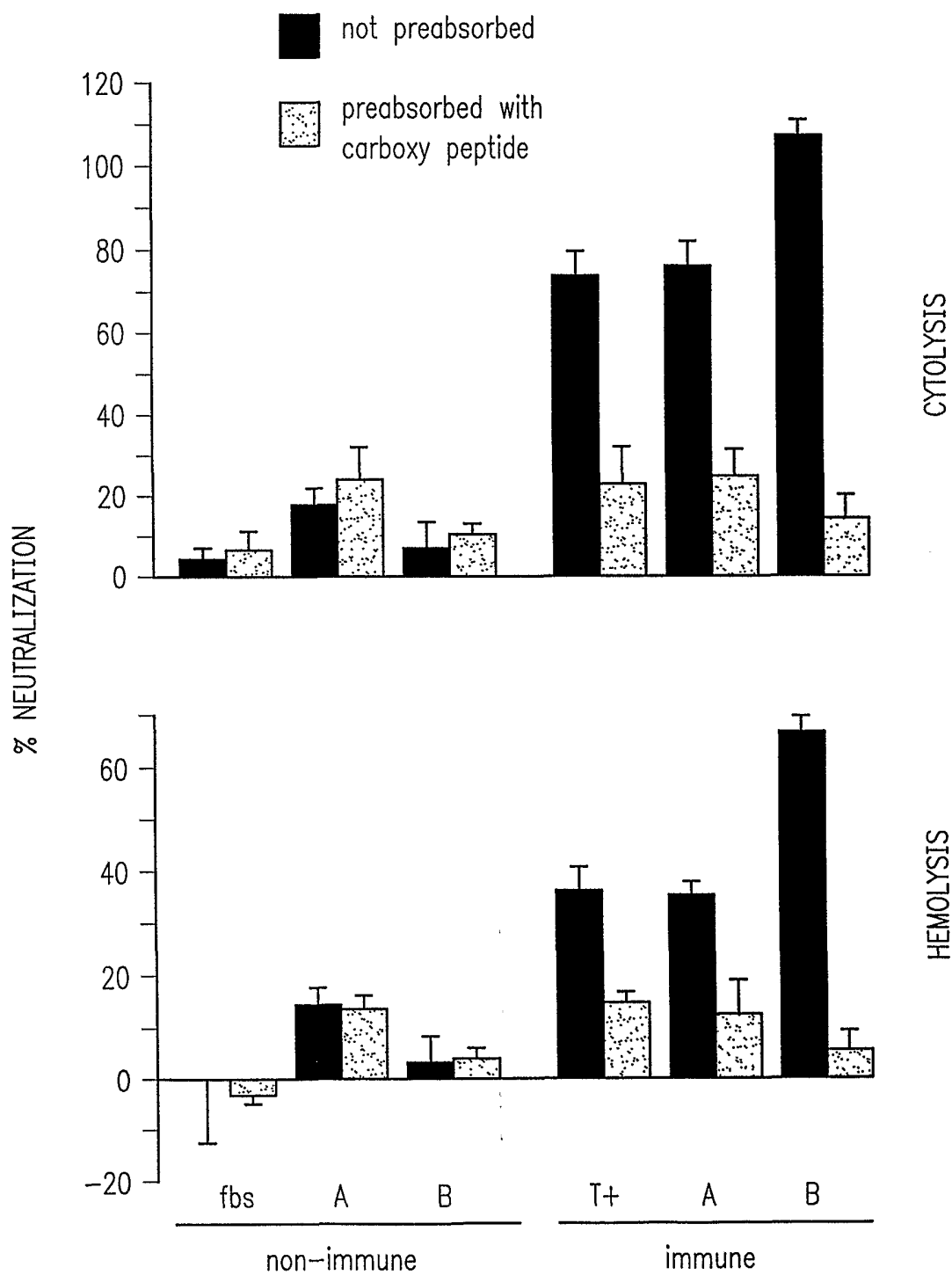


FIG. 7

FIG. 8-1

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1 ATGGGTGGTGATACTTCTTTAATTAGACTTAATTTACAAACCCTTAATAGTAATTTAGTT 60
1 M G G D T S L I R N L Q T L N S N L V 20

61 ATGATAGATTATGCTCAACAACCTGCTCTATCTGCTCTGGTTATCCTTGCCAAATACTAT 120
21 M I D Y A Q Q P A L S A L V I L A K Y Y 40

121 GGTATTTCTGCAAGTCCAGCAGACATTATGCATCAGTTTTCTGATAATACAAAAGGAGAC 180
41 G I S A S P A D I M H Q F S D N T K G D 60

181 CTGAATGAAATTGAATGGATGTTGGCAGCAAAGAAATTAGAATTAAAGGTAAAGATTATA 240
61 L N E I E W M L A A K K L E L K V K I I 80

241 AAACAGCCTTTAACTCGATTGTCAATGATAACACTTCCTGCTTTGGTGTGGTGTGATAAT 300
81 K Q P L T R L S M I T L P A L V W C D N 100

301 AAGCCCGATTTAGATCAAAATTTAACTCTCATTTTATACTAACTAAAATTGATGGGGTG 360
101 K P D L D Q N L N S H F I L T K I D G V 120

361 GGATCTGCTGCAAAATATCTCATCTACGATTTGATTGAGAATCGTCCCATAAATATTAGAT 420
121 G S A A K Y L I Y D L I E N R P I I L D 140

421 GCAAGTGAGTTTTCTGAAAGATATTCTGGTAAGTTAATGCTAGTAACTTCCCGTGCGTCA 480
141 A S E F S E R Y S G K L M L V T S R A S 160

481 ATATTGGGTTTCATTGGCTAAATTTGATTTTACTTGGTTTATTCCTGCGGTAATCAAATAT 540
161 I L G S L A K F D F T W F I P A V I K Y 180

541 CGTTATATTTTTTTTGAAGTCATCGTTATTTTCAGTGGTGCTACAGATTTTTGCTCTGATT 600
181 R Y I F F E V I V I S V V L Q I F A L I 200

601 ACGCCATTGTTTTTTTCAGGTTGTGATGGATAAGGTATTGGTGCATCGTGGTTTTTCTACT 660
201 T P L F F Q V V M D K V L V H R G F S T 220

661 CTGGATGTGGTAGCGATTGCCTTGTTGGTAGTAAGTTTATTTGAAGTCATTTTAAGTGGT 720
221 L D V V A I A L L V V S L F E V I L S G 240

721 CTACGCACTTATATTTTTGCTCATACAACCTCTCGAATTGATGTAGAGCTAGGAGCACGA 780
241 L R T Y I F A H T T S R I D V E L G A R 260

781 TTATTTTCGTCATCTATTAGCTCTACCGCTTGCTTATTTTGAGAGTAGAAGAGTAGGCGAT 840
261 L F R H L L A L P L A Y F E S R R V G D 280

841 ACAGTTGCACGTATACGTGAATTGGAACATATCCGCAATTTCTTAACTGGTCAAGCTCTC 900
281 T V A R I R E L E H I R N F L T G Q A L 300

901 ACTTCAGTTTTAGATTTGGTGTCTTTTATATTCTTGTTTGTAATGTGGTATTACAGC 960
301 T S V L D L V F S F I F L F V M W Y Y S 320

FIG. 8-2

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961 CCTACTTTAACTGGTAGTTTTGGCATCATTACCAATATATGCGTTTTGGTCTGCCTTT 1020
321 P T L T L V V L A S L P I Y A F W S A F 340

1021 ATTAGCCCAATTTTACGCACTCGACTAAATGATCAATTTGCACGCAATGCAGATAATCAA 1080
341 I S P I L R T R L N D Q F A R N A D N Q 360

1081 TCTTTTTTAGTGAAAAGTATTACTGCGGTTGGTACGGTAAAAGCAATGGCAGTTGAACCT 1140
361 S F L V E S I T A V G T V K A M A V E P 380

1141 CAAATGACCCGTCGCTGGGATAATCAATTAGCAGCTTATGTGGTTTCTAGTTTTCGGGTA 1200
381 Q M T R R W D N Q L A A Y V V S S F R V 400

1201 GCTAAGTTGGCAATGGTTGGGCAGCAAGGAGTACAACCTATTCAAAGATGGTTATTGTG 1260
401 A K L A M V G Q Q G V Q L I Q K M V I V 420

1261 GCAACTCTATGGATTGGTGCAAAATTGGTAATTGAAGGCAAGCTATCGGTAGGTCAATTA 1320
421 A T L W I G A K L V I E G K L S V G Q L 440

1321 ATAGCATTTAATATGCTGGCAGGTGAGGTGGCCGCTCCTGTTATCCGCCTGGCACAGCTA 1380
441 I A F N M L A G Q A A P V I R L A Q L 460

1381 TGGCAAGATTTTCAGCAAGTAGGTATTTGAGTGGCGAGATTGGGTGATATTTAAATACT 1440
461 W Q D F Q Q V G I S V A R L G D I L N T 480

1441 CCAACTGAGCATTCTACATCTCGCTTAACCTTTACCTGATATTAAGGGTGATATTACATTT 1500
481 P T E H S T S R L T L P D I K G D I T F 500

1501 GAAAATGTTGATTTTCGCTACAAAATAGATGGGCATTTAATATTACAGAATTTAAATTTA 1560
501 E N V D F R Y K I D G H L I L Q N L N L 520

1561 CAGATTAACGCTGGAGAGATACTAGGTATCGTAGGACGCTCTGGTTCAGGTAAATCAACA 1620
521 Q I N A G E I L G I V G R S G S G K S T 540

1621 TTGACAAAATTAGTACAGCGTTTATATGTACCAGAAAATGGGCGAATATTAGTTGATGGA 1680
541 L T K L V Q R L Y V P E N G R I L V D G 560

1681 AACGATTTGGCATTAGCTGATCCCGCTTGGCTGCGTCGCCAAGTGGGTGTTGTTTTGCAG 1740
561 N D L A L A D P A W L R R Q V G V V L Q 580

1741 GAAAATGTGTTACTCAATCGTAGTATTCGAGATAATATTGCCCTAACTGATACGGGCATG 1800
581 E N V L L N R S I R D N I A L T D T G M 600

1801 TCATTAGAGTTTATTATCCAGGCTGCCAAGATGTCTGGGGCACATGACTTTATTATGGAA 1860
601 S L E F I I Q A A K M S G A H D F I M E 620

1861 TTGCCTGAGGGTTATGATACGATTGTTGGAGAGCAAGGTGCAGGCTTGTCAGGTGGACAA 1920
621 L P E G Y D T I V G E Q G A G L S G G Q 640

*FIG. 8-3**12/22*

1921 CGCCAGCGTATCGCTATTGCGCGTGCTTTAATTACCAATCCGCGTATTTTGATTTTGGAT 1980
641 R Q R I A I A R A L I T N P R I L I F D 660

1981 GAAGCTACTAGTGCATTAGACTATGAGTCGAAAGGGCTATTATGCAAAATATGCAGGCA 2040
661 E A T S A L D Y E S E R A I M Q N M Q A 680

2041 ATTTGCCAAGGTAGAACAGTGTTGATTATTGCACATCGCTTATCTACCGTAAAAATGGCA 2100
681 I C Q G R T V L I I A H R L S T V K M A 700

2101 CATCGCATTATTGCAATGGACAAGGGGAAAATTGTAGAGCAAGGCACACATCAAGAATTG 2160
701 H R I I A M D K G K I V E Q G T H Q E L 720

2161 TTGCAAAAAGAAGATGGTTACTATCGTTATTTATATGATTTGCAGAATGGATAAA 2215
721 L Q K E D G Y Y R Y L Y D L Q N G *

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MbxB	MAQQPA[SA]	LVLLAKNYG	TSASPADIM	HOESDNTKG	DNEIEMML	AAKKEEL	55
LktB	MEANRQND	L....GLVA	LSLNPEETK	HKFDEGKG	.S...TAWL	AAKSEA	56
ApxB	MEFYRE.ED	V....GLYA	LAVNPEELK	HKFDEGKG	.D...TAWL	AAKSEEL	55
HlyB	MDSC[HK.ID]	V....GLYA	VSVNPEETK	HREDTGGTG	.GG...SWL	AAKSEEL	55
MbxB	KVRIIRQPL	TRUSMETP	DLQNLNSH	ELFKRIQGV	GSAKYLHY	DLENRP	116
LktB	KAKHIKKEL	SREHLVNP	GKH	ELVKVVD..	TNNRYLYY	NLEQDA	107
ApxB	KAKQVKKAL	DRALALP	GKH	ELFKRID..	NEAKKYLIF	DLETHNP	106
HlyB	KVKQVKKTL	DRENELSEP	GRH	ELFKVKS..	KEANRYLIF	DLEQRPD	106
MbxB	ITLDASEFS	ERNSGKML	SLAKEDFTM	FIPAVIKYR	YEFEEVENI	SMVGGEE	177
LktB	QKISTDEFE	ACVGGQLIL	QLAKEDFTM	FIPAVIKYR	REFLETLIN	SIFLOLE	168
ApxB	RLLEQAEFE	SLVGGKLL	KLAKEDFTM	FIPAVIKYR	KIFENELIV	SIFLOLE	167
HlyB	RVLQOSEFE	ALVGGHLL	KLAKEDFTM	FIPAVIKYR	REFLETLIN	SMFLOLE	167
MbxB	ALITPLFFQ	VWMDKVLVH	ALACLVMSE	FEVILSGLR	FYTFAHITS	RIDVELG	238
LktB	ALITPLFFQ	VWMDKVLVH	EVALAMIT	FELVSGLR	FYVFSHSTS	RIDVELG	229
ApxB	ALITPLFFQ	VWMDKVLVH	EVALAMIL	FELVNGLR	FYTFAHSTS	RIDVELG	228
HlyB	ALITPLFFQ	VWMDKVLVH	EVALSVMV	FETILSGLR	FYTFAHSTS	RIDVELG	228
MbxB	ARLERHLA	EPPLAVFESR	RELEHIRNF	LTGOALTSV	DLVFSLE	LFVMMVY	299
LktB	AKLERHLS	EPISYFEENR	RELDQIRNF	LTGOALTSV	DLEFSLE	FAVMVY	290
ApxB	ARLERHLA	EPISYFEENR	RELDQIRNF	LTGOALTSV	DLEFSLE	FAVMVY	289
HlyB	AKLERHLA	EPISYFEENR	RELDQIRNF	LTGOALTSV	DLEFSLE	FAVMVY	289
MbxB	SPILTLNWL	ASRLIYAFM	TRENDQFAR	NAGNOSTFLY	ESITAVGTFM	KAMAVEP	360
LktB	SPKLTLYTL	GSIRCYILM	RREDEKPAR	SADNQAFLX	ESVTATNMT	KAMAVAP	351
ApxB	SPKLTLYTL	GSIDPYMGW	RREDEKPAR	GADNQSFLY	ESVTATNMT	KALAVTP	350
HlyB	SPKLTLYTL	FSLPCYAFM	RREDDKFSR	NADNQSFLY	ESVTATNMT	KAMAVSP	350

FIG. 9-1

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MbxB	QMTTRMDNQ	LAAYVSSSE	RVAKEAMVR	QQGVOLIOK	MVLVATLWE	GAKEVLEGK	LSVGQEL	421
LktB	QMTDMDKO	LAAYVSSSE	RVTVEATIG	QQGVOLIOK	TVMVTINLWE	GAHLVISGD	LSIGQL	412
ApxIB	QMTNMDKO	LAAYVSSAGE	RVTVEATIG	QQGVOLIOK	VVMVITLWE	GAHLVISGD	LSIGQL	411
HlYB	QMTNMDKO	LAGVMAAGE	KVTVEATIG	QQQIQEIOK	TVMVTINLWE	GAHLVISGD	LSIGQL	411
MbxB	AFNMEAGQV	APVIRLEAO	LWQDFQOVG	TSVIRLGDV	ENIPTSTHST	SRLTLPDIK	GDIPTEN	482
LktB	AFNMSQGV	TAPVIRLEAO	LWQDFQOVG	TSVIRLGDV	LNSPTSTQVQ	GKLSLPEIK	GDISEKN	473
ApxIB	AFNMSGGV	TAPVIRLEAO	LWQDFQOVG	TSVIRLGDV	LNSPTSTYQ	GKLAALPEIK	GDIPTERN	472
HlYB	AFNMEAGQI	MARVIRLEAO	LWQDFQOVG	TSVIRLGDV	LNSPTSTYH	GKLAALPEIN	GDIPTERN	472
MbxB	VDTRYKIDG	HLITONLINE	QINAGEILL	TVGRSGSGK	STLTKEVMQR	LNVIPENGRI	LVDGNDE	543
LktB	TRERYKPD	PTLENNVNL	ETROGEVIG	TVGRSGSGK	STLTKEVLQR	RYTIPENGQV	LTDGHD	534
ApxIB	TRERYKPD	PVLENDVNE	STQOGEVIG	TVGRSGSGK	STLTKEVMQR	EYIPENGQV	LTDGHD	533
HlYB	TRERYKPD	PVLENDVNL	STQOGEVIG	TVGRSGSGK	STLTKEVMQR	EYIPENGQV	LTDGHD	533
MbxB	ALADPNIWR	ROVGWVLOE	NVLNRSIR	DNIALIDPG	MSLEFIDQA	AKMSGAHDE	IMEPEEG	604
LktB	ALADPNWLR	ROIGWVLOD	NVLNRSIR	DNIALIDPG	MPMERVITYA	AKLAGAHDE	ISELREG	595
ApxIB	ALADPNWLR	ROVGWVLOD	NVLNRSIR	DNIALIDPG	MPMERVITYA	AKLAGAHDE	ISELREG	594
HlYB	ALADPNWLR	ROVGWVLOD	NVLNRSIR	DNIALIDPG	MPMERVITYA	AKLAGAHDE	ISELREG	594
MbxB	YDITVGEQG	AGLSGGGORG	RTATARALI	TNPKILLFD	EATSALDYE	SERATIMQNM	QATGQGR	665
LktB	YNTIVGEQG	AGLSGGGORG	RTATARALY	NNPKILLFD	EATSALDYE	SEHTIMQNM	QKIQQGR	656
ApxIB	YNTIVGEQG	AGLSGGGORG	RTATARALY	NNPKILLFD	EATSALDYE	SEHTIMQNM	HQICKGR	655
HlYB	YNTIVGEQG	AGLSGGGORG	RTATARALY	NNPKILLFD	EATSALDYE	SEHTIMQNM	HKICKGR	655
MbxB	TVITTAHRL	STVKMAHRT	TAMDKGGIV	EQGTHQELL	QKEDGYVRM	LYDQNG	717	
LktB	TVITTAHRL	STVKNAORI	TYMEKGEIN	EQGKHHEL	QNSNGLYSY	CHQLOLN	708	
ApxIB	TVITTAHRL	STVKNAORI	TYMEKGGIV	EQGKHHEL	ADPNGLYH	HQLOSE	707	
HlYB	TVITTAHRL	STVKNAORI	TYMEKGGIV	EQGKHHEL	SEPELSYSY	EYQLOSD	707	

FIG. 9-2

FIG. 10

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1	ATGACGAAAAAGTTTGCAGAGCTAGGTTTAATTGCATGGCTTTGGTCTAACTCTGATATG	60
1	M T K K F A E L G L I A W L W S N S D M	20
61	CATAAACATTGGACGTTGTCTTTGTTTGCACCAATGTTATTCCGGCAATTGAGACAGGT	120
21	H K H W T L S L F A T N V I P A I E T G	40
121	CAATATGTTATATTGAAAAGAGAAGATATGCCTGTAGCATATTGTAGTTGGGCTAAACTT	180
41	Q Y V I L K R E D M P V A Y C S W A K L	60
181	AGTTTAGAAAACGAGGTTAAATATATTAACGATGTTACTTCTCTTAAGTTAGATGACTGG	240
61	S L E N E V K Y I N D V T S L K L D D W	80
241	CAGTCAGGTGACCGAAACTGGTTTATTGACTGGATTGCTCCATTTGGCGATAGTCTTACA	300
81	Q S G D R N W F I D W I A P F G D S L T	100
301	CTCACAAAACACATGAGAACGTTATTTTCAGATGAATTGTTTAGAGCGATTTCGTGTAGAT	360
101	L T K H M R T L F S D E L F R A I R V D	120
361	GGAAATTCATCGCATGGTAAGATATCTGAATTTTATGGAAAGTCTGTTGATTCAAAATTA	420
121	G N S S H G K I S E F Y G K S V D S K L	140
421	GCCTCAAGAATATTTGCACAATATCACGAAGATTTGACGAGCAAATTGTCAACTCAGAAT	480
141	A S R I F A Q Y H E D L T S K L S T Q N	160
481	AATTTTATTATATCTAAAGATAATTAA	507
161	N F I I S K D N *	169

MbxC	~MTKKFAE	EGLIAMNIN	SDMKHMTLS	LFATNVI PAT	ETGOY	42
LktC	~MNQSYENL	EGNITMWMN	SSLHKEMSC	LFARNVI PAT	ENEQY	43
ApxIC	MSKKINGFEV	LGEVAAILWAS	SPLHRKAPLS	LFATNVI PAT	ESNGY	45
H1yC	~MNRNIPLEV	LGHVSALWAS	SPLHRNAPVS	LFATNVI PAT	RANQY	44
MbxC	VILKREDMPV	AYCSWAKESL	ENEMKVIIDV	TSEKLDDWQS	GDRNW	87
LktC	MLIDNGTPI	AYCSWADEN	ETEMKYIKDI	NSLTPEEMQS	GDRRW	88
ApxIC	VALKRDGFRI	AFCSWANLINE	ENETKVLDDV	ASLVADDWTS	GDRRW	90
H1yC	ALITRONYPV	AYCSWANESL	ENETKVLDDV	TSEVAEDWTS	GDRKW	89
MbxC	FIDWIAPEGD	SLTITIKHMRT	LFSDEELFRAT	RVEGNSS.HG	KISET	131
LktC	ITIDWIAPEGH	SOLLYKMKCQ	KYPMIVIRSI	REYDKQKELG	KIAYF	133
ApxIC	FIDWIAPEGD	SALYKHMED	NENELFRAT	RVEDPSR.VG	KISET	134
H1yC	FIDWIAPEGD	NGALYKMKRK	KFDEELFRAT	RVEDPKTH.VG	KVSEF	133
MbxC	YBKSVDKLA	SRIFAQYHED	TSKISTONN	FATISKDN~	168	
LktC	KGCKLIDKTA	KKREDITYCEE	ATAKNEFN	EIKK~~~~	167	
ApxIC	HGGKIDKLA	SKLEDOYHFE	LMSELKMKON	EKESLVNS	172	
H1yC	HGGKIDKLA	NKTFKQYHFE	ITTEVKNMSD	FNESLITG~	170	

FIG. 11

FIG. 12-1

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1	ATGTTTATACAAGCACTTAAAGATTTTTTTTATTCGCTATATAACCGTTTGGCGCAATACA	60
1	M F I Q A L K D F I R Y I T V W R N T	
61	TGGGCAGTTCGAGACCAACTAACCCCTCCTAAGCGTACTAAAGAAGAACTCGCTTTTCTT	120
21	W A V R D Q L T P P K R T K E E L A F L	40
121	CCTGCACATCTAGAACTCACTGACACACCTGTATCCAGATCTTCTAAGTGGACAGCTAGA	180
41	P A H L E L T D T P V S R S S K W T A R	60
181	ATAATCATGATATTTGTCCTATTTGCTTTGCTATGGTCTTGGGTTGGACAGATTGACATT	240
61	I I M I F V L F A L L W S W V G Q I D I	80
241	GTTGCTACAGCTTCAGGTAATAATTTCTTCAGGTAGCCGTAGCAAGACTATTCAATCTTTG	300
81	V A T A S G K I S S G S R S K T I Q S L	100
301	GAAACAGCGATAGTTAAAGCAGTTTATGTACGTGATGGTCAAAATGTTCAACAAGGTGAA	360
101	E T A I V K A V Y V R D G Q N V Q Q G E	120
361	ATATTAGTAGATTTAGTGGGAATCGGTTTCAGATAGTGATGTTGCTCAGTCCGAGAAAGCC	420
121	I L V D L V G I G S D S D V A Q S E K A	140
421	CTTCGAGCAGCGCAATTATCTAAGCTACGCCTTGAAGCAATTTTATCAGCATTAAATCAC	480
141	L R A A Q L S K L R L E A I L S A L N H	160
481	CGTATTAATCCTCAGATTGATGTAGCATATGCAAAGTCTTTAAATATTTTCAAGATCGGAA	540
161	R I N P Q I D V A Y A K S L N I S E S E	180
541	ATTAATGAAGCTCAAACTTTAGCCCAAAATCAATATCAAGCATGGTTAGCACAAGATGAA	600
181	I N E A Q T L A Q N Q Y Q A W L A Q D E	200
601	CAACTAAAATTAACCTTAAAAGGACATCAAGCAGAATTACAATCTGCTCGATCCCAAGAA	660
201	Q L K L T L K G H Q A E L Q S A R S Q E	220
661	CAAAAGTTGGTTTCAGTTGGTGCAATTGAACATCAAAAGACTGATGATTATCGGAGTCTC	720
221	Q K L V S V G A I E H Q K T D D Y R S L	240
721	AAAGCAGAAAATTTTATATCTGAGCATGCTTATCTAGAACAAGAAAGCAAATTACTTAGC	780
241	K A E N F I S E H A Y L E Q E S K L L S	260
781	AATCAAAATGATTTACAAAGTACACGTAGTCAGATTCAAAAAATACAGGCTGCAATCATG	840
261	N Q N D L Q S T R S Q I Q K I Q A A I M	280
841	CAAGCTGAACAGAACCGTATGTTATATACTCAAAATCTAAAACGTGATACATTAGAATCT	900
281	Q A E Q N R M L Y T Q N L K R D T L E S	300
901	TTACGCCAAACCAATGAACAGATTAATCAATATACTGGTCAAACTAATAAAGCTAAGCAG	960
301	L R Q T N E Q I N Q Y T G Q T N K A K Q	320

FIG. 12-2

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961 CGACAGAAATTGCTGAGTATTAATCACCTGTTAATGGTACTATACAAGAGCTAACAGCT 1020
321 R Q K L L S I K S P V N G T I Q E L T A 340

1021 TATACTTTAGGTGGAGTTGTACAAGCAGCACAAAAAATTATGGTTGTGGCACCTAACGAT 1080
341 Y T L G G V V Q A A Q K I M V V A P N D 360

1081 AATCAAGTGGAAGTAGAGGTATTAGTGCTAAATAAAGATATCGGCTTTGTAAAAGCTGGG 1140
361 N Q V E V E V L V L N K D I G F V K A G 380

1141 CAGAATGTTATCATCAAAATCGAGAGTTTTCTTATACACGTTATGGTTATTTAACAGGT 1200
381 Q N V I I K I E S F P Y T R Y G Y L T G 400

1201 AAAATAAAAAGTATTAGTCATGATGCTATAGAACATCAACATTTAGGTCTAGTGTATACT 1260
401 K I K S I S H D A I E H Q H L G L V Y T 420

1261 GCACTTGTTTTCTCTTGATAAAAGCACATTAAATATAGATGGAGTAACAATCAACTTAACG 1320
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1321 CCAGGAATGAATGTTACTGCTGAAATTTAAACAGGTAAACGTCGTGTTTTGGATTATATA 1380
441 P G M N V T A E I T G K R R V L D Y I 461

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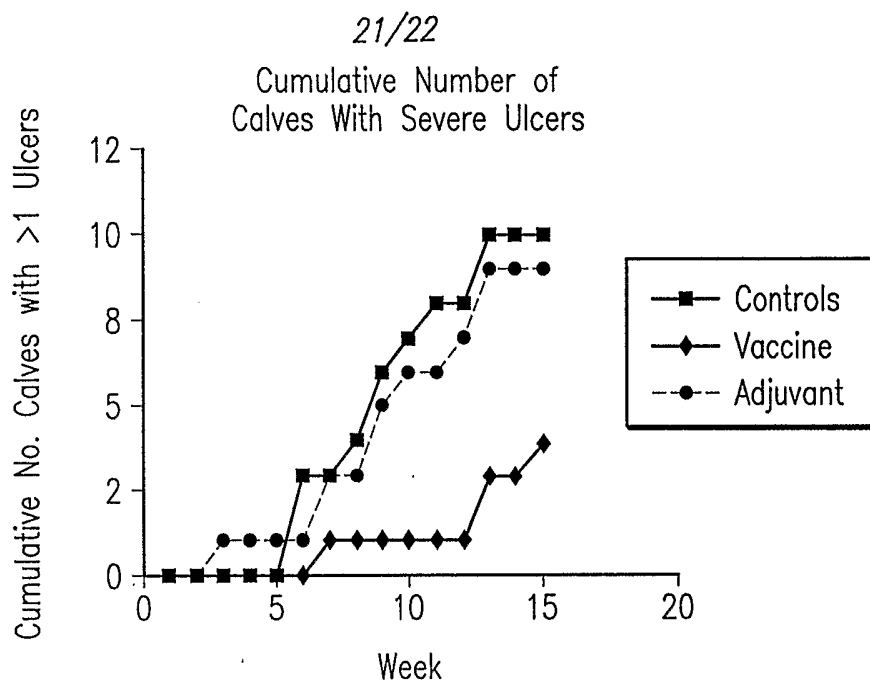
19/22

FIG. 13-1

MbxD	~MFIQAQKND	EEIRYIIVVHR	NETAVRDQII	PPKRTKEELA	ELPAHLEELID	48
Lktd	MKIMLSGIYE	FFRYKNIWA	EVVKIRKELD	HNRKKDESE	ELPAHLEELI	50
ApxD	MKTWLMGLYE	FFQRYKTYWT	EIMKTRHQID	TPDREKDENE	ELPAHLEELI	50
HlyD	MKTWLMGFSE	FLERYKLIWS	EIMKIRKQID	TPVREKDENE	ELPAHLEELI	50
MbxD	TPVSRSSKWT	ARTIMTIVLF	ALLWSWVGII	DIVATASGKI	SSGSRSKITIQ	98
Lktd	TPVSKKPRRI	AYLIMLEFV	AIMVASVSKV	EIVATAPGKI	TFSGRSKEIK	100
ApxD	TPVSKKPRRI	AYLIMLEFEL	ALVISIVSHV	EIVATATGKI	AESDRSKEIK	100
HlyD	TPVSRPRREIV	AYFIMGEELI	AFDLSVLGQV	EIVATANGKI	TLSGRSKEIK	100
MbxD	SLEIATVAV	YVRDGNVQQ	GETLVDEIVGI	GSBSEVAQSE	KAEIRAAQISK	148
Lktd	PIENATVOEI	FVKDGFVEK	GOLIVSEPAL	GSBADIKKTIM	ASLSLAKLEN	150
ApxD	PIENALVKEI	EVQDGFVEK	DQLLHIFAL	GADAPQOKIK	SSLSLTKLER	150
HlyD	PDENSTVKEI	IVKEGESVIRK	GDVLLKLTAL	CAEADTLKTIQ	SSSLQARLEIQ	150
MbxD	LRLEATISAL	NHRINPQIDV	AYAKSLNIS	ESEINEAQTII	AONQYQAWLA	197
Lktd	YRYQTLEITAI	EKESERVIDI	.SRTEKDDSS	EEDRLRKHE	TEEOYTTMOK	199
ApxD	YRYETLEAV	AADREPLIEI	.TKDEFKHAT	EEDKTRIRYL	ITTEQFEAWOK	199
HlyD	TRYQINSRSI	ELNKLPELKE	PDEPYEQNVIS	EFEVLRITSL	HKKEQFSTWGN	200
MbxD	QDEQLKETEK	GHQABEQSAR	SQEQKLVSIG	ATIEHQKTDDV	RSEKAENFIS	247
Lktd	QKTKTLAYK	RKEAEKQITF	AYMRKVEGAT	RIIEQEKKQOF	KALYKQKSL	249
ApxD	QKYQKELAQ	RREAEKQIV	ANERKYEGIS	RVENERKDL	KKLFNSKSTS	249
HlyD	QKYQKELNID	KKRAERLITL	ARNRVENYS	RVEKSRDDEE	RSL LHKQATA	250
MbxD	EHAIVIEQESK	EILSNQNDIQS	TRSQTIQKIQ	AIMQAEQNRM	QYTONLKRDIT	297
Lktd	KHELLAQENK	LTEAGNAVAV	YRSKINELIN	DLINVEELE	LITOFFKSDM	299
ApxD	KHDVITQENR	HTEAVNELAV	YKSRLNEVES	DLROAKKEITH	ELITOLFRADL	299
HlyD	KHAYLEQENK	YVIEAANEIRV	YKSQLEQIES	EIESAKKEHYQ	LVITQLEKNEI	300

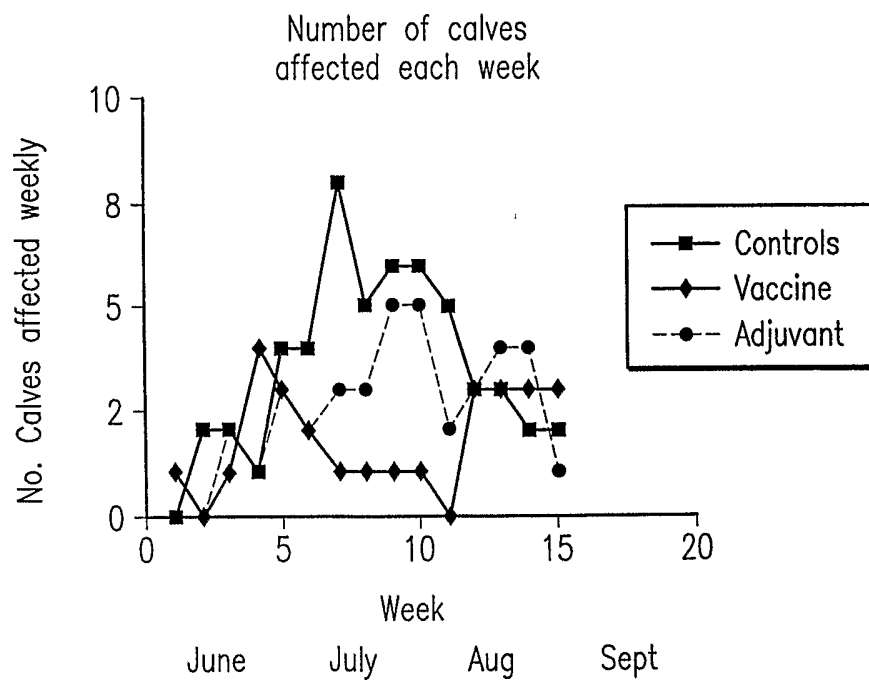
FIG. 13-2

MbxD	LESERQTN	EQ	INQMTGQ	TNK	AKQRQKLLS	KSPVNGT	IQE	ETAYT	GGV	347
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ApxD	LEKQKNV	EA	EKQSS	EE	NEORQIASV	RAPVSGT	VQO	LEKQKH	GGV	349
HlyD	LDKERQ	TIDS	IELTE	EE	NEERQOASV	RAPVSGT	VQO	LEKQKH	GGV	350
MbxD	QAACKIMW	MA	PNDNQV	EEV	LVLNKD	IGF	V	KAGQV	ITEKI	397
Lktd	TTAETIM	IV	PEDDV	EA	LVLPNK	QIGF	V	AAGQEV	ITEKI	399
ApxD	TTAETIM	IV	PEDDV	EA	LVLPNK	QIGF	V	EVGDAM	ITEKI	399
HlyD	TTAETIM	IV	PEDDV	EA	LVLPNK	QIGF	V	NVGONAL	ITEKI	400
MbxD	LTGKIK	KS	SH	DATE	HQ	LEG	MTAT	MS	LDK	446
Lktd	LTGRIKH	SP	DA	E	QPNV	GV	EN	AT	IS	449
ApxD	LVGKV	KN	IT	L	LE	EN	ST	IS	IS	449
HlyD	LVGKV	KN	IT	L	LE	EN	ST	IS	IS	449
MbxD	AEIKG	K	R	V	LDY	IL	SP	LQ		475
Lktd	AEIKG	R	S	V	MS	LL	SP	LE		478
ApxD	AEIKG	R	S	V	TS	Y	LL	SP		478
HlyD	AEIKG	R	S	V	TS	Y	LL	SP		478



Number of calves with ulcers with clinical scores >+2

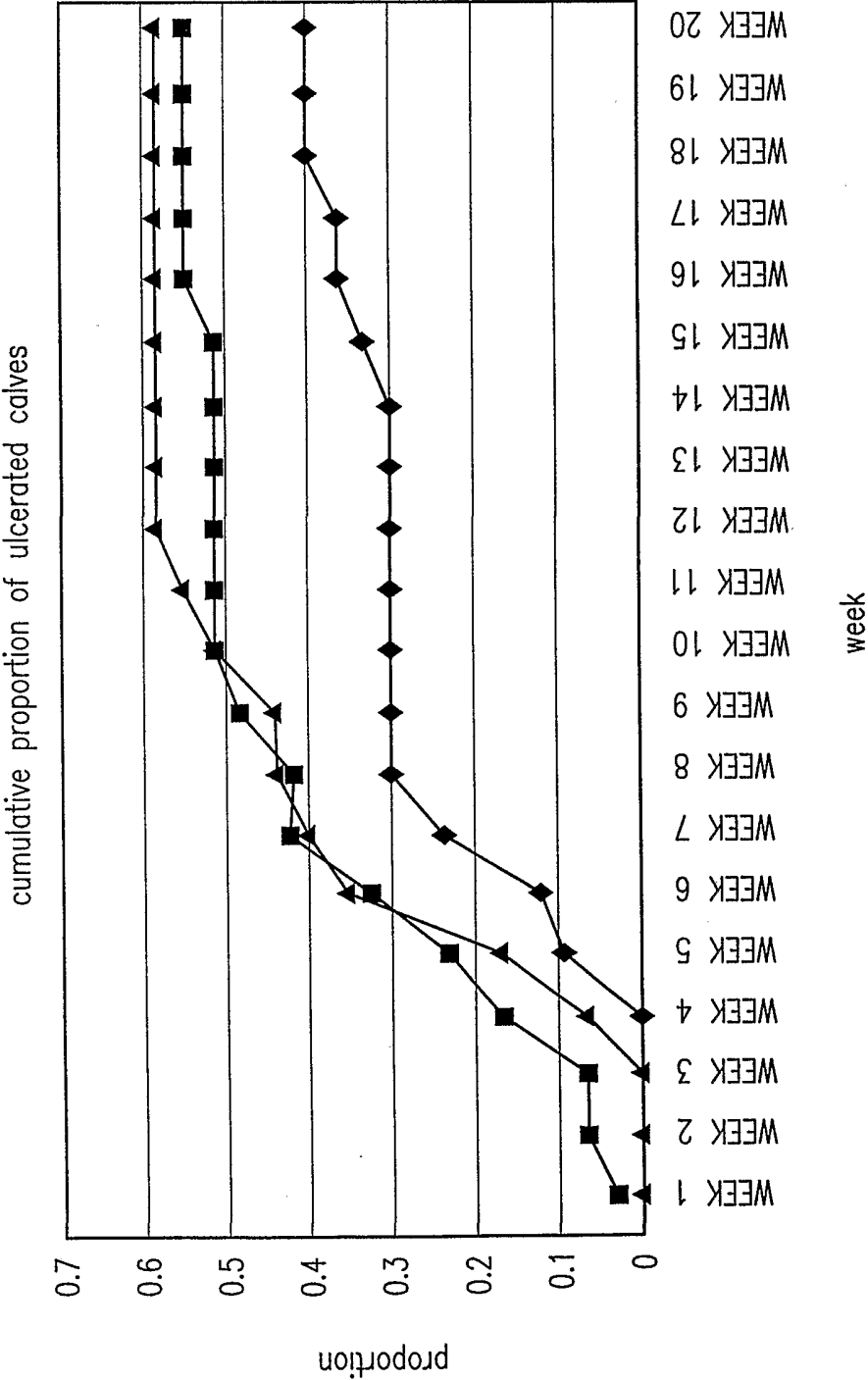
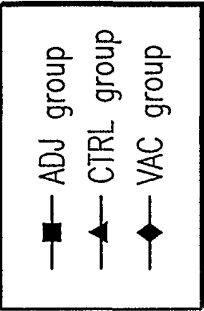
FIG. 14



Number of calves affected weekly in 1 group of vaccinated calves and in controls.

FIG. 15

FIG. 16



SEQUENCE LISTING

<110> GEORGE, LISLE W
ANGELOS, JOHN A
HESS, JOHN F

<120> MORAXELLA BOVIS CYTOTOXIN, CYTOTOXIN GENE, ANTIBODIES
AND VACCINES FOR PREVENTION AND TREATMENT OF MORAXELLA
BOVIS INFECTIONS

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<140> 09/884,696

<141> 2001-06-19

<160> 41

<170> PatentIn Ver. 2.1

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Ser Thr Lys Ser Gly Leu Lys Asn Leu Tyr Leu Ala Ile Pro Lys Asp	
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Tyr Asp Pro Gln Lys Gly Gly Thr Leu Asn Asp Phe Ile Lys Ala Ala	
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gat gaa tta ggt att gct cgt tta gca gaa gag cct aat cac act gaa	192
Asp Glu Leu Gly Ile Ala Arg Leu Ala Glu Glu Pro Asn His Thr Glu	
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Thr Ala Lys Lys Ser Val Asp Thr Val Asn Gln Phe Leu Ser Leu Thr	
65 70 75 80	
caa act ggt att gct att tct gca aca aaa tta gaa aag ttc tta caa	288
Gln Thr Gly Ile Ala Ile Ser Ala Thr Lys Leu Glu Lys Phe Leu Gln	
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Lys His Ser Thr Asn Lys Leu Ala Lys Gly Leu Asp Ser Val Glu Asn	
100 105 110	

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Ile Asp Arg Lys Leu Gly Lys Ala Ser Asn Val Leu Ser Thr Leu Ser	
115 120 125	
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Ser Phe Leu Gly Thr Ala Leu Ala Gly Ile Glu Leu Asp Ser Leu Ile	
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Lys Lys Gly Asp Ala Ala Pro Asp Ala Leu Ala Lys Ala Ser Ile Asp	
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Ala Lys Gly Phe Ser Asn Ile Gly Asn Lys Leu Gln Asn Leu Asn Phe	
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Thr Lys Ala Ile Ser Ser Tyr Val Leu Ala Gln Arg Val Ala Ala Gly	
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Leu Ser Thr Thr Gly Ala Val Ala Ala Leu Ile Thr Ser Ser Ile Met	
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 Ala Lys Leu Gly Glu Gly Asp Asp Asn Val Phe Val Gly Ser Gly Thr
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Gly Asn Tyr Gly Ala Leu Thr Ile Asp Ala Thr Lys Glu Thr Glu Gln
 645 650 655

Gly Ser Tyr Thr Val Asn Arg Phe Val Glu Thr Gly Lys Ala Leu His
 660 665 670

Glu Val Thr Ser Thr His Thr Ala Leu Val Gly Asn Arg Glu Glu Lys
 675 680 685

Ile Glu Tyr Arg His Ser Asn Asn Gln His His Ala Gly Tyr Tyr Thr
 690 695 700

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 740 745 750

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 835 840 845

Glu Val Pro Asn Tyr Lys Ala Thr Lys Asp Glu Lys Ile Glu Glu Ile
 850 855 860

Ile Gly Gln Asn Gly Glu Arg Ile Thr Ser Lys Gln Val Asp Asp Leu
 865 870 875 880

Ile Ala Lys Gly Asn Gly Lys Ile Thr Gln Asp Glu Leu Ser Lys Val
 885 890 895

Val Asp Asn Tyr Glu Leu Leu Lys His Ser Lys Asn Val Thr Asn Ser
 900 905 910

Leu Asp Lys Leu Ile Ser Ser Val Ser Ala Phe Thr Ser Ser Asn Asp
 915 920 925

Ser Arg Asn Val Leu Val Ala Pro Thr Ser Met Leu Asp Gln Ser Leu
 930 935 940

12

Ser Ser Leu Gln Phe Ala Arg Ala Ala
945 950

<210> 4

<211> 956

<212> PRT

<213> Actinobacillus pleuropneumoniae

<400> 4

Met Ser Lys Ile Thr Leu Ser Ser Leu Lys Ser Ser Leu Gln Gln Gly
1 5 10 15

Leu Lys Asn Gly Lys Asn Lys Leu Asn Gln Ala Gly Thr Thr Leu Lys
20 25 30

Asn Gly Leu Thr Gln Thr Gly His Ser Leu Gln Asn Gly Ala Lys Lys
35 40 45

Leu Ile Leu Tyr Ile Pro Gln Gly Tyr Asp Ser Gly Gln Gly Asn Gly
50 55 60

Val Gln Asp Leu Val Lys Ala Ala Asn Asp Leu Gly Ile Glu Val Trp
65 70 75 80

Arg Glu Glu Arg Ser Asn Leu Asp Ile Ala Lys Thr Ser Phe Asp Thr
85 90 95

Thr Gln Lys Ile Leu Gly Phe Thr Asp Arg Gly Ile Val Leu Phe Ala
100 105 110

Pro Gln Leu Asp Asn Leu Leu Lys Lys Asn Pro Lys Ile Gly Asn Thr
115 120 125

Leu Gly Ser Ala Ser Ser Ile Ser Gln Asn Ile Gly Lys Ala Asn Thr
130 135 140

Val Leu Gly Gly Ile Gln Ser Ile Leu Gly Ser Val Leu Ser Gly Val
145 150 155 160

Asn Leu Asn Glu Leu Leu Gln Asn Lys Asp Pro Asn Gln Leu Glu Leu
165 170 175

Ala Lys Ala Gly Leu Glu Leu Thr Asn Glu Leu Val Gly Asn Ile Ala
180 185 190

Ser Ser Val Gln Thr Val Asp Ala Phe Ala Glu Gln Ile Ser Lys Leu
195 200 205

Gly Ser His Leu Gln Asn Val Lys Gly Leu Gly Gly Leu Ser Asn Lys
210 215 220

Leu Gln Asn Leu Pro Asp Leu Gly Lys Ala Ser Leu Gly Leu Asp Ile
225 230 235 240

Ile Ser Gly Leu Leu Ser Gly Ala Ser Ala Gly Leu Ile Leu Ala Asp
245 250 255

Lys Glu Ala Ser Thr Glu Lys Lys Ala Ala Ala Gly Val Glu Phe Ala
 260 265 270
 Asn Gln Ile Ile Gly Asn Val Thr Lys Ala Val Ser Ser Tyr Ile Leu
 275 280 285
 Ala Gln Arg Val Ala Ser Gly Leu Ser Ser Thr Gly Pro Val Ala Ala
 290 295 300
 Leu Ile Ala Ser Thr Val Ala Leu Ala Val Ser Pro Leu Ser Phe Leu
 305 310 315 320
 Asn Val Ala Asp Lys Phe Lys Gln Ala Asp Leu Ile Lys Ser Tyr Ser
 325 330 335
 Glu Arg Phe Gln Lys Leu Gly Tyr Asp Gly Asp Arg Leu Leu Ala Asp
 340 345 350
 Phe His Arg Glu Thr Gly Thr Ile Asp Ala Ser Val Thr Thr Ile Asn
 355 360 365
 Thr Ala Leu Ala Ala Ile Ser Gly Gly Val Gly Ala Ala Ser Ala Gly
 370 375 380
 Ser Leu Val Gly Ala Pro Val Ala Leu Leu Val Ala Gly Val Thr Gly
 385 390 395 400
 Leu Ile Thr Thr Ile Leu Glu Tyr Ser Lys Gln Ala Met Phe Glu His
 405 410 415
 Val Ala Asn Lys Val His Asp Arg Ile Val Glu Trp Glu Lys Lys His
 420 425 430
 Asn Lys Asn Tyr Phe Glu Gln Gly Tyr Asp Ser Arg His Leu Ala Asp
 435 440 445
 Leu Gln Asp Asn Met Lys Phe Leu Ile Asn Leu Asn Lys Glu Leu Gln
 450 455 460
 Ala Glu Arg Val Val Ala Ile Thr Gln Gln Arg Trp Asp Asn Gln Ile
 465 470 475 480
 Gly Asp Leu Ala Ala Ile Ser Arg Arg Thr Asp Lys Ile Ser Ser Gly
 485 490 495
 Lys Ala Tyr Val Asp Ala Phe Glu Glu Gly Gln His Gln Ser Tyr Asp
 500 505 510
 Ser Ser Val Gln Leu Asp Asn Lys Asn Gly Ile Ile Asn Ile Ser Asn
 515 520 525
 Thr Asn Arg Lys Thr Gln Ser Val Leu Phe Arg Thr Pro Leu Leu Thr
 530 535 540
 Pro Gly Glu Glu Asn Arg Glu Arg Ile Gln Glu Gly Lys Asn Ser Tyr
 545 550 555 560

14

Ile	Thr	Lys	Leu	His	Ile	Gln	Arg	Val	Asp	Ser	Trp	Thr	Val	Thr	Asp	
				565					570						575	
Gly	Asp	Ala	Ser	Ser	Ser	Val	Asp	Phe	Thr	Asn	Val	Val	Gln	Arg	Ile	
			580					585					590			
Ala	Val	Lys	Phe	Asp	Asp	Ala	Gly	Asn	Ile	Ile	Glu	Ser	Lys	Asp	Thr	
		595					600					605				
Lys	Ile	Ile	Ala	Asn	Leu	Gly	Ala	Gly	Asn	Asp	Asn	Val	Phe	Val	Gly	
	610					615					620					
Ser	Ser	Thr	Thr	Val	Ile	Asp	Gly	Gly	Asp	Gly	His	Asp	Arg	Val	His	
625					630					635					640	
Tyr	Ser	Arg	Gly	Glu	Tyr	Gly	Ala	Leu	Val	Ile	Asp	Ala	Thr	Ala	Glu	
				645					650					655		
Thr	Glu	Lys	Gly	Ser	Tyr	Ser	Val	Lys	Arg	Tyr	Val	Gly	Asp	Ser	Lys	
			660					665					670			
Ala	Leu	His	Glu	Thr	Ile	Ala	Thr	His	Gln	Thr	Asn	Val	Gly	Asn	Arg	
		675					680					685				
Glu	Glu	Lys	Ile	Glu	Tyr	Arg	Arg	Glu	Asp	Asp	Arg	Phe	His	Thr	Gly	
	690					695				700						
Tyr	Thr	Val	Thr	Asp	Ser	Leu	Lys	Ser	Val	Glu	Glu	Ile	Ile	Gly	Ser	
705					710					715					720	
Gln	Phe	Asn	Asp	Ile	Phe	Lys	Gly	Ser	Gln	Phe	Asp	Asp	Val	Phe	His	
				725					730					735		
Gly	Gly	Asn	Gly	Val	Asp	Thr	Ile	Asp	Gly	Asn	Asp	Gly	Asp	Asp	His	
			740					745					750			
Leu	Phe	Gly	Gly	Ala	Gly	Asp	Asp	Val	Ile	Asp	Gly	Gly	Asn	Gly	Asn	
		755					760					765				
Asn	Phe	Leu	Val	Gly	Gly	Thr	Gly	Asn	Asp	Ile	Ile	Ser	Gly	Gly	Lys	
		770				775					780					
Asp	Asn	Asp	Ile	Tyr	Val	His	Lys	Thr	Gly	Asp	Gly	Asn	Asp	Ser	Ile	
785					790					795					800	
Thr	Asp	Ser	Gly	Gly	Gln	Asp	Lys	Leu	Ala	Phe	Ser	Asp	Val	Asn	Leu	
				805					810					815		
Lys	Asp	Leu	Thr	Phe	Lys	Lys	Val	Asp	Ser	Ser	Leu	Glu	Ile	Ile	Asn	
			820					825					830			
Gln	Lys	Gly	Glu	Lys	Val	Arg	Ile	Gly	Asn	Trp	Phe	Leu	Glu	Asp	Asp	
		835					840					845				
Leu	Ala	Ser	Thr	Val	Ala	Asn	Tyr	Lys	Ala	Thr	Asn	Asp	Arg	Lys	Ile	
	850					855					860					

15

Glu Glu Ile Ile Gly Lys Gly Gly Glu Arg Ile Thr Ser Glu Gln Val
 865 870 875 880
 Asp Lys Leu Ile Lys Glu Gly Asn Asn Gln Ile Ser Ala Glu Ala Leu
 885 890 895
 Ser Lys Val Val Asn Asp Tyr Asn Thr Ser Lys Asp Arg Gln Asn Val
 900 905 910
 Ser Asn Ser Leu Ala Lys Leu Ile Ser Ser Val Gly Ser Phe Thr Ser
 915 920 925
 Ser Ser Asp Phe Arg Asn Asn Leu Gly Thr Tyr Val Pro Ser Ser Ile
 930 935 940
 Asp Val Ser Asn Asn Ile Gln Leu Ala Arg Ala Ala
 945 950 955

<210> 5
 <211> 1023
 <212> PRT
 <213> Escherichia coli

<400> 5
 Met Pro Thr Ile Thr Ala Ala Gln Ile Lys Ser Thr Leu Gln Ser Ala
 1 5 10 15
 Lys Gln Ser Ala Ala Asn Lys Leu His Ser Ala Gly Gln Ser Thr Lys
 20 25 30
 Asp Ala Leu Lys Lys Ala Ala Glu Gln Thr Arg Asn Ala Gly Asn Arg
 35 40 45
 Leu Ile Leu Leu Ile Pro Lys Asp Tyr Lys Gly Gln Gly Ser Ser Leu
 50 55 60
 Asn Asp Leu Val Arg Thr Ala Asp Glu Leu Gly Ile Glu Val Gln Tyr
 65 70 75 80
 Asp Glu Lys Asn Gly Thr Ala Ile Thr Lys Gln Val Phe Gly Thr Ala
 85 90 95
 Glu Lys Leu Ile Gly Leu Thr Glu Arg Gly Val Thr Ile Phe Ala Pro
 100 105 110
 Gln Leu Asp Lys Leu Leu Gln Lys Tyr Gln Lys Ala Gly Asn Lys Leu
 115 120 125
 Gly Gly Ser Ala Glu Asn Ile Gly Asp Asn Leu Gly Lys Ala Gly Ser
 130 135 140
 Val Leu Ser Thr Phe Gln Asn Phe Leu Gly Thr Ala Leu Ser Ser Met
 145 150 155 160
 Lys Ile Asp Glu Leu Ile Lys Lys Gln Lys Ser Gly Gly Asn Val Ser
 165 170 175

Ser Ser Glu Leu Ala Lys Ala Ser Ile Glu Leu Ile Asn Gln Leu Val
 180 185 190
 Asp Thr Ala Ala Ser Leu Asn Asn Val Asn Ser Phe Ser Gln Gln Leu
 195 200 205
 Asn Lys Leu Gly Ser Val Leu Ser Asn Thr Lys His Leu Asn Gly Val
 210 215 220
 Gly Asn Lys Leu Gln Asn Leu Pro Asn Leu Asp Asn Ile Gly Ala Gly
 225 230 235 240
 Leu Asp Thr Val Ser Gly Ile Leu Ser Ala Ile Ser Ala Ser Phe Ile
 245 250 255
 Leu Ser Asn Ala Asp Ala Asp Thr Gly Thr Lys Ala Ala Ala Gly Val
 260 265 270
 Glu Leu Thr Thr Lys Val Leu Gly Asn Val Gly Lys Gly Ile Ser Gln
 275 280 285
 Tyr Ile Ile Ala Gln Arg Ala Ala Gln Gly Leu Ser Thr Ser Ala Ala
 290 295 300
 Ala Ala Gly Leu Ile Ala Ser Val Val Thr Leu Ala Ile Ser Pro Leu
 305 310 315 320
 Ser Phe Leu Ser Ile Ala Asp Lys Phe Lys Arg Ala Asn Lys Ile Glu
 325 330 335
 Glu Tyr Ser Gln Arg Phe Lys Lys Leu Gly Tyr Asp Gly Asp Ser Leu
 340 345 350
 Leu Ala Ala Phe His Lys Glu Thr Gly Ala Ile Asp Ala Ser Leu Thr
 355 360 365
 Arg Ile Ser Thr Val Leu Ala Ser Val Ser Ser Gly Ile Ser Ala Ala
 370 375 380
 Ala Thr Thr Ser Leu Val Gly Ala Pro Val Ser Ala Leu Val Gly Ala
 385 390 395 400
 Val Thr Gly Ile Ile Ser Gly Ile Leu Glu Ala Ser Lys Gln Ala Met
 405 410 415
 Phe Glu His Val Ala Ser Lys Met Ala Asp Val Ile Ala Glu Trp Glu
 420 425 430
 Lys Lys His Gly Lys Asn Tyr Phe Glu Asn Gly Tyr Asp Ala Arg His
 435 440 445
 Ala Ala Phe Leu Glu Asp Asn Phe Lys Ile Leu Ser Gln Tyr Asn Lys
 450 455 460
 Glu Tyr Ser Val Glu Arg Ser Val Leu Ile Thr Gln Gln His Trp Asp
 465 470 475 480

Thr Leu Ile Gly Glu Leu Ala Gly Val Thr Arg Asn Gly Asp Lys Thr
 485 490 495
 Leu Ser Gly Lys Ser Tyr Ile Asp Tyr Tyr Glu Glu Gly Lys Arg Leu
 500 505 510
 Glu Lys Lys Pro Asp Glu Phe Gln Lys Gln Val Phe Asp Pro Leu Lys
 515 520 525
 Gly Asn Ile Asp Leu Ser Asp Ser Lys Ser Ser Thr Leu Leu Lys Phe
 530 535 540
 Val Thr Pro Leu Leu Thr Pro Gly Glu Glu Ile Arg Glu Arg Arg Gln
 545 550 555 560
 Ser Gly Lys Tyr Glu Tyr Ile Thr Glu Leu Leu Val Lys Gly Val Asp
 565 570 575
 Lys Trp Thr Val Lys Gly Val Gln Asp Lys Gly Ser Val Tyr Asp Tyr
 580 585 590
 Ser Asn Leu Ile Gln His Ala Ser Val Gly Asn Asn Gln Tyr Arg Glu
 595 600 605
 Ile Arg Ile Glu Ser His Leu Gly Asp Gly Asp Asp Lys Val Phe Leu
 610 615 620
 Ser Ala Gly Ser Ala Asn Ile Tyr Ala Gly Lys Gly His Asp Val Val
 625 630 635 640
 Tyr Tyr Asp Lys Thr Asp Thr Gly Tyr Leu Thr Ile Asp Gly Thr Lys
 645 650 655
 Ala Thr Glu Ala Gly Asn Tyr Thr Val Thr Arg Val Leu Gly Gly Asp
 660 665 670
 Val Lys Val Leu Gln Glu Val Val Lys Glu Gln Glu Val Ser Val Gly
 675 680 685
 Lys Arg Thr Glu Lys Thr Gln Tyr Arg Ser Tyr Glu Phe Thr His Ile
 690 695 700
 Asn Gly Lys Asn Leu Thr Glu Thr Asp Asn Leu Tyr Ser Val Glu Glu
 705 710 715 720
 Leu Ile Gly Thr Thr Arg Ala Asp Lys Phe Phe Gly Ser Lys Phe Ala
 725 730 735
 Asp Ile Phe His Gly Ala Asp Gly Asp Asp His Ile Glu Gly Asn Asp
 740 745 750
 Gly Asn Asp Arg Leu Tyr Gly Asp Lys Gly Asn Asp Thr Leu Ser Gly
 755 760 765
 Gly Asn Gly Asp Asp Gln Leu Tyr Gly Gly Asp Gly Asn Asp Lys Leu
 770 775 780

18

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Ile Gly Gly Ala Gly Asn Asn Tyr Leu Asn Gly Gly Asp Gly Asp Asp
785                               790                               795                               800

Glu Leu Gln Val Gln Gly Asn Ser Leu Ala Lys Asn Val Leu Ser Gly
805                               810                               815

Gly Lys Gly Asn Asp Lys Leu Tyr Gly Ser Glu Gly Ala Asp Leu Leu
820                               825                               830

Asp Gly Gly Glu Gly Asn Asp Leu Leu Lys Gly Gly Tyr Gly Asn Asp
835                               840                               845

Ile Tyr Arg Tyr Leu Ser Gly Tyr Gly His His Ile Ile Asp Asp Asp
850                               855                               860

Gly Gly Lys Asp Asp Lys Leu Ser Leu Ala Asp Ile Asp Phe Arg Asp
865                               870                               875                               880

Val Ala Phe Arg Arg Glu Gly Asn Asp Leu Ile Met Tyr Lys Ala Glu
885                               890                               895

Gly Asn Val Leu Ser Ile Gly His Lys Asn Gly Ile Thr Phe Lys Asn
900                               905                               910

Trp Phe Glu Lys Glu Ser Gly Asp Ile Ser Asn His Gln Ile Glu Gln
915                               920                               925

Ile Phe Asp Lys Asp Gly Arg Val Ile Thr Pro Asp Ser Leu Lys Lys
930                               935                               940

Ala Leu Glu Tyr Gln Gln Ser Asn Asn Lys Ala Ser Tyr Val Tyr Gly
945                               950                               955                               960

Asn Asp Ala Leu Ala Tyr Gly Ser Gln Gly Asn Leu Asn Pro Leu Ile
965                               970                               975

Asn Glu Ile Ser Lys Ile Ile Ser Ala Ala Gly Asn Phe Asp Val Lys
980                               985                               990

Glu Glu Arg Ala Ala Ala Ser Leu Leu Gln Leu Ser Gly Asn Ala Ser
995                               1000                               1005

Asp Phe Ser Tyr Gly Arg Asn Ser Ile Thr Leu Thr Ala Ser Ala
1010                               1015                               1020

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<210> 6
<211> 12
<212> PRT
<213> Moraxella bovis

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<400> 6
Phe Leu Ser Glu Leu Asn Lys Glu Leu Glu Ala Glu
  1                               5                               10

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<210> 7
<211> 12

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19

<212> PRT

<213> *Pasteurella haemolytica*

<400> 7

Phe	Leu	Leu	Asn	Leu	Asn	Lys	Glu	Leu	Gln	Ala	Glu
1				5					10		

<210> 8

<211> 12

<212> PRT

<213> *Escherichia coli*

<400> 8

Ile	Leu	Ser	Gln	Tyr	Asn	Lys	Glu	Tyr	Ser	Val	Glu
1				5					10		

<210> 9

<211> 12

<212> PRT

<213> *Actinobacillus pleuropneumoniae*

<400> 9

Phe	Leu	Ile	Asn	Leu	Asn	Lys	Glu	Leu	Gln	Ala	Glu
1				5					10		

<210> 10

<211> 12

<212> PRT

<213> *Actinobacillus pleuropneumoniae*

<400> 10

Leu	Leu	Ser	Gln	Tyr	Asn	Lys	Glu	Tyr	Ser	Val	Glu
1				5					10		

<210> 11

<211> 12

<212> PRT

<213> *Actinobacillus suis*

<400> 11

Phe	Leu	Ile	Asn	Leu	Asn	Lys	Glu	Leu	Gln	Ala	Glu
1				5					10		

<210> 12

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: consensus
sequence

20

<220>
<221> MOD_RES
<222> (3)
<223> L, Y, A or V

<220>
<221> MOD_RES
<222> (7)..(8)
<223> L, Y, A or V

<400> 12
Phe Leu Xaa Asn Lys Glu Xaa Xaa Glu
1 5

<210> 13
<211> 14
<212> PRT
<213> Moraxella bovis

<400> 13
Phe Asn Asp Ile Phe His Ser Gly Glu Gly Asp Asp Leu Leu
1 5 10

<210> 14
<211> 14
<212> PRT
<213> Actinobacillus pleuropneumoniae

<400> 14
Phe Arg Asp Ile Phe His Gly Ala Asp Gly Asp Asp Leu Leu
1 5 10

<210> 15
<211> 14
<212> PRT
<213> Actinobacillus actinomycetemcomitans

<400> 15
Phe Asn Asp Val Phe His Gly His Asp Gly Asp Asp Leu Ile
1 5 10

<210> 16
<211> 14
<212> PRT
<213> Actinobacillus pleuropneumoniae

<400> 16
Phe Arg Asp Ile Phe His Gly Ala Asp Gly Asp Asp Leu Leu
1 5 10

<210> 17
<211> 11

21

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: consensus
sequence

<220>

<221> MOD_RES

<222> (11)

<223> L or I

<400> 17

Phe	Asp	Phe	His	Gly	Ala	Asp	Gly	Asp	Asp	Xaa
1				5					10	

<210> 18

<211> 737

<212> PRT

<213> Moraxella bovis

<400> 18

Met	Gly	Gly	Asp	Thr	Ser	Leu	Ile	Arg	Leu	Asn	Leu	Gln	Thr	Leu	Asn
1					5				10					15	

Ser	Asn	Leu	Val	Met	Ile	Asp	Tyr	Ala	Gln	Gln	Pro	Ala	Leu	Ser	Ala
		20						25					30		

Leu	Val	Ile	Leu	Ala	Lys	Tyr	Tyr	Gly	Ile	Ser	Ala	Ser	Pro	Ala	Asp
	35						40					45			

Ile	Met	His	Gln	Phe	Ser	Asp	Asn	Thr	Lys	Gly	Asp	Leu	Asn	Glu	Ile
	50					55					60				

Glu	Trp	Met	Leu	Ala	Ala	Lys	Lys	Leu	Glu	Leu	Lys	Val	Lys	Ile	Ile
65					70					75					80

Lys	Gln	Pro	Leu	Thr	Arg	Leu	Ser	Met	Ile	Thr	Leu	Pro	Ala	Leu	Val
				85					90					95	

Trp	Cys	Asp	Asn	Lys	Pro	Asp	Leu	Asp	Gln	Asn	Leu	Asn	Ser	His	Phe
			100					105					110		

Ile	Leu	Thr	Lys	Ile	Asp	Gly	Val	Gly	Ser	Ala	Ala	Lys	Tyr	Leu	Ile
	115						120					125			

Tyr	Asp	Leu	Ile	Glu	Asn	Arg	Pro	Ile	Ile	Leu	Asp	Ala	Ser	Glu	Phe
	130					135					140				

Ser	Glu	Arg	Tyr	Ser	Gly	Lys	Leu	Met	Leu	Val	Thr	Ser	Arg	Ala	Ser
145						150				155					160

Ile	Leu	Gly	Ser	Leu	Ala	Lys	Phe	Asp	Phe	Thr	Trp	Phe	Ile	Pro	Ala
				165					170					175	

Val	Ile	Lys	Tyr	Arg	Tyr	Ile	Phe	Phe	Glu	Val	Ile	Val	Ile	Ser	Val
						180		185					190		

Val Leu Gln Ile Phe Ala Leu Ile Thr Pro Leu Phe Phe Gln Val Val
 195 200 205
 Met Asp Lys Val Leu Val His Arg Gly Phe Ser Thr Leu Asp Val Val
 210 215 220
 Ala Ile Ala Leu Leu Val Val Ser Leu Phe Glu Val Ile Leu Ser Gly
 225 230 235 240
 Leu Arg Thr Tyr Ile Phe Ala His Thr Thr Ser Arg Ile Asp Val Glu
 245 250 255
 Leu Gly Ala Arg Leu Phe Arg His Leu Leu Ala Leu Pro Leu Ala Tyr
 260 265 270
 Phe Glu Ser Arg Arg Val Gly Asp Thr Val Ala Arg Ile Arg Glu Leu
 275 280 285
 Glu His Ile Arg Asn Phe Leu Thr Gly Gln Ala Leu Thr Ser Val Leu
 290 295 300
 Asp Leu Val Phe Ser Phe Ile Phe Leu Phe Val Met Trp Tyr Tyr Ser
 305 310 315 320
 Pro Thr Leu Thr Leu Val Val Leu Ala Ser Leu Pro Ile Tyr Ala Phe
 325 330 335
 Trp Ser Ala Phe Ile Ser Pro Ile Leu Arg Thr Arg Leu Asn Asp Gln
 340 345 350
 Phe Ala Arg Asn Ala Asp Asn Gln Ser Phe Leu Val Glu Ser Ile Thr
 355 360 365
 Ala Val Gly Thr Val Lys Ala Met Ala Val Glu Pro Gln Met Thr Arg
 370 375 380
 Arg Trp Asp Asn Gln Leu Ala Ala Tyr Val Val Ser Ser Phe Arg Val
 385 390 395 400
 Ala Lys Leu Ala Met Val Gly Gln Gln Gly Val Gln Leu Ile Gln Lys
 405 410 415
 Met Val Ile Val Ala Thr Leu Trp Ile Gly Ala Lys Leu Val Ile Glu
 420 425 430
 Gly Lys Leu Ser Val Gly Gln Leu Ile Ala Phe Asn Met Leu Ala Gly
 435 440 445
 Gln Val Ala Ala Pro Val Ile Arg Leu Ala Gln Leu Trp Gln Asp Phe
 450 455 460
 Gln Gln Val Gly Ile Ser Val Ala Arg Leu Gly Asp Ile Leu Asn Thr
 465 470 475 480
 Pro Thr Glu His Ser Thr Ser Arg Leu Thr Leu Pro Asp Ile Lys Gly
 485 490 495

23

Asp Ile Thr Phe Glu Asn Val Asp Phe Arg Tyr Lys Ile Asp Gly His
 500 505 510
 Leu Ile Leu Gln Asn Leu Asn Leu Gln Ile Asn Ala Gly Glu Ile Leu
 515 520 525
 Gly Ile Val Gly Arg Ser Gly Ser Gly Lys Ser Thr Leu Thr Lys Leu
 530 535 540
 Val Gln Arg Leu Tyr Val Pro Glu Asn Gly Arg Ile Leu Val Asp Gly
 545 550 555 560
 Asn Asp Leu Ala Leu Ala Asp Pro Ala Trp Leu Arg Arg Gln Val Gly
 565 570 575
 Val Val Leu Gln Glu Asn Val Leu Leu Asn Arg Ser Ile Arg Asp Asn
 580 585 590
 Ile Ala Leu Thr Asp Thr Gly Met Ser Leu Glu Phe Ile Ile Gln Ala
 595 600 605
 Ala Lys Met Ser Gly Ala His Asp Phe Ile Met Glu Leu Pro Glu Gly
 610 615 620
 Tyr Asp Thr Ile Val Gly Glu Gln Gly Ala Gly Leu Ser Gly Gly Gln
 625 630 635 640
 Arg Gln Arg Ile Ala Ile Ala Arg Ala Leu Ile Thr Asn Pro Arg Ile
 645 650 655
 Leu Ile Phe Asp Glu Ala Thr Ser Ala Leu Asp Tyr Glu Ser Glu Arg
 660 665 670
 Ala Ile Met Gln Asn Met Gln Ala Ile Cys Gln Gly Arg Thr Val Leu
 675 680 685
 Ile Ile Ala His Arg Leu Ser Thr Val Lys Met Ala His Arg Ile Ile
 690 695 700
 Ala Met Asp Lys Gly Lys Ile Val Glu Gln Gly Thr His Gln Glu Leu
 705 710 715 720
 Leu Gln Lys Glu Asp Gly Tyr Tyr Arg Tyr Leu Tyr Asp Leu Gln Asn
 725 730 735

Gly

<210> 19

<211> 708

<212> PRT

<213> Pasteurella haemolytica

<400> 19

Met Glu Ala Asn His Gln Arg Asn Asp Leu Gly Leu Val Ala Leu Thr
 1 5 10 15

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Met Leu Ala Gln Tyr His Asn Ile Ser Leu Asn Pro Glu Glu Ile Lys
      20                      25                      30

His Lys Phe Asp Leu Asp Gly Lys Gly Leu Ser Leu Thr Ala Trp Leu
      35                      40                      45

Leu Ala Ala Lys Ser Leu Ala Leu Lys Ala Lys His Ile Lys Lys Glu
      50                      55                      60

Ile Ser Arg Leu His Leu Val Asn Leu Pro Ala Leu Val Thr Gln Asp
      65                      70                      75                      80

Asn Gly Lys His Phe Leu Leu Val Lys Val Asp Thr Asp Asn Asn Arg
      85                      90                      95

Tyr Leu Thr Tyr Asn Leu Glu Gln Asp Ala Pro Gln Ile Leu Ser Thr
      100                     105                     110

Asp Glu Phe Glu Ala Cys Tyr Gln Gly Gln Leu Ile Leu Val Thr Ser
      115                     120                     125

Arg Ala Ser Val Val Gly Gln Leu Ala Lys Phe Asp Phe Thr Trp Phe
      130                     135                     140

Ile Pro Ala Val Ile Lys Tyr Arg Lys Ile Phe Leu Glu Thr Leu Ile
      145                     150                     155                     160

Val Ser Ile Phe Leu Gln Ile Phe Ala Leu Ile Thr Pro Leu Phe Phe
      165                     170                     175

Gln Val Val Met Asp Lys Val Leu Val His Arg Gly Phe Ser Thr Leu
      180                     185                     190

Asn Ile Ile Thr Val Ala Leu Ala Ile Val Ile Ile Phe Glu Ile Val
      195                     200                     205

Leu Ser Gly Leu Arg Thr Tyr Val Phe Ser His Ser Thr Ser Arg Ile
      210                     215                     220

Asp Val Glu Leu Gly Ala Lys Leu Phe Arg His Leu Leu Ser Leu Pro
      225                     230                     235                     240

Ile Ser Tyr Phe Glu Asn Arg Arg Val Gly Asp Thr Val Ala Arg Val
      245                     250                     255

Arg Glu Leu Asp Gln Ile Arg Asn Phe Leu Thr Gly Gln Ala Leu Thr
      260                     265                     270

Ser Val Leu Asp Leu Leu Phe Ser Phe Ile Phe Phe Ala Val Met Trp
      275                     280                     285

Tyr Tyr Ser Pro Lys Leu Thr Leu Val Ile Leu Gly Ser Leu Pro Cys
      290                     295                     300

Tyr Ile Leu Trp Ser Ile Phe Ile Ser Pro Ile Leu Arg Arg Arg Leu
      305                     310                     315                     320

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Asp Glu Lys Phe Ala Arg Ser Ala Asp Asn Gln Ala Phe Leu Val Glu
 325 330 335
 Ser Val Thr Ala Ile Asn Met Ile Lys Ala Met Ala Val Ala Pro Gln
 340 345 350
 Met Thr Asp Thr Trp Asp Lys Gln Leu Ala Ser Tyr Val Ser Ser Ser
 355 360 365
 Phe Arg Val Thr Val Leu Ala Thr Ile Gly Gln Gln Gly Val Gln Leu
 370 375 380
 Ile Gln Lys Thr Val Met Val Ile Asn Leu Trp Leu Gly Ala His Leu
 385 390 395 400
 Val Ile Ser Gly Asp Leu Ser Ile Gly Gln Leu Ile Ala Phe Asn Met
 405 410 415
 Leu Ser Gly Gln Val Ile Ala Pro Val Ile Arg Leu Ala Gln Leu Trp
 420 425 430
 Gln Asp Phe Gln Gln Val Gly Ile Ser Val Thr Arg Leu Gly Asp Val
 435 440 445
 Leu Asn Ser Pro Thr Glu Gln Tyr Gln Gly Lys Leu Ser Leu Pro Glu
 450 455 460
 Ile Lys Gly Asp Ile Ser Phe Lys Asn Ile Arg Phe Arg Tyr Lys Pro
 465 470 475 480
 Asp Ala Pro Thr Ile Leu Asn Asn Val Asn Leu Glu Ile Arg Gln Gly
 485 490 495
 Glu Val Ile Gly Ile Val Gly Arg Ser Gly Ser Gly Lys Ser Thr Leu
 500 505 510
 Thr Lys Leu Leu Gln Arg Phe Tyr Ile Pro Glu Asn Gly Gln Val Leu
 515 520 525
 Ile Asp Gly His Asp Leu Ala Leu Ala Asp Pro Asn Trp Leu Arg Arg
 530 535 540
 Gln Ile Gly Val Val Leu Gln Asp Asn Val Leu Leu Asn Arg Ser Ile
 545 550 555 560
 Arg Glu Asn Ile Ala Leu Ser Asp Pro Gly Met Pro Met Glu Arg Val
 565 570 575
 Ile Tyr Ala Ala Lys Leu Ala Gly Ala His Asp Phe Ile Ser Glu Leu
 580 585 590
 Arg Glu Gly Tyr Asn Thr Ile Val Gly Glu Gln Gly Ala Gly Leu Ser
 595 600 605
 Gly Gly Gln Arg Gln Arg Ile Ala Ile Ala Arg Ala Leu Val Asn Asn
 610 615 620

26

Pro Lys Ile Leu Ile Phe Asp Glu Ala Thr Ser Ala Leu Asp Tyr Glu
 625 630 635 640

Ser Glu His Ile Ile Met Gln Asn Met Gln Lys Ile Cys Gln Gly Arg
 645 650 655

Thr Val Ile Leu Ile Ala His Arg Leu Ser Thr Val Lys Asn Ala Asp
 660 665 670

Arg Ile Ile Val Met Glu Lys Gly Glu Ile Val Glu Gln Gly Lys His
 675 680 685

His Glu Leu Leu Gln Asn Ser Asn Gly Leu Tyr Ser Tyr Leu His Gln
 690 695 700

Leu Gln Leu Asn
 705

<210> 20

<211> 707

<212> PRT

<213> Actinobacillus pleuropneumoniae

<400> 20

Met Asp Phe Tyr Arg Glu Glu Asp Tyr Gly Leu Tyr Ala Leu Thr Ile
 1 5 10 15

Leu Ala Gln Tyr His Asn Ile Ala Val Asn Pro Glu Glu Leu Lys His
 20 25 30

Lys Phe Asp Leu Glu Gly Lys Gly Leu Asp Leu Thr Ala Trp Leu Leu
 35 40 45

Ala Ala Lys Ser Leu Glu Leu Lys Ala Lys Gln Val Lys Lys Ala Ile
 50 55 60

Asp Arg Leu Ala Phe Ile Ala Leu Pro Ala Leu Val Trp Arg Glu Asp
 65 70 75 80

Gly Lys His Phe Ile Leu Thr Lys Ile Asp Asn Glu Ala Lys Lys Tyr
 85 90 95

Leu Ile Phe Asp Leu Glu Thr His Asn Pro Arg Ile Leu Glu Gln Ala
 100 105 110

Glu Phe Glu Ser Leu Tyr Gln Gly Lys Leu Ile Leu Val Ala Ser Arg
 115 120 125

Ala Ser Ile Val Gly Lys Leu Ala Lys Phe Asp Phe Thr Trp Phe Ile
 130 135 140

Pro Ala Val Ile Lys Tyr Arg Lys Ile Phe Ile Glu Thr Leu Ile Val
 145 150 155 160

Ser Ile Phe Leu Gln Ile Phe Ala Leu Ile Thr Pro Leu Phe Phe Gln
 165 170 175

Val Val Met Asp Lys Val Leu Val His Arg Gly Phe Ser Thr Leu Asn
 180 185 190
 Val Ile Thr Val Ala Leu Ala Ile Val Val Leu Phe Glu Ile Val Leu
 195 200 205
 Asn Gly Leu Arg Thr Tyr Ile Phe Ala His Ser Thr Ser Arg Ile Asp
 210 215 220
 Val Glu Leu Gly Ala Arg Leu Phe Arg His Leu Leu Ala Leu Pro Ile
 225 230 235 240
 Ser Tyr Phe Glu Asn Arg Arg Val Gly Asp Thr Val Ala Arg Val Arg
 245 250 255
 Glu Leu Asp Gln Ile Arg Asn Phe Leu Thr Gly Gln Ala Leu Thr Ser
 260 265 270
 Val Leu Asp Leu Met Phe Ser Phe Ile Phe Phe Ala Val Met Trp Tyr
 275 280 285
 Tyr Ser Pro Lys Leu Thr Leu Val Ile Leu Gly Ser Leu Pro Phe Tyr
 290 295 300
 Met Gly Trp Ser Ile Phe Ile Ser Pro Ile Leu Arg Arg Arg Leu Asp
 305 310 315 320
 Glu Lys Phe Ala Arg Gly Ala Asp Asn Gln Ser Phe Leu Val Glu Ser
 325 330 335
 Val Thr Ala Ile Asn Thr Ile Lys Ala Leu Ala Val Thr Pro Gln Met
 340 345 350
 Thr Asn Thr Trp Asp Lys Gln Leu Ala Ser Tyr Val Ser Ala Gly Phe
 355 360 365
 Arg Val Thr Thr Leu Ala Thr Ile Gly Gln Gln Gly Val Gln Phe Ile
 370 375 380
 Gln Lys Val Val Met Val Ile Thr Leu Trp Leu Gly Ala His Leu Val
 385 390 395 400
 Ile Ser Gly Asp Leu Ser Ile Gly Gln Leu Ile Ala Phe Asn Met Leu
 405 410 415
 Ser Gly Gln Val Ile Ala Pro Val Ile Arg Leu Ala Gln Leu Trp Gln
 420 425 430
 Asp Phe Gln Gln Val Gly Ile Ser Val Thr Arg Leu Gly Asp Val Leu
 435 440 445
 Asn Ser Pro Thr Glu Ser Tyr Gln Gly Lys Leu Ala Leu Pro Glu Ile
 450 455 460
 Lys Gly Asp Ile Thr Phe Arg Asn Ile Arg Phe Arg Tyr Lys Pro Asp
 465 470 475 480

28

Ala Pro Val Ile Leu Asn Asp Val Asn Leu Ser Ile Gln Gln Gly Glu
 485 490 495

Val Ile Gly Ile Val Gly Arg Ser Gly Ser Gly Lys Ser Thr Leu Thr
 500 505 510

Lys Leu Ile Gln Arg Phe Tyr Ile Pro Glu Asn Gly Gln Val Leu Ile
 515 520 525

Asp Gly His Asp Leu Ala Leu Ala Asp Pro Asn Trp Leu Arg Arg Gln
 530 535 540

Val Gly Val Val Leu Gln Asp Asn Val Leu Leu Asn Arg Ser Ile Arg
 545 550 555 560

Asp Asn Ile Ala Leu Ala Asp Pro Gly Met Pro Met Glu Lys Ile Val
 565 570 575

His Ala Ala Lys Leu Ala Gly Ala His Glu Phe Ile Ser Glu Leu Arg
 580 585 590

Glu Gly Tyr Asn Thr Ile Val Gly Glu Gln Gly Ala Gly Leu Ser Gly
 595 600 605

Gly Gln Arg Gln Arg Ile Ala Ile Ala Arg Ala Leu Val Asn Asn Pro
 610 615 620

Lys Ile Leu Ile Phe Asp Glu Ala Thr Ser Ala Leu Asp Tyr Glu Ser
 625 630 635 640

Glu His Ile Ile Met Arg Asn Met His Gln Ile Cys Lys Gly Arg Thr
 645 650 655

Val Ile Ile Ile Ala His Arg Leu Ser Thr Val Lys Asn Ala Asp Arg
 660 665 670

Ile Ile Val Met Glu Lys Gly Gln Ile Val Glu Gln Gly Lys His Lys
 675 680 685

Glu Leu Leu Ala Asp Pro Asn Gly Leu Tyr His Tyr Leu His Gln Leu
 690 695 700

Gln Ser Glu
 705

<210> 21

<211> 707

<212> PRT

<213> Escherichia coli

<400> 21

Met Asp Ser Cys His Lys Ile Asp Tyr Gly Leu Tyr Ala Leu Glu Ile
 1 5 10 15

Leu Ala Gln Tyr His Asn Val Ser Val Asn Pro Glu Glu Ile Lys His
 20 25 30

Arg Phe Asp Thr Asp Gly Thr Gly Leu Gly Leu Thr Ser Trp Leu Leu
 35 40 45
 Ala Ala Lys Ser Leu Glu Leu Lys Val Lys Gln Val Lys Lys Thr Ile
 50 55 60
 Asp Arg Leu Asn Phe Ile Ser Leu Pro Ala Leu Val Trp Arg Glu Asp
 65 70 75 80
 Gly Arg His Phe Ile Leu Thr Lys Val Ser Lys Glu Ala Asn Arg Tyr
 85 90 95
 Leu Ile Phe Asp Leu Glu Gln Arg Asn Pro Arg Val Leu Glu Gln Ser
 100 105 110
 Glu Phe Glu Ala Leu Tyr Gln Gly His Ile Ile Leu Ile Ala Ser Arg
 115 120 125
 Ser Ser Val Ala Gly Lys Leu Ala Lys Phe Asp Phe Thr Trp Phe Ile
 130 135 140
 Pro Ala Ile Ile Lys Tyr Arg Arg Ile Phe Ile Glu Thr Leu Val Val
 145 150 155 160
 Ser Val Phe Leu Gln Leu Phe Ala Leu Ile Thr Pro Leu Phe Phe Gln
 165 170 175
 Val Val Met Asp Lys Val Leu Val His Arg Gly Phe Ser Thr Leu Asn
 180 185 190
 Val Ile Thr Val Ala Leu Ser Val Val Val Val Phe Glu Ile Ile Leu
 195 200 205
 Ser Gly Leu Arg Thr Tyr Ile Phe Ala His Ser Thr Ser Arg Ile Asp
 210 215 220
 Val Glu Leu Gly Ala Lys Leu Phe Arg His Leu Leu Ala Leu Pro Ile
 225 230 235 240
 Ser Tyr Phe Glu Ser Arg Arg Val Gly Asp Thr Val Ala Arg Val Arg
 245 250 255
 Glu Leu Asp Gln Ile Arg Asn Phe Leu Thr Gly Gln Ala Leu Thr Ser
 260 265 270
 Val Leu Asp Leu Leu Phe Ser Phe Ile Phe Phe Ala Val Met Trp Tyr
 275 280 285
 Tyr Ser Pro Lys Leu Thr Leu Val Ile Leu Phe Ser Leu Pro Cys Tyr
 290 295 300
 Ala Ala Trp Ser Val Phe Ile Ser Pro Ile Leu Arg Arg Arg Leu Asp
 305 310 315 320
 Asp Lys Phe Ser Arg Asn Ala Asp Asn Gln Ser Phe Leu Val Glu Ser
 325 330 335

Val Thr Ala Ile Asn Thr Ile Lys Ala Met Ala Val Ser Pro Gln Met
 340 345 350
 Thr Asn Ile Trp Asp Lys Gln Leu Ala Gly Tyr Val Ala Ala Gly Phe
 355 360 365
 Lys Val Thr Val Leu Ala Thr Ile Gly Gln Gln Gly Ile Gln Leu Ile
 370 375 380
 Gln Lys Thr Val Met Ile Ile Asn Leu Trp Leu Gly Ala His Leu Val
 385 390 395 400
 Ile Ser Gly Asp Leu Ser Ile Gly Gln Leu Ile Ala Phe Asn Met Leu
 405 410 415
 Ala Gly Gln Ile Val Ala Pro Val Ile Arg Leu Ala Gln Ile Trp Gln
 420 425 430
 Asp Phe Gln Gln Val Gly Ile Ser Val Thr Arg Leu Gly Asp Val Leu
 435 440 445
 Asn Ser Pro Thr Glu Ser Tyr His Gly Lys Leu Ala Leu Pro Glu Ile
 450 455 460
 Asn Gly Asp Ile Thr Phe Arg Asn Ile Arg Phe Arg Tyr Lys Pro Asp
 465 470 475 480
 Ser Pro Val Ile Leu Asp Asn Ile Asn Leu Ser Ile Lys Gln Gly Glu
 485 490 495
 Val Ile Gly Ile Val Gly Arg Ser Gly Ser Gly Lys Ser Thr Leu Thr
 500 505 510
 Lys Leu Ile Gln Arg Phe Tyr Ile Pro Glu Asn Gly Gln Val Leu Ile
 515 520 525
 Asp Gly His Asp Leu Ala Leu Ala Asp Pro Asn Trp Leu Arg Arg Gln
 530 535 540
 Val Gly Val Val Leu Gln Asp Asn Val Leu Leu Asn Arg Ser Ile Ile
 545 550 555 560
 Asp Asn Ile Ser Leu Ala Asn Pro Gly Met Ser Val Glu Lys Val Ile
 565 570 575
 Tyr Ala Ala Lys Leu Ala Gly Ala His Asp Phe Ile Ser Glu Leu Arg
 580 585 590
 Glu Gly Tyr Asn Thr Ile Val Gly Glu Gln Gly Ala Gly Leu Ser Gly
 595 600 605
 Gly Gln Arg Gln Arg Ile Ala Ile Ala Arg Ala Leu Val Asn Asn Pro
 610 615 620
 Lys Ile Leu Ile Phe Asp Glu Ala Thr Ser Ala Leu Asp Tyr Glu Ser
 625 630 635 640

31

Glu His Ile Ile Met Arg Asn Met His Lys Ile Cys Lys Gly Arg Thr
 645 650 655

Val Ile Ile Ile Ala His Arg Leu Ser Thr Val Lys Asn Ala Asp Arg
 660 665 670

Ile Ile Val Met Glu Lys Gly Lys Ile Val Glu Gln Gly Lys His Lys
 675 680 685

Glu Leu Leu Ser Glu Pro Glu Ser Leu Tyr Ser Tyr Leu Tyr Gln Leu
 690 695 700

Gln Ser Asp
 705

<210> 22
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer

<400> 22
 aatgacgata tctttgttgg tcaaggtaaa

30

<210> 23
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer

<220>
 <221> modified_base
 <222> (18)
 <223> a, t, c, g, other or unknown

<400> 23
 aayaaagart trgargcnga r

21

<210> 24
 <211> 32
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer

<220>
 <221> modified_base
 <222> (6)
 <223> a, t, c, g, other or unknown

32

<400> 24
ccytncrcrc trtgraadat rtctttraat tt

32

<210> 25
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<220>
<221> modified_base
<222> (15)
<223> a, t, c, g, other or unknown

<220>
<221> modified_base
<222> (18)
<223> a, t, c, g, other or unknown

<220>
<221> modified_base
<222> (24)
<223> a, t, c, g, other or unknown

<400> 25
athgaytgga thgcnccntt yggngay

27

<210> 26
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 26
actttatcca tcacracttg raaraa

26

<210> 27
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 27
gatcatatgt ccaatataaa tgtaattaaa tctaa

35

<210> 28
<211> 30

33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 28

atcactagtt ccataatcta taaccaatga

30

<210> 29

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: consensus
sequence

<220>

<221> MOD_RES

<222> (1)..(2)

<223> Any amino acid

<220>

<221> MOD_RES

<222> (5)

<223> Any amino acid

<220>

<221> MOD_RES

<222> (7)

<223> N or D

<220>

<221> MOD_RES

<222> (9)

<223> Any amino acid

<400> 29

Xaa Xaa Gly Gly Xaa Gly Xaa Asp Xaa

1

5

<210> 30

<211> 2215

<212> DNA

<213> Moraxella bovis

<220>

<221> CDS

<222> (1)..(2211)

<400> 30

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Met Gly Gly Asp Thr Ser Leu Ile Arg Leu Asn Leu Gln Thr Leu Asn

1

5

10

15

34

agt aat tta gtt atg ata gat tat gct caa caa cct gct cta tct gct Ser Asn Leu Val Met Ile Asp Tyr Ala Gln Gln Pro Ala Leu Ser Ala	96
20 25 30	
ctg gtt atc ctt gcc aaa tac tat ggt att tct gca agt cca gca gac Leu Val Ile Leu Ala Lys Tyr Tyr Gly Ile Ser Ala Ser Pro Ala Asp	144
35 40 45	
att atg cat cag ttt tct gat aat aca aaa gga gac ctg aat gaa att Ile Met His Gln Phe Ser Asp Asn Thr Lys Gly Asp Leu Asn Glu Ile	192
50 55 60	
gaa tgg atg ttg gca gca aag aaa tta gaa tta aag gta aag att ata Glu Trp Met Leu Ala Ala Lys Lys Leu Glu Leu Lys Val Lys Ile Ile	240
65 70 75 80	
aaa cag cct tta act cga ttg tca atg ata aca ctt cct gct ttg gtg Lys Gln Pro Leu Thr Arg Leu Ser Met Ile Thr Leu Pro Ala Leu Val	288
85 90 95	
tgg tgt gat aat aag ccc gat tta gat caa aat tta aac tct cat ttt Trp Cys Asp Asn Lys Pro Asp Leu Asp Gln Asn Leu Asn Ser His Phe	336
100 105 110	
ata cta act aaa att gat ggg gtg gga tct gct gca aaa tat ctc atc Ile Leu Thr Lys Ile Asp Gly Val Gly Ser Ala Ala Lys Tyr Leu Ile	384
115 120 125	
tac gat ttg att gag aat cgt ccc ata ata tta gat gca agt gag ttt Tyr Asp Leu Ile Glu Asn Arg Pro Ile Ile Leu Asp Ala Ser Glu Phe	432
130 135 140	
tct gaa aga tat tct ggt aag tta atg cta gta act tcc cgt gcg tca Ser Glu Arg Tyr Ser Gly Lys Leu Met Leu Val Thr Ser Arg Ala Ser	480
145 150 155 160	
ata ttg ggt tca ttg gct aaa ttt gat ttt act tgg ttt att cct gcg Ile Leu Gly Ser Leu Ala Lys Phe Asp Phe Thr Trp Phe Ile Pro Ala	528
165 170 175	
gta atc aaa tat cgt tat att ttt ttt gaa gtc atc gtt att tca gtg Val Ile Lys Tyr Arg Tyr Ile Phe Phe Glu Val Ile Val Ile Ser Val	576
180 185 190	
gtg cta cag att ttt gct ctg att acg cca ttg ttt ttt cag gtt gtg Val Leu Gln Ile Phe Ala Leu Ile Thr Pro Leu Phe Phe Gln Val Val	624
195 200 205	
atg gat aag gta ttg gtg cat cgt ggt ttt tct act ctg gat gtg gta Met Asp Lys Val Leu Val His Arg Gly Phe Ser Thr Leu Asp Val Val	672
210 215 220	
gcg att gcc ttg ttg gta gta agt tta ttt gaa gtc att tta agt ggt Ala Ile Ala Leu Leu Val Val Ser Leu Phe Glu Val Ile Leu Ser Gly	720
225 230 235 240	

35

cta cgc act tat att ttt gct cat aca acc tct cga att gat gta gag	768
Leu Arg Thr Tyr Ile Phe Ala His Thr Thr Ser Arg Ile Asp Val Glu	
245 250 255	
cta gga gca cga tta ttt cgt cat cta tta gct cta ccg ctt gct tat	816
Leu Gly Ala Arg Leu Phe Arg His Leu Leu Ala Leu Pro Leu Ala Tyr	
260 265 270	
ttt gag agt aga aga gta ggc gat aca gtt gca cgt ata cgt gaa ttg	864
Phe Glu Ser Arg Arg Val Gly Asp Thr Val Ala Arg Ile Arg Glu Leu	
275 280 285	
gaa cat atc cgc aat ttc tta act ggt caa gct ctc act tca gtt tta	912
Glu His Ile Arg Asn Phe Leu Thr Gly Gln Ala Leu Thr Ser Val Leu	
290 295 300	
gat ttg gtg ttt tct ttt ata ttc ttg ttt gta atg tgg tat tac agc	960
Asp Leu Val Phe Ser Phe Ile Phe Leu Phe Val Met Trp Tyr Tyr Ser	
305 310 315 320	
cct act tta aca ctg gta gtt ttg gca tca tta cca ata tat gcg ttt	1008
Pro Thr Leu Thr Leu Val Val Leu Ala Ser Leu Pro Ile Tyr Ala Phe	
325 330 335	
tgg tct gcc ttt att agc cca att tta cgc act cga cta aat gat caa	1056
Trp Ser Ala Phe Ile Ser Pro Ile Leu Arg Thr Arg Leu Asn Asp Gln	
340 345 350	
ttt gca cgc aat gca gat aat caa tct ttt tta gtg gaa agt att act	1104
Phe Ala Arg Asn Ala Asp Asn Gln Ser Phe Leu Val Glu Ser Ile Thr	
355 360 365	
gcg gtt ggt acg gta aaa gca atg gca gtt gaa cct caa atg acc cgt	1152
Ala Val Gly Thr Val Lys Ala Met Ala Val Glu Pro Gln Met Thr Arg	
370 375 380	
cgc tgg gat aat caa tta gca gct tat gtg gtt tct agt ttt cgg gta	1200
Arg Trp Asp Asn Gln Leu Ala Ala Tyr Val Val Ser Ser Phe Arg Val	
385 390 395 400	
gct aag ttg gca atg gtt ggg cag caa gga gta caa ctc att caa aag	1248
Ala Lys Leu Ala Met Val Gly Gln Gln Gly Val Gln Leu Ile Gln Lys	
405 410 415	
atg gtt att gtg gca act cta tgg att ggt gca aaa ttg gta att gaa	1296
Met Val Ile Val Ala Thr Leu Trp Ile Gly Ala Lys Leu Val Ile Glu	
420 425 430	
ggc aag cta tcg gta ggt caa tta ata gca ttt aat atg ctg gca ggt	1344
Gly Lys Leu Ser Val Gly Gln Leu Ile Ala Phe Asn Met Leu Ala Gly	
435 440 445	
cag gtg gcc gct cct gtt atc cgc ctg gca cag cta tgg caa gat ttt	1392
Gln Val Ala Ala Pro Val Ile Arg Leu Ala Gln Leu Trp Gln Asp Phe	
450 455 460	

36

cag	caa	gta	ggg	att	tca	gtg	gcg	aga	ttg	ggg	gat	att	tta	aat	act	1440
Gln	Gln	Val	Gly	Ile	Ser	Val	Ala	Arg	Leu	Gly	Asp	Ile	Leu	Asn	Thr	
465					470					475					480	
cca	act	gag	cat	tct	aca	tct	cgc	tta	act	tta	cct	gat	att	aag	ggg	1488
Pro	Thr	Glu	His	Ser	Thr	Ser	Arg	Leu	Thr	Leu	Pro	Asp	Ile	Lys	Gly	
				485					490					495		
gat	att	aca	ttt	gaa	aat	gtt	gat	ttt	cgc	tac	aaa	ata	gat	ggg	cat	1536
Asp	Ile	Thr	Phe	Glu	Asn	Val	Asp	Phe	Arg	Tyr	Lys	Ile	Asp	Gly	His	
			500					505					510			
tta	ata	tta	cag	aat	tta	aat	tta	cag	att	aac	gct	gga	gag	ata	cta	1584
Leu	Ile	Leu	Gln	Asn	Leu	Asn	Leu	Gln	Ile	Asn	Ala	Gly	Glu	Ile	Leu	
		515					520					525				
ggg	atc	gta	gga	cgc	tct	ggg	tca	ggg	aaa	tca	aca	ttg	aca	aaa	tta	1632
Gly	Ile	Val	Gly	Arg	Ser	Gly	Ser	Gly	Lys	Ser	Thr	Leu	Thr	Lys	Leu	
	530					535					540					
gta	cag	cgt	tta	tat	gta	cca	gaa	aat	ggg	cga	ata	tta	gtt	gat	gga	1680
Val	Gln	Arg	Leu	Tyr	Val	Pro	Glu	Asn	Gly	Arg	Ile	Leu	Val	Asp	Gly	
545					550					555					560	
aac	gat	ttg	gca	tta	gct	gat	ccc	gct	tggt	ctg	cgt	cgc	caa	gtg	ggg	1728
Asn	Asp	Leu	Ala	Leu	Ala	Asp	Pro	Ala	Trp	Leu	Arg	Arg	Gln	Val	Gly	
			565					570						575		
gtt	gtt	ttg	cag	gaa	aat	gtg	tta	ctc	aat	cgt	agt	att	cga	gat	aat	1776
Val	Val	Leu	Gln	Glu	Asn	Val	Leu	Leu	Asn	Arg	Ser	Ile	Arg	Asp	Asn	
		580					585						590			
att	gcc	cta	act	gat	acg	ggc	atg	tca	tta	gag	ttt	att	atc	cag	gct	1824
Ile	Ala	Leu	Thr	Asp	Thr	Gly	Met	Ser	Leu	Glu	Phe	Ile	Ile	Gln	Ala	
		595					600					605				
gcc	aag	atg	tct	ggg	gca	cat	gac	ttt	att	atg	gaa	ttg	cct	gag	ggg	1872
Ala	Lys	Met	Ser	Gly	Ala	His	Asp	Phe	Ile	Met	Glu	Leu	Pro	Glu	Gly	
	610					615					620					
tat	gat	acg	att	gtt	gga	gag	caa	ggg	gca	ggc	ttg	tca	ggg	gga	caa	1920
Tyr	Asp	Thr	Ile	Val	Gly	Glu	Gln	Gly	Ala	Gly	Leu	Ser	Gly	Gly	Gln	
625					630					635					640	
cgc	cag	cgt	atc	gct	att	gcg	cgt	gct	tta	att	acc	aat	ccg	cgt	att	1968
Arg	Gln	Arg	Ile	Ala	Ile	Ala	Arg	Ala	Leu	Ile	Thr	Asn	Pro	Arg	Ile	
			645						650					655		
ttg	att	ttt	gat	gaa	gct	act	agt	gca	tta	gac	tat	gag	tcg	gaa	agg	2016
Leu	Ile	Phe	Asp	Glu	Ala	Thr	Ser	Ala	Leu	Asp	Tyr	Glu	Ser	Glu	Arg	
			660					665					670			
gct	att	atg	caa	aat	atg	cag	gca	att	tgc	caa	ggg	aga	aca	gtg	ttg	2064
Ala	Ile	Met	Gln	Asn	Met	Gln	Ala	Ile	Cys	Gln	Gly	Arg	Thr	Val	Leu	
		675					680					685				

37

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att att gca cat cgc tta tct acc gta aaa atg gca cat cgc att att 2112
Ile Ile Ala His Arg Leu Ser Thr Val Lys Met Ala His Arg Ile Ile
690 695 700

gca atg gac aag ggg aaa att gta gag caa ggc aca cat caa gaa ttg 2160
Ala Met Asp Lys Gly Lys Ile Val Glu Gln Gly Thr His Gln Glu Leu
705 710 715 720

ttg caa aaa gaa gat ggt tac tat cgt tat tta tat gat ttg cag aat 2208
Leu Gln Lys Glu Asp Gly Tyr Tyr Arg Tyr Leu Tyr Asp Leu Gln Asn
725 730 735

gga taaa 2215
Gly

<210> 31
<211> 507
<212> DNA
<213> Moraxella bovis

<220>
<221> CDS
<222> (1)..(504)

<400> 31
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Met Thr Lys Lys Phe Ala Glu Leu Gly Leu Ile Ala Trp Leu Trp Ser
1 5 10 15

aac tct gat atg cat aaa cat tgg acg ttg tct ttg ttt gcg acc aat 96
Asn Ser Asp Met His Lys His Trp Thr Leu Ser Leu Phe Ala Thr Asn
20 25 30

gtt att ccg gca att gag aca ggt caa tat gtt ata ttg aaa aga gaa 144
Val Ile Pro Ala Ile Glu Thr Gly Gln Tyr Val Ile Leu Lys Arg Glu
35 40 45

gat atg cct gta gca tat tgt agt tgg gct aaa ctt agt tta gaa aac 192
Asp Met Pro Val Ala Tyr Cys Ser Trp Ala Lys Leu Ser Leu Glu Asn
50 55 60

gag gtt aaa tat att aac gat gtt act tct ctt aag tta gat gac tgg 240
Glu Val Lys Tyr Ile Asn Asp Val Thr Ser Leu Lys Leu Asp Asp Trp
65 70 75 80

cag tca ggt gac cga aac tgg ttt att gac tgg att gct cca ttt ggc 288
Gln Ser Gly Asp Arg Asn Trp Phe Ile Asp Trp Ile Ala Pro Phe Gly
85 90 95

gat agt ctt aca ctc aca aaa cac atg aga acg tta ttt tca gat gaa 336
Asp Ser Leu Thr Leu Thr Lys His Met Arg Thr Leu Phe Ser Asp Glu
100 105 110

ttg ttt aga gcg att cgt gta gat gga aat tca tcg cat ggt aag ata 384
Leu Phe Arg Ala Ile Arg Val Asp Gly Asn Ser Ser His Gly Lys Ile
115 120 125

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38

tct gaa ttt tat gga aag tct gtt gat tca aaa tta gcc tca aga ata 432
 Ser Glu Phe Tyr Gly Lys Ser Val Asp Ser Lys Leu Ala Ser Arg Ile
 130 135 140

ttt gca caa tat cac gaa gat ttg acg agc aaa ttg tca act cag aat 480
 Phe Ala Gln Tyr His Glu Asp Leu Thr Ser Lys Leu Ser Thr Gln Asn
 145 150 155 160

aat ttt att ata tct aaa gat aat taa 507
 Asn Phe Ile Ile Ser Lys Asp Asn
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<210> 32

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<213> Moraxella bovis

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Asp Met Pro Val Ala Tyr Cys Ser Trp Ala Lys Leu Ser Leu Glu Asn
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Glu Val Lys Tyr Ile Asn Asp Val Thr Ser Leu Lys Leu Asp Asp Trp
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Gln Ser Gly Asp Arg Asn Trp Phe Ile Asp Trp Ile Ala Pro Phe Gly
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Asp Ser Leu Thr Leu Thr Lys His Met Arg Thr Leu Phe Ser Asp Glu
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Leu Phe Arg Ala Ile Arg Val Asp Gly Asn Ser Ser His Gly Lys Ile
 115 120 125

Ser Glu Phe Tyr Gly Lys Ser Val Asp Ser Lys Leu Ala Ser Arg Ile
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<213> Pasteurella haemolytica

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Trp Gln Ser Gly Asp Arg Arg Trp Ile Ile Asp Trp Val Ala Pro Phe
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Gly His Ser Gln Leu Leu Tyr Lys Lys Met Cys Gln Lys Tyr Pro Asp
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Lys Ile Ala Tyr Phe Lys Gly Gly Lys Leu Asp Lys Lys Thr Ala Lys
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Glu Asn Glu Ile Lys Tyr Leu Asn Asp Val Thr Ser Leu Val Ala Glu
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 Thr Pro Val Ser Arg Ser Ser Lys Trp Thr Ala Arg Ile Ile Met Ile
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 Phe Val Leu Phe Ala Leu Leu Trp Ser Trp Val Gly Gln Ile Asp Ile
 65 70 75 80

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 Gly Ser Asp Ser Asp Val Ala Gln Ser Glu Lys Ala Leu Arg Ala Ala
 130 135 140

caa tta tct aag cta cgc ctt gaa gca att tta tca gca tta aat cac 480
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 145 150 155 160

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 165 170 175

tca gaa tcg gaa att aat gaa gct caa act tta gcc caa aat caa tat 576
 Ser Glu Ser Glu Ile Asn Glu Ala Gln Thr Leu Ala Gln Asn Gln Tyr
 180 185 190

caa gca tgg tta gca caa gat gaa caa cta aaa tta acc tta aaa gga	624
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cat caa gca gaa tta caa tct gct cga tcc caa gaa caa aag ttg gtt	672
His Gln Ala Glu Leu Gln Ser Ala Arg Ser Gln Glu Gln Lys Leu Val	
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aaa tta ctt agc aat caa aat gat tta caa agt aca cgt agt cag att	816
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Tyr Thr Gln Asn Leu Lys Arg Asp Thr Leu Glu Ser Leu Arg Gln Thr	
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Val Leu Asn Lys Asp Ile Gly Phe Val Lys Ala Gly Gln Asn Val Ile	
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Lys Ile Lys Ser Ile Ser His Asp Ala Ile Glu His Gln His Leu Gly	
405 410 415	

43

cta gtg tat act gca ctt gtt tct ctt gat aaa agc aca tta aat ata 1296
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gat gga gta aca atc aac tta acg cca gga atg aat gtt act gct gaa 1344
 Asp Gly Val Thr Ile Asn Leu Thr Pro Gly Met Asn Val Thr Ala Glu
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att aaa aca ggt aaa cgt cgt gtt ttg gat tat ata tta agt cca ttg 1392
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 35 40 45

Thr Pro Val Ser Arg Ser Ser Lys Trp Thr Ala Arg Ile Ile Met Ile
 50 55 60

Phe Val Leu Phe Ala Leu Leu Trp Ser Trp Val Gly Gln Ile Asp Ile
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Val Ala Thr Ala Ser Gly Lys Ile Ser Ser Gly Ser Arg Ser Lys Thr
 85 90 95

Ile Gln Ser Leu Glu Thr Ala Ile Val Lys Ala Val Tyr Val Arg Asp
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Gly Gln Asn Val Gln Gln Gly Glu Ile Leu Val Asp Leu Val Gly Ile
 115 120 125

Gly Ser Asp Ser Asp Val Ala Gln Ser Glu Lys Ala Leu Arg Ala Ala
 130 135 140

Gln Leu Ser Lys Leu Arg Leu Glu Ala Ile Leu Ser Ala Leu Asn His
 145 150 155 160

Arg Ile Asn Pro Gln Ile Asp Val Ala Tyr Ala Lys Ser Leu Asn Ile
 165 170 175

Ser Glu Ser Glu Ile Asn Glu Ala Gln Thr Leu Ala Gln Asn Gln Tyr
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Gln Ala Trp Leu Ala Gln Asp Glu Gln Leu Lys Leu Thr Leu Lys Gly
 195 200 205
 His Gln Ala Glu Leu Gln Ser Ala Arg Ser Gln Glu Gln Lys Leu Val
 210 215 220
 Ser Val Gly Ala Ile Glu His Gln Lys Thr Asp Asp Tyr Arg Ser Leu
 225 230 235 240
 Lys Ala Glu Asn Phe Ile Ser Glu His Ala Tyr Leu Glu Gln Glu Ser
 245 250 255
 Lys Leu Leu Ser Asn Gln Asn Asp Leu Gln Ser Thr Arg Ser Gln Ile
 260 265 270
 Gln Lys Ile Gln Ala Ala Ile Met Gln Ala Glu Gln Asn Arg Met Leu
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 Asn Glu Gln Ile Asn Gln Tyr Thr Gly Gln Thr Asn Lys Ala Lys Gln
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 Glu Leu Thr Ala Tyr Thr Leu Gly Gly Val Val Gln Ala Ala Gln Lys
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 385 390 395 400
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 Asp Gly Val Thr Ile Asn Leu Thr Pro Gly Met Asn Val Thr Ala Glu
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<211> 478

<212> PRT

<213> Pasteurella haemolytica

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Ile Glu Thr Pro Val Ser Lys Lys Pro Arg Leu Ile Ala Tyr Leu Ile
      50           55           60

Met Leu Phe Leu Val Val Ala Ile Val Leu Ala Ser Val Ser Lys Val
      65           70           75           80

Glu Ile Val Ala Thr Ala Pro Gly Lys Leu Thr Phe Ser Gly Arg Ser
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Lys Glu Ile Lys Pro Ile Glu Asn Ala Ile Val Gln Glu Ile Phe Val
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Ala Leu Gly Ser Asp Ala Asp Ile Lys Lys Thr Met Ala Ser Leu Ser
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Tyr Glu Gly Ala Thr Arg Ile Glu Gln Glu Lys Leu Lys Asp Phe Lys
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 Glu Thr Leu Met Ile Ile Val Pro Glu Asp Asp Val Leu Glu Ala Thr
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 Val Ile Ile Lys Val Glu Thr Phe Pro Tyr Thr Arg Tyr Gly Tyr Leu
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 Val Gly Leu Val Phe Asn Ala Thr Ile Ala Ile Asp Arg Lys Asn Leu
 420 425 430
 Thr Ser Pro Asp Gly Arg Lys Ile Asp Leu Ser Ser Gly Met Thr Ile
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<212> PRT

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 35 40 45
 Ile Glu Thr Pro Val Ser Lys Lys Pro Arg Leu Ile Ala Tyr Leu Ile
 50 55 60

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Met Leu Phe Leu Phe Leu Ala Leu Val Ile Ser Ile Val Ser His Val
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 Glu Ile Val Ala Thr Ala Thr Gly Lys Leu Ala Phe Ser Asp Arg Ser
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 Lys Glu Ile Lys Pro Ile Glu Asn Ala Leu Val Lys Glu Ile Phe Val
 100 105 110
 Gln Asp Gly Gln Phe Val Glu Lys Asp Gln Leu Leu Leu His Leu Thr
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 Glu Gln Arg Gln Ile Ala Ser Val Ile Arg Ala Pro Val Ser Gly Thr
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 Val Gln Gln Leu Lys Thr His Thr Val Gly Gly Val Val Thr Thr Ala
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 Glu Thr Leu Met Val Ile Ala Pro Glu Asp Asp Val Leu Glu Val Thr
 355 360 365

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Ala Leu Ile Gln Asn Lys Asp Ile Gly Phe Ile Glu Val Gly Gln Asp
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Ala Val Ile Lys Val Glu Thr Phe Pro Tyr Thr Arg Tyr Gly Tyr Leu
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Met Gly Lys Val Lys Asn Ile Thr Leu Glu Ala Ile Glu His Pro Gln
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Leu Gly Leu Val Phe Asn Ser Ile Ile Ser Ile Asp Arg Lys Thr Leu
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Ser Gly Lys Asp Gly Lys Glu Ile Glu Leu Gly Ser Gly Met Ser Val
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Ile Glu Thr Pro Val Ser Arg Arg Pro Arg Leu Val Ala Tyr Phe Ile
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Met Gly Phe Leu Val Ile Ala Phe Ile Leu Ser Val Leu Gly Gln Val
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Lys Glu Gly Glu Ser Val Arg Lys Gly Asp Val Leu Leu Lys Leu Thr
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Ala Leu Gly Ala Glu Ala Asp Thr Leu Lys Thr Gln Ser Ser Leu Leu
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Gln Ala Arg Leu Glu Gln Ile Arg Tyr Gln Ile Leu Ser Arg Ser Ile
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 Glu Gln Phe Ser Thr Trp Gln Asn Gln Lys Tyr Gln Lys Glu Leu Asn
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 Leu Asp Lys Lys Arg Ala Glu Arg Leu Thr Ile Leu Ala Arg Ile Asn
 210 215 220
 Arg Tyr Glu Asn Val Ser Arg Val Glu Lys Ser Arg Leu Asp Asp Phe
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 Arg Ser Leu Leu His Lys Gln Ala Ile Ala Lys His Ala Val Leu Glu
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 260 265 270
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 Ala Glu Thr Leu Met Val Ile Val Pro Glu Asp Asp Thr Leu Glu Val
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 370 375 380
 Asn Ala Ile Ile Lys Val Glu Ala Phe Pro Tyr Thr Arg Tyr Gly Tyr
 385 390 395 400
 Leu Val Gly Lys Val Lys Asn Ile Asn Leu Asp Ala Ile Glu Asp Gln
 405 410 415
 Lys Leu Gly Leu Val Phe Asn Val Ile Val Ser Val Glu Glu Asn Asp
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 Leu Ser Thr Gly Asn Lys His Ile Pro Leu Ser Ser Gly Met Ala Val
 435 440 445
 Thr Ala Glu Ile Lys Thr Gly Met Arg Ser Val Ile Ser Tyr Leu Leu
 450 455 460

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/18268

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/02; C07K 14/22

US CL : 580/350; 424/251.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 580/350; 424/251.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/16172 A1 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION, UNIVERSITY OF MELBOURNE) 08 March 2001, see Figure 6, sequence listing 5, pages 25-28, pages 11-13, page 7, lines 29-36, page 5, lines 25-33	1-15, 23-25, 26-33
Y		16-22
X	WO 90/07525 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 12 JULY 1990, see all claims and entire document.	1-7, 16-18, 23-25, 30-33
X	US 5,766,607 A (FENWICK et al) 16 June 1998, see abstract, title, claims, see entire document.	23-25, 16-18
A	US 4,522,803 A (LENK et al), 11 june 1985, see title, abstract, claim 36, and entire document.	1-33

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

09 JULY 2002

Date of mailing of the international search report

05 SEP 2002

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

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Authorized officer

GINNY PORTNER

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/18263

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,083,512 A (ROBERTS) 04 July 2000, see claims, abstract, and entire document.	1-33
A	US 4,675,176 A (GERBER) 23 June 1987, see title, abstract, claims and entire document.	1-33

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/18263

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

DIALOG, WEST, MEDLINE, DIALINDEX-ALL SCIENCE (290+ FILES)

search terms: Moraxella bovis, cytotoxin, toxin, hemolysin, leukotoxin, kda or kilodalton