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(56) Related Art
Waller et al (2007) Vaccine, April, 25(18):3629-3635
Flock et al (2004) Infection and Immunity, June, 72(6):3228-3236
Flock et al (2006) Vaccine, May, 24(9):4144-4151
US 2006/0140980 A1 (Guss et al.) 29 June 2006
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(54) Title: IMPROVED IMMUNIZING COMPOSITION

(57) Abstract: The present invention is concerned with an antigenic composition comprising at least one antigen that comprises at least one antigenic epitope or antigenic determinant derived from a protein present in one or both of *S. equi subsp. equi* and subsp. *zooepidemicus* and use thereof for immunization of non-human mammals against *S. equisubsp. equi* and/or *subsp. zooepidemicus*.The present invention also discloses a vaccine composition comprising the aforesaid antigenic composition as immunizing component.

WO 2009/075646 A1

IMPROVED IMMUNIZING COMPOSITION

Background of the Invention

1. Field of the Invention

This invention is generally related to antigenic or immunogenic compositions and use thereof for immunization of non-human mammals, e.g. horses, against *Streptococcus equi.*

2. Background of the Invention

Streptococcal infections in horses are mainly caused by the species *Streptococcus equi*, which is classified as a Lancefield Group C *Streptococcus* and comprises two subspecies designated *equi* and *zooepidemicus,* respectively.

Streptococcus equi subsp. *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. Moreover, since strangles is a highly contagious disease, not only infected animals but also all other members of e.g. an afflicted stud must be isolated for as long as up to three months.

S. equi subsp. *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, subsp. *zooepidemicus* infects not only horses but also a wide range of other animals, like pigs, dogs, cats, and cows. Even human cases of infection due to subsp. *zooepidemicus* have been reported. This subspecies has been implicated as the primary pathogen in conditions such as endometritis, cervicitis, abortion, mastitis, pneumonia, abscesses and joint infections.

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.

3. 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 vaccines or immunizing preparations are available, neither for the subspecies *equi* nor for the subspecies *zooepidemicus*.

Existing vaccines against strangles are based on inactivated, e. g. heat-killed, or attenuated strains of *S. equi* subsp. *equi* or acid extracts/mutanolysin enriched in M-protein(s), i.e. immunogenic protein(s) produced by *S. equi.* A vaccine against *S. equi* subsp. *zooepidemicus* based on an M-like protein is disclosed in US-A-5,583,014. In WO 87/00436, an avirulent strain of *S. equi* is disclosed for use as a vaccine against *S. equi* that stimulates an antibody response in the nasopharyngeal mucosa after administration thereof to a horse.

Recently, a commercial vaccine against strangles, Equilis StrepE from IntervetVET, UK, has been released in Great Britain (November 2004), which vaccine also has been used throughout Europe and in South Africa and South America. However, the safety and efficacy of this vaccine, which is based on an attenuated (living, deletion mutated) strain of *S. equi* subsp. *equi*, can be questioned.

Since the previously developed vaccines or immunizing preparations are hampered by side-effects and, moreover, 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.

It is well known that attachment to eukaryotic cell surfaces is an essential step in the establishment of infection and colonization by bacterial pathogens. Accordingly, streptococcal surface proteins, that interact with and/or bind to different components of the Extracellular Matrix (ECM) or plasma proteins of the host cell, are potential candidates for use as active component(s) for immunizing purposes.

This is illustrated by the vaccines based on M-like proteins mentioned above or disclosed in the literature, i.a. in WO 98/01561. The binding of fibrinogen and complement factor H to M-proteins is assumed to be important for the ability of streptococci to resist phagocytosis.

Another mechanism used by streptococci for attachment to host cells involves binding to the ECM component fibronectin (Fn) (Ref. 21, 22). Binding between Fn-

binding bacterial cell-surface proteins and immobilized Fn promotes internalization of streptococci by epithelial cells (Ref. 2, 23, 24). Fibronectin is a dimeric glycoprotein found both in plasma and in a fibrillar form in the extracellular matrix. The main function of Fn is to mediate substrate adhesion of eukaryotic cells, which involves the binding of specific cell-surface receptors to certain domains of the Fn molecule. Furthermore, it also interacts with several other macromolecules, such as DNA, heparin, fibrin, and collagen.

Accordingly, Fn-binding proteins from different streptococcal species have been cloned and sequenced previously. For instance, from *S. equi*, one Fn-binding protein has been cloned and characterized, which is a Fn-binding cell-surface protein of subsp. *zooepidemicus*, that has been designated FNZ (Lindmark et al., 1996, Ref. 9). Another Fn-binding protein from *S. equi* subsp. *equi,* has been cloned and characterized by Lindmark and Guss (1999) (Ref. 12). This latter protein that is designated SFS and its potential use as an active component in a vaccine for protection of horses against strangles are disclosed in WO 00/37496.

In Jonsson et al. (1995) (Ref. 8), a protein designated ZAG has been cloned and characterized from *S. equi* subsp. *zooepidemicus* that mediates binding to the plasma proteinase inhibitor α_2M. It is speculated therein that this protein is similar in function to streptococcal M proteins. This protein, ZAG, is also disclosed in WO 95/07296, where its α_2M-binding properties are indicated. However, immunogenic properties or potential use thereof as an active component in a vaccine for protection of e.g. horses against strangles are not disclosed therein. The gene *zag* encoding ZAG is also disclosed in these references.

A gene that is similar to the aforesaid *zag* gene from *S. equi* subsp. *zooepidemicus* but is present in subsp. *equi* has been described by Lindmark et al. (1999) (Ref. 11) and Lindmark (1999) (Ref.13). This gene is hereafter designated *eag* and encodes a protein designated EAG.

In WO 2004/032957 A1, antigenic compositions are disclosed which comprise at least one antigen derived from a protein designated EAG, which protein is present in *S. equi,* and which composition suitably comprises at least one further antigen selected from a group of proteins which are present in *S. equi* and are designated FNZ, SFS, SEC and SclC, respectively.

In WO 2007/115059 A2, subunit immunogenic or vaccine compositions are disclosed which comprise at least one polypeptide of *S. equi* having a specific amino

acid sequence as shown in the sequence listing attached to said publication or an analog thereof or a fragment thereof which is a part of said polypeptide and contains at least one epitope. However, no results as regards immunizing of horses against strangles are provided in this document.

In the study reported in Lannergard, J., Frykberg, L. and Guss, B. (2003) FEMS Microbiol Lett 222: 69-74, (Ref. 28), a new gene designated cne has been isolated and the corresponding protein CNE has been characterized.

In Flock, M., Jacobsson, K., Frykberg, L., Hirst, T., R., Franklin, A., Guss, B. and Flock, J.-I. (2004) Infect Immun 72:3228-3236 (Ref. 5), 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.

In Lannergard, J. and Guss, B. (2006) FEMS Microbiol Lett 262: 230-235, (Ref. 26), two new proteins, IdeE and IdeZ, from *S. equi* subspecies *equi* and *zooepidemicus*, respectively, have been characterized as regards enzymatic activities.

In Vaccine (Timoney et al.; 2007) it is reported that a great number of recombinant extracellular proteins of S. equi, including CNE (also designated SEC) and Se 44.2 (also designated IdeE2) are useless as vaccine components. It is speculated therein that earlier results for SEC/CNE obtained for mice are not applicable to horses. Thus, it is not obvious that recombinant forms of surface localized proteins necessarily are likely candidates for vaccine components.

In Waller, A., Flock, M., Smith, K., Robinson, C, Mitchell, Z., Karlström, A., Lannergard, J., Bergman, R., Guss, B. and Flock, J.-I. (2007) Vaccine 25: 3629-3635, (Ref. 27), vaccination of horses against strangles using the recombinant antigens EAG, CNE and SclC from *S. equi* subspecies *equi* is reported. In this study, vaccinated horses showed, after challenge with *S. equi* subspecies *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 of **WO** 2004/032957 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.

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

4. Brief Summary of the Invention

The present invention is based on an antigenic, suitably an immunogenic, composition comprising at least one antigen, suitably an immunogen, that comprises at least one antigenic epitope or antigenic determinant derived from a protein present in one or both of *S. equi* subsp. *equi* and subsp. *zooepidemicus* and use thereof for immunization of non-human mammals against *S. equi* subsp. *equi* and/or subsp. *zooepidemicus*.

The present invention is also directed to a vaccine composition comprising the afore-said 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* in non-human mammals; and to methods for prophylactic or therapeutic treatment of *S. equi* infection in non-human mammals. When used generally, the expression *S. equi* refers to one or both of subsp. *equi* and subsp. *zooepidemicus*.

In a first aspect, the present invention relates to an isolated antigenic composition comprising at least two antigens, wherein each antigen comprises at least part of a protein or polypeptide of *Streptococcus equi* subsp. *equi* or subsp. *zooepidemicus* and said at least part of said protein or polypeptide comprises at least one antigenic epitope or antigenic determinant of *Streptococcus equi,* wherein the antigens comprise:

an antigen comprising at least part of a protein or polypeptide which is designated EAG and has an amino acid sequence as shown in SEQ ID NO: 13; and

an antigen comprising at least part of a protein or polypeptide which is designated IdeE and has an amino acid sequence as shown in SEQ ID NO: 10.

In a second aspect, the present invention relates to an antigenic composition which comprises at least one recombinant vector and at least one polynucleotide inserted therein that encodes the proteins or polypeptides of the antigenic composition according to the first aspect, and wherein said at least one recombinant vector is able to express said proteins or polypeptides *in vivo* in a non-human mammal susceptible to infection with *S. equi*.

In a third aspect, the present invention relates to a vaccine composition for protecting a non-human mammal against infection of *Streptococcus equi,* said vaccine composition comprising the antigenic composition according to the first and/or second aspect, and a pharmaceutically acceptable carrier.

In a fourth aspect, the present invention relates to a method for preparation of a vaccine composition according to the third aspect, said method comprising mixing anantigenic composition according to the first and/or second aspect and a pharmaceutically acceptable carrier.

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In a fifth aspect, the present invention relates to the use of an antigenic composition according to the first and/or second aspect in the preparation of a vaccine for protection against S. *equi* infection inclusive of strangles caused by subsp. *equi* infection in horses.

In a sixth aspect, the present invention relates to a method for the production of an antiserum, said method comprising administering an antigenic composition according to the first and/or second aspect to a non-human animal host to produce antibodies in said animal host and recovering antiserum containing said antibodies produced in said animal host.

In a seventh aspect, the present invention relates to a vaccine composition when produced by a method according the fourth aspect.

In an eighth aspect, the present invention relates to an antiserum when produced by a method according to the sixth aspect.

According to a suitable embodiment, the present invention is directed to a vaccine that protects equines, such as horses, against strangles.

In the context of infections caused by *S. equi* subsp. *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 ninnies. Camels and dromedaries are also encompassed therein.

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

Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise”, “comprising”, and the 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”.

Brief Description of the Drawings

In the following, the present invention is described in closer detail with reference to the drawings, where:

Fig. 1 shows weight loss of mice given experimental infection with S. equi subsp. equi strain 1866 after vaccination with the polypeptides Eq5 and Eq8 (open symbols) or non-vaccinated (filled symbols);

Fig. 2 shows nasal growth in mice given experimental infection with S. equi subsp. equi strain 1866 after vaccination with the polypeptides Eq5 and Eq8 (open symbols) or non-vaccinated (filled symbols);

Fig. 3 shows weight loss of mice given experimental infection with S. equi subsp. equi strain 1866 after vaccination with the polypeptide EAG (filled squares),

the polypeptides EAG+IdeE+IdeE2 (open circles) or non-vaccinated controls (filled circles);

Fig. 4 shows nasal growth in mice given experimental infection with *S. equi* subsp. *equi* strain 1866 after vaccination with the polypeptide EAG (filled squares), the polypeptides EAG+IdeE+IdeE2 (open circles) or non-vaccinated controls (filled circles).

Fig. 5a and 5b show weight loss and nasal growth in mice immunized with EAG+CNE+SclC i.n. (filled squares), Eq5+Eq8 i.n. (filled circles) and the control (open circles).

In these figures 1-5, mean values and standard errors are indicated.

Fig. 6 shows growth of challenge inoculum (*S. equi* subsp. *equi* strain 4047);

Fig. 7 shows mean pony temperatures during the vaccination phase;

Fig. 8 shows mean nasal score during the vaccination phase;

Fig. 9 shows mean lymph node score during the vaccination phase;

Fig. 10 shows mean counts of *S. zooepidemicus* in nasal washes during the vaccination phase;

Fig. 11 shows mean pony temperatures after challenge;

Fig. 12 shows mean fibrinogen levels during the challenge phase;

Fig. 13 shows mean neutrophil levels during the challenge phase;

Fig. 14 shows mean lymph node score during the challenge phase;

Fig. 15 shows mean nasal score during the challenge phase;

Fig. 16 shows mean *S. zooepidemicus* counts during challenge phase;

Fig. 17 shows mean pathology score on post mortem examination; and

Fig. 18 shows mean histopathology scores.

Fig. 19 shows ELISA measurements of IgG antibodies in nasal washings of seven immunized horses. The log dilution of sera required to give an absorbance value at a cut-off of 1.0 was calculated for each individual nasal wash sample. Mean values (n=7) with standard errors are shown. Samples taken before (pre imm. day 1) and twelve days after the third immunization are shown (day 86). The horses were immunized with EAG, CNE and SclC.

Fig. 20 shows ELISA measurements of IgG antibodies in sera of seven immunized horses. The log dilution of sera required to give an absorbance value at a cut-off of 1.5 was calculated for each individual serum sample. Mean values (n=7) with standard errors are shown. Sample taken before (day 1), after V2 (day71), and after V3 (day 86) are shown.

Fig. 21 shows ELISA measurements of IgG antibodies in sera of immunized horses (Pentavac). The log dilution of sera required to give an absorbance value at a

cut-off of 1.5 was calculated for each individual serum sample. Mean values (n=7) with standard errors are shown. Sample taken before (day 1), after V2 (day 71), and after V3 (day 86) and samples taken between V3 and V4 (day 270) are shown.

Brief description of the sequence listing

SEQ ID NO 1 shows the amino acid sequence of the protein IdeE2.

SEQ ID NO 2 shows the amino acid sequence of the recombinant protein IdeE2.

SEQ ID NO 3 shows the amino acid sequence of the protein Eq5.

SEQ ID NO 4 shows the amino acid sequence of the recombinant protein Eq5.

SEQ ID NO 5 shows the amino acid sequence of the protein Eq8.

SEQ ID NO 6 shows the amino acid sequence of the recombinant protein Eq8.

SEQ ID NO 7 shows the amino acid sequence of the protein IdeZ2 from subsp. *zooepidemicus*.

SEQ ID NO 8 shows the amino acid sequence of the protein Eqz5 from subsp. *zooepidemicus*.

SEQ ID NO 9 shows the amino acid sequence of the protein Eqz8 from subsp. *zooepidemicus*.

SEQ ID NO 10 shows the amino acid sequence of the protein IdeE.

SEQ ID NO 11 shows the amino acid sequence of the protein IdeZ from subsp. *zooepidemicus*.

SEQ ID NOS 12 and **13** shows, respectively, the nucleotide sequence of the gene *eag* and the amino acid sequence of the protein EAG4B, which protein is usually designated EAG in connection with the present invention.

SEQ ID NO 14 shows the nucleotide sequence of the gene *ideE2*.

SEQ ID NO 15 shows the nucleotide sequence of the gene *eq5*.

SEQ ID NO 16 shows the nucleotide sequence of the gene *eq8*.

SEQ ID NO 17 shows the nucleotide sequence of the gene *ideZ2* from subsp. *zooepidemicus*.

SEQ ID NO 18 shows the nucleotide sequence of the gene *eqz5* from subsp. *zooepidemicus*.

SEQ ID NO 19 shows the nucleotide sequence of the gene *eqz8* from subsp. *zooepidemicus*.

SEQ ID NO 20 shows the nucleotide sequence of the gene *ideE*.

SEQ ID NO 21 shows the nucleotide sequence of the gene *ideZ* from subsp. *zooepidemicus*.

SEQ ID NOS 22-27 show nucleotide sequences of oligonucleotide primers.

SEQ ID NO 28 shows the amino acid sequence of the protein CNE (or SEC 2.16).

SEQ ID NO 29 shows the amino acid sequence of the protein SclC.

SEQ ID NO 30 shows the amino acid sequence of the recombinant IdeE used for immunization.

SEQ ID NO 31-32 shows the nucleotide sequence of primers.

Detailed Description of the Invention

The present invention is concerned with identification of polypeptides or proteins of *S. equi* that are able to elicit an antigenic, suitably an immunogenic, response, when administered to a non-human 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, genes of *S. equi* encoding extracellular proteins were identified and, subsequently, the corresponding products were expressed and evaluated in vaccine studies. The present invention is at least partly based on such studies.

Accordingly, the present invention relates to an antigenic composition comprising at least one antigen, wherein said at least one antigen comprises at least part of a protein of *Streptococcus equi* subsp. *equi* or subsp. *zooepidemicus,* and said at least part of said protein comprises at least one antigenic epitope or antigenic determinant of *Streptococcus equi.*

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

a protein or polypeptide which is designated EAG and has an amino acid sequence as shown in SEQ ID NO: 13;

a protein or polypeptide which is designated IdeE and has an amino acid sequence as shown in SEQ ID NO: 10;

a protein or polypeptide which is designated IdeE2 and has an amino acid sequence as shown in SEQ ID NO: 1;

a protein or polypeptide which is designated Eq5 and has an amino acid sequence as shown in SEQ ID NO: 3;

a protein or polypeptide which is designated Eq8 and has an amino acid sequence as shown in SEQ ID NO: 5;

a protein or polypeptide which is designated IdeZ2 and has an amino acid sequence as shown in SEQ ID NO: 7;

a protein or polypeptide which is designated Eqz5 and has an amino acid sequence as shown in SEQ ID NO: 8; and

a protein or polypeptide which is designated Eqz8 and has an amino acid sequence as shown in SEQ ID NO: 9;

or an analog or a fragment thereof, and wherein a composition which comprises EAG, comprises at least one further antigen, which is a protein or polypeptide, which is selected from the group comprising IdeE, IdeE2, Eq5, Eq8, IdeZ2, Eqz5, and Eqz8.

For convenience, the polypeptides having amino acid sequences as shown in the sequence listing are frequently only designated 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* subsp. *equi* and IdeZ, IdeZ2, Eqz5, and Eqz8 designate proteins that can be found in *S. equi* subsp. *zooepidemicus*.

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. For instance, an N-terminal fragment of EAG is used according to various embodiments of the present invention.

According to one embodiment, the present invention is related to an antigenic or immunogenic composition which contains at least 2 or 3 antigens or immunogens selected from the group consisting of EAG, IdeE, IdeE2, Eq5, Eq8, IdeZ, IdeZ2, Eqz5, and Eqz8.

According to a specific embodiment, the present invention is related to an antigenic or immunogenic composition which contains at least 2 or 3 antigens or immunogens selected from the group consisting of EAG, IdeE, IdeE2, Eq5, and Eq8.

Suitably this composition also comprises one or both of the previously described antigens SclC (SEQ ID NO: 29) and CNE (SEQ ID NO: 28) (also designated SEC e.g. SEC 2.16). A further embodiment is related to an antigenic composition comprising EAG, SclC, CNE, Eq5, and Eq8.

A suitable composition contains 2 antigens or immunogens which are comprised of Eq5 and Eq8, respectively. According to a further embodiment, the present invention is directed to a composition that contains 3 antigens or immunogens, which suitably are comprised of EAG, IdeE, and IdeE2. The present invention is also related to compositions that comprise one or both of IdeE and IdeE2.

The present invention is also related to an antigenic composition, wherein said at least one protein or polypeptide is selected from the group consisting of EAG, Eq5 and Eq8 and which composition further comprises at least one antigen, which is selected from the group comprising a protein or a polypeptide designated CNE (or SEC), which has an amino acid sequence as shown in SEQ ID NO: 28, and a protein or a polypeptide designated SclC, which has an amino acid sequence as shown in SEQ ID NO: 29. Suitably, said at least one protein or polypeptide is selected from the group comprising IdeE and IdeE2.

Antigenic compositions of the present invention, which have been shown to be useful in vaccine compositions, comprise according to one embodiment, the antigens EAG, SclC, CNE (or SEC), Eq5, Eq8, IdeE and IdeE2, and according to another embodiment, the antigens EAG, SclC, CNE (or SEC), Eq5, and Eq8.

The present invention is also related to an antigenic composition, wherein said at least one protein or polypeptide is selected from the group consisting of EAG, Eq8, and IdeE2 and which composition comprises at least one further antigen which is selected from the group comprising IdeE, Eq5, IdeZ2, Eqz5 and Eqz8 and/or SclC and CNE (or SEC).

According to the present invention, the antigenic composition suitably comprises at least one antigen which is recombinantly produced and/or at least one antigen which is an isolated or purified antigen.

From the above, it is evident that the present antigens or immunogens that are derived from proteins of *Streptococcus equi* may comprise the entire protein, a fragment of said protein or an analog of said protein which is antigenic or 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.*

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.

A further embodiment of the present invention is concerned with a vaccine composition for protecting non-human mammals against infection of *Streptococcus 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 2, 3 or more of the present antigens or immunogens as immunizing components. Optionally, one or more of these antigens or immunogens are comprised of analogs of said proteins or fragments thereof, e.g. N-terminal or C-terminal fragments.

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.

A suitable adjuvant for use according to the present invention is the adjuvant Abisco 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. Quillaia saponins mixed with cholesterol, and phospholipids under specific stoichiometry 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 adjuvant 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 stimulates 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 *Streptococcus equi* subsp. *equi.*

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

Suitably, the vaccine composition of the present invention stimulates serum, mucosal and/or bronchial lavage antibody responses directed to *Streptococcus equi* antigens in mammals susceptible to *Streptococcus equi*, 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

(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, said 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 technique.

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* infection in non-human 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 *Streptococcus equi* infection, which method comprises inoculating a horse intramuscular, subcutaneously or intranasally, or a combination of e.g. both subcutaneously and intranasally, with a vaccine composition of the present invention to induce an immune response against *Streptococcus equi* in said horse. Suitably, an immune response, in the form of IgG and/or IgA and/or IgM antibodies in the nasopharyngeal mucus, 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 *Streptococcus equi* or infected by *Streptococcus equi.*

The present invention describes 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 can in protein consisting of parts from different antigens. This fusion strategy could also be suitable for introducing 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. Furthermore, other hosts for expressing the recombinant antigens addition to E. coli also

be other suitable species of bacteria and viruses. Today many different systems for expression of heterologus expression are well known in the field of molecular biology.

Yet another implication of this invention is that it can be used to design specific attenuated mutants of *S. equi* that lack or have inactivated genes important for survival (i.e. mutations causing deficiency in metabolic pathways) in the host but retain or overproduce the antigens of the present invention.

EXPERIMENTAL PART

The DNA sequence of the genome of *S. equi* subsp. *equi* and subsp. *zooepidemicus* have been determined (www.sanger.ac.uk/) but not yet annotated. By screening open reading frames a great number of genes encoding extracellular proteins were identified. Among these genes a selected number were chosen and recombinant proteins were produced and evaluated in vaccine studies. The cloning and expression of these genes is described below. Furthermore, the use of these proteins as antigens will also be described.

Example 1. Constructions of clones harboring the genes *ideE, ideE2, eq5* and *eq8* from subsp. *equi*.

Chromosomal DNA from *S. equi* subspecies *equi* strain 1866 (PCT/SE03/01587, Lannergård and Guss 2007) was used as a template to amplify potential genes encoding IdeE2, Eq5 and Eq8 (the nucleotide- and protein-sequences are presented in the sequence listing further below). To identify the predicted signal sequences, the computer program SignalP (http://www.cbs.dtu.dk/services/SignalP/) was used. The sequences of primers used to amplify the genes or part of the genes *ideE, ideE2* , *eq5* and *eq8* are listed in the Primer Table. Cleavage sites for the restriction enzymes *Nco*I and *Xho*I were included in the primer sequences to match the cloning sites in the plasmid vector pTYB4 (New England Biolabs). The PCR amplifications were performed using the primers (20 pmol/µl) and the ReadyToGo™ PCR beads (GE Healthcare) 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 46 °C; 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). Cleavage with the restriction enzymes was performed over night whereupon the fragments were purified one additional time using the same kit.

Primer Table: The primer sequences used to PCR amplify the genes *ideE, ideE2, eq5* and *eq8*. The nucleotides underlined correspond to the introduced restriction cleavage sites *Nco*I and *Xho*I.

Gene	Primer	Primer sequence
ideE2	Forward primer	5'- CATGCCATGGAGGTAGTTGAAGTTTGGCCTAAT- 3' (SEQ ID NO: 22)
ideE2	Reverse primer	5'- CCGCTCGAGTTTTTCTGTCTTGTTGAAGTAATCTGC- 3' (SEQ ID NO: 23)
eq5	Forward primer Eqp51:	5'- GTAGCCATGGAAACGACTACTGCTAGTGCA-3' (SEQ ID NO: 24)
eq5	Reverse primer Eqp52:	5'CTGGCTCGAGCGGTTTAGCAACCAAGGCT - 3' (SEQ ID NO: 25)
eq8	Forward primer Eqp81:	5' CATGCCATGGCGACTACCCTAGCAGGACAAA – 3' (SEQ ID NO: 26)
eq8	Reverse primer Eqp82:	5'CTAGCTCGAGGTGCTTAAGCTTTTCAATCTG - 3' (SEQ ID NO: 27)
ideE	Forward primer IdEG1:	5'-TACTGGATCCGACGATTACCAAAGGAATGCTAC -3' (SeQ ID NO: 31)
ideE	Reverse primer IdEG2:	TGATCTCGAGTTAGCTCAGTTTCTGCCATATG (SEQ ID NO: 32)

To clone and produce recombinant proteins in *E. coli* the IMPACT™ Protein Purification System (New England Biolabs) was used. *E. coli* strain ER2566 containing the pTYB4 vector (New England Biolabs) was grown according to the manufacturer's instructions, and the vector was purified using the QIAprep Spin Miniprep (Qiagen). Purified vector was digested using restriction endonucleases *Nco*I and *Xho*I. After digestion, the vector was treated with the enzyme alkaline phosphatase to reduce the background of re-ligated vector in the later ligation step. For the ligation of the vector and the respective PCR product, the ReadyToGo T4DNA Ligase (GE Healthcare) was used. After ligation, the respective sample were transformed into competent cells of *E. coli* strain ER2566 using electroporation, and spread on LA-Amp plates (Luria-Bertani broth agar plates supplemented with ampicillin, final conc. 50 µg/ml) and incubated over night at 37 °C.

Next day colonies were counted and four colonies per construct were cultivated and used for further experiments. To verify the presence of an insert in the respective constructs, plasmids were purified and additional PCR analyses were performed using the respective primer combination. The sequence of the respective insert was also determined by DNA sequencing using primers that hybridise in the vector (T7 universal forward primer and a reverse primer located in the intein coding region).

Cloning of the *ideE* gene of *S. equi* subsp. *equi* strain 1866 has been reported previously by Lannergård and Guss (2006). The GenBank accession number of *ideE* is DQ508733. The part of the gene used to obtain the recombinant IdeE protein used for immunization was cloned using the primers IdEG1 and IdEG2 listed in the Primer Table. After PCR amplification the DNA fragment was digested with restriction enzymes *BamH*I and *Xho*I and ligated into the vector pGEX6-P-1 (GE Healthcare), previously digested with the same enzymes.

Example 2. Preparation of antigens CNE, SclC, EAG4B, IdeE, IdeE2, Eq5 and Eq8

The vector used is a part of an *E. coli* expression and purification system called IMPACT™ T7 (NEB Inc.) Briefly, following the manufacturer's instructions the clones expressing recombinant IdeE2, Eq5 and Eq8, respectively were grown at 37 °C in Luria Bertani growth 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. 0.4 mM) and the growth temperature shifted to 20 °C. After incubation over night the cells were harvested and resuspended in a buffer [20 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1 mM EDTA, and 0.1 % Triton X100] and lysed by freezing and thawing. After centrifugation, the supernatant was sterile filtrated and applied onto a chitin column. The columns were extensively washed using the same buffer and subsequently treated with cleavage buffer [20 mM Tris-HCl (pH 8.0), 50 mM NaCl, 0.1 mM EDTA, and 30 mM dithiothreitol (DTT)]. In the cleavage buffer, the reducing conditions induce an intein-mediated self-cleavage that releases the antigen part from the column while the intein-chitin-binding part is still bound. The eluted samples containing the antigens were dialysed against phosphate-buffered saline [PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.4 mM KH_2PO_4 (pH 7.4)] and concentrated. The amounts of antigens obtained were determined and the quality was checked using SDS-PAGE. The recombinant IdeE protein was produced and purified using the GST-affinity chromatography system according to the procedure recommended by the manufacturer

(GE Healthcare). The description of and production of the recombinant proteins CNE(SEC), SclC, and EAG4B antigens have been described previously (WO 2004/032957 (PCT/SE03/01587), Waller et al 2007). In the following examples, the EAG4B protein is simply called EAG.

Example 3. Recombinant IdE2 cleaves IgG

IdE has previously been shown to be a protease that specifically cleaves IgG from various species (Lannegård and Guss 2006). To test if recombinant IdeE2 also cleaves antibodies, IgG from human, horse and mouse were incubated in PBS at 37 °C for one hour. Purified recombinant IdeE was used as a positive control and the negative control was pure IgG. After cleavage, the samples were analysed using 8-25% gradient SDS-PAGE. The result showed that recombinant IdeE2 cleaves cleaves horse IgG much more efficiently than IdeE does.

Example 4. Presence of the genes *ideE*, *ideE2*, *eq5*, and *eq8* in *S. equi* subsp. *zooepidemicus*

Previously the presence of a homologous subsp. *equi ideE* gene in subsp. *zooepidemicus* has been reported (Lannegård and Guss 2006). Using the *S. zooepidemicus* genome database (www.sanger.ac.uk/), the presence of similar genes to *ideE2, eq5* and *eq8* in subspecies *zooepidemicus* was analysed using BLAST search. The results showed that genes encoding similar proteins were detected. The sequence of these genes called *ideZ2, eqz5* and *eqz8* along with amino acid sequences IdeZ2, Eqz5 and Eqz8 are shown in the list of sequences in the experimental part of this specification.

Example 5. Immunisation of mice with Eq5 and Eq8

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). Fifteen animals were immunised with antigen (Eq5 and Eq8) and 15 were only given Abisco 300 adjuvant to serve as a negative control. The total volume was kept to less than 27 µl and applied into the nostrils twice with 30 minutes interval of mice anaesthetized with Isoflovet (Abbot Laboratories, England). Immunisations were given on days 0, 13 and 32.

Example 6. Immunisation of mice with EAG, IdeE and IdeE2

Immunisation with EAG, IdeE and IdeE2 was performed essentially as for Eq5 and Eq8. However, animals were divided into three groups, with ten mice in each group. These were given EAG+IdeE+IdeE2 or EAG only and one group with only adjuvans, Abisco 300, as negative control. Immunisations were given on days 0, 21 and 53. Experimental infection was given on day 60.

Example 7. Experimental infection with *Streptococcus equi* subsp. *equi*

Experimental infection was given on day 43 (10 days after last time of immunisation) for Eq5 + Eq8 and on day 60 (10 days after last immunisation) for EAG+/-IdeE+IdeE2. *S. equi* supsp. *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 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 (THB) with 1% yeast extract (THY). The culture was 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 THY and grown without shaking over night. The cultures 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 blood agar 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 8. Experimental results of vaccination

Mice were immunised with both Eq5 and Eq8 and the percentage weight loss over time was determined. Figure 1 shows that vaccinated animals (n=15) lost less weight that control animals (n=15). P-values = 0.0001 for all days (Student's t-test). Nasal growth of *S. equi* was also determined daily on a four graded scale. Figure 2 shows that the vaccinated animals had much less nasal growth than the control group. The frequency of

animals grossly colonised nasally with bacteria (scoring 2-3) on day 5 was significantly different between the two groups; p= 0.002 (Fisher's exact test).

In the next experiment, mice were vaccinated with EAG (n=10), with EAG+IdeE+IdeE2 (n=10) or non-vaccinated (n=10). The percentage weight loss over time was determined. Figure 3 shows that animals vaccinated with EAG+IdeE+IdeE2 lost less weight that control animals. P values were 0.0013, 0.0008 and 0.0009 for days 3, 5 and 6 respectively (Student's t-test). Animals vaccinated with EAG alone also lost weight to a similar magnitude as control animals. Nasal growth of *S. equi* was also determined daily on a four graded scale. Figure 4 shows that the animals vaccinated with EAG+IdeE+IdeE2 had much less nasal growth than the control group. Again, vaccination with only EAG showed no protection.

Example 9. Immunisation of mice with Eq5, Eq8, and EAG, CNE, SclC

Immunisation i.n. with Eq5+ Eq8 and EAG+CNE+SclC was performed as above with three groups with ten mice in each group. One group with Eq5+Eq8 and one with EAG+CNE+SclC. The third group was the control with Abisco-300. Immunisations were given on days 0, 14 and 22. Challenge was given on day 29. The experimental results are shown in Fig. 5a and Fig. 5b. Figure 5a and b show significant protection for EAG+CNE+SclC (n=10). P-values were 0.04 and 0.09 for day 2 and 5. The protection with Eq5+Eq8 was even more pronounced where p-values were 0.005 and 0.009 for these days.

LIST OF SEQUENCES

(1) SEQ ID NO: 1 and **SEQ ID NO: 14** are combined to show the amino acid sequence of the IdeE2 protein **(SEQ ID NO: 1)** under the nucleotide sequence of *ideE2* **(SEQ ID NO: 14)**

```
atgatgaaaaaacaa
 M  M  K  K  Q
tcattcacacactcacgtaaacctaaattcggtatgagaaaattatctattggccttgcc
 S  F  T  H  S  R  K  P  K  F  G  M  R  K  L  S  I  G  L  A
tcatgtatgctaggaatgatgttcctaacaacaggacatgtttctggtgaggtagttgaa
 S  C  M  L  G  M  M  F  L  T  T  G  H  V  S  G  E  V  V  E
gtttggcctaatgggcaaaatcctaatggtaaaatagaaattctaagtcaaactgagcac
 V  W  P  N  G  Q  N  P  N  G  K  I  E  I  L  S  Q  T  E  H
tctgagcatttacagaaattacgcgatattgaagatttccaagctcaaaagcaagctgat
 S  E  H  L  Q  K  L  R  D  I  E  D  F  Q  A  Q  K  Q  A  D
catgttcgttacactaaatggttagatggggtaactgttgatgagcatgaattcagaaaa
 H  V  R  Y  T  K  W  L  D  G  V  T  V  D  E  H  E  F  R  K
atcaaggaatatgacacagaatattatgtaacacctcttttaagtggtaaaggttactat
 I  K  E  Y  D  T  E  Y  Y  V  T  P  L  L  S  G  K  G  Y  Y
gatatcaataaagatttcaatcaagatagtgataaatgtgctgccgctgtagcggctaat
 D  I  N  K  D  F  N  Q  D  S  D  K  C  A  A  A  V  A  A  N
atgttccattattggtttgatagaaatagagacagtattaatcgtttcttaagtcaaagt
 M  F  H  Y  W  F  D  R  N  R  D  S  I  N  R  F  L  S  Q  S
ccaggtgaaaatggtgttattaaacttgaaaatgaaaaaacaatagaagtatcaaaattt
 P  G  E  N  G  V  I  K  L  E  N  E  K  T  I  E  V  S  K  F
ttagaaacttaccgtagtgatggtgattatcttgataaaagtccgtttttgaccttatc
 L  E  T  Y  R  S  D  G  D  Y  L  D  K  S  P  F  F  D  L  I
agtaacagctttaaaggtcctgtttgggcaaataagctattggatgcttacattaacggc
 S  N  S  F  K  G  P  V  W  A  N  K  L  L  D  A  Y  I  N  G
tatggttatatccataaatttgctaaaaatactccacattctaaaaataataatagtaaa
 Y  G  Y  I  H  K  F  A  K  N  T  P  H  S  K  N  N  N  S  K
tttaatttctttaaaaaagtatttgatggtaatctcttgacagatattcaccaaatttttt
 F  N  F  F  K  K  V  F  D  G  N  L  L  T  D  I  H  Q  I  F
gattataacacttttcagataaattaagtgaggctctctatactggtaaagccattgga
 D  Y  N  T  F  S  D  K  L  S  E  A  L  Y  T  G  K  A  I  G
ttggcctacggacctggagacttgcgtcgttcactaggtcatattatttctgtctgggga
 L  A  Y  G  P  G  D  L  R  R  S  L  G  H  I  I  S  V  W  G
gctgatcttgacgatcagaatcgcgtggtagctatttatgtaactgattctgatgataaa
 A  D  L  D  D  Q  N  R  V  V  A  I  Y  V  T  D  S  D  D  K
aagttaactataggaaatgagagagttggtttgaagcgatataaagtatctagcgatgat
 K  L  T  I  G  N  E  R  V  G  L  K  R  Y  K  V  S  S  D  D
caaggtcgtgctcgtctgacgactcgtgataaagataacacaggtggtgaaattcgatct
 Q  G  R  A  R  L  T  T  R  D  K  D  N  T  G  G  E  I  R  S
attgaaacattagatatgggtacacaagagtgggcagattacttcaacaagacagaaaaa
 I  E  T  L  D  M  G  T  Q  E  W  A  D  Y  F  N  K  T  E  K
taa
```

(2) SEQ ID NO: 2 shows the recombinant IdeE2 protein sequence. The amino acids in bold are those that corresponds to the amino acids encoded by the pTYB4 vector while the rest originates from the IdeE2 protein.

MEVVEVWPNGQNPNGKIEILSQTEHSEHLQKLRDIEDFQAQKQADHVRYTKWLDGVTVDE
HEFRKIKEYDTEYYVTPLLSGKGYYDINKDFNQDSDKCAAAVAANMFHYWFDRNRDSINR
FLSQSPGENGVIKLENEKTIEVSKFLETYRSDGDYLDKSPFFDLISNSFKGPVWANKLLD
AYINGYGYIHKFAKNTPHSKNNNSKFNFFKKVFDGNLLTDIHQIFDYNTFSDKLSEALYT
GKAIGLAYGPGDLRRSLGHIISVWGADLDDQNRVVAIYVTDSDDKKLTIGNERVGLKRYK
VSSDDQGRARLTTRDKDNTGGEIRSIETLDMGTQEWADYFNKTEK**LEPG**

(3) SEQ ID NO: 3 and **SEQ ID NO: 15** are combined to show the amino acid sequence of the Eq5 protein **(SEQ ID NO: 3)** under the nucleotide sequence of *eq5* gene **(SEQ ID NO: 15)**

```
atgaagaaattcacgaaacggtgtcttaagggctgtggtcttgttggattagttttcagc
 M  K  K  F  T  K  R  C  L  K  G  C  G  L  V  G  L  V  F  S
acaggattggttgccttgtcggataatattgatagcgctttaacagtaggggcggaaacg
 T  G  L  V  A  L  S  D  N  I  D  S  A  L  T  V  G  A  E  T
actactgctagtgcatttgaaaataatgggacaggtcaacatctgaactggcacatagat
 T  T  A  S  A  F  E  N  N  G  T  G  Q  H  L  N  W  H  I  D
attccacaagaatatacagttgaattaggagaaccaattactatctcagatcttatgagt
 I  P  Q  E  Y  T  V  E  L  G  E  P  I  T  I  S  D  L  M  S
caaattacggttactcgtaaaggtagtaatgggactgttaatgatggagatacttttgac
 Q  I  T  V  T  R  K  G  S  N  G  T  V  N  D  G  D  T  F  D
tttatttcgaatggagatggttcaagaggaattgatacccctggagtaaaaatatggttt
 F  I  S  N  G  D  G  S  R  G  I  D  T  P  G  V  K  I  W  F
gacttttacaatgctgcgggtacttccttttaactgatgaaatgttagcttcgcctaca
 D  F  Y  N  A  A  G  T  S  F  L  T  D  E  M  L  A  S  P  T
tatgctgtaccgggggatcttatactattaaagcttgggtattctatgggaaaaatgat
 Y  A  V  P  G  G  S  Y  T  I  K  A  W  V  F  Y  G  K  N  D
accaaaaagctcttcacatttaaactaaaaaattccaacagcaataaaactgagttaagg
 T  K  K  L  F  T  F  K  L  K  N  S  N  S  N  K  T  E  L  R
aagtcgttagaggaggctaagctaaaactcagccagcctgaaggaacgtattctgatgaa
 K  S  L  E  E  A  K  L  K  L  S  Q  P  E  G  T  Y  S  D  E
tcactgcaagccttgcaatcagcggttactcttggtaagacctatttaaacagtgaccct
 S  L  Q  A  L  Q  S  A  V  T  L  G  K  T  Y  L  N  S  D  P
gatcaaaatacagtagatcaatctgttactactattgattccgctattactagtcttgtt
 D  Q  N  T  V  D  Q  S  V  T  T  I  D  S  A  I  T  S  L  V
aatcttaatgctttaaatgaagctattaatcaagctacacctttataacagatggcaaa
 N  L  N  A  L  N  E  A  I  N  Q  A  T  P  F  I  T  D  G  K
gagtatcctaaagaagcgtatgacggtcttgtgcaaaagcttgcagcggcagctaagctt
 E  Y  P  K  E  A  Y  D  G  L  V  Q  K  L  A  A  A  A  K  L
caaaattcatttggtccttcacaaggagatgttgataaggctgcgactgatttaacgcaa
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 Q  N  S  F  G  P  S  Q  G  D  V  D  K  A  A  T  D  L  T  Q
gctcttacgacgcttaagactgctgtagcgcatgaagccttagatcaagccttggctaag
 A  L  T  T  L  K  T  A  V  A  H  E  A  L  D  Q  A  L  A  K
ctgttagagctttaccgagaaaatccaaatcttgctttgacatcagagtctttgaaggaa
 L  L  E  L  Y  R  E  N  P  N  L  A  L  T  S  E  S  L  K  E
ttgtacaataaggccattgaagcagcaggtaccttctatagaactgttaacaaggataaa
 L  Y  N  K  A  I  E  A  A  G  T  F  Y  R  T  V  N  K  D  K
gagagaaaagacatttccctttatgagctagagcgctacactacagaaacaaattcagtt
 E  R  K  D  I  S  L  Y  E  L  E  R  Y  T  T  E  T  N  S  V
gttgatactattttaaaggtaaaggctgcgattgccgaagaaggaaaggcaaaattgcgt
 V  D  T  I  L  K  V  K  A  A  I  A  E  E  G  K  A  K  L  R
tctgctttagaccaattaaatgctcttatcggagaaaatctagacctatctccatataca
 S  A  L  D  Q  L  N  A  L  I  G  E  N  L  D  L  S  P  Y  T
gcagcttctgctcaagcctatacagaccagctagctaaggctaaggaggtcgcagcagcg
 A  A  S  A  Q  A  Y  T  D  Q  L  A  K  A  K  E  V  A  A  A
ggtgagacagcttatgctcaggagacagaaccgacagctattactaacagcttggttaag
 G  E  T  A  Y  A  Q  E  T  E  P  T  A  I  T  N  S  L  V  K
gtgttaaatgctaagaaatccctctcagatgccaaggcagccttggttgctaaaccggtc
 V  L  N  A  K  K  S  L  S  D  A  K  A  A  L  V  A  K  P  V
gatccagtagatccagtagacccagtggatccggtagacccagtagatccggtagaccca
 D  P  V  D  P  V  D  P  V  D  P  V  D  P  V  D  P  V  D  P
gtggatccggtagacccagtggatccagtagacccagtagacccagtagacccagtggat
 V  D  P  V  D  P  V  D  P  V  D  P  V  D  P  V  D  P  V  D
ccggtagacccagtggatccggtagacccggtcgatccaatcgacccagcggatccagta
 P  V  D  P  V  D  P  V  D  P  V  D  P  I  D  P  A  D  P  V
aaaccatcagatcctgaggttaagccagagcctaaaccagaatctaagcctgaagctaag
 K  P  S  D  P  E  V  K  P  E  P  K  P  E  S  K  P  E  A  K
aaggaggacaagaaagcagctgataagcagcaagtgcttccggcaactgctgatacagct
 K  E  D  K  K  A  A  D  K  Q  Q  V  L  P  A  T  A  D  T  A
aatccattctttacagcagcagctcttgcagttattgcttgtgcaggccagcttgctatt
 N  P  F  F  T  A  A  A  L  A  V  I  A  C  A  G  Q  L  A  I
gtgtcaagacgcaaagaatcaaattaactgtaggcgatgattttccccctttaattaatt
 V  S  R  R  K  E  S  N  -  L  -  A  M  I  F  P  L  -  L  I
```

(4) SEQ ID NO: 4 shows the recombinant Eq 5 protein sequence: The amino acids in bold are those that corresponds to the amino acids encoded by the pTYB4 vector while the rest originates from the Eq5 protein.

METTTASAFENNGTGQHLNWHIDIPQEYTVELGEPITISDLMSQITVTRKGSNGTVNDGD
TFDFISNGDGSRGIDTPGVKIWFDFYNAAGTSFLTDEMLASPTYAVPGGSYTIKAWVFYG
KNDTKKLFTFKLKNSNSNKTELRKSLEEAKLKLSQPEGTYSDESLQALQSAVTLGKTYLN
SDPDQNTVDQSVTTIDSAITSLVNLNALNEAINQATPFITDGKEYPKEAYDGLVQKLAAA
AKLQNSFGPSQGDVDKAATDLTQALTTLKTAVAHEALDQALAKLLELYRENPNLALTSES
LKELYNKAIEAAGTFYRTVNKDKERKDISLYELERYTTETNSVVDTILKVKAAIAEEGKA
KLRSALDQLNALIGENLDLSPYTAASAQAYTDQLAKAKEVAAAGETAYAQETEPTAITNS
LVKVLNAKKSLSDAKAALVAKP**LEPG**

(5) SEQ ID NO: 5 and **SEQ ID NO: 16** are combined to show the amino acid sequence of the Eq8 protein **(SEQ ID NO: 5)** under the nucleotide sequence of *eq8* gene **(SEQ ID NO: 16)**

```
atgaacaaaaaatcagcaagacgcaggcgtaagaatcttattacgaagcttgcgatgaca
 M  N  K  K  S  A  R  R  R  R  K  N  L  I  T  K  L  A  M  T
agtgccttaaccctgggtgtaggcgcagcgactaccctagcaggacaaacagaagtacgg
 S  A  L  T  L  G  V  G  A  A  T  T  L  A  G  Q  T  E  V  R
gctgataatatcttacgcttagatatgacagataaagaagcagttgaaaaattcgctaac
 A  D  N  I  L  R  L  D  M  T  D  K  E  A  V  E  K  F  A  N
gagcttaaaaatgaagtccataaaaactatcgtggtagtaatacttggcaaaagcttacc
 E  L  K  N  E  V  H  K  N  Y  R  G  S  N  T  W  Q  K  L  T
cttatacttaatggttatcaaaaccttagagaacaaatagagaccgagctaaaaaatagt
 L  I  L  N  G  Y  Q  N  L  R  E  Q  I  E  T  E  L  K  N  S
gaacaaaaagtaaaagagcttaatgataaggttaatagtgaaactcaaggaaaacaagag
 E  Q  K  V  K  E  L  N  D  K  V  N  S  E  T  Q  G  K  Q  E
ttacagaatcagcttgagaaagaaaaagaagagttagaaacactaaaaaaagagcttgaa
 L  Q  N  Q  L  E  K  E  K  E  E  L  E  T  L  K  K  E  L  E
gctgagaaggctaaaggaactggagaaacagagaagcttcaaaaggaaattgaagcaaaa
 A  E  K  A  K  G  T  G  E  T  E  K  L  Q  K  E  I  E  A  K
aatgcaatgatttctgacctacaaaaacagcttgaggaaactaagcaaagggttcaagag
 N  A  M  I  S  D  L  Q  K  Q  L  E  E  T  K  Q  R  V  Q  E
tttgaagctgaagtaggtaaattaatggccgaaaaggcagacctacaaacaaaattaaat
 F  E  A  E  V  G  K  L  M  A  E  K  A  D  L  Q  T  K  L  N
gaacaagagcagcttaacgctaagcttcaaaaagaaattgaagacttaaaggctcagatt
 E  Q  E  Q  L  N  A  K  L  Q  K  E  I  E  D  L  K  A  Q  I
gaaaagcttaagcactgtcaagatacacctaagccagagcctaagccagagcctaagcca
 E  K  L  K  H  C  Q  D  T  P  K  P  E  P  K  P  E  P  K  P
gagcctaagccagagcctaagccagagcctaagccagagcctaagccagagcctaagcca
 E  P  K  P  E  P  K  P  E  P  K  P  E  P  K  P  E  P  K  P
gagcctaagccagggcctaagccagagcctaagccagagcctaagccagggcctaagcca
 E  P  K  P  G  P  K  P  E  P  K  P  E  P  K  P  G  P  K  P
gagcctaagccagagcctaagccagggcctaagccagggcctaagccagagcctaagcca
 E  P  K  P  E  P  K  P  G  P  K  P  G  P  K  P  E  P  K  P
gggcctaagccagagcctaagccagagcctaagccagagcctaagcctgaagctaagaag
 G  P  K  P  E  P  K  P  E  P  K  P  E  P  K  P  E  A  K  K
cctgaacaacctaaaccaatgactaaaccaggagctaagaagcctgagcaatcacttcca
 P  E  Q  P  K  P  M  T  K  P  G  A  K  K  P  E  Q  S  L  P
tcaactggtgacatcagaaatccattcttcacgcctgcagctattgctattatgatcgca
 S  T  G  D  I  R  N  P  F  F  T  P  A  A  I  A  I  M  I  A
gcaggtaccattgccattccaaaacgcaaggaagaagattaaacaaattaacaatcccca
 A  G  T  I  A  I  P  K  R  K  E  E  D  -  T  N  -  Q  S  P
```

(6) SEQ ID NO: 6 shows the recombinant Eq8 protein sequence: The amino acids in bold are those that corresponds to the amino acids encoded by the pTYB4 vector while the rest originates from the Eq8 protein.

MATTLAGQTEVRADNILRLDMTDKEAVEKFANELKNEVHKNYRGSNTWQKLTLILNGYQN
LREQIETELKNSEQKVKELNDKVNSETQGKQELQNQLEKEKEELETLKKELEAEKAKGTG
ETEKLQKEIEAKNAMISDLQKQLEETKQRVQEFEAEVGKLMAEKADLQTKLNEQEQLNAK
LQKEIEDLKAQIEKLKH**LEPG**

(7) SEQ ID NO: 7 and **SEQ ID NO: 17** are combined to show the amino acid sequence of the IdeZ2 protein **(SEQ ID NO: 7)** under the nucleotide sequence of the *ideZ2* gene **(SEQ ID NO: 17)**from *S. equi* subsp. *zooepidemicus*

```
atgatgaaaaaacaatcattcacacactcacgtaaacctaaattcggtatgagaaaatta
 M  M  K  K  Q  S  F  T  H  S  R  K  P  K  F  G  M  R  K  L
tctattggccttgcctcatgtatgctaggaatgatgttcctaacaacaagccatgtttct
 S  I  G  L  A  S  C  M  L  G  M  M  F  L  T  T  S  H  V  S
ggtgaggtagttgaagtttggccttatgggcaagatcctaatgataaaatagaagtttta
 G  E  V  V  E  V  W  P  Y  G  Q  D  P  N  D  K  I  E  V  L
agtcaatctgagtattccgaatatttacagagattacacgatgttgaagatttccaagct
 S  Q  S  E  Y  S  E  Y  L  Q  R  L  H  D  V  E  D  F  Q  A
gaaaagaaaaaagaaggagttgtccgtacacaatggttagagggtgtgaacgttactgac
 E  K  K  K  E  G  V  V  R  T  Q  W  L  E  G  V  N  V  T  D
catgacttccggaaaatcactgatggtggtagtgtttattatgccacacctcttttaaat
 H  D  F  R  K  I  T  D  G  G  S  V  Y  Y  A  T  P  L  L  N
gatagaggctattatgatatcaacaagaatttcaatcaagacagtgataaatgtgctgct
 D  R  G  Y  Y  D  I  N  K  N  F  N  Q  D  S  D  K  C  A  A
gctgtggcagttaatatgttccattattggcttgataggaataaagataatgtagctaag
 A  V  A  V  N  M  F  H  Y  W  L  D  R  N  K  D  N  V  A  K
tttcttagtcaaagtccagaccatggttttgttgaaggtgaacctacttttaacttagta
 F  L  S  Q  S  P  D  H  G  F  V  E  G  E  P  T  F  N  L  V
gattttcaatatacatatgcatctccatatgaagaaggaggatatagggacaatagtaaa
 D  F  Q  Y  T  Y  A  S  P  Y  E  E  G  G  Y  R  D  N  S  K
ctcttcgactttattagcaaggcttttaataagcctctttgggcaaataaattgttagat
 L  F  D  F  I  S  K  A  F  N  K  P  L  W  A  N  K  L  L  D
gcttacattaatggctatggctatatcgacagatacgttaaaaataccccgcattctgga
 A  Y  I  N  G  Y  G  Y  I  D  R  Y  V  K  N  T  P  H  S  G
caaaataatagtaaatttaatttctttaaaaaagtatttgatggcaagctcttgacagat
 Q  N  N  S  K  F  N  F  F  K  K  V  F  D  G  K  L  L  T  D
attcaacaaatttttgattattatactttatcgtctgagctacgtgaagctcttgatact
 I  Q  Q  I  F  D  Y  Y  T  L  S  S  E  L  R  E  A  L  D  T
ggcaaagctattggtttagcctatggacctggagatttacgccgttctctgggacatatt
 G  K  A  I  G  L  A  Y  G  P  G  D  L  R  R  S  L  G  H  I
atctccgtctggggagctgacattaatgaagatggaaatgtcgtggctatttatgtgact
 I  S  V  W  G  A  D  I  N  E  D  G  N  V  V  A  I  Y  V  T
```

```
gattccgatgataaaaaattaactatagggaataaaaaagaccgaattggtttgaagcga
 D  S  D  D  K  K  L  T  I  G  N  K  K  D  R  I  G  L  K  R
tacaaactgtattctgataacgtgggacgagctcgcctaacagcctatgctacagaaaac
 Y  K  L  Y  S  D  N  V  G  R  A  R  L  T  A  Y  A  T  E  N
caacaaactggtggtgaagttcgagggattgaaactttagatatggctacacaagattgg
 Q  Q  T  G  G  E  V  R  G  I  E  T  L  D  M  A  T  Q  D  W
gcagattattttagcaggacagacgaagcagaacaataa
 A  D  Y  F  S  R  T  D  E  A  E  Q  -
```

(8) SEQ ID NO: 8 and **SEQ ID NO: 18** are combined to show the amino acid sequence of the Eqz5 protein **(SEQ ID NO: 8)** under the nucleotide sequence of the *eqz5* gene **(SEQ ID NO: 18)**from *S. equi* subsp. *zooepidemicus*

```
atgaagaaattcacgaaacggtgtctt
 M  K  K  F  T  K  R  C  L
aagggctgcggtcttgttggattagttttcagcacaggattggttgccttgtcggataat
 K  G  C  G  L  V  G  L  V  F  S  T  G  L  V  A  L  S  D  N
attgatagcgctttaacagtaggggcggaaacggctactactgctaatgcatttgaagaa
 I  D  S  A  L  T  V  G  A  E  T  A  T  T  A  N  A  F  E  E
agtggtgaccaacaacataaaaattggcatatttatattccagaggtttatactgttaaa
 S  G  D  Q  Q  H  K  N  W  H  I  Y  I  P  E  V  Y  T  V  K
gtcggtcagccaatcaccattgaggatatcttaagtcagattacgattactcgtaaggga
 V  G  Q  P  I  T  I  E  D  I  L  S  Q  I  T  I  T  R  K  G
gaagattcgcaaggtaaaacatctcccggaatgatctatacttatgaagaataccctaaa
 E  D  S  Q  G  K  T  S  P  G  M  I  Y  T  Y  E  E  Y  P  K
gtacgaggaattgaagtttcagcaggaactatttggtttgatttttataattctggaaac
 V  R  G  I  E  V  S  A  G  T  I  W  F  D  F  Y  N  S  G  N
tgggtaaataatgatgttttagctaccttcaacgaacctggaggaacttataccttatct
 W  V  N  N  D  V  L  A  T  F  N  E  P  G  G  T  Y  T  L  S
gcttgggcatactatgctaacgaaaatgtaaaaaaacaatttgttttcaaacttcaagtt
 A  W  A  Y  Y  A  N  E  N  V  K  K  Q  F  V  F  K  L  Q  V
gaaaatagtgataagcgtgcattagaacaatctcttgctactgctaacgaaaagttacag
 E  N  S  D  K  R  A  L  E  Q  S  L  A  T  A  N  E  K  L  Q
gctcctgaaggaacgtattctgatgaatcactgcaacgtttacaagaatcagttttcctt
 A  P  E  G  T  Y  S  D  E  S  L  Q  R  L  Q  E  S  V  F  L
ggtcaaacttatttgaacagggatcctgagcaacaagaagtggacgatatgaaggcaact
 G  Q  T  Y  L  N  R  D  P  E  Q  Q  E  V  D  D  M  K  A  T
attgattctgctgtttctggtcttgttgatcttactgtcttaaataccgcagttgaaaca
 I  D  S  A  V  S  G  L  V  D  L  T  V  L  N  T  A  V  E  T
gcaacaccattgttaacagatggtaaggagtatcctaaagaagcgtatgatagccttgtt
 A  T  P  L  L  T  D  G  K  E  Y  P  K  E  A  Y  D  S  L  V
caaaagcttgcagcagcagctaagcttcaaaattcctttaacccatcacaagaagaagtt
 Q  K  L  A  A  A  A  K  L  Q  N  S  F  N  P  S  Q  E  E  V
aacgaggctgcgactgatttaacgcaagctcttacgacgcttaagactgctgtagcgcat
 N  E  A  A  T  D  L  T  Q  A  L  T  T  L  K  T  A  V  A  H
gaagccttagatcaagccttggctaagctgttagagctttaccgagaaaatccaaacctt
```

```
 E  A  L  D  Q  A  L  A  K  L  L  E  L  Y  R  E  N  P  N  L
gctttgacatcagagcctttgaaggaattgtacaataaggccattgaagcagcaggcacc
 A  L  T  S  E  P  L  K  E  L  Y  N  K  A  I  E  A  A  G  T
ttctatagaactgttagcaaggataaagagagaaaaggcatttccctttatgagctagag
 F  Y  R  T  V  S  K  D  K  E  R  K  G  I  S  L  Y  E  L  E
cgttacactacagaaacaaactcagttgttgatactattttaaaggtaaaggctgcaatt
 R  Y  T  T  E  T  N  S  V  V  D  T  I  L  K  V  K  A  A  I
gccgaagaaggaaaggcaaaattgcgttctgctttagaccaattaaatgctcttatcgga
 A  E  E  G  K  A  K  L  R  S  A  L  D  Q  L  N  A  L  I  G
gaaaatctagacctatctccatatacagcagcttctgctcaagcctatacagaccagcta
 E  N  L  D  L  S  P  Y  T  A  A  S  A  Q  A  Y  T  D  Q  L
gctaaggctaaggaggttgcagcagcgggtgagacagcttatgctcaggagacagaaccg
 A  K  A  K  E  V  A  A  A  G  E  T  A  Y  A  Q  E  T  E  P
acagctattactaacagcttgattaaggtgctaaatgctaagaaatccctctcagatgcc
 T  A  I  T  N  S  L  I  K  V  L  N  A  K  K  S  L  S  D  A
aaggcagcattggttgctaaaccggtagatccggtagacccagtagatccggtagaccca
 K  A  A  L  V  A  K  P  V  D  P  V  D  P  V  D  P  V  D  P
gtggatccggtagacccaattgatccagtagatccagtaaaaccagtcgatcctgaggtt
 V  D  P  V  D  P  I  D  P  V  D  P  V  K  P  V  D  P  E  V
aagccagagcctaaaccagaatctaagcctgaagctaagaaggaggacaagaaagcagct
 K  P  E  P  K  P  E  S  K  P  E  A  K  K  E  D  K  K  A  A
gataagcagcaagtgcttccggcaactgctgatacagctaacccattctttacagcagca
 D  K  Q  Q  V  L  P  A  T  A  D  T  A  N  P  F  F  T  A  A
gctcttgcagttattgcttgtgcaggccagcttgctattgtgtcaagacgcaaagaatca
 A  L  A  V  I  A  C  A  G  Q  L  A  I  V  S  R  R  K  E  S
aattaa
 N  -
```

(9) SEQ ID NO: 9 and **SEQ ID NO: 19** are combined to show the amino acid sequence of the Eqz8 protein **(SEQ ID NO: 9)** under the nucleotide sequence of the *eqz8* gene **(SEQ ID NO: 19)** from *S. equi* subsp. *zooepidemicus*

```
atgaacaaaaaatcagca
 M  N  K  K  S  A
agacgcaagcgtaaggatcttatcacgaagcttgcgatgacaagtgccttaaccctgggt
 R  R  K  R  K  D  L  I  T  K  L  A  M  T  S  A  L  T  L  G
gtaggcgcagcagctaccatagcaggacaaacagaagtacgggctgaggttctaaccttа
 V  G  A  A  A  T  I  A  G  Q  T  E  V  R  A  E  V  L  T  L
aatatgaaagataaagctaaagttgaagaattcgctaataagcttaaagattacgcaaag
 N  M  K  D  K  A  K  V  E  E  F  A  N  K  L  K  D  Y  A  K
caaaagaaatctggccaaattactttgcaagaactttcccttatacttgatgggtacaga
 Q  K  K  S  G  Q  I  T  L  Q  E  L  S  L  I  L  D  G  Y  R
aatattagggagcagatagaacaagacttagctactacagaaaaaactaaaaatttctat
 N  I  R  E  Q  I  E  Q  D  L  A  T  T  E  K  T  K  N  F  Y
ggagaacagttaattcttactgataaactttatcagtctgaaaaagaaaagaaagaaaag
 G  E  Q  L  I  L  T  D  K  L  Y  Q  S  E  K  E  K  K  E  K
```

```
ctagaagctgagctacaactaagccaacaaaaaattcatgaccttgatgaaaaacatcaa
 L  E  A  E  L  Q  L  S  Q  Q  K  I  H  D  L  D  E  K  H  Q
aaagagaaattagagctacaagaacaacttgaggcttcaaatcaaaagattaaagagctt
 K  E  K  L  E  L  Q  E  Q  L  E  A  S  N  Q  K  I  K  E  L
gaaatggcaaagagcacagctgaagctgaaataaatagactaacagctgaaaaaaatgga
 E  M  A  K  S  T  A  E  A  E  I  N  R  L  T  A  E  K  N  G
ttacaagaaaaattaaataatcaagaaaagcttaatgctgagttacaagcaaaattagct
 L  Q  E  K  L  N  N  Q  E  K  L  N  A  E  L  Q  A  K  L  A
aagcaagaagagcttaacgctaagcttcaaaaggaaattgacgaattaaatgctcagctt
 K  Q  E  E  L  N  A  K  L  Q  K  E  I  D  E  L  N  A  Q  L
gaaaagcttaagcattgtcaagatacacctaagccagagcctaagccagagcctaagcca
 E  K  L  K  H  C  Q  D  T  P  K  P  E  P  K  P  E  P  K  P
gagcctaagccagagcctaagccagagcctaagccagagcctaagccagagcctaagcca
 E  P  K  P  E  P  K  P  E  P  K  P  E  P  K  P  E  P  K  P
gagcctaagccagagcctaagccagagcctaagccagagcctaagccagagcctaagcca
 E  P  K  P  E  P  K  P  E  P  K  P  E  P  K  P  E  P  K  P
gagcctaagccagagcctaagccagagcctaagccagagcctaagccagagcctaagcca
 E  P  K  P  E  P  K  P  E  P  K  P  E  P  K  P  E  P  K  P
gagcctaagccagagcctaagccagagcctaagccagagcctaagccagagcctaagcca
 E  P  K  P  E  P  K  P  E  P  K  P  E  P  K  P  E  P  K  P
gagcctaagccagagcctaagccagagcctaagccagagcctaagccagagcctaagcca
 E  P  K  P  E  P  K  P  E  P  K  P  E  P  K  P  E  P  K  P
gagcctaagccagagcctaagcctgaagctaaaaagcctgaacaacctaaaccaatgact
 E  P  K  P  E  P  K  P  E  A  K  K  P  E  Q  P  K  P  M  T
aaaccaggggctaagaagcctgagcaatcacttccatcaactggtgacatcagaaatcca
 K  P  G  A  K  K  P  E  Q  S  L  P  S  T  G  D  I  R  N  P
ttcttcacacctgcagctattgctattatgatcgcagcaggtaccattgcaattccaaaa
 F  F  T  P  A  A  I  A  I  M  I  A  A  G  T  I  A  I  P  K
cgcaaggaagaagactaa
 R  K  E  E  D  -
```

(10) SEQ ID NO: 10 and **SEQ ID NO: 20** are combined to show the amino acid sequence of the IdeE protein **(SEQ ID NO: 10)** under the nucleotide sequence of the *ideE* gene **(SEQ ID NO: 20).**

The nucleotide sequence of the *ideE* gene (GenBank DQ508733) and the amino acid sequence of the IdeE protein from *S. equi* subsp. *equi* are shown.

```
atgaaaacaatagcttatccaaataaacctcactccttatcagctggtctcttaactgct
 M  K  T  I  A  Y  P  N  K  P  H  S  L  S  A  G  L  L  T  A
atagctattttagcctggcgagttcaaacattacttatgctgacgattaccaaaggaat
 I  A  I  F  S  L  A  S  S  N  I  T  Y  A  D  D  Y  Q  R  N
gctacggaagcttatgccaaagaagtaccacatcagatcacttctgtatggaccaaaggt
 A  T  E  A  Y  A  K  E  V  P  H  Q  I  T  S  V  W  T  K  G
gttacaccactaacacccgagcagtttcgatataataacgaagatgtgatccatgcgcca
```

```
 V  T  P  L  T  P  E  Q  F  R  Y  N  N  E  D  V  I  H  A  P
tatcttgctcatcaaggctggtacgatatcaccaaggccttcgatgggaaggataatctc
 Y  L  A  H  Q  G  W  Y  D  I  T  K  A  F  D  G  K  D  N  L
ttgtgtggcgcagcaacggcaggtaatatgctgcattggtggtttgatcaaaataaaaca
 L  C  G  A  A  T  A  G  N  M  L  H  W  W  F  D  Q  N  K  T
gagattgaagcctatttaagtaaacaccctgaaaagcaaaaaatcatttttaacaaccaa
 E  I  E  A  Y  L  S  K  H  P  E  K  Q  K  I  I  F  N  N  Q
gagctatttgatttgaaagctgctatcgataccaaggacagtcaaaccaatagtcagctt
 E  L  F  D  L  K  A  A  I  D  T  K  D  S  Q  T  N  S  Q  L
tttaattattttagagataaagcctttccaaatctatcagcacgtcaactcggggttatg
 F  N  Y  F  R  D  K  A  F  P  N  L  S  A  R  Q  L  G  V  M
cctgatcttgttctagacatgtttatcaatggttactacttaaatgtgtttaaaacacag
 P  D  L  V  L  D  M  F  I  N  G  Y  Y  L  N  V  F  K  T  Q
tctactgatgtcaatcgaccttatcaggacaaggacaaacgaggtggtattttcgatgct
 S  T  D  V  N  R  P  Y  Q  D  K  D  K  R  G  G  I  F  D  A
gttttcaccagaggagatcagacaacgctcttgacagctcgtcatgatttaaaaaataaa
 V  F  T  R  G  D  Q  T  T  L  L  T  A  R  H  D  L  K  N  K
ggactaaatgacatcagcaccattatcaagcaagaactgactgaaggaagagcccttgct
 G  L  N  D  I  S  T  I  I  K  Q  E  L  T  E  G  R  A  L  A
ttatcacatacctacgccaatgttagcattagccatgtgattaacttgtggggagctgat
 L  S  H  T  Y  A  N  V  S  I  S  H  V  I  N  L  W  G  A  D
tttaatgctgaaggaaaccttgaggccatctatgtcacagactcagatgctaatgcgtct
 F  N  A  E  G  N  L  E  A  I  Y  V  T  D  S  D  A  N  A  S
attggtatgaaaaaatattttgtcggcattaatgctcatagacatgtcgccatttctgcc
 I  G  M  K  K  Y  F  V  G  I  N  A  H  R  H  V  A  I  S  A
aagaaaatagaaggagaaaacattggcgctcaagtattaggcttatttacgctttccagt
 K  K  I  E  G  E  N  I  G  A  Q  V  L  G  L  F  T  L  S  S
ggcaaggacatatggcagaaactgagctaa
 G  K  D  I  W  Q  K  L  S  -
```

(11) SEQ ID NO: 11 and **SEQ ID NO: 21** are combined to show the amino acid sequence of the IdeZ protein **(SEQ ID NO: 11)** under the nucleotide sequence of the *ideZ* gene **(SEQ ID NO: 21).**

The nucleotide sequence of the *ideZ* gene (Genbank DQ826037) and the amino acid sequence of the IdeZ protein from *S. equi* subsp. *zooepidemicus* are shown.

```
atgaaaacaatagcttatccaaataaacctcactccttatcagctggtctcttaactgct
 M  K  T  I  A  Y  P  N  K  P  H  S  L  S  A  G  L  L  T  A
atagctatttttagcctggcgagttcaaacattacttatgctgacgattaccaaaggaat
 I  A  I  F  S  L  A  S  S  N  I  T  Y  A  D  D  Y  Q  R  N
gctgcggaagtttatgccaaagaagtaccacatcagatcacttctgtatggaccaaaggt
 A  A  E  V  Y  A  K  E  V  P  H  Q  I  T  S  V  W  T  K  G
gttacaccactaacacccgagcagtttcgatataataacgaagatgtgatccatgcgcca
 V  T  P  L  T  P  E  Q  F  R  Y  N  N  E  D  V  I  H  A  P
```

```
tatcttgctcatcaaggctggtacgatatcaccaaggtcttcgatgggaaggataatctc
 Y  L  A  H  Q  G  W  Y  D  I  T  K  V  F  D  G  K  D  N  L
ttgtgtggcgcagcaacggcaggtaatatgctgcattggtggtttgatcaaaataaaaca
 L  C  G  A  A  T  A  G  N  M  L  H  W  W  F  D  Q  N  K  T
gagattgaagcctatttaagtaaacaccctgaaaagcaaaaaatcatttttaacaaccaa
 E  I  E  A  Y  L  S  K  H  P  E  K  Q  K  I  I  F  N  N  Q
gagctatttgatttgaaagctgctatcgataccaaggacagtcaaaccaatagtcagctt
 E  L  F  D  L  K  A  A  I  D  T  K  D  S  Q  T  N  S  Q  L
tttaattattttagagataaagcctttccaaatctatcagcacgtcaactcggggttatg
 F  N  Y  F  R  D  K  A  F  P  N  L  S  A  R  Q  L  G  V  M
cctgatcttgttctagacatgtttatcaatggttactacttaaatgtgtttaaaacacag
 P  D  L  V  L  D  M  F  I  N  G  Y  Y  L  N  V  F  K  T  Q
tctactgatgtcaatcgaccttatcaggacaaggacaaacgaggtggtattttcgatgct
 S  T  D  V  N  R  P  Y  Q  D  K  D  K  R  G  G  I  F  D  A
gttttcaccagaggagatcagacaacgctcttgacagctcgtcatgatttaaaaaataaa
 V  F  T  R  G  D  Q  T  T  L  L  T  A  R  H  D  L  K  N  K
ggactaaatgacatcagcaccattatcaagcaggaactgactgaaggaagagcccttgct
 G  L  N  D  I  S  T  I  I  K  Q  E  L  T  E  G  R  A  L  A
ttatcacatacctacgccaatgttagcattagccatgtgattaacttgtggggagctgat
 L  S  H  T  Y  A  N  V  S  I  S  H  V  I  N  L  W  G  A  D
tttaatgctgaaggaaaccttgaggccatctatgtcacagactcagatgctaatgcgtct
 F  N  A  E  G  N  L  E  A  I  Y  V  T  D  S  D  A  N  A  S
attggtatgaaaaaatattttgtcggcattaatgctcatggacatgtcgccatttctgcc
 I  G  M  K  K  Y  F  V  G  I  N  A  H  G  H  V  A  I  S  A
aagaaaatagaaggagaaaacattggcgctcaagtattaggcttatttacgctttccagt
 K  K  I  E  G  E  N  I  G  A  Q  V  L  G  L  F  T  L  S  S
ggcaaggacatatggcagaaactgagctaa
 G  K  D  I  W  Q  K  L  S  -
```

(12) SEQ ID NO: 12

Nucleotide sequence of the *eag* gene

```
    1 aaataattttgtttaactttaagaaggagatataaccatggctctagatg
   51 ctacaacggtgttagagcctacaacagccttcattagagaagctgttagg
  101 gaaatcaatcagctgagtgatgactacgctgacaatcaagagcttcaggc
  151 tgttcttgctaatgctggagttgaggcacttgctgcagatactgttgatc
  201 aggctaaagcagctcttgacaaagcaaaggcagctgttgctggtgttcag
  251 cttgatgaagcaagacgtgaggcttacagaacaatcaatgccttaagtga
  301 tcagcacaaaagcgatcaaaaggttcagctagctctagttgctgcagcag
  351 ctaaggtggcagatgctgcttcagttgatcaagtgaatgcagccattaat
  401 gatgctcatacagctattgcggacattacaggagcagccttgttggaggc
  451 taaagaagctgctatcaatgaactaaagcagtatggcattagtgattact
  501 atgtgaccttaatcaacaaagccaaaactgttgaaggtgtcaatgcgctt
  551 aaggcaaagattttatcagctctaccgtagctcgagccgggtgctttgc
```

(13) SEQ ID NO: 13

Amino acid sequence of the EAG4B protein

```
1   MALDATTVLE PTTAFIREAV REINQLSDDY ADNQELQAVL ANAGVEALAA DTVDQAKAAL
61  DKAKAAVAGV QLDEARREAY RTINALSDQH KSDQKVQLAL VAAAAKVADA ASVDQVNAAI
121 NDAHTAIADI TGAALLEAKE AAINELKQYG ISDYYVTLIN KAKTVEGVNA LKAKILSALP
```

(14) SEQ ID NO: 28

Protein sequence of SEC2.16 (CNE)

```
Met Ala Thr Asn Leu Ser Asp Asn Ile Thr Ser Leu Thr Val Ala Ser
1               5                  10                  15

Ser Ser Leu Arg Asp Gly Glu Arg Thr Thr Val Lys Val Ala Phe Asp
            20                  25                  30

Asp Lys Lys Gln Lys Ile Lys Ala Gly Asp Thr Ile Glu Val Thr Trp
        35                  40                  45

Pro Thr Ser Gly Asn Val Tyr Ile Gln Gly Phe Asn Lys Thr Ile Pro
    50                  55                  60

Leu Asn Ile Arg Gly Val Asp Val Gly Thr Leu Glu Val Thr Leu Asp
65                  70                  75                  80

Lys Ala Val Phe Thr Phe Asn Gln Asn Ile Glu Thr Met His Asp Val
                85                  90                  95

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

Thr Ala Glu Thr Ser Gly Thr Thr Thr Val Lys Val Gly Asn Arg Thr
        115                 120                 125

Ala Thr Ile Thr Val Thr Lys Pro Glu Ala Gly Thr Gly Thr Ser Ser
    130                 135                 140

Phe Tyr Tyr Lys Thr Gly Asp Ile Gln Pro Asn Asp Thr Glu Arg Val
145                 150                 155                 160
```

Arg Trp Phe Leu Leu Ile Asn Asn Asn Lys Glu Trp Val Ala Asn Thr
165 170 175

Val Thr Val Glu Asp Asp Ile Gln Gly Gly Gln Thr Leu Asp Met Ser
180 185 190

Ser Phe Asp Ile Thr Val Ser Gly Tyr Arg Asn Glu Arg Phe Val Gly
195 200 205

Glu Asn Ala Leu Thr Glu Phe His Thr Thr Phe Pro Asn Ser Val Ile
210 215 220

Thr Ala Thr Asp Asn His Ile Ser Val Arg Leu Asp Gln Tyr Asp Ala
225 230 235 240

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

Asp Gln Lys Glu Phe Ala Asn Asn Ser Lys Ile Trp Tyr Gln Ile Leu
260 265 270

Tyr Lys Asp Gln Val Ser Gly Gln Glu Ser Asn His Gln Val Ala Asn
275 280 285

Ile Asn Ala Asn Gly Gly Val Asp Gly Ser Arg Tyr Thr Ser Phe Thr
290 295 300

Val Lys Lys Ile Trp Asn Asp Lys Glu Asn Gln Asp Gly Lys Arg Pro
305 310 315 320

Lys Thr Ile Thr Val Gln Leu Tyr Ala Asn Asp Gln Lys Val Asn Asp
325 330 335

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

Lys Leu Asp Lys Tyr Asp Ser Gln Asn Gln Lys Ile Thr Tyr Ser Val
355 360 365

Lys Glu Val Met Val Pro Val Gly Tyr Gln Ser Gln Val Glu Gly Asp
370 375 380

```
Ser Gly Val Gly Phe Thr Ile Thr Asn Thr Tyr Thr Pro Glu Val Ile
385                 390                 395                 400

Ser Ile Thr Gly Gln Lys Thr Trp Asp Asp Arg Glu Asn Gln Asp Gly
                405                 410                 415

Lys Arg Pro Lys Glu Ile Thr Val Arg Leu Leu Ala Asn Asp Ala Ala
            420                 425                 430

Thr Asp Lys Val Ala Thr Ala Ser Glu Gln Thr Gly Trp Lys Tyr Thr
        435                 440                 445

Phe Thr Asn Leu Pro Lys Tyr Lys Asp Gly Lys Gln Ile Thr Tyr Thr
    450                 455                 460

Ile Gln Glu Asp Pro Val Ala Asp Tyr Thr Thr Thr Ile Gln Gly Phe
465                 470                 475                 480

Asp Ile Thr Asn His His Glu Val Ala Leu Thr Ser Leu Lys Val Ile
                485                 490                 495

Lys Val Trp Asn Asp Lys Asp Asp Tyr Tyr His Lys Arg Pro Lys Glu
            500                 505                 510

Ile Thr Ile Leu Leu Lys Ala Asp Gly Lys Val Ile Arg Glu His Gln
        515                 520                 525

Met Thr Pro Asp Gln Gln Gly Lys Trp Glu Tyr Thr Phe Asp Gln Leu
    530                 535                 540

Pro Val Tyr Gln Ala Gly Lys Lys Ile Ser Tyr Ser Ile Glu Glu Lys
545                 550                 555                 560

Gln Val Ala Gly Tyr Gln Ala Pro Val Tyr Glu Val Asp Glu Gly Leu
                565                 570                 575

Lys Gln Val Thr Val Thr Asn Thr Leu Asn Pro Ser Tyr Lys Leu Glu
            580                 585                 590

Pro Gly
```

(15) SEQ ID NO 29

Protein sequence of SclC

```
Met Thr Asn Lys Thr Lys Arg Thr Gly Leu Val Arg Lys Tyr Gly Ala
1               5                  10                  15

Cys Ser Ala Ala Ile Ala Leu Ala Ala Leu Ala Ser Leu Gly Ala Gly
            20                  25                  30

Lys Ala Val Lys Ala Asp Gln Pro Ala Ala Leu Lys Tyr Pro Glu Pro
        35                  40                  45

Arg Asp Tyr Phe Leu His Thr Arg Glu Gly Asp Val Ile Tyr Asp Glu
    50                  55                  60

Asp Ile Lys Arg Tyr Phe Glu Asp Leu Glu Ala Tyr Leu Thr Ala Arg
65                  70                  75                  80

Leu Gly Gly Ile Asp Lys Lys Val Glu Glu Ala Ala Gln Lys Pro Gly
                85                  90                  95

Ile Pro Gly Pro Thr Gly Pro Gln Gly Pro Lys Gly Asp Lys Gly Asp
            100                 105                 110

Pro Gly Ala Pro Gly Glu Arg Gly Pro Ala Gly Pro Lys Gly Asp Thr
        115                 120                 125

Gly Glu Ala Gly Pro Arg Gly Glu Gln Gly Pro Ala Gly Gln Ala Gly
    130                 135                 140

Glu Arg Gly Pro Lys Gly Asp Pro Gly Ala Pro Gly Pro Lys Gly Glu
145                 150                 155                 160

Lys Gly Asp Thr Gly Ala Val Gly Pro Lys Gly Glu Lys Gly Asp Thr
                165                 170                 175

Gly Ala Thr Gly Pro Lys Gly Asp Lys Gly Glu Arg Gly Glu Lys Gly
            180                 185                 190

Glu Gln Gly Gln Arg Gly Glu Lys Gly Glu Gln Gly Gln Arg Gly Glu
        195                 200                 205
```

```
Lys Gly Glu Gln Lys Pro Lys Gly Asp Gln Gly Lys Asp Thr Lys Pro
    210                 215                 220

Ser Ala Pro Lys Ala Pro Glu Lys Ala Pro Ala Pro Lys Ala Pro Lys
225                 230                 235                 240

Ala Ser Glu Gln Ser Ser Asn Pro Lys Ala Pro Ala Pro Lys Ser Ala
                245                 250                 255

Pro Ser Lys Ser Ala Ala Pro Thr Gly Gln Lys Ala Ala Leu Pro Ala
            260                 265                 270

Thr Gly Glu Ile Asn His Pro Phe Phe Thr Leu Ala Ala Leu Ser Val
        275                 280                 285

Ile Ala Ser Val Gly Val Leu Thr Leu Lys Gly Lys Lys Asp
    290                 295                 300
```

(16) SEQ ID NO 30. Recombinant protein IdeE

GPLGSDDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFRYNNEDVIHAPYLA
HQGWYDITKAFDGKDNLLCGAATAGNMLHWWFDQNKTEIEAYLSKHPEKQKII
FNNQELF
DLKAAIDTKDSQTNSQLFNYFRDKAFPNLSARQLGVMPDLVLDMFINGYYLNVF
KTQSTD
VNRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIIKQELTEGRA
LALSH
TYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHRHVAI
SAKKI
EGENIGAQVLGLFTLSSGKDIWQKLS

Amino acids in bold originates from the vector.

Example 10. Vaccination study

The objective of this study was to determine the level of protection conferred on vaccination with Intervacc's new multi-component subunit vaccine following intranasal challenge with wild type *S. equi* strain 4047 in Welsh Mountain ponies. The study has been performed by Animal Health Trust, UK. The vaccines used therein, which are designated Nordostrep Septavac or Nordostrep Pentavac A (or only Septavac or Pentavac) are disclosed below.

Methods

The ponies were initially randomised into 3 groups for the vaccination period.

Table 1: Vaccination groups.

Group	Vaccine	No per group	Route
1	Nordostrep Septavac	7	IN + SC
2	Nordostrep Pentavac A	7	IN + SC
3	Placebo	7	IN + SC

In the first trial groups 1 and 3 were taken through to challenge. (The challenge of second trial group 2 (Pentavac A) is described in section 9). The decision as to which vaccine group to challenge was taken by Intervacc one week prior to challenge.

The Nordostrep Pentavac A formulation

The Pentavac vaccine consisted of the following five *S. equi* recombinant proteins: EAG, SclC, CNE, Eq5 and Eq8. For subcutaneous vaccination, the five proteins were mixed in PBS (50 µg/ml of the respective protein), divided in aliquots of 1 ml in vials and stored at -20° C. Immediately before vaccination, the vial was thawed and mixed with 1ml adjuvant (Abisco 200, 375 µg/dose, Isconova AB, Sweden). For intranasal vaccination the five proteins were mixed in PBS (150 µg/ml of respective protein) and divided in aliquots of 2 ml in vials and stored at -20°C. Immediately before vaccination the vial was thawed and mixed with 2 ml adjuvant (Abisco 300, 500 µg/dose, Isconova AB, Sweden). In the placebo formulations the *S. equi* proteins were omitted. Thus, the placebo for subcutaneous vaccination only contained PBS and Abisco 200, 375 µg/dose and for intranasal vaccination, the placebo contained only PBS and Abisco 300, 500 µg/dose.

The Nordostrep Septavac formulation

The Septavac vaccine consisted of the following seven *S. equi* recombinant proteins: EAG, SclC, CNE, Eq5, Eq8, IdeE and IdeE2. For subcutaneous vaccination, the seven

proteins were mixed in PBS (50 µg/ml of respective protein) and divided in aliquots of 1 ml in vials and stored at -20°C. Immediately before vaccination the vial was thawed and mixed with 1ml adjuvant (Abisco 200, 375 µg/dose, Isconova AB, Sweden). For intranasal vaccination, the seven proteins were mixed in PBS (150 µg/ml of the respective protein) and divided in aliquots of 2 ml in vials and stored at -20°C. Immediately before vaccination, the vial was thawed and mixed with 2 ml adjuvant (Abisco 300, 500 µg/dose, Isconova AB, Sweden). In the placebo formulations, the *S. equi* proteins were omitted. Thus, the placebo for subcutaneous vaccination only contained PBS and Abisco 200, 375 µg/dose, and for intranasal vaccination, it only contained PBS and Abisco 300, 500 µg/dose.

In these formulations, EAG is comprised of the fragment EAG4B and CNE is the fragment designated 2.16.

SHORT SUMMARY OF RESULTS

This study evaluated the efficacy of a new multi-component subunit vaccine for the prevention of strangles. The Septavac vaccine induced pyrexia in ponies for one day after first and second vaccinations. However, there were no other adverse reactions and this vaccine appears to be very well tolerated.

All ponies were challenged with an identical dose of $1x10^8$ cfu of *S. equi* strain 4047, which was split and administered via both nostrils. All seven control ponies developed pyrexia and multiple lymph node abscesses (100%). Only one vaccinated pony developed pyrexia (which could have been due to an ongoing *S. zooepidemicus* infection) and only one developed lymph node abscesses (14%). Statistically, vaccinated ponies were significantly protected from *S. equi* as measured by temperature, post mortem score, and fibrinogen and neutrophil levels.

Overall, the Septavac vaccine was a safe and effective vaccine for the prevention of strangles. However, the invention is not restricted to the Septavac and Pentavac vaccines which have been studied in this Example but many combinations of the present antigens/immunogens are possible candidates for use in vaccine compositions for prevention of strangles.

1 Procedure

Two earlier studies (WO 2004/032957 A1 and ref. 27) demonstrated that Intervacc vaccines conferred some protection against *S. equi* challenge. All four

vaccinated groups across the two studies showed reduced guttural pouch empyema. The present study was designed to compare the immunogenicity of two Nordvacc vaccines: one containing five (Pentavac) and one containing seven (Septavac) *S. equi* proteins.

Blood and nasal wash samples were taken according to the protocol to determine the equine immune responses to the vaccine subunits. Based on immunogenicity data, one vaccinated group was challenged to quantify the level of protection conferred.

Each pony was challenged with a total challenge dose of $1x10^8$ cfu of *S. equi* strain 4047 administered via the spraying of a 2ml culture containing $5x10^7$ cfu into both nostrils. This dose regime is believed to optimise the infection rate whilst avoiding overwhelming the host immune response.

Ponies were carefully monitored for the onset of clinical signs of disease over a period of three weeks post challenge by regular checks, daily physical examination, monitoring of body temperature, the taking of sera to determine seroconversion and the taking of nasal washes for bacteriological analysis. All ponies were subjected to post mortem examination following abscessation or reaching the study endpoint at 3 weeks post challenge to determine the severity of disease pathology according to a scoring system developed at the AHT. Histopathological examination of tissues recovered from the study ponies was used to identify early signs of *S. equi* infection that were not obvious on post mortem (PM) examination.

Table 2: Sampling Schedule

Day of study	Day of week	date	procedure	Volume of sera to be taken	Sample/Analysis/comment
day -10	Thurs	31/01/2008	veterinary examination		
day 1	Mon	11/02/2008	Obs/temps, NW, BL	40 ml normal, 20 ml EDTA	IgG and CFU from NW; ELISA for IgG from sera
day 2	Tues	12/02/2008	Obs/temps		
day 3	Wed	13/02/2008	Obs/temps		
day 4	Thurs	14/02/2008	Obs/temps, V1		7 contr and 7 vaccinated
day 5 to 18		15/02/2008 to 28/02/2008	Obs/temps		
day 50	Mon	31/03/2008	Obs/temps, NW, BL	20ml normal	IgG and CFU from NW; ELISA for IgG from sera
day 51	Tues	01/04/2008	Obs/temps		
day 52 to 59		02/04/2008 to 09/04/2008			
day 60	Wed	10/04/2008	Obs/temps		
day 61 to 68	Thurs	11/04/2008 to 18/04/2008	Obs/temps, V2		7 contr and 7 vaccinated
day 71	Fri	21/04/2008	Obs/temps		
day 72	Mon	22/04/2008	Obs/temps, NW, BL	20ml normal	IgG and CFU from NW; ELISA for IgG from sera
day 73	Tues	23/04/2008	Obs/temps		
day 74	Wed	24/04/2008	Obs/temps		
day 75 to 81	Thurs	25/04/2008 to 2/05/2008	Obs/temps, V3		7 contr and 7 vaccinated
day 86	Fri	06/05/2008	Obs/temps		
day 87	Tues	07/05/2008	Obs/temps, NW, BL	40 ml normal, 20 ml EDTA	IgG and CFU from NW; ELISA for IgG from sera; fibrinogen and neutrophil levels to be quantified Move to Allen Centre
day 88	Wed	08/05/2008	Obs/temps		
day 89	Thurs	09/05/2008	Obs/temps, Challange		
day 90	Fri	10/05/2008	Obs/temps, NW, BL	20 ml normal, 10 ml EDTA	IgG and CFU from NW; ELISA for IgG from sera; fibrinogen and neutrophil levels to be quantified
day 91	Sat	11/05/2008	Obs/temps		
	Sun		Obs/temps		

day 92	Mon	12/05/2008	Obs/temps, NW, BL	20 ml normal, 10 ml EDTA	IgG and CFU from NW; ELISA for IgG from sera; fibrinogen and neutrophil levels to be quantified
day 93	Tues	13/05/2008	Obs/temps		
day 94	Wed	14/05/2008	Obs/temps, NW, BL	20 ml normal, 10 ml EDTA	IgG and CFU from NW; ELISA for IgG from sera; fibrinogen and neutrophil levels to be quantified
day 95	Thurs	15/05/2008	Obs/temps		
day 96	Fri	16/05/2008	Obs/temps, NW, BL	20 ml normal, 10 ml EDTA	IgG and CFU from NW; ELISA for IgG from sera; fibrinogen and neutrophil levels to be quantified
day 97	Sat	17/05/2008	Obs/temps		
day 98	Sun	18/05/2008	Obs/temps		
day 99	Mon	19/05/2008	Obs/temps, NW, BL	20 ml normal, 10 ml EDTA	IgG and CFU from NW; ELISA for IgG from sera; fibrinogen and neutrophil levels to be quantified
day 100	Tues	20/05/2008	Obs/temps		
day 101	Wed	21/05/2008	Obs/temps, NW, BL	20 ml normal, 10 ml EDTA	IgG and CFU from NW; ELISA for IgG from sera; fibrinogen and neutrophil levels to be quantified
day 102	Thurs	22/05/2008	Obs/temps		
day 103	Fri	23/05/2008	Obs/temps, NW, BL	20 ml normal, 10 ml EDTA	IgG and CFU from NW; ELISA for IgG from sera; fibrinogen and neutrophil levels to be quantified
day 104	Sat	24/05/2008	Obs/temps		
day 105	Sun	25/05/2008	Obs/temps		
day 106	Mon	26/05/2008	Obs/temps, NW, BL	30ml	IgG and CFU from NW; ELISA for IgG from sera; fibrinogen and neutrophil levels to be quantified
day 107	Tues	27/05/2008	Obs/temps		
day 108	Wed	28/05/2008	Obs/temps, NW, BL	30ml	IgG and CFU from NW; ELISA for IgG from sera; fibrinogen and neutrophil levels to be quantified
day 109	Thurs	29/05/2008	Obs/temps		

Comments:
NW = Nasal washings
BL = Blood sample
1. Nasal immunisation in both nostrils at all three occasions (2x 2 ml in each nostril = 4 ml/vaccination)
2. Subcutaneous immunisation near submandibular lymph nodes (1 ml)
3. Two groups of seven (7) horses will be vaccinated and seven (7) unvaccinated/controls
4. IgG in sera analysed by Intervacc AS; IgG in NW by AHT (7 antigens)

2.1 Vaccine

Nordostrep vaccines for horses

Group 1: 7 ponies vaccinated with Nordostrep Pentavac A

2ml subcutaneous injection (1ml on each side of the head)

4ml intranasal injection (2ml in each nostril)

Day 4; 60; 74

Group 2: 7 ponies vaccinated with Nordostrep Septavac

2ml subcutaneous injection (1ml on each side of the head)

4ml intranasal injection (2ml in each nostril)

Day 4; 60; 74

Group 3: 7 ponies vaccinated with Placebo

2ml subcutaneous injection (1ml on each side of the head)

4ml intranasal injection (2ml in each nostril)

Day 4; 60; 74

The vaccine vials were received by the AHT prior to the first vaccination and stored at -20°C until use in freezer number EQ No. 2305. Placebo (containing no antigens) and adjuvant vials were stored at 4°C until use in fridge number EQ No. 44.

At the time of vaccination, vaccines and adjuvants were mixed as stated in the protocol in situ by A Waller, L Prowse or C Robinson at AHT.

2.2 Challenge bacterium

S. equi 4047 was prepared from fresh plates as described in SOP/BACT/25.

The bacteria grew as expected and the 1:40 diluted culture was harvested when the OD_{600nm} reached 0.3. The growth of the challenge inoculum is shown in Figure 6. The following results were obtained.

Plating results: $1/10^5$ dilution

37 colonies
35 colonies
33 colonies
32 colonies

Mean = 34.25 in 100µl
Therefore actual dose per pony = $4x34.25x10^5x10$
= $1.37x10^8$ cfu/dose

3 ANIMAL MANAGEMENT

3.1 Supply

Twenty one Welsh Mountain ponies originally supplied by Mr Beedles, Shropshire, UK, were used. Ponies were approximately 8 months of age at the time of the first vaccination.

3.2 Identification/allocation

Ponies were identified by a microchip in the neck. The 21 ponies were randomly assigned to vaccination groups (Table 3).

Table 3: Vaccination groups and pony chip IDs

Group	Vaccine	Pony Chip ID's
1	Septavac	00012, 00159, 00833, 00976, 99123, 99668, 99794
2	Pentavac	01298, 01605, 01724, 99223, 99229, 99773, 99919
3	Placebo	00173, 00427, 01635, 02078, 99549, 99776, 99886

3.3 Husbandry

Prior to challenge, ponies were kept at pasture on grass at Lanwades Park, Kentford, UK and Kirtling, Newmarket, UK. These sites have been approved by the Home Office for this type of work. Drinking water was available *ad libitum.*

Ponies in groups 1 and 3 were transferred to the ACVS (Allen Centre), three days prior to challenge to allow acclimatisation. Ponies were separated into two animal rooms according to their vaccination groups, so that ponies from each vaccination group were kept together.

4 METHODS

4.1 Vaccination

Vaccinations were given by subcutaneous injection near the retropharyngeal lymph nodes according to AHT SOP/EQU/03 or via intranasal spray according to AHT SOP/EQU/07.

4.1.2 Preliminary clinical examination

A veterinarian clinically examined all ponies before the first vaccination, before V2 (due to *S. zoo* infection) and before V3. Only healthy ponies in good clinical condition were included in the study (SOP/EQU/08).

4.1.3 Vaccination

Ponies received vaccinations according to Table 4. With the exception that pony 9229 was pyrexic on 14/02/08 due to an ongoing *S. zooepidemicus* infection. This pony recovered over the weekend and was vaccinated on 18/04/08.

Table 4: Vaccination routes and dates

Group	Vaccine	V1	V2*	V3*
A	Septavac	14/2/08	10/4/08	24/4/08
B	Pentavac	14/2/08	10/4/08	24/4/08
C	Placebo	14/2/08	10/4/08	24/4/08

*Delayed by 7 days due to *S. zooepidemicus* infection.

4.1.4 Clinical observations around vaccinations

Clinical observations were performed daily after vaccination. If adverse reactions occurred, then additional checks were made as required.

4.2 Experimental challenge with *S. equi* 4047

4.2.1 Preliminary clinical examination

Prior to transfer to the ACVS, a veterinarian clinically examined the challenge ponies. Only healthy ponies in good clinical condition were subjected to the challenge.

4.2.2 Challenge

Two weeks after the third vaccination (08/05/08), each pony was challenged by intranasal administration of 2ml of a fresh *S. equi* 4047 culture into both nostrils using

a flexible tube and spray nozzle according to AHT SOP/BACT32. Such a challenge dose was predicted to contain a total of $1x10^8$ cfu of *S. equi* 4047.

No problems were encountered during the administration of the challenge dose. Spare inocula were used to quantify the actual challenge dose administered, which was found to be $1.37x10^8$ cfu/dose.

4.3 Post challenge monitoring

4.3.1 Clinical examination

Ponies were examined according to AHT SOP/EQU/02. Each pony was examined clinically on the day of challenge, and on each of the following 21 days for the occurrence of symptoms associated with *S. equi* infection (demeanor, nasal discharge, lymph node swelling and abscessation, signs of coughing, difficulty swallowing and feeding, and ocular signs).

4.3.2 Rectal temperatures

Individual rectal temperatures were taken at around 9.00am from the day of challenge through to day 21 after challenge.

4.4 Blood sampling

Blood samples were taken from the jugular vein according to AHT SOP/EQU/01 and according to the study protocol schedule. Serum was prepared according to AHT SOP/EQU/01 and stored frozen at -20°C or below until use.

4.5 Processing of blood samples

Processing of blood samples was carried out by Leah Prowse under the responsibility of Andrew Waller at the Animal Health Trust.

4.6 Processing of nasal wash samples

Individual nasal washes were taken according to AHT SOP/EQU/02 as stated in the study protocol schedule.

A 500µl sample of the nasal wash was added to 500µl of Todd-Hewitt Broth in situ at the time of sampling for transportation to the lab to allow quantification of the number of β-haemolytic streptococci per ml according to AHT SOP/BACT/02. The remaining nasal wash sample was centrifuged and the supernatant decanted into a clean 5 ml polypropylene tube and stored at

-70°C until use for quantification of mucosal antibodies.

4.7 Post mortem examination

Provision was made for a complete post mortem examination to be carried out by the Animal Health Trust on all ponies following euthanasia as a result of abscessation or on reaching the study end point 21 days post challenge.

Tissue samples were preserved in phosphate buffered formalin and subjected to microscopic examination according to standard techniques and provision of a full and formal report. Tissue swabs were taken and the results recorded and used to evaluate the level of *S. equi* infection. Charcoal swabs were taken from each of the areas as stated in the protocol and processed on COBA Streptococcal selective plates to determine the presence of *S. equi*.

Strangles pathology was scored using the system in Table 5.

Table 5: Pathology scoring system

Pathology	Score
Retropharyngeal or submandibular lymph node abscess:	15
Retropharyngeal or submandibular lymph node microabscess:	10
Empyaema of guttural pouch:	5
Scarring of guttural pouch:	5
Enlarged lymph node:	1
Follicular hyperplasia of guttural pouch:	1

4.8 Histopathological examination

Tissue samples taken from ponies at post mortem examination were fixed in formalin, cut into sections and sent to Professor Ken Smith at the Royal Veterinary College for analysis. Professor Smith prepared a report for the samples from each pony and his observations were scored according to Table 6.

Table 6: Histopathology scoring system

Histopathology	Score
Rhinitis	1
Lymphadenitis	1
Pharyngitis	1
Lymph node abscessation	5
Guttural pouch empyema	5

DEVIATIONS

The study was performed in accordance with the study protocol no. 08.C001.P and subsequent amendments, with the following deviations from the agreed study protocol:

- Pony 9229 was pyrexic on 14/02/08 due to an ongoing S. zooepidemicus infection. This pony recovered over the weekend and was vaccinated on 18/04/08.
- Date of V2 delayed 7 days due to S. zoo infection in 45% of ponies. This had a knock on effect on V3 and challenge which were also delayed 7 days.
- A delay of one day occurred on sampling ponies due to staff shortages. Ponies due to be sampled on day 85 were actually sampled on day 86.
- 20 ml of EDTA blood was taken on day 86 instead of 10 ml to enable purification of the ponies' DNA for archiving.
- Nordvacc decided to retain the unchallenged Pentavac group (2) for a 6-month period to monitor the duration of antibody response.

6 FATE OF PONIES AT THE END OF THE STUDY

All ponies in groups 1 and 3 were euthanased and subjected to post mortem examination. Ponies in group 2 were retained for 6 months to monitor the duration of antibody responses.

7 ARCHIVING

The raw data have been archived by Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU.

8 SUMMARY OF RESULTS

8.1 Responses following the first and second vaccinations

8.1.1 Clinical Responses

All ponies responded well to first vaccination. No injection site reactions were observed in any of the groups. However, a rise in rectal temperature was observed in the vaccinated groups (Figure 7). This was most pronounced in the Septavac group with 4 of 7 ponies developing pyrexia (temperature >38.9°C) one day post V1 and 5 of 7 ponies developing pyrexia one day post V2. In comparison, 2 of 7 ponies of the Pentavac group and none of the controls were pyrexic post V1, and 3 ponies of the Pentavac group and no controls were pyrexic post V2. Interestingly, only 1 Septavac, 2 Pentavac and 1 control pony developed pyrexia post V3. This could be due to the high level of antibodies induced post V2, which may have neutralized the antigens in the vaccine more effectively.

There were no obvious differences in nasal score (Figure 8), lymph node score (Figure 9) or *S. zooepidemicus* counts (Figure 10) between the study groups during the vaccination phase, with the exception of some ponies that had ongoing *S. zooepidemicus* infections typical of ponies of this age. This resulted in a rise in mean rectal temperature around the original date for V2 (03/04/08) as demonstrated in Figure 7. Ponies were allowed to recover from this *S. zooepidemicus* infection and all ponies were vaccinated on 10/04/08.

8.2 Responses following challenge

The preparation and conduct of both challenges went extremely well and all ponies received the required dose of *S. equi* without incident on the 8/5/08.

Earliest onset of pyrexia was at day 4 post challenge in control pony 2078. Two more ponies developed pyrexia on day 5, another on day 6 and 7 and the final control pony developed pyrexia on day 10 (Figure 11). The mean number of days that control ponies were pyrexic was 4.2 days compared with 0.7 days for vaccinated ponies (Table 7). However, it should be noted that control ponies were euthanased on welfare grounds from day 8 post challenge and all control ponies had been euthanased by day 13 post challenge. This has had a knock on effect on the mean temperatures, observation scores, fibrinogen and neutrophil levels and observation scores for control ponies, which decline as ponies succumbing to *S. equi* infection were euthanased.

Overall, there was a significant difference in the mean temperatures of the two groups from day 5 to day 11 post challenge (Figure 11). Of the Septavac ponies only pony 0976 developed pyrexia on day 8 (Table 7). However, this may have been due to the ongoing *S. zooepidemicus* infection that was evident in this pony.

Fibrinogen levels were significantly different between the two study groups on days 6, 8 and 11 post challenge (Figure 12). All controls developed elevated fibrinogen levels, but only 2 vaccinates (ponies 0976 and 9794) had higher levels.

Neutrophil levels were also significantly different between the two study groups on days 6, 8 and 11 post challenge (Figure 13). All controls developed elevated neutrophil levels, but only 1 vaccinate (pony 9794) had higher levels.

There was an increased level of submandibular lymph node swelling in control ponies, although this did not appear to be statistically significant (Figure 14). There were no differences in nasal discharge (Figure 15) or *S. zooepidemicus* counts (Figure 16) between the study groups.

On post mortem examination, all controls were found to have multiple lymph node abscesses, while only one vaccinated pony, 9794, was found to have lymph node abscesses

(Tables 8 and 9). Overall the mean pathology score for controls and 11.7, respectively indicating that a significant level of protection had been induced by the Septavac vaccine (Figure 17). *S. equi* was isolated from the lymph nodes of all control ponies, but only 2 vaccinates (0976 and 9794) (Table 10). These findings were strengthened by histopathological examination, which confirmed that only one Septavac pony had developed abscesses in at least two of their lymph nodes (Table 11 and Figure 18).

Furthermore, the IgG levels in nasal washings and serum samples of the septavac group were measured using ELISA (Figs. 19 and 20) showing that the antigens generate mucosal and serum antibodies.

Table 7: Number of days pyrexic after challenge

Group	Pony ID	Number of days
Septavac	0012	0
Septavac	0159	0
Septavac	0833	0
Septavac	0976	5
Septavac	9123	0
Septavac	9668	0
Septavac	9794	0
Control	0173	2
Control	0427	4
Control	1635	5
Control	2078	4
Control	9549	4
Control	9776	5
Control	9886	6

Mean Septavac = 0.7 days

Mean control = 4.2 days*

* All control ponies were euthanased by day 13 post-challenge, but most would have continued to have elevated temperatures had they not been euthanased on welfare grounds.

Table 8: Post Mortem Analysis after Challenge

Group	Pony ID	Total PM score
Septavac	0012	6
Septavac	0159	3
Septavac	0833	5
Septavac	0976	6
Septavac	9123	4
Septavac	9668	1
Septavac	9794	57
Control	0173	42
Control	0427	53
Control	1635	66
Control	2078	49
Control	9549	57
Control	9776	43
Control	9886	42

Table 9: Number and Location of Abscesses on Post Morte

Group	Pony ID	SMLN		RPLN	
		L	R	L	R
Septavac	0012	-	-	-	-
Septavac	0159	-	-	-	-
Septavac	0833	-	-	-	-
Septavac	0976	-	-	-	-
Septavac	9123	-	-	-	-
Septavac	9668	-	-	-	-
Septavac	9794	-	✓	✓	✓
Control	0173	-	-	✓	✓
Control	0427	✓	-	✓	✓
Control	1635	✓	✓	✓	✓
Control	2078	-	✓	✓	✓
Control	9549	✓	-	✓	✓
Control	9776	-	-	✓	✓
Control	9886	-	-	✓	✓

SMLN – Submandibular Lymph Node

RPLN – Retropharyngeal Lymph Node

✓ = abscess

Table 10: *S.equi* Counts Found in the Lymph Nodes on Post Mortem

Pony ID	SMLN		RPLN		Cervical LN	Tracheal /Broncheal LN	*S.equi* Confirmed by sugar test
	L	R	L	R			
0012	-	-	-	-	-	-	-
0159	-	-	-	-	-	-	-
0833	-	-	-	-	-	-	-
0976	-	Sparse	-	-	-	-	Yes
9123	-	-	-	-	-	-	-
9668	-	-	-	-	-	-	-
9794	-	Con	Con	Con	-	-	Yes
0173	Sparse	Sparse	Con	Con	-	-	Yes
0427	Con	Con	Con	Con	-	-	Yes
1635	Con	Con	Con	Con	-	-	Yes
2078	Con	Con	Con	Con	Con	-	Yes
9549	Con	Con	Con	Con	-	-	Yes
9776	Sparse	Sparse	Con	Con	-	-	Yes
9886	-	Sparse	Con	Con	-	-	Yes

Ponies 0833 and 0159 showed sparse *S.equi* in areas other than the lymph node. Ponies 0012, 9123 and 9668 showed no *S.equi.*

Con – confluent

SMLN – Submandibular Lymph Node

RPLN – Retropharyngeal Lymph Node

Table 11: Histopathology Scores

Pony Chip ID	0012	0159	0833	0976	9123	9668	9794	0173	0427	1635	2078	9549	9776	9886
Identity	Sep	Sep	Sep	Sep	Sep	Sep	Sep	Con	Con	Con	Con	Con	Con	Con
Nasal turbinate	0	1	1	0	0	0	0	0	0	0	0	0	1	0
Nasopharynx	0	0	0	1	0	0	0	0	0	0	0	0	0	0
SMLN – L	0	0	0	0	0	0	0	0	5	5	0	5	0	0
SMLN – R	0	0	0	0	0	0	5	0	5	5	5	0	0	0
RPLN – L	0	0	0	1	0	0	5	5	5	5	5	5	5	5
RPLN – R	0	0	0	1	0	0	5	5	5	5	5	5	5	5
Gut pouch – L	5	5	0	0	0	0	5	5	5	5	5	5	5	5
Gut pouch - R	5	0	0	0	0	0	5	5	5	5	5	5	5	5
Lung	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brain	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	10	6	1	3	0	0	25	20	30	30	25	25	21	20

Rhinitis: 1 Pharyngitis: 1 Lymphadenitis: 1 Lymph node abscessation: 5
Guttural pouch empyaema: 5 Sep = Septavac Con = Control

9. Pentavac A vaccination study

In the second trial the seven horses of group 2 (section 3.2, table 3) where after vaccination V3 (Table 4) kept at pasture on grass and blood samples where taken regularly to measure IgG antibody titers in ELISA against the five antigens present in the Pentavac A formulation (Figure 21). In Day 270 (November 6, 2008) a booster dose of Pentavac A was given according to the procedure described in section 4.1. Before challenge the group was transferred to ACVS and fourteen days post booster the group was experimentally challenged with S. equi 4047 as described in section 4.2 and monitored essentially as described in section 4.3.

9.1 Brief summary of the Pentavac A vaccination study

The Pentavac A study revealed that after vaccination a significant antibody response against the individual antigens remains for at least six months (Figure 21).

The Pentavac A vaccine delayed the onset of infection upon challenge with S. equi and that one of the ponies in the group did not developed strangles.

Further applications

One implication of the present invention is that enzymes degrading immunoglobulins can be used as antigens in a vaccine to protect the target animal from infection. Therefore one embodiment of the present invention is that concerning the human pathogenic group A streptococci (GAS) it is possible to construct a vaccine composition which protects humans from infections caused by this bacterium. In strains of GAS there are several reported extracellular immunoglobulin degrading proteins (called Sib35, IdeS or Mac-proteins) which share amino acid sequence homologies to IdeE and IdeE2 and therefore in light of the present invention can be purified and used as antigens in a vaccine separately or in combination with other purified extracellular proteins (like M-proteins or M-like proteins or fragments thereof) from group A strains. As in the present invention another implication is that the invention can be used to develop specific antisera, polyclonal or monoclonal antibodies to be used for diagnostic purposes or to be used in passive immunisations of the target animal including humans.

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CLAIMS

1. An isolated antigenic composition comprising at least two antigens, wherein each antigen comprises at least part of a protein or polypeptide of *Streptococcus equi* subsp. *equi* or subsp. *zooepidemicus* and said at least part of said protein or polypeptide comprises at least one antigenic epitope or antigenic determinant of *Streptococcus equi,* wherein the antigens comprise:

an antigen comprising at least part of a protein or polypeptide which is designated EAG and has an amino acid sequence as shown in SEQ ID NO: 13; and

an antigen comprising at least part of a protein or polypeptide which is designated IdeE and has an amino acid sequence as shown in SEQ ID NO: 10.

2. The antigenic composition of claim 1, further comprising at least one further antigen which is at least part of a protein or polypeptide selected from the group consisting of:

a protein or polypeptide which is designated IdeE2 and has an amino acid sequence set forth in SEQ ID NO: 1;

a protein or polypeptide which is designated Eq5 and has an amino acid sequence set forth in SEQ ID NO: 3;

a protein or polypeptide which is designated Eq8 and has an amino acid sequence set forth in SEQ ID NO: 5;

a protein or polypeptide which is designated IdeZ2 and has an amino acid sequence set forth in SEQ ID NO: 7;

a protein or polypeptide which is designated Eqz5 and has an amino acid sequence set forth in SEQ ID NO: 8; and

a protein or polypeptide which is designated Eqz8 and has an amino acid sequence set forth in SEQ ID NO: 9.

3. The antigenic composition of claim 1, further comprising an antigen comprising at least part of a protein or polypeptide which is designated IdeE2 and has an amino acid sequence as shown in SEQ ID NO: 1.

4. The antigenic composition of claim 1, further comprising an antigen comprising at least part of a protein or polypeptide which is designated Eq5 and has

an amino acid sequence set forth in SEQ ID NO: 3, said composition optionally further comprising at least one antigen which is selected from the group consisting of:

at least part of a protein or a polypeptide designated CNE (or SEC), which has an amino acid sequence set forth in SEQ ID NO: 28; and

at least part of a protein or a polypeptide designated SclC, which has an amino acid sequence set forth in SEQ ID NO: 29.

5. The antigenic composition of claim 1, further comprising:

an antigen comprising at least part of a protein or polypeptide designated SclC, which has an amino acid sequence set forth in SEQ ID NO: 29;

an antigen comprising at least part of a protein or polypeptide designated CNE (or SEC), which has an amino acid sequence set forth in SEQ ID NO: 28;

an antigen comprising at least part of a protein or polypeptide designated Eq5, which has an amino acid sequence set forth in SEQ ID NO: 3;

an antigen comprising at least part of a protein or polypeptide designated Eq8, which has an amino acid sequence set forth in SEQ ID NO: 5; and

an antigen comprising at least part of a protein or polypeptide designated IdeE2, which has an amino acid sequence set forth in SEQ ID NO: 1.

6. The antigenic composition according to any one of claims 1 to 5, wherein the at least part of a protein or polypeptide which is designated IdeE comprises amino acids 6 to 320 of an amino acid sequence set forth in SEQ ID NO: 30.

7. The antigenic composition according to any one of claims 1 to 6, wherein at least one antigen is recombinantly produced.

8. An antigenic composition which comprises at least one recombinant vector and at least one polynucleotide inserted therein that encodes the proteins or polypeptides of the antigenic composition of any one of claims 1 to 7, and wherein said at least one recombinant vector is able to express said proteins or polypeptides *in vivo* in a non-human mammal susceptible to infection with *S. equi*.

9. The antigenic composition of claim 8 , wherein the recombinant vector is an expression vector which is a plasmid or a viral vector, and wherein the polynucleotide has a nucleotide sequence set forth in SEQ ID NOS: 12 and 14-21.

10. The antigenic composition according to any one of claims 1 to 9, wherein said antigenic composition is an immunogenic composition.

11. A vaccine composition for protecting a non-human mammal against infection of *Streptococcus equi,* said vaccine composition comprising the antigenic composition of any one of claims 1 to 10 as an immunizing component, and a pharmaceutically acceptable carrier.

12. The vaccine composition according to claim 11, further comprising an adjuvant.

13. The vaccine composition according to claim 11 or claim 12, wherein said vaccine composition is a vaccine that protects a susceptible mammal against strangles caused by *Streptococcus equi* subsp. *equi.*

14. The vaccine composition according to any one of claims 11 to 13, which is provided in a physiologically administrable form.

15. The vaccine composition according to any one of claims 11 to 14, wherein said composition is administrable by subcutaneous or intranasal inoculation.

16. The vaccine composition according to any one of claims 13 to 15, wherein said compositions stimulates serum, mucosal and/or bronchial lavage antibody responses directed to *Streptococcus equi* antigens in a mammal susceptible to *Streptococcus equi.*

17. The vaccine composition according to claim 13 or claim 16, wherein the susceptible mammal is an equine mammal.

18. A method for preparation of a vaccine composition according to any one of claims 11 to 17, said method comprising mixing anantigenic composition according to any one of claims 1 to 10 and a pharmaceutically acceptable carrier.

19. The method according to claim 18, further comprising preparing each antigen of the antigenic composition by a method comprising:

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

(b) introducing said expression vector containing said DNA fragment into a non-human compatible host cell;

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

(d) isolating the expressed protein or polypeptide product from the cultured host cell, and, optionally,

(e) purifying the isolated protein or polypeptide product from step (d) by affinity chromatography or other chromatographic methods known in the art.

20. Use of an antigenic composition according to any one of claims 1 to 10 in the preparation of a vaccine for protection against S. *equi* infection inclusive of strangles caused by subsp. *equi* infection in horses.

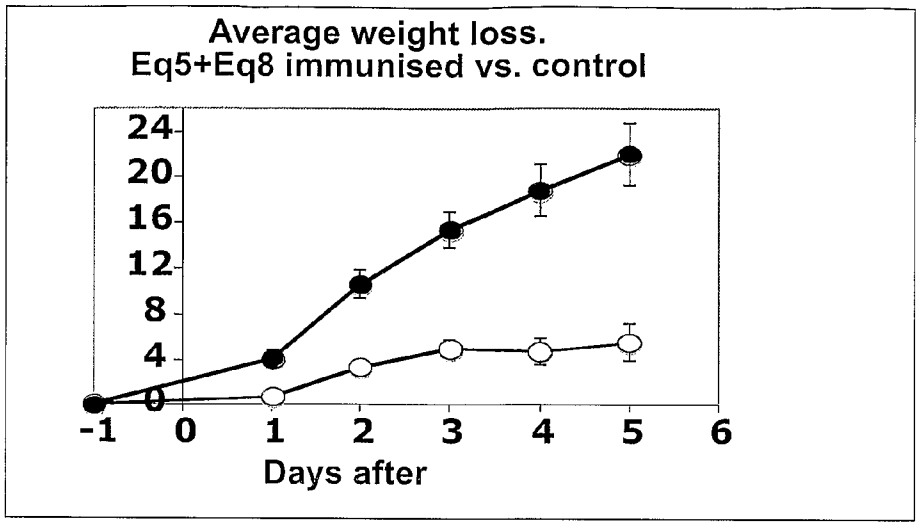
21. A method for the production of an antiserum, said method comprising administering an antigenic composition according to any one of claims 1 to 10 to a non-human animal host to produce antibodies in said animal host and recovering antiserum containing said antibodies produced in said animal host.

22. A vaccine composition when produced by the method according to claim 18 or claim 19.

23. An antiserum when produced by the method according to claim 21.

24. An antigenic composition according to any one of claims 1 to 10; a vaccine composition according to any one of claims 11 to 17; a method according to any one of claims 18 or 19 or 21; use according to claim 20; a vaccine composition

according to claim 22; or an antiserum according to claim 23, substantially as hereinbefore described with reference to any one or more of the examples but excluding comparative examples.



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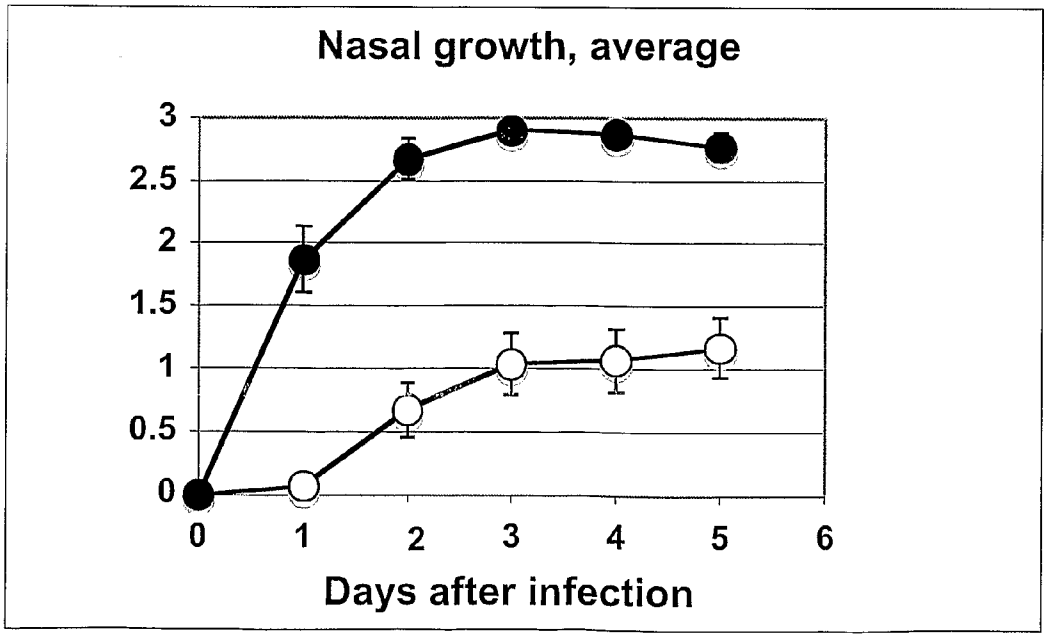

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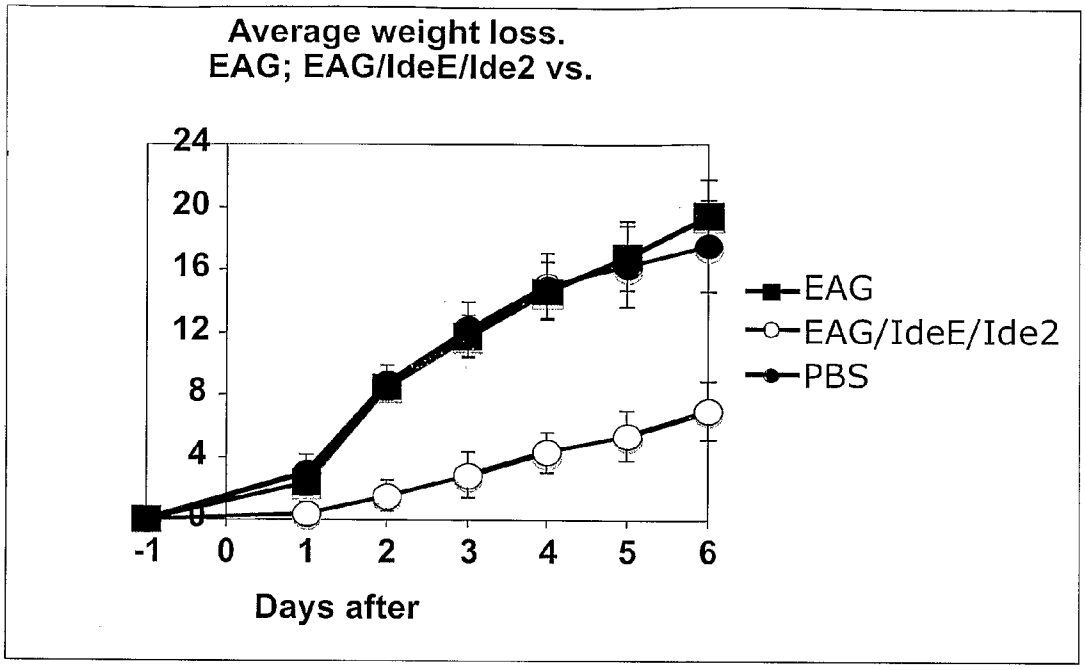

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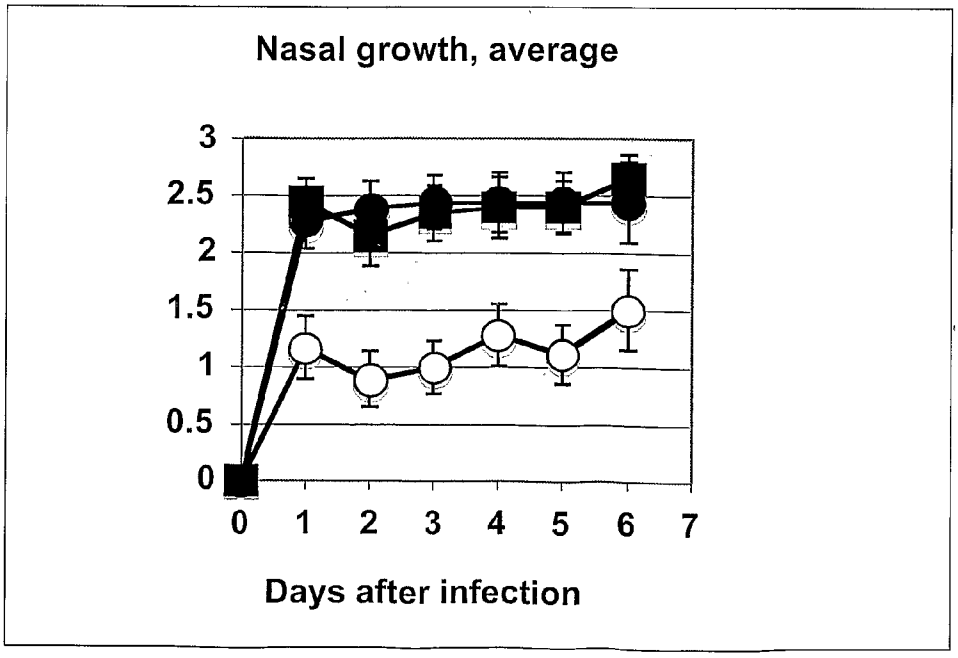

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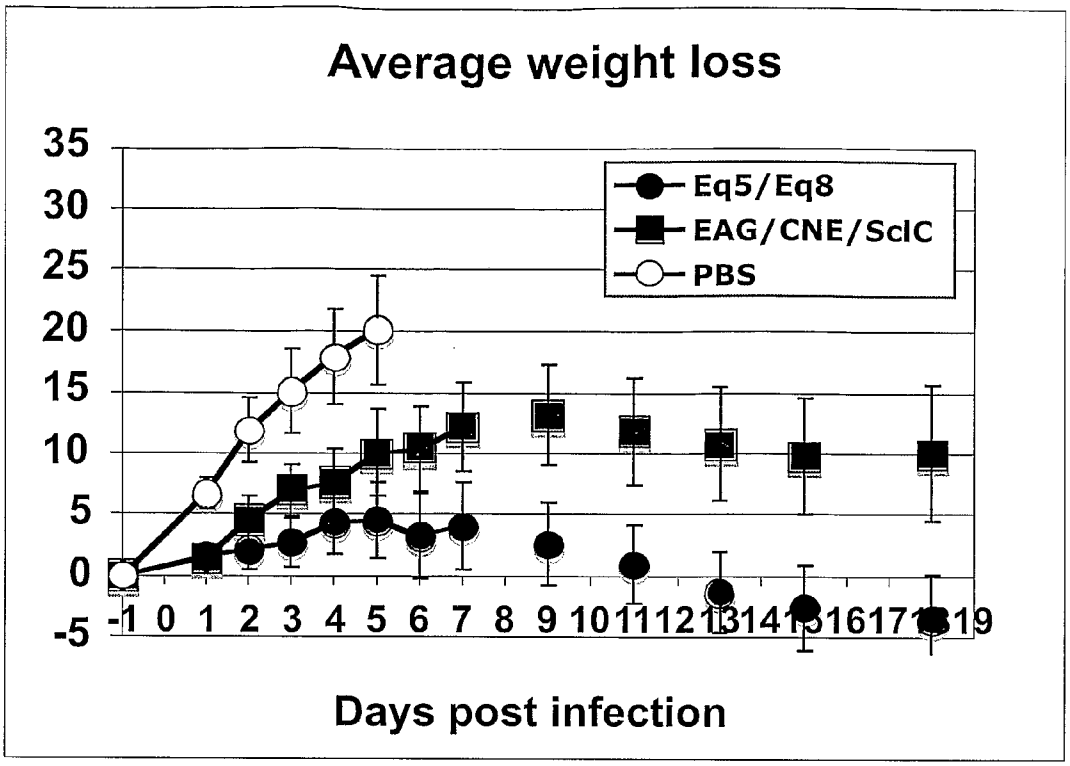

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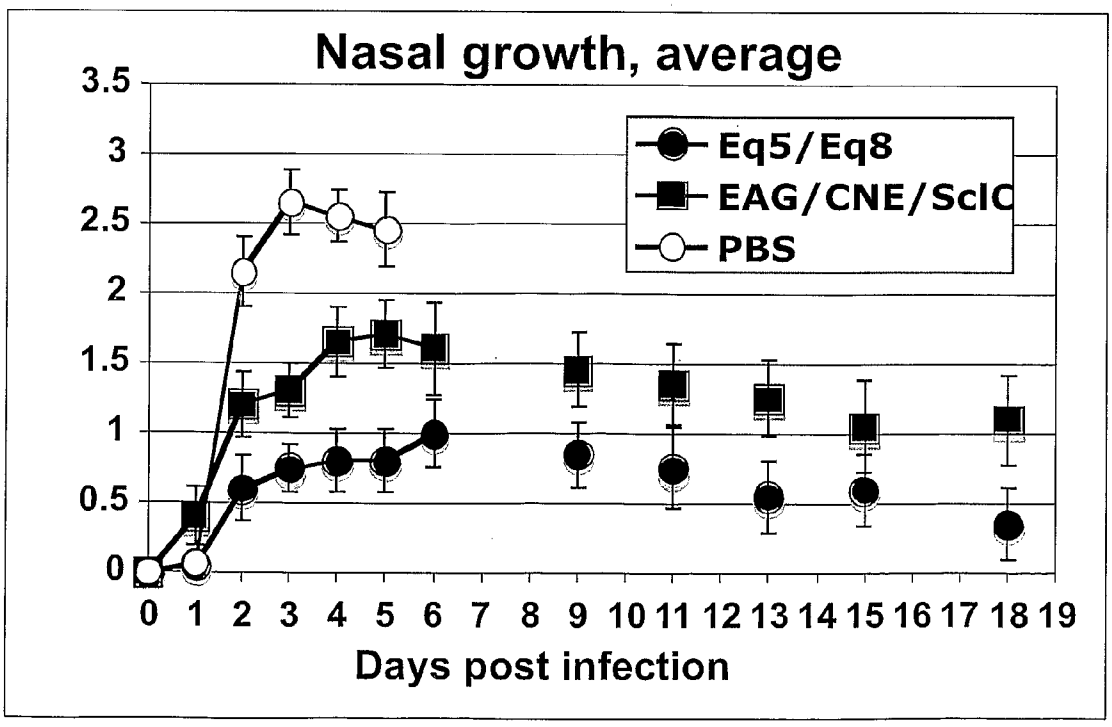


Figure 5b

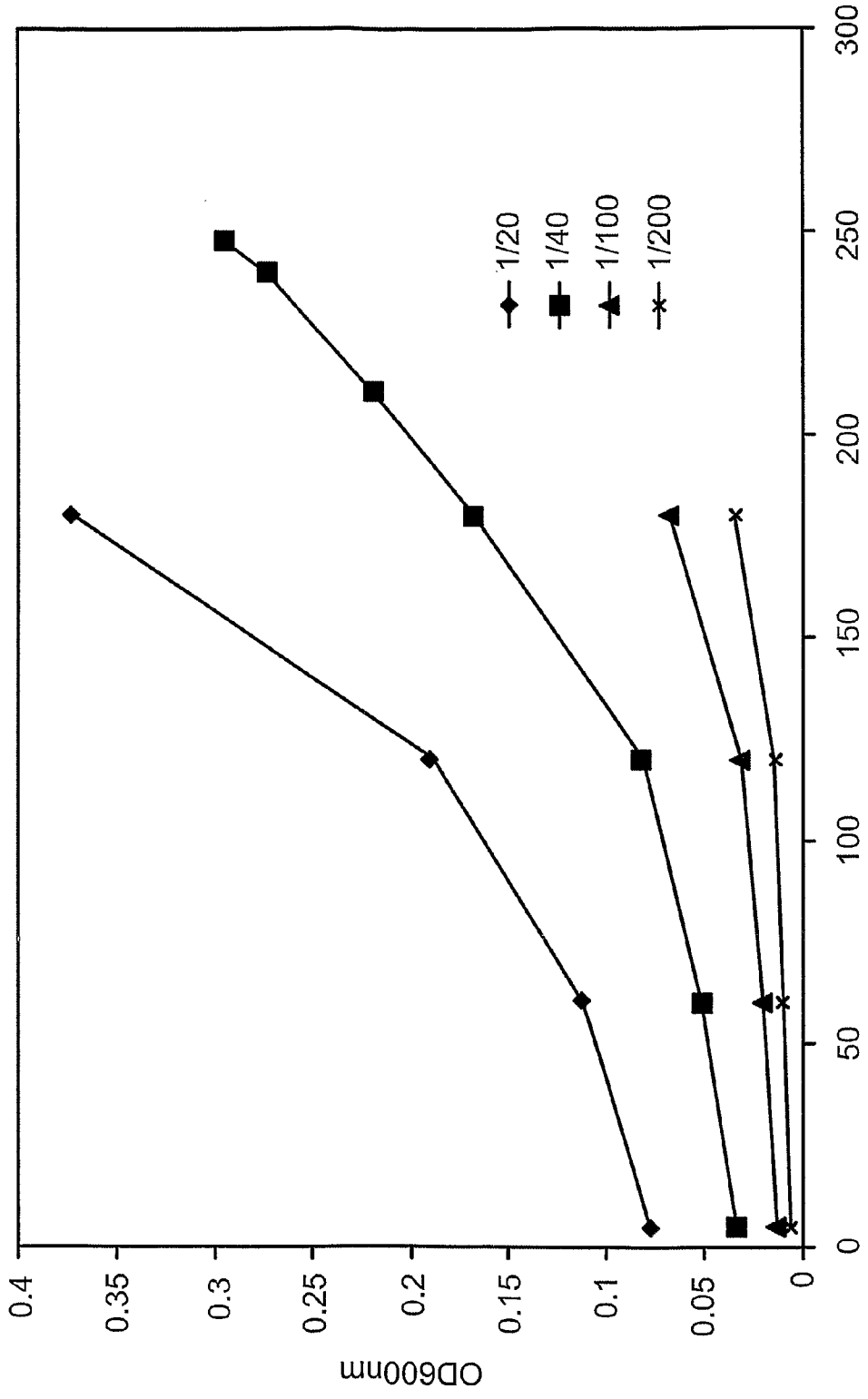


Figure 6: GROWTH OF CHALLENGE INCOLUM 08/5/08

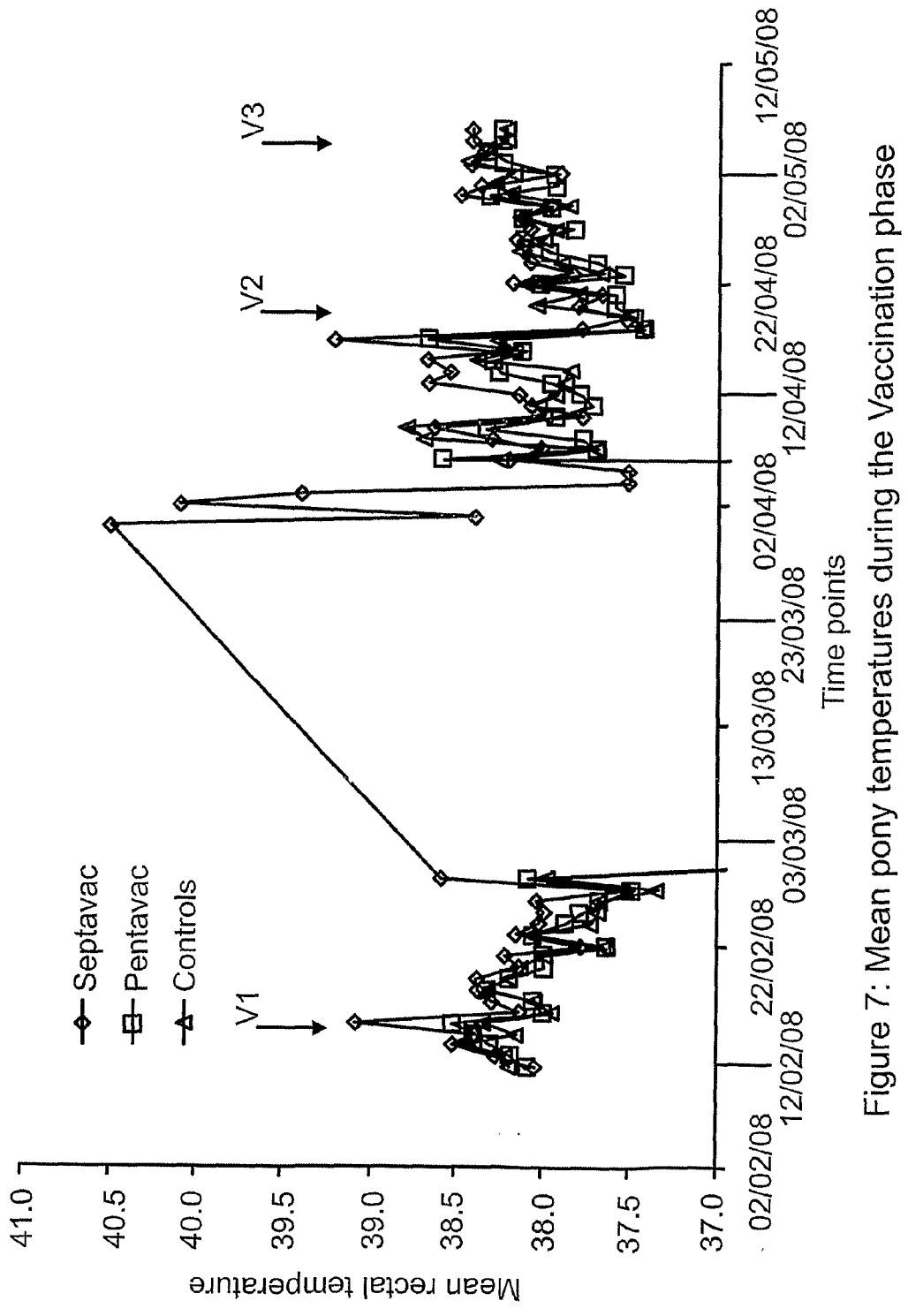


Figure 7: Mean pony temperatures during the Vaccination phase

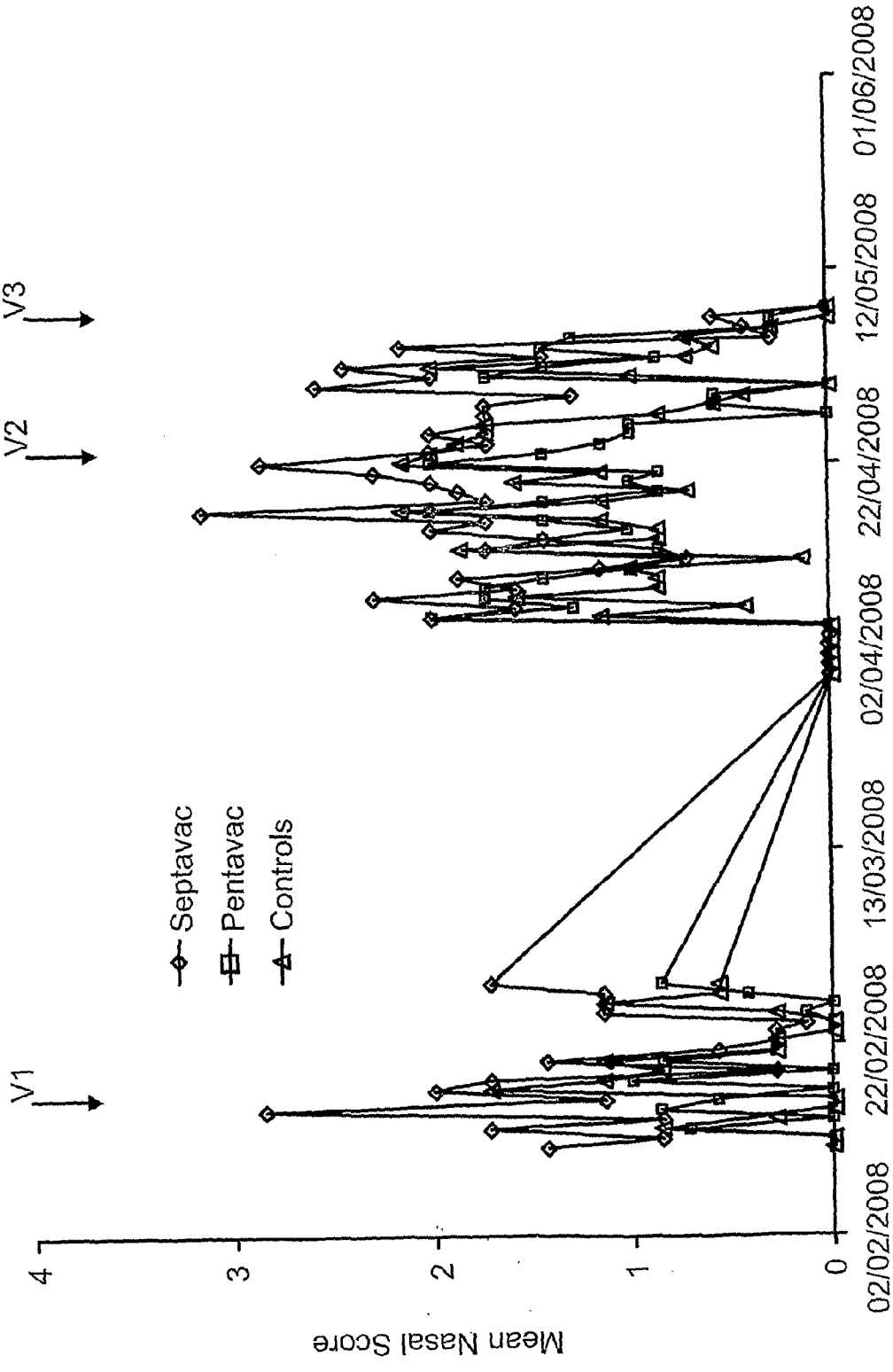


Figure 8: Mean nasal score during the vaccination phase

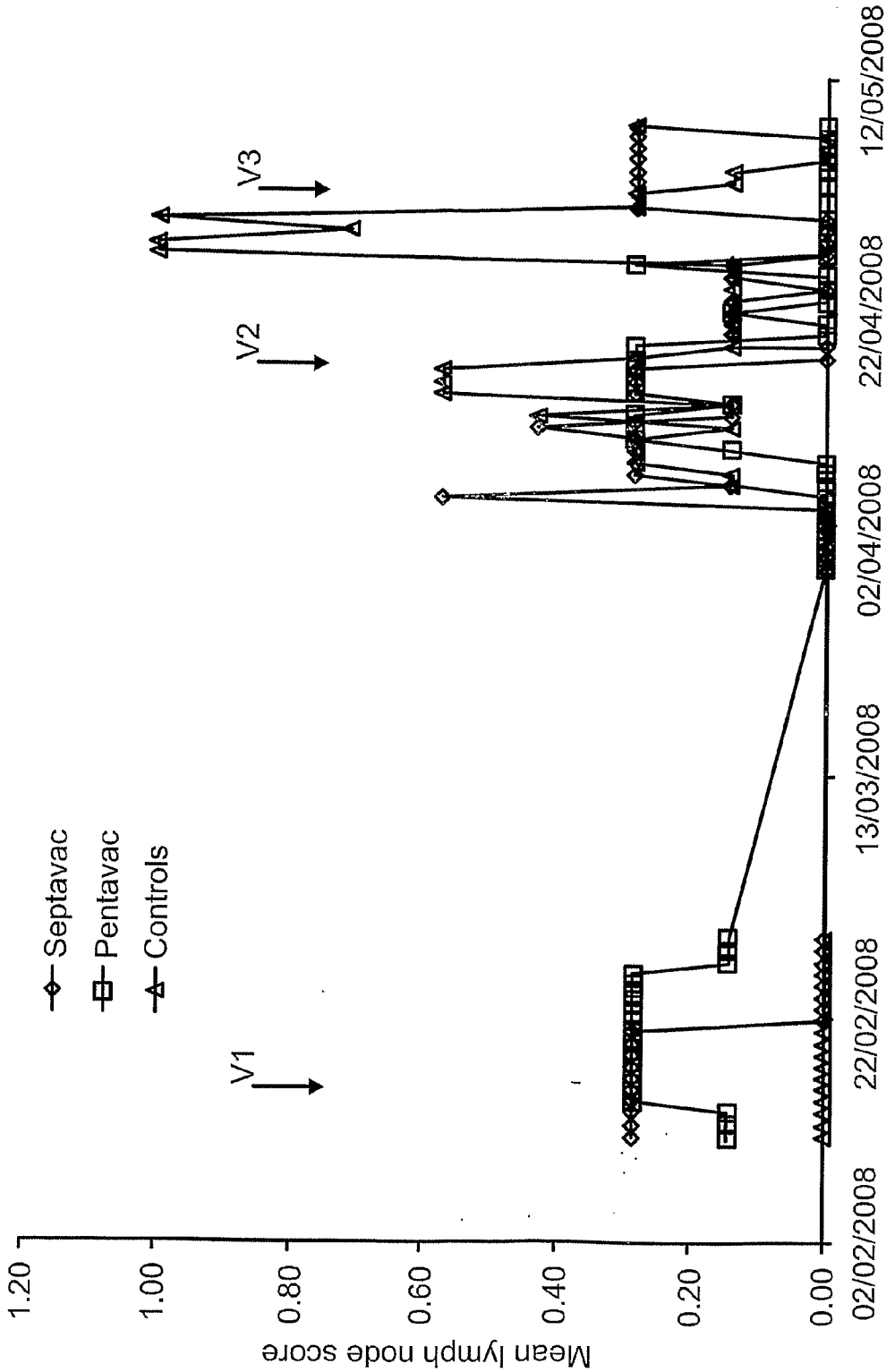


Figure 9: Mean lymph node score during the vaccination phase

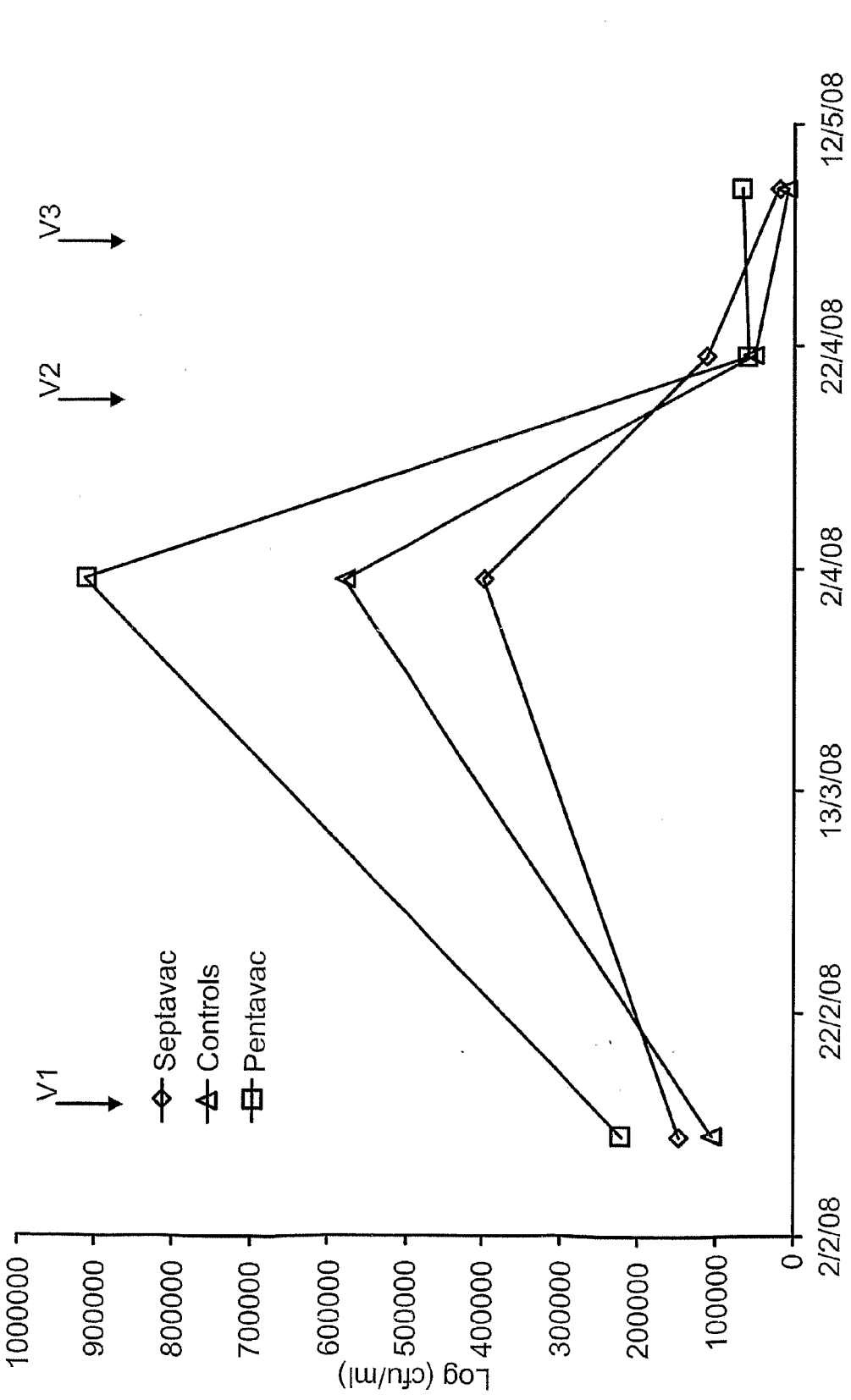
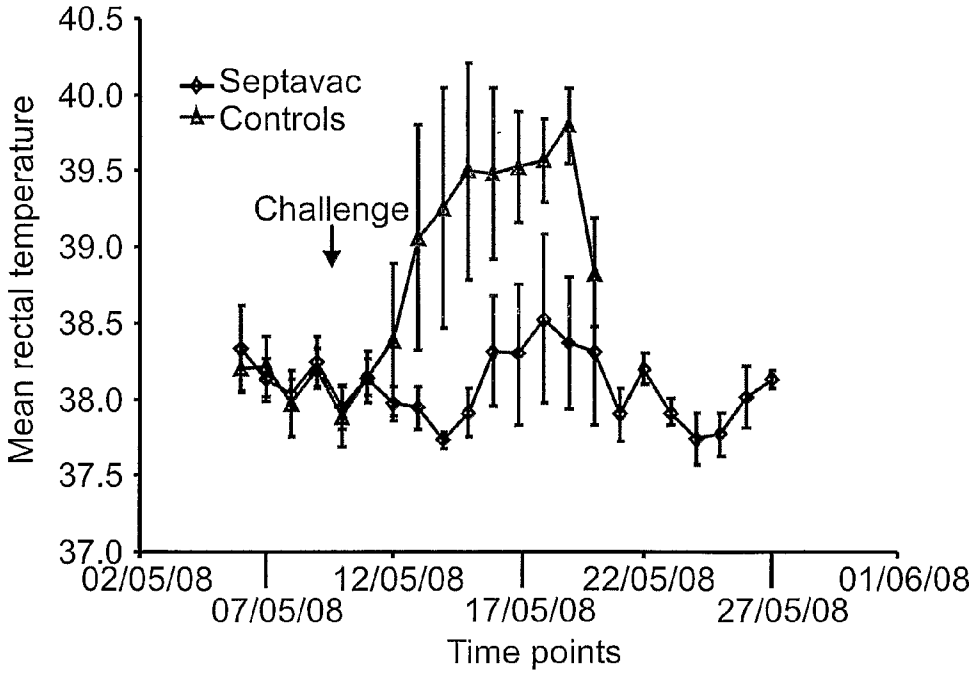


Figure 10: Mean counts of *S. zooepidemicus* in nasal washes during the vaccination phase



* All control ponies were euthanased by day 13 of the study, but most would have continued to have elevated temperatures had they not been euthanased on welfare grounds.

Figure 11: Mean temperatures after challange

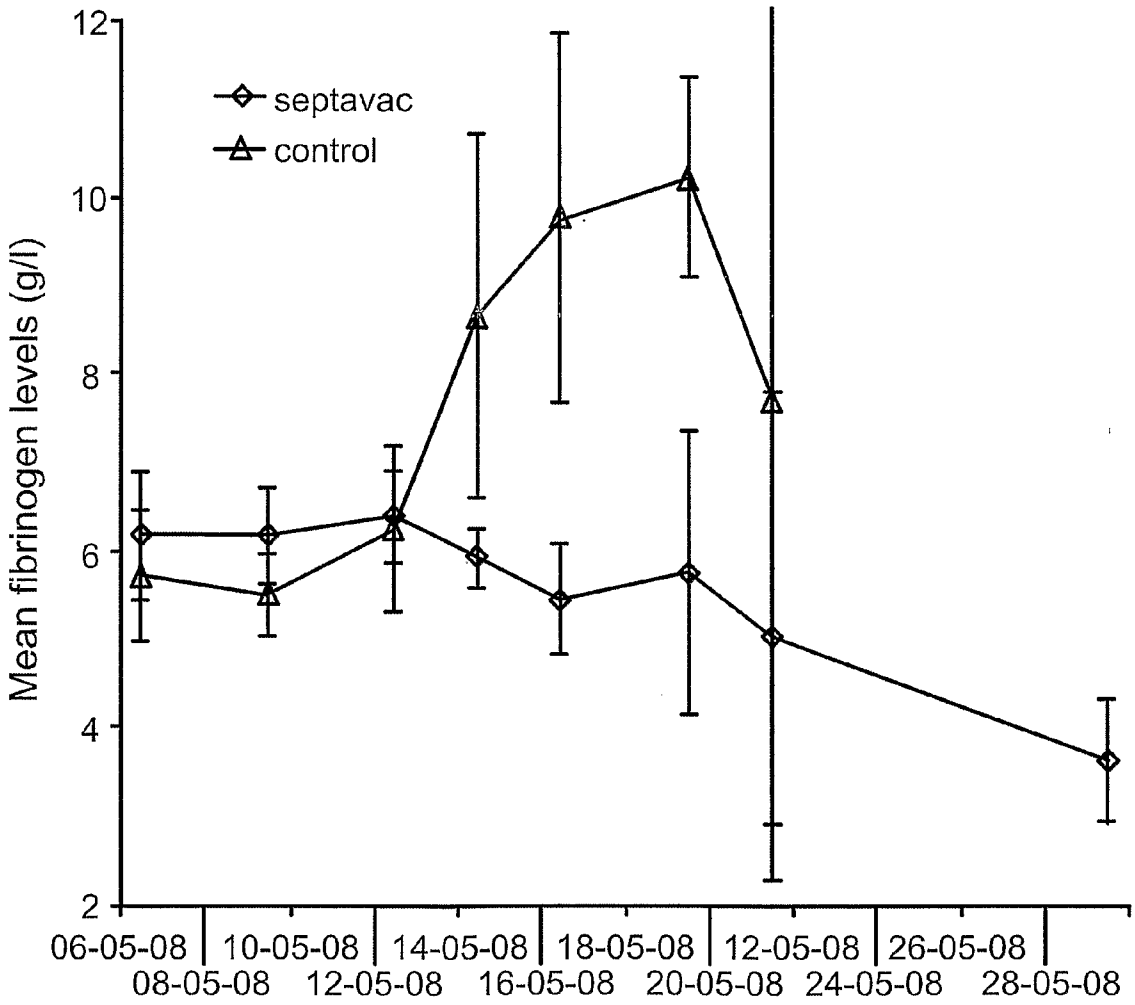
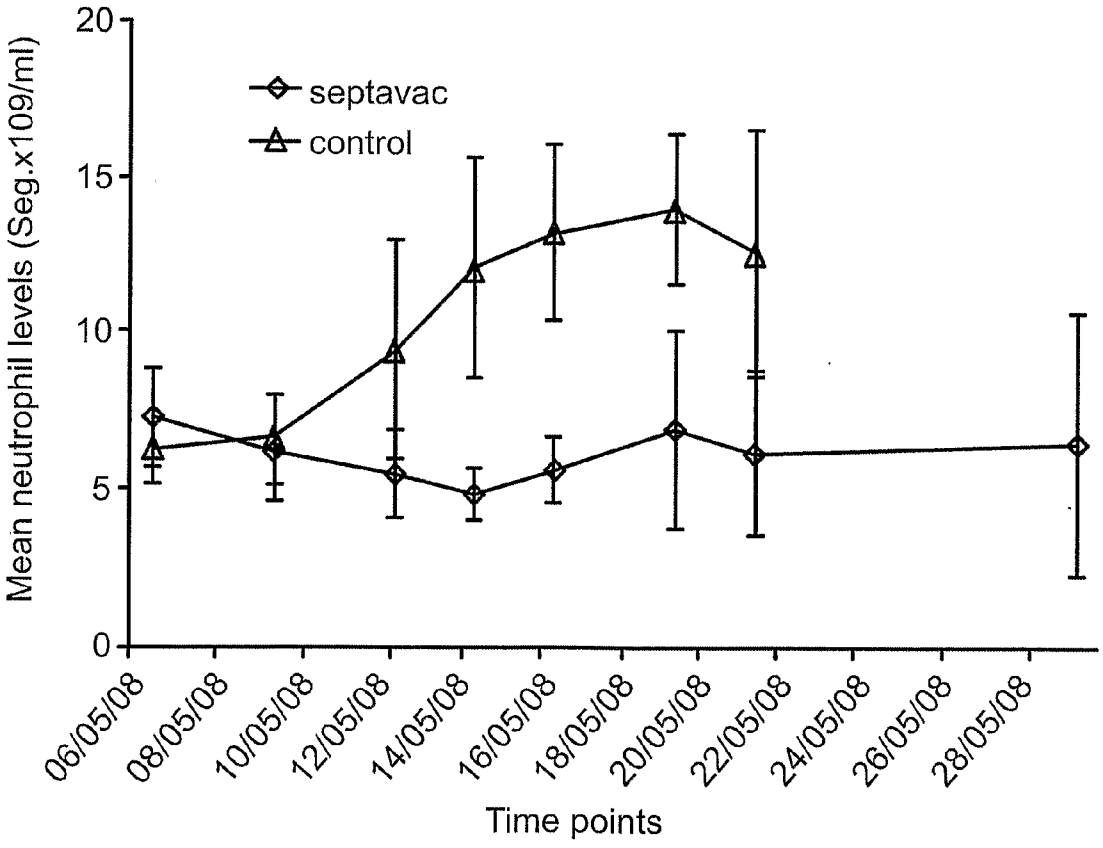
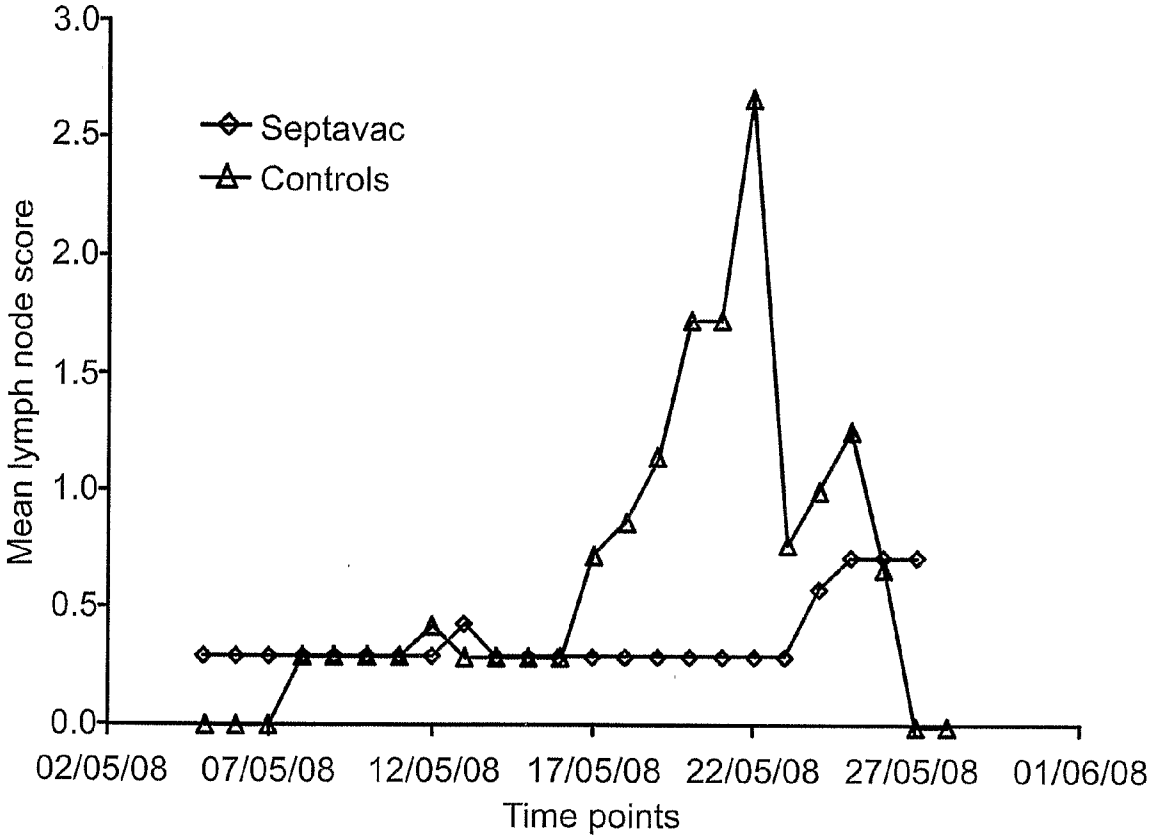


Figure 12: Mean fibrinogen levels during the challenge phase



* All control ponies were euthanased by day 13 post-challenge, but most would have continued to have elevated neutrophil levels had they not been euthanased on welfare grounds.

Figure 13: Neutrophil levels during challenge phase



* All control ponies were euthanased by day 13 post-challenge, but most would have continued to have elevated lymph node scores had they not been euthanased on welfare grounds.

Figure 14: Mean lymph node score during challenge phase

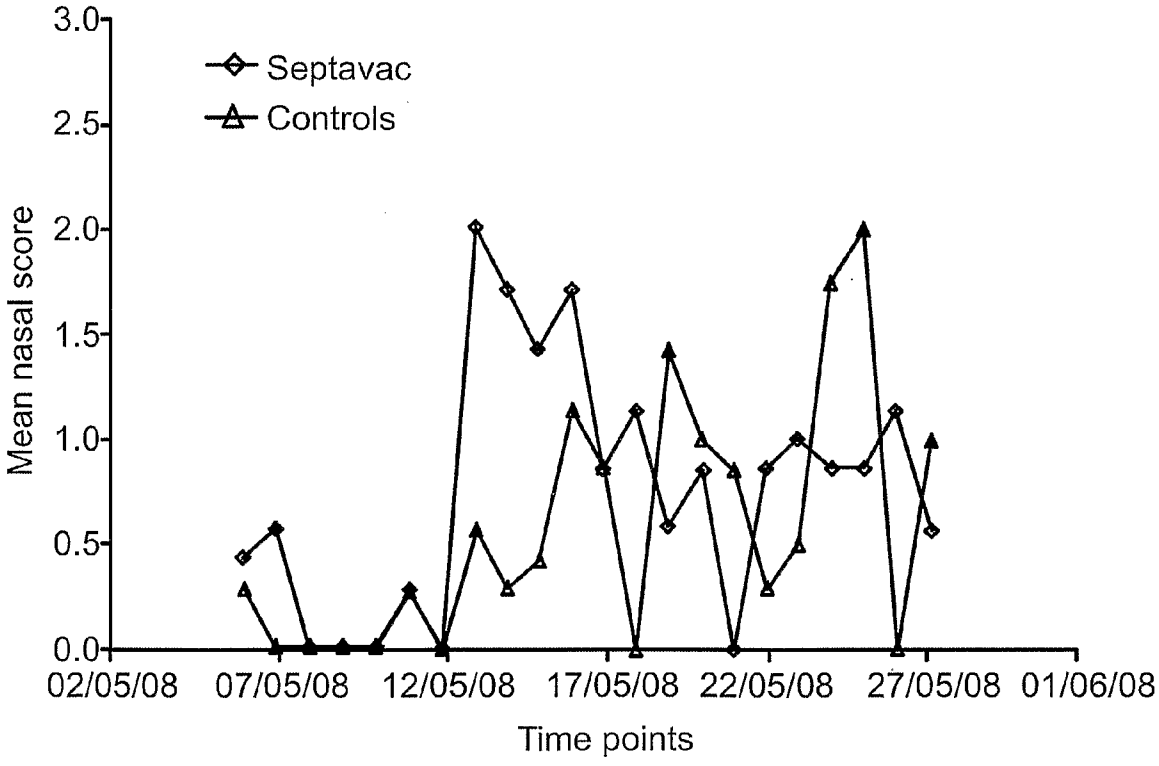


Figure 15: Mean nasal score during challenge phase

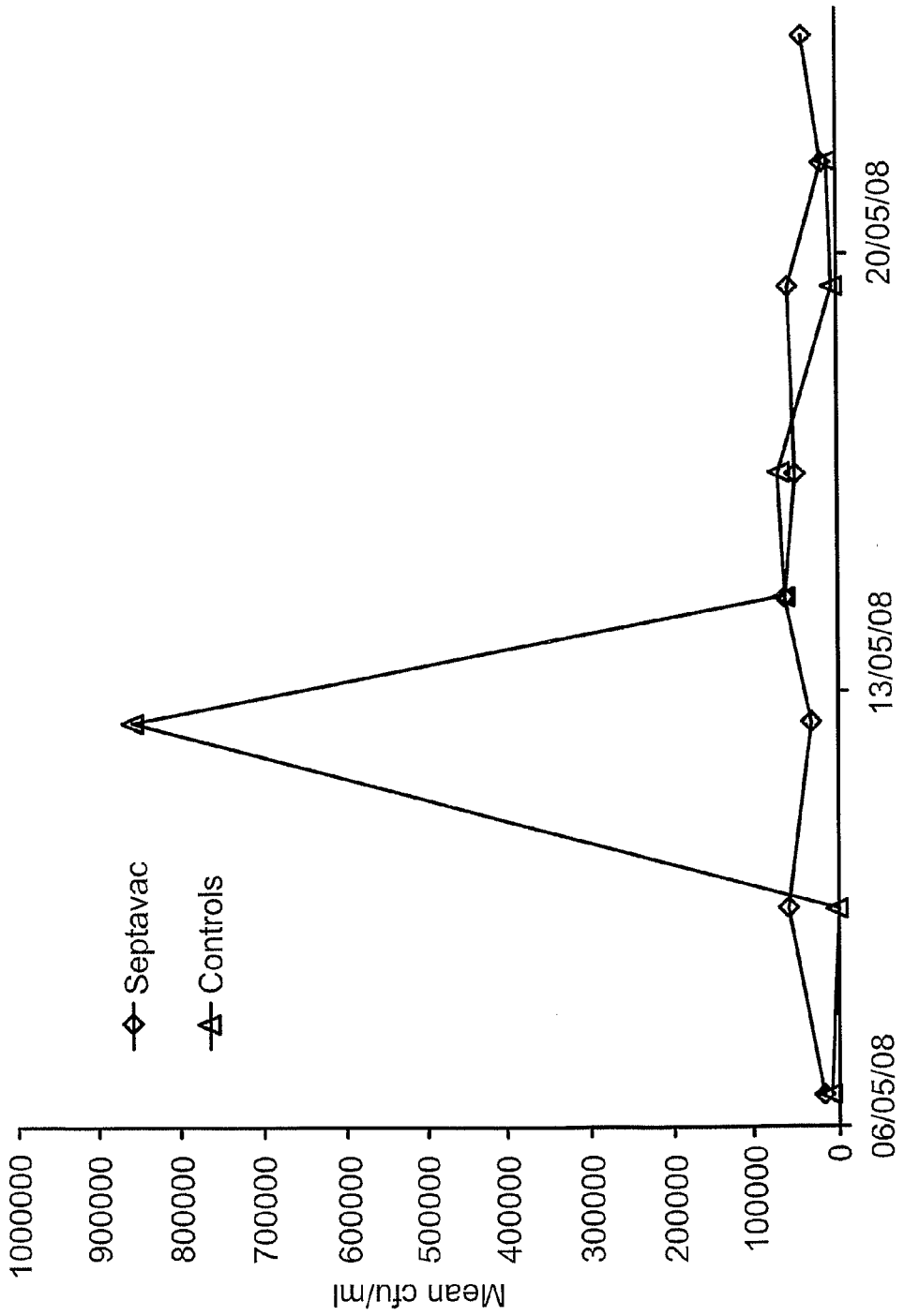


Figure 16: Mean *S. zooepidemicus* counts during challenge phase

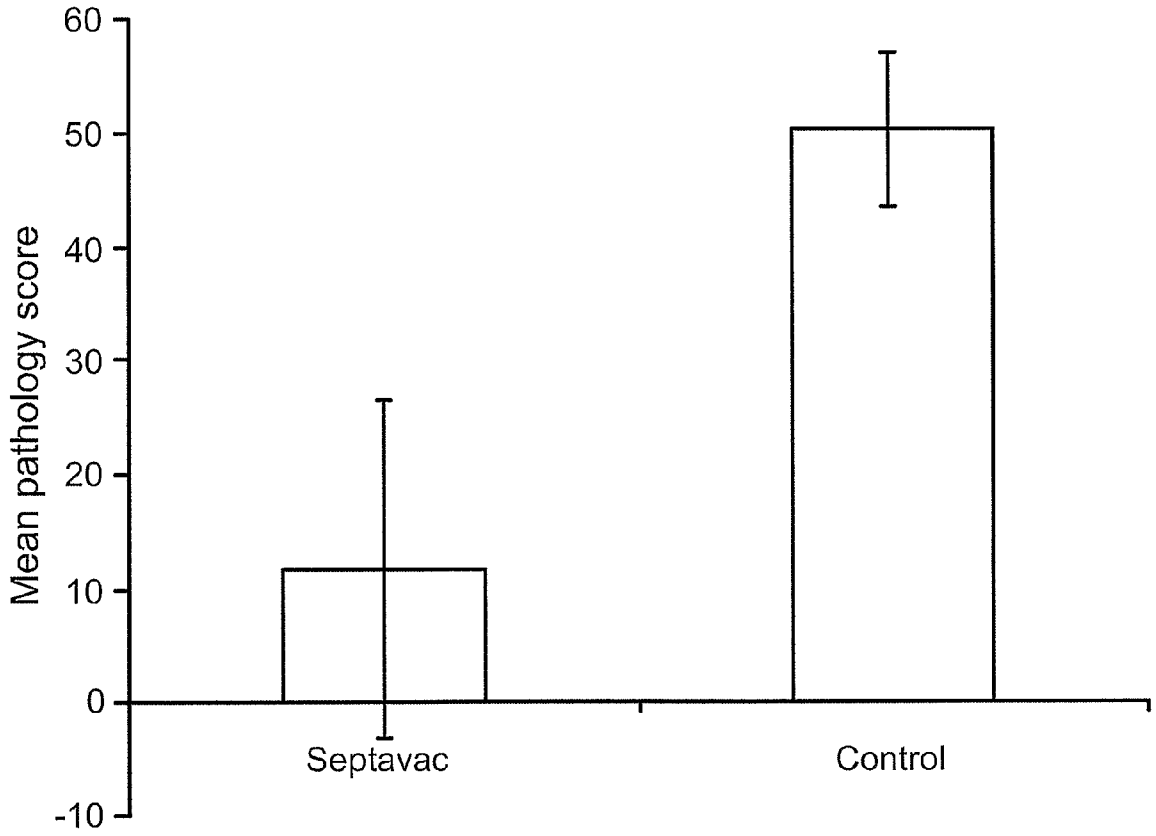


Figure 17: Mean pathology score on post mortem examination

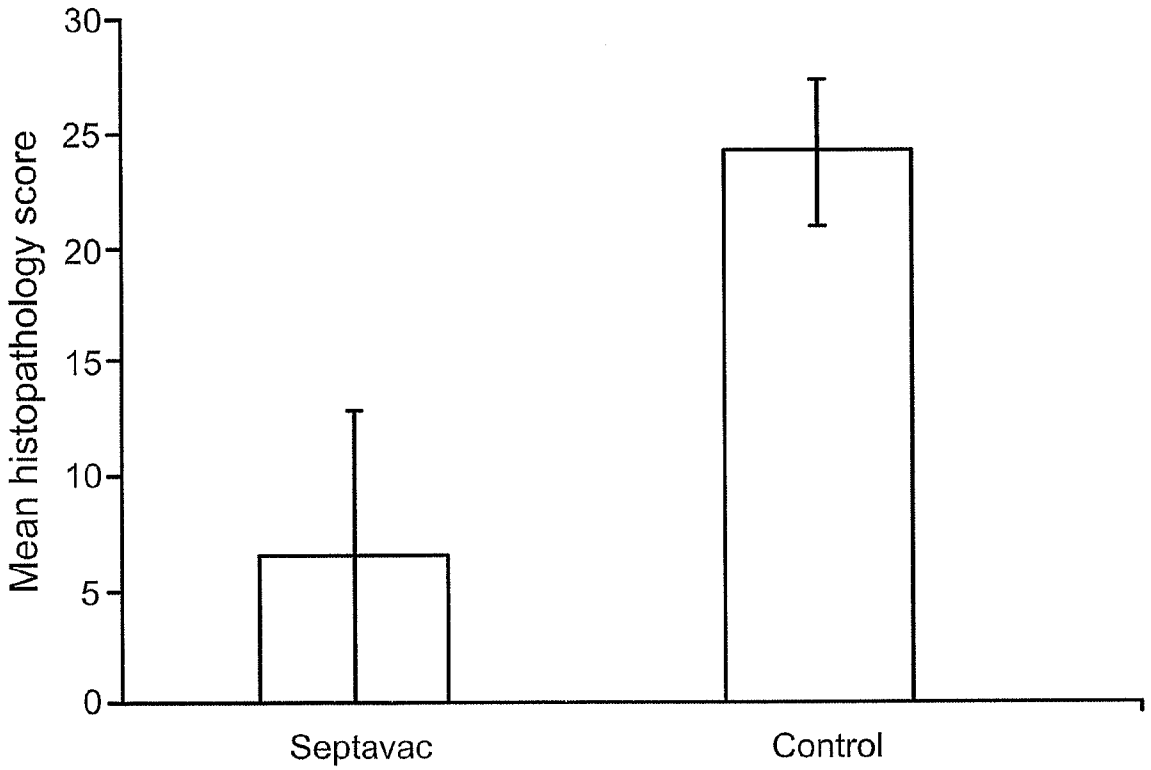
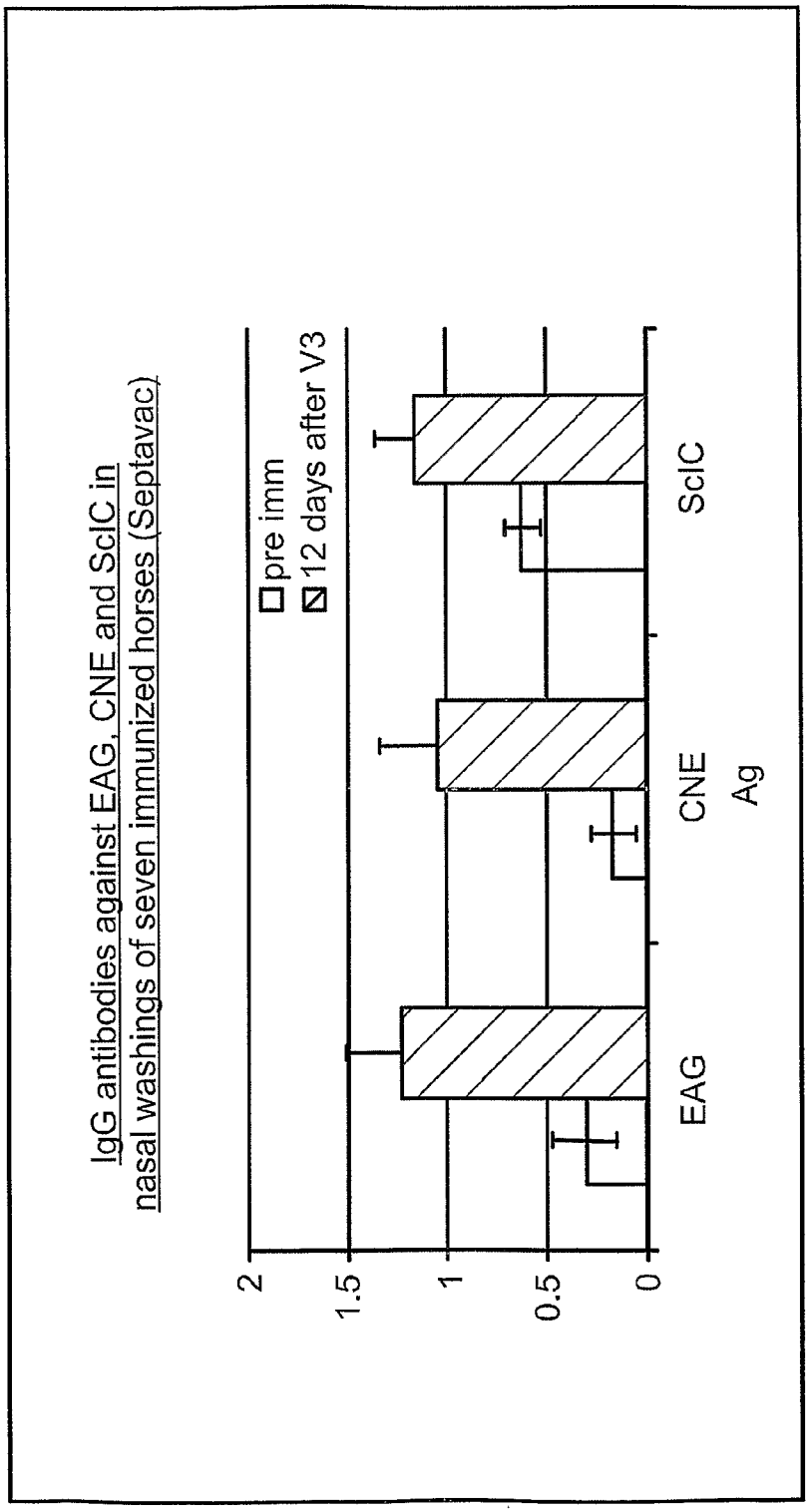


Figure 18: Mean Histopathology Scores


IgG antibodies against EAG, CNE and ScIC in
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12 days after V3
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1
0.5
0
EAG
CNE
ScIC
Ag

Figure 19

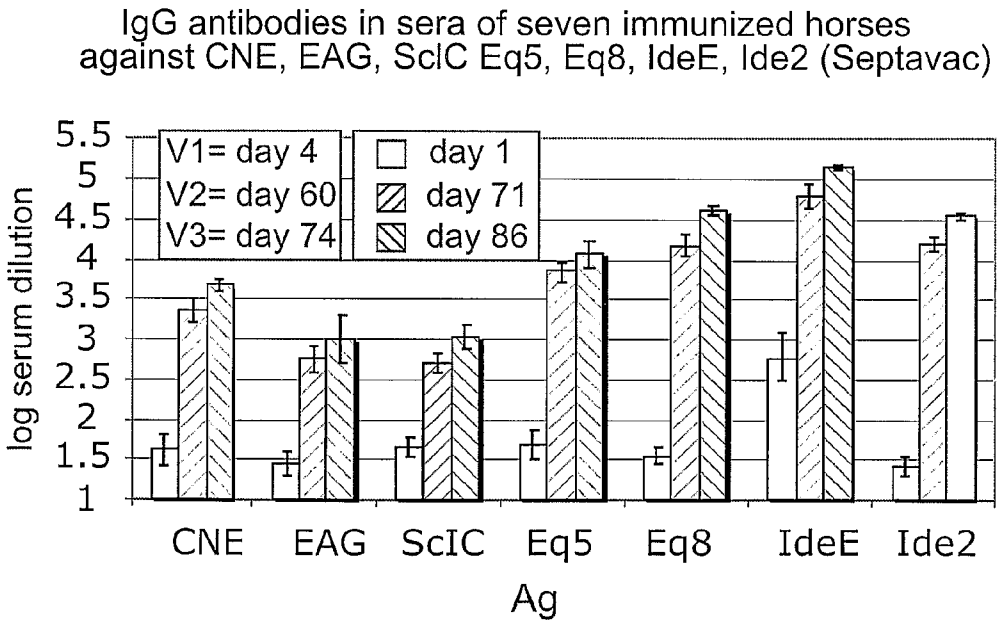
IgG antibodies in sera of seven immunized horses against CNE, EAG, SclC Eq5, Eq8, IdeE, Ide2 (Septavac)
log serum dilution
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
V1= day 4
V2= day 60
V3= day 74
day 1
day 71
day 86
CNE
EAG
ScIC
Eq5
Eq8
IdeE
Ide2
Ag

Figure 20

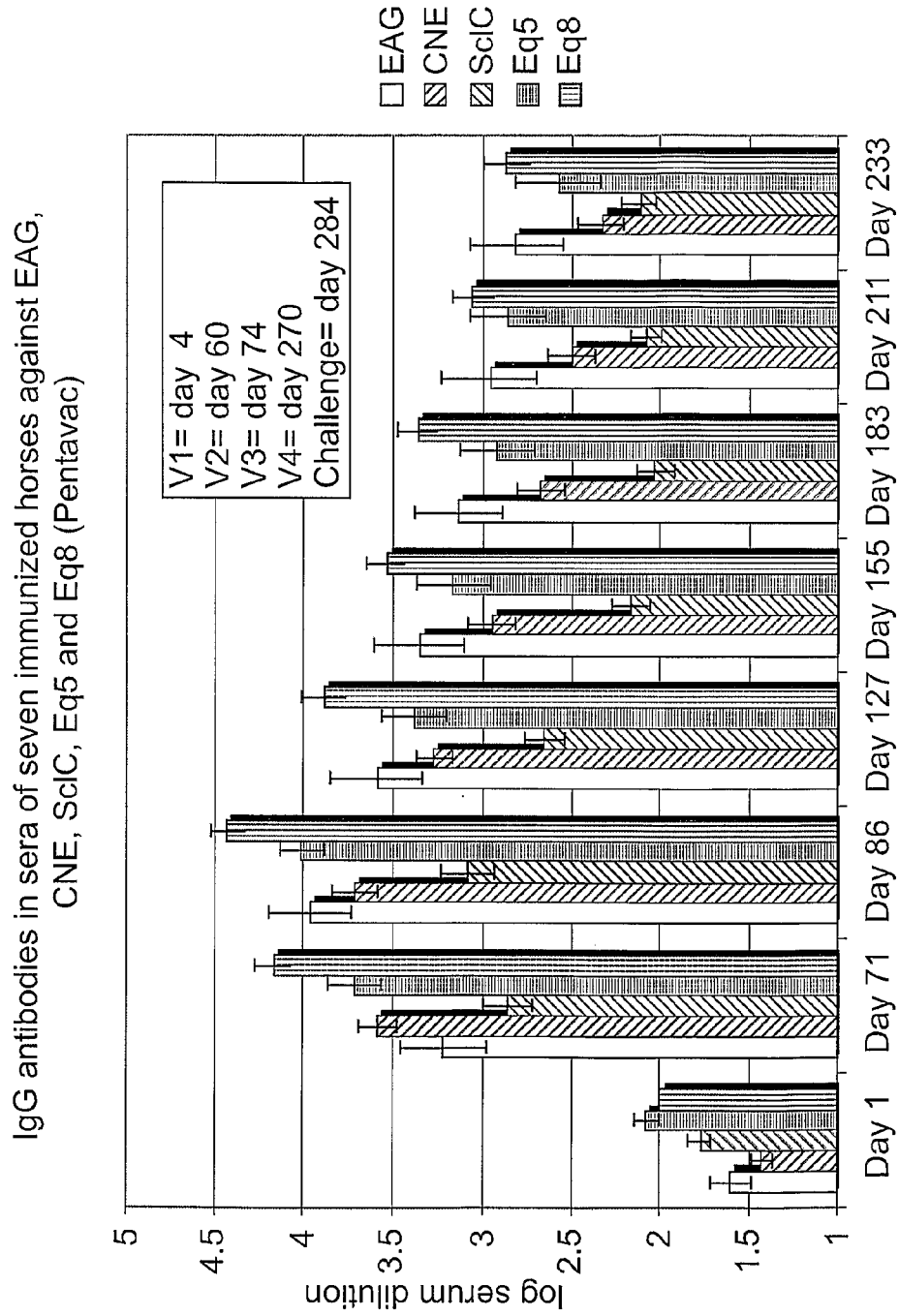
IgG antibodies in sera of seven immunized horses against EAG, CNE, SclC, Eq5 and Eq8 (Pentavac)
EAG
CNE
SclC
Eq5
Eq8
V1= day 4
V2= day 60
V3= day 74
V4= day 270
Challenge= day 284
log serum dilution
5
4.5
4
3.5
3
2.5
2
1.5
1
Day 1
Day 71
Day 86
Day 127
Day 155
Day 183
Day 211
Day 233

Figure 21

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<223> Eq5 protein having one N-terminal and four C-terminal amino acids originating from the pTYB4 vector

<400> 6

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

Leu Arg Leu Asp Met Thr Asp Lys Glu Ala Val Glu Lys Phe Ala Asn
20 25 30

Glu Leu Lys Asn Glu Val His Lys Asn Tyr Arg Gly Ser Asn Thr Trp
35 40 45

Gln Lys Leu Thr Leu Ile Leu Asn Gly Tyr Gln Asn Leu Arg Glu Gln
50 55 60

Ile Glu Thr Glu Leu Lys Asn Ser Glu Gln Lys Val Lys Glu Leu Asn
65 70 75 80

Asp Lys Val Asn Ser Glu Thr Gln Gly Lys Gln Glu Leu Gln Asn Gln
85 90 95

Leu Glu Lys Glu Lys Glu Glu Leu Glu Thr Leu Lys Lys Glu Leu Glu
100 105 110

Ala Glu Lys Ala Lys Gly Thr Gly Glu Thr Glu Lys Leu Gln Lys Glu
115 120 125

Ile Glu Ala Lys Asn Ala Met Ile Ser Asp Leu Gln Lys Gln Leu Glu
130 135 140

Glu Thr Lys Gln Arg Val Gln Glu Phe Glu Ala Glu Val Gly Lys Leu
145 150 155 160

Met Ala Glu Lys Ala Asp Leu Gln Thr Lys Leu Asn Glu Gln Glu Gln
165 170 175

Leu Asn Ala Lys Leu Gln Lys Glu Ile Glu Asp Leu Lys Ala Gln Ile
180 185 190

Glu Lys Leu Lys His Leu Glu Pro Gly
195 200

<210> 7
<211> 392
<212> PRT
<213> Streptococcus zooepidemicus

<400> 7

Met Met Lys Lys Gln Ser Phe Thr His Ser Arg Lys Pro Lys Phe Gly
1 5 10 15

Met Arg Lys Leu Ser Ile Gly Leu Ala Ser Cys Met Leu Gly Met Met
20 25 30

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

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

Tyr Ser Glu Tyr Leu Gln Arg Leu His Asp Val Glu Asp Phe Gln Ala
65 70 75 80

Glu Lys Lys Lys Glu Gly Val Val Arg Thr Gln Trp Leu Glu Gly Val

85 90 95

Asn Val Thr Asp His Asp Phe Arg Lys Ile Thr Asp Gly Gly Ser Val
100 105 110

Tyr Tyr Ala Thr Pro Leu Leu Asn Asp Arg Gly Tyr Tyr Asp Ile Asn
115 120 125

Lys Asn Phe Asn Gln Asp Ser Asp Lys Cys Ala Ala Ala Val Ala Val
130 135 140

Asn Met Phe His Tyr Trp Leu Asp Arg Asn Lys Asp Asn Val Ala Lys
145 150 155 160

Phe Leu Ser Gln Ser Pro Asp His Gly Phe Val Glu Gly Glu Pro Thr
165 170 175

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

Gly Gly Tyr Arg Asp Asn Ser Lys Leu Phe Asp Phe Ile Ser Lys Ala
195 200 205

Phe Asn Lys Pro Leu Trp Ala Asn Lys Leu Leu Asp Ala Tyr Ile Asn
210 215 220

Gly Tyr Gly Tyr Ile Asp Arg Tyr Val Lys Asn Thr Pro His Ser Gly
225 230 235 240

Gln Asn Asn Ser Lys Phe Asn Phe Phe Lys Lys Val Phe Asp Gly Lys
245 250 255

Leu Leu Thr Asp Ile Gln Gln Ile Phe Asp Tyr Tyr Thr Leu Ser Ser
260 265 270

Glu Leu Arg Glu Ala Leu Asp Thr Gly Lys Ala Ile Gly Leu Ala Tyr
275 280 285

Gly Pro Gly Asp Leu Arg Arg Ser Leu Gly His Ile Ile Ser Val Trp
290 295 300

Gly Ala Asp Ile Asn Glu Asp Gly Asn Val Val Ala Ile Tyr Val Thr
305 310 315 320

Asp Ser Asp Asp Lys Lys Leu Thr Ile Gly Asn Lys Lys Asp Arg Ile
325 330 335

Gly Leu Lys Arg Tyr Lys Leu Tyr Ser Asp Asn Val Gly Arg Ala Arg
340 345 350

Leu Thr Ala Tyr Ala Thr Glu Asn Gln Gln Thr Gly Gly Glu Val Arg
355 360 365

Gly Ile Glu Thr Leu Asp Met Ala Thr Gln Asp Trp Ala Asp Tyr Phe
370 375 380

Ser Arg Thr Asp Glu Ala Glu Gln
385 390

<210> 8
<211> 570
<212> PRT
<213> Streptococcus zooepidemicus

<400> 8

Met Lys Lys Phe Thr Lys Arg Cys Leu Lys Gly Cys Gly Leu Val Gly
1 5 10 15

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

Ala Leu Thr Val Gly Ala Glu Thr Ala Thr Thr Ala Asn Ala Phe Glu
35 40 45

Glu Ser Gly Asp Gln Gln His Lys Asn Trp His Ile Tyr Ile Pro Glu
50 55 60

Val Tyr Thr Val Lys Val Gly Gln Pro Ile Thr Ile Glu Asp Ile Leu
65 70 75 80

Ser Gln Ile Thr Ile Thr Arg Lys Gly Glu Asp Ser Gln Gly Lys Thr
85 90 95

Ser Pro Gly Met Ile Tyr Thr Tyr Glu Glu Tyr Pro Lys Val Arg Gly
100 105 110

Ile Glu Val Ser Ala Gly Thr Ile Trp Phe Asp Phe Tyr Asn Ser Gly
115 120 125

Asn Trp Val Asn Asn Asp Val Leu Ala Thr Phe Asn Glu Pro Gly Gly
130 135 140

Thr Tyr Thr Leu Ser Ala Trp Ala Tyr Tyr Ala Asn Glu Asn Val Lys
145 150 155 160

Lys Gln Phe Val Phe Lys Leu Gln Val Glu Asn Ser Asp Lys Arg Ala
165 170 175

Leu Glu Gln Ser Leu Ala Thr Ala Asn Glu Lys Leu Gln Ala Pro Glu
180 185 190

Gly Thr Tyr Ser Asp Glu Ser Leu Gln Arg Leu Gln Glu Ser Val Phe
195 200 205

Leu Gly Gln Thr Tyr Leu Asn Arg Asp Pro Glu Gln Gln Glu Val Asp
210 215 220

Asp Met Lys Ala Thr Ile Asp Ser Ala Val Ser Gly Leu Val Asp Leu
225 230 235 240

Thr Val Leu Asn Thr Ala Val Glu Thr Ala Thr Pro Leu Leu Thr Asp
245 250 255

Gly Lys Glu Tyr Pro Lys Glu Ala Tyr Asp Ser Leu Val Gln Lys Leu
260 265 270

Ala Ala Ala Ala Lys Leu Gln Asn Ser Phe Asn Pro Ser Gln Glu Glu
275 280 285

Val Asn Glu Ala Ala Thr Asp Leu Thr Gln Ala Leu Thr Thr Leu Lys
290 295 300

Thr Ala Val Ala His Glu Ala Leu Asp Gln Ala Leu Ala Lys Leu Leu
305 310 315 320

Glu Leu Tyr Arg Glu Asn Pro Asn Leu Ala Leu Thr Ser Glu Pro Leu
325 330 335

Lys Glu Leu Tyr Asn Lys Ala Ile Glu Ala Ala Gly Thr Phe Tyr Arg
340 345 350

Thr Val Ser Lys Asp Lys Glu Arg Lys Gly Ile Ser Leu Tyr Glu Leu
355 360 365

Glu Arg Tyr Thr Thr Glu Thr Asn Ser Val Val Asp Thr Ile Leu Lys
370 375 380

Val Lys Ala Ala Ile Ala Glu Glu Gly Lys Ala Lys Leu Arg Ser Ala
385 390 395 400

Leu Asp Gln Leu Asn Ala Leu Ile Gly Glu Asn Leu Asp Leu Ser Pro
405 410 415

Tyr Thr Ala Ala Ser Ala Gln Ala Tyr Thr Asp Gln Leu Ala Lys Ala
420 425 430

Lys Glu Val Ala Ala Ala Gly Glu Thr Ala Tyr Ala Gln Glu Thr Glu
435 440 445

Pro Thr Ala Ile Thr Asn Ser Leu Ile Lys Val Leu Asn Ala Lys Lys
450 455 460

Ser Leu Ser Asp Ala Lys Ala Ala Leu Val Ala Lys Pro Val Asp Pro
465 470 475 480

Val Asp Pro Val Asp Pro Val Asp Pro Val Asp Pro Val Asp Pro Ile
485 490 495

Asp Pro Val Asp Pro Val Lys Pro Val Asp Pro Glu Val Lys Pro Glu
500 505 510

Pro Lys Pro Glu Ser Lys Pro Glu Ala Lys Lys Glu Asp Lys Lys Ala
515 520 525

Ala Asp Lys Gln Gln Val Leu Pro Ala Thr Ala Asp Thr Ala Asn Pro
530 535 540

Phe Phe Thr Ala Ala Ala Leu Ala Val Ile Ala Cys Ala Gly Gln Leu
545 550 555 560

Ala Ile Val Ser Arg Arg Lys Glu Ser Asn
565 570

<210> 9
<211> 411
<212> PRT
<213> Streptococcus zooepidemicus

<400> 9

Met Asn Lys Lys Ser Ala Arg Arg Lys Arg Lys Asp Leu Ile Thr Lys
1 5 10 15

Leu Ala Met Thr Ser Ala Leu Thr Leu Gly Val Gly Ala Ala Ala Thr
20 25 30

Ile Ala Gly Gln Thr Glu Val Arg Ala Glu Val Leu Thr Leu Asn Met
35 40 45

Lys Asp Lys Ala Lys Val Glu Glu Phe Ala Asn Lys Leu Lys Asp Tyr

50 55 60

Ala Lys Gln Lys Lys Ser Gly Gln Ile Thr Leu Gln Glu Leu Ser Leu
65 70 75 80

Ile Leu Asp Gly Tyr Arg Asn Ile Arg Glu Gln Ile Glu Gln Asp Leu
85 90 95

Ala Thr Thr Glu Lys Thr Lys Asn Phe Tyr Gly Glu Gln Leu Ile Leu
100 105 110

Thr Asp Lys Leu Tyr Gln Ser Glu Lys Glu Lys Lys Glu Lys Leu Glu
115 120 125

Ala Glu Leu Gln Leu Ser Gln Gln Lys Ile His Asp Leu Asp Glu Lys
130 135 140

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

Gln Lys Ile Lys Glu Leu Glu Met Ala Lys Ser Thr Ala Glu Ala Glu
165 170 175

Ile Asn Arg Leu Thr Ala Glu Lys Asn Gly Leu Gln Glu Lys Leu Asn
180 185 190

Asn Gln Glu Lys Leu Asn Ala Glu Leu Gln Ala Lys Leu Ala Lys Gln
195 200 205

Glu Glu Leu Asn Ala Lys Leu Gln Lys Glu Ile Asp Glu Leu Asn Ala
210 215 220

Gln Leu Glu Lys Leu Lys His Cys Gln Asp Thr Pro Lys Pro Glu Pro
225 230 235 240

Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro
245 250 255

Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro
260 265 270

Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro
275 280 285

Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro
290 295 300

Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro
305 310 315 320

Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro
325 330 335

Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro Lys Pro Glu Pro
340 345 350

Lys Pro Glu Ala Lys Lys Pro Glu Gln Pro Lys Pro Met Thr Lys Pro
355 360 365

Gly Ala Lys Lys Pro Glu Gln Ser Leu Pro Ser Thr Gly Asp Ile Arg
370 375 380

Asn Pro Phe Phe Thr Pro Ala Ala Ile Ala Ile Met Ile Ala Ala Gly
385 390 395 400

Thr Ile Ala Ile Pro Lys Arg Lys Glu Glu Asp
405 410

<210> 10
<211> 349
<212> PRT
<213> Streptococcus equi

<400> 10

Met Lys Thr Ile Ala Tyr Pro Asn Lys Pro His Ser Leu Ser Ala Gly
1 5 10 15

Leu Leu Thr Ala Ile Ala Ile Phe Ser Leu Ala Ser Ser Asn Ile Thr
20 25 30

Tyr Ala Asp Asp Tyr Gln Arg Asn Ala Thr Glu Ala Tyr Ala Lys Glu
35 40 45

Val Pro His Gln Ile Thr Ser Val Trp Thr Lys Gly Val Thr Pro Leu
50 55 60

Thr Pro Glu Gln Phe Arg Tyr Asn Asn Glu Asp Val Ile His Ala Pro
65 70 75 80

Tyr Leu Ala His Gln Gly Trp Tyr Asp Ile Thr Lys Ala Phe Asp Gly
85 90 95

Lys Asp Asn Leu Leu Cys Gly Ala Ala Thr Ala Gly Asn Met Leu His
100 105 110

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

His Pro Glu Lys Gln Lys Ile Ile Phe Asn Asn Gln Glu Leu Phe Asp
130 135 140

Leu Lys Ala Ala Ile Asp Thr Lys Asp Ser Gln Thr Asn Ser Gln Leu
145 150 155 160

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

Leu Gly Val Met Pro Asp Leu Val Leu Asp Met Phe Ile Asn Gly Tyr
180 185 190

Tyr Leu Asn Val Phe Lys Thr Gln Ser Thr Asp Val Asn Arg Pro Tyr
195 200 205

Gln Asp Lys Asp Lys Arg Gly Gly Ile Phe Asp Ala Val Phe Thr Arg
210 215 220

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

Gly Leu Asn Asp Ile Ser Thr Ile Ile Lys Gln Glu Leu Thr Glu Gly
245 250 255

Arg Ala Leu Ala Leu Ser His Thr Tyr Ala Asn Val Ser Ile Ser His
260 265 270

Val Ile Asn Leu Trp Gly Ala Asp Phe Asn Ala Glu Gly Asn Leu Glu
275 280 285

Ala Ile Tyr Val Thr Asp Ser Asp Ala Asn Ala Ser Ile Gly Met Lys
290 295 300

Lys Tyr Phe Val Gly Ile Asn Ala His Arg His Val Ala Ile Ser Ala
305 310 315 320

Lys Lys Ile Glu Gly Glu Asn Ile Gly Ala Gln Val Leu Gly Leu Phe
325 330 335

Thr Leu Ser Ser Gly Lys Asp Ile Trp Gln Lys Leu Ser
340 345

<210> 11
<211> 349
<212> PRT
<213> Streptococcus zooepidemicus

<400> 11

Met Lys Thr Ile Ala Tyr Pro Asn Lys Pro His Ser Leu Ser Ala Gly
1 5 10 15

Leu Leu Thr Ala Ile Ala Ile Phe Ser Leu Ala Ser Ser Asn Ile Thr
20 25 30

Tyr Ala Asp Asp Tyr Gln Arg Asn Ala Ala Glu Val Tyr Ala Lys Glu
35 40 45

Val Pro His Gln Ile Thr Ser Val Trp Thr Lys Gly Val Thr Pro Leu
50 55 60

Thr Pro Glu Gln Phe Arg Tyr Asn Asn Glu Asp Val Ile His Ala Pro
65 70 75 80

Tyr Leu Ala His Gln Gly Trp Tyr Asp Ile Thr Lys Val Phe Asp Gly
85 90 95

Lys Asp Asn Leu Leu Cys Gly Ala Ala Thr Ala Gly Asn Met Leu His
100 105 110

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

His Pro Glu Lys Gln Lys Ile Ile Phe Asn Asn Gln Glu Leu Phe Asp
130 135 140

Leu Lys Ala Ala Ile Asp Thr Lys Asp Ser Gln Thr Asn Ser Gln Leu
145 150 155 160

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

Leu Gly Val Met Pro Asp Leu Val Leu Asp Met Phe Ile Asn Gly Tyr
180 185 190

Tyr Leu Asn Val Phe Lys Thr Gln Ser Thr Asp Val Asn Arg Pro Tyr
195 200 205

Gln Asp Lys Asp Lys Arg Gly Gly Ile Phe Asp Ala Val Phe Thr Arg
210 215 220

Gly Asp Gln Thr Thr Leu Leu Thr Ala Arg His Asp Leu Lys Asn Lys

225 230 235 240

Gly Leu Asn Asp Ile Ser Thr Ile Ile Lys Gln Glu Leu Thr Glu Gly
245 250 255

Arg Ala Leu Ala Leu Ser His Thr Tyr Ala Asn Val Ser Ile Ser His
260 265 270

Val Ile Asn Leu Trp Gly Ala Asp Phe Asn Ala Glu Gly Asn Leu Glu
275 280 285

Ala Ile Tyr Val Thr Asp Ser Asp Ala Asn Ala Ser Ile Gly Met Lys
290 295 300

Lys Tyr Phe Val Gly Ile Asn Ala His Gly His Val Ala Ile Ser Ala
305 310 315 320

Lys Lys Ile Glu Gly Glu Asn Ile Gly Ala Gln Val Leu Gly Leu Phe
325 330 335

Thr Leu Ser Ser Gly Lys Asp Ile Trp Gln Lys Leu Ser
340 345

<210> 12
<211> 600
<212> DNA
<213> Streptococcus equi

<400> 12
aaataatttt gtttaacttt aagaaggaga tataaccatg gctctagatg ctacaacggt 60

gttagagcct acaacagcct tcattagaga agctgttagg gaaatcaatc agctgagtga 120

tgactacgct gacaatcaag agcttcaggc tgttcttgct aatgctggag ttgaggcact 180

tgctgcagat actgttgatc aggctaaagc agctcttgac aaagcaaagg cagctgttgc 240

tggtgttcag cttgatgaag caagacgtga ggcttacaga acaatcaatg ccttaagtga 300

tcagcacaaa agcgatcaaa aggttcagct agctctagtt gctgcagcag ctaaggtggc 360

agatgctgct tcagttgatc aagtgaatgc agccattaat gatgctcata cagctattgc 420

ggacattaca ggagcagcct tgttggaggc taaagaagct gctatcaatg aactaaagca 480

gtatggcatt agtgattact atgtgacctt aatcaacaaa gccaaaactg ttgaaggtgt 540

caatgcgctt aaggcaaaga ttttatcagc tctaccgtag ctcgagcccg ggtgctttgc 600

<210> 13
<211> 180
<212> PRT
<213> Streptococcus equi

<400> 13

Met Ala Leu Asp Ala Thr Thr Val Leu Glu Pro Thr Thr Ala Phe Ile
1 5 10 15

Arg Glu Ala Val Arg Glu Ile Asn Gln Leu Ser Asp Asp Tyr Ala Asp
20 25 30

Asn Gln Glu Leu Gln Ala Val Leu Ala Asn Ala Gly Val Glu Ala Leu
35 40 45

Ala Ala Asp Thr Val Asp Gln Ala Lys Ala Ala Leu Asp Lys Ala Lys
50 55 60

Ala Ala Val Ala Gly Val Gln Leu Asp Glu Ala Arg Arg Glu Ala Tyr
65 70 75 80

Arg Thr Ile Asn Ala Leu Ser Asp Gln His Lys Ser Asp Gln Lys Val
85 90 95

Gln Leu Ala Leu Val Ala Ala Ala Ala Lys Val Ala Asp Ala Ala Ser
100 105 110

Val Asp Gln Val Asn Ala Ala Ile Asn Asp Ala His Thr Ala Ile Ala
115 120 125

Asp Ile Thr Gly Ala Ala Leu Leu Glu Ala Lys Glu Ala Ala Ile Asn
130 135 140

Glu Leu Lys Gln Tyr Gly Ile Ser Asp Tyr Tyr Val Thr Leu Ile Asn
145 150 155 160

Lys Ala Lys Thr Val Glu Gly Val Asn Ala Leu Lys Ala Lys Ile Leu
165 170 175

Ser Ala Leu Pro
180

<210> 14
<211> 1158
<212> DNA
<213> Streptococcus equi

<400> 14
atgatgaaaa aacaatcatt cacacactca cgtaaaccta aattcggtat gagaaaatta 60

tctattggcc ttgcctcatg tatgctagga atgatgttcc taacaacagg acatgtttct 120

ggtgaggtag ttgaagtttg gcctaatggg caaaatccta atggtaaaat agaaattcta 180

agtcaaactg agcactctga gcatttacag aaattacgcg atattgaaga tttccaagct 240

caaaagcaag ctgatcatgt tcgttacact aaatggttag atggggtaac tgttgatgag 300

catgaattca gaaaaatcaa ggaatatgac acagaatatt atgtaacacc tcttttaagt 360

ggtaaaggtt actatgatat caataaagat ttcaatcaag atagtgataa atgtgctgcc 420

gctgtagcgg ctaatatgtt ccattattgg tttgatagaa atagagacag tattaatcgt 480

ttcttaagtc aaagtccagg tgaaaatggt gttattaaac ttgaaaatga aaaaacaata 540

gaagtatcaa aatttttaga aacttaccgt agtgatggtg attatcttga taaaagtccg 600

ttttttgacc ttatcagtaa cagctttaaa ggtcctgttt gggcaaataa gctattggat 660

gcttacatta acggctatgg ttatatccat aaatttgcta aaaatactcc acattctaaa 720

aataataata gtaaatttaa tttctttaaa aaagtatttg atggtaatct cttgacagat 780

attcaccaaa tttttgatta taacactttt tcagataaat taagtgaggc tctctatact 840

ggtaaagcca ttggattggc ctacggacct ggagacttgc gtcgttcact aggtcatatt 900

atttctgtct ggggagctga tcttgacgat cagaatcgcg tggtagctat ttatgtaact 960

gattctgatg ataaaaagtt aactatagga aatgagagag ttggtttgaa gcgatataaa 1020

gtatctagcg atgatcaagg tcgtgctcgt ctgacgactc gtgataaaga taacacaggt 1080

ggtgaaattc gatctattga aacattagat atgggtacac aagagtgggc agattacttc 1140

aacaagacag aaaaataa 1158

<210> 15
<211> 1860
<212> DNA
<213> Streptococcus equi

<400> 15
atgaagaaat tcacgaaacg gtgtcttaag ggctgtggtc ttgttggatt agttttcagc 60

acaggattgg ttgccttgtc ggataatatt gatagcgctt taacagtagg ggcggaaacg 120

actactgcta gtgcatttga aaataatggg acaggtcaac atctgaactg gcacatagat 180

attccacaag aatatacagt tgaattagga gaaccaatta ctatctcaga tcttatgagt 240

caaattacgg ttactcgtaa aggtagtaat gggactgtta atgatggaga tacttttgac 300

tttatttcga atggagatgg ttcaagagga attgataccc ctggagtaaa aatatggttt 360

gacttttaca atgctgcggg tacttccttt ttaactgatg aaatgttagc ttcgcctaca 420

tatgctgtac cgggggggatc ttatactatt aaagcttggg tattctatgg gaaaaatgat 480

accaaaaagc tcttcacatt taaactaaaa aattccaaca gcaataaaac tgagttaagg 540

aagtcgttag aggaggctaa gctaaaactc agccagcctg aaggaacgta ttctgatgaa 600

tcactgcaag ccttgcaatc agcggttact cttggtaaga cctatttaaa cagtgaccct 660

gatcaaaata cagtagatca atctgttact actattgatt ccgctattac tagtcttgtt 720

aatcttaatg ctttaaatga agctattaat caagctacac cttttataac agatggcaaa 780

gagtatccta aagaagcgta tgacggtctt gtgcaaaagc ttgcagcggc agctaagctt 840

caaaattcat ttggtccttc acaaggagat gttgataagg ctgcgactga tttaacgcaa 900

gctcttacga cgcttaagac tgctgtagcg catgaagcct tagatcaagc cttggctaag 960

ctgttagagc tttaccgaga aaatccaaat cttgctttga catcagagtc tttgaaggaa 1020

ttgtacaata aggccattga agcagcaggt accttctata gaactgttaa caaggataaa 1080

gagagaaaag acatttccct ttatgagcta gagcgctaca ctacagaaac aaattcagtt 1140

gttgatacta ttttaaaggt aaaggctgcg attgccgaag aaggaaaggc aaaattgcgt 1200

tctgctttag accaattaaa tgctcttatc ggagaaaatc tagacctatc tccatataca 1260

gcagcttctg ctcaagccta tacagaccag ctagctaagg ctaaggaggt cgcagcagcg 1320

ggtgagacag cttatgctca ggagacagaa ccgacagcta ttactaacag cttggttaag 1380

gtgttaaatg ctaagaaatc cctctcagat gccaaggcag ccttggttgc taaaccggtc 1440

gatccagtag atccagtaga cccagtggat ccggtagacc cagtagatcc ggtagaccca 1500

gtggatccgg tagacccagt ggatccagta gacccagtag acccagtaga cccagtggat 1560

ccggtagacc cagtggatcc ggtagacccg gtcgatccaa tcgacccagc ggatccagta 1620

aaaccatcag atcctgaggt taagccagag cctaaaccag aatctaagcc tgaagctaag 1680

aaggaggaca agaaagcagc tgataagcag caagtgcttc cggcaactgc tgatacagct 1740

aatccattct ttacagcagc agctcttgca gttattgctt gtgcaggcca gcttgctatt 1800

gtgtcaagac gcaaagaatc aaattaactg taggcgatga ttttcccct ttaattaatt 1860

<210> 16
<211> 1140
<212> DNA
<213> Streptococcus equi

<400> 16
atgaacaaaa aatcagcaag acgcaggcgt aagaatctta ttacgaagct tgcgatgaca 60

agtgccttaa ccctgggtgt aggcgcagcg actaccctag caggacaaac agaagtacgg 120

gctgataata tcttacgctt agatatgaca gataaagaag cagttgaaaa attcgctaac 180

gagcttaaaa atgaagtcca taaaaactat cgtggtagta atacttggca aaagcttacc 240

cttatactta atggttatca aaaccttaga gaacaaatag agaccgagct aaaaaatagt 300

gaacaaaaag taaaagagct taatgataag gttaatagtg aaactcaagg aaaacaagag 360

ttacagaatc agcttgagaa agaaaaagaa gagttagaaa cactaaaaaa agagcttgaa 420

gctgagaagg ctaaaggaac tggagaaaca gagaagcttc aaaaggaaat tgaagcaaaa 480

aatgcaatga tttctgacct acaaaaacag cttgaggaaa ctaagcaaag ggttcaagag 540

tttgaagctg aagtaggtaa attaatggcc gaaaaggcag acctacaaac aaaattaaat 600

gaacaagagc agcttaacgc taagcttcaa aaagaaattg aagacttaaa ggctcagatt 660

gaaaagctta agcactgtca agatacacct aagccagagc ctaagccaga gcctaagcca 720

gagcctaagc cagagcctaa gccagagcct aagccagagc ctaagccaga gcctaagcca 780

gagcctaagc cagggcctaa gccagagcct aagccagagc ctaagccagg gcctaagcca 840

gagcctaagc cagagcctaa gccagggcct aagccagggc ctaagccaga gcctaagcca 900

gggcctaagc cagagcctaa gccagagcct aagccagagc ctaagcctga agctaagaag 960

cctgaacaac ctaaaccaat gactaaacca ggagctaaga agcctgagca atcacttcca 1020

tcaactggtg acatcagaaa tccattcttc acgcctgcag ctattgctat tatgatcgca 1080

gcaggtacca ttgccattcc aaaacgcaag gaagaagatt aaacaaatta acaatcccca 1140

<210> 17
<211> 1179
<212> DNA
<213> Streptococcus zooepidemicus

<400> 17
atgatgaaaa aacaatcatt cacacactca cgtaaaccta aattcggtat gagaaaatta 60

tctattggcc ttgcctcatg tatgctagga atgatgttcc taacaacaag ccatgtttct 120

ggtgaggtag ttgaagtttg gccttatggg caagatccta atgataaaat agaagtttta 180

agtcaatctg agtattccga atatttacag agattacacg atgttgaaga tttccaagct 240

gaaaagaaaa aagaaggagt tgtccgtaca caatggttag agggtgtgaa cgttactgac 300

catgacttcc ggaaaatcac tgatggtggt agtgtttatt atgccacacc tcttttaaat 360

gatagaggct attatgatat caacaagaat ttcaatcaag acagtgataa atgtgctgct 420

gctgtggcag ttaatatgtt ccattattgg cttgatagga ataaagataa tgtagctaag 480

tttcttagtc aaagtccaga ccatggtttt gttgaaggtg aacctacttt taacttagta 540

gattttcaat atacatatgc atctccatat gaagaaggag gatataggga caatagtaaa 600

ctcttcgact ttattagcaa ggcttttaat aagcctcttt gggcaaataa attgttagat 660

gcttacatta atggctatgg ctatatcgac agatacgtta aaaatacccc gcattctgga 720

caaaataata gtaaatttaa tttctttaaa aaagtatttg atggcaagct cttgacagat 780

attcaacaaa tttttgatta ttatacttta tcgtctgagc tacgtgaagc tcttgatact 840

ggcaaagcta ttggtttagc ctatggacct ggagatttac gccgttctct gggacatatt 900

atctccgtct ggggagctga cattaatgaa gatggaaatg tcgtggctat ttatgtgact 960

gattccgatg ataaaaaatt aactataggg aataaaaaag accgaattgg tttgaagcga 1020

tacaaactgt attctgataa cgtgggacga gctcgcctaa cagcctatgc tacagaaaac 1080

caacaaactg gtggtgaagt tcgagggatt gaaactttag atatggctac acaagattgg 1140

gcagattatt ttagcaggac agacgaagca gaacaataa 1179

<210> 18
<211> 1713
<212> DNA
<213> Streptococcus zooepidemicus

<400> 18
atgaagaaat tcacgaaacg gtgtcttaag ggctgcggtc ttgttggatt agttttcagc 60

acaggattgg ttgccttgtc ggataatatt gatagcgctt taacagtagg ggcggaaacg 120

gctactactg ctaatgcatt tgaagaaagt ggtgaccaac aacataaaaa ttggcatatt 180

tatattccag aggtttatac tgttaaagtc ggtcagccaa tcaccattga ggatatctta 240

agtcagatta cgattactcg taagggagaa gattcgcaag gtaaaacatc tcccggaatg 300

atctatactt atgaagaata ccctaaagta cgaggaattg aagtttcagc aggaactatt 360

tggtttgatt tttataattc tggaaactgg gtaaataatg atgttttagc taccttcaac 420

gaacctggag gaacttatac cttatctgct tgggcatact atgctaacga aaatgtaaaa 480

aaacaatttg ttttcaaact tcaagttgaa aatagtgata agcgtgcatt agaacaatct 540

cttgctactg ctaacgaaaa gttacaggct cctgaaggaa cgtattctga tgaatcactg 600

caacgtttac aagaatcagt tttccttggt caaacttatt tgaacaggga tcctgagcaa 660

caagaagtgg acgatatgaa ggcaactatt gattctgctg tttctggtct tgttgatctt 720

actgtcttaa ataccgcagt tgaaacagca acaccattgt taacagatgg taaggagtat 780

cctaaagaag cgtatgatag ccttgttcaa aagcttgcag cagcagctaa gcttcaaaat 840

tcctttaacc catcacaaga agaagttaac gaggctgcga ctgatttaac gcaagctctt 900

acgacgctta agactgctgt agcgcatgaa gccttagatc aagccttggc taagctgtta 960

gagctttacc gagaaaatcc aaaccttgct ttgacatcag agcctttgaa ggaattgtac 1020

aataaggcca ttgaagcagc aggcaccttc tatagaactg ttagcaagga taaagagaga 1080

aaaggcattt ccctttatga gctagagcgt tacactacag aaacaaactc agttgttgat 1140

actattttaa aggtaaaggc tgcaattgcc gaagaaggaa aggcaaaatt gcgttctgct 1200

ttagaccaat taaatgctct tatcggagaa aatctagacc tatctccata tacagcagct 1260

tctgctcaag cctatacaga ccagctagct aaggctaagg aggttgcagc agcgggtgag 1320

acagcttatg ctcaggagac agaaccgaca gctattacta acagcttgat taaggtgcta 1380

aatgctaaga aatccctctc agatgccaag gcagcattgg ttgctaaacc ggtagatccg 1440

gtagacccag tagatccggt agacccagtg gatccggtag acccaattga tccagtagat 1500

ccagtaaaac cagtcgatcc tgaggttaag ccagagccta aaccagaatc taagcctgaa 1560

gctaagaagg aggacaagaa agcagctgat aagcagcaag tgcttccggc aactgctgat 1620

acagctaacc cattctttac agcagcagct cttgcagtta ttgcttgtgc aggccagctt 1680

gctattgtgt caagacgcaa agaatcaaat taa 1713

<210> 19
<211> 1236
<212> DNA
<213> Streptococcus zooepidemicus

<400> 19
atgaacaaaa aatcagcaag acgcaagcgt aaggatctta tcacgaagct tgcgatgaca 60

agtgccttaa ccctgggtgt aggcgcagca gctaccatag caggacaaac agaagtacgg 120

gctgaggttc taaccttaaa tatgaaagat aaagctaaag ttgaagaatt cgctaataag 180

cttaaagatt acgcaaagca aaagaaatct ggccaaatta ctttgcaaga actttccctt 240

atacttgatg ggtacagaaa tattagggag cagatagaac aagacttagc tactacagaa 300

aaaactaaaa atttctatgg agaacagtta attcttactg ataaacttta tcagtctgaa 360

aaagaaaaga aagaaaagct agaagctgag ctacaactaa gccaacaaaa aattcatgac 420

cttgatgaaa aacatcaaaa agagaaatta gagctacaag aacaacttga ggcttcaaat 480

caaaagatta aagagcttga aatggcaaag agcacagctg aagctgaaat aaatagacta 540

acagctgaaa aaaatggatt acaagaaaaa ttaaataatc aagaaaagct taatgctgag 600

ttacaagcaa aattagctaa gcaagaagag cttaacgcta agcttcaaaa ggaaattgac 660

gaattaaatg ctcagcttga aaagcttaag cattgtcaag atacacctaa gccagagcct 720

aagccagagc ctaagccaga gcctaagcca gagcctaagc cagagcctaa gccagagcct 780

aagccagagc ctaagccaga gcctaagcca gagcctaagc cagagcctaa gccagagcct 840

aagccagagc ctaagccaga gcctaagcca gagcctaagc cagagcctaa gccagagcct 900

aagccagagc ctaagccaga gcctaagcca gagcctaagc cagagcctaa gccagagcct 960

aagccagagc ctaagccaga gcctaagcca gagcctaagc cagagcctaa gccagagcct 1020

aagccagagc ctaagccaga gcctaagcca gagcctaagc ctgaagctaa aaagcctgaa 1080

caacctaaac caatgactaa accaggggct aagaagcctg agcaatcact tccatcaact 1140

ggtgacatca gaaatccatt cttcacacct gcagctattg ctattatgat cgcagcaggt 1200

accattgcaa ttccaaaacg caaggaagaa gactaa 1236

<210> 20

<211> 1050
<212> DNA
<213> Streptococcus equi

<400> 20
atgaaaacaa tagcttatcc aaataaacct cactccttat cagctggtct cttaactgct 60

atagctattt ttagcctggc gagttcaaac attacttatg ctgacgatta ccaaaggaat 120

gctacggaag cttatgccaa agaagtacca catcagatca cttctgtatg gaccaaaggt 180

gttacaccac taacacccga gcagtttcga tataataacg aagatgtgat ccatgcgcca 240

tatcttgctc atcaaggctg gtacgatatc accaaggcct tcgatgggaa ggataatctc 300

ttgtgtggcg cagcaacggc aggtaatatg ctgcattggt ggtttgatca aaataaaaca 360

gagattgaag cctatttaag taaacaccct gaaaagcaaa aaatcatttt taacaaccaa 420

gagctatttg atttgaaagc tgctatcgat accaaggaca gtcaaaccaa tagtcagctt 480

tttaattatt ttagagataa agcctttcca aatctatcag cacgtcaact cggggttatg 540

cctgatcttg ttctagacat gtttatcaat ggttactact taaatgtgtt taaaacacag 600

tctactgatg tcaatcgacc ttatcaggac aaggacaaac gaggtggtat tttcgatgct 660

gttttcacca gaggagatca gacaacgctc ttgacagctc gtcatgattt aaaaaataaa 720

ggactaaatg acatcagcac cattatcaag caagaactga ctgaaggaag agcccttgct 780

ttatcacata cctacgccaa tgttagcatt agccatgtga ttaacttgtg gggagctgat 840

tttaatgctg aaggaaacct tgaggccatc tatgtcacag actcagatgc taatgcgtct 900

attggtatga aaaaatattt tgtcggcatt aatgctcata gacatgtcgc catttctgcc 960

aagaaaatag aaggagaaaa cattggcgct caagtattag gcttatttac gctttccagt 1020

ggcaaggaca tatggcagaa actgagctaa 1050

<210> 21
<211> 1050
<212> DNA
<213> Streptococcus zooepidemicus

<400> 21
atgaaaacaa tagcttatcc aaataaacct cactccttat cagctggtct cttaactgct 60

atagctattt ttagcctggc gagttcaaac attacttatg ctgacgatta ccaaaggaat 120

gctgcggaag tttatgccaa agaagtacca catcagatca cttctgtatg gaccaaaggt 180

gttacaccac taacacccga gcagtttcga tataataacg aagatgtgat ccatgcgcca 240

tatcttgctc atcaaggctg gtacgatatc accaaggtct tcgatgggaa ggataatctc 300

ttgtgtggcg cagcaacggc aggtaatatg ctgcattggt ggtttgatca aaataaaaca 360

gagattgaag cctatttaag taaacaccct gaaaagcaaa aaatcatttt taacaaccaa 420

gagctatttg atttgaaagc tgctatcgat accaaggaca gtcaaaccaa tagtcagctt 480

tttaattatt ttagagataa agcctttcca aatctatcag cacgtcaact cggggttatg 540

cctgatcttg ttctagacat gtttatcaat ggttactact taaatgtgtt taaaacacag 600

tctactgatg tcaatcgacc ttatcaggac aaggacaaac gaggtggtat tttcgatgct 660

gttttcacca gaggagatca gacaacgctc ttgacagctc gtcatgattt aaaaaataaa 720

ggactaaatg acatcagcac cattatcaag caggaactga ctgaaggaag agcccttgct 780

ttatcacata cctacgccaa tgttagcatt agccatgtga ttaacttgtg gggagctgat 840

tttaatgctg aaggaaacct tgaggccatc tatgtcacag actcagatgc taatgcgtct 900

attggtatga aaaaatattt tgtcggcatt aatgctcatg gacatgtcgc catttctgcc 960

aagaaaatag aaggagaaaa cattggcgct caagtattag gcttatttac gctttccagt 1020

ggcaaggaca tatggcagaa actgagctaa 1050

<210> 22
<211> 33
<212> DNA
<213> Artificial

<220>
<223> Oligonucleotides as PCR primer

<400> 22
catgccatgg aggtagttga agtttggcct aat 33

<210> 23
<211> 36
<212> DNA

<213> Artificial

<220>
<223> Oligonucleotides as PCR primer

<400> 23
ccgctcgagt ttttctgtct tgttgaagta atctgc 36

<210> 24
<211> 30
<212> DNA
<213> Artificial

<220>
<223> Oligonucleotides as PCR primer

<400> 24
gtagccatgg aaacgactac tgctagtgca 30

<210> 25
<211> 29
<212> DNA
<213> Artificial

<220>
<223> Oligonucleotides as PCR primer

<400> 25
ctggctcgag cggtttagca accaaggct 29

<210> 26
<211> 31
<212> DNA
<213> Artificial

<220>
<223> Oligonucleotides as PCR primer

<400> 26
catgccatgg cgactaccct agcaggacaa a 31

<210> 27
<211> 31
<212> DNA
<213> Artificial

<220>
<223> Oligonucleotides as PCR primer

<400> 27
ctagctcgag gtgcttaagc ttttcaatct g 31

<210> 28
<211> 594
<212> PRT
<213> Streptococcus equi

<400> 28

Met Ala Thr Asn Leu Ser Asp Asn Ile Thr Ser Leu Thr Val Ala Ser
1 5 10 15

Ser Ser Leu Arg Asp Gly Glu Arg Thr Thr Val Lys Val Ala Phe Asp
20 25 30

Asp Lys Lys Gln Lys Ile Lys Ala Gly Asp Thr Ile Glu Val Thr Trp
35 40 45

Pro Thr Ser Gly Asn Val Tyr Ile Gln Gly Phe Asn Lys Thr Ile Pro
50 55 60

Leu Asn Ile Arg Gly Val Asp Val Gly Thr Leu Glu Val Thr Leu Asp
65 70 75 80

Lys Ala Val Phe Thr Phe Asn Gln Asn Ile Glu Thr Met His Asp Val
85 90 95

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

Thr Ala Glu Thr Ser Gly Thr Thr Thr Val Lys Val Gly Asn Arg Thr
115 120 125

Ala Thr Ile Thr Val Thr Lys Pro Glu Ala Gly Thr Gly Thr Ser Ser
130 135 140

Phe Tyr Tyr Lys Thr Gly Asp Ile Gln Pro Asn Asp Thr Glu Arg Val
145 150 155 160

Arg Trp Phe Leu Leu Ile Asn Asn Asn Lys Glu Trp Val Ala Asn Thr
165 170 175

Val Thr Val Glu Asp Asp Ile Gln Gly Gly Gln Thr Leu Asp Met Ser
180 185 190

Ser Phe Asp Ile Thr Val Ser Gly Tyr Arg Asn Glu Arg Phe Val Gly
195 200 205

Glu Asn Ala Leu Thr Glu Phe His Thr Thr Phe Pro Asn Ser Val Ile
210 215 220

Thr Ala Thr Asp Asn His Ile Ser Val Arg Leu Asp Gln Tyr Asp Ala
225 230 235 240

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

Asp Gln Lys Glu Phe Ala Asn Asn Ser Lys Ile Trp Tyr Gln Ile Leu
260 265 270

Tyr Lys Asp Gln Val Ser Gly Gln Glu Ser Asn His Gln Val Ala Asn
275 280 285

Ile Asn Ala Asn Gly Gly Val Asp Gly Ser Arg Tyr Thr Ser Phe Thr
290 295 300

Val Lys Lys Ile Trp Asn Asp Lys Glu Asn Gln Asp Gly Lys Arg Pro
305 310 315 320

Lys Thr Ile Thr Val Gln Leu Tyr Ala Asn Asp Gln Lys Val Asn Asp
325 330 335

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

Lys Leu Asp Lys Tyr Asp Ser Gln Asn Gln Lys Ile Thr Tyr Ser Val
355 360 365

Lys Glu Val Met Val Pro Val Gly Tyr Gln Ser Gln Val Glu Gly Asp
370 375 380

Ser Gly Val Gly Phe Thr Ile Thr Asn Thr Tyr Thr Pro Glu Val Ile
385 390 395 400

Ser Ile Thr Gly Gln Lys Thr Trp Asp Asp Arg Glu Asn Gln Asp Gly
405 410 415

Lys Arg Pro Lys Glu Ile Thr Val Arg Leu Leu Ala Asn Asp Ala Ala
420 425 430

Thr Asp Lys Val Ala Thr Ala Ser Glu Gln Thr Gly Trp Lys Tyr Thr
435 440 445

Phe Thr Asn Leu Pro Lys Tyr Lys Asp Gly Lys Gln Ile Thr Tyr Thr
450 455 460

Ile Gln Glu Asp Pro Val Ala Asp Tyr Thr Thr Thr Ile Gln Gly Phe
465 470 475 480

Asp Ile Thr Asn His His Glu Val Ala Leu Thr Ser Leu Lys Val Ile
485 490 495

Lys Val Trp Asn Asp Lys Asp Asp Tyr Tyr His Lys Arg Pro Lys Glu
500 505 510

Ile Thr Ile Leu Leu Lys Ala Asp Gly Lys Val Ile Arg Glu His Gln
515 520 525

Met Thr Pro Asp Gln Gln Gly Lys Trp Glu Tyr Thr Phe Asp Gln Leu
530 535 540

Pro Val Tyr Gln Ala Gly Lys Lys Ile Ser Tyr Ser Ile Glu Glu Lys
545 550 555 560

Gln Val Ala Gly Tyr Gln Ala Pro Val Tyr Glu Val Asp Glu Gly Leu
565 570 575

Lys Gln Val Thr Val Thr Asn Thr Leu Asn Pro Ser Tyr Lys Leu Glu
580 585 590

Pro Gly

<210> 29
<211> 302
<212> PRT
<213> Streptococcus equi

<400> 29

Met Thr Asn Lys Thr Lys Arg Thr Gly Leu Val Arg Lys Tyr Gly Ala
1 5 10 15

Cys Ser Ala Ala Ile Ala Leu Ala Ala Leu Ala Ser Leu Gly Ala Gly
20 25 30

Lys Ala Val Lys Ala Asp Gln Pro Ala Ala Leu Lys Tyr Pro Glu Pro
35 40 45

Arg Asp Tyr Phe Leu His Thr Arg Glu Gly Asp Val Ile Tyr Asp Glu
50 55 60

Asp Ile Lys Arg Tyr Phe Glu Asp Leu Glu Ala Tyr Leu Thr Ala Arg
65 70 75 80

Leu Gly Gly Ile Asp Lys Lys Val Glu Glu Ala Ala Gln Lys Pro Gly
85 90 95

Ile Pro Gly Pro Thr Gly Pro Gln Gly Pro Lys Gly Asp Lys Gly Asp
100 105 110

Pro Gly Ala Pro Gly Glu Arg Gly Pro Ala Gly Pro Lys Gly Asp Thr
115 120 125

Gly Glu Ala Gly Pro Arg Gly Glu Gln Gly Pro Ala Gly Gln Ala Gly
130 135 140

Glu Arg Gly Pro Lys Gly Asp Pro Gly Ala Pro Gly Pro Lys Gly Glu
145 150 155 160

Lys Gly Asp Thr Gly Ala Val Gly Pro Lys Gly Glu Lys Gly Asp Thr
165 170 175

Gly Ala Thr Gly Pro Lys Gly Asp Lys Gly Glu Arg Gly Glu Lys Gly
180 185 190

Glu Gln Gly Gln Arg Gly Glu Lys Gly Glu Gln Gly Gln Arg Gly Glu
195 200 205

Lys Gly Glu Gln Lys Pro Lys Gly Asp Gln Gly Lys Asp Thr Lys Pro
210 215 220

Ser Ala Pro Lys Ala Pro Glu Lys Ala Pro Ala Pro Lys Ala Pro Lys
225 230 235 240

Ala Ser Glu Gln Ser Ser Asn Pro Lys Ala Pro Ala Pro Lys Ser Ala
245 250 255

Pro Ser Lys Ser Ala Ala Pro Thr Gly Gln Lys Ala Ala Leu Pro Ala
260 265 270

Thr Gly Glu Ile Asn His Pro Phe Phe Thr Leu Ala Ala Leu Ser Val
275 280 285

Ile Ala Ser Val Gly Val Leu Thr Leu Lys Gly Lys Lys Asp
290 295 300

<210> 30

<211> 320
<212> PRT
<213> Artificial

<220>
<223> Recombinant protein IdeE

<400> 30

Gly Pro Leu Gly Ser Asp Asp Tyr Gln Arg Asn Ala Thr Glu Ala Tyr
1 5 10 15

Ala Lys Glu Val Pro His Gln Ile Thr Ser Val Trp Thr Lys Gly Val
20 25 30

Thr Pro Leu Thr Pro Glu Gln Phe Arg Tyr Asn Asn Glu Asp Val Ile
35 40 45

His Ala Pro Tyr Leu Ala His Gln Gly Trp Tyr Asp Ile Thr Lys Ala
50 55 60

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

Met Leu His Trp Trp Phe Asp Gln Asn Lys Thr Glu Ile Glu Ala Tyr
85 90 95

Leu Ser Lys His Pro Glu Lys Gln Lys Ile Ile Phe Asn Asn Gln Glu
100 105 110

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

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

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

Asn Gly Tyr Tyr Leu Asn Val Phe Lys Thr Gln Ser Thr Asp Val Asn

165 170 175

Arg Pro Tyr Gln Asp Lys Asp Lys Arg Gly Gly Ile Phe Asp Ala Val
180 185 190

Phe Thr Arg Gly Asp Gln Thr Thr Leu Leu Thr Ala Arg His Asp Leu
195 200 205

Lys Asn Lys Gly Leu Asn Asp Ile Ser Thr Ile Ile Lys Gln Glu Leu
210 215 220

Thr Glu Gly Arg Ala Leu Ala Leu Ser His Thr Tyr Ala Asn Val Ser
225 230 235 240

Ile Ser His Val Ile Asn Leu Trp Gly Ala Asp Phe Asn Ala Glu Gly
245 250 255

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

Gly Met Lys Lys Tyr Phe Val Gly Ile Asn Ala His Arg His Val Ala
275 280 285

Ile Ser Ala Lys Lys Ile Glu Gly Glu Asn Ile Gly Ala Gln Val Leu
290 295 300

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

<210> 31
<211> 33
<212> DNA
<213> Artificial

<220>
<223> primer

<400> 31
tactggatcc gacgattacc aaaggaatgc tac 33

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

<220>
<223> primer

<400> 32
tgatctcgag ttagctcagt ttctgccata tg 32