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(54) **Title**: VACCINE AGAINST STREPTOCOCCAL INFECTIONS BASED ON RECOMBINANT PROTEINS

(57) **Abstract**: An antigenic composition comprises several antigenic components derived from antigens of *Streptococcus equi* subsp. equi or subsp. *zooepidemicus*, wherein at least one component is a fusion protein or polypeptide comprising two or more such antigens or fragments thereof. The antigenic composition may be used for immunization of mammals against *S. equi* subsp. *equi* and/or subsp. *zooepidemicus*. A vaccine composition comprising the antigenic composition as immunizing component is also disclosed.

WO 2011/149419 A1

VACCINE AGAINST STREPTOCOCCAL INFECTIONS BASED ON RECOMBINANT PROTEINS

Field of the Invention

This invention relates to subunit immunogenic or vaccine compositions and use thereof for immunization of mammals susceptible to streptococcal infections. The invention also relates to methods for preparing, formulating and administrating such compositions.

Background of the Invention

Streptococcal infections in horses are mainly caused by the species *Streptococcus equi*, which comprises three subspecies designated *equi*, *zooepidemicus,* and *ruminatorium* respectively hereafter simply called *S. equi*, *S. zooepidemicus* and *S. ruminatorium* (Refs. 15, 24, 40).

S. equi which is virtually confined to horses is the causative agent of strangles, a world-wide distributed and highly contagious serious disease of the upper respiratory tract of the Equidae. Strangles is one of the most frequently reported equine diseases world-wide and is characterized by fever, nasal discharge, and abscess formation in the retropharyngeal and mandibular lymph nodes. In some cases the disease shows a metastatic course in the body, so called "bastard strangles". The disease has a world-wide distribution and causes great economic losses (Ref. 39).

S. zooepidemicus is considered as an opportunistic commensal often occurring in the upper respiratory tract of healthy horses. However, after stress or virus infection, it can cause a secondary infection, which results in strangles-like symptoms. Moreover, *S. zooepidemicus* infects not only horses but also a wide range of other animals, like pigs, goats, dogs, cats, and cows. Even human cases of infection due to subsp. *zooepidemicus* have been reported (Ref. 5). This subspecies has been implicated as the primary pathogen in conditions such as endometritis, cervicitis, abortion, mastitis, pneumonia, abscesses and joint infections.

S. ruminatorium has been isolated from milk of sheep and goats with mastitis (Ref. 10).

Streptococcus pyogenes is an important human pathogen which causes a variety of diseases e.g. impetigo, pharyngitis, necrotizing fasciitis and toxic shock syndrome.

Although it is possible to treat and cure these streptococcal infections with antibiotics, such as penicillin, tetracycline or gentamicin, an effective prophylactic agent that could prevent outbursts of such infections and obviate or reduce the risk for development of resistant strains associated with antibiotic treatment, would be appreciated.

Description of the Related Art

However, although many attempts have been made to develop prophylactic agents such as vaccines against *S. equi,* at the present time no efficient and safe vaccines are available on the market, neither for the subsp. *equi* nor for the subsp. *zooepidemicus,* subsp. *ruminatorium* or *S. pyogenes.*

Existing vaccines against strangles are based on inactivated, e. g. heat-killed, or attenuated strains of *S. equi* or acid extracts/mutanolysin enriched in M-protein(s), i.e. immunogenic protein(s) produced by *S. equi.* A vaccine against *S. zooepidemicus* based on an M-like protein is disclosed in US-A-5,583,014. In WO 87/00436, Ref. 17 and WO 2009/093014 A2 attenuated strains of *S. equi* are disclosed for use as a vaccine against infections caused by *S. equi.*

A commercial vaccine against strangles, Equilis StrepE from Intervet, UK, was released in 2004. However, the safety and efficacy of this vaccine, which is based on an attenuated (living, deletion mutated) strain of *S. equi* can be questioned (Refs. 23, 35).

Since the previously developed vaccines or immunizing preparations based on living or inactivated bacteria are hampered by side-effects and may provide insufficient protection there is a need for efficient and safe prophylactic agents, such as vaccines, that protect against *S. equi* infections and/or prevent spread thereof without giving rise to undesirable side-effects.

For years, streptococcal surface proteins, that interact with and/or bind to different components of the Extracellular Matrix (ECM) or plasma proteins of the host cell have been identified and characterized. Examples of extracellular surface proteins of *S. equi* and *S. zooepidemicus* that have been characterized are FNZ (Ref. 29), EAG (Ref. 27), the collagen-like proteins (SclC, SclD, SclE, SclF, SclG, SclH and SclI) (Refs. 21, 22), CNE (also called Sec) (Ref. 25), ZAG (Ref. 18 and WO 95/07296). Furthermore, examples of *S. equi* extracellular proteins that are supposed to be released into the surrounding medium are SFS (Ref. 28), IdeE and IdeZ (Ref. 26), IdeE2 and IdeZ2 (Ref. 16). These types of proteins are potential candidates for use as active component(s) for immunizing purposes.

The uses of this type of proteins as components in a potential vaccine for protection of horses against strangles are disclosed in WO 2004/032957 A1, WO 00/37496, WO 2007/115059 A2, WO 98/01561 and WO 2009/075646 A1.

In Flock, M., et al (2004) (Ref. 11), it is reported that in a mouse model of equine strangles, parts of the proteins designated FNZ, SFS and EAG, respectively, were used to immunize mice. FNZ and EAG were considered as promising candidates for development of a safe and efficacious vaccine against strangles.

Timoney et al (2007) (Ref. 42) reported that recombinant DNA produced extracellular proteins of subsp. *equi* are useless as vaccine components. It was speculated therein that earlier reported results for some *S. equi* proteins produced by recombinant DNA technology, showing protection in mice experiments, are not applicable to horses. Thus, it is not obvious that recombinant forms of extracellular localized *S. equi* proteins necessarily are functional as vaccine components.

In Ref. 45, vaccination of horses against strangles using the recombinant antigens EAG, CNE and SclC from *S. equi* is reported. In this study, vaccinated horses showed, after challenge with *S. equi,* significantly reduced recovery of bacteria and significantly lower levels of nasal discharge.

Although many efforts have been made to develop efficient vaccines and some of the immunizing components presented in Refs. 14 and 15, WO 2004/032957 A1, WO2009/075646 A1, are promising candidates for use in a vaccine that protects against *S. equi* infection, development of safe vaccines having a high degree of immunogenicity and exhibiting limited or no side effects is still desirable.

The human pathogen *Streptococcus pyogenes* also expresses a great number of extracellular proteins interacting with ECM and/or blood components of the host (Refs. 6, 7, 9, 33). Among these are an endoglycosidase, called EndoS that has the ability to hydrolyse the chitobiose core of the asparagine-linked glycan on human immunoglobulin G (IgG) (Ref. 8). EndoS has been further characterized in a series of articles, describing e.g. enzymatic properties, specificity etc (Refs. 1, 2, 3, 4, 34). The use of EndoS in treating or preventing diseases mediated by IgG antibodies such as autoimmune diseases is disclosed in WO/2008/071418 A2 and the in vitro use of EndoS to isolate and analyse IgG in WO 2009/033670 A2. The use of EndoSe of *Streptococcus equi* subsp. *equi* and EndoSz of *Streptococcus equi* subsp. *zooepidemicus*, or fragments thereof, as a component in a vaccine

against bacterial infections or to elicit an immunogenic response or a protective immune response is disclosed in WO 2011/059385 A1 (the entire disclosure of which is incorporated by reference herein).

Brief Summary of the Invention

The present invention is based on an antigenic, suitably an immunogenic, composition comprising multiple antigens, suitably immunogens that comprise at least one antigenic epitope or antigenic determinant derived from a protein present in one or both of *S. equi* and *S. zooepidemicus* and use thereof for immunization of non-human mammals against *S. equi* and/or *S. zooepidemicus.* According to the invention, at least one component of the composition is a fusion protein or polypeptide comprising two or more antigens or fragments thereof.

The present invention is also directed to a subunit immunogen or vaccine composition comprising the aforesaid antigenic composition as immunizing component; to methods to prepare said antigenic, suitably immunogenic, composition or vaccine composition; to methods to induce an immune response against *S. equi* and/or *S. zooepidemicus* in non-human mammals; and to methods for prophylactic or therapeutic treatment of *S. equi* and/or *S. zooepidemicus* infection in non-human mammals.

The invention is also directed to specific antigenic fusion polypeptides *per se.*

According to a suitable embodiment, the present invention is directed to a vaccine that protects equines, such as horses, against diseases caused by *S. equi*, e.g. strangles, upper respiratory tract infections, wound infections and endometritis. The word "protects" is a general term including anything between full protection and reduction of the severity of infection. The degree of protection can be measured in various ways, concerning e.g. *S. equi* infections in horses the effect of the vaccine can be reduced clinical symptoms and reduced clinical disease, where reduced increase in temperature, reduced swelling of lympnodes and reduced dissemination of bacteria from infected animals etc can be observed. Methods and procedures how to measure the efficacy of an immunizing composition after challenge can be obtained from e.g. Ref. 14, and WO 2009/075646 A1.

For various reasons, before performing vaccination and challenge experiments in horses, the evaluation of novel antigens to be used in a vaccine are studied in a small animal model. Concerning upper respiratory tract infections caused by subsp. *equi* a suitable and well

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established vaccination and experimental model has been described (Refs. 11, 12, 13, 14, 16, 43, WO 2004/032957 A1, WO 2009/075646 A1). This model has been used with a high degree of reliability to screen and evaluate S. Equi antigens with a potential to provoke a protective immunogenic response in horses (Refs. 13, 14).

According to a first aspect, the present invention provides an antigenic composition comprising a plurality of antigenic components derived from antigens of *Streptococcus equi* subsp. *equi* or subsp. *zooepidemicus*, the antigenic components comprising:

(i) a first fusion polypeptide comprising at least part of a protein designated EAG and at least a part of protein designated CNE; and

(ii) at least one additional polypeptide selected from

(a) a second fusion polypeptide comprising at least part of a protein designated Eq5 and at least a part of a protein designated Eq8,

(b) a third fusion polypeptide comprising at least part of a protein designated IdeE and at least a part of a protein designated Eq5,

(c) at least a part of a protein designated EndoSe or a protein designated EndoSz,

(d) at least part of a protein designated IdeE,

(e) at least part of a protein designated IdeE2,

(f) at least part of a protein designated Eq27,

(g) at least part of a protein designated Eq54,

(h) at least part of a protein of a protein family designated Scl.

wherein each said at least part of said proteins comprises at least one antigenic epitope and wherein the first fusion polypeptide comprises an amino acid sequence selected from the amino acid sequence as shown in SEQ ID NO: 24, starting from amino acid 12; the amino acid sequence as shown in SEQ ID NO: 28, starting from amino acid 12; and the amino acid sequence as shown in SEQ ID NO: 34, starting from amino acid 12; and/or

the second fusion polypeptide comprises the amino acid sequence as shown in SEQ ID NO: 22, starting from amino acid 12; and/or

the third fusion polypeptide comprises the amino acid sequence as shown in SEQ ID NO: 30, starting from amino acid 12.

According to a second aspect, the present invention provides an antigenic fusion polypeptide selected from the group consisting of:

a polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 24, starting from amino acid 12;

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a polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 28, starting from amino acid 12; and

a polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 34, starting from amino acid 12.

According to a third aspect, the present invention provides an antigenic composition comprising at least one of the fusion polypeptides defined in the second aspect.

According to a fourth aspect, the present invention provides a vaccine composition for protecting non-human mammals against infection of *Streptococcus equi*, which comprises the antigenic composition of according to the first or third aspect as an immunizing component, a pharmaceutically acceptable carrier, and preferably an adjuvant, and which preferably provided in a physiologically administrable form, preferably a form that is administrable by intramuscular, intradermal, subcutaneous or intranasal inoculation.

According to a fifth aspect, the present invention provides a vaccine composition for protecting non-human mammals against infection of *Streptococcus equi*, which comprises at least one recombinant vector with polynucleotides inserted therein which encode the antigenic components of the compositions according to the first or third aspect, which vector or vectors are capable of expressing said antigenic components *in vivo* in a mammal susceptible to infection with *Streptococcus equi*, wherein the vector preferably is an expression vector in the form of a plasmid or a viral vector, and which composition preferably is provided in a physiologically administrable form, preferably a form that is administrable by intramuscular, intradermal, subcutaneous or intranasal inoculation.

According to a sixth aspect, the present invention provides a method for producing an antigenic composition according to the first or third aspect which method comprises:

(a) providing DNA fragments encoding said antigen components and introducing said fragments into at least one expression vector;

(b) introducing said at least one vector, which contains said DNA fragments, into a at least one compatible host cell;

(c) culturing said at least one host cell provided in step (b) under conditions required for expression of the products encoded by said DNA fragments; and

(d) isolating the expressed products from each cultured host cell, and, optionally,

(e) purifying the isolated products from step (d), preferably by a chromatographic method.

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According to a seventh aspect, the present invention provides a method for the preparation of a vaccine composition according to the fourth or fifth aspect, which vaccine composition comprises as an immunizing component an antigenic composition according to the first or third aspect, wherein the method comprises mixing the antigenic composition with a pharmaceutically acceptable carrier.

According to an eighth aspect, the present invention provides use of an antigenic composition according to the first or third aspect, in the preparation of a vaccine protecting against infection with *Streptococcus equi*, especially against strangles caused by subsp. *equi* infection in horses.

According to a ninth aspect, the present invention provides use of an antigenic composition according to the first or third aspect for the prophylactic or therapeutic treatment of infections caused by *Streptococcus equi*.

According to a tenth aspect, the present invention provides a method for the production of an antiserum, which method comprises administering an antigenic composition according to the first or third aspect to a non-human mammal host to produce antibodies in said host and recovering antiserum containing said antibodies produced in said host.

According to an eleventh aspect, the present invention provides a method of prophylactic or therapeutic treatment of *Streptococcus equi* infection in a mammal, especially a horse, comprising administering to said mammal an immunologically effective amount of a vaccine composition according to the fourth or fifth aspect, or of an antiserum produced according to a ninth aspect.

According to a twelfth aspect, the present invention provides a method of protecting horses against *Streptococcus equi* infection, which comprises inoculating a horse subcutaneously, intradermally, intramuscularly or intranasally with a vaccine composition according to the fourth or fifth aspect, to induce an immune response against *Streptococcus equi* in said horse, wherein the immune response preferably is induced in the form of IgG and/or IgA and/or IgM antibodies in serum and/or the nasopharyngeal mucus in said horse.

According to a thirteenth aspect, the present invention provides an antibody preparation comprising at least one, and preferably at least two monoclonal or polyclonal antibodies, or antibody fragments, specific for an antigen of the antigenic composition according to the first or

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third aspect, wherein the antibody preparation preferably is to be used prophylactically or therapeutically to provide passive immunization when administered to a mammal susceptible to infection by *Streptococcus equi*.

Acording to a fourteenth aspect, the present invention provides a method of passive immunization which comprises administering to a mammal an antibody preparation according to the twelfth aspect.

According to a fifteenth aspect, the present invention provides an antigenic composition produced by the method of the sixth aspect.

According to a sixteenth aspect, the present invention provides an antiserum produced by the method of the ninth aspect. In the context of infection caused by *S. Equi*, the expression "non-human mammals" primarily refers to animals belonging to the family Equidae that consists of horses, donkeys and zebras and to hybrids thereof, such as mules and himmies. Camels and dromedaries are also encompassed therein.

In connection with infection cause by *S. zooepidemicus*, the expression "non-human mammals in addition refers also to other mammals such as cows, pigs, dogs and cats.

The above-mentioned aspects of the invention, and preferred embodiments thereof, are defined in the appended claims.

In particular embodiments, the present invention makes use of one or more polypeptides selected from the amino acid sequences SEQ ID NOS: 22, 24, 26, 28, 30, 32, 34, 38, 42 and one or more nucleotide sequences selected from the nucleotide sequences SEQ ID NOS: 21, 23, 25, 27, 29, 31, 33, 37, 41.

The practice of the present invention will employ, unless otherwise indicated conventional techniques of microbiology, recombinant DNA technology and molecular biology and immunology, which are within the skills of the art. Such techniques are explained in literature e.g. Sambrook et al (2001) Molecular Cloning: A laboratory manual, 3rd ed. Cold Spring Harbour Press. Unless defined otherwise, all scientific and technical terms used herein have the same meaning as commonly understood by a person with ordinary skill in the art to which the invention pertains.

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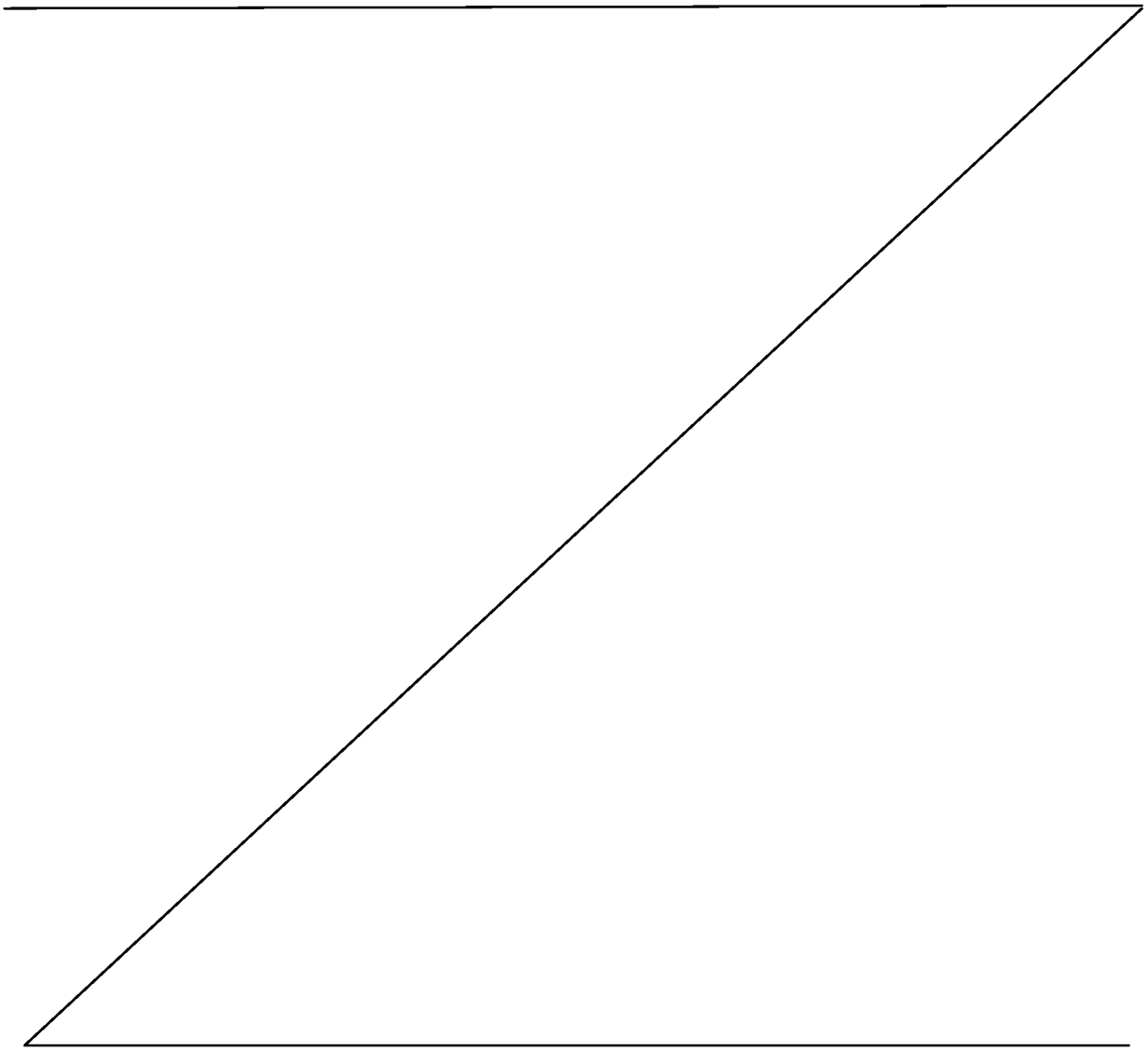
A “fragment” of a molecule such as a protein or nucleic acid is meant to refer to a portion of the amino acid or nucleotide sequence.

The term “analog” refers to a nucleic acid or amino acid sequence variant having a sequence homology (“identity”) of 80% or more, especially 90% or more, with the reference sequence. In general, “identity” refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectfully. Techniques for determining nucleic acid sequence identity are well known in the art, and software programs for calculating identity between sequences are available.

Unless the contact clearly requires otherwise, throughout the description and the claims, he words “comprise”, “comprising” and he like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”



Many of the *S. equi* proteins have different designations in various reports. To facilitate the reading of this application to previous reports/patent applications references and alternative designation is shown in Table 1 below.

Table 1

Present application	**References**	**Alternative designation**
CNE	WO 2004/032957 A1, WO 2009/075646 A1 Ref. 25	SEC
EAG	WO 2004/032957 A1, WO 2009/075646 A1 Ref. 11	
Eq8	WO 2009/075646 A1 Ref. 14	SEQ0402
Eq5	WO 2009/075646 A1 Ref. 14	SEQ0256
A21	Ref. 22	SclF
A36	Ref. 22	SclI
A42	WO 2004/032957 A1, WO 2009/075646 A1 Ref. 22	SclC
IdeE	WO 2009/075646 A1 Refs. 16, 26	
EndoSe	WO 2011/059385 A1	
Eq54	Ref. 14	SEQ0939
Eq27	Ref. 14	SEQ0944

Brief description of the drawings

Figure 1A is diagrams showing rectal temperature versus days post challenge in ponies vaccinated in Study I with Strangvacc 2, Strangvacc 3/4 or placebo for Panels A, B and C.

Figure 1B is diagrams showing rectal temperature versus days post challenge in ponies vaccinated in Study II with Strangvacc 3/4, 5 or 7 for Panels D, E and F.

Figure 1C is diagrams showing rectal temperature versus days post challenge in ponies vaccinated in Study II with Strangvacc 8 or placebo for Panels G and H.

Figure 2 is a diagram showing accumulated post mortem values for individual ponies vaccinated in Studies I and II.

Figure 3 is diagrams showing antibody levels in ponies vaccinated with Strangvacc I (containing single antigens) or Strangvacc 2 and 3/4 (data combined together)(containing fusion proteins). Top diagram (panel) shows antibodies against CNE, and bottom diagram (panel) shows antibodies against Eq5 (SEQ0256).

Figure 4A is a diagram showing antibody titer against Eq54 (n=10) and Eq27. IgG titers in sera from mice immunized with Eq54 or Eq27, or left non-vaccinated are shown. Mean values and standard errors of log values of dilutions required to get an absorbance of 1.5 in ELISA are shown. Values from non-vaccinated mice are included.

Figure 4B is a diagram showing weight loss of infected mice. The average weight loss over time of mice infected with *S.equi* subsp. *equi* is shown. Mice (n=3x10) had previously been vaccinated with antigens as indicated. Mean values and standard errors are shown.

Figure 4C is a diagram showing nasal colonisation of infected mice. The nasal growth of *S.equi* subsp. *equi* over time of mice infected with *S.equi* subsp. *equi* is shown. Mice (n=3x10) had previously been vaccinated with antigens as indicated. Mean values and standard errors are shown.

Detailed Description of the Invention

As mentioned above, the present invention is generally concerned with identification of polypeptides or proteins of *S. equi* or *S. zooepidemicus* that are able to elicit an immunogenic response, when administered to a mammal; and to the identification of polynucleotides or genes encoding these polypeptides or proteins.

The present invention is also concerned with fragments or analogs of said polypeptides or proteins or of said polynucleotides or genes.

More specifically, the invention discloses how gene fragments of *S. equi* or *S. zooepidemicus* encoding various extracellular proteins can be combined by gene fusion technology, expressed in a suitable host and used as antigens in a vaccine against

streptococcal infections in mammals. While based on such studies, the present invention is not limited to the specific combinations disclosed. Basically, the individual antigens represented in each fusion protein can be arranged in various number, order or combinations. In principal, an order of the antigens can e.g. be *N-terminus*-A-B-C-D-E-*C-terminus*, but the position of each individual antigen can be changed and the number thereof varied. Further, the invention also discloses how fusion proteins can be combined in a vaccine with non-fusion proteins to obtain efficient vaccine compositions.

In the following, reference will be made to various patent and literature references, the relevant disclosures of which are incorporated by reference herein.

According to one embodiment, the present invention is directed to an antigenic composition comprising several antigens, wherein each antigen comprises at least part of a protein or polypeptide of *S. equi* or *S. zooepidemicus*, and said at least part of said protein or polypeptide comprises at least one antigenic epitope or antigenic determinant of *S. equi* or *S. zooepidemicus,* and wherein said at least part of a protein or polypeptide is selected from the group comprising:

a protein or polypeptide which is designated Eq85 and has an amino acid sequence as shown in SEQ ID NO: 22;

a protein or polypeptide which is designated CCE and has an amino acid sequence as shown in SEQ ID NO: 24;

a protein or polypeptide which is designated IdeE and has an amino acid sequence as shown in SEQ ID NO: 26;

a protein or polypeptide which is designated CNEEAG and has an amino acid sequence as shown in SEQ ID NO: 28;

a protein or polypeptide which is designated IE5 and has an amino acid sequence as shown in SEQ ID NO: 30;

a protein or polypeptide which is designated EndoSe and has an amino acid sequence as shown in SEQ ID NO: 32;

a protein or polypeptide which is designated CPCE and has an amino acid sequence as shown in SEQ ID NO: 34;

a protein or polypeptide which is designated Eq54 and has an amino acid sequence as shown in SEQ ID NO: 38;

a protein or polypeptide which is designated Eq27 and has an amino acid sequence as shown in SEQ ID NO: 42;

and fragments and analogs thereof;

wherein at least one antigen is a fusion protein or polypeptide.

The above-mentioned antigen or antigens may further be combined with at least a part of a protein or polypeptide selected from the group comprising:

a protein or polypeptide which is designated CNE and has an amino acid sequence as shown in WO 2004/032957 A1, *SEQ ID NO: 4*;

a protein or polypeptide which is designated FNZ and has an amino acid sequence as shown in WO 2004/032957 A1, *SEQ ID NO: 2*;

a protein or polypeptide which is designated SFS and has an amino acid sequence as shown in WO 2004/032957 A1, *SEQ ID NO: 3*;

a protein or polypeptide which is designated SclC and has an amino acid sequence as shown in WO 2004/032957 A1, *SEQ ID NO: 23*;

a protein or polypeptide which is designated EAG and has an amino acid sequence as shown in WO 2004/032957 A1, *SEQ ID NO: 1*, and WO 2009/075646 A1, *SEQ ID NO: 13*;

a protein or polypeptide which is designated IdeE and has an amino acid sequence as shown in WO 2009/075646 A1, *SEQ ID NO: 10*;

a protein or polypeptide which is designated IdeE2 and has an amino acid sequence as shown in WO 2009/075646 A1, *SEQ ID NO: 1*;

a protein or polypeptide which is designated Eq5 and has an amino acid sequence as shown in WO 2009/075646 A1, *SEQ ID NO: 3*;

a protein or polypeptide which is designated Eq8 and has an amino acid sequence as shown in WO 2009/075646 A1, *SEQ ID NO: 5*;

a protein or polypeptide which is designated IdeZ2 and has an amino acid sequence as shown in WO 2009/075646 A1, *SEQ ID NO: 7*;

a protein or polypeptide which is designated Eqz5 and has an amino acid sequence as shown in WO 2009/075646 A1, *SEQ ID NO: 8*; and

a protein or polypeptide which is designated Eqz8 and has an amino acid sequence as shown in WO 2009/075646 A1, *SEQ ID NO: 9*;

or an analog or a fragment thereof.

For convenience, the polypeptides having amino acid sequences as shown in the sequence listing of WO 2009/075646 A1 and WO 2004/032957 A1 mentioned above are frequently only designated CNE, FNZ, SclC, SFS, EAG, IdeE, IdeE2, Eq5, Eq8, IdeZ2, Eqz5, and Eqz8, respectively. EAG, IdeE, IdeE2, Eq5, and Eq8 designate proteins that can be found in *S. equi* and IdeZ2, Eqz5, and Eqz8 designate proteins that can be found in *S. zooepidemicus*. Other examples are the M or M-like proteins e.g. SeM described in Ref. 42.

Further examples of antigens that may be included in the antigenic composition of the invention comprise the SclC proteins SclD-SclI (genbank acc. nos. DQ158080, DQ158081, DQ158082, DQ158083, DQ158084, DQ158085), FNE (acc. no. AF360373), FNEB (acc. no AY898649), FNEC-FNEF (Ref.24), SeM (acc. no. U73162 also called FBP acc.no. YP002747233), SzPSe (acc. no. U73162), seeH (acc. no. AF186180), seeM (acc. no. AJ583528), seeI (GenBank, Gene ID7697191, SEQ_2037, Ref. 15), seeL (acc. no. AJ583527), Se51.9 (acc. no. AF521601), Se46.8 (acc. no. AF521600), Se24.3 (acc. no. AY137521), Se75.3 (acc. no. AY137528), Se110.0 (acc. no. AY137519), Se24.3 (AY137521), Se42.0 (acc. no AY137521), Se117.0 (acc.no. AY137523), Se18.9 (acc. no. DQ068464), ZAG (acc. no. U25852), slaA (acc. no. CAW93317), slaB (acc. no. CAW95519), sagA (acc. no. ACG61862), streptolysin S biosynthesis proteins (CW92800, CW92802, CW92798), streptolysin S precursor (CW92796), SpyCEP (acc. no. DQ413032), the SpyCEP similar proteins SeCEP and SzoCEP (Ref. 43).

However, the proteins or polypeptide fragments that may be included in the antigenic compositions of the invention are not restricted to those listed above. In general, the invention can be used in principle with any extracellular protein or fragments thereof expressed on the surface or proteins transported into the environment of pathogenic streptococci, e.g. different subsp. of *S. equi* or *S. pyogenes*. By DNA sequence analysis of the genome of these bacteria e.g. http://www.sanger.ac.uk/Projects/S_equi/; http://www.sanger.ac.uk/Projects/S_zooepidemicus/; http://www.sanger.ac.uk/Projects/S_pyogenes/, open reading frames can be identified coding for extracellular proteins. These proteins are usually characterized by harboring an N-terminal signal sequence responsible for the transport across the membrane after translation. A particular interesting group of protein for vaccine development is proteins which in addition to harboring the signal sequence also display an easily recognized C-terminal domain including an amino acid motif generally defined as e.g. LPXTG, important for anchoring an

extracellular protein to the peptidoglycan structure of the bacterial cell wall (Ref. 37). How to identify such proteins by bioinformatics methods, e.g. computer program SignalP (http://www.cbs.dtu.dk/services/SignalP/), (Refs.19, 38), is well known to people skilled in the art.

The antigens or immunogens of the present antigenic or immunogenic compositions may comprise the entire amino acid sequence of said protein or polypeptide or may comprise a fragment, e.g. a C-terminal or N-terminal fragment thereof, or an analog thereof. These antigens may be used alone or in combinations. According to the invention, they may also by gene fusion technology be fused in various combinations and used as antigens in a vaccine. Furthermore, these fusion combinations may be used alone or in combination with other fusion combinations and/or in combination with single antigens.

According to the present invention, the antigenic compositions may comprise at least one antigen which is produced by recombinant technology and/or at least one antigen which is an isolated or purified antigen, or fragment thereof, such as the native forms produced by the streptococcal bacteria (or overproducing mutants). The native forms may be isolated from cells or growth media from bacteria grown in suitable media resulting in high production of the respective protein. In addition, after finding the optimal growth conditions (including physiological conditions) to obtain the native proteins it is also possible to construct overproducing streptococcal strains. Using methods well known for people skilled in the art there are several ways to generate and isolate overproducing strains, e.g. by site directed mutagenesis, chemical mutagenesis, ultraviolet light etc. The procedure of purifying and isolating an extracellullar protein from growth media is well known for people skilled in the art.

From the above, it is evident that the present antigens or immunogens that are derived from proteins of *S. equi,* or *S. zooepidemicus* may comprise the entire protein, a fragment of said protein or an analog of said protein (like for instance synthetic peptides) which is immunogenic. Thus, the present invention is not limited to the fragments of proteins that are specifically disclosed herein.

The antigenic composition of the present invention may comprise at least one recombinant vector and at least one polynucleotide inserted therein that encodes said at least one protein or polypeptide, and which vector is able to express said polypeptide in vivo in a non-human mammal susceptible to infection with *S. equi* and/ or *S. zooepidemicus.*

According to one embodiment of the present invention, the vector is an expression vector which is a plasmid or a viral vector and wherein said polynucleotide has a nucleotide sequence that encodes an antigen of the present invention.

The application of the present invention is not restricted to the usage of *E. coli* and vectors suitable for this bacterium as vehicles and tools to express recombinant polypeptides. Other hosts and vectors are well known in the art and can be found in literature and in literature cited in WO 2007/115059 A2. Furthermore, the application of the present application is not restricted to the specific nucleotide sequences of the antigens disclosed in the invention since it may be necessary to adapt the codon usage of the specific nucleotide sequences to the production host to be used. The technique to synthesize and adapt the codon usage is well known for people skilled in the art.

A further embodiment of the present invention is concerned with a vaccine composition for protecting non-human mammals against infection of *S. equi,* which comprises an antigenic composition as disclosed above as immunizing component, and a pharmaceutically acceptable carrier.

Suitably, the present vaccine composition comprises an antigenic or immunogenic composition that contains one or more of the present antigens or immunogens as immunizing component(s). Optionally, one or more of these antigens or immunogens are comprised of analogs of said proteins or fragments thereof.

The vaccine composition may comprise further components, such as an adjuvant. Suitably, said adjuvant stimulates systemic or mucosal immunity. Such adjuvants are well known in the art.

Suitable adjuvants for use according to the present invention comprise (1) polymers of acrylic or methacrylic acid, maleic anhydride and alkenyl derivative polymers, (2) immunostimulating sequences (ISS), (3) an oil in water emulsion, (4) cation lipids containing a quaternary ammonium salt, (5) cytokines, (6) aluminum hydroxide or aluminum phosphate, (7) saponin or (8) nanoparticles or (9) any combinations or mixtures thereof. Further examples of suitable adjuvants may also be found in literature cited in WO 2007/115059 A2.

A suitable adjuvant for use according to the present invention is the adjuvants Abisco , Matrix C and Matrix Q from Isconova AB, Sweden. The key components of ISCOMS are Quillaia saponins derived from the bark of the Chilean soap bark tree Quillaia saporinaria molina. Quillaia saponins are well known for their ability to activate the immune system (Ref.

32). Quillaia saponins mixed with cholesterol, and phospholipids under specific stochiometry form spherical open cage like structures known as ISCOMS.

Another suitable adjuvant is Ginseng. Ginseng is a dry extract prepared from the root of the plant Panax ginseng, C.A. Meyer. Ginseng contains a number of active substances named ginsenosides that are a kind of saponins, chemically tri-terpenoid glycosides of the dammaran series. The ginsenosides have adjuvant properties and one of the most active adjuvants is the fraction named Rb1. It has been proved that the fraction Rb1 elicits a balanced Th1 and Th2 immune response as determined by measuring the levels of the cytokines IFN- γ, IL-2, IL-4, IL-10 secreted post vaccination with a Rb1 adjuvanted vaccine. In addition ginseng and the fraction Rb1 stimulate a strong antigen specific antibody response.

According to a suitable embodiment, the vaccine composition is a vaccine that protects susceptible mammals, suitably horses, against strangles caused by *S. equi* and against infections caused by *S. zooepidemicus*.

The vaccine composition of the present invention is provided in a physiologically administrable form. Suitably, it is administrable by intramuscular, subcutaneous, intradermal or intranasal inoculation.

Suitably, the vaccine composition of the present invention stimulates serum, mucosal and/or bronchial antibody responses directed to *S. equi* and/or *S. zooepidemicus* antigens in mammals susceptible to these bacteria, suitably horses.

The present invention is also related to a method for producing an antigen or immunogen to be used in an antigenic or immunogenic composition of the present invention, which method comprises the steps of

(a) providing a DNA fragment encoding said antigen and introducing said fragment into an expression vector;

(b) introducing said vector, which contains said DNA fragment, into a compatible host cell;

(c) culturing said host cell provided in step (b) under conditions required for expression of the product encoded by said DNA fragment; and

(d) isolating the expressed product from the cultured host cell.

Preferably, the method further comprises a step (e) wherein the isolated product from step (d) is purified, e.g. by affinity chromatography or other chromatographic methods known in the art.

Accordingly, the antigens of the present invention are usually produced according to recombinant techniques.

A further embodiment of the present invention is concerned with a method for preparation of a vaccine of the present invention, which vaccine contains as immunizing component an antigenic or immunogenic composition as disclosed above, said method comprising mixing said antigenic composition and a pharmaceutically acceptable carrier.

The present invention is also related to a method for the production of an antiserum, said method comprising administering an antigenic preparation of the present invention to an animal host to produce antibodies in said animal host and recovering antiserum containing said antibodies produced in said animal host.

Moreover, the present invention is concerned with a method of prophylactic or therapeutic treatment of *S. equi* and/or *S. zooepidemicus* infection in mammals, suitably horses, comprising administering to said mammal an immunologically effective amount of a vaccine or an antiserum of the present invention.

Accordingly, the present invention is related to a method for protecting horses against *S. equi* infection, which method comprises inoculating a horse subcutaneously, intranasally, intradermally, orally or intramuscularly, or any combination thereof with a vaccine composition of the present invention to induce an immune response against *S. equi* in said horse. Suitably, an immune response, in the form of IgG and/or IgA and/or IgM antibodies in the nasopharyngeal mucus, and/or serum is induced in said horse.

The present invention also relates to an antibody preparation comprising at least one, and suitably at least two, antibodies specific for a protein or a polypeptide of the present antigenic composition, which antibody/antibodies is/are polyclonal or monoclonal; or which preparation comprises a fragment of said antibodies.

The antibody preparation of the present invention could be used prophylactically or therapeutically against strangles and provides passive immunization when administered to a non-human mammal susceptible to infection by *S. equi* or infected by *S. equi*.

The present invention provides a vaccine composition comprising one or several antigen components which have been prepared according to the present method using *E. coli*

as host cells. The source of these antigens might also be the native bacteria, if methods are developed for expression and purification thereof. Alternatively, the antigens of the present invention can also be produced according to methods that are based on fusion strategies where various parts of the respective antigen are recombined resulting in a fusion protein consisting of parts from different antigens. This fusion strategy could also be suitable for introducing an immune reactive part(s), e.g. T-cell epitopes or attenuated toxins (or parts thereof), thereby introducing other features suitable for optimizing the antigen presentation or localization.

The present invention may also be used in other vaccines or subunit immunogenic compositions, where the invention can be combined with one or more immunogens, antigens or epitopes selected from other pathogenic microorganisms or viruses to form multivalent subunit immunogenic compositions or vaccines. For example, concerning equine, such a multivalent subunit immunogenic composition or vaccine may comprise at least one polypeptide according to the present invention and at least one immunogen, antigen, or epitope from WEEV, EEV, VEEV, equine influenza virus, EHV-1, EHV-4, EAV, WNV, tetanus, *Rhodococcus*.

The present invention also provides diagnostic methods to measure antibodies against the various proteins (or fragments thereof) included in the vaccine composition. For instance, these types of methods may be used to determine antibody titers in sera before and/or after immunization or to determine antibody titers in infected mammals. The methods may also be applied to screen individual mammals to detect infected or chronical carriers of *S. equi* and *S. zooepidemicus*. Furthermore, the invention also provides a method to determine antibodies with neutralizing activity against the antigens in the vaccine thereby making it possible to measure the effect of e.g. immunization procedures or to identify individuals who lack antibodies that neutralize the antigens.

EXPERIMENTAL PART

Example 1. PCR amplifications and constructions of *E. coli* clones

S. equi subspecies *equi* strain 1866 (obtained from Nordvacc Läkemedel AB, Sweden), (WO 2004/032957 A1, Ref. 25) was used as source of DNA for cloning. Chromosomal DNA from subspecies *equi* strain 1866 was prepared and used as a template to amplify various gene fragments presented in the Examples 2-8 and 16 further below. The

sequences of primers used to amplify the various gene fragments are listed in Tables 2, 4 and 5. Cleavage sites for the restriction enzymes were included in the primer sequences. The plasmid vector pGEX-6P-1 (GE Healthcare, Uppsala, Sweden) (alternatively the pTYB4 vector, New England Biolabs, was used) was used for cloning and expression. The PCR amplifications were performed using the primers (20 pmol/µl) and FideliTaq™ PCR Master Mix (USB Corporation, Cleveland, Ohio) using the following programme: Step 1, pre-heat 1 minute at 95 °C, DNA strand separation; Step 2, 30 seconds at 95 °C; Step 3, annealing 15 seconds at 5 degrees below the respective primer combination melting point; and Step 4, elongation for 2 minutes at 72 °C, Steps 2 – 4 were run for 26 cycles. The PCR products were analysed on a 1 % agarose gel, and thereafter purified using the QIAquick PCR Purification Kit™ (Qiagen). After cleavage with the restriction enzymes the fragments were purified one additional time using the same kit. After purification the fragments were ligated into the vector using ReadyToGo T4DNA Ligase (GE Healthcare, Uppsala, Sweden). After ligation, the respective sample were transformed into competent cells of *E. coli* strainTG1 using electroporation, and spread on LA-Amp plates (Luria-Bertani broth agar (15g/L) plates supplemented with ampicillin, final conc. 50 µg/ml) and incubated over night at 37 °C. Next day colonies were picked and analysed by PCR using the respective primer combination. Clones with the expected insert were grown and plasmid prepared. The sequence of the respective insert was also determined by DNA sequencing. Correct clones were transformed into competent cells of *E. coli* strain BL21 (DE3) pLys for protein expression.

Table 2

Primer sequences 5′-3′

SEQ ID NO: 1. CneBam: ggttggatccactaatcttagtgacaacatcac

SEQ ID NO: 2. CneSac: TCCAGAGCTCCTTGACAGTAAAGCTGGTATAG

SEQ ID NO: 3. EagSac: agtggagctcttagacgcagcaacagtg

SEQ ID NO: 4. EagXho: CACCCTCGAGTTATTTGGCTTTGTTGATTAAGGTC

SEQ ID NO: 5. Eqc9: cgta**gagctc**tcggaacccaatccatatc

SEQ ID NO: 6. Eqc10: GAGGTCTAGAAGGACCTTGTTTGCCATTT

SEQ ID NO: 7. Eqc11: agca**tctaga**ttatctggtccgccagga

SEQ ID NO: 8. Eqc12: GAGGCTGCAGTGGACCTCGGGTACCGCCTT

SEQ ID NO: 9. Eqc13: agta**ctgcag**gaccagccagcagcactaa

SEQ ID NO: 10. ScSac: TGCAGAGCTCTGGCTTTTGGGCAGCTTCTTC

SEQ ID NO: 11. Eq8Bam: catg**ggatcc**gcgactaccctagcaggac

SEQ ID NO: 12. Eq8Nco: CTAG**CCATGG**GTGCTTAAGCTTTTCAATCTG

SEQ ID NO: 13. 85Nco: agta**ccatgg**gaaacgactactgctagtgc

SEQ ID NO: 14. Eq5C2: CTGG**CTCGAG**TTATTTAGCAACCAAGGCTGC

SEQ ID NO: 15. IdEG1: tact**ggatcc**gacgattaccaaaggaatgctac

SEQ ID NO: 16. IdEG2: TGAT**CTCGAG**TTAGCTCAGTTTCTGCCATATG

SEQ ID NO: 17. Eq61p1: gtc**ggatcc**gaggataaggttgtgcaaactag

SEQ ID NO: 18. Eq61p6: GCCT**CTCGAG**GGATAAGCTAGTCTGTCTTTGG

SEQ ID NO: 19. 54Sac: ggca**gagctc**gatacagcaagctataccatcac

SEQ ID NO: 20. 54Xba: TATTTCTAGAAGTTTTATAGGTGAAAACGATAACC

Example 2. Construction of a clone expressing fusion protein Eq85

A gene fragment of *eq8* was PCR amplified using primer pairs eq8Bam and eq8Nco. After amplification and purification the fragment was digested with BamHI and NcoI. Also a gene fragment of *eq5* was PCR amplified using primer pairs 85Nco and eq5C2. After amplification and purification the fragment was digested with NcoI and XhoI. Both fragments were ligated into the BamHI and XhoI cleaved vector pGEX-6P-1.

SEQ ID NO: 21. Showing the nucleotide sequence of the gene fusion fragment encoding Eq85 inserted in the pGEX-6P-1vector. The BamHI and XhoI sites are indicated in bold and the vector sequences are underlined. Note that the nucleotide A in bold and italics is different in this position compared to the published sequence in http://www.sanger.ac.uk/Projects/S_equi/;

TCTGTTCCAGGGGCCCCTG**GGATCC**GCGACTACCCTAGCAGGACAAACAGAAGTACGGGC
TGATAATATCTTACGCTTAGATATGACAGATAAAGAAGCAGTTGAAAAATTCGCTAACGA
GCTTAAAAATGAAGTCCATAAAAACTATCGTGGTAGTAATACTTGGCAAAAGCTTACCCT
TATACTTAATGGTTATCAAAACCTTAGAGAACAAATAGAGACCGAGCTAAAAAATAGTGA
ACAAAAAGTAAAAGAGCTTAATGATAAGGTTAATAGTGAAACTCAAGGAAAACAAGAGTT
ACAGAATCAGCTTGAGAAAGAAAAAGAAGAGTTAGAAACACTAAAAAAAGAGCTTGAAGC
TGAGAAGGCTAAAGGAACTGGAGAAACAGAGAAGCTTCAAAAGGAAATTGAAGCAAAAAA
TGCAATGATTTCTGACCTACAAAAACAGCTTGAGGAAACTAAGCAAAGGGTTCAAGAGTT

TGAAGCTGAAGTAGGTAAATTAATGGCCGAAAAGGCAGACCTACAAACAAAATTAAATGA
ACAAGAGCAGCTTAACGCTAAGCTTCAAAAAGAAATTGAAGACTTAAAGGCTCAGATTGA
AAAGCTTAAGCACCCATGGGAAACGACTACTGCTAGTGCATTTGAAAATAATGGGACAGG
TCAACATCTGAACTGGCACATAGATATTCCACAAGAATATACAGTTGAATTAGGAGAACC
AATTACTATCTCAGATCTTATGAGTCAAATTACGGTTACTCGTAAAGGTAGTAATGGGAC
TGTTAATGATGGAGATACTTTTGACTTTATTTCGAATGGAGATGGTTCAAGAGGAATTGA
TACCCCTGGAGTAAAAATATGGTTTGACTTTTACAATGCTGCGGGTACTTCCTTTTTAAC
TGATGAAATGTTAGCTTCGCCTACATATGCTGTACCGGGGGGATCTTATACTATTAAAGC
TTGGGTATTCTATGGGAAAAATGATACCAAAAAGCTCTTCACATTTAAACTAAAAAATTC
CAACAGCAATAAAACTGAGTTAAGGAAGTCGTTAGAGGAGGCTAAGCTAAAACTCAGCCA
GCCTGAAGGAACGTATTCTGATGAATCACTGCAAGCCTTGCAATCAGCGGTTACT**A**TTGG
TAAGACCTATTTAAACAGTGACCCTGATCAAAATACAGTAGATCAATCTGTTACTACTAT
TGATTCCGCTATTACTAGTCTTGTTAATCTTAATGCTTTAAATGAAGCTATTAATCAAGC
TACACCTTTTTATAACAGATGGCAAAGAGTATCCTAAAGAAGCGTATGACGGTCTTGTGCA
AAAGCTTGCAGCGGCAGCTAAGCTTCAAAATTCATTTGGTCCTTCACAAGGAGATGTTGA
TAAGGCTGCGACTGATTTAACGCAAGCTCTTACGACGCTTAAGACTGCTGTAGCGCATGA
AGCCTTAGATCAAGCCTTGGCTAAGCTGTTAGAGCTTTACCGAGAAAATCCAAATCTTGC
TTTGACATCAGAGTCTTTGAAGGAATTGTACAATAAGGCCATTGAAGCAGCAGGTACCTT
CTATAGAACTGTTAACAAGGATAAAGAGAGAAAAGACATTTCCCTTTATGAGCTAGAGCG
CTACACTACAGAAACAAATTCAGTTGTTGATACTATTTTAAAGGTAAAGGCTGCGATTGC
CGAAGAAGGAAAGGCAAAATTGCGTTCTGCTTTAGACCAATTAAATGCTCTTATCGGAGA
AAATCTAGACCTATCTCCATATACAGCAGCTTCTGCTCAAGCCTATACAGACCAGCTAGC
TAAGGCTAAGGAGGTCGCAGCAGCGGGTGAGACAGCTTATGCTCAGGAGACAGAACCGAC
AGCTATTACTAACAGCTTGGTTAAGGTGTTAAATGCTAAGAAATCCCTCTCAGATGCCAA
GGCAGCCTTGGTTGCTAAATAA**CTCGAG**CGGCCGCATCGTG

SEQ ID NO: 22. Eq85 fusion protein. Underlined amino acids indicate the sequence originating from the vector. The * indicates a scissor protease cleavage site. Note that the amino acids in bold originate from the construction work of the fusion protein and that these amino acids could be changed or even absent if another fusion strategy is used. Note that the amino acid Ile (I) in bold and italics in this position is different compared to the published sequence in http://www.sanger.ac.uk/Projects/S_equi/;

LEVLFQ*GPLGSATTLAGQTEVRADNILRLDMTDKEAVEKFANELKNEVHKNYRGSNTWQK
LTLILNGYQNLREQIETELKNSEQKVKELNDKVNSETQGKQELQNQLEKEKEELETLKKE

LEAEKAKGTGETEKLQKEIEAKNAMISDLQKQLEETKQRVQEFEAEVGKLMAEKADLQTK
LNEQEQLNAKLQKEIEDLKAQIEKLKH**PW**ETTTASAFENNGTGQHLNWHIDIPQEYTVEL
GEPITISDLMSQITVTRKGSNGTVNDGDTFDFISNGDGSRGIDTPGVKIWFDFYNAAGTS
FLTDEMLASPTYAVPGGSYTIKAWVFYGKNDTKKLFTFKLKNSNSNKTELRKSLEEAKLK
LSQPEGTYSDESLQALQSAVT***I***GKTYLNSDPDQNTVDQSVTTIDSAITSLVNLNALNEAI
NQATPFITDGKEYPKEAYDGLVQKLAAAAKLQNSFGPSQGDVDKAATDLTQALTTLKTAV
AHEALDQALAKLLELYRENPNLALTSESLKELYNKAIEAAGTFYRTVNKDKERKDISLYE
LERYTTETNSVVDTILKVKAAIAEEGKAKLRSALDQLNALIGENLDLSPYTAASAQAYTD
QLAKAKEVAAAGETAYAQETEPTAITNSLVKVLNAKKSLSDAKAALVAK

Example 3. Construction of a clone expressing fusion protein CCE

This gene fusion construct is made of five different *S. equi* gene fragments (*cne, eq21, eq36, eq42* and *eag*). First a gene fragment of *cne* was PCR amplified using primer pairs CneBam and CneSac. After amplification and purification the fragment was digested with BamHI and SacI. Second, a gene fragment of *eag* was PCR amplified using primer pairs EagSac and EagXho. After amplification and purification the fragment was digested with SacI and XhoI. The purified *cne* and *eag* fragments were ligated into the BamHI and XhoI cleaved vector pGEX-6P-1. After transformation into *E. coli* a correct clone was identified and denoted pCNEEAG. Thereafter, a gene fragment of *eq21* was PCR amplified using primer pairs eqc9 and eqc10. After amplification and purification the fragment was digested with SacI and XbaI. The gene fragment of *eq36* was PCR amplified using primer pairs eqc11 and eqc12. After amplification and purification the fragment was digested with XbaI and PstI. The gene fragment of *eq42* was PCR amplified using primer pairs eqc13 and ScSac. After amplification and purification the fragment was digested with PstI and SacI. The three cleaved fragments (*eq21*, *eq36* and *eq42*) were ligated together and a new PCR was performed using primer pairs eqc9 and ScSac. The obtained PCR product was cleaved with SacI and ligated into SacI cleaved pCNEEAG generating pCCE harboring the gene fragments in the following order *cne–eq21–eq36–eq42–eag*.

SEQ ID NO: 23. Showing the nucleotide sequence of the gene fusion fragment of *cne–eq21–eq36–eq42–eag* inserted in the pGEX-6P-1vector. The BamHI and XhoI sites are indicated in bold and the vector sequences are underlined.

CTGGAAGTTCTGTTCCAGGGGCCCCTG**GGATCC**ACTAATCTTAGTGACAACATCACATCA
TTGACGGTTGCTTCTTCATCACTCCGAGATGGAGAGAGAACGACGGTAAAGGTTGCGTTT
GATGACAAAAAACAGAAAATCAAGGCAGGGGATACGATAGAGGTCACCTGGCCTACAAGT
GGTAATGTCTACATTCAGGGCTTTAATAAAACCATACCGCTTAATATTAGAGGGGTAGAT
GTTGGTACCTTGGAGGTCACGCTAGACAAGGCTGTTTTCACATTCAATCAAAATATTGAA
ACAATGCATGATGTCTCTGGTTGGGGAGAGTTTGATATTACTGTTAGAAATGTGACACAA
ACCACCGCTGAAACATCAGGAACGACCACAGTAAAGGTAGGCAATCGCACTGCTACTATC
ACTGTTACTAAGCCTGAGGCAGGCACTGGTACCAGCTCATTTTATTATAAGACTGGTGAT
ATGCAGCCCAATGATACTGAGCGTGTGAGATGGTTCCTGCTGATTAACAACAACAAGGAA
TGGGTGGCCAATACTGTTACAGTCGAAGACGATATTCAAGGTGGTCAAACCTTGGATATG
AGCAGCTTTGACATCACCGTATCTGGTTATCGTAACGAGCGCTTCGTTGGGGAAAACGCT
CTGACAGAGTTTCATACAACATTTCCAAATTCTGTCATTACGGCAACAGATAATCACATT
AGTGTGCGGTTAGATCAATATGATGCCTCACAAAACACTGTCAACATTGCTTATAAGACA
AAGATAACGGACTTTGACCAAAAAGAATTTGCCAACAACAGTAAAATCTGGTACCAGATT
TTATACAAGGATCAGGTATCGGGTCAAGAGTCAAACCACCAAGTAGCCAATATCAATGCT
AACGGCGGGGTTGATGGCAGTCGCTATACCAGCTTTACTGTCAAGGAGCTCTCGGAACCC
AATCCATATCCAGATGTGAGGCGTTTCCTTGATGAGAAGTACGATGGAGATGTGGATAAA
TTATCTAAACAACTTCAAGGTTATTTTGGTAGTTTAAGAGAGTATATAGAGTTTGAACTT
AAAAATGGCAAACAAGGTCCTTCTAGATTATCTGGTCCGCCAGGATACCCACTTACTCGT
GATTTCTCCCGTAACTTCCTAGAAGAAAATACTGCAAAATATTTAGATCAATTAAGAGAA
CATCTACAGCACAGATTTAGTGAACTTGAGAGCTTAACAAGAAAATTAGAGAAAGAAGGC
GGTACCCGAGGTCCACTGCAGGACCAGCCAGCAGCACTAAAATATCCAGAACCTAGAGAC
TATTTTCTTCATACTCGTGAAGGTGATGTTATTTATGATGAGGATATAAAAAGATATTTT
GAGGATTTAGAAGCCTATTTAACAGCTAGACTTGGTGGGATTGATAAAAAAGTAGAAGAA
GCTGCCCAAAAGCCAGAGCTCTTAGACGCAGCAACAGTGTTAGAGCCTACAACAGCCTTC
ATTAGAGAAGCTGTTAGGGAAATCAATCAGCTGAGTGATGACTACGCTGACAATCAAGAG
CTTCAGGCTGTTCTTGCTAATGCTGGAGTTGAGGCACTTGCTGCAGATACTGTTGATCAG
GCTAAAGCAGCTCTTGACAAAGCAAAGGCAGCTGTTGCTGGTGTTCAGCTTGATGAAGCA
AGACGTGAGGCTTACAGAACAATCAATGCCTTAAGTGATCAGCACAAAAGCGATCAAAAG
GTTCAGCTAGCTCTAGTTGCTCCAGCAGCTAAGGTGGCAGATGCTGCTTCAGTTGATCAA
GTGAATGCAGCCATTAATGATGCTCATACAGCTATTGCGGACATTACAGGAGCAGCCTTG
TTGGAGGCTAAAGAAGCTGCTATCAATGAACTAAAGCAGTATGGCATTAGTGATTACTAT
GTGACCTTAATCAACAAAGCCAAATAA**CTCGAG**CGGCCGCAT

SEQ ID NO: 24. CCE fusion protein. Underlined amino acids indicate the sequence originating from the vector. The * indicates a scissor protease cleavage site. Note that the amino acids in bold originate from the construction work of the fusion protein and that these amino acids could be changed or even absent if another fusion strategy is used.

LEVLFQ*GPLGSTNLSDNITSLTVASSSLRDGERTTVKVAFDDKKQKIKAGDTIEVTWPTS
GNVYIQGFNKTIPLNIRGVDVGTLEVTLDKAVFTFNQNIETMHDVSGWGEFDITVRNVTQ
TTAETSGTTTVKVGNRTATITVTKPEAGTGTSSFYYKTGDMQPNDTERVRWFLLINNNKE
WVANTVTVEDDIQGGQTLDMSSFDITVSGYRNERFVGENALTEFHTTFPNSVITATDNHI
SVRLDQYDASQNTVNIAYKTKITDFDQKEFANNSKIWYQILYKDQVSGQESNHQVANINA
NGGVDGSRYTSFTVK**EL**SEPNPYPDVRRFLDEKYDGDVDKLSKQLQGYFGSLREYIEFEL
KNGKQGP**SR**LSGPPGYPLTRDFSRNFLEENTAKYLDQLREHLQHRFSELESLTRKLEKEG
GTRGP**LQ**DQPAALKYPEPRDYFLHTREGDVIYDEDIKRYFEDLEAYLTARLGGIDKKVEE
AAQKP**EL**LDAATVLEPTTAFIREAVREINQLSDDYADNQELQAVLANAGVEALAADTVDQ
AKAALDKAKAAVAGVQLDEARREAYRTINALSDQHKSDQKVQLALVAAAAKVADAASVDQ
VNAAINDAHTAIADITGAALLEAKEAAINELKQYGISDYYVTLINKAK

Example 4. Construction of a clone expressing IdeE

A gene fragment of the *ideE* gene was PCR amplified using primer pairs IdEG1 and IdEG2. After amplification and purification the fragment was digested with BamHI and XhoI and ligated into the BamHI and XhoI cleaved vector pGEX-6P-1.

SEQ ID NO: 25. The nucleotide sequence of the *ideE* gene inserted in the pGEX-6P-1 vector. The BamHI and XhoI sites are indicated in bold and the vector sequences are underlined.

CTGGAAGTTCTGTTCCAGGGGCCCCTG**GGATCC**GACGATTACCAAAGGAATGCTACGGAA
GCTTATGCCAAAGAAGTACCACATCAGATCACTTCTGTATGGACCAAAGGTGTTACACCA
CTAACACCCGAGCAGTTTCGATATAATAACGAAGATGTGATCCATGCGCCATATCTTGCT
CATCAAGGCTGGTACGATATCACCAAGGCCTTCGATGGGAAGGATAATCTCTTGTGTGGC
GCAGCAACGGCAGGTAATATGCTGCATTGGTGGTTTGATCAAAATAAAACAGAGATTGAA
GCCTATTTAAGTAAACACCCTGAAAAGCAAAAAATCATTTTTAACAACCAAGAGCTATTT
GATTTGAAAGCTGCTATCGATACCAAGGACAGTCAAACCAATAGTCAGCTTTTTAATTAT
TTTAGAGATAAAGCCTTTCCAAATCTATCAGCACGTCAACTCGGGGTTATGCCTGATCTT
GTTCTAGACATGTTTATCAATGGTTACTACTTAAATGTGTTTAAAACACAGTCTACTGAT
GTCAATCGACCTTATCAGGACAAGGACAAACGAGGTGGTATTTTCGATGCTGTTTTCACC

AGAGGAGATCAGACAACGCTCTTGACAGCTCGTCATGATTTAAAAAATAAAGGACTAAAT
GACATCAGCACCATTATCAAGCAAGAACTGACTGAAGGAAGAGCCCTTGCTTTATCACAT
ACCTACGCCAATGTTAGCATTAGCCATGTGATTAACTTGTGGGGAGCTGATTTTAATGCT
GAAGGAAACCTTGAGGCCATCTATGTCACAGACTCAGATGCTAATGCGTCTATTGGTATG
AAAAAATATTTTGTCGGCATTAATGCTCATAGACATGTCGCCATTTCTGCCAAGAAAATA
GAAGGAGAAAACATTGGCGCTCAAGTATTAGGCTTATTTACGCTTTCCAGTGGCAAGGAC
ATATGGCAGAAACTGAGCTAA**CTCGAG**CGGCCGCAT

SEQ ID NO: 26. IdeE protein. Underlined amino acids indicate the sequence originating from the vector. The * indicates a scissor protease cleavage site.

LEVLFQ*GPLGSDDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFRYNNEDVIHAPYLA
HQGWYDITKAFDGKDNLLCGAATAGNMLHWWFDQNKTEIEAYLSKHPEKQKIIFNNQELF
DLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLDMFINGYYLNVFKTQSTD
VNRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIIKQELTEGRALALSH
TYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHRHVAISAKKI
EGENIGAQVLGLFTLSSGKDIWQKLS

Example 5. Construction of a clone expressing fusion protein CNEEAG

A gene fragment of *cne* was PCR amplified using primer pairs CneBam and CneSac. After amplification and purification the fragment was digested with BamHI and SacI. Also gene fragment of *eag* was PCR amplified using primer pairs EagSac and EagXho. After amplification and purification the fragment was digested with SacI and XhoI. Both fragments were ligated into the BamHI and XhoI cleaved vector pGEX-6P-1.

SEQ ID NO: 27. Showing the nucleotide sequence of the gene fusion fragment *cne-eag* encoding CNEEAG inserted in the pGEX-6P-1vector. The BamHI and XhoI sites are indicated in bold and the vector sequences are underlined.

CTGGAAGTTCTGTTCCAGGGGCCCCTG**GGATCC**ACTAATCTTAGTGACAACATCACATCA
TTGACGGTTGCTTCTTCATCACTCCGAGATGGAGAGAGAACGACGGTAAAGGTTGCGTTT
GATGACAAAAAACAGAAAATCAAGGCAGGGGATACGATAGAGGTCACCTGGCCTACAAGT
GGTAATGTCTACATTCAGGGCTTTAATAAAACCATACCGCTTAATATTAGAGGGGTAGAT
GTTGGTACCTTGGAGGTCACGCTAGACAAGGCTGTTTTCACATTCAATCAAAATATTGAA
ACAATGCATGATGTCTCTGGTTGGGGAGAGTTTGATATTACTGTTACAAATGTGACACAA

ACCACCGCTGAAACATCAGGAACGACCACAGTAAAGGTAGGCAATCGCACTGCTACTATC
ACTGTTACTAAGCCTGAGGCAGGCACTGGTACCAGCTCATTTTATTATAAGACTGGTGAT
ATGCAGCCCAATGATACTGAGCGTGTGAGATGGTTCCTGCTGATTAACAACAACAAGGAA
TGGGTGGCCAATACTGTTACAGTCGAAGACGATATTCAAGGTGGTCAAACCTTGGATATG
AGCAGCTTTGACATCACCGTATCTGGTTATCGTAACGAGCGCTTCGTTGGGGAAAACGCT
CTGACAGAGTTTCATACAACATTTCCAAATTCTGTCATTACGGCAACAGATAATCACATT
AGTGTGCGGTTAGATCAATATGATGCCTCACAAAACACTGTCAACATTGCTTATAAGACA
AAGATAACGGACTTTGACCAAAAAGAATTTGCCAACAACAGTAAAATCTGGTACCAGATT
TTATACAAGGATCAGGTATCGGGTCAAGAGTCAAACCACCAAGTAGCCAATATCAATGCT
AACGGCGGGGGTTGATGGCAGTCGCTATACCAGCTTTACTGTCAAGGAGCTCTTAGACGCA
GCAACAGTGTTAGAGCCTACAACAGCCTTCATTAGAGAAGCTGTTAGGGAAATCAATCAG
CTGAGTGATGACTACGCTGACAATCAAGAGCTTCAGGCTGTTCTTGCTAATGCTGGAGTT
GAGGCACTTGCTGCAGATACTGTTGATCAGGCTAAAGCAGCTCTTGACAAAGCAAAGGCA
GCTGTTGCTGGTGTTCAGCTTGATGAAGCAAGACGTGAGGCTTACAGAACAATCAATGCC
TTAAGTGATCAGCACAAAAGCGATCAAAAGGTTCAGCTAGCTCTAGTTGCTGCAGCAGCT
AAGGTGGCAGATGCTGCTTCAGTTGATCAAGTGAATGCAGCCATTAATGATGCTCATACA
GCTATTGCGGACATTACAGGAGCAGCCTTGTTGGAGGCTAAAGAAGCTGCTATCAATGAA
CTAAAGCAGTATGGCATTAGTGATTACTATGTGACCTTAATCAACAAAGCCAAATAA**CTC**
GAGCGGCCGCAT

SEQ ID NO: 28. CNEEAG fusion protein. Underlined amino acids indicate the sequence originating from the vector. The * indicates a scissor protease cleavage site. Note that the amino acids in bold originate from the construction work of the fusion protein and that these amino acids could be changed or even absent if another fusion strategy is used.

LEVLFQ*GPLGSTNLSDNITSLTVASSSLRDGERTTVKVAFDDKKQKIKAGDTIEVTWPTS
GNVYIQGFNKTIPLNIRGVDVGTLEVTLDKAVFTFNQNIETMHDVSGWGEFDITVRNVTQ
TTAETSGTTTVKVGNRTATITVTKPEAGTGTSSFYYKTGDMQPNDTERVRWFLLINNNKE
WVANTVTVEDDIQGGQTLDMSSFDITVSGYRNERFVGENALTEFHTTFPNSVITAIDNHI
SVRLDQYDASQNTVNIAYKTKITDFDQKEFANNSKIWYQILYKDQVSGQESNHQVANINA
NGGVDGSRYTSFTVK**EL**LDAATVLEPTTAFIREAVREINQLSDDYADNQELQAVLANAGV
EALAADTVDQAKAALDKAKAAVAGVQLDEARREAYRTINALSDQHKSDQKVQLALVAAAA
KVADAASVDQVNAAINDAHTAIADITGAALLEAKEAAINELKQYGISDYYVTLINKAK

Example 6. Construction of a clone expressing fusion protein IE5

A gene fragment of the *ideE* gene was PCR amplified using primer pairs IdEG1 and IENco. After amplification and purification the fragment was digested with BamHI and NcoI. Also gene fragment of *eq5* was PCR amplified using primer pairs 85Nco and eq5C2. After amplification and purification the fragment was digested with NcoI and XhoI. Both fragments were ligated into the BamHI and XhoI cleaved vector pGEX-6P-1.

SEQ ID NO: 29. The nucleotide sequence of the *ideE – eq5* fusion inserted in the pGEX-6P-1 vector. The BamHI and XhoI sites are indicated in bold and the vector sequences are underlined.

CTGGAAGTTCTGTTCCAGGGGCCCCTG**GGATCC**GACGATTACCAAAGGAATGCTACGGAA
GCTTATGCCAAAGAAGTACCACATCAGATCACTTCTGTATGGACCAAAGGTGTTACACCA
CTAACACCCGAGCAGTTTCGATATAATAACGAAGATGTGATCCATGCGCCATATCTTGCT
CATCAAGGCTGGTACGATATCACCAAGGCCTTCGATGGGAAGGATAATCTCTTGTGTGGC
GCAGCAACGGCAGGTAATATGCTGCATTGGTGGTTTGATCAAAATAAAACAGAGATTGAA
GCCTATTTAAGTAAACACCCTGAAAAGCAAAAAATCATTTTTAACAACCAAGAGCTATTT
GATTTGAAAGCTGCTATCGATACCAAGGACAGTCAAACCAATAGTCAGCTTTTTAATTAT
TTTAGAGATAAAGCCTTTCCAAATCTATCAGCACGTCAACTCGGGGTTATGCCTGATCTT
GTTCTAGACATGTTTATCAATGGTTACTACTTAAATGTGTTTAAAACACAGTCTACTGAT
GTCAATCGACCTTATCAGGACAAGGACAAACGAGGTGGTATTTTCGATGCTGTTTTCACC
AGAGGAGATCAGACAACGCTCTTGACAGCTCGTCATGATTTAAAAAATAAAGGACTAAAT
GACATCAGCACCATTATCAAGCAAGAACTGACTGAAGGAAGAGCCCTTGCTTTATCACAT
ACCTACGCCAATGTTAGCATTAGCCATGTGATTAACTTGTGGGGAGCTGATTTTAATGCT
GAAGGAAACCTTGAGGCCATCTATGTCACAGACTCAGATGCTAATGCGTCTATTGGTATG
AAAAAATATTTTGTCGGCATTAATGCTCATAGACATGTCGCCATTTCTGCCAAGAAAATA
GAAGGAGAAAACATTGGCGCTCAAGTATTAGGCTTATTTACGCTTTCCAGTGGCAAGGAC
ATATGGCAGAAACTGAGCCCATGGGAAACGACTACTGCTAGTGCATTTGAAAATAATGGG
ACAGGTCAACATCTGAACTGGCACATAGATATTCCACAAGAATATACAGTTGAATTAGGA
GAACCAATTACTATCTCAGATCTTATGAGTCAAATTACGGTTACTCGTAAAGGTAGTAAT
GGGACTGTTAATGATGGAGATACTTTTGACTTTATTTCGAATGGAGATGGTTCAAGAGGA
ATTGATACCCCTGGAGTAAAAATATGGTTTGACTTTTACAATGCTGCGGGTACTTCCTTT
TTAACTGATGAAATGTTAGCTTCGCCTACATATGCTGTACCGGGGGGATCTTATACTATT
AAAGCTTGGGTATTCTATGGGAAAAATGATACCAAAAAGCTCTTCACATTTAAACTAAAA
AATTCCAACAGCAATAAAACTGAGTTAAGGAAGTCGTTAGAGGAGGCTAAGCTAAAACTC

AGCCAGCCTGAAGGAACGTATTCTGATGAATCACTGCAAGCCTTGCAATCAGCGGTTACT
ATTGGTAAGACCTATTTAAACAGTGACCCTGATCAAAATACAGTAGATCAATCTGTTACT
ACTATTGATTCCGCTATTACTAGTCTTGTTAATCTTAATGCTTTAAATGAAGCTATTAAT
CAAGCTACACCTTTTATAACAGATGGCAAAGAGTATCCTAAAGAAGCGTATGACGGTCTT
GTGCAAAAGCTTGCAGCGGCAGCTAAGCTTCAAAATTCATTTGGTCCTTCACAAGGAGAT
GTTGATAAGGCTGCGACTGATTTAACGCAAGCTCTTACGACGCTTAAGACTGCTGTAGCG
CATGAAGCCTTAGATCAAGCCTTGGCTAAGCTGTTAGAGCTTTACCGAGAAAATCCAAAT
CTTGCTTTGACATCAGAGTCTTTGAAGGAATTGTACAATAAGGCCATTGAAGCAGCAGGT
ACCTTCTATAGAACTGTTAACAAGGATAAAGAGAGAAAAGACATTTCCCTTTATGAGCTA
GAGCGCTACACTACAGAAACAAATTCAGTTGTTGATACTATTTTAAAGGTAAAGGCTGCG
ATTGCCGAAGAAGGAAAGGCAAAATTGCGTTCTGCTTTAGACCAATTAAATGCTCTTATC
GGAGAAAATCTAGACCTATCTCCATATACAGCAGCTTCTGCTCAAGCCTATACAGACCAG
CTAGCTAAGGCTAAGGAGGTCGCAGCAGCGGGTGAGACAGCTTATGCTCAGGAGACAGAA
CCGACAGCTATTACTAACAGCTTGGTTAAGGTGTTAAATGCTAAGAAATCCCTCTCAGAT
GCCAAGGCAGCCTTGGTTGCTAAATAA**CTCGAG**CGGCCGCAT

SEQ ID NO: 30. IE5 fusion protein. Underlined amino acids indicate the sequence originating from the vector. The * indicates a scissor protease cleavage site. Note that the amino acids in bold originate from the construction work of the fusion protein and that these amino acids could be changed or even absent if another fusion strategy is used.
Note that the amino acid Ile (I) in bold and italics in this position is different compared to the published sequence in http://www.sanger.ac.uk/Projects/S_equi/;

LEVLFQ*GPLGSDDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFRYNNEDVIHAPYLA
HQGWYDITKAFDGKDNLLCGAATAGNMLHWWFDQNKTEIEAYLSKHPEKQKIIFNNQELF
DLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLDMFINGYYLNVFKIQSTD
VNRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIIKQELTEGRALALSH
TYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHRHVAISAKKI
EGENIGAQVLGLFTLSSGKDIWQKLS**PW**ETTTASAFENNGTGQHLNWHIDIPQEYTVELG
EPITISDLMSQITVTRKGSNGTVNDGDTFDFISNGDGSRGIDTPGVKIWFDFYNAAGTSF
LTDEMLASPTYAVPGGSYTIKAWVFYGKNDTKKLFTFKLKNSNSNKTELRKSLEEAKLKL
SQPEGTYSDESLQALQSAVT***I***GKTYLNSDPDQNTVDQSVTTIDSAITSLVNLNALNEAIN
QATPFITDGKEYPKEAYDGLVQKLAAAAKLQNSFGPSQGDVDKAATDLTQALTTLKTAVA
HEALDQALAKLLELYRENPNLALTSESLKELYNKAIEAAGTFYRTVNKDKERKDISLYEL
ERYTTETNSVVDTILKVKAAIAEEGKAKLRSALDQLNALIGENLDLSPYTAASAQAYTDQ

LAKAKEVAAAGETAYAQETEPTAITNSLVKVLNAKKSLSDAKAALVAK

Example 7. Construction of a clone expressing EndoSe

A gene fragment of the *endoSe* gene was PCR amplified using primer pairs eq61p1 and eq61p6. After amplification and purification the fragment was digested with BamHI and XhoI and ligated into the BamHI and XhoI cleaved vector pGEX-6P-1.

SEQ ID NO: 31. The nucleotide sequence of the *endoSe* gene inserted in the pGEX-6P-1 vector. The BamHI and XhoI sites are indicated in bold and the vector sequences are underlined.

CTGGAAGTTCTGTTCCAGGGGCCCCTG**GGATCC**GAGGATAAGGTTGTGCAAACTAGTCCA
TCAGTCTCTGCTATTGATGACCTACATTACCTGTCGGAAAACAGTAAAAAAGAATTTAAG
GAGGGGTTATCAAAGGCAGGAGAAGTACCTGAAAAGCTAAAGGATATTTTATCCAAGGCA
CAGCAGGCAGATAAGCAGGCAAAGGTTCTTGCAGAAATGAAGGTTCCTGAAAAAATAGCC
ATGAAGCCTTTAAAGGGGCCTCTTTATGGTGGCTATTTTAGGACTTGGCATGATAAAACA
TCAGATCCGGCTGAAAAGGATAAGGTTAATTCTATGGGAGAATTGCCTAAGGAGGTTGAC
TTAGCCTTTGTTTTCCATGATTGGACCAAGGATTATAGCTTTTTCTGGCAAGAATTGGCG
ACCAAGCATGTGCCAACGCTGAACAAGCAGGGAACACGTGTGATTCGTACCATTCCATGG
CGGTTCCTTGCAGGCGGTGATCATAGTGGTATTGCTGAAGATACGCAAAAATACCCAAAT
ACTCCAGAGGGAAATAAGGCCTTGGCAAAGGCTATTGTAGATGAATACGTTTATAAATAT
AATCTTGATGGTTTAGATGTTGATATTGAGCGGGATAGCATTCCAAAAGTAAATGGAAAA
GAGAGTAACGAAAATATTCAGCGCTCTATTGCTGTTTTTGAAGAAATTGGCAAGCTTATT
GGGCCAAAGGGCGCTGACAAGTCACGTTTGTTCATTATGGATAGCACCTACATGGCTGAC
AAGAACCCATTGATTGAGCGCGGTGCCCAATATATTGATTTGCTGCTTGTGCAGGTTTAT
GGCACTCAAGGTGAGAAGGGAGATTGGGATCCAGTCGCTAGAAAACCTGAAAAGACAATG
GAGGAACGTTGGGAATCGTATAGCAAATACATTCGTCCTGAGCAGTACATGGTTGGTTTT
TCTTTCTATGAGGAATATGCGGGCAGTGGTAACCTCTGGTATGATATTAATGAGAGGAAA
GATGATCATAATCCGTTAAATTCAGAGATAGCTGGTACTCGTGCTGAGCGTTATGCAAAA
TGGCAGCCTAAGACAGGTGGTGTCAAGGGAGGGATTTTCTCTTATGCGATTGATCGCGAT
GGTGTAGCGCATCAACCTAAAAAAGTCTCAGATGATGAGAAAAGAACTAACAAGGCTATA
AAGGATATAACAGATGGTATTGTCAAATCAGATTATAAGGTTTCTAAGGCCTTGAAGAAG
GTTATGGAAAATGACAAATCCTATGAGCTGATTGATCAGAAAGATTTTCCAGACAAGGCT
TTGCGAGAAGCAGTTATTGCACAGGTTGGAAGCAGAAGAGGGGATTTAGAGCGGTTCAAT
GGAACCCTGCGCTTAGACAATCCGGATATCAAGAGTTTAGAAGGCCTGAATAAGCTTAAA

AAACTAGCTAAGCTAGAGCTAATCGGTCTATCACAAATCACAAAGCTGGATAGCTTAGTC
CTACCTGCAAATGCTAAGCCGACCAAGGATACGCTGGCCAATGTTCTTGAAGCCTACGAC
AGCGCTAAGAAGGAAGAGACTAAGGCGATTCCACAGGTGGCTCTGACCATTTCTGGTCTA
ACTGGCTTGAAGGAATTAAATCTTGCTGGCTTTGATCGTGATAGCTTGGCTGGAATTGAC
GCAGCTAGCCTAACCTCTCTTGAAAAGGTGGATCTCTCTAGTAATAAGCTGGACTTAGCA
GCTGGTACGGAAAATCGTCAGATTCTTGATACCATGCTGGCAACAGTGACTAAGCATGGC
GGTGTTAGCGAAAAGACGTTTGTATTTGATCATCAAAAGCCTACTGGTCTTTATCCTGAT
ACTTATGGCACTAAGAGCCTTCAGTTACCAGTAGCAAATGATACAATTGATTTGCAGGCT
AAGCTTTTATTTGGAACAGTTACCAATCAGGGCACGCTAATCAATAGCGAAGCTGACTAT
AAGGCTTATCAGGAGCAGGAAATAGCAGGTCACCGTTTTGTTGATTCAAGCTATGATTAC
AAAGCCTTTGCAGTGACCTACAAGGACTATAAGATCAAGGTGACTGACTCAACCTTAGGT
GTCACTGATCACAAGGACTTATCCACTAGCAAGGAGGAGACCTACAAGGTTGAATTCTTT
AGCCCTACTAATAGCACTAAGCCTGTGCATGAGGCTAAGGTTGTCGTTGGTGCGGAAAAA
ACCATGATGGTTAACCTAGCAGAGGGAGCAACTGTGATTGGTGGTGATGCAGATCCAACA
AATGCAAAAAAAGTGTTTGATGGTTTGCTCAATAATGATACAACAATTCTGTCAACTAGC
AATAAAGCTTCTATCATTTTTGAACTTAAAGAGCCTGGCTTAGTCAAGTATTGGCGTTTC
TTTAATGACAGCAAAATTAGTAAAGCTGACTGTATTAAGGAGGCCAAGCTTGAAGCCTTT
GTTGGCCATCTTGAAGCTGGCTCAAAGGTAAAGGATAGCTTGGAAAAATCATCAAAATGG
GTAACAGTTTCAGATTATTCAGGAGAGGACCAAGAGTTTAGCCAGCCGTTAAACAACATT
GGTGCCAAATATTGGAGAATAACAGTTGATACTAAGGGAGGACGTTACAATTGGCCATCA
CTTCCTGAGCTTCAAATCATTGGTTATCAATTACCGGCTGCGGATCTTGTGATGGCAATG
CTAGCTACTGCAGAGGAGCTATCTCAGCAAAAAGACAAGTTCTCTCAAGAGCAGCTTAAG
GAGCTCGAAGTCAAAATAGCTGCCTTAAAGGCTGCTTTAGATAGTAAGATGTTTAATGCC
GATGCTATTAACGCTAGTACTGCTGATCTGAAGGCTTATGTTGATAAGCTTTTAGCTGAT
AGAACTGATCAGGAAAAAGTAGCTAAAGCAGCTAAAGTTGAGCAGCCTGTGGCTACTGAC
ATAAAAGAAAATACTGAGCCAGAAAATCCAAAGACAGACTAGCTTATCC**CTCGAG**CGGCC
GCAT

SEQ ID NO: 32. EndoSe protein. Underlined amino acids indicate the sequence originating from the vector. The * indicates a scissor protease cleavage site.
Note that the amino acid Y in bold and italics is different in this position compared to the published sequence in http://www.sanger.ac.uk/Projects/S_equi/;

LEVLFQ*GPLGSEDKVVQTSPSVSAIDDLHYLSENSKKEFKEGLSKAGEVPEKLKDILSKA
QQADKQAKVLAEMKVPEKIAMKPLKGPLYGGYFRTWHDKTSDPAEKDKVNSMGELPKEVD
LAFVFHDWTKDYSFFWQELATKHVPTLNKQGTRVIRTIPWRFLAGGDHSGIAEDTQKYPN

TPEGNKALAKAIVDEYVYKYNLDGLDVDIERDSIPKVNGKESNENIQRSIAVFEEIGKLI
GPKGADKSRLFIMDSTYMADKNPLIERGAQYIDLLLVQVYGTQGEKGDWDPVARKPEKTM
EERWESYSKYIRPEQYMVGFSFYEE**Y**AGSGNLWYDINERKDDHNPLNSEIAGTRAERYAK
WQPKTGGVKGGIFSYAIDRDGVAHQPKKVSDDEKRTNKAIKDITDGIVKSDYKVSKALKK
VMENDKSYELIDQKDFPDKALREAVIAQVGSRRGDLERFNGTLRLDNPDIKSLEGLNKLK
KLAKLELIGLSQITKLDSLVLPANAKPTKDTLANVLEAYDSAKKEETKAIPQVALTISGL
TGLKELNLAGFDRDSLAGIDAASLTSLEKVDLSSNKLDLAAGTENRQILDTMLATVTKHG
GVSEKTFVFDHQKPTGLYPDTYGTKSLQLPVANDTIDLQAKLLFGTVTNQGTLINSEADY
KAYQEQEIAGHRFVDSSYDYKAFAVTYKDYKIKVTDSTLGVTDHKDLSTSKEETYKVEFF
SPTNSTKPVHEAKVVVGAEKTMMVNLAEGATVIGGDADPTNAKKVFDGLLNNDTTILSTS
NKASIIFELKEPGLVKYWRFFNDSKISKADCIKEAKLEAFVGHLEAGSKVKDSLEKSSKW
VTVSDYSGEDQEFSQPLNNIGAKYWRITVDTKGGRYNWPSLPELQIIGYQLPAADLVMAM
LATAEELSQQKDKFSQEQLKELEVKIAALKAALDSKMFNADAINASTADLKAYVDKLLAD
RTDQEKVAKAAKVEQPVATDIKENTEPENPKTD

Example 8. Construction of a clone expressing fusion protein CPCE

This gene fusion construct is made of five different *S. equi* gene fragments (*cne, eq54, eq36, eq42* and *eag*). The gene fragment of *eq54* was PCR amplified using primer pairs 54Sac and 54XbaI. After amplification and purification the fragment was digested with SacI and XbaI. The *eq36-eq42* fragment was obtained by PCR using primer pairs eqc11 and ScSac with the DNA from construct CCE as template. After amplification and purification the fragment was digested with SacI and XbaI. The two cleaved DNA fragments were ligated into the construct CNEEAG previously cleaved with SacI, generating a clone harboring the gene fragments in the following order *cne–eq54–eq36–eq42–eag*.

SEQ ID NO: 33. Showing the nucleotide sequence of the gene fusion fragment of *cne–eq54–eq36–eq42–eag* inserted in the pGEX-6P-1vector. The BamHI and XhoI sites are indicated in bold and the vector sequences are underlined.

CTGGAAGTTCTGTTCCAGGGGCCCCTG**GGATCC**ACTAATCTTAGTGACAACATCACATCA
TTGACGGTTGCTTCTTCATCACTCCGAGATGGAGAGAGAACGACGGTAAAGGTTGCGTTT
GATGACAAAAAACAGAAAATCAAGGCAGGGGATACGATAGAGGTCACCTGGCCTACAAGT
GGTAATGTCTACATTCAGGGCTTTAATAAAACCATACCGCTTAATATTAGAGGGGTAGAT
GTTGGTACCTTGGAGGTCACGCTAGACAAGGCTGTTTTCACATTCAATCAAAATATTGAA

ACAATGCATGATGTCTCTGGTTGGGGAGAGTTTGATATTACTGTTAGAAATGTGACACAA
ACCACCGCTGAAACATCAGGAACGACCACAGTAAAGGTAGGCAATCGCACTGCTACTATC
ACTGTTACTAAGCCTGAGGCAGGCACTGGTACCAGCTCATTTTATTATAAGACTGGTGAT
ATGCAGCCCAATGATACTGAGCGTGTGAGATGGTTCCTGCTGATTAACAACAACAAGGAA
TGGGTGGCCAATACTGTTACAGTCGAAGACGATATTCAAGGTGGTCAAACCTTGGATATG
AGCAGCTTTGACATCACCGTATCTGGTTATCGTAACGAGCGCTTCGTTGGGGAAAACGCT
CTGACAGAGTTTCATACAACATTTCCAAATTCTGTCATTACGGCAACAGATAATCACATT
AGTGTGCGGTTAGATCAATATGATGCCTCACAAAACACTGTCAACATTGCTTATAAGACA
AAGATAACGGACTTTGACCAAAAAGAATTTGCCAACAACAGTAAAATCTGGTACCAGATT
TTATACAAGGATCAGGTATCGGGTCAAGAGTCAAACCACCAAGTAGCCAATATCAATGCT
AACGGCGGGGTTGATGGCAGTCGCTATACCAGCTTTACTGTCAAGGAGCTCGATACAGCA
AGCTATACCATCACTGTTGAGGGAGCTACAGCAGGTCACACCTATGAGGCTTATCAGATT
TTCAAGGGTGACTTGTTTGACAGTACCCTATCAAACATCACATGGGGAGGTGGTGTTACA
CCTTTTGAATTTGATGGTTCAAAAGACGCTGCTAAGATTGCAGAGGGATTGAAGGAAGCA
AATGCAGCTGCCTTTGCCAAGGAAGCAGGTAAGCACTTGACAGCAACCATTGCAGGAACA
GGAACACATGCAATCACCGTTAACGAGGCTGGCTACTACCTCATCAAGGACAAAAATGAT
TCTCAAACAGGCAAGCATGACGCCTACACCTCATTTGTCCTGAAGGTTGTTAAAAACACC
AGCTTCAAACCAAAATCTGCTATCCCAACAGTCCTTAAAAAGGTCAAGGACCGTAATGAC
AAGACAGGTCTTGAGACAGGCTGGCAAGATTCAGCTGACTATGACAAAAATGACAAGGTG
CCATTCCAGCTAACCGCAACCCTACCGTCAAATTACGATGCCTTTCAAGAATACTACCTT
GAATTTGTAGATACCTTATCAAAAGGGCTAAGCTACAACAAAGACGCCAAGGTCTATGTG
GTTAATGGAGATACTCGTCAAGATATTACTAATTCATTTACAGTTAGTGAAGATGGTTCA
TCTTTTAAAATCAATAACCTAAAGGCTGTTCAGGGAGTAACAATAACAGCTACCAGTAAG
ATCGTTGTCGAATACACTGCTACCCTCAATGACCAAGCGGCCATCGGCAAAAAAGGAAAT
CCAAACGAAGTTGCTTTGAAATACTCAAACGATCCAAACGCTCTTGGAAAAGGAGAGGAG
TCTCCAAAAGGGGAGACACCAAAAGACAAGGTTATCGTTTTCACCTATAAAACTTCTAGA
TTATCTGGTCCGCCAGGATACCCACTTACTCGTGATTTCTCCCGTAACTTCCTAGAAGAA
AATACTGCAAAATATTTAGATCAATTAAGAGAACATCTACAGCACAGATTTAGTGAACTT
GAGAGCTTAACAAGAAAATTAGAGAAAGAAGGCGGTACCCGAGGTCCACTGCAGGACCAG
CCACCAGCACTAAAATATCCACAACCTAGAGACTATTTTCTTCATACTCGTGAAGGTGAT
GTTATTTATGATGAGGATATAAAAAGATATTTTGAGGATTTAGAAGCCTATTTAACAGCT
AGACTTGGTGGGATTGATAAAAAAGTAGAAGAAGCTGCCCAAAAGCCAGAGCTCTTAGAC
GCAGCAACAGTGTTAGAGCCTACAACAGCCTTCATTAGAGAAGCTGTTAGGGAAATCAAT
CAGCTGAGTGATGACTACGCTGACAATCAAGAGCTTCAGGCTGTTCTTGCTAATGCTGGA
GTTGAGGCACTTGCTGCAGATACTGTTGATCAGGCTAAAGCAGCTCTTGACAAAGCAAAG
GCAGCTGTTGCTGGTGTTCAGCTTGATGAAGCAAGACGTGAGGCTTACAGAACAATCAAT

GCCTTAAGTGATCAGCACAAAAGCGATCAAAAGGTTCAGCTAGCTCTAGTTGCTGCAGCA
GCTAAGGTGGCAGATGCTGCTTCAGTTGATCAAGTGAATGCAGCCATTAATGATGCTCAT
ACAGCTATTGCGGACATTACAGGAGCAGCCTTGTTGGAGGCTAAAGAAGCTGCTATCAAT
GAACTAAAGCAGTATGGCATTAGTGATTACTATGTGACCTTAATCAACAAAGCCAAATAA
CTCGAGCGGCCGCAT

SEQ ID NO: 34. CPCE fusion protein. Underlined amino acids indicate the sequence originating from the vector. The * indicates a scissor protease cleavage site. Note that the amino acids in bold originate from the construction work of the fusion protein and that these amino acids could be changed or even absent if another fusion strategy is used.

LEVLFQ*GPLGSTNLSDNITSLTVASSSLRDGERTTVKVAFDDKKQKIKAGDTIEVTWPTS
GNVYIQGFNKTIPLNIRGVDVGTLEVTLDKAVFTFNQNIETMHDVSGWGEFDITVRNVTQ
TTAETSGTTTVKVGNRTATITVTKPEAGTGTSSFYYKTGDMQPNDTERVRWFLLINNNKE
WVANTVTVEDDIQGGQTLDMSSFDITVSGYRNERFVGENALTEFHTTFPNSVITAIDNHI
SVRLDQYDASQNTVNIAYKTKITDFDQKEFANNSKIWYQILYKDQVSGQESNHQVANINA
NGGVDGSRYTSFTVK**EL**DTASYTITVEGATAGHTYEAYQIFKGDLFDSTLSNITWGGGVT
PFEFDGSKDAAKIAEGLKEANAAAFAKEAGKHLTATIAGTGTHAITVNEAGYYLIKDKND
SQTGKHDAYTSFVLKVVKNTSFKPKSAIPTVLKKVKDRNDKTGLETGWQDSADYDKNDKV
PFQLTATLPSNYDAFQEYYLEFVDTLSKGLSYNKDAKVYVVNGDTRQDITNSFTVSEDGS
SFKINNLKAVQGVTITATSKIVVEYTATLNDQAAIGKKGNPNFVALKYSNDPNALGKGEE
SPKGETPKDKVIVFTYKT**SR**LSGPPGYPLTRDFSRNFLEENTAKYLDQLREHLQHRFSEL
ESLTRKLEKEGGTRGP**LQ**DQPAALKYPEPRDYFLHTREGDVIYDEDIKRYFEDLEAYLTA
RLGGIDKKVEEAAQKP**EL**LDAATVLEPTTAFIREAVREINQLSDDYADNQELQAVLANAG
VEALAADTVDQAKAALDKAKAAVAGVQLDEARREAYRTINALSDQHKSDQKVQLALVAAA
AKVADAASVDQVNAAINDAHTAIADITGAALLEAKEAAINELKQYGISDYYVTLINKAK

Example 9. Purification of recombinant proteins

The pGEX-6P-1 vector used is a part of an *E. coli* expression and purification system called GST-glutathione affinity system (GE Healthcare, Uppsala, Sweden). Briefly, following the manufacturer´s instructions the clones encoding recombinant proteins were grown at 37 °C in Luria Bertani Broth medium supplemented with ampicillin (final conc. 50 µg/ml). At an optical density (OD_{600}) ~ 0.6, the growth medium was supplemented with IPTG (final conc. 0.2 mM) and the growth temperature shifted to 15 °C. After incubation over night the *E. coli*

cells were harvested and resuspended in a PBS phosphate-buffered saline [137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.4 mM KH_2PO_4 (pH 7.4)] supplemented with TWEEN 20, final conc. 0.1% (v/v) (PBST) and lysozyme was added (final conc. 50 µg/ml) whereupon the cells were lysed by freezing and thawing. After centrifugation, the supernatant was sterile filtrated and batch purified with Glutathione-Sepharose™ beads. After extensive washing using PBST the fusion protein was treated with scissor protease to release the recombinant proteins. The eluted samples containing the antigens were dialysed against PBS and concentrated. Finally, the amounts of antigens obtained were determined using spectrophotometer and the quality analyzed by SDS-PAGE (performed under reducing conditions) whereupon the gels were coomassie brilliant blue stained. The proteins were stored finally at -20°C. It should be noted that each protein produced in this system (SEQ ID NOS: 22, 24, 26, 28, 30, 32 and 34) contains five additional N- terminal amino acids, Gly-Pro-Leu-Gly-Ser, which are derived from the vector. The C-terminal end of each protein is as stated since a stop codon was added in the primer sequence.

Another *E. coli* expression and purification system used was the IMPACT system from New England Biolabs. The use of this system to produce *S. equi* recombinant proteins has previously been described (e.g. Ref. 14). It should be noted that each protein produced in this system (SEQ ID NOS: 38 and 42) contains five additional amino acids, one Met in the N-terminal part and four Leu-Glu-Pro-Gly at the C-terminal which are derived from the vector.

Example 10. Formulation of Strangvacc vaccines for horse immunizations

The recombinant proteins described in the examples were after purification (Example 9) mixed in the following combinations.

Strangvacc 1.

This combination of seven recombinant proteins (earlier called Septavacc) consists of proteins (fragments of) EAG, CNE, SclC, IdeE, IdeE2, SEQ0256 (Eq5), SEQ0402 (Eq8) and has previously been described in WO 2009/075646 (A1) and Refs. 13 and 14.

Strangvacc 2.

This combination consists of four recombinant proteins IdeE2, IdeE, Eq85 and CCE of which two are fusion proteins (Eq85 and CCE).

Strangvacc 3/ 4.

This combination consists of three recombinant proteins IdeE, Eq85 and CCE of which two are fusion proteins (Eq85 and CCE).

Strangvacc 5.

This combination consists of three recombinant proteins CNEEAG, IE5 and EndoSe of which two are fusion proteins (CNEEAG and IE5).

Strangvacc 7.

This combination consists of two recombinant fusion proteins CPCE and IE5.

Strangvacc 8.

This combination consists of three recombinant proteins CPCE, IE5 and EndoSe of which two are fusion proteins (CPCE and IE5).

Each of the Strangvacc vaccines (1-8) was briefly formulated as follows:

For subcutaneous immunization each dose contained 75 μg of each protein mixed with 375 μg/dose of the adjuvant Matrix C. (Except for Strangvacc 1 where each dose contained 50 μg of each protein). Dose volume was 2 ml which was subcutaneously injected 1ml + 1ml close to the retropharyngeal lymph nodes on each side.

For intranasal immunization each dose contained 225 μg of each protein mixed with 500 μg/dose of the adjuvant Matrix Q. (Except for Strangvacc 1 where each dose contained 150 μg of each protein). Dose volume was 4ml which was intranasal injected 2ml+2ml in each nostril.

For intramuscular immunization each dose contained 300 μg of each protein mixed with 375 μg/dose of the adjuvant Matrix C. Dose volume 2 ml that was injected at one location intramuscular.

To adjust the volumes in resp. Strangvacc preparation PBS was used. In the placebo samples the recombinant proteins were omitted. Matrix C and Q was obtained from Isconova AB, Uppsala, Sweden.

Horses were vaccinated three times. Time between first and second vaccination was seven weeks. The time between second and third vaccination was two weeks and horses were challenged two weeks after the last vaccination.

Example 11. Immunization and challenge studies in horses

Study I. This vaccination and challenge study was performed at Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU, UK sponsored by Intervacc AB, Sweden (study identification B009/001). Study II (study identification B009/002) was also performed at the same location. The objective of these studies were to determine the level of protection conferred on vaccination with variants of Intervacc's new multi-component subunit vaccine following intranasal challenge with wild type *S. equi* strain 4047 in Welsh Mountain ponies.

Briefly, all procedures for immunisation, experimental infection and clinical assessment of the horses were as described in PLoS Path, Guss et al (2009), Ref. 14, and WO 2009/075646 A1. However, study II was extended to also include a separate group of horses (group 6) that were only vaccinated intramuscularly (three vaccinations). Briefly, in the vaccination and challenge studies several parameters were monitored such as clinical symptoms, rectal temperature, injection site observations, and swelling of lymph nodes etc. The number of *S. equi* and *S. zooepidemicus* bacteria were also monitored. Furthermore, blood samples were also taken and used to determine e.g. neutrophils and fibrinogen levels and antibody response against antigens present in the respective vaccine. After completion of the vaccination/challenges studies the horses were euthanized and post mortem (PM) examinations were performed.

Table 3: Vaccination groups. IN means intranasal immunization. SC means subcutan immunization. Matrix is an adjuvant of Isconova AB, Uppsala, Sweden.

Study I

Group	Vaccine	Pony Chip ID's	Route	PM
1	Strangvacc 2 + Matrix	2691, 2695, 2703, 2711, 2717, 2811	IN + SC	17
2	Strangvacc 3/4 + Matrix	2849, 2900, 2901, 2930, 3028, 3060	IN + SC	21
3	Placebo + adjuvants	3156, 3250, 3693, 3736, 3844, 9624	IN + SC	48

Study II

Group	Vaccine	Pony Chip ID's	Route	PM
1	Strangvac 3/4 + Matrix	0278,0594,0481 0504,1529	IN + SC	2
2	Strangvacc 5 + Matrix	1567,2226,2259 2277,2417,2749	IN + SC	21
3	Strangvacc 7 + Matrix	2227,2633,2775 3305,3329,3611	IN + SC	18
4	Strangvacc 8 + Matrix	2720,3062,3680 3773,3942,3954	IN + SC	26
5	Placebo + Matrix	3839,9893,4069 9170,9408	IN + SC	38
6	Strangvacc 8 + Matrix	3596,3730,3762 2799,2991,9240 9807	Intramuscular	28

PM= Mean value from pathology score as taken at post mortem examination.

Example 12.

Welsh Mountain Ponies were vaccinated with Strangvacc 2 (n=6), Strangvacc 3/4 (n=6) and placebo (n=6) in Study I. In Study II ponies were vaccinated with Strangvacc 3/4 (n=5), Strangvacc 5 (n=6), Strangvacc 7 (n=6), Strangvacc 8 (n= 6) and placebo (n=5). Ponies given placebo serve as controls and were given adjuvant only. Immunisations were done at three occasions intranasally and subcutaneously on both sides. All ponies were experimentally infected with *S. equi* to cause strangles. The ponies were subjected to clinical examinations daily and rectal temperatures were monitored. Pyrexia is a typical sign of strangles and correlates very well with inflammatory parameters, such as elevated fibrinogen level and neutrophil counts in blood. All procedures for immunisation, experimental infection and clinical assessment of the horses were as described in PLoS Path, Guss et al (2009).

Figure 1 (Figures 1A-C) includes 8 panels, each showing the temperature of individual ponies for each group vaccinated as indicated on top of each panel. It is clear from the graphs that different formulations result in different levels of protection. As an example, vaccination

with Strangvacc 3/4 results in only one out of eleven ponies (Studies I and II combined) with pathological pyrexia, defined as temperature exceeding 39°C. Strangvacc 8, on the other hand, although protective, results in 3-4 out of six ponies with pyrexia.

Example 13.

Ponies vaccinated as described for Example 12 above were subjected to post mortem analysis at the end point of the experiments. The endpoint is defined as pyrexia for 3 days, obvious clinical signs of infection with suffering or at the end of study (day 21 in study I or day 25 in Study II). A scoring system was used for various post mortem observations as described in PLoS Path, Guss et al (2009), Ref 14. The added score is shown in Figure 2 where each dot represents an individual pony. Of the eleven ponies vaccinated with Strangvacc 3/4 only three displayed a pathological high score. The highest score (46) is the same individual as the one with pyrexia. Strangvacc 8 resulted in two ponies fully protected and one intermediate, based on the post mortem scoring. Using a Mann Whitney statistical analysis of the groups in comparison with the combined placebo groups (n=11), the following p-values were obtained: Strangvacc 2, 0.0019; Strangvacc 3/4, 0.00027; Strangvacc 5 , 0.0048; Strangvacc 7, 0.00064; Strangvacc 8, 0.078. A good correlation was found between parameters such as time to pyrexia and post mortem score; short time to pyrexia was found in ponies with high post mortem score. As obvious from the graph, different combinations of fusion proteins in the vaccines result in different level of protection.

Example 14.

Antibody responses in vaccinated ponies were determined as described in PLoS Path, Guss et al (2009), Ref. 14. Briefly, a conventional ELISA test was used where serum samples were diluted in two-fold series. The log values of the dilution required to give an absorbance value of 1.0 were determined for each sample. Sera were analysed from ponies immunised in a previous study where a vaccine, Septavacc (also called Strangvacc 1) was used. Strangvacc 1 contains seven recombinant proteins as single proteins. Sera were also analysed from ponies vaccinated with various fusion proteins.

All ponies vaccinated with any of the Strangvacc vaccines responded immunologically. This is the case both for Strangvacc 1, where antigens are single antigens, and for the other Strangvacc vaccines with fusion proteins.

In no case did a fusion protein result in an encompassed protein becoming non-immunogenic, due to unfavourable folding or exposure to the immune system.

Figure 3 shows instead that immunogenicity are in some cases significantly enhanced by using fusion proteins. Top panel in Figure 3 shows that antibodies in ponies vaccinated with Strangvacc 2 and Strangvacc 3/4 have significantly ($p=0.04$) better anti CNE antibodies than ponies vaccinated with Strangvacc 1. CNE is in Strangvacc 2 and 3/4 included in the same fusion as EAG. In Strangvacc 1, CNE is included as a single protein. Similarly, bottom panel in Figure 3 shows that antibodies against Eq5 (=SEQ0256) are significantly higher ($p= 0.0008$) in ponies vaccinated with fusion proteins than with Eq5 as a single protein.

Example 15. Intramuscular vaccination using Strangvacc 8.

The intramuscular vaccination using Strangvacc 8 (group 6 in study II) resulted in a protection level similar to group 4 in study II.

Example 16. Construction of clones expressing Eq54 and Eq27 protein fragments to be used as recombinant antigens in vaccination of mice against *S. equi* infection

A gene fragment of the *eq54* gene was PCR amplified using primer pairs Eq54F and Eq54R. After amplification and purification the fragment was digested with NcoI and XhoI and ligated into the NcoI and XhoI cleaved vectorpTYB4 obtained from New England Biolabs Inc., USA (NEB).

Table4. Primers used to clone *eq54* gene fragment

SEQ ID 35. Eq54F 5′-gcat**ccatgg**atacagcaagctatacca-3′

SEQ ID 36. Eq54R 3′-caattatttttt**cccagatag**gagctcagct-5′

SEQ ID NO: 37. The nucleotide sequence of the *eq54* gene inserted in the pTYB4 vector. The NcoI and XhoI sites are indicated in bold and the vector sequences are underlined.

CCATGGATACAGCAAGCTATACCATCACTGTTGAGGGAGCTACAGCAGGTCACACC
TATGAGGCTTATCAGATTTTCAAGGGTGACTTGTTTGACAGTACCCTATCAAACATCACA
TGGGGAGGTGGTGTTACACCTTTTGAATTTGATGGTTCAAAAGACGCTGCTAAGATTGCA
GAGGGATTGAAGGAAGCAAATGCAGCTGCCTTTGCCAAGGAAGCAGGTAAGCACTTGACA
GCAACCATTGCAGGAACAGGAACACATGCAATCACCGTTAACGAGGCTGGCTACTACCTC
ATCAAGGACAAAAATGATTCTCAAACAGGCAAGCATGACGCCTACACCTCATTTGTCCTG

AAGGTTGTTAAAAACACCAGCTTCAAACCAAAATCTGCTATCCCAACAGTCCTTAAAAAG
GTCAAGGACCGTAATGACAAGACAGGTCTTGAGACAGGCTGGCAAGATTCAGCTGACTAT
GACAAAAATGACAAGGTGCCATTCCAGCTAACCGCAACCCTACCGTCAAATTACGATGCC
TTTCAAGAATACTACCTTGAATTTGTAGATACCTTATCAAAAGGGCTAAGCTACAACAAA
GACGCCAAGGTCTATGTGGTTAATGGAGATACTCGTCAAGATATTACTAATTCATTTACA
GTTAGTGAAGATGGTTCATCTTTTAAAATCAATAACCTAAAGGCTGTTCAGGGAGTAACA
ATAACAGCTACCAGTAAGATCGTTGTCGAATACACTGCTACCCTCAATGACCAAGCGGCC
ATCGGCAAAAAAGGAAATCCAAACGAAGTTGCTTTGAAATACTCAAACGATCCAAACGCT
CTTGGAAAAGGAGAGGAGTCTCCAAAAGGGGAGACACCAAAAGACAAGGTTATCGTTTTC
ACCTATAAAACTATCATCAATAAGGTTGATCAAGATCAAAAAGCCCTAAAAGGTGCAGGC
TTTACCCTTTATAAGCTGGTCAAAGGTGATAATGGCGAGGAAAAATATCAAATAGTCCAA
GAAATTAAAGCAGGGGATACAACTAGCTTTGAGTTTGTTGGACTTGACGCTGGTGATTAC
AAGCTCAGCGAAACAACAACACCTGGCGGTTACAACACTATTGCAGATGTCATGTTCAGC
ATTGTAGCGCAGCATGAAACCGAGTCAGACGATCCTCAGTTGACTAGCCTAACCGTTGAC
AAAGCAACTGGCTTCACTGCTGATACAGAAGCTGGTACCGTATCCGCAACTATTGTTAAT
AAAAGGTCTATC**CTCGAG**CCCGGGTGC

SEQ ID NO: 38. Eq54 protein expressed using the IMPACT™ –system (NEB). Note that N-terminal amino acid Met and the four C-terminal amino acids Leu-Glu-Pro-Gly originate from the vector.

MDTASYTITVEGATAGHTYEAYQIFKGDLFDSTLSNITWGGGVTPFEFDGSKDAAKIAEG
LKEANAAAFAKEAGKHLTATIAGTGTHAITVNEAGYYLIKDKNDSQTGKHDAYTSFVLKV
VKNTSFKPKSAIPTVLKKVKDRNDKTGLETGWQDSADYDKNDKVPFQLTATLPSNYDAFQ
EYYLEFVDTLSKGLSYNKDAKVYVVNGDTRQDITNSFTVSEDGSSFKINNLKAVQGVTIT
ATSKIVVEYTATLNDQAAIGKKGNPNEVALKYSNDPNALGKGEESPKGETPKDKVIVFTY
KTIINKVDQDQKALKGAGFTLYKLVKGDNGEEKYQIVQEIKAGDTTSFEFVGLDAGDYKL
SETTTPGGYNTIADVMFSIVAQHETESDDPQLTSLTVDKATGFTADTEAGTVSATIVNKR
SI**LEPG**

The IMPACT-system was also used to clone and express a fragment of the Eq27 protein. A gene fragment of the *eq27* gene was PCR amplified using primer pairs Eqp271 and Eqp272. After amplification and purification the fragment was digested with NcoI and XhoI and ligated into the NcoI and XhoI cleaved vectorpTYB4 obtained from New England Biolabs Inc., USA (NEB)

Table 5. Primers (5′-3′) used to clone *eq27* gene fragment.

SEQ ID NO: 39. Eqp271: gcag**ccatgg**agagtctgacgagtgttga

SEQ ID NO: 40. Eqp272: TCACCTCGAGTCCTAGCTCACCGTCATAAGC

SEQ ID NO: 41. The nucleotide sequence of the *eq27* gene inserted in the pTYB4 vector. The NcoI and XhoI sites are indicated in bold and the vector sequences are underlined.

CCATGGAGAGTCTGACGAGTGTTGAGCCTGCTGATGGTGCGGTCATGGTCAAGTCAGAGG
CTGCTGACCAAGGCTCAAATGAGCTACCAGAAGCTACTGACATTAGTGATATTGCTGGTA
TTTCTGATGTGACTAAGGTGTCAGCTGCTGTCAATGCTGATACTGTCAAGGAAGTTCAGC
CAGTAGCTGTACCTCTTGTAGAGGATCAGGCGCATGAGGAAACTACAGACCAGTCTCAGC
CTTCATCATCGATAGTGTCTGTTACGACAGACAGCTCTCTAGAGACACCAGAAGCTACAA
GCTCAGAGGAGCCGATAGCGGAGCAGACCTTGCGGCTGCATTTCAAGACCCTGCCAGCTC
AAGACCTATCCTCGCTTGGTCTTTGGGTGTGGGACGATGTTGAGACACCATCTGATCAGC
TGGGAGGCTGGCCGACTGGGGCTACCAATTTTAGTCTAGCGAAGACAGATGACTATGGCT
ATTACATGGACGTTAAGCTTTCAGCCAATCAAGCCAATAAGGTTAGCTTTTTGATCAATA
ACACTAAGGGAGACAATCTGACGGGCGATCGAACCATAGACCTTCTCAGCCCTAAGATGA
ATGAGGTCTGGATTGATGGCCAGGAGCTGTCTTACTATCGGCCGCTGGCTCAGGGCTATA
TCCGTATCAATTATTATCGCAGTGATGGCCATTATGACAACAAATCGCTCTGGCTTTGGG
GAAGTGCTGATGCGTCAATGACTAGTCAGCAGGGCGCTTGGCCAGATGGTATTGATTTTA
AGCAGGTCGGTCGATATGGTGCTTATATAGATGTCAAGCTAGCTGATACCAATGAGCTAG
GCTTTCTCTTGCTAGATGAGCGTCAGACAGGTGACGCTGTTAAAATTCAGCCCAATGATT
ATATTTTTAAAGATTTAAAGAATCACACCCAAATTTTCTTGAAAGACGAGGATCCAACCA
TTTATACGAACCCTTATTTTGTTAATACAGTTAGATTAATCGGTGCTCAGCAGGTCAGCC
CAAGCAGTATTGAGGCGAGCTTTACGACTCTAGCAGATGTGGATAAGGAAAGCCTTTTAA
AAGAATTAAAAATCAGCACTGACAGTAAGGAAGCAGTTGCTATTACTGATATCACCTTAG
ATGAAAAGACTCATAAGGCTGTCATCACAGGTGATTTTAGTCAAGCAGTGGCCACTTATA
CGGTGACCTTTCATCATGAGAGCTTCCAGGCTAGGCCAAATTGGCAATACAAGGATAGCC
TGTATGCTTATGACGGTGAGCTAGGA**CTCGAG**CCCGGGTGC

SEQ ID NO: 42. Eq27 protein fragment expressed using the IMPACT^{TM} –system (NEB). Note that N-terminal amino acid Met and the four C-terminal amino acids Leu-Glu-Pro-Gly originate from the vector.

MESLTSVEPADGAVMVKSEAADQGSNELPEATDISDIAGISDVTKVSAAVNADTVKEVQP
VAVPLVEDQAHEETTDQSQPSSSIVSVTTDSSLETPEATSSEEPIAEQTLRLHFKTLPAQ
DLSSLGLWVWDDVETPSDQLGGWPTGATNFSLAKTDDYGYYMDVKLSANQANKVSFLINN
TKGDNLTGDRTIDLLSPKMNEVWIDGQELSYYRPLAQGYIRINYYRSDGHYDNKSLWLWG
SADASMTSQQGAWPDGIDFKQVGRYGAYIDVKLADTNELGFLLLDERQTGDAVKIQPNDY
IFKDLKNHTQIFLKDEDPTIYTNPYFVNTVRLIGAQQVSPSSIEASFTTLADVDKESLLK
ELKISTDSKEAVAITDITLDEKTHKAVITGDFSQAVATYTVTFHHESFQARPNWQYKDSL
YAYDGELG**LEPG**

Intranasal vaccination with Eq 54 and Eq27 followed by challenge with *Streptococcus equi*

Example 17. Immunisation of mice with Eq54 and Eq27

Mice (NMRI) weighting approximately 23-25 g were kept in cages of five animals in each. The mice were immunised intranasally with 12 micrograms of each antigen and 10 microgram of Abisco 300 (Isconova AB, Sweden). Ten animals were immunised with Eq54, 10 animals were immunised with Eq27 and 10 were given Abisco 300 adjuvant only to serve as a negative control. Immunisations were given on days 0, 31 and 45.

Example 18. Experimental infection with *Streptococcus equi*

Experimental infection was given on day 52 (7 days after last time of immunisation). *S. equi* strain 1866 from a clinical case of strangles was used. The strain was first passed through an animal by inoculating ca 10^6 CFU into the nostrils of an anaesthetized mouse. Bacteria were recovered after 7 days from the nose of the mouse and grown on BG plates (agar plates containing 5% sheep blood 0.01% gentiana violet) at 37°C in 5% CO_2. A single colony was grown on BG plates overnight at 37°C and resuspended in Todd Hewitt Broth (Oxoid, Basingstoke, Hampshire, United Kingdom) (THB) with 1% yeast extract (THY). The bacteria were kept at -80°C in vials and a new vial was used for each experiment. To infect mice, bacteria were grown on BG plates at 37°C in 5% CO_2 overnight, followed by inoculation into THB supplemented with 1% Yeast extract (THY) and grown without shaking over night. The culture was then diluted 10 times into THY and 10% horse serum (Sigma) and grown for 4 hours at 37°C in 5% CO_2. The culture was centrifuged and resuspended in THB. A dose containing $1x10^6$ CFU in 10µl was used for all *S. equi* infections of mice. The animals were followed daily. Bacterial nasal growth was scored on a four-graded scale from 0

to +++ by gently pressing the nose of the animal onto a BG plate in a reproducible manner. The nasal sample was then spread out onto the entire surface of the plate. One + means 5-100 colonies; two + means more than 100 and three + means confluent growth. The weight was determined every day and the percentage of weight-loss was calculated.

Example 19. Experimental results of vaccination with Eq54 or Eq27.

Three groups of mice (n=3x10) were immunised with 1) Eq54 2) Eq27 and 3) non-immunised group where the antigen was replaced with PBS, but still containing the adjuvant.

A typical sign of infection in mice infected with *S. equi* subsp. *equi* is the loss of weight. The percentage weight loss over time was thus determined. Figure 4B shows that animals vaccinated with Eq54 or Eq27 were protected from infection, reflected by a milder loss of weight compared with control animals. Animals that lost more than 20% weight were killed. It can be seen in Figure 4B that non-vaccinated animals lost more weight than the vaccinated animals. On day 2 to 4, $p<0.05$ for Eq54 and for Eq27 compared with controls.

Another sign of persistent infection of mice with *S. equi* subsp. *equi* is the colonisation of bacteria in the upper respiratory airways. Nasal growth of *S. equi* was therefore determined daily on a four graded scale. Figure 4C shows that after 2 to 3 days, the non-vaccinated control animals were heavily colonized with bacteria. Mice vaccinated with Eq54 or Eq27 were significantly ($p<0.05$) less colonized compared with the control group on days 2 and 3.

Example 20. Determination of antibody levels in immunized mice

Mice were immunized as described above. Serum samples were collected 5 days after last vaccination. Standard Enzyme Linked Immuno Sorbent Assay (ELISA) was used to determine levels of IgG specifically directed against Eq54 and Eq27. Briefly, microtiter plates were coated with 100 µl over night at room temperature with either protein at 9 µg/ml in Phosphate Buffered Saline (PBS). Bovine Serum Albumin, 100 µl at 2%, was added (1 hour at 37°C). The plates were washed with PBS with 0.05% Tween (PBST). Serum samples were added at serial dilutions, starting at a 40-fold dilution (1 hour at 37°C) followed by washing. The specific binding of IgG to the antigens was monitored by adding anti mouse IgG antibodies raised in rabbit conjugated with Horse Radish Peroxidase (Sigma Chemical Co, Mo, USA); 100 µl per well at 1000-fold dilution. After washing in PBST, binding of the conjugate was measured by adding OPD substrate according to the instructions provided by

the manufacturer (Dako, Glostrup, Denmark). The coloration was determined at 492 nm in a standard ELISA spectrophotometer. The obtained absorbance values were plotted as a function of serum dilution. For each sample, the 10log values of the dilution required to bring down the absorbance value to 1.5 were determined. I.e., if a sample requires a 2000 fold dilution to give an absorbance of 1.5, a value of 3.30 is assigned to that sample. Figure 4A shows antibody titers against Eq54 and Eq27 in mice immunized with these antigens.

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CLAIMS

1. An antigenic composition comprising a plurality of antigenic components derived from antigens of *Streptococcus equi* subsp. *equi* or subsp. *zooepidemicus*, the antigenic components comprising:

(i) a first fusion polypeptide comprising at least part of a protein designated EAG and at least a part of protein designated CNE; and

(ii) at least one additional polypeptide selected from

(a) a second fusion polypeptide comprising at least part of a protein designated Eq5 and at least a part of a protein designated Eq8,

(b) a third fusion polypeptide comprising at least part of a protein designated IdeE and at least a part of a protein designated Eq5,

(c) at least a part of a protein designated EndoSe or a protein designated EndoSz,

(d) at least part of a protein designated IdeE,

(e) at least part of a protein designated IdeE2,

(f) at least part of a protein designated Eq27,

(g) at least part of a protein designated Eq54,

(h) at least part of a protein of a protein family designated Scl.

wherein each said at least part of said proteins comprises at least one antigenic epitope, and wherein the first fusion polypeptide comprises an amino acid sequence selected from the amino acid sequence as shown in SEQ ID NO: 24, starting from amino acid 12; the amino acid sequence as shown in SEQ ID NO: 28, starting from amino acid 12; and the amino acid sequence as shown in SEQ ID NO: 34, starting from amino acid 12; and/or

the second fusion polypeptide comprises the amino acid sequence as shown in SEQ ID NO: 22, starting from amino acid 12; and/or

the third fusion polypeptide comprises the amino acid sequence as shown in SEQ ID NO: 30, starting from amino acid 12.

2. The antigenic composition according to claim 1, comprising:

(i) a fusion polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 22 (starting from amino acid 12); (ii) a fusion polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 24 (starting from amino acid 12); and (iii) a polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 26 (starting from amino acid 12).

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3. The antigenic composition according to claim 1, comprising:

(i) a fusion polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 28 (starting from amino acid 12); (ii) a fusion polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 30 (starting from amino acid 12); and (iii) a polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 32 (starting from amino acid 12).

4. The antigenic composition according to claim 1, comprising:

(i) a fusion polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 34 (starting from amino acid 12); (ii) a fusion polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 30 (starting from amino acid 12); and (iii) a polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 32 (starting from amino acid 12).

5. The antigenic composition according to claim 1, comprising:

(i) a fusion polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 34 (starting from amino acid 12); and (ii) a fusion polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 30 (starting from amino acid 12).

6. An antigenic fusion polypeptide selected from the group consisting of:

a polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 24, starting from amino acid 12;

a polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 28, starting from amino acid 12; and

a polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 34, starting from amino acid 12.

7. An antigenic composition comprising at least one of the fusion polypeptides defined in claim 6.

8. A vaccine composition for protecting non-human mammals against infection of *Streptococcus equi*, which comprises the antigenic composition of any one of claims 1 to 5 and 7 as an immunizing component, a pharmaceutically acceptable carrier, and preferably an adjuvant, and which preferably is provided in a physiologically administrable form, preferably a

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form that is administrable by intramuscular, intradermal, subcutaneous or intranasal inoculation.

9. A vaccine composition for protecting non-human mammals against infection of *Streptococcus equi*, which comprises at least one recombinant vector with polynucleotides inserted therein which encode the antigenic components of the compositions of any one of claims 1 to 5 and 7, which vector or vectors are capable of expressing said antigenic components *in vivo* in a mammal susceptible to infection with *Streptococcus equi*, wherein the vector preferably is an expression vector in the form of a plasmid or a viral vector, and which composition preferably is provided in a physiologically administrable form, preferably a form that is administrable by intramuscular, intradermal, subcutaneous or intranasal inoculation.

10 The vaccine composition according to claim 8 or 9, wherein the vaccine composition is capable of protecting susceptible mammals against infection with *Streptococcus equi*, especially horses against strangles caused by *Streptococcus equi* subsp. *equi*, and which preferably is capable of stimulating serum, mucosal and/or bronchial antibody responses directed against *Streptococcus equi* antigens in mammals susceptible to *Streptococcus equi*, especially horses.

11. A method for producing an antigenic composition of any one of claims 1 to 5 and 7, which method comprises:

(a) providing DNA fragments encoding said antigen components and introducing said fragments into at least one expression vector;

(b) introducing said at least one vector, which contains said DNA fragments, into a at least one compatible host cell;

(c) culturing said at least one host cell provided in step (b) under conditions required for expression of the products encoded by said DNA fragments; and

(d) isolating the expressed products from each cultured host cell, and, optionally,

(e) purifying the isolated products from step (d), preferably by a chromatographic method.

12. A method for the preparation of a vaccine composition according to any one of claims 8 to 10, which vaccine composition comprises as an immunizing component an antigenic composition of any one of claims 1 to 5 and 7, wherein the method comprises mixing the antigenic composition with a pharmaceutically acceptable carrier.

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13. Use of an antigenic composition according to any one of claims 1 to 5 and 7 in the preparation of a vaccine protecting against infection with *Streptococcus equi*, especially against strangles caused by subsp. *equi* infection in horses.

14. Use of an antigenic composition according to any one of claims 1 to 5 and 7 for the prophylactic or therapeutic treatment of infections caused by *Streptococcus equi*.

15. A method for the production of an antiserum, which method comprises administering an antigenic composition according to any one of claims 1 to 5 and 7 to a non-human mammal host to produce antibodies in said host and recovering antiserum containing said antibodies produced in said host.

16. A method of prophylactic or therapeutic treatment of *Streptococcus equi* infection in a mammal, especially a horse, comprising administering to said mammal an immunologically effective amount of a vaccine composition of any one of claims 8 to 10, or of an antiserum produced according to claim 15.

17. A method of protecting horses against *Streptococcus equi* infection, which comprises inoculating a horse subcutaneously, intradermally, intramuscularly or intranasally with a vaccine composition of any one of claims 8 to 10 to induce an immune response against *Streptococcus equi* in said horse, wherein the immune response preferably is induced in the form of IgG and/or IgA and/or IgM antibodies in serum and/or the masopharyngeal mucus in said horses.

18. An antibody preparation comprising at least one, and preferably at least two monoclonal or polyclonal antibodies, or antibody fragments, specific for an antigen of the antigenic composition according to any one of claims 1 to 5 and 7, wherein the antibody preparation preferably is to be used prophylactically or therapeutically to provide passive immunization when administered to a mammal susceptible to infection by *Streptococcus equi*.

19. A method of passive immunization which comprises administering to a mammal an antibody preparation of claim 18.

20. An antigenic composition produced by the method of claim 11.

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21. An antiserum produced by the method of claim 15.

22. An antigenic composition according to claim 1 or claim 7, an antigenic fusion polypeptide according to claim 6; a vaccine composition according to claim 8 or claim 9; a method for producing an antigenic composition according to claim 11; a method for the preparation of a vaccine composition according to claim 12; use of an antigenic composition according to claim 13 or claim 14; a method for the production of an antiserum according to claim 15; a method of prophylactic or therapeutic treatment according to claim 16; a method of protecting horses according to claim 17; an antibody preparation according to claim 18; a method of passive immunization according to claim 19; an antigenic composition according to claim 20; or an antiserum according to claim 21, substantially as herein described with reference to any one or more of the examples but excluding comparative examples.

Figure 1A
(Panels A, B and C)

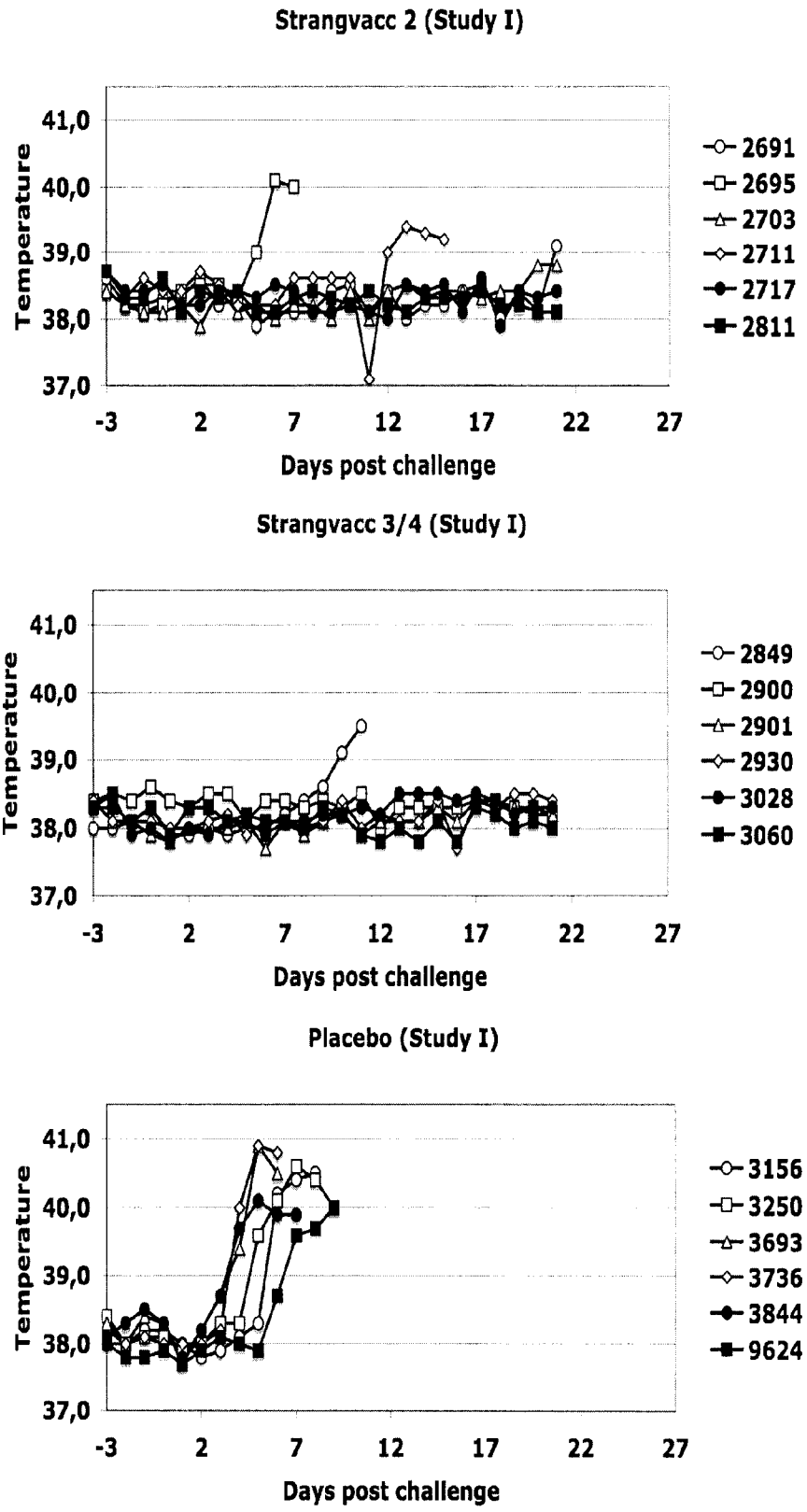

Figure 1B
(Panels D, E and F)

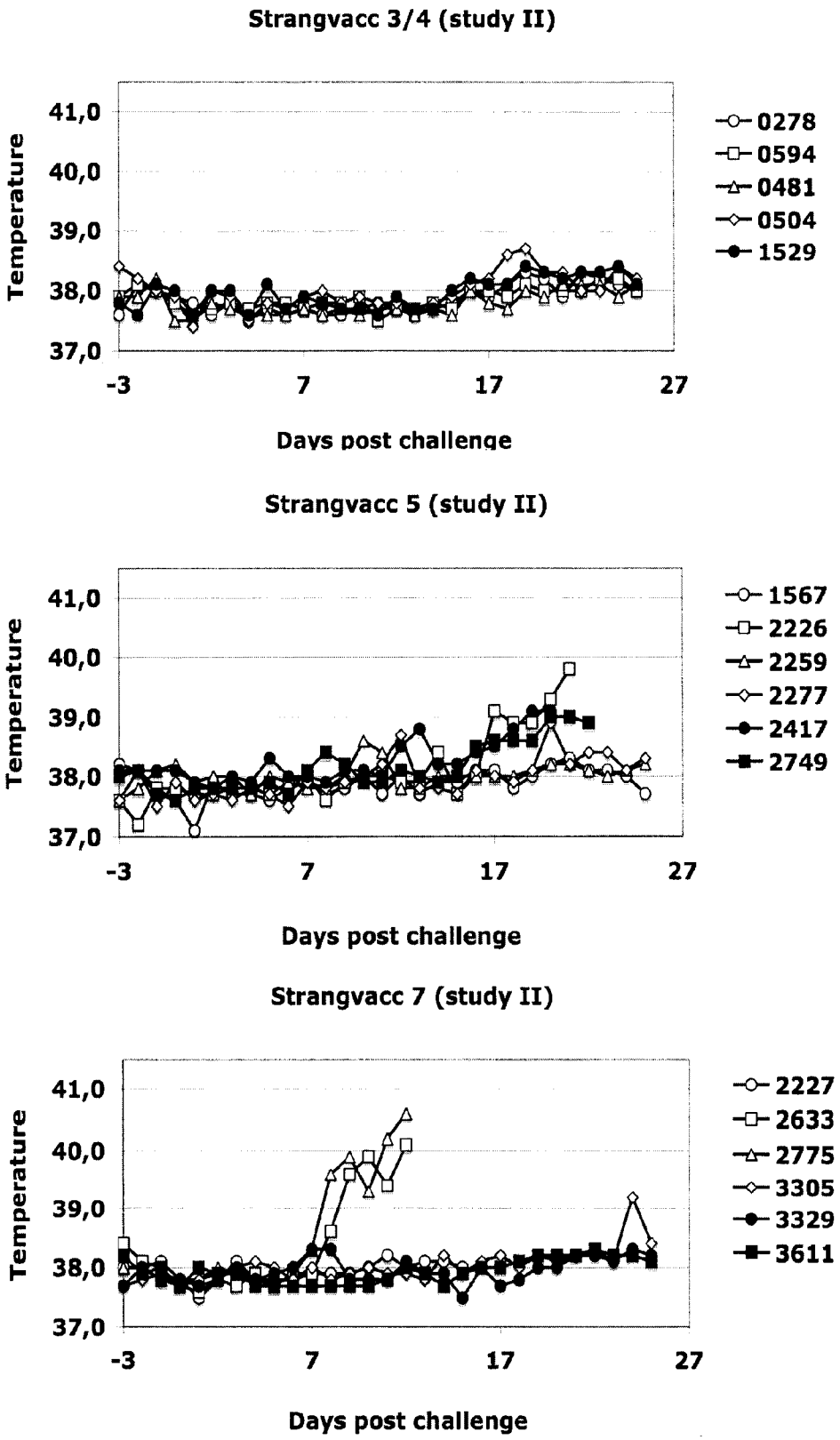

Figure 1C
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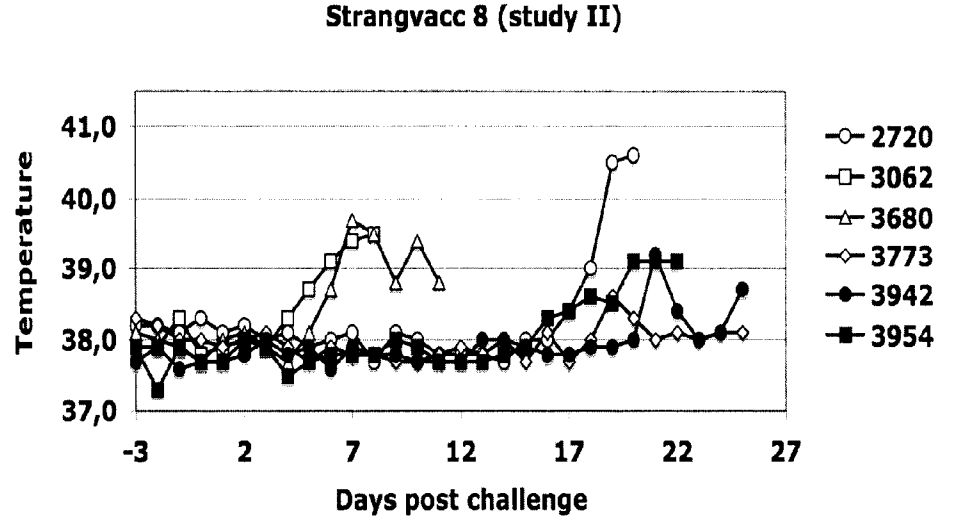

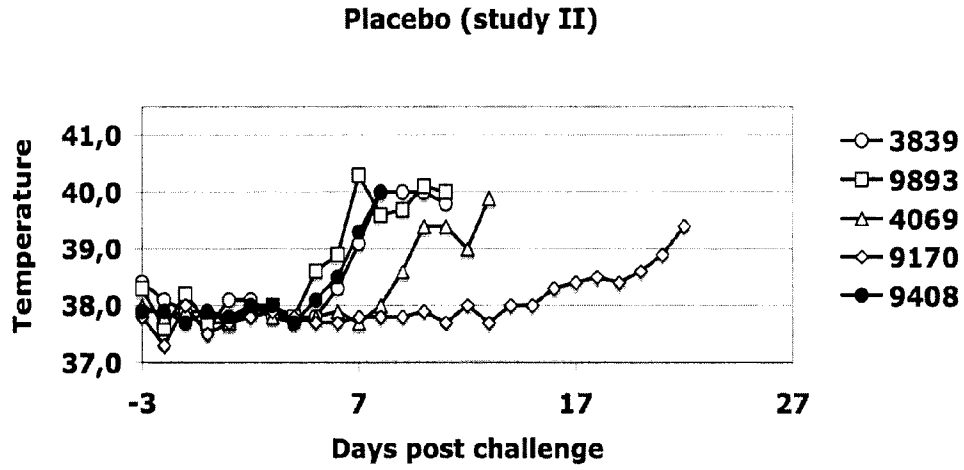

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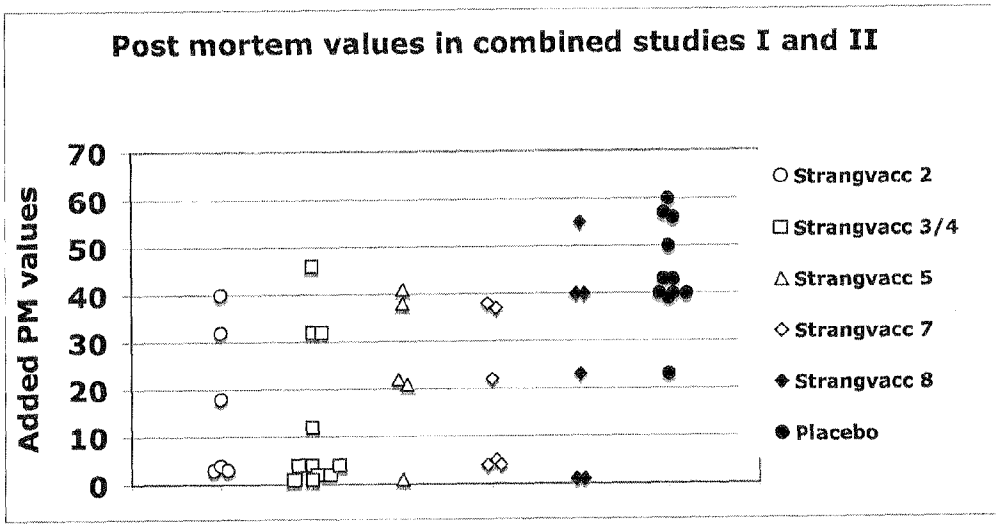
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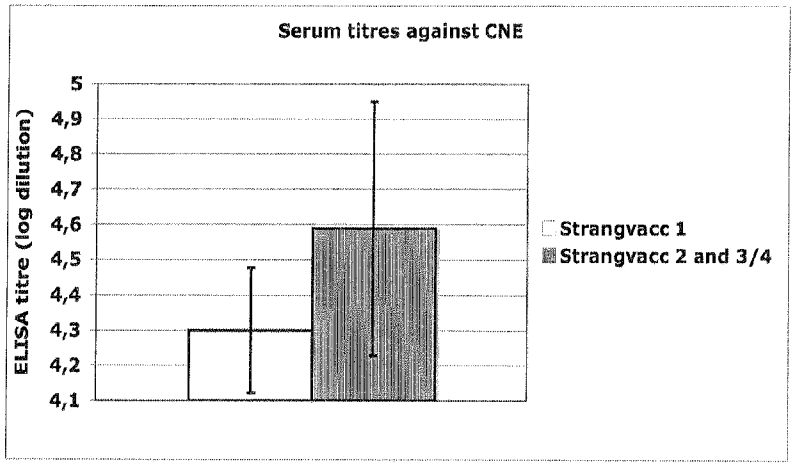
Placebo

Figure 3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ELISA titre (log dilution)
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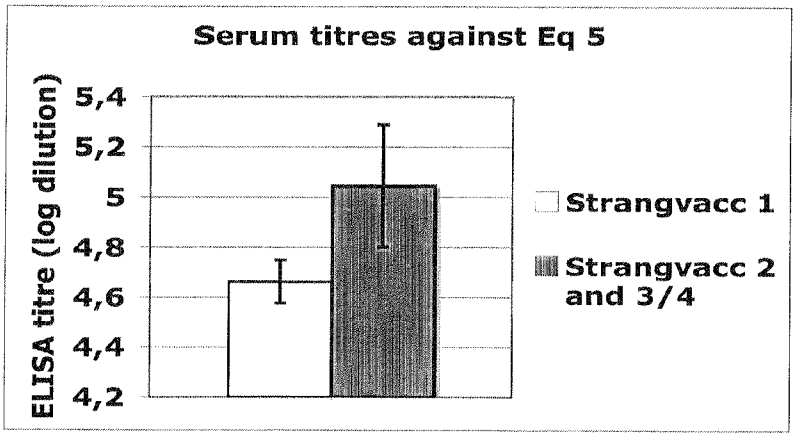
Strangvacc 1
Strangvacc 2 and 3/4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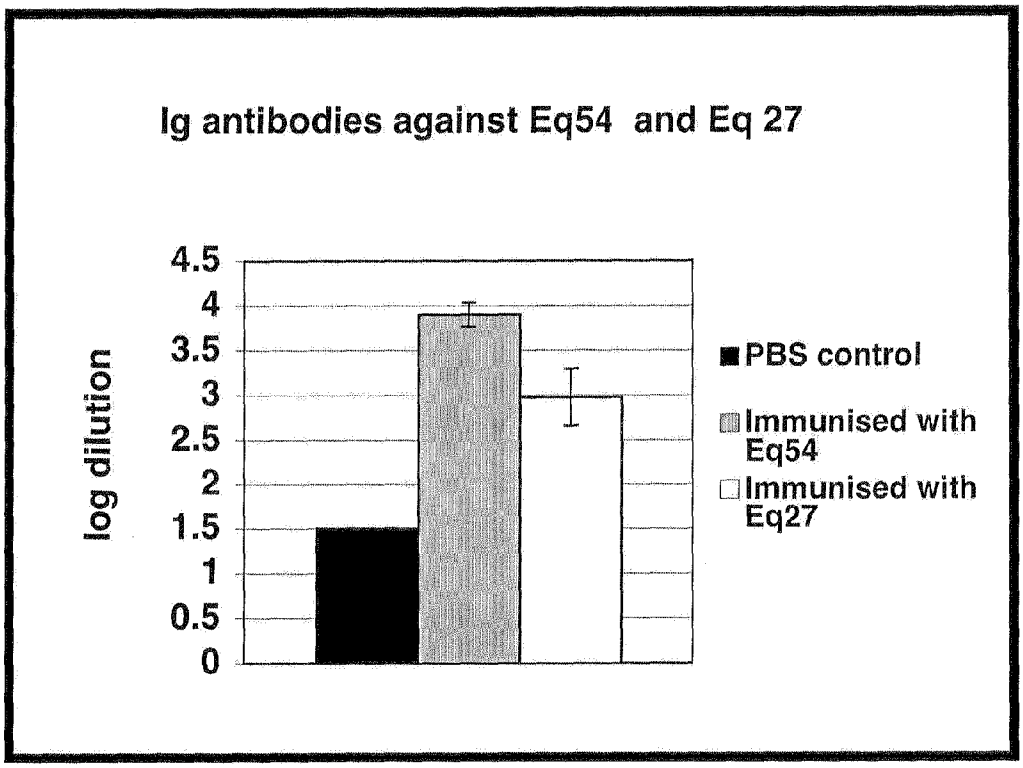
Strangvacc 2 and 3/4

Figure 4A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Immunised with Eq27

Figure 4B

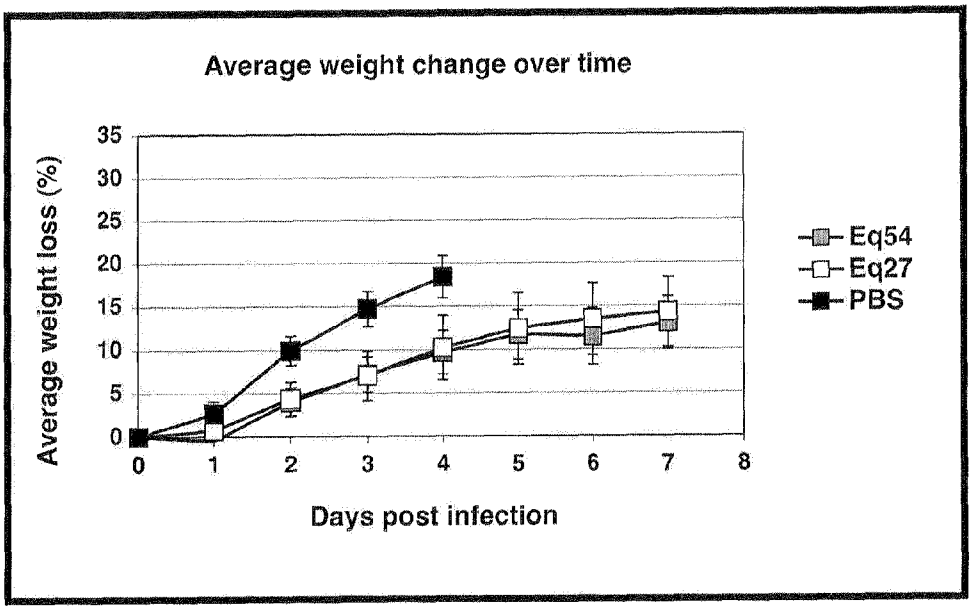
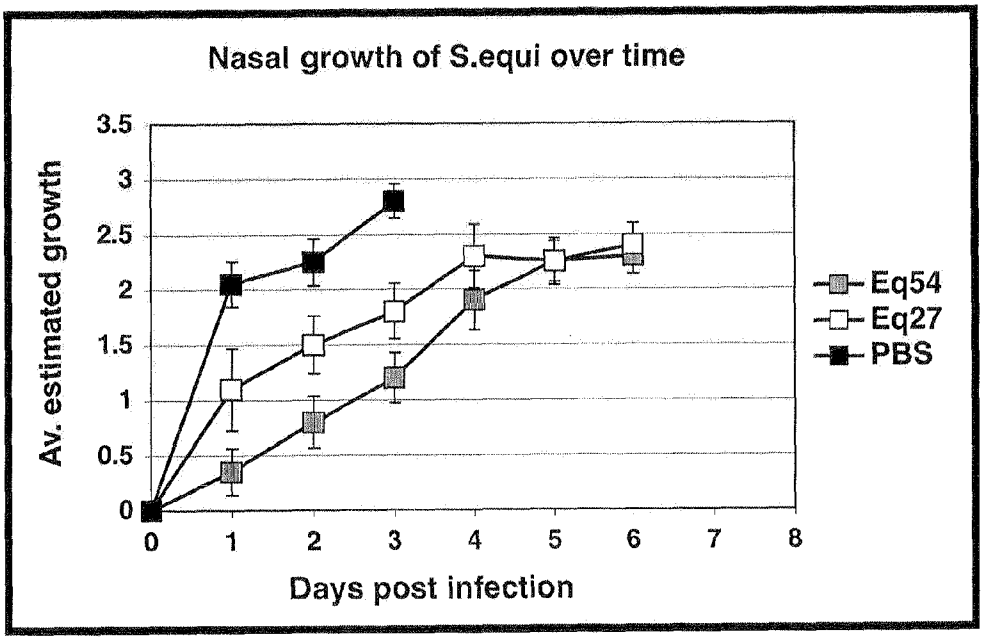


Figure 4C



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<400> 23
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gatgacaaaa aacagaaaat caaggcaggg gatacgatag aggtcacctg gcctacaagt 180

ggtaatgtct acattcaggg ctttaataaa accataccgc ttaatattag aggggtagat 240

gttggtacct tggaggtcac gctagacaag gctgttttca cattcaatca aaatattgaa 300

acaatgcatg atgtctctgg ttggggagag tttgatatta ctgttagaaa tgtgacacaa 360

accaccgctg aaacatcagg aacgaccaca gtaaaggtag gcaatcgcac tgctactatc 420

actgttacta agcctgaggc aggcactggt accagctcat tttattataa gactggtgat 480

atgcagccca atgatactga gcgtgtgaga tggttcctgc tgattaacaa caacaaggaa 540

tgggtggcca atactgttac agtcgaagac gatattcaag gtggtcaaac cttggatatg 600

agcagctttg acatcaccgt atctggttat cgtaacgagc gcttcgttgg ggaaaacgct 660

ctgacagagt ttcatacaac atttccaaat tctgtcatta cggcaacaga taatcacatt 720

agtgtgcggt tagatcaata tgatgcctca caaaacactg tcaacattgc ttataagaca 780

aagataacgg actttgacca aaaagaattt gccaacaaca gtaaaatctg gtaccagatt 840

ttatacaagg atcaggtatc gggtcaagag tcaaaccacc aagtagccaa tatcaatgct 900

aacggcgggg ttgatggcag tcgctataccagctttactg tcaaggagct ctcggaaccc 960

aatccatatc cagatgtgag gcgtttcctt gatgagaagt acgatggaga tgtggataaa 1020

ttatctaaac aacttcaagg ttatttttggt agtttaagag agtatatagaa gtttgaactt 1080

aaaaatggca aacaaggtcc ttctagatta tctggtccgc caggataccc acttactcgt 1140

gatttctccc gtaacttcct agaagaaaat actgcaaaat atttagatca attaagagaa 1200

catctacagc acagatttag tgaacttgag agcttaacaa gaaaattaga gaaagaaggc 1260

ggtacccgag gtccactgca ggaccagcca gcagcactaa aatatccaga acctagagac 1320

tattttcttc atactcgtga aggtgatgtt atttatgatg aggatataaa aagatatttt 1380

gaggatttag aagcctattt aacagctaga cttggtggga ttgataaaaa agtagaagaa 1440

gctgcccaaa agccagagct cttagacgca gcaacagtgt tagagcctac aacagccttc 1500

attagagaag ctgttaggga aatcaatcag ctgagtgatg actacgctga caatcaagag 1560

cttcaggctg ttcttgctaa tgctggagtt gaggcacttg ctgcagatac tgttgatcag 1620

gctaaagcag ctcttgacaa agcaaaggca gctgttgctg gtgttcagct tgatgaagca 1680

agacgtgagg cttacagaac aatcaatgcc ttaagtgatc agcacaaaag cgatcaaaag 1740

gttcagctag ctctagttgc tgcagcagct aaggtggcag atgctgcttc agttgatcaa 1800

gtgaatgcag ccattaatga tgctcataca gctattgcgg acattacagg agcagccttg 1860

ttggaggcta aagaagctgc tatcaatgaa ctaaagcagt atggcattag tgattactat 1920

gtgaccttaa tcaacaaagc caaataactc gagcggccgc at 1962

<210> 24
<211> 648
<212> PRT
<213> Artificial Sequence

<220>
<223> Recombinant fusion protein

<400> 24

Leu Glu Val Leu Phe Gln Gly Pro Leu Gly Ser Thr Asn Leu Ser Asp
1 5 10 15

Asn Ile Thr Ser Leu Thr Val Ala Ser Ser Ser Leu Arg Asp Gly Glu
20 25 30

Arg Thr Thr Val Lys Val Ala Phe Asp Asp Lys Lys Gln Lys Ile Lys
35 40 45

Ala Gly Asp Thr Ile Glu Val Thr Trp Pro Thr Ser Gly Asn Val Tyr
50 55 60

Ile Gln Gly Phe Asn Lys Thr Ile Pro Leu Asn Ile Arg Gly Val Asp
65 70 75 80

Val Gly Thr Leu Glu Val Thr Leu Asp Lys Ala Val Phe Thr Phe Asn
85 90 95

Gln Asn Ile Glu Thr Met His Asp Val Ser Gly Trp Gly Glu Phe Asp
100 105 110

Ile Thr Val Arg Asn Val Thr Gln Thr Thr Ala Glu Thr Ser Gly Thr
115 120 125

Thr Thr Val Lys Val Gly Asn Arg Thr Ala Thr Ile Thr Val Thr Lys
130 135 140

Pro Glu Ala Gly Thr Gly Thr Ser Ser Phe Tyr Tyr Lys Thr Gly Asp
145 150 155 160

Met Gln Pro Asn Asp Thr Glu Arg Val Arg Trp Phe Leu Leu Ile Asn
165 170 175

Asn Asn Lys Glu Trp Val Ala Asn Thr Val Thr Val Glu Asp Asp Ile
180 185 190

Gln Gly Gly Gln Thr Leu Asp Met Ser Ser Phe Asp Ile Thr Val Ser
195 200 205

Gly Tyr Arg Asn Glu Arg Phe Val Gly Glu Asn Ala Leu Thr Glu Phe
210 215 220

His Thr Thr Phe Pro Asn Ser Val Ile Thr Ala Thr Asp Asn His Ile
225 230 235 240

Ser Val Arg Leu Asp Gln Tyr Asp Ala Ser Gln Asn Thr Val Asn Ile
245 250 255

Ala Tyr Lys Thr Lys Ile Thr Asp Phe Asp Gln Lys Glu Phe Ala Asn
260 265 270

Asn Ser Lys Ile Trp Tyr Gln Ile Leu Tyr Lys Asp Gln Val Ser Gly
275 280 285

Gln Glu Ser Asn His Gln Val Ala Asn Ile Asn Ala Asn Gly Gly Val
290 295 300

Asp Gly Ser Arg Tyr Thr Ser Phe Thr Val Lys Glu Leu Ser Glu Pro
305 310 315 320

Asn Pro Tyr Pro Asp Val Arg Arg Phe Leu Asp Glu Lys Tyr Asp Gly
325 330 335

Asp Val Asp Lys Leu Ser Lys Gln Leu Gln Gly Tyr Phe Gly Ser Leu
340 345 350

Arg Glu Tyr Ile Glu Phe Glu Leu Lys Asn Gly Lys Gln Gly Pro Ser
355 360 365

Arg Leu Ser Gly Pro Pro Gly Tyr Pro Leu Thr Arg Asp Phe Ser Arg
370 375 380

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

His Leu Gln His Arg Phe Ser Glu Leu Glu Ser Leu Thr Arg Lys Leu
405 410 415

Glu Lys Glu Gly Gly Thr Arg Gly Pro Leu Gln Asp Gln Pro Ala Ala
420 425 430

Leu Lys Tyr Pro Glu Pro Arg Asp Tyr Phe Leu His Thr Arg Glu Gly
435 440 445

Asp Val Ile Tyr Asp Glu Asp Ile Lys Arg Tyr Phe Glu Asp Leu Glu
450 455 460

Ala Tyr Leu Thr Ala Arg Leu Gly Gly Ile Asp Lys Lys Val Glu Glu
465 470 475 480

Ala Ala Gln Lys Pro Glu Leu Leu Asp Ala Ala Thr Val Leu Glu Pro
485 490 495

Thr Thr Ala Phe Ile Arg Glu Ala Val Arg Glu Ile Asn Gln Leu Ser
500 505 510

Asp Asp Tyr Ala Asp Asn Gln Glu Leu Gln Ala Val Leu Ala Asn Ala
515 520 525

Gly Val Glu Ala Leu Ala Ala Asp Thr Val Asp Gln Ala Lys Ala Ala
530 535 540

Leu Asp Lys Ala Lys Ala Ala Val Ala Gly Val Gln Leu Asp Glu Ala
545 550 555 560

Arg Arg Glu Ala Tyr Arg Thr Ile Asn Ala Leu Ser Asp Gln His Lys
565 570 575

Ser Asp Gln Lys Val Gln Leu Ala Leu Val Ala Ala Ala Ala Lys Val
580 585 590

Ala Asp Ala Ala Ser Val Asp Gln Val Asn Ala Ala Ile Asn Asp Ala
595 600 605

His Thr Ala Ile Ala Asp Ile Thr Gly Ala Ala Leu Leu Glu Ala Lys
610 615 620

Glu Ala Ala Ile Asn Glu Leu Lys Gln Tyr Gly Ile Ser Asp Tyr Tyr
625 630 635 640

Val Thr Leu Ile Asn Lys Ala Lys
645

<210> 25
<211> 996
<212> DNA
<213> Streptococcus equi

<400> 25
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gcttatgcca aagaagtacc acatcagatc acttctgtat ggaccaaagg tgttacacca 120

ctaacacccg agcagttttcg atataataac gaagatgtga tccatgcgcc atatcttgct 180

catcaaggct ggtacgatat caccaaggcc ttcgatggga aggataatct cttgtgtggc 240

gcagcaacgg caggtaatat gctgcattgg tggttttgatc aaaataaaac agagattgaa 300

gcctatttaa gtaaacaccc tgaaaagcaa aaaatcattt ttaacaacca agagctattt 360

gattttgaaag ctgctatcga taccaaggac agtcaaacca atagtcagct ttttaattat 420

ttttagagata aagcctttcc aaatctatca gcacgtcaac tcggggttat gcctgatctt 480

gttctagaca tgttttatcaa tggttactac ttaaatgtgt ttaaaacaca gtctactgat 540

gtcaatcgac cttatcagga caaggacaaa cgaggtggta ttttcgatgc tgttttcacc 600

agaggagatc agacaacgct cttgacagct cgtcatgatt taaaaaataa aggactaaat 660

gacatcagca ccattatcaa gcaagaactg actgaaggaa gagcccttgc tttatcacat 720

acctacgcca atgttagcat tagccatgtg attaacttgt ggggagctga ttttaatgct 780

gaaggaaacc ttgaggccat ctatgtcaca gactcagatg ctaatgcgtc tattggtatg 840

aaaaaatatt ttgtcggcat taatgctcat agacatgtcg ccatttctgc caagaaaata 900

gaaggagaaa acattggcgc tcaagtatta ggcttattta cgctttccag tggcaaggac 960

atatggcaga aactgagcta actcgagcgg ccgcat 996

<210> 26
<211> 326
<212> PRT
<213> Streptococcus equi

<400> 26

Leu Glu Val Leu Phe Gln Gly Pro Leu Gly Ser Asp Asp Tyr Gln Arg
1 5 10 15

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

Val Trp Thr Lys Gly Val Thr Pro Leu Thr Pro Glu Gln Phe Arg Tyr
35 40 45

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Asn Asn Glu Asp Val Ile His Ala Pro Tyr Leu Ala His Gln Gly Trp
    50                  55                  60

Tyr Asp Ile Thr Lys Ala Phe Asp Gly Lys Asp Asn Leu Leu Cys Gly
65                  70                  75                  80

Ala Ala Thr Ala Gly Asn Met Leu His Trp Trp Phe Asp Gln Asn Lys
                85                  90                  95

Thr Glu Ile Glu Ala Tyr Leu Ser Lys His Pro Glu Lys Gln Lys Ile
            100                 105                 110

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

Lys Asp Ser Gln Thr Asn Ser Gln Leu Phe Asn Tyr Phe Arg Asp Lys
    130                 135                 140

Ala Phe Pro Asn Leu Ser Ala Arg Gln Leu Gly Val Met Pro Asp Leu
145                 150                 155                 160

Val Leu Asp Met Phe Ile Asn Gly Tyr Tyr Leu Asn Val Phe Lys Thr
                165                 170                 175

Gln Ser Thr Asp Val Asn Arg Pro Tyr Gln Asp Lys Asp Lys Arg Gly
            180                 185                 190

Gly Ile Phe Asp Ala Val Phe Thr Arg Gly Asp Gln Thr Thr Leu Leu
        195                 200                 205

Thr Ala Arg His Asp Leu Lys Asn Lys Gly Leu Asn Asp Ile Ser Thr
    210                 215                 220

Ile Ile Lys Gln Glu Leu Thr Glu Gly Arg Ala Leu Ala Leu Ser His
225                 230                 235                 240

Thr Tyr Ala Asn Val Ser Ile Ser His Val Ile Asn Leu Trp Gly Ala
                245                 250                 255

Asp Phe Asn Ala Glu Gly Asn Leu Glu Ala Ile Tyr Val Thr Asp Ser
            260                 265                 270

Asp Ala Asn Ala Ser Ile Gly Met Lys Lys Tyr Phe Val Gly Ile Asn
        275                 280                 285

Ala His Arg His Val Ala Ile Ser Ala Lys Lys Ile Glu Gly Glu Asn
    290                 295                 300

Ile Gly Ala Gln Val Leu Gly Leu Phe Thr Leu Ser Ser Gly Lys Asp
305                 310                 315                 320
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Ile Trp Gln Lys Leu Ser
325

<210> 27
<211> 1452
<212> DNA
<213> Artificial Sequence

<220>
<223> Recombinant gene fusion fragment

<400> 27
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ttgacggttg cttcttcatc actccgagat ggagagagaa cgacggtaaa ggttgcgttt 120

gatgacaaaa aacagaaaat caaggcaggg gatacgatag aggtcacctg gcctacaagt 180

ggtaatgtct acattcaggg ctttaataaa accataccgc ttaatattag aggggtagat 240

gttggtacct tggaggtcac gctagacaag gctgttttca cattcaatca aaatattgaa 300

acaatgcatg atgtctctgg ttggggagag tttgatatta ctgttagaaa tgtgacacaa 360

accaccgctg aaacatcagg aacgaccaca gtaaaggtag gcaatcgcac tgctactatc 420

actgttacta agcctgaggc aggcactggt accagctcat tttattataa gactggtgat 480

atgcagccca tgatactga gcgtgtgaga tggttcctgc tgattaacaa caacaaggaa 540

tgggtggcca tactgttac agtcgaagac gatattcaag gtggtcaaac cttggatatg 600

agcagctttg acatcaccgt atctggttat cgtaacgagc gcttcgttgg ggaaaacgct 660

ctgacagagt ttcatacaac atttccaaat tctgtcatta cggcaacaga taatcacatt 720

agtgtgcggt tagatcaata tgatgcctca caaaacactg tcaacatttgc ttataagaca 780

aagataacgg actttgacca aaaagaattt gccaacaaca gtaaaatctg gtaccagatt 840

ttatacaagg atcaggtatc gggtcaagag tcaaaccacc aagtagccaa tatcaatgct 900

aacggcgggg ttgatggcag tcgctatacc agctttactg tcaaggagct cttagacgca 960

gcaacagtgt tagagcctac aacagccttc attagagaag ctgttaggga aatcaatcag 1020

ctgagtgatg actacgctga caatcaagag ctttaggctg ttcttgctaa tgctggagtt 1080

gaggcacttg ctgcagatac tgttgatcag gctaaagcag ctcttgacaa agcaaaggca 1140

gctgttgctg gtgttcagct tgatgaagca agacgtgagg cttacagaac aatcaatgcc 1200

ttaagtgatc agcacaaaag cgatcaaaag gttcagctag ctctagttgc tgcagcagct 1260

aaggtggcag atgctgcttc agttgatcaa gtgaatgcag ccattaatga tgctcataca 1320

gctattgcgg acattacagg agcagccttg ttggaggcta aagaagctgc tatcaatgaa 1380

ctaaagcagt atggcattag tgattactat gtgaccttaa tcaacaaagc caaataactc 1440

gagcggccgc at 1452

<210> 28
<211> 478
<212> PRT
<213> Artificial Sequence

<220>
<223> Recombinant fusion protein

<400> 28

Leu Glu Val Leu Phe Gln Gly Pro Leu Gly Ser Thr Asn Leu Ser Asp
1 5 10 15

Asn Ile Thr Ser Leu Thr Val Ala Ser Ser Ser Leu Arg Asp Gly Glu
20 25 30

Arg Thr Thr Val Lys Val Ala Phe Asp Asp Lys Lys Gln Lys Ile Lys
35 40 45

Ala Gly Asp Thr Ile Glu Val Thr Trp Pro Thr Ser Gly Asn Val Tyr
50 55 60

Ile Gln Gly Phe Asn Lys Thr Ile Pro Leu Asn Ile Arg Gly Val Asp
65 70 75 80

Val Gly Thr Leu Glu Val Thr Leu Asp Lys Ala Val Phe Thr Phe Asn
85 90 95

Gln Asn Ile Glu Thr Met His Asp Val Ser Gly Trp Gly Glu Phe Asp
100 105 110

Ile Thr Val Arg Asn Val Thr Gln Thr Thr Ala Glu Thr Ser Gly Thr
115 120 125

Thr Thr Val Lys Val Gly Asn Arg Thr Ala Thr Ile Thr Val Thr Lys
130 135 140

Pro Glu Ala Gly Thr Gly Thr Ser Ser Phe Tyr Tyr Lys Thr Gly Asp
145 150 155 160

Met Gln Pro Asn Asp Thr Glu Arg Val Arg Trp Phe Leu Leu Ile Asn
165 170 175

Asn Asn Lys Glu Trp Val Ala Asn Thr Val Thr Val Glu Asp Asp Ile
180 185 190

Gln Gly Gly Gln Thr Leu Asp Met Ser Ser Phe Asp Ile Thr Val Ser
195 200 205

Gly Tyr Arg Asn Glu Arg Phe Val Gly Glu Asn Ala Leu Thr Glu Phe
210 215 220

His Thr Thr Phe Pro Asn Ser Val Ile Thr Ala Thr Asp Asn His Ile
225 230 235 240

Ser Val Arg Leu Asp Gln Tyr Asp Ala Ser Gln Asn Thr Val Asn Ile
245 250 255

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Ala Tyr Lys Thr Lys Ile Thr Asp Phe Asp Gln Lys Glu Phe Ala Asn
            260                 265                 270

Asn Ser Lys Ile Trp Tyr Gln Ile Leu Tyr Lys Asp Gln Val Ser Gly
        275                 280                 285

Gln Glu Ser Asn His Gln Val Ala Asn Ile Asn Ala Asn Gly Gly Val
    290                 295                 300

Asp Gly Ser Arg Tyr Thr Ser Phe Thr Val Lys Glu Leu Leu Asp Ala
305                 310                 315                 320

Ala Thr Val Leu Glu Pro Thr Thr Ala Phe Ile Arg Glu Ala Val Arg
                325                 330                 335

Glu Ile Asn Gln Leu Ser Asp Asp Tyr Ala Asp Asn Gln Glu Leu Gln
            340                 345                 350

Ala Val Leu Ala Asn Ala Gly Val Glu Ala Leu Ala Ala Asp Thr Val
        355                 360                 365

Asp Gln Ala Lys Ala Ala Leu Asp Lys Ala Lys Ala Ala Val Ala Gly
    370                 375                 380

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

Leu Ser Asp Gln His Lys Ser Asp Gln Lys Val Gln Leu Ala Leu Val
                405                 410                 415

Ala Ala Ala Ala Lys Val Ala Asp Ala Ala Ser Val Asp Gln Val Asn
            420                 425                 430

Ala Ala Ile Asn Asp Ala His Thr Ala Ile Ala Asp Ile Thr Gly Ala
        435                 440                 445

Ala Leu Leu Glu Ala Lys Glu Ala Ala Ile Asn Glu Leu Lys Gln Tyr
    450                 455                 460

Gly Ile Ser Asp Tyr Tyr Val Thr Leu Ile Asn Lys Ala Lys
465                 470                 475


<210>  29
<211>  2322
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Recombinant gene fusion fragment

<400>  29
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ct aacacccg agcagt t t cg at at aat aac gaagat gt ga t ccat gcgcc at at ct t gct 180

cat caaggct ggt acgat at caccaaggcc t t cgat ggga aggat aat ct ct t gt gt ggc 240

gcagcaacgg caggt aat at gct gcat t gg t ggt t t gat c aaaat aaaac agagat t gaa 300

gcct at t t aa gt aaacaccc t gaaaagcaa aaaat cat t t t t aacaacca agagct at t t 360

gat t t gaaag ct gct at cga t accaaggac agt caaacca at agt cagct t t t t aat t at 420

t t t agagat a aagcct t t cc aaat ct at ca gcacgt caac t cggggt t at gcct gat ct t 480

gt t ct agaca t gt t t at caa t ggt t act ac t t aaat gt gt t t aaaacaca gt ct act gat 540

gt caat cgac ct t at cagga caaggacaaa cgaggt ggt a t t t t cgat gc t gt t t t cacc 600

agaggagat c agacaacgct ct t gacagct cgt cat gat t t aaaaaat aa aggact aaat 660

gacat cagca ccat t at caa gcaagaact g act gaaggaa gagccct t gc t t t at cacat 720

acct acgcca at gt t agcat t agccat gt g at t aact t gt ggggagct ga t t t t aat gct 780

gaaggaaacc t t gaggccat ct at gt caca gact cagat g ct aat gcgt c t at t ggt at g 840

aaaaaat at t t t gt cggcat t aat gct cat agacat gt cg ccat t t ct gc caagaaaat a 900

gaaggagaaa acat t ggcgc t caagt at t a ggct t at t t a cgct t t ccag t ggcaaggac 960

at at ggcaga aact gagccc at gggaaacg act act gct a gt gcat t t ga aaat aat ggg 1020

acaggt caac at ct gaact g gcacat agat at t ccacaag aat at acagt t gaat t agga 1080

gaaccaat t a ct at ct caga t ct t at gagt caaat t acgg t t act cgt aa aggt agt aat 1140

gggact gt t a at gat ggaga t act t t t gac t t t at t t cga at ggagat gg t t caagagga 1200

at t gat accc ct ggagt aaa aat at ggt t t gact t t t aca at gct gcggg t act t cct t t 1260

t t aact gat g aaat gt t agc t t cgcct aca t at gct gt ac cgggggat c t t at act at t 1320

aaagct t ggg t at t ct at gg gaaaaat gat accaaaaagc t ct t cacat t t aaact aaaa 1380

aat t ccaaca gcaat aaaac t gagt t aagg aagt cgt t ag aggaggct aa gct aaaact c 1440

agccagcct g aaggaacgt a t t ct gat gaa t cact gcaag cct t gcaat c agcggt t act 1500

at t ggt aaga cct at t t aaa cagt gaccct gat caaaat a cagt agat ca at ct gt t act 1560

act at t gat t ccgct at t ac t agt ct t gt t aat ct t aat g ct t t aaat ga agct at t aat 1620

caagct acac ct t t t at aac agat ggcaaa gagt at cct a aagaagcgt a t gacggt ct t 1680

gt gcaaaagc t t gcagcggc agct aagct t caaaat t cat t t ggt cct t c acaaggagat 1740

gt t gat aagg ct gcgact ga t t t aacgcaa gct ct t acga cgct t aagac t gct gt agcg 1800

cat gaagcct t agat caagc ct t ggct aag ct gt t agagc t t t accgaga aaat ccaaat 1860

ct t gct t t ga cat cagagt c t t t gaaggaa t t gt acaat a aggccat t ga agcagcaggt 1920

acct t ct at a gaact gt t aa caaggat aaa gagagaaaag acat t t ccct t t at gagct a 1980

gagcgct aca ct acagaaac aaat t cagt t gt t gat act a t t t t aaaggt aaaggct gcg 2040

at t gccgaag aaggaaaggc aaaat t gcgt t ct gct t t ag accaat t aaa t gct ct t at c 2100

ggagaaaatc tagacctatc tccatataca gcagcttctg ctcaagccta tacagaccag 2160

ctagctaagg ctaaggaggt cgcagcagcg ggtgagacag cttatgctca ggagacagaa 2220

ccgacagcta ttactaacag cttggttaag gtgttaaatg ctaagaaatc cctctcagat 2280

gccaaggcag ccttggttgc taaataactc gagcggccgc at 2322

<210> 30
<211> 768
<212> PRT
<213> Artificial Sequence

<220>
<223> Recombinant fusion protein

<400> 30

Leu Glu Val Leu Phe Gln Gly Pro Leu Gly Ser Asp Asp Tyr Gln Arg
1 5 10 15

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

Val Trp Thr Lys Gly Val Thr Pro Leu Thr Pro Glu Gln Phe Arg Tyr
35 40 45

Asn Asn Glu Asp Val Ile His Ala Pro Tyr Leu Ala His Gln Gly Trp
50 55 60

Tyr Asp Ile Thr Lys Ala Phe Asp Gly Lys Asp Asn Leu Leu Cys Gly
65 70 75 80

Ala Ala Thr Ala Gly Asn Met Leu His Trp Trp Phe Asp Gln Asn Lys
85 90 95

Thr Glu Ile Glu Ala Tyr Leu Ser Lys His Pro Glu Lys Gln Lys Ile
100 105 110

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

Lys Asp Ser Gln Thr Asn Ser Gln Leu Phe Asn Tyr Phe Arg Asp Lys
130 135 140

Ala Phe Pro Asn Leu Ser Ala Arg Gln Leu Gly Val Met Pro Asp Leu
145 150 155 160

Val Leu Asp Met Phe Ile Asn Gly Tyr Tyr Leu Asn Val Phe Lys Thr
165 170 175

Gln Ser Thr Asp Val Asn Arg Pro Tyr Gln Asp Lys Asp Lys Arg Gly
180 185 190

Gly Ile Phe Asp Ala Val Phe Thr Arg Gly Asp Gln Thr Thr Leu Leu
195 200 205

Thr Ala Arg His Asp Leu Lys Asn Lys Gly Leu Asn Asp Ile Ser Thr
210 215 220

Ile Ile Lys Gln Glu Leu Thr Glu Gly Arg Ala Leu Ala Leu Ser His
225 230 235 240

Thr Tyr Ala Asn Val Ser Ile Ser His Val Ile Asn Leu Trp Gly Ala
245 250 255

Asp Phe Asn Ala Glu Gly Asn Leu Glu Ala Ile Tyr Val Thr Asp Ser
260 265 270

Asp Ala Asn Ala Ser Ile Gly Met Lys Lys Tyr Phe Val Gly Ile Asn
275 280 285

Ala His Arg His Val Ala Ile Ser Ala Lys Lys Ile Glu Gly Glu Asn
290 295 300

Ile Gly Ala Gln Val Leu Gly Leu Phe Thr Leu Ser Ser Gly Lys Asp
305 310 315 320

Ile Trp Gln Lys Leu Ser Pro Trp Glu Thr Thr Thr Ala Ser Ala Phe
325 330 335

Glu Asn Asn Gly Thr Gly Gln His Leu Asn Trp His Ile Asp Ile Pro
340 345 350

Gln Glu Tyr Thr Val Glu Leu Gly Glu Pro Ile Thr Ile Ser Asp Leu
355 360 365

Met Ser Gln Ile Thr Val Thr Arg Lys Gly Ser Asn Gly Thr Val Asn
370 375 380

Asp Gly Asp Thr Phe Asp Phe Ile Ser Asn Gly Asp Gly Ser Arg Gly
385 390 395 400

Ile Asp Thr Pro Gly Val Lys Ile Trp Phe Asp Phe Tyr Asn Ala Ala
405 410 415

Gly Thr Ser Phe Leu Thr Asp Glu Met Leu Ala Ser Pro Thr Tyr Ala
420 425 430

Val Pro Gly Gly Ser Tyr Thr Ile Lys Ala Trp Val Phe Tyr Gly Lys
435 440 445

Asn Asp Thr Lys Lys Leu Phe Thr Phe Lys Leu Lys Asn Ser Asn Ser
450 455 460

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Asn Lys Thr Glu Leu Arg Lys Ser Leu Glu Glu Ala Lys Leu Lys Leu
465                 470                 475                 480

Ser Gln Pro Glu Gly Thr Tyr Ser Asp Glu Ser Leu Gln Ala Leu Gln
                485                 490                 495

Ser Ala Val Thr Ile Gly Lys Thr Tyr Leu Asn Ser Asp Pro Asp Gln
            500                 505                 510

Asn Thr Val Asp Gln Ser Val Thr Thr Ile Asp Ser Ala Ile Thr Ser
        515                 520                 525

Leu Val Asn Leu Asn Ala Leu Asn Glu Ala Ile Asn Gln Ala Thr Pro
    530                 535                 540

Phe Ile Thr Asp Gly Lys Glu Tyr Pro Lys Glu Ala Tyr Asp Gly Leu
545                 550                 555                 560

Val Gln Lys Leu Ala Ala Ala Ala Lys Leu Gln Asn Ser Phe Gly Pro
                565                 570                 575

Ser Gln Gly Asp Val Asp Lys Ala Ala Thr Asp Leu Thr Gln Ala Leu
            580                 585                 590

Thr Thr Leu Lys Thr Ala Val Ala His Glu Ala Leu Asp Gln Ala Leu
        595                 600                 605

Ala Lys Leu Leu Glu Leu Tyr Arg Glu Asn Pro Asn Leu Ala Leu Thr
    610                 615                 620

Ser Glu Ser Leu Lys Glu Leu Tyr Asn Lys Ala Ile Glu Ala Ala Gly
625                 630                 635                 640

Thr Phe Tyr Arg Thr Val Asn Lys Asp Lys Glu Arg Lys Asp Ile Ser
                645                 650                 655

Leu Tyr Glu Leu Glu Arg Tyr Thr Thr Glu Thr Asn Ser Val Val Asp
            660                 665                 670

Thr Ile Leu Lys Val Lys Ala Ala Ile Ala Glu Glu Gly Lys Ala Lys
        675                 680                 685

Leu Arg Ser Ala Leu Asp Gln Leu Asn Ala Leu Ile Gly Glu Asn Leu
    690                 695                 700

Asp Leu Ser Pro Tyr Thr Ala Ala Ser Ala Gln Ala Tyr Thr Asp Gln
705                 710                 715                 720

Leu Ala Lys Ala Lys Glu Val Ala Ala Ala Gly Glu Thr Ala Tyr Ala
                725                 730                 735
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Gln Glu Thr Glu Pro Thr Ala Ile Thr Asn Ser Leu Val Lys Val Leu
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Asn Ala Lys Lys Ser Leu Ser Asp Ala Lys Ala Ala Leu Val Ala Lys
755 760 765

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<211> 3004
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<213> Streptococcus equi

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gaggggttat caaaggcagg agaagtacct gaaaagctaa aggatattta tatccaaggca 180
cagcaggcag ataagcaggc aaaggttctt gcagaaatga aggttcctga aaaaatagcc 240
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accaagcatg tgccaacgct gaacaagcag ggaacacgtg tgattcgtac cattccatgg 480
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aatcttgatg gtttagatgt tgatattgag cgggatagca ttccaaaagt aaatggaaaa 660
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aagaacccat tgattgagcg cggtgcccaa tatattgatt tgctgcttgt gcaggttttat 840
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gaggaacgtt gggaatcgta tagcaaatac attcgtcctg agcagtacat ggttggtttt 960
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agcgctaaga aggaagagac taaggcgatt ccacaggtgg ctctgaccat ttctggtcta 1620

actggcttga aggaattaaa tcttgctggc tttgatcgtg atagcttggc tggaattgac 1680

gcagctagcc taacctctct tgaaaaggtg gatctctcta gtaataagct ggacttagca 1740

gctggtacgg aaaatcgtca gattcttgat accatgctgg caacagtgac taagcatggc 1800

ggtgttagcg aaaagacgtt tgtatttgat catcaaaagc ctactggtct ttatcctgat 1860

acttatggca ctaagagcct tcagttacca gtagcaaatg atacaatttga tttgcaggct 1920

aagctttttat ttggaacagt taccaatcag ggcacgctaa tcaatagcga agctgactat 1980

aaggcttatc aggagcagga aatagcaggt caccgttttg ttgattcaag ctatgattac 2040

aaagcctttg cagtgacctta caaggactat aagatcaagg tgactgactc aaccttaggt 2100

gtcactgatc acaaggactt atccactagc aaggaggaga cctacaaggt tgaattctttt 2160

agccctactа atagcactaa gcctgtgcat gaggctaagg ttgtcgttgg tgcggaaaaa 2220

accatgatgg ttaacctagc agagggagca actgtgattg gtggtgatgc agatccaaca 2280

aatgcaaaaa aagtgttga tggttgctc aataatgata caacaattct gtcaactagc 2340

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tttaatgaca gcaaaattag taaagctgac tgtattaagg aggccaagct tgaagccttt 2460

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agaactgatc aggaaaaagt agctaaagca gctaaagttg agcagcctgt ggctactgac 2940

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gcat 3004

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<211> 993
<212> PRT
<213> Streptococcus equi

<400> 32

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20 25 30

Glu Asn Ser Lys Lys Glu Phe Lys Glu Gly Leu Ser Lys Ala Gly Glu
35 40 45

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

Lys Gln Ala Lys Val Leu Ala Glu Met Lys Val Pro Glu Lys Ile Ala
65                  70                  75                  80

Met Lys Pro Leu Lys Gly Pro Leu Tyr Gly Gly Tyr Phe Arg Thr Trp
                85                  90                  95

His Asp Lys Thr Ser Asp Pro Ala Glu Lys Asp Lys Val Asn Ser Met
            100                 105                 110

Gly Glu Leu Pro Lys Glu Val Asp Leu Ala Phe Val Phe His Asp Trp
        115                 120                 125

Thr Lys Asp Tyr Ser Phe Phe Trp Gln Glu Leu Ala Thr Lys His Val
    130                 135                 140

Pro Thr Leu Asn Lys Gln Gly Thr Arg Val Ile Arg Thr Ile Pro Trp
145                 150                 155                 160

Arg Phe Leu Ala Gly Gly Asp His Ser Gly Ile Ala Glu Asp Thr Gln
                165                 170                 175

Lys Tyr Pro Asn Thr Pro Glu Gly Asn Lys Ala Leu Ala Lys Ala Ile
            180                 185                 190

Val Asp Glu Tyr Val Tyr Lys Tyr Asn Leu Asp Gly Leu Asp Val Asp
        195                 200                 205

Ile Glu Arg Asp Ser Ile Pro Lys Val Asn Gly Lys Glu Ser Asn Glu
    210                 215                 220

Asn Ile Gln Arg Ser Ile Ala Val Phe Glu Glu Ile Gly Lys Leu Ile
225                 230                 235                 240

Gly Pro Lys Gly Ala Asp Lys Ser Arg Leu Phe Ile Met Asp Ser Thr
                245                 250                 255

Tyr Met Ala Asp Lys Asn Pro Leu Ile Glu Arg Gly Ala Gln Tyr Ile
            260                 265                 270

Asp Leu Leu Leu Val Gln Val Tyr Gly Thr Gln Gly Glu Lys Gly Asp
        275                 280                 285

Trp Asp Pro Val Ala Arg Lys Pro Glu Lys Thr Met Glu Glu Arg Trp
    290                 295                 300

Glu Ser Tyr Ser Lys Tyr Ile Arg Pro Glu Gln Tyr Met Val Gly Phe
305                 310                 315                 320
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Ser Phe Tyr Glu Glu Tyr Ala Gly Ser Gly Asn Leu Trp Tyr Asp Ile
325 330 335

Asn Glu Arg Lys Asp Asp His Asn Pro Leu Asn Ser Glu Ile Ala Gly
340 345 350

Thr Arg Ala Glu Arg Tyr Ala Lys Trp Gln Pro Lys Thr Gly Gly Val
355 360 365

Lys Gly Gly Ile Phe Ser Tyr Ala Ile Asp Arg Asp Gly Val Ala His
370 375 380

Gln Pro Lys Lys Val Ser Asp Asp Glu Lys Arg Thr Asn Lys Ala Ile
385 390 395 400

Lys Asp Ile Thr Asp Gly Ile Val Lys Ser Asp Tyr Lys Val Ser Lys
405 410 415

Ala Leu Lys Lys Val Met Glu Asn Asp Lys Ser Tyr Glu Leu Ile Asp
420 425 430

Gln Lys Asp Phe Pro Asp Lys Ala Leu Arg Glu Ala Val Ile Ala Gln
435 440 445

Val Gly Ser Arg Arg Gly Asp Leu Glu Arg Phe Asn Gly Thr Leu Arg
450 455 460

Leu Asp Asn Pro Asp Ile Lys Ser Leu Glu Gly Leu Asn Lys Leu Lys
465 470 475 480

Lys Leu Ala Lys Leu Glu Leu Ile Gly Leu Ser Gln Ile Thr Lys Leu
485 490 495

Asp Ser Leu Val Leu Pro Ala Asn Ala Lys Pro Thr Lys Asp Thr Leu
500 505 510

Ala Asn Val Leu Glu Ala Tyr Asp Ser Ala Lys Lys Glu Glu Thr Lys
515 520 525

Ala Ile Pro Gln Val Ala Leu Thr Ile Ser Gly Leu Thr Gly Leu Lys
530 535 540

Glu Leu Asn Leu Ala Gly Phe Asp Arg Asp Ser Leu Ala Gly Ile Asp
545 550 555 560

Ala Ala Ser Leu Thr Ser Leu Glu Lys Val Asp Leu Ser Ser Asn Lys
565 570 575

Leu Asp Leu Ala Ala Gly Thr Glu Asn Arg Gln Ile Leu Asp Thr Met
580 585 590

Leu Ala Thr Val Thr Lys His Gly Gly Val Ser Glu Lys Thr Phe Val
595 600 605

Phe Asp His Gln Lys Pro Thr Gly Leu Tyr Pro Asp Thr Tyr Gly Thr
610 615 620

Lys Ser Leu Gln Leu Pro Val Ala Asn Asp Thr Ile Asp Leu Gln Ala
625 630 635 640

Lys Leu Leu Phe Gly Thr Val Thr Asn Gln Gly Thr Leu Ile Asn Ser
645 650 655

Glu Ala Asp Tyr Lys Ala Tyr Gln Glu Gln Glu Ile Ala Gly His Arg
660 665 670

Phe Val Asp Ser Ser Tyr Asp Tyr Lys Ala Phe Ala Val Thr Tyr Lys
675 680 685

Asp Tyr Lys Ile Lys Val Thr Asp Ser Thr Leu Gly Val Thr Asp His
690 695 700

Lys Asp Leu Ser Thr Ser Lys Glu Glu Thr Tyr Lys Val Glu Phe Phe
705 710 715 720

Ser Pro Thr Asn Ser Thr Lys Pro Val His Glu Ala Lys Val Val Val
725 730 735

Gly Ala Glu Lys Thr Met Met Val Asn Leu Ala Glu Gly Ala Thr Val
740 745 750

Ile Gly Gly Asp Ala Asp Pro Thr Asn Ala Lys Lys Val Phe Asp Gly
755 760 765

Leu Leu Asn Asn Asp Thr Thr Ile Leu Ser Thr Ser Asn Lys Ala Ser
770 775 780

Ile Ile Phe Glu Leu Lys Glu Pro Gly Leu Val Lys Tyr Trp Arg Phe
785 790 795 800

Phe Asn Asp Ser Lys Ile Ser Lys Ala Asp Cys Ile Lys Glu Ala Lys
805 810 815

Leu Glu Ala Phe Val Gly His Leu Glu Ala Gly Ser Lys Val Lys Asp
820 825 830

Ser Leu Glu Lys Ser Ser Lys Trp Val Thr Val Ser Asp Tyr Ser Gly
835 840 845

Glu Asp Gln Glu Phe Ser Gln Pro Leu Asn Asn Ile Gly Ala Lys Tyr
850 855 860

Trp Arg Ile Thr Val Asp Thr Lys Gly Gly Arg Tyr Asn Trp Pro Ser
865 870 875 880

Leu Pro Glu Leu Gln Ile Ile Gly Tyr Gln Leu Pro Ala Ala Asp Leu
885 890 895

Val Met Ala Met Leu Ala Thr Ala Glu Glu Leu Ser Gln Gln Lys Asp
900 905 910

Lys Phe Ser Gln Glu Gln Leu Lys Glu Leu Glu Val Lys Ile Ala Ala
915 920 925

Leu Lys Ala Ala Leu Asp Ser Lys Met Phe Asn Ala Asp Ala Ile Asn
930 935 940

Ala Ser Thr Ala Asp Leu Lys Ala Tyr Val Asp Lys Leu Leu Ala Asp
945 950 955 960

Arg Thr Asp Gln Glu Lys Val Ala Lys Ala Ala Lys Val Glu Gln Pro
965 970 975

Val Ala Thr Asp Ile Lys Glu Asn Thr Glu Pro Glu Asn Pro Lys Thr
980 985 990

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<211> 2715
<212> DNA
<213> Artificial Sequence

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gatgacaaaa aacagaaaat caaggcaggg gatacgatag aggtcacctg gcctacaagt 180

ggtaatgtct acattcaggg ctttaataaa accataccgc ttaatattag aggggtagat 240

gttggtacct tggaggtcac gctagacaag gctgttttca cattcaatca aaatattgaa 300

acaatgcatg atgtctctgg ttggggagag tttgatatta ctgttagaaa tgtgacacaa 360

accaccgctg aaacatcagg aacgaccaca gtaaaggtag gcaatcgcac tgctactatc 420

actgttacta agcctgaggc aggcactggt accagctcat tttattataa gactggtgat 480

atgcagccca tgatactga gcgtgtgaga tggttcctgc tgattaacaa caacaaggaa 540

tgggtggcca tactgttac agtcgaagac gatattcaag gtggtcaaac cttggatatg 600

agcagctttg acatcaccgt atctggttat cgtaacgagc gcttcgttgg ggaaaacgct 660

ctgacagagt ttcatacaac atttccaaat tctgtcatta cggcaacaga taatcacatt 720

agt gt gcggt t agat caat a t gat gcct ca caaaacact g t caacat t gc t t at aagaca 780

aagat aacgg act t t gacca aaaagaat t t gccaacaaca gt aaaat ct g gt accagat t 840

t t at acaagg at caggt at c gggt caagag t caaaccacc aagt agccaa t at caat gct 900

aacggcgggg t t gat ggcag t cgct at acc agct t t act g t caaggagct cgat acagca 960

agct at acca t cact gt t ga gggagct aca gcaggt caca cct at gaggc t t at cagat t 1020

t t caagggt g act t gt t t ga cagt accct a t caaacat ca cat ggggagg t ggt gt t aca 1080

cct t t t gaat t t gat ggt t c aaaagacgct gct aagat t g cagagggat t gaaggaagca 1140

aat gcagct g cct t t gccaa ggaagcaggt aagcact t ga cagcaaccat t gcaggaaca 1200

ggaacacat g caat caccgt t aacgaggct ggct act acc t cat caagga caaaaat gat 1260

t ct caaacag gcaagcat ga cgcct acacc t cat t t gt cc t gaaggt t gt t aaaaacacc 1320

agct t caaac caaaat ct gc t at cccaaca gt cct t aaaa aggt caagga ccgt aat gac 1380

aagacaggt c t t gagacagg ct ggcaagat t cagct gact at gacaaaaa t gacaaggt g 1440

ccat t ccagc t aaccgcaac cct accgt ca aat t acgat g cct t t caaga at act acct t 1500

gaat t t gt ag at acct t at c aaaagggct a agct acaaca aagacgccaa ggt ct at gt g 1560

gt t aat ggag at act cgt ca agat at t act aat t cat t t a cagt t agt ga agat ggt t ca 1620

t ct t t t aaaa t caat aacct aaaggct gt t cagggagt aa caat aacagc t accagt aag 1680

at cgt t gt cg aat acact gc t accct caat gaccaagcgg ccat cggcaa aaaaggaaat 1740

ccaaacgaag t t gct t t gaa at act caaac gat ccaaacg ct ct t ggaaa aggagaggag 1800

t ct ccaaaag gggagacacc aaaagacaag gt t at cgt t t t cacct at aa aact t ct aga 1860

t t at ct ggt c cgccaggat a cccact t act cgt gat t t ct cccgt aact t cct agaagaa 1920

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gagagct t aa caagaaaat t agagaaagaa ggcggt accc gaggt ccact gcaggaccag 2040

ccagcagcac t aaaat at cc agaacct aga gact at t t t c t t cat act cg t gaaggt gat 2100

gt t at t t at g at gaggat at aaaaagat at t t t gaggat t t agaagcct a t t t aacagct 2160

agact t ggt g ggat t gat aa aaaagt agaa gaagct gccc aaaagccaga gct ct t agac 2220

gcagcaacag t gt t agagcc t acaacagcc t t cat t agag aagct gt t ag ggaaat caat 2280

cagct gagt g at gact acgc t gacaat caa gagct t cagg ct gt t ct t gc t aat gct gga 2340

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gcagct gt t g ct ggt gt t ca gct t gat gaa gcaagacgt g aggct t acag aacaat caat 2460

gcct t aagt g at cagcacaa aagcgat caa aaggt t cagc t agct ct agt t gct gcagca 2520

gct aaggt gg cagat gct gc t t cagt t gat caagt gaat g cagccat t aa t gat gct cat 2580

acagct at t g cggacat t ac aggagcagcc t t gt t ggagg ct aaagaagc t gct at caat 2640

gaact aaagc agt at ggcat t agt gat t ac t at gt gacct t aat caacaa agccaaat aa 2700

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<211> 899
<212> PRT
<213> Artificial Sequence

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20 25 30

Arg Thr Thr Val Lys Val Ala Phe Asp Asp Lys Lys Gln Lys Ile Lys
35 40 45

Ala Gly Asp Thr Ile Glu Val Thr Trp Pro Thr Ser Gly Asn Val Tyr
50 55 60

Ile Gln Gly Phe Asn Lys Thr Ile Pro Leu Asn Ile Arg Gly Val Asp
65 70 75 80

Val Gly Thr Leu Glu Val Thr Leu Asp Lys Ala Val Phe Thr Phe Asn
85 90 95

Gln Asn Ile Glu Thr Met His Asp Val Ser Gly Trp Gly Glu Phe Asp
100 105 110

Ile Thr Val Arg Asn Val Thr Gln Thr Thr Ala Glu Thr Ser Gly Thr
115 120 125

Thr Thr Val Lys Val Gly Asn Arg Thr Ala Thr Ile Thr Val Thr Lys
130 135 140

Pro Glu Ala Gly Thr Gly Thr Ser Ser Phe Tyr Tyr Lys Thr Gly Asp
145 150 155 160

Met Gln Pro Asn Asp Thr Glu Arg Val Arg Trp Phe Leu Leu Ile Asn
165 170 175

Asn Asn Lys Glu Trp Val Ala Asn Thr Val Thr Val Glu Asp Asp Ile
180 185 190

Gln Gly Gly Gln Thr Leu Asp Met Ser Ser Phe Asp Ile Thr Val Ser
195 200 205

Gly Tyr Arg Asn Glu Arg Phe Val Gly Glu Asn Ala Leu Thr Glu Phe
210 215 220

His Thr Thr Phe Pro Asn Ser Val Ile Thr Ala Thr Asp Asn His Ile
225 230 235 240

Ser Val Arg Leu Asp Gln Tyr Asp Ala Ser Gln Asn Thr Val Asn Ile
245 250 255

Ala Tyr Lys Thr Lys Ile Thr Asp Phe Asp Gln Lys Glu Phe Ala Asn
260 265 270

Asn Ser Lys Ile Trp Tyr Gln Ile Leu Tyr Lys Asp Gln Val Ser Gly
275 280 285

Gln Glu Ser Asn His Gln Val Ala Asn Ile Asn Ala Asn Gly Gly Val
290 295 300

Asp Gly Ser Arg Tyr Thr Ser Phe Thr Val Lys Glu Leu Asp Thr Ala
305 310 315 320

Ser Tyr Thr Ile Thr Val Glu Gly Ala Thr Ala Gly His Thr Tyr Glu
325 330 335

Ala Tyr Gln Ile Phe Lys Gly Asp Leu Phe Asp Ser Thr Leu Ser Asn
340 345 350

Ile Thr Trp Gly Gly Gly Val Thr Pro Phe Glu Phe Asp Gly Ser Lys
355 360 365

Asp Ala Ala Lys Ile Ala Glu Gly Leu Lys Glu Ala Asn Ala Ala Ala
370 375 380

Phe Ala Lys Glu Ala Gly Lys His Leu Thr Ala Thr Ile Ala Gly Thr
385 390 395 400

Gly Thr His Ala Ile Thr Val Asn Glu Ala Gly Tyr Tyr Leu Ile Lys
405 410 415

Asp Lys Asn Asp Ser Gln Thr Gly Lys His Asp Ala Tyr Thr Ser Phe
420 425 430

Val Leu Lys Val Val Lys Asn Thr Ser Phe Lys Pro Lys Ser Ala Ile
435 440 445

Pro Thr Val Leu Lys Lys Val Lys Asp Arg Asn Asp Lys Thr Gly Leu
450 455 460

Glu Thr Gly Trp Gln Asp Ser Ala Asp Tyr Asp Lys Asn Asp Lys Val
465 470 475 480

Pro Phe Gln Leu Thr Ala Thr Leu Pro Ser Asn Tyr Asp Ala Phe Gln
485 490 495

Glu Tyr Tyr Leu Glu Phe Val Asp Thr Leu Ser Lys Gly Leu Ser Tyr
500 505 510

Asn Lys Asp Ala Lys Val Tyr Val Val Asn Gly Asp Thr Arg Gln Asp
515 520 525

Ile Thr Asn Ser Phe Thr Val Ser Glu Asp Gly Ser Ser Phe Lys Ile
530 535 540

Asn Asn Leu Lys Ala Val Gln Gly Val Thr Ile Thr Ala Thr Ser Lys
545 550 555 560

Ile Val Val Glu Tyr Thr Ala Thr Leu Asn Asp Gln Ala Ala Ile Gly
565 570 575

Lys Lys Gly Asn Pro Asn Glu Val Ala Leu Lys Tyr Ser Asn Asp Pro
580 585 590

Asn Ala Leu Gly Lys Gly Glu Glu Ser Pro Lys Gly Glu Thr Pro Lys
595 600 605

Asp Lys Val Ile Val Phe Thr Tyr Lys Thr Ser Arg Leu Ser Gly Pro
610 615 620

Pro Gly Tyr Pro Leu Thr Arg Asp Phe Ser Arg Asn Phe Leu Glu Glu
625 630 635 640

Asn Thr Ala Lys Tyr Leu Asp Gln Leu Arg Glu His Leu Gln His Arg
645 650 655

Phe Ser Glu Leu Glu Ser Leu Thr Arg Lys Leu Glu Lys Glu Gly Gly
660 665 670

Thr Arg Gly Pro Leu Gln Asp Gln Pro Ala Ala Leu Lys Tyr Pro Glu
675 680 685

Pro Arg Asp Tyr Phe Leu His Thr Arg Glu Gly Asp Val Ile Tyr Asp
690 695 700

Glu Asp Ile Lys Arg Tyr Phe Glu Asp Leu Glu Ala Tyr Leu Thr Ala
705 710 715 720

Arg Leu Gly Gly Ile Asp Lys Lys Val Glu Glu Ala Ala Gln Lys Pro
725 730 735

Glu Leu Leu Asp Ala Ala Thr Val Leu Glu Pro Thr Thr Ala Phe Ile
740 745 750

Arg Glu Ala Val Arg Glu Ile Asn Gln Leu Ser Asp Asp Tyr Ala Asp
755 760 765

Asn Gln Glu Leu Gln Ala Val Leu Ala Asn Ala Gly Val Glu Ala Leu
770 775 780

Ala Ala Asp Thr Val Asp Gln Ala Lys Ala Ala Leu Asp Lys Ala Lys
785 790 795 800

Ala Ala Val Ala Gly Val Gln Leu Asp Glu Ala Arg Arg Glu Ala Tyr
805 810 815

Arg Thr Ile Asn Ala Leu Ser Asp Gln His Lys Ser Asp Gln Lys Val
820 825 830

Gln Leu Ala Leu Val Ala Ala Ala Ala Lys Val Ala Asp Ala Ala Ser
835 840 845

Val Asp Gln Val Asn Ala Ala Ile Asn Asp Ala His Thr Ala Ile Ala
850 855 860

Asp Ile Thr Gly Ala Ala Leu Leu Glu Ala Lys Glu Ala Ala Ile Asn
865 870 875 880

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

Lys Ala Lys

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<223> PCR primer

<400> 35
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<210> 36
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<220>
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<210> 37
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gaggtggtgt tacacctttt gaatttgatg gttcaaaaga cgctgctaag attgcagagg 180

gatttgaagga agcaaatgca gctgccttttg ccaaggaagc aggtaagcac ttgacagcaa 240

ccattgcagg aacaggaaca catgcaatca ccgttaacga ggctggctac tacctcatca 300

aggacaaaaa tgattctcaa acaggcaagc atgacgccta cacctcattt gtcctgaagg 360

ttgttaaaaa caccagcttc aaaccaaaat ctgctatccc aacagtcctt aaaaaggtca 420

aggaccgtaa tgacaagaca ggtcttgaga caggctggca agatttcagct gactatgaca 480

aaaatgacaa ggtgccattc cagctaaccg caaccctacc gtcaaatttac gatgccttttc 540

aagaatactta ccttgaatttt gtagatacct tatcaaaagg gctaagctac aacaaagacg 600

ccaaggtctta tgtggttaat ggagatactc gtcaagatat tactaatttca ttttacagttta 660

gtgaagatgg ttcatctttt aaaatcaatta acctaaaggc tgttcaggga gtaacaataa 720

cagctaccag taagatcgtt gtcgaatacta ctgctaccct caatgaccaa gcggccatcg 780

gcaaaaaagg aaatccaaac gaagttgctt tgaaatactc aaacgatcca aacgctcttg 840

gaaaaggaga ggagtctcca aaaggggaga caccaaaaga caaggttatc gttttcacct 900

ataaaactat catcaataag gttgatcaag atcaaaaagc cctaaaaggt gcaggctttta 960

ccctttataa gctggtcaaa ggtgataatg gcgaggaaaa atatcaaata gtccaagaaa 1020

ttaaagcagg ggatacaact agctttgagt ttgttggact tgacgctggt gattacaagc 1080

tcagcgaaac aacaacacct ggcggttaca acactattgc agatgtcatg ttcagcattg 1140

tagcgcagca tgaaaccgag tcagacgatc ctcagttgac tagcctaacc gttgacaaag 1200

caactggctt cactgctgat acagaagctg gtaccgtatc cgcaactatt gttaataaaa 1260

ggtctatcct cgagcccggg tgc 1283

<210> 38
<211> 426
<212> PRT
<213> Streptococcus equi

<400> 38

Met Asp Thr Ala Ser Tyr Thr Ile Thr Val Glu Gly Ala Thr Ala Gly
1 5 10 15

His Thr Tyr Glu Ala Tyr Gln Ile Phe Lys Gly Asp Leu Phe Asp Ser
20 25 30

Thr Leu Ser Asn Ile Thr Trp Gly Gly Gly Val Thr Pro Phe Glu Phe
35 40 45

Asp Gly Ser Lys Asp Ala Ala Lys Ile Ala Glu Gly Leu Lys Glu Ala
50 55 60

Asn Ala Ala Ala Phe Ala Lys Glu Ala Gly Lys His Leu Thr Ala Thr
65 70 75 80

Ile Ala Gly Thr Gly Thr His Ala Ile Thr Val Asn Glu Ala Gly Tyr
85 90 95

Tyr Leu Ile Lys Asp Lys Asn Asp Ser Gln Thr Gly Lys His Asp Ala
100 105 110

Tyr Thr Ser Phe Val Leu Lys Val Val Lys Asn Thr Ser Phe Lys Pro
115 120 125

Lys Ser Ala Ile Pro Thr Val Leu Lys Lys Val Lys Asp Arg Asn Asp
130 135 140

Lys Thr Gly Leu Glu Thr Gly Trp Gln Asp Ser Ala Asp Tyr Asp Lys
145 150 155 160

Asn Asp Lys Val Pro Phe Gln Leu Thr Ala Thr Leu Pro Ser Asn Tyr
165 170 175

Asp Ala Phe Gln Glu Tyr Tyr Leu Glu Phe Val Asp Thr Leu Ser Lys
180 185 190

Gly Leu Ser Tyr Asn Lys Asp Ala Lys Val Tyr Val Val Asn Gly Asp
195 200 205

Thr Arg Gln Asp Ile Thr Asn Ser Phe Thr Val Ser Glu Asp Gly Ser
210 215 220

Ser Phe Lys Ile Asn Asn Leu Lys Ala Val Gln Gly Val Thr Ile Thr
225 230 235 240

Ala Thr Ser Lys Ile Val Val Glu Tyr Thr Ala Thr Leu Asn Asp Gln
245 250 255

Ala Ala Ile Gly Lys Lys Gly Asn Pro Asn Glu Val Ala Leu Lys Tyr
260 265 270

Ser Asn Asp Pro Asn Ala Leu Gly Lys Gly Glu Glu Ser Pro Lys Gly
275 280 285

Glu Thr Pro Lys Asp Lys Val Ile Val Phe Thr Tyr Lys Thr Ile Ile
290 295 300

Asn Lys Val Asp Gln Asp Gln Lys Ala Leu Lys Gly Ala Gly Phe Thr
305 310 315 320

Leu Tyr Lys Leu Val Lys Gly Asp Asn Gly Glu Glu Lys Tyr Gln Ile
325 330 335

Val Gln Glu Ile Lys Ala Gly Asp Thr Thr Ser Phe Glu Phe Val Gly
340 345 350

Leu Asp Ala Gly Asp Tyr Lys Leu Ser Glu Thr Thr Thr Pro Gly Gly
355 360 365

Tyr Asn Thr Ile Ala Asp Val Met Phe Ser Ile Val Ala Gln His Glu
370 375 380

Thr Glu Ser Asp Asp Pro Gln Leu Thr Ser Leu Thr Val Asp Lys Ala
385 390 395 400

Thr Gly Phe Thr Ala Asp Thr Glu Ala Gly Thr Val Ser Ala Thr Ile
405 410 415

Val Asn Lys Arg Ser Ile Leu Glu Pro Gly
420 425

<210> 39
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 39
gcagccatgg agagtctgac gagtgttga 29

<210> 40
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 40
tcacctcgag tcctagctca ccgtcataag c 31

<210> 41
<211> 1301
<212> DNA
<213> Streptococcus equi

<400> 41
ccatggagag tctgacgagt gttgagcctg ctgatggtgc ggtcatggtc aagtcagagg 60

ctgctgacca aggctcaaat gagctaccag aagctactga cattagtgat attgctggta 120

tttctgatgt gactaaggtg tcagctgctg tcaatgctga tactgtcaag gaagttcagc 180

cagtagctgt acctcttgta gaggatcagg cgcatgagga aactacagac cagtctcagc 240

cttcatcatc gatagtgtct gttacgacag acagctctct agagacacca gaagctacaa 300

gctcagagga gccgatagcg gagcagacct tgcggctgca tttcaagacc ctgccagctc 360

aagacctatc ctcgcttggt ctttgggtgt gggacgatgt tgagacacca tctgatcagc 420

tgggaggctg gccgactggg gctaccaatt ttagtctagc gaagacagat gactatggct 480

attacatgga cgttaagctt tcagccaatc aagccaataa ggttagcttt ttgatcaata 540

acactaaggg agacaatctg acgggcgatc gaaccataga cctttctcagc cctaagatga 600

atgaggtctg gatttgatggc caggagctgt cttactatcg gccgctggct cagggctata 660

tccgtatcaa ttattatcgc agtgatggcc attatgacaa caaatcgctc tggctttggg 720

gaagtgctga tgcgtcaatg actagtcagc agggcgcttg gccagatggt attgatttta 780

agcaggtcgg tcgatatggt gcttatatag atgtcaagct agctgatacc aatgagctag 840

gctttctctt gctagatgag cgtcagacag gtgacgctgt taaaatttcag cccaatgatt 900

atattttaa agatttaaag aatcacaccc aaatttttctt gaaagacgag gatccaacca 960

tttatacgaa ccctttattt gttaatacag ttagattaat cggtgctcag caggtcagcc 1020

caagcagtat tgaggcgagc tttacgactc tagcagatgt ggataaggaa agccttttaa 1080

aagaatttaaa aatcagcact gacagtaagg aagcagttgc tatttactgat atcaccttag 1140

atgaaaagac tcataaggct gtcatcacag gtgatttttag tcaagcagtg gccacttata 1200

cggtgacctt tcatcatgag agcttccagg ctaggccaaa ttggcaatac aaggatagcc 1260

tgtatgcttta tgacggtgag ctaggactcg agcccgggtg c 1301

<210> 42
<211> 432
<212> PRT
<213> Streptococcus equi

<400> 42

Met Glu Ser Leu Thr Ser Val Glu Pro Ala Asp Gly Ala Val Met Val
1 5 10 15

Lys Ser Glu Ala Ala Asp Gln Gly Ser Asn Glu Leu Pro Glu Ala Thr
20 25 30

Asp Ile Ser Asp Ile Ala Gly Ile Ser Asp Val Thr Lys Val Ser Ala
35 40 45

Ala Val Asn Ala Asp Thr Val Lys Glu Val Gln Pro Val Ala Val Pro
50 55 60

Leu Val Glu Asp Gln Ala His Glu Glu Thr Thr Asp Gln Ser Gln Pro
65 70 75 80

Ser Ser Ser Ile Val Ser Val Thr Thr Asp Ser Ser Leu Glu Thr Pro
85 90 95

Glu Ala Thr Ser Ser Glu Glu Pro Ile Ala Glu Gln Thr Leu Arg Leu
100 105 110

His Phe Lys Thr Leu Pro Ala Gln Asp Leu Ser Ser Leu Gly Leu Trp
115 120 125

Val Trp Asp Asp Val Glu Thr Pro Ser Asp Gln Leu Gly Gly Trp Pro
130 135 140

Thr Gly Ala Thr Asn Phe Ser Leu Ala Lys Thr Asp Asp Tyr Gly Tyr
145 150 155 160

Tyr Met Asp Val Lys Leu Ser Ala Asn Gln Ala Asn Lys Val Ser Phe
165 170 175

Leu Ile Asn Asn Thr Lys Gly Asp Asn Leu Thr Gly Asp Arg Thr Ile
180 185 190

Asp Leu Leu Ser Pro Lys Met Asn Glu Val Trp Ile Asp Gly Gln Glu
195 200 205

Leu Ser Tyr Tyr Arg Pro Leu Ala Gln Gly Tyr Ile Arg Ile Asn Tyr
210 215 220

Tyr Arg Ser Asp Gly His Tyr Asp Asn Lys Ser Leu Trp Leu Trp Gly
225 230 235 240

Ser Ala Asp Ala Ser Met Thr Ser Gln Gln Gly Ala Trp Pro Asp Gly
245 250 255

Ile Asp Phe Lys Gln Val Gly Arg Tyr Gly Ala Tyr Ile Asp Val Lys
260 265 270

Leu Ala Asp Thr Asn Glu Leu Gly Phe Leu Leu Leu Asp Glu Arg Gln
275 280 285

Thr Gly Asp Ala Val Lys Ile Gln Pro Asn Asp Tyr Ile Phe Lys Asp
290 295 300

Leu Lys Asn His Thr Gln Ile Phe Leu Lys Asp Glu Asp Pro Thr Ile
305 310 315 320

Tyr Thr Asn Pro Tyr Phe Val Asn Thr Val Arg Leu Ile Gly Ala Gln
325 330 335

Gln Val Ser Pro Ser Ser Ile Glu Ala Ser Phe Thr Thr Leu Ala Asp
340 345 350

Val Asp Lys Glu Ser Leu Leu Lys Glu Leu Lys Ile Ser Thr Asp Ser
355 360 365

Lys Glu Ala Val Ala Ile Thr Asp Ile Thr Leu Asp Glu Lys Thr His
370 375 380

Lys Ala Val Ile Thr Gly Asp Phe Ser Gln Ala Val Ala Thr Tyr Thr
385 390 395 400

Val Thr Phe His His Glu Ser Phe Gln Ala Arg Pro Asn Trp Gln Tyr
405 410 415

Lys Asp Ser Leu Tyr Ala Tyr Asp Gly Glu Leu Gly Leu Glu Pro Gly
420 425 430