



(86) Date de dépôt PCT/PCT Filing Date: 2008/12/12
(87) Date publication PCT/PCT Publication Date: 2009/06/18
(85) Entrée phase nationale/National Entry: 2010/06/07
(86) N° demande PCT/PCT Application No.: SE 2008/051445
(87) N° publication PCT/PCT Publication No.: 2009/075646
(30) Priorités/Priorities: 2007/12/13 (US61/013,495);
2008/07/21 (US61/082,281)

(51) Cl.Int./Int.Cl. *A61K 39/09* (2006.01),
C07K 14/315 (2006.01), *A61K 39/40* (2006.01),
C07K 16/12 (2006.01)

(71) Demandeur/Applicant:
INTERVACC AB, SE

(72) Inventeurs/Inventors:
GUSS, BENGT, SE;
FLOCK, JAN-INGMAR, SE;
FRYKBERG, LARS, SE;
FLOCK, MARGARETA, SE

(74) Agent: CASSAN MACLEAN

(54) Titre : COMPOSITION D'IMMUNISATION AMELIOREE
(54) Title: IMPROVED IMMUNIZING COMPOSITION

(57) **Abrégé/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. equi* subsp. *equi* and/or subsp. *zooepidemicus*. The present invention also discloses a vaccine composition comprising the aforesaid antigenic composition as immunizing component.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
18 June 2009 (18.06.2009)

PCT

(10) International Publication Number
WO 2009/075646 A1

(51) International Patent Classification:

A61K 39/09 (2006.01) A61K 39/40 (2006.01)
C07K 14/315 (2006.01) C07K 16/12 (2006.01)

(21) International Application Number:

PCT/SE2008/051445

(22) International Filing Date:

12 December 2008 (12.12.2008)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/013,495 13 December 2007 (13.12.2007) US
61/082,281 21 July 2008 (21.07.2008) US

(71) Applicants and

(72) Inventors: **GUSS, Bengt** [SE/SE]; Dag Hammar-skölds väg 238 B, S-756 52 Uppsala (SE). **FLOCK, Jan-Ingmar** [SE/SE]; Sångarvägen 2, S-167 67 Bromma (SE). **FRYK-BERG, Lars** [SE/SE]; Villavägen 22, S-743 91 Storvreta (SE). **FLOCK, Margareta** [SE/SE]; Sångarvägen 2, S-167 67 Bromma (SE).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

(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. equi subsp. 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

5 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*.

10 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
15 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
20 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
25 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
30 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

5 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*.

10 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.

15 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.

20 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.

25 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.

30 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_2 M. 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_2 M-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 ScIC, 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.

5 In the study reported in Lannergård, 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.

10 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.

15 In Lannergård, 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.

20 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.

25 In Waller, A., Flock, M., Smith, K., Robinson, C., Mitchell, Z., Karlström, Å., Lannergård, 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.

30 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.

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*.

5 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.

10 When used generally, the expression "*S. equi*" refers to one or both of subsp. *equi* and subsp. *zooepidemicus*.

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

15 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 hinnies. 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
20 cows, pigs, dogs and cats.

Brief Description of the Drawings

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

25 **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);

30 **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

- 5 **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.
- 10 **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.
- 15 **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.
- 20 *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*.
- 25 **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.
- 30 *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.

5 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.

10

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.

15 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*.

25 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:

30 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;

5 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;

10 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;

15 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, 20 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 25 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 30 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 ScIC (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, ScIC, CNE, Eq5, and Eq8.

5 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.

10 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
15 designated ScIC, 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
20 EAG, ScIC, CNE (or SEC), Eq5, Eq8, IdeE and IdeE2, and according to another embodiment, the antigens EAG, ScIC, 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
25 selected from the group comprising IdeE, Eq5, IdeZ2, Eqz5 and Eqz8 and/or ScIC 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.

30 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*.

5 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*,
10 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
15 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.

20 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.

25 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
30 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.

5 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.

10 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
15 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.

20 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*.

25 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
30 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 heterologous 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 *NcoI* and *XhoI* 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 *NcoI* and *XhoI*.

5

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)

10

15

20

25

30

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 *NcoI* and *XhoI*. 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
5 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
10 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 *Bam*HI and *Xho*I and ligated into the vector pGEX6-P-1 (GE Healthcare), previously digested with the same enzymes.

15 **Example 2. Preparation of antigens CNE, ScIC, 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
20 density (OD₆₀₀) ~ 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
25 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
30 dialysed against phosphate-buffered saline [PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.4 mM KH₂PO₄ (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), ScIC, 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.

5

Example 3. Recombinant IdeE2 cleaves IgG

IdeE 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 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.

25

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.

30

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 1×10^6 CFU in 10 μl 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 than 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).

5 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 than 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
10 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, ScIC

15 Immunisation i.n. with Eq5+ Eq8 and EAG+CNE+ScIC was performed as above with three groups with ten mice in each group. One group with Eq5+Eq8 and one with EAG+CNE+ScIC. 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
20 EAG+CNE+ScIC ($n=10$). 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)

```

5  atgatgaaaaaaciaa
   M M K K Q
   tcattcacacactcacgtaaaccctaaattcgggtatgagaaaattatctattggccttgcc
   S F T H S R K P K F G M R K L S I G L A
   tcatgtatgctaggaatgatgttcctaacaacaggacatgtttctggtgaggtagttgaa
10  S C M L G M M F L T T G H V S G E V V E
   gtttggcctaattgggcaaaatcctaattggtaaaatagaaattctaagtcaaactgagcac
   V W P N G Q N P N G K I E I L S Q T E H
   tctgagcattttacagaaattacgcgatattgaagatttccaagctcaaaagcaagctgat
   S E H L Q K L R D I E D F Q A Q K Q A D
15  catgttcggttacactaaatgggttagatggggtaactgttgatgagcatgaattcagaaaa
   H V R Y T K W L D G V T V D E H E F R K
   atcaaggaatatgacacagaatattatgtaacacctcttttaagtggtaaaagggttactat
   I K E Y D T E Y Y V T P L L S G K G Y Y
   gatatcaataaagattttcaatcaagatagtgataaatgtgctgccgctgtagcggctaata
20  D I N K D F N Q D S D K C A A A V A A N
   atgttccattattgggtttgatagaaatagagacagatttaaatcgtttcttaagtcaaagt
   M F H Y W F D R N R D S I N R F L S Q S
   ccaggtgaaaatgggtgttattaaacttgaaaatgaaaaaacaatagaagtatcaaaattt
   P G E N G V I K L E N E K T I E V S K F
25  ttagaaacttaccgtagtgatgggtgattatcttgataaaagtcggttttttgaccttattc
   L E T Y R S D G D Y L D K S P F F D L I
   agtaacagcttttaaagggtcctgtttgggcaaaataagctattggatgcttacattaacggc
   S N S F K G P V W A N K L L D A Y I N G
   tatgggttatatccataaattttgctaaaaataactccacattctaaaaataataatagtaaa
30  Y G Y I H K F A K N T P H S K N N N S K
   ttttaattttcttttaaaaaaagtatgttgatggtaatctcttgacagatattcaccaaattttt
   F N F F K K V F D G N L L T D I H Q I F
   gattataacacttttttcagataaattaagtgaggctctctataactggtaaaagccattgga
   D Y N T F S D K L S E A L Y T G K A I G
35  ttggcctacggacctggagacttgcgctcgttcactagggtcatattatttctgtctgggga
   L A Y G P G D L R R S L G H I I S V W G
   gctgatcttgacgatcagaatcgcgtaggttagctatttatgtaactgattctgatgataaa
   A D L D D Q N R V V A I Y V T D S D D K
   aagttaactataggaaatgagagagttgggtttgaagcgatataaagtatctagcgatgat
40  K L T I G N E R V G L K R Y K V S S D D
   caaggctcgtgctcgtctgacgactcgtgataaagataacacaggtgggtgaaattcgatct
   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
45  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.

5

MEVVEVWPNGQNPNGKIEILSQTEHSEHLQKLRLDIEDFQAQKQADHVRYTKWLDGVTVD
HEFRKIKEYDTEYYVTPLLSGKGYDINKDFNQDSKCAAAVAANMFHYWFDNRNRSINR
FLSQSPGENGVIKLENEKTI EVSKFLETYRSDGDYLDKSPFFDLISNSFKGPVWANKLLD
AYINGYGYIHKFAKNTPHSKNNNSKFNFFKKVFDGNLLTDIHQIFDYNTFSDKLSEALYT
10 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**)

15

atgaagaaattcacgaaacggtgtccttaagggctgtggtccttggttgattagttttcagc
M K K F T K R C L K G C G L V G L V F S
acaggattggttgcccttgctcggataatattgatagcgctttaacagtaggggcggaacg
20 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
25 caaattacggttactcgtaaaggtagtaatgggactgttaatgatggagatacttttgac
Q I T V T R K G S N G T V N D G D T F D
tttattttcgaatggagatgggttcaagaggaattgatacccctggagtaaaaatatgggtt
F I S N G D G S R G I D T P G V K I W F
gactttttacaatgctgcgggtacttccttttttaactgatgaaatgtagcttcgcctaca
30 D F Y N A A G T S F L T D E M L A S P T
tatgctgtaccgggggggatcttatactattaaagcttgggtattctatgggaaaaatgat
Y A V P G G S Y T I K A W V F Y G K N D
accaaaaagctcttcacatttaaactaaaaaattccaacagcaataaaaactgagttaagg
T K K L F T F K L K N S N S N K T E L R
35 aagtcgtagaggaggctaagctaaaactcagccagcctgaaggaacgtattctgatgaa
K S L E E A K L K L S Q P E G T Y S D E
tcactgcaagccttgcaatcagcgggttactccttggttaagacctatttaaacagtgaccct
S L Q A L Q S A V T L G K T Y L N S D P
gatcaaaatacagtagatcaatctgttactactattgattccgctattactagtcttggt
40 D Q N T V D Q S V T T I D S A I T S L V
aatcttaatgcttttaaataagctattaatcaagctacaccttttataacagatggcaaa
N L N A L N E A I N Q A T P F I T D G K
gagtatcctaagaagcgtatgacgggtccttggtgcaaaagccttgacgcggcagctaagctt
E Y P K E A Y D G L V Q K L A A A A K L
45 caaaattcatttggtccttcacaaggagatggttgataaggctgcgactgatttaacgcaa

22

Q N S F G P S Q G D V D K A A T D L T Q
 gctcttacgacgcttaagactgctgtagcgcatgaagccttagatcaagccttggttaag
 A L T T L K T A V A H E A L D Q A L A K
 ctgttagagctttaccgagaaaaatccaaatcttgctttgacatcagagtcctttgaaggaa
 5 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
 gagagaaaagacatttcccttttatgagctagagcgctacactacagaaacaaattcagtt
 E R K D I S L Y E L E R Y T T E T N S V
 10 gttgataactattttaaaggtaaaggctgcgattgccgaagaaggaaaggcaaaattgcgt
 V D T I L K V K A A I A E E G K A K L R
 tctgcttttagaccaattaatgctcttatcggagaaaatctagacctatctccatataca
 S A L D Q L N A L I G E N L D L S P Y T
 gcagcttctgctcaagcctatacagaccagctagctaaggctaaggagggtcgcagcagcg
 15 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
 gtgttaaatagctaagaaatccctctcagatgccaaaggcagccttggttgctaaaccgggtc
 V L N A K K S L S D A K A A L V A K P V
 20 gatccagtagatccagtagaccagtggtatccggtagaccagtagatccggtagaccga
 D P V D P V D P V D P V D P V D P V D P V D P
 gtggatccggtagaccagtggtatccagtagaccagtagaccagtagaccagtggtat
 V D P V D P V D P V D P V D P V D P V D P V D
 ccggtagaccagtggtatccggtagaccggtcgatccaatcgaccagcggatccagta
 25 P V D P V D P V D P V D P I D P A D P V
 aaaccatcagatcctgagggttaagccagagcctaaaccagaatctaagcctgaagctaag
 K P S D P E V K P E P K P E S K P E A K
 aaggaggacaagaaagcagctgataagcagcaagtgccttccggcaactgctgatacagct
 K E D K K A A D K Q Q V L P A T A D T A
 30 aatccattctttacagcagcagctcttgagttattgcttgtgcaggccagcttgctatt
 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

35 **(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.

40 METTTASAFENNGTGQHLNWHIDIPQEYTVELGEPITISDLMSQITVTRKGSNGTVNDGD
 TFDIFSNGDGSRGIDTPGVKIWFDFYNAAGTSFLTDEMLASPTYAVPGGSYTIKAWVFYG
 KNDTKKLFTFKLKNSNSNKTRELKRSLEEAKLKLSQPEGTYSDSLQALQSAVTLGKTYLN
 SDPDQNTVDQSVTTIDSAITSLVNLNALNEAINQATPFITDGKEYPKEAYDGLVQKLAAA
 AKLQNSFGPSQGDVDKAATDLTQALTTLKTAVAHEALDQALAKLLELYRENPNLALTSES
 LKELYNKAI EAAGTFYRTVNKDKERKDISLYELERYTTETNSVVDITILKVKAIAEEGKA
 45 KLRSALDQLNALIGENLDLSPYTAASAQAYTDQLAKAKEVAAAGETAYAQETEPTAITNS
 LVKVLNAKKSLSDAKAALVAKPLEPG

(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)

```

5  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
10 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
   cttatacttaatgggttatcaaaaccttagagaacaaatagagaccgagctaaaaaatagt
15 L I L N G Y Q N L R E Q I E T E L K N S
   gaacaaaaagtaaaagagcttaatgataagggttaatagtgaactcaaggaaaacaagag
   E Q K V K E L N D K V N S E T Q G K Q E
   ttacagaatcagcttgagaaagaaaaagaagagttagaacactaaaaaaagagcttgaa
   L Q N Q L E K E K E E L E T L K K E L E
20 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
   tttgaagctgaagtaggtaaatattggccgaaaaggcagacctacaaacaaaattaaat
25 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
30 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
35 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
40 tcaactgggtgacatcagaaatccattcttcacgcctgcagctattgctattatgatcgca
   S T G D I R N P F F T P A A I A I M I A
   gcaggtaccattgccattccaaaacgcaaggaagaagattaacaaattaacaatcccca
   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.

5 **MATTL**AGQTEVRADN**I**LRLD**M**TDKEAVE**K**FANEL**K**NEV**H**KNYRGSNTWQ**K**LTL**I**LNGYQ**N**
LREQ**I**ETEL**K**NSE**Q**KVKELNDK**V**NSET**Q**G**K**QELQ**N**QLEKEKEELET**L**KKELEAE**K**AKGT**G**
ETE**K**LQKE**I**EAK**N**AM**I**SDLQ**K**QLEET**K**Q**R**VQEF**E**AEV**G**KLMAEKAD**L**QTK**L**NEQEQ**L**NA**K**
LQKE**I**ED**L**KA**Q**I**E**KL**K**H**L**EP**G**

10 (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*

atgatgaaaaaacaatcattcacacactcacgtaaaccctaaatttcggtatgagaaaatta
 15 M M K K Q S F T H S R K P K F G M R K L
 tctattggccttgccctcatgtatgctaggaatgatgttcctaacaacaagccatgtttct
 S I G L A S C M L G M M F L T T S H V S
 ggtgaggtagttgaagtttggccttatgggcaagatcctaatagataaaatagaagtttta
 G E V V E V W P Y G Q D P N D K I E V L
 20 agtcaatctgagtatccgaatatccacagagattacacgatgttgaagatttccaagct
 S Q S E Y S E Y L Q R L H D V E D F Q A
 gaaaagaaaaaagaaggagttgtccgtacacaatgggttagagggtgtgaacgttactgac
 E K K K E G V V R T Q W L E G V N V T D
 catgacttccggaaaatcactgatgggtggttagtgtttattatgccacacctcttttaaat
 25 H D F R K I T D G G S V Y Y A T P L L N
 gatagaggctattatgatatcaacaagaatttcaatcaagacagtataaatgtgctgct
 D R G Y Y D I N K N F N Q D S D K C A A
 gctgtggcagttaatatgttccattattggccttgataggaataaagataatgtagctaag
 A V A V N M F H Y W L D R N K D N V A K
 30 tttcttagtcaaagtcagaccatgggttttgttgaagggtgaacctacttttaacttagta
 F L S Q S P D H G F V E G E P T F N L V
 gatatttcaatatacatatgcatctccatatgaagaaggaggatatagggacaatagtaaa
 D F Q Y T Y A S P Y E E G G Y R D N S K
 ctcttcgactttatttagcaaggccttttaataagcctctttgggcaaataaattggttagat
 35 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
 caaaataatagtaaattttaatttcttttaaaaaagtatgttgatggcaagctcttgacagat
 Q N N S K F N F F K K V F D G K L L T D
 40 attcaacaaatttttgattattatactttatcgtctgagctacgtgaagctcttgatact
 I Q Q I F D Y Y T L S S E L R E A L D T
 ggcaaagctattgggttagcctatggacctggagatttacgccgttctctgggacatatt
 G K A I G L A Y G P G D L R R S L G H I
 atctccgtctggggagctgacattaatgaagatggaaatgtcgtggctatttatgtgact
 45 I S V W G A D I N E D G N V V A I Y V T

25

gattccgatgataaaaaattaactataggggaataaaaaagaccgaattggtttgaagcga
 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
 5 caacaaactgggtgggtgaagttcgagggattgaaacttttagatatggctacacaagattgg
 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 -

- 10 **(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
 15 M K K F T K R C L
 aagggctgcggtcttggattagttttcagcacaggattggttgccttgtcggataat
 K G C G L V G L V F S T G L V A L S D N
 attgatagcgcctttaacagtaggggacgaaacggctactactgctaatagcatttgaagaa
 I D S A L T V G A E T A T T A N A F E E
 20 agtgggtgaccaacaacataaaaaattggcatatattatattccagagggtttataactgttaaa
 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
 gaagattcgcaaggtaaaacatctcccgaatgatctatacttatgaagaataccctaaa
 25 E D S Q G K T S P G M I Y T Y E E Y P K
 gtacgaggaattgaagtttcagcaggaactatgttggtttgattttttataattctggaaac
 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
 30 gcttgggcatactatgctaacgaaaatgtaaaaaaacaatttggttttcaaacttcaagtt
 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
 gctcctgaaggaacgtattctgatgaatcactgcaacggtttacaagaatcagttttcctt
 35 A P E G T Y S D E S L Q R L Q E S V F L
 ggtcaaacttatttgaacagggatcctgagcaacaagaagtgagcagatatgaaggcaact
 G Q T Y L N R D P E Q Q E V D D M K A T
 attgattctgctgtttctgggtcttggatgcttactgtcttaataaccgcagttgaaaca
 I D S A V S G L V D L T V L N T A V E T
 40 gcaacaccattgttaacagatggtaaggagatcctaaagaagcgtatgatagccttggt
 A T P L L T D G K E Y P K E A Y D S L V
 caaaagcttgcagcagcagctaagcttcaaaattcctttaaccatcacagaagaagtt
 Q K L A A A A K L Q N S F N P S Q E E V
 aacgaggctgcgactgatttaacgcaagctcttacgacgcttaagactgctgtagcgc
 45 N E A A T D L T Q A L T T L K T A V A H
 gaagccttagatcaagccttgggctaagctgttagagctttaccgagaaaatccaaacctt

26

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
 ttctatagaactgtagcaaggataaagagagagaaaaggcatttccctttatgagctagag
 5 F Y R T V S K D K E R K G I S L Y E L E
 cgttacactacagaaacaaactcagttggtgatactattttaaaggtaaggctgcaatt
 R Y T T E T N S V V D T I L K V K A A I
 gccgaagaaggaaaggcaaaattgcgttctgcttttagaccaattaaatgctccttatcgga
 A E E G K A K L R S A L D Q L N A L I G
 10 gaaaatctagacctatctccatatacagcagcttctgctcaagcctatacagaccagcta
 E N L D L S P Y T A A S A Q A Y T D Q L
 gctaaggctaaggagggttgagcagcgggtgagacagcttatgctcaggagacagaaccg
 A K A K E V A A A G E T A Y A Q E T E P
 acagctattactaacagcttgattaaggtgctaaatgctaagaaatccctctcagatgcc
 15 T A I T N S L I K V L N A K K S L S D A
 aaggcagcattgggttgctaaaccggtagatccggtagaccagtagatccggtagacca
 K A A L V A K P V D P V D P V D P V D P
 gtggatccggtagaccaattgatccagtagatccagtaaaaccagtcgacccctgaggtt
 V D P V D P I D P V D P V K P V D P E V
 20 aagccagagcctaaaccagaatctaagcctgaagctaagaaggaggacaagaaagcagct
 K P E P K P E S K P E A K K E D K K A A
 gataagcagcaagtgcttccggcaactgctgatacagctaaccattctttacagcagca
 D K Q Q V L P A T A D T A N P F F T A A
 gctcttgagcttattgcttgtgcaggccagcttgctattgtgtcaagacgcaaagaatca
 25 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
 30 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
 35 agacgcaagcgtaaggatcttatcacgaagcttgcgatgacaagtgccttaaccctgggt
 R R K R K D L I T K L A M T S A L T L G
 gtaggcgcagcagctaccatagcaggacaaacagaagtacgggctgaggttctaaccctta
 V G A A A T I A G Q T E V R A E V L T L
 aatatgaaagataaagctaaagttgaagaattcgctaataagcttaagattacgcaaag
 40 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
 45 ggagaacagttaattcttactgataaactttatcagtctgaaaaagaaaagaaaag
 G E Q L I L T D K L Y Q S E K E K K E K

27

ctagaagctgagctacaactaagccaacaaaaaattcatgaccttgatgaaaaacatcaa
 L E A E L Q L S Q Q K I H D L D E K H Q
 aaagagaaattagagctacaagaacaacttgaggcttcaaatacaaaagattaaagagctt
 K E K L E L Q E Q L E A S N Q K I K E L
 5 gaaatggcaaagagcacagctgaagctgaaataaatagactaacagctgaaaaaatgga
 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
 10 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
 15 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
 20 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
 gagcctaagccagagcctaagcctgaagctaaaaagcctgaacaacctaataacaaatgact
 E P K P E P K P E A K K P E Q P K P M T
 25 aaaccaggggctaagaagcctgagcaatcacttccatcaactgggtgacatcagaaatcca
 K P G A K K P E Q S L P S T G D I R N P
 ttcttcacacctgcagctattgctattatgatcgagcaggtaccattgcaattccaaaa
 F F T P A A I A I M I A A G T I A I P K
 cgcaaggaagaagactaa
 30 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**).

35

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.

40

45

atgaaaacaatagcttatccaaataaacctcactccttatcagctgggtctcttaactgct
 M K T I A Y P N K P H S L S A G L L T A
 atagctatTTTTtagcctggcgagttcaaacattacttatgctgacgattaccaaggaat
 I A I F S L A S S N I T Y A D D Y Q R N
 gctacggaagcttatgccaaagaagtaccacatcagatcacttctgtatggaccaaggt
 A T E A Y A K E V P H Q I T S V W T K G
 45 gttacaccactaacaccccgagcagtttcgatataataacgaagatgtgatccatgcgcca

28

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
 ttgtgtggcgcagcaacggcaggtaatatgctgcattgggtgggtttgatcaaaataaaaca
 5 L C G A A T A G N M L H W W F D Q N K T
 gagattgaagcctattttaagtaaacaccctgaaaagcaaaaaaatcattttttaacaaccaa
 E I E A Y L S K H P E K Q K I I F N N Q
 gagctattttgatttgaaagctgctatcgataccaaggacagtcaaaccaatagtcagcctt
 E L F D L K A A I D T K D S Q T N S Q L
 10 tttaattatttttagagataaagcctttccaaatctatcagcacgtcaactcgggggttatg
 F N Y F R D K A F P N L S A R Q L G V M
 cctgatcttggttctagacatgtttatcaatgggttactacttaaattgtgtttaaaacacag
 P D L V L D M F I N G Y Y L N V F K T Q
 tctactgatgtcaatcgaccttatcaggacaaggacaaacgaggtgggtatttttcgatgct
 15 S T D V N R P Y Q D K D K R G G I F D A
 gttttcaccagaggagatcagacaacgctcttgacagctcgtcatgatttaaaaaataaaa
 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
 20 ttatcacatacctacgccaatgtagcattagccatgtgattaacttggtggggagctgat
 L S H T Y A N V S I S H V I N L W G A D
 tttaatgctgaaggaaaccttgaggccatctatgtcacagactcagatgctaatagcgtct
 F N A E G N L E A I Y V T D S D A N A S
 attgggtatgaaaaaatattttgtcggcattaatgctcatagacatgtcgcctatttctgcc
 25 I G M K K Y F V G I N A H R H V A I S A
 aagaaaatagaaggagaaaacattggcgcgtcaagtattaggcttatttacgctttccagt
 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 -

30

(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**).

35

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.

40

45

atgaaaacaatagcttatccaaataaacctcactccttatcagctgggtctctttaactgct
 M K T I A Y P N K P H S L S A G L L T A
 atagctatttttagcctggcgcaggttcaaacattacttatgctgacgattaccaaggaat
 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
 gttacaccactaacacccgagcagtttctgatataataacgaagatgtgatccatgcgcc
 45 V T P L T P E Q F R Y N N E D V I H A P

tatcttgctcatcaaggctggtacgatatcaccaaggctcttcgatgggaaggataatctc
 Y L A H Q G W Y D I T K V F D G K D N L
 ttgtgtggcgcagcaacggcaggtaatatgctgcattgggtgggtttgatcaaaataaaaca
 L C G A A T A G N M L H W W F D Q N K T
 5 gagattgaagcctattttaagtaaaccacctgaaaagcaaaaaatcattttttaacaaccaa
 E I E A Y L S K H P E K Q K I I F N N Q
 gagctattttgatttgaaagctgctatcgataccaaggacagtcaaaccaatagtcagctt
 E L F D L K A A I D T K D S Q T N S Q L
 ttttaattatttttagagataaagcctttccaaatctatcagcacgtcaactcgggggttatg
 10 F N Y F R D K A F P N L S A R Q L G V M
 cctgatcttggttctagacatgtttatcaatgggttactacttaaatgtgtttaaaacacag
 P D L V L D M F I N G Y Y L N V F K T Q
 tctactgatgtcaatcgaccttatcaggacaaggacaaacgaggtgggtattttcgatgct
 S T D V N R P Y Q D K D K R G G I F D A
 15 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
 ttatcacatacctacgccaatgtagcattagccatgtgattaacttgtggggagctgat
 20 L S H T Y A N V S I S H V I N L W G A D
 tttaatgctgaaggaaaccttgaggccatctatgtcacagactcagatgctaatacgctct
 F N A E G N L E A I Y V T D S D A N A S
 attgggtatgaaaaaatattttgtcggcattaatgctcatggacatgtcgccatttctgcc
 I G M K K Y F V G I N A H G H V A I S A
 25 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 -

30 (12) SEQ ID NO: 12

Nucleotide sequence of the *eag* gene

1 aaataattttgttttaactttaagaaggagatataaccatggctctagatg
 51 ctacaacggtgtagagcctacaacagccttcattagagaagctgtagg
 35 101 gaaatcaatcagctgagtgatgactacgctgacaatcaagagcttcaggc
 151 tgttcttgctaatactggtgaggtgagggcacttgctgcagatactggtgatc
 201 aggctaaagcagctcttgacaaagcaaaggcagctggtgctggtggtcag
 251 cttgatgaagcaagacgtgaggcttacagaacaatcaatgccttaagtga
 301 tcagcacaaaagcgatcaaaagggttcagctagctctagttgctgcagcag
 40 351 ctaagggtggcagatgctgcttcagttgatcaagtgaatgcagccattaat
 401 gatgctcatacagctattgaggacattacaggagcagccttggtggaggc
 451 taaagaagctgctatcaatgaactaaagcagtatggcattagtgattact
 501 atgtgaccttaatacaaaagccaaaactggtgaagggtgcaatgcgctt
 551 aaggcaaagattttatcagctctaccgtagctcgagcccggtgctttgc

30

(13) SEQ ID NO: 13

Amino acid sequence of the EAG4B protein

5 1 MALDATTVLE PTTAFIREAV REINQLSDDY ADNQELQAVL ANAGVEALAA DTVDQAKAAL
 61 DKAKAAVAGV QLDEARREAY RTINALSDQH KSDQKVQLAL VAAAANKVADA ASVDQVNAAI
 121 NDAHTAIADI TGAALLEAKE AAINELKQYG ISDYVTLIN KAKTVEGVNA LKAKILSALP

(14) SEQ ID NO: 28

Protein sequence of SEC2.16 (CNE)

15 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 20 25 30

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

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

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

35 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
 40 100 105 110

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

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

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

31

	Arg	Trp	Phe	Leu	Leu	Ile	Asn	Asn	Asn	Lys	Glu	Trp	Val	Ala	Asn	Thr
				165						170					175	
5	Val	Thr	Val	Glu	Asp	Asp	Ile	Gln	Gly	Gly	Gln	Thr	Leu	Asp	Met	Ser
				180					185					190		
10	Ser	Phe	Asp	Ile	Thr	Val	Ser	Gly	Tyr	Arg	Asn	Glu	Arg	Phe	Val	Gly
			195					200					205			
15	Glu	Asn	Ala	Leu	Thr	Glu	Phe	His	Thr	Thr	Phe	Pro	Asn	Ser	Val	Ile
	210						215					220				
20	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	
25	Asp	Gln	Lys	Glu	Phe	Ala	Asn	Asn	Ser	Lys	Ile	Trp	Tyr	Gln	Ile	Leu
				260					265					270		
30	Tyr	Lys	Asp	Gln	Val	Ser	Gly	Gln	Glu	Ser	Asn	His	Gln	Val	Ala	Asn
			275					280					285			
35	Ile	Asn	Ala	Asn	Gly	Gly	Val	Asp	Gly	Ser	Arg	Tyr	Thr	Ser	Phe	Thr
	290						295					300				
40	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	
45	Lys	Thr	Ile	Glu	Leu	Ser	Asp	Thr	Asn	Ser	Trp	Gln	Ala	Ser	Phe	Gly
				340				345						350		
50	Lys	Leu	Asp	Lys	Tyr	Asp	Ser	Gln	Asn	Gln	Lys	Ile	Thr	Tyr	Ser	Val
			355					360					365			
55	Lys	Glu	Val	Met	Val	Pro	Val	Gly	Tyr	Gln	Ser	Gln	Val	Glu	Gly	Asp
	370						375					380				

32

	Ser	Gly	Val	Gly	Phe	Thr	Ile	Thr	Asn	Thr	Tyr	Thr	Pro	Glu	Val	Ile
	385					390					395					400
5	Ser	Ile	Thr	Gly	Gln	Lys	Thr	Trp	Asp	Asp	Arg	Glu	Asn	Gln	Asp	Gly
					405					410					415	
10	Lys	Arg	Pro	Lys	Glu	Ile	Thr	Val	Arg	Leu	Leu	Ala	Asn	Asp	Ala	Ala
				420					425					430		
15	Thr	Asp	Lys	Val	Ala	Thr	Ala	Ser	Glu	Gln	Thr	Gly	Trp	Lys	Tyr	Thr
			435					440					445			
20	Phe	Thr	Asn	Leu	Pro	Lys	Tyr	Lys	Asp	Gly	Lys	Gln	Ile	Thr	Tyr	Thr
	450						455					460				
25	Ile	Gln	Glu	Asp	Pro	Val	Ala	Asp	Tyr	Thr	Thr	Thr	Ile	Gln	Gly	Phe
	465					470					475					480
30	Asp	Ile	Thr	Asn	His	His	Glu	Val	Ala	Leu	Thr	Ser	Leu	Lys	Val	Ile
					485					490					495	
35	Lys	Val	Trp	Asn	Asp	Lys	Asp	Asp	Tyr	Tyr	His	Lys	Arg	Pro	Lys	Glu
				500					505					510		
40	Ile	Thr	Ile	Leu	Leu	Lys	Ala	Asp	Gly	Lys	Val	Ile	Arg	Glu	His	Gln
			515					520					525			
45	Met	Thr	Pro	Asp	Gln	Gln	Gly	Lys	Trp	Glu	Tyr	Thr	Phe	Asp	Gln	Leu
	530						535					540				
50	Pro	Val	Tyr	Gln	Ala	Gly	Lys	Lys	Ile	Ser	Tyr	Ser	Ile	Glu	Glu	Lys
	545					550					555					560
55	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														

33

(15) SEQ ID NO 29

Protein sequence of SclC

5 Met Thr Asn Lys Thr Lys Arg Thr Gly Leu Val Arg Lys Tyr Gly Ala
 1 5 10 15
 10 Cys Ser Ala Ala Ile Ala Leu Ala Ala Leu Ala Ser Leu Gly Ala Gly
 20 25 30
 15 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
 20 Asp Ile Lys Arg Tyr Phe Glu Asp Leu Glu Ala Tyr Leu Thr Ala Arg
 65 70 75 80
 25 Leu Gly Gly Ile Asp Lys Lys Val Glu Glu Ala Ala Gln Lys Pro Gly
 85 90 95
 30 Ile Pro Gly Pro Thr Gly Pro Gln Gly Pro Lys Gly Asp Lys Gly Asp
 100 105 110
 35 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
 40 Glu Arg Gly Pro Lys Gly Asp Pro Gly Ala Pro Gly Pro Lys Gly Glu
 145 150 155 160
 45 Lys Gly Asp Thr Gly Ala Val Gly Pro Lys Gly Glu Lys Gly Asp Thr
 165 170 175
 50 Gly Ala Thr Gly Pro Lys Gly Asp Lys Gly Glu Arg Gly Glu Lys Gly
 180 185 190
 55 Glu Gln Gly Gln Arg Gly Glu Lys Gly Glu Gln Gly Gln Arg Gly Glu
 195 200 205

34

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

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

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

15 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

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

25 **(16) SEQ ID NO 30.** Recombinant protein IdeE
GPLGSDDYQRNATEAYAKEVPHQITSVWTKGVTPLTPEQFRYNNEDVIHAPYL
A
 HQGWYDITKAFDGKDNLLCGAATAGNMLHWWFDQNKTEIEAYLSKHPEKQKII
 30 FNNQELF
 DLKAAIDTKDSQTNSQLFNIFRDKAFPNLSARQLGVMPDLVLDMFINGYYLNVF
 KTQSTD
 VNRPYQDKDKRGGIFDAVFTRGDQTTLLTARHDLKNKGLNDISTIHKQELTEGRA
 LALSH
 35 TYANVSISHVINLWGADFNAEGNLEAIYVTDSDANASIGMKKYFVGINAHHRHVAI
 SAKKI
 EGENIGAQVLGLFTLSSGKDIWQKLS

Amino acids in bold originates from the vector.

40

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
 45 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.

5 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

10 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

15 The Pentavac vaccine consisted of the following five *S. equi* recombinant proteins: EAG, ScIC, 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
20 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
25 µg/dose.

The Nordostrep Septavac formulation

The Septavac vaccine consisted of the following seven *S. equi* recombinant proteins: EAG, ScIC, 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
5 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
10 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

15 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 1×10^8 cfu of *S. equi*
20 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
25 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
30 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.

5 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.

10 Each pony was challenged with a total challenge dose of 1×10^8 cfu of *S. equi* strain 4047 administered via the spraying of a 2ml culture containing 5×10^7 cfu into both nostrils. This dose regime is believed to optimise the infection rate whilst avoiding overwhelming the host immune response.

15 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
20 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	Fri	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	Wed	02/04/2008 to 09/04/2008	Obs/temps		
day 60	Thurs	10/04/2008	Obs/temps, V2		7 contr and 7 vaccinated
day 61 to 68	Fri	11/04/2008 to 18/04/2008	Obs/temps		
day 71	Mon	21/04/2008	Obs/temps, NW, BL	20ml normal	IgG and CFU from NW; ELISA for IgG from sera
day 72	Tues	22/04/2008	Obs/temps		
day 73	Wed	23/04/2008	Obs/temps		
day 74	Thurs	24/04/2008	Obs/temps, V3		7 contr and 7 vaccinated
day 75 to 81	Fri	25/04/2008 to 2/05/2008	Obs/temps		
day 86	Tues	06/05/2008	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
day 87	Wed	07/05/2008	Obs/temps		Move to Allen Centre
day 88	Thurs	08/05/2008	Obs/temps, Challenge		
day 89	Fri	09/05/2008	Obs/temps, NW, BL		IgG and CFU from NW; ELISA for IgG from sera; fibrinogen and neutrophil levels to be quantified
day 90	Sat	10/05/2008	Obs/temps	20 ml normal, 10 ml EDTA	
day 91	Sun	11/05/2008	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

5 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

15 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

25 2ml subcutaneous injection (1ml on each side of the head)

4ml intranasal injection (2ml in each nostril)

Day 4; 60; 74

30 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
35 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
40 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 $= 4 \times 34.25 \times 10^5 \times 10$
 $= 1.37 \times 10^8$ cfu/dose

5

3 ANIMAL MANAGEMENT

3.1 Supply

10

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

15

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

20

25

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

30

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*.

35

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 pyrexia 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 1×10^8 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 1.37×10^8 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 500 μl sample of the nasal wash was added to 500 μl 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.

5 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
10 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
Empyema of guttural pouch:	5
Scarring of guttural pouch:	5
Enlarged lymph node:	1
Follicular hyperplasia of guttural pouch:	1

4.8 Histopathological examination

15 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.
20

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:

- 5 • Pony 9229 was pyrexia 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
10 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.

15 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

20 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

25 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 pyrexia post V1, and 3 ponies of the Pentavac group and no controls were
30 pyrexia 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 pyrexia 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
 5 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.

10 **Table 7: Number of days pyrexia 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
 15 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

5 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	<i>S.equi</i> 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

5

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

10

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 empyema: 5

Sep = Septavac

Con = Control

9. Pentavac A vaccination study

5 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
10 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

15 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

20 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
25 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
30 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.

REFERENCES

1. Courtney, H. S., Y. Li, J. B. Dale, and D. L. Hasty. 1994. Cloning, sequencing and expression of a fibronectin/fibrinogen-binding protein from group A streptococci. *Infect. Immun.* **62**:3937-3946.
- 5 2. Cue, D., P. E. Dombek, H. Lam, and P.P. Cleary. 1998. *Streptococcus pyogenes* serotype M1 encodes multiple pathways for entry into human epithelial cells. *Infect. Immun.* **66**:4593-4601.
3. Barnham, M., A. Ljunggren, and M. McIntyre. 1987. Human infection with *Streptococcus zooepidemicus* (Lancefield group C): three case reports. *Epidem. Inf.* **98**: 183-190.
- 10 4. Galán, J.E., and J. F. Timoney. 1988. Immunologic and genetic comparison of *Streptococcus equi* isolates from the United States and Europe. *J. Clin. Microbiol.* **26**:1142-1146.
5. Flock, M., Jacobsson, K., Frykberg, L., Hirst, T., R., Franklin, A., Guss, B. and Flock, J.-I. (2004) *Infect Immun* 72:3228-3236.
- 15 6. Engvall, E., E. Ruoslahti, and J. M. Miller. 1978. Affinity of fibronectin to collagen of different genetic types and to fibronogen. *J. Exp. Med.* **147**:1584-1595.
7. Patti, J. M., Jonsson, H., Guss, B., Switalski, L.M., Wiberg, K., Lindberg, M., and Höök, M. (1992) Molecular characterization and expression of a gene encoding a *Staphylococcus aureus* collagen adhesin. *J. Biol. Chem.* **267**:4766-4772.
- 20 8. Jonsson, H., Lindmark, H., and Guss, B. (1995) A protein G related cell surface protein in *Streptococcus zooepidemicus*. *Infect Immun* **63**:2968-2975.
9. Lindmark, H., Jacobsson, K., Frykberg, L., and Guss, B. (1996) Fibronectin-binding protein of *Streptococcus equi* subspecies *zooepidemicus*. *Infect Immun* **64**:3993-3999.
- 25 10. Jacobsson, K., Jonsson, H., Lindmark, H., Guss, B., Lindberg, M., and Frykberg, L. (1997) Shot-gun phage display mapping of two streptococcal cell-surface proteins. *Microbiol Res.* **152**:1-8.
11. Lindmark, H., Jonsson, P., Olsson-Engvall, E., and Guss, B. (1999) Pulsed-field gel electrophoresis and distribution of the genes *zag* and *fnz* in isolates of *Streptococcus equi*. *Res Vet Sci.* **66**:93-99.
- 30 12. Lindmark, H., and Guss, B. (1999) SFS, a novel fibronectin-binding protein from *Streptococcus equi*, inhibits the binding between fibronectin and collagen. *Infect. Immun.* **67**: 2383-2388.
13. Lindmark, H. (1999) Characterization of adhesive extracellular proteins from *Streptococcus equi*. (Doctoral thesis) Acta Universitatis Agriculturae Sueciae, Agraria 139. ISBN 91-576-5488-3.
- 35

14. Lindmark, H., Nilsson, M., and Guss, B. (2001) Comparison of the fibronectin-binding protein FNE from *Streptococcus equi* subspecies *equi* with FNZ from *S. equi* subspecies *zooepidemicus* reveals a major and conserved difference. **69**: 3159-3163.
15. Schneewind, O., Fowler, A. and Faull, K.F. (1995) Structure of the cell wall anchor of surface proteins in *Staphylococcus aureus*. *Science* 268:103-106.
16. Anton Mayr et al. Handbuch der Schutzimpfungen in der Tiermedizin.3.3.4. p. 196-200. Verlag Paul Parey. Berlin and Hamburg. 11984
17. Elson, C.O., and M.T. Dertzbaugh. 1999. p. 817-838, Mucosal immunology, 2nd ed. Of Academic Press, New York, N.Y.
18. Winner et al. 1991. *Infect. Immun.* **59**:997-982
19. Ogra et al. *Clinical Microbiology Reviews*, Apr. 2002, p. 430-445.
20. B. Morein and Karin Lövgren Bengtsson 1998, **76**:295-299. *Immunology and Cellbiology*
21. Hanski, E., and M.G. Caparon. 1992. Protein F, a fibronectin-binding protein, is an adhesin of the group A streptococcus *Streptococcus pyogenes*. *Proc. Natl. Acad. Sci. USA* **89**:6172-6176.
22. Hanski, E., P.A. Horwitz, and M.G. Caparon. 1992. Expression of protein F, the Fibronectin-binding protein of *Streptococcus pyogenes* JRS4, in heterologous streptococcal and enterococcal strains promotes their adherence to respiratory epithelial cells. *Infect. Immun.* **60**:5119-5125.
23. Jadoun, J., V. Ozeri, E. Burstein, E. Skutelsky, E. Hanski, and S. Sela. 1998. Protein F1 required for efficient entry of *Streptococcus pyogenes* into epithelial cells. *J. Infect. Dis.* **178**:147-158.
24. Molinari, G., S. R. Talay, P. Valentin-Weigand, M. Rohde, and G.S. Chhatwal. 1997. The fibronectin-binding protein of *Streptococcus pyogenes* SfbI, is involved in the internalization of group A streptococci by epithelial cells. *Infect. Immun.* **65**:1357-1363.
25. Lannergård, J., Frykberg, L. and Guss B. (2003) CNE, a collagen-binding protein of *Streptococcus equi*. *FEMS Microbiol. Lett.* **222**:69-74.
26. Lannergård, J. and Guss, B. (2006) *FEMS Microbiol Lett* **262**: 230-235.
27. Waller, A., Flock, M., Smith, K., Robinson, C., Mitchell, Z., Karlström, Å., Lannergård, J., Bergman, R., Guss, B. and Flock, J.-I. (2007) *Vaccine* **25**: 3629-3635.
28. WO 92/07002
29. WO 00/37496
30. WO 2007/115059

CLAIMS

1. 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;
- 5 a protein or polypeptide which is designated IdeE and has an amino acid sequence as shown in SEQ ID NO: 10;
- 10 a protein or polypeptide which is designated IdeE2 and has an amino acid sequence as shown in SEQ ID NO: 1;
- 15 a protein or polypeptide which is designated Eq5 and has an amino acid sequence as shown in SEQ ID NO: 3;
- 20 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;
- 25 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 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.
2. The antigenic composition of claim 1, wherein said at least one protein or polypeptide is selected from the group consisting of IdeE and IdeE2 and wherein a composition that comprises IdeE2 comprises at least one further antigen.
- 30 3. The antigenic composition of claim 1 or 2, wherein said at least one protein or polypeptide is selected from the group consisting of Eq5 and Eq8.
4. The antigenic composition of claim 1, 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.
- 35

5. The antigenic composition of claim 4, wherein said at least one protein or polypeptide is selected from the group comprising IdeE and IdeE2.

6. The antigenic composition of claim 1, which comprises the antigens EAG, SclC, CNE (or SEC), Eq5, Eq8, IdeE and IdeE2, or which composition comprises the
5 antigens EAG, SclC, CNE (or SEC), Eq5, and Eq8.

7. The antigenic composition of claim 1, 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.

8. The antigenic composition of any preceding claim, wherein at least one antigen is recombinantly produced.

9. The antigenic composition of any one of claims 1-8, wherein at least one antigen is an isolated or purified antigen.

10. The antigenic composition of any preceding claim, which comprises at least
15 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*.

11. The antigenic composition of claim 10, wherein the vector is an expression
20 vector which is a plasmid or a viral vector and wherein said polynucleotide has a nucleotide sequence as shown in SEQ ID NOS: 12 and 14-21.

12. The antigenic composition of any one of claims 1-11, which is an immunogenic composition.

13. A vaccine composition for protecting non-human mammals against infection
25 of *Streptococcus equi*, which comprises the antigenic composition of any one of claims 1-12 as immunizing component, and a pharmaceutically acceptable carrier.

14. The vaccine composition of claim 13, which further comprises an adjuvant.

15. The vaccine composition of any one of claims 13-14, which is a vaccine that protects susceptible mammals, suitably horses, against strangles caused by *Streptococcus*
30 *equi* subsp. *equi*.

16. The vaccine composition of claim 13, which is provided in a physiologically administrable form, and suitably is administrable by subcutaneous or intranasal inoculation.

17. The vaccine composition of claim 15 or 16, which stimulates serum, mucosal and/or bronchial lavage antibody responses directed to *Streptococcus equi* antigens
35 in mammals susceptible to *Streptococcus equi*, suitably horses.

18. A method for producing an antigen or an immunogen of an antigenic composition of any one of claims 1-12, 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, and, optionally,
(e) purifying the isolated product from step (d) by affinity chromatography or other chromatographic methods known in the art.

19. A method for preparation of a vaccine composition according to any one of claims 13-17, which vaccine composition contains as immunizing component, an antigenic or immunogenic composition of any one of claims 1-12, said method comprising mixing said antigenic or immunogenic composition and a pharmaceutically acceptable carrier.

20. Use of an antigenic or immunogenic composition of any one of claims 1-12 in the preparation of a vaccine protecting 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 preparation of any one of claims 1-12 to an animal host to produce antibodies in said animal host and recovering antiserum containing said antibodies produced in said animal host.

22. 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 composition of any one of claim 13-17 or an antiserum produced according to claim 21.

23. A method for protecting horses against *Streptococcus equi* infection, which comprises inoculating a horse subcutaneously or intranasally with a vaccine composition of any one of claims 13-17 to induce an immune response against *Streptococcus equi* in said horse.

24. The method of claim 23, wherein an immune response in the form of IgG and/or IgA and/or IgM antibodies in the nasopharyngeal mucus is induced in said horse.

25. An antibody preparation comprising at least one, and suitably at least two, antibodies specific for a protein or polypeptide of the composition of any one of claims 1-12, which antibody/antibodies is/are polyclonal or monoclonal; or which preparation comprises a fragment of said antibodies.

26. The antibody preparation of claim 25 which is 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*.

1/19

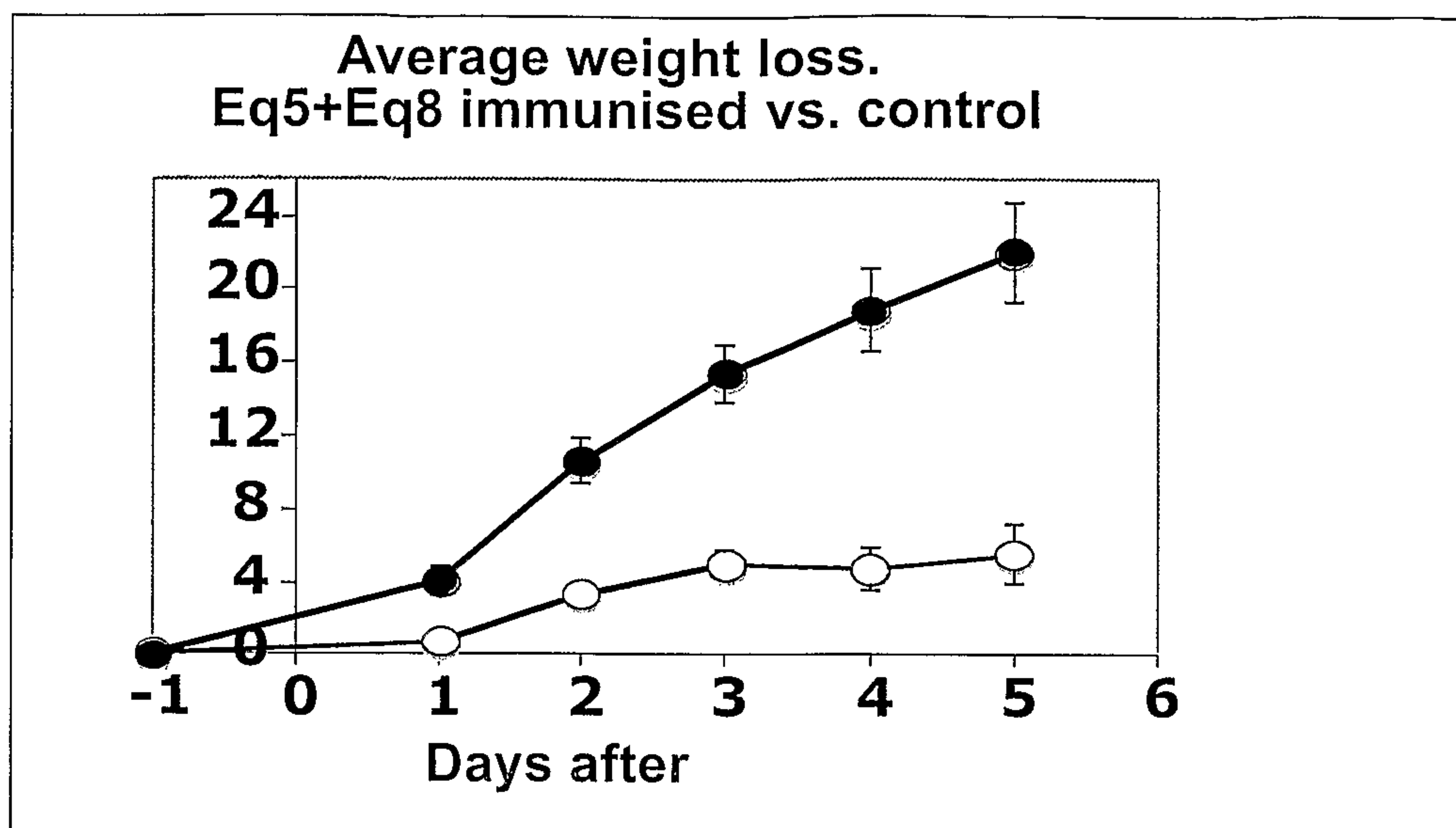


Figure 1

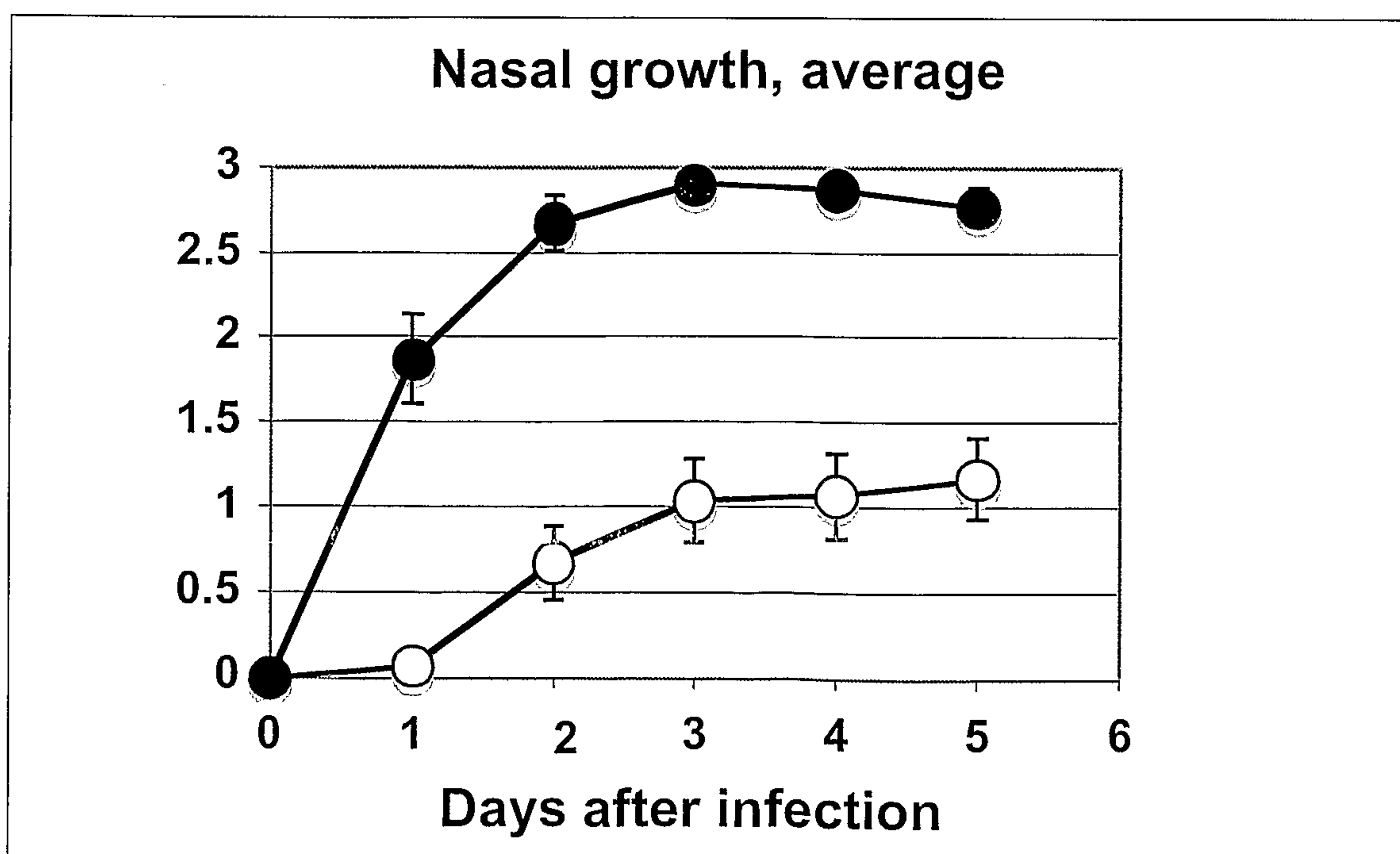


Figure 2

2/19

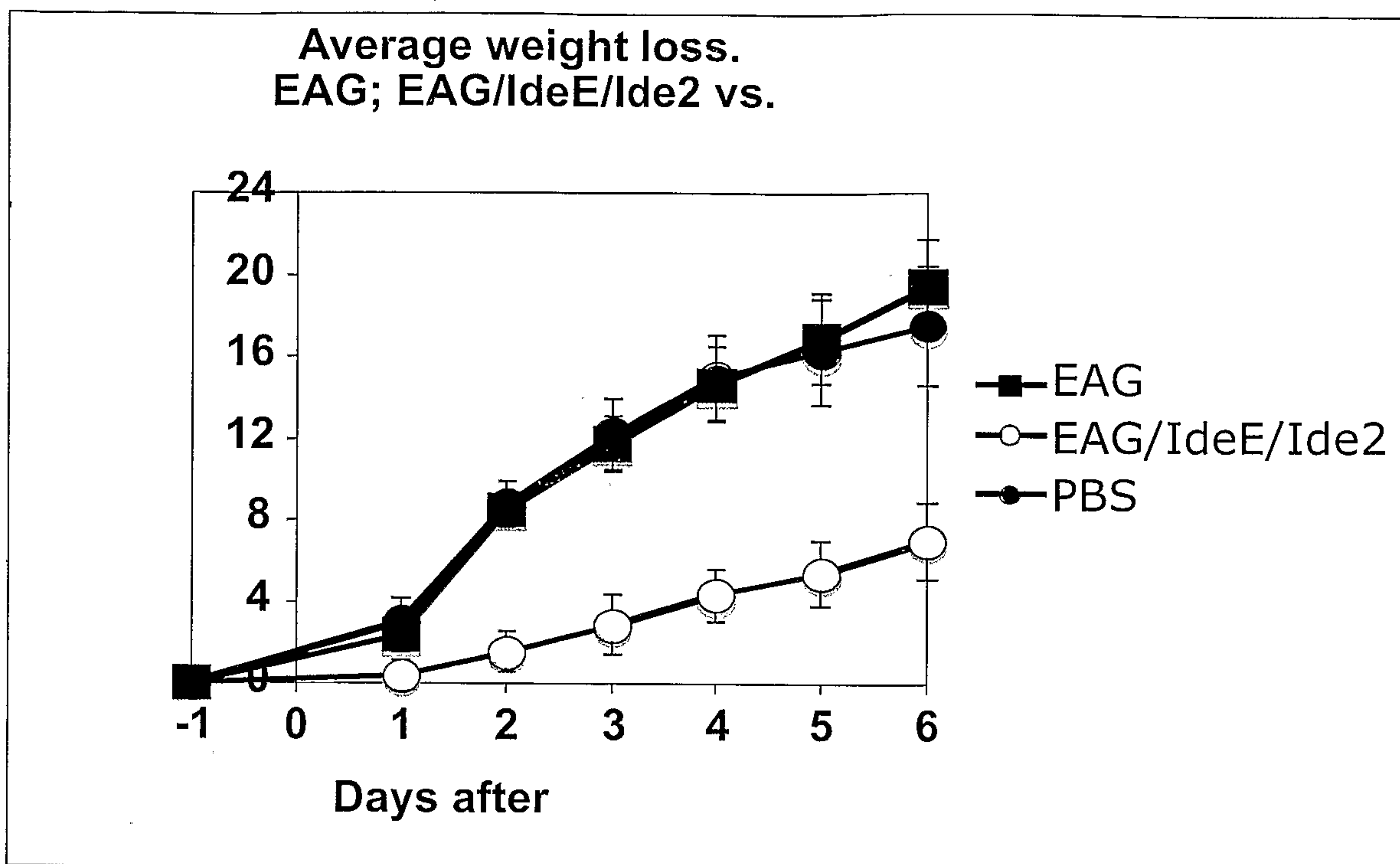


Figure 3

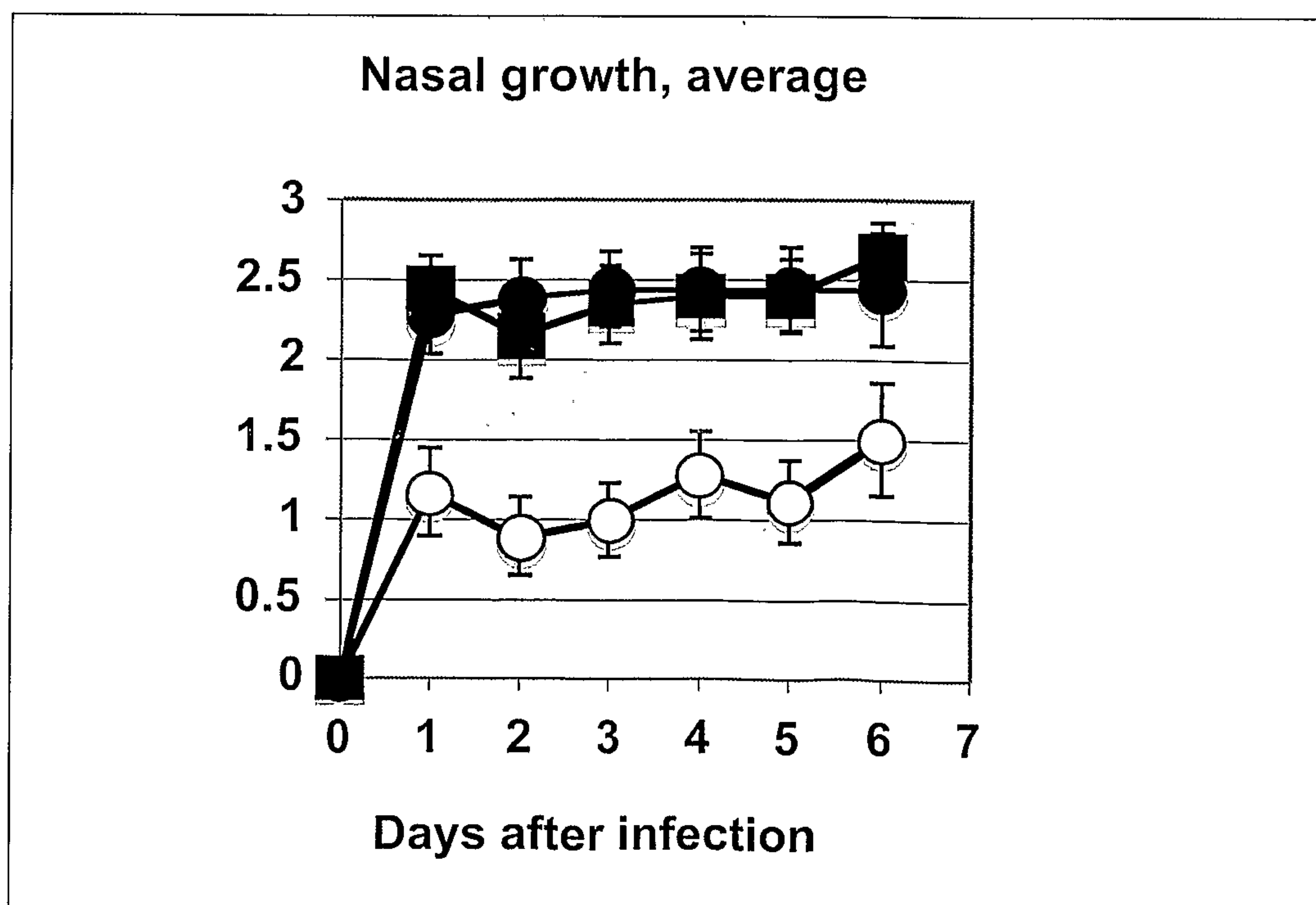


Figure 4

3/19

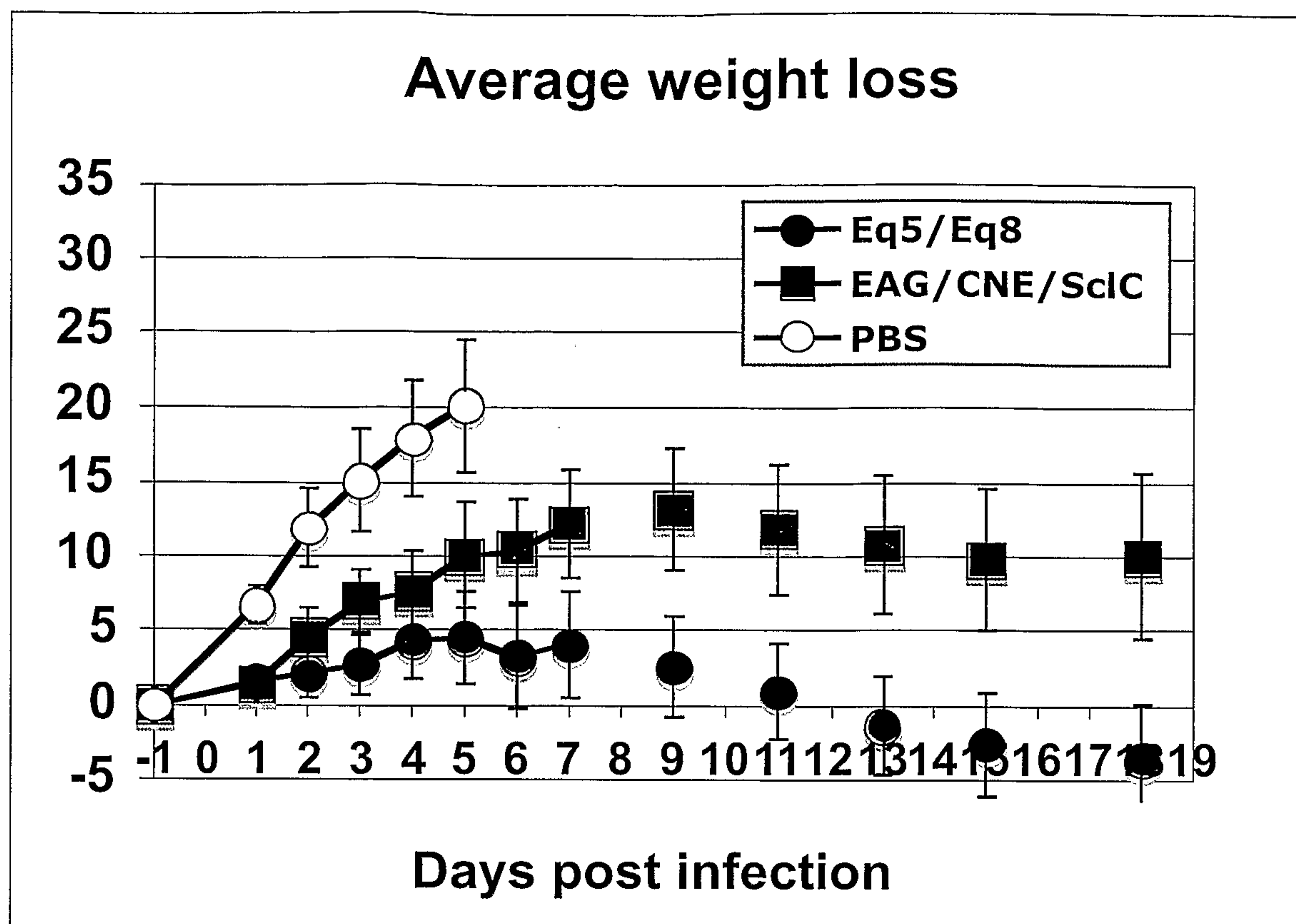


Figure 5a

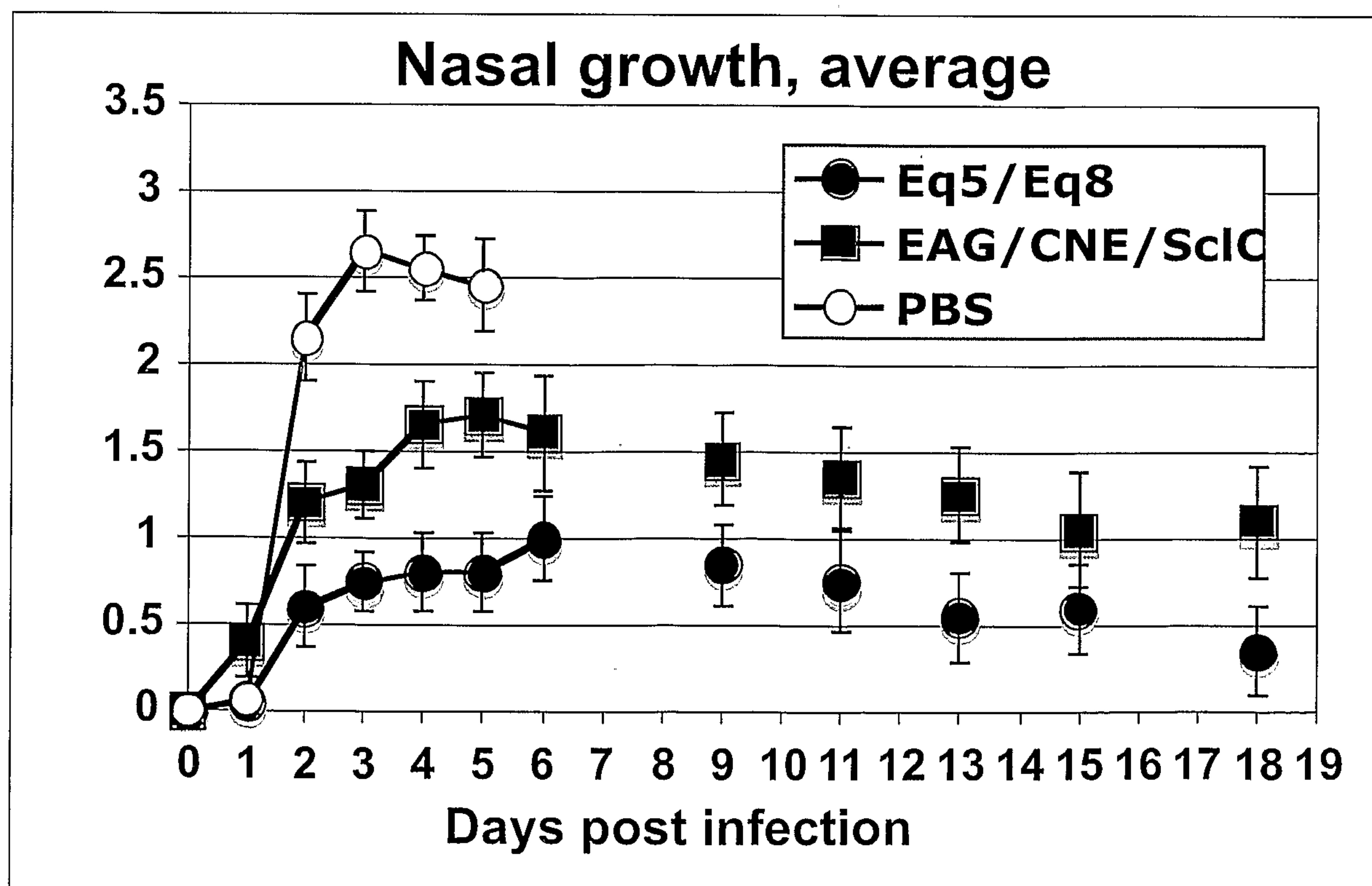


Figure 5b

4/19

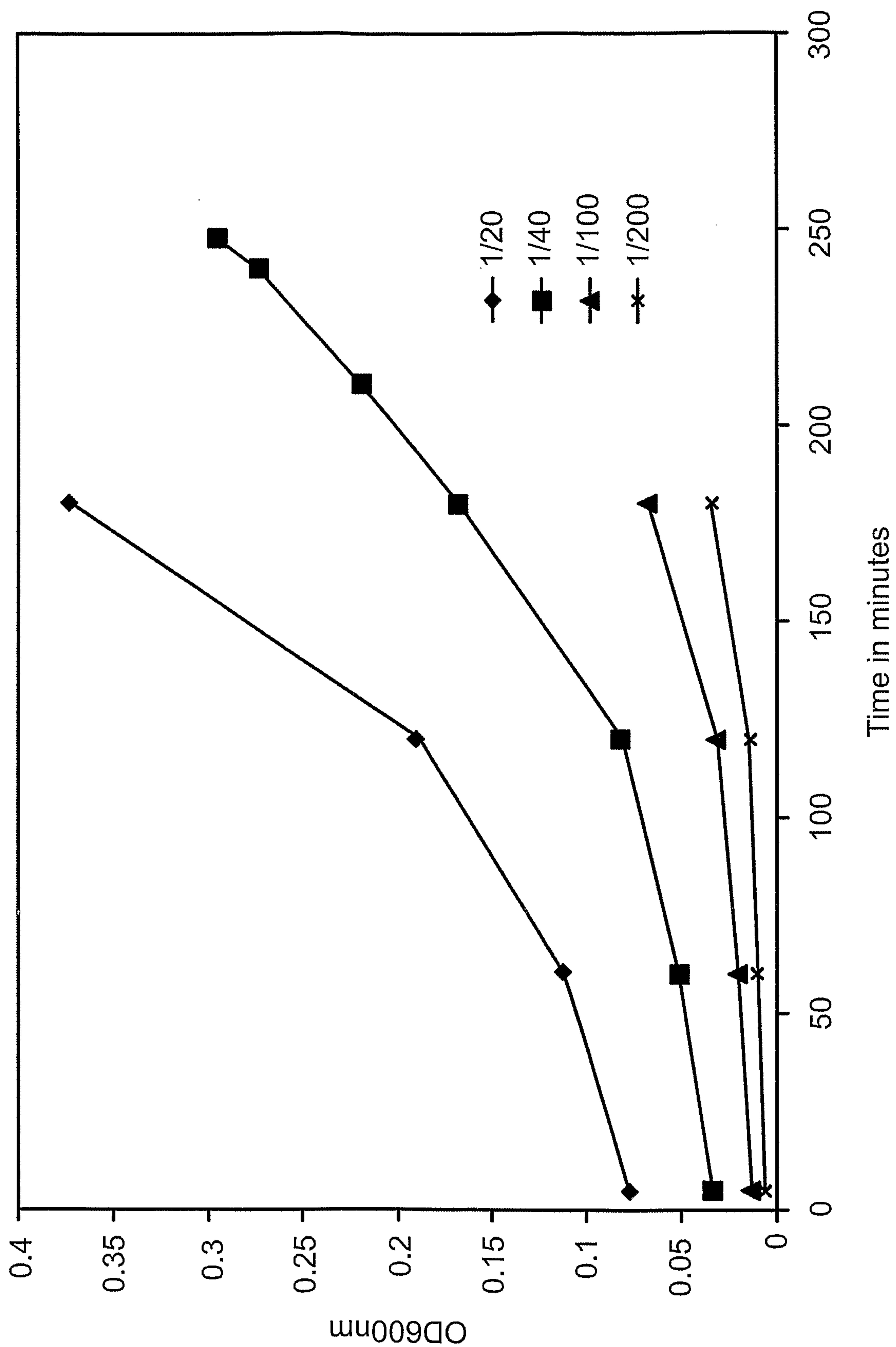


Figure 6: GROWTH OF CHALLENGE INCOLUM 08/5/08

5/19

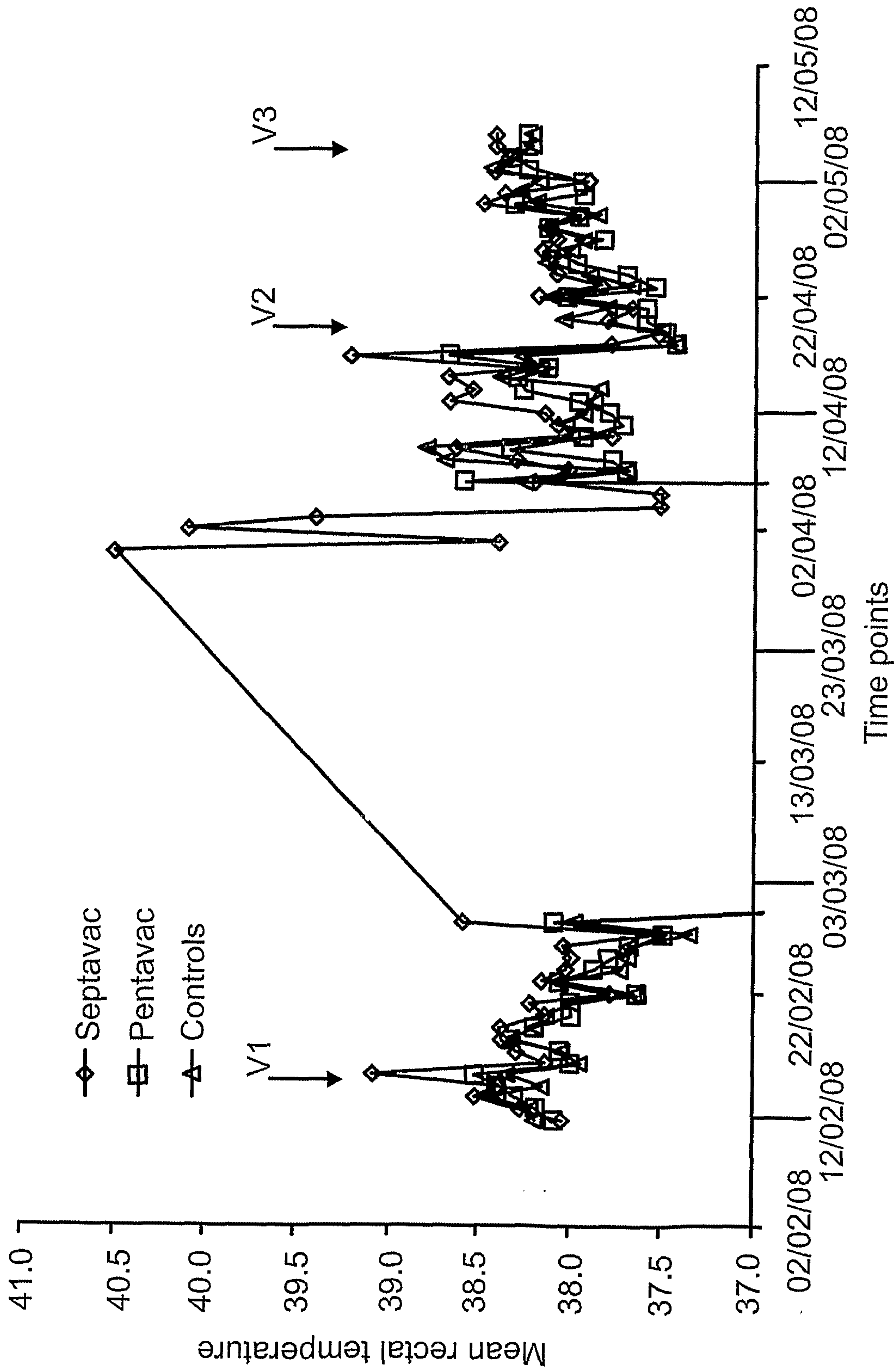


Figure 7: Mean pony temperatures during the Vaccination phase

6/19

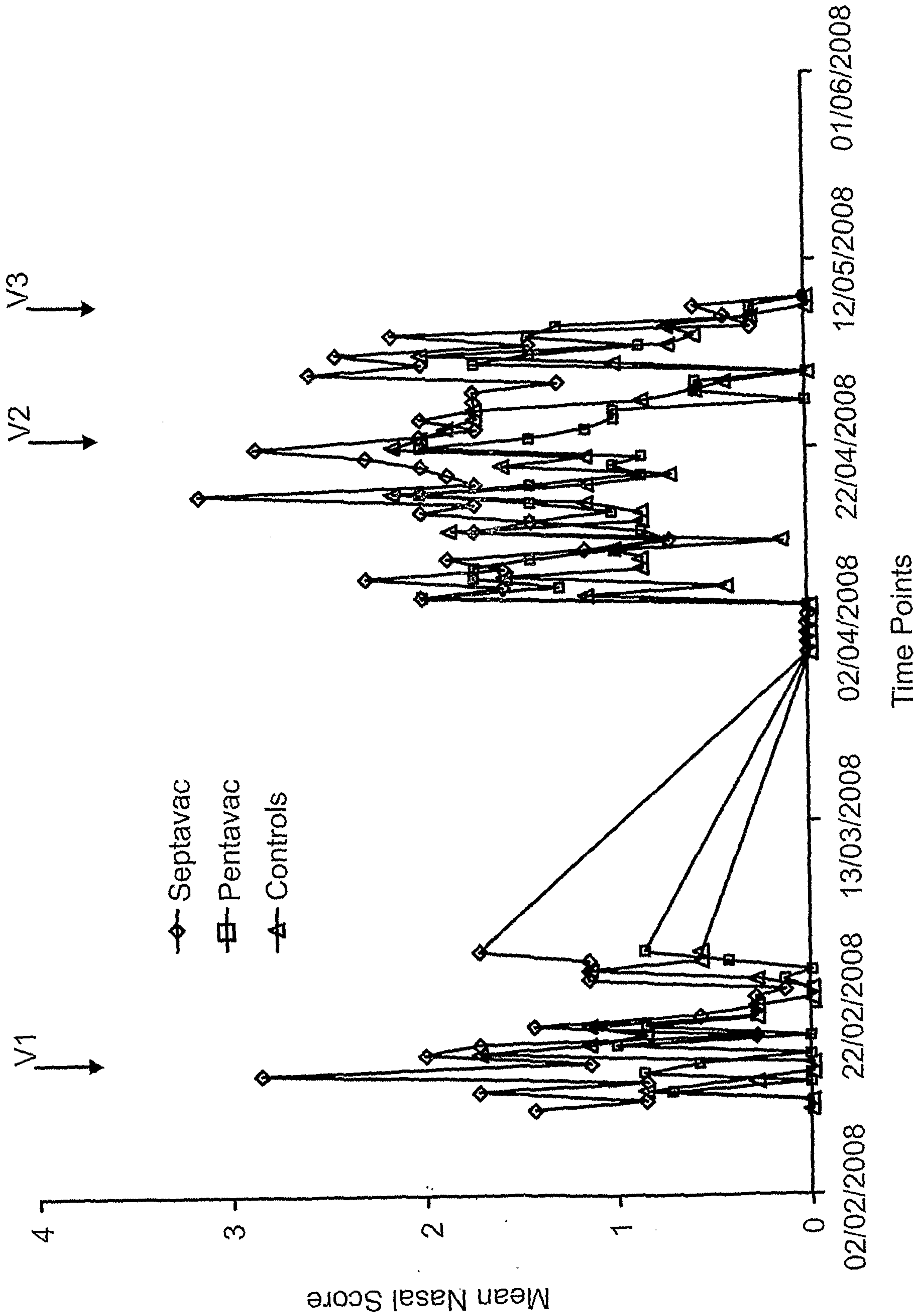


Figure 8: Mean nasal score during the vaccination phase

7/19

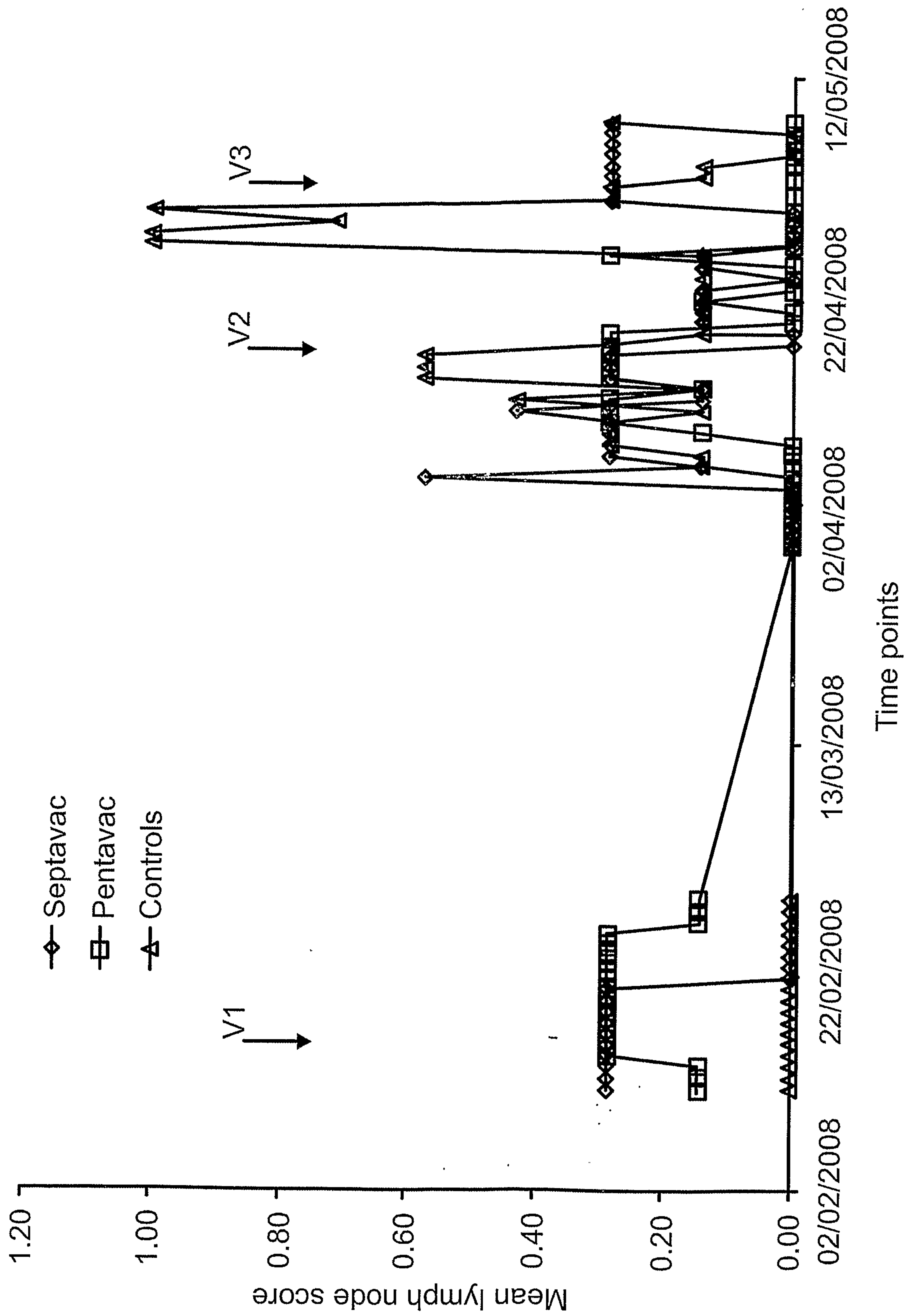
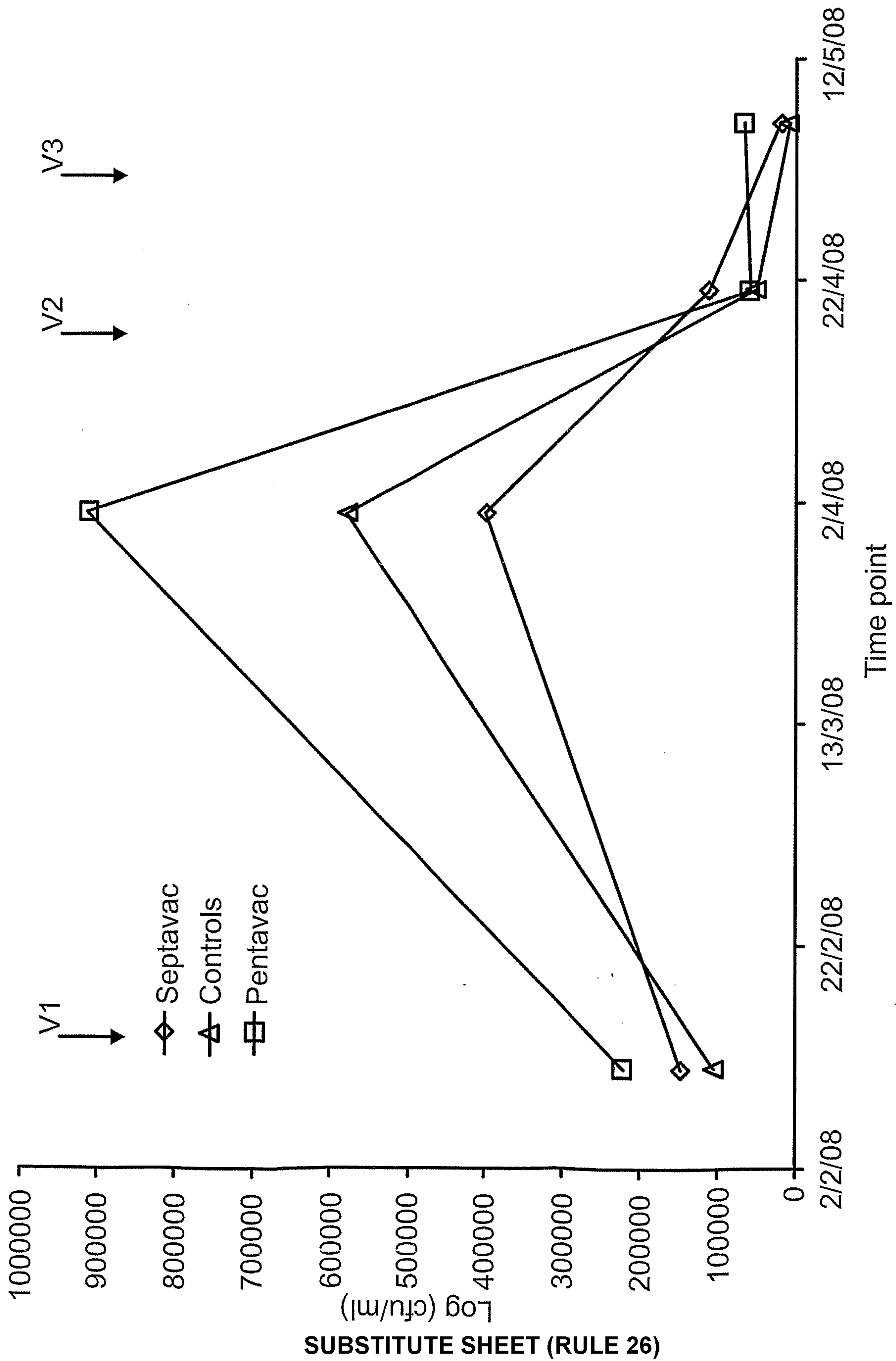
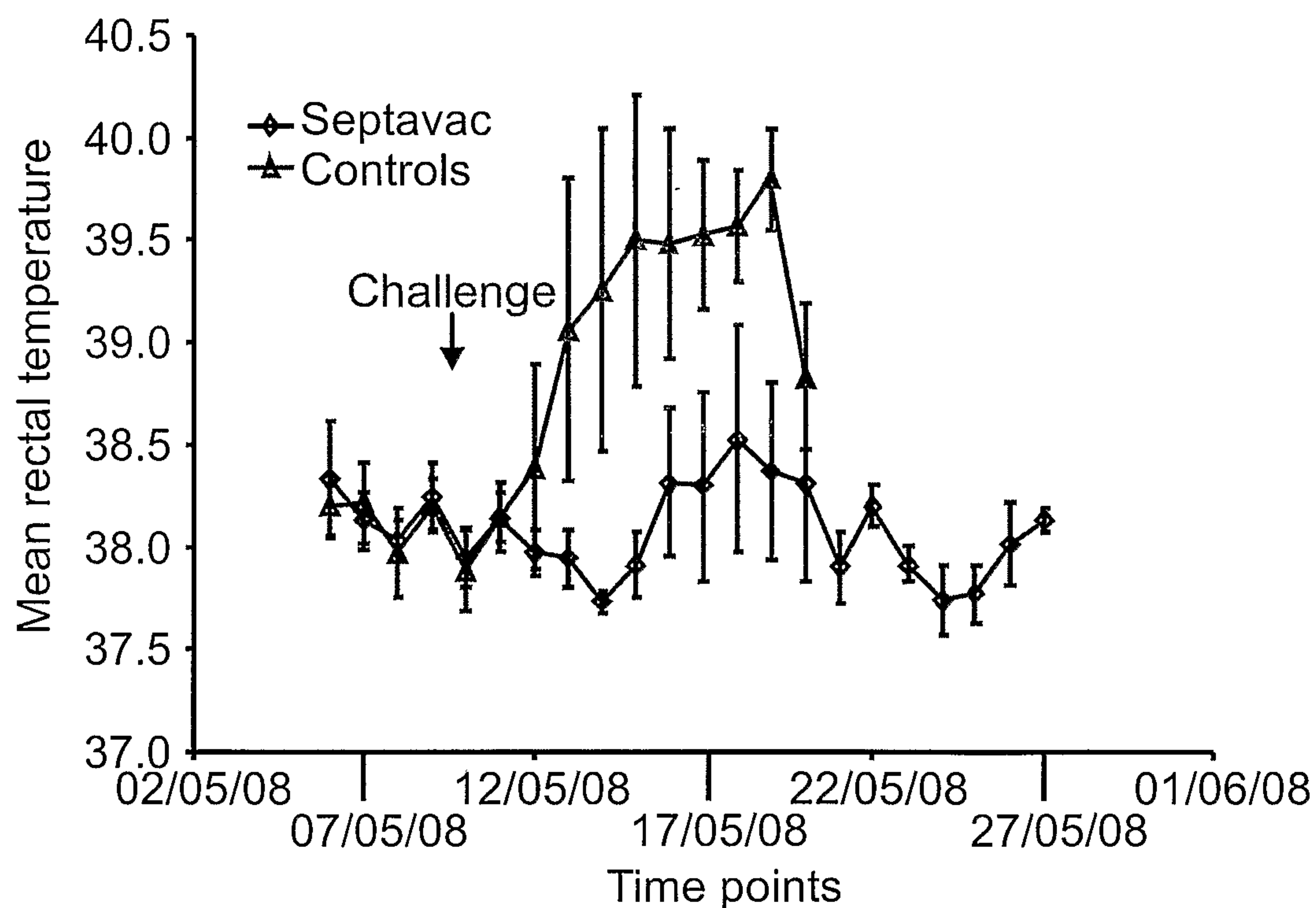


Figure 9: Mean lymph node score during the vaccination phase

8/19

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

9/19



* 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 challenge

10/19

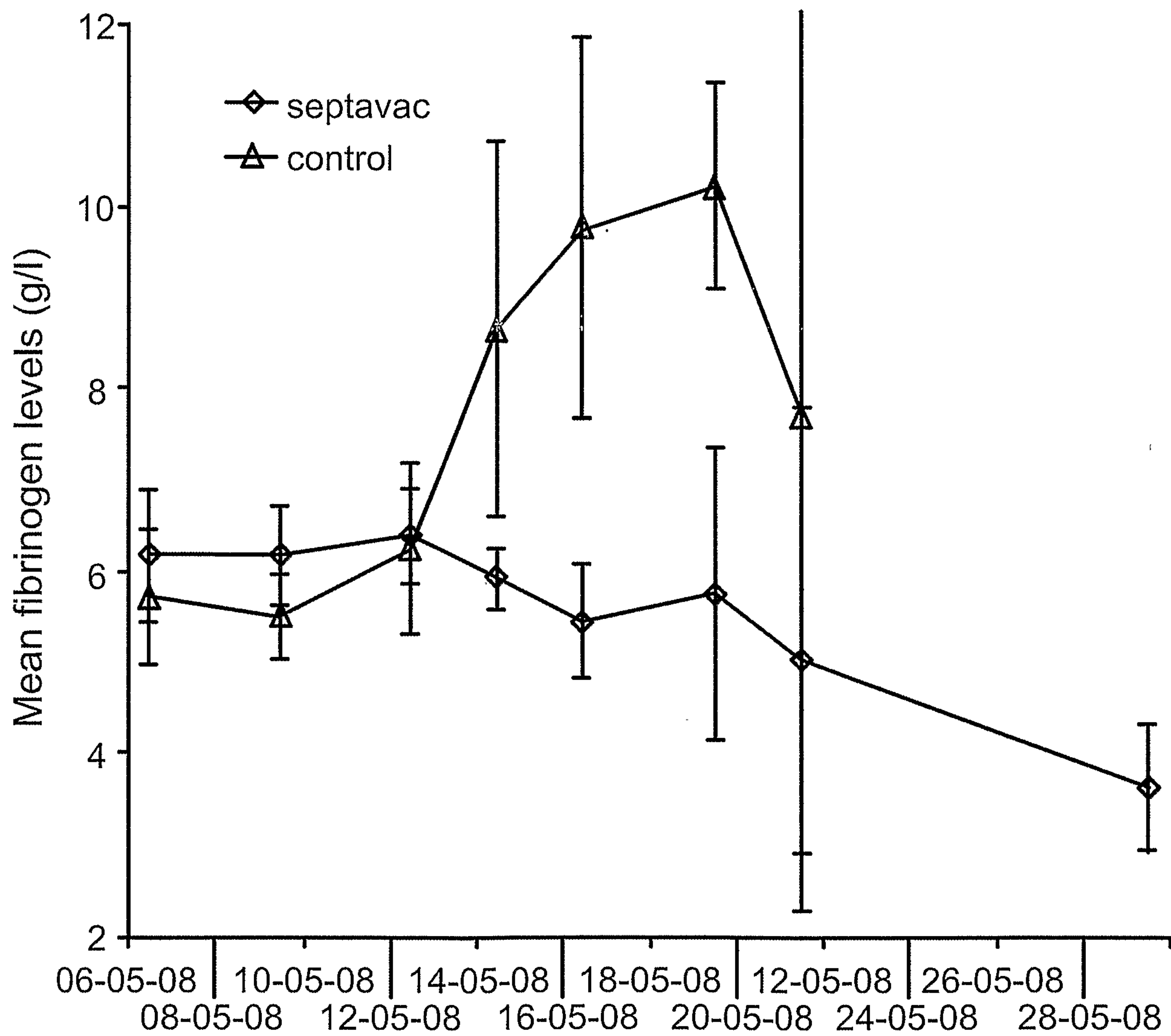
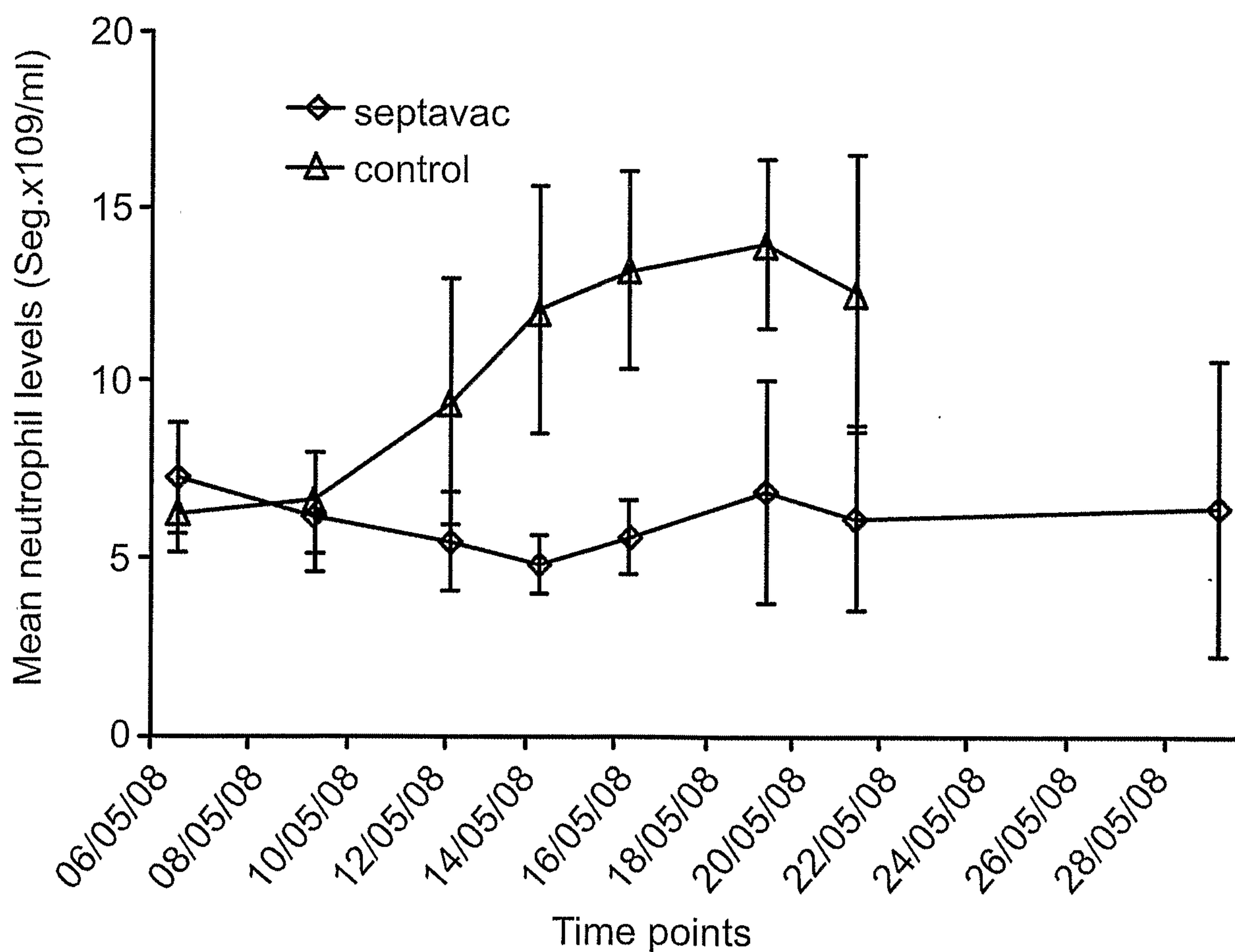


Figure 12: Mean fibrinogen levels during the challenge phase

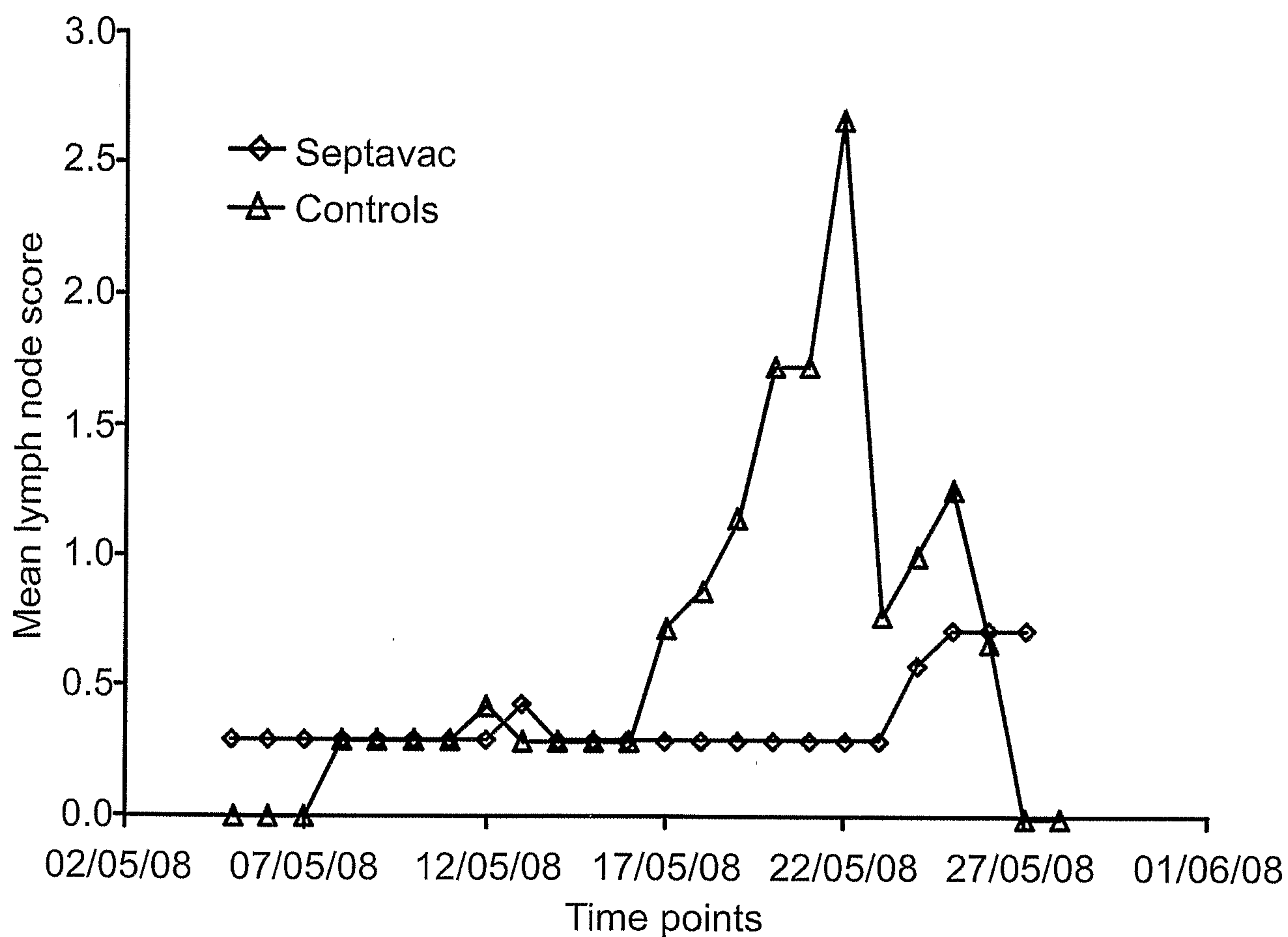
11/19



* 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

12/19



* 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

13/19

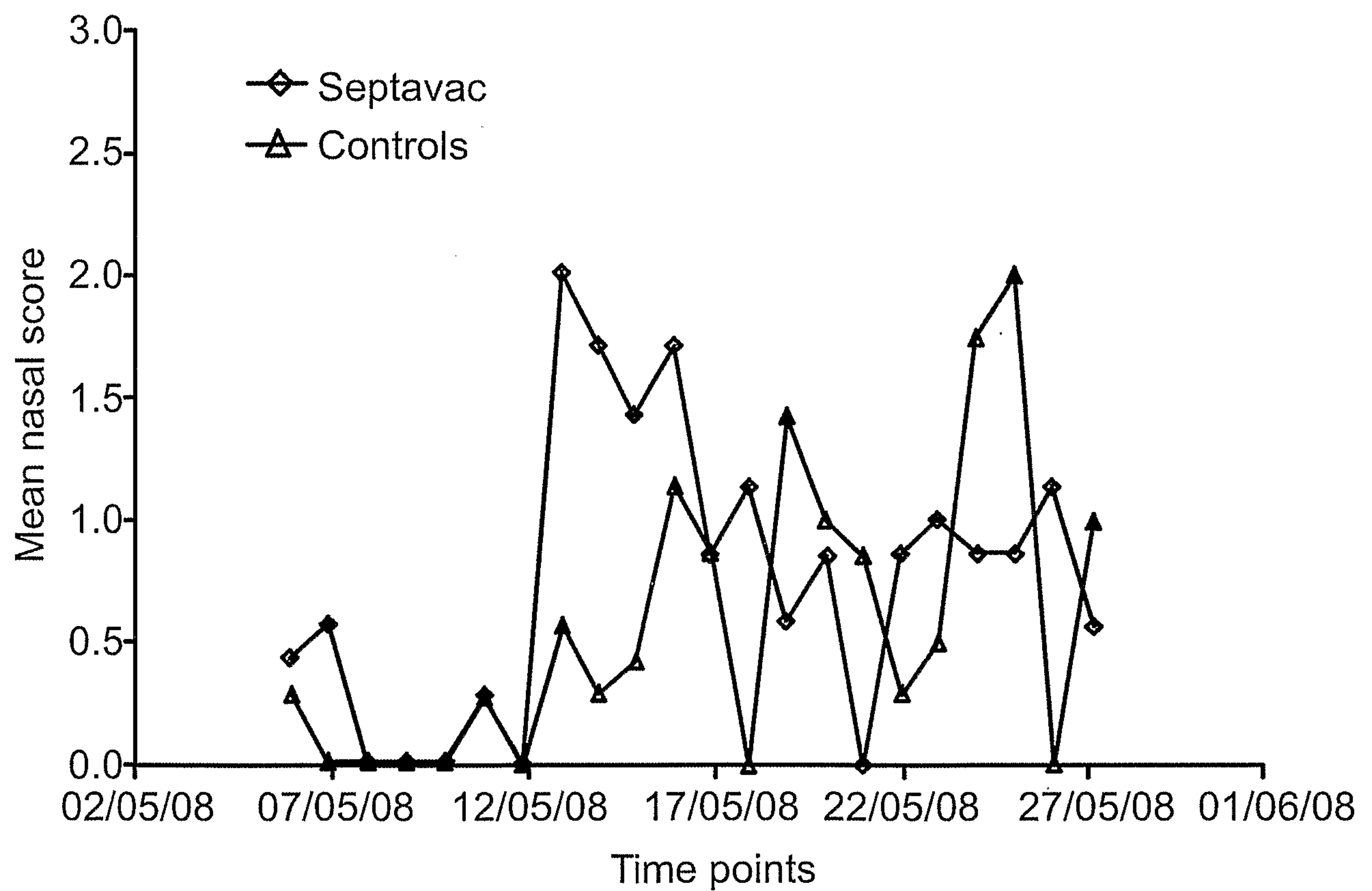


Figure 15: Mean nasal score during challenge phase

14/19

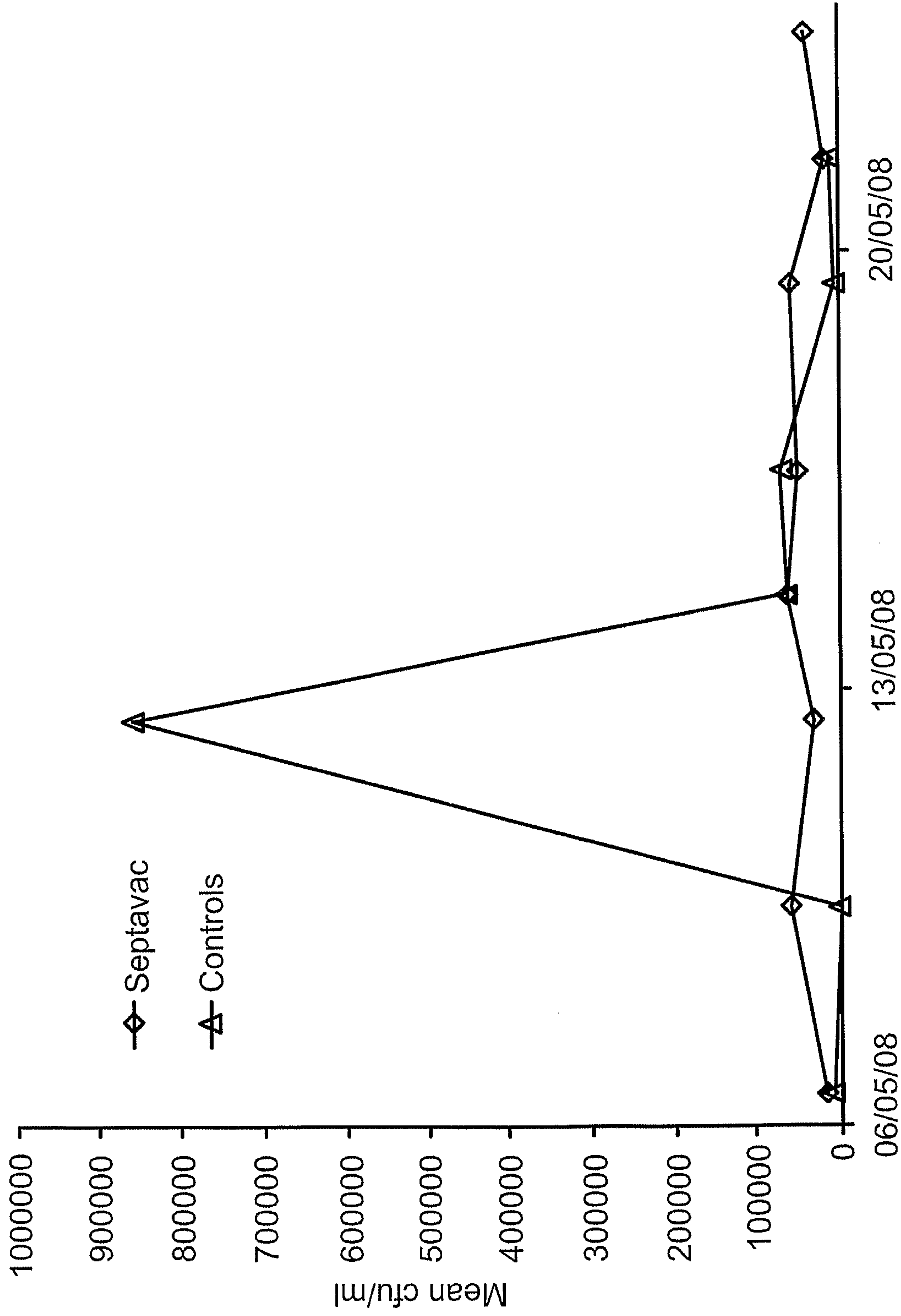


Figure 16: Mean *S. zooepidemicus* counts during challenge phase

15/19

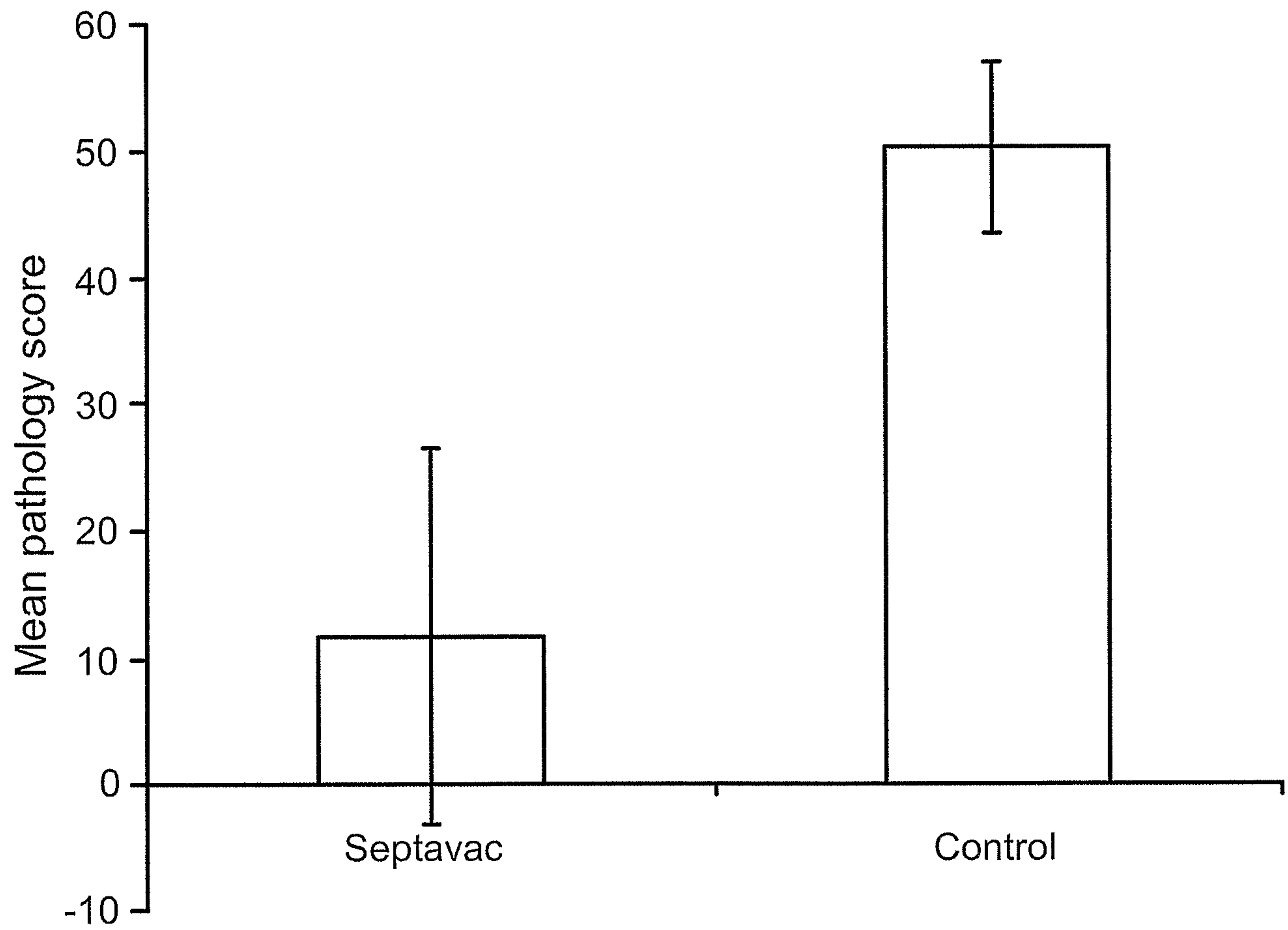


Figure 17: Mean pathology score on post mortem examination

16/19

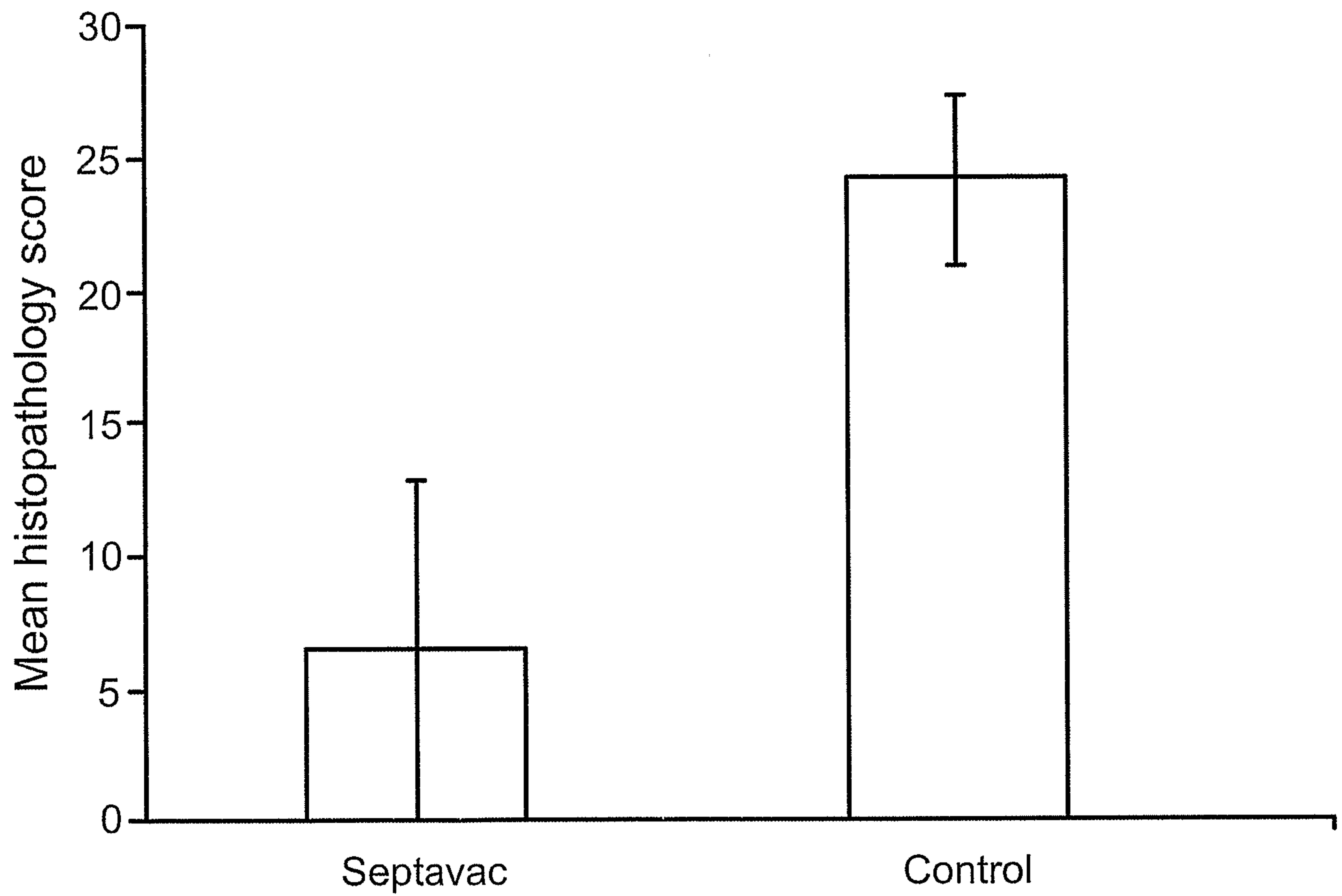


Figure 18: Mean Histopathology Scores

IgG antibodies against EAG, CNE and ScIC in
nasal washings of seven immunized horses (Septavac)

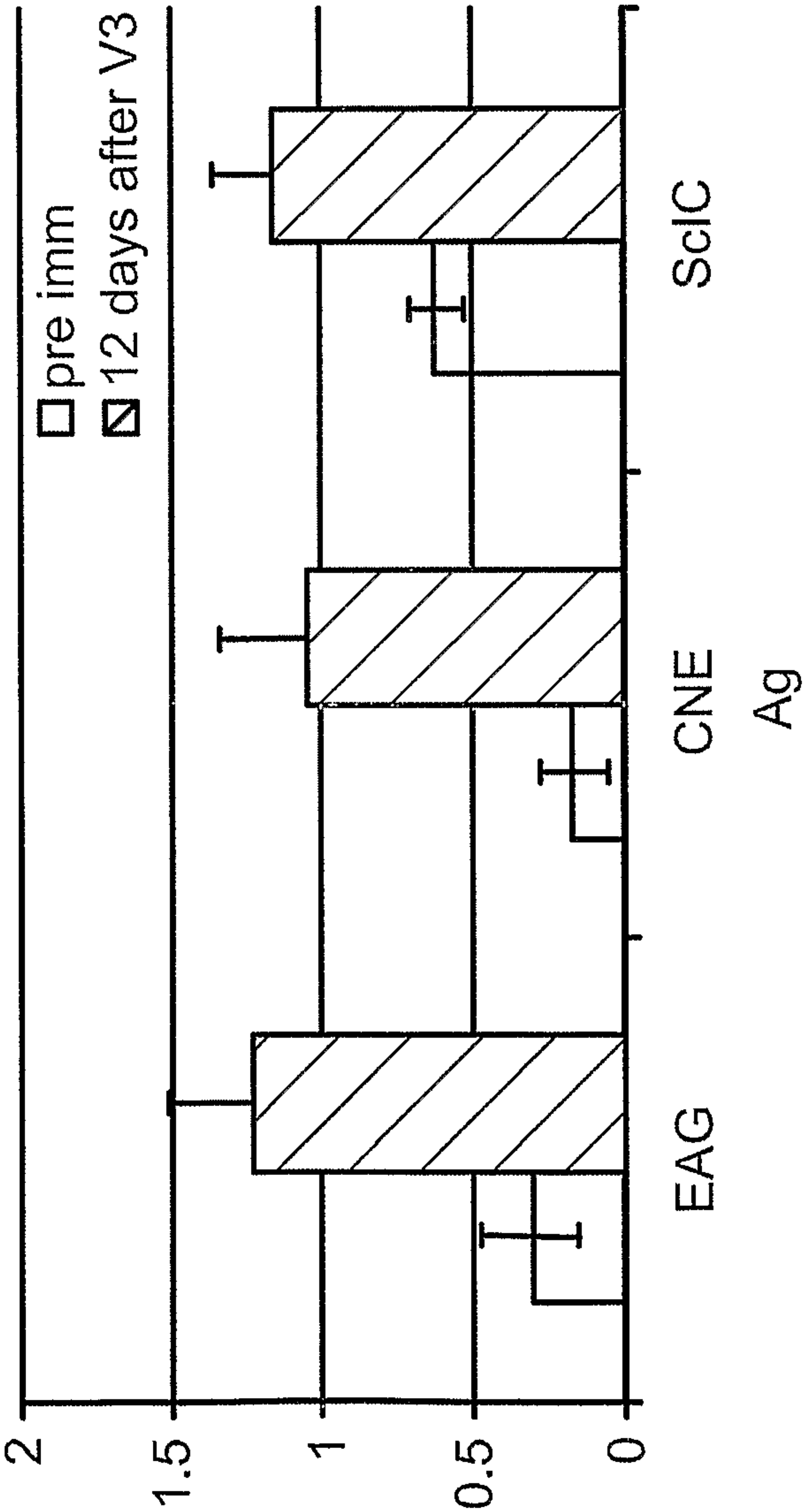


Figure 19

18/19

IgG antibodies in sera of seven immunized horses
against CNE, EAG, ScIC Eq5, Eq8, IdeE, Ide2 (Septavac)

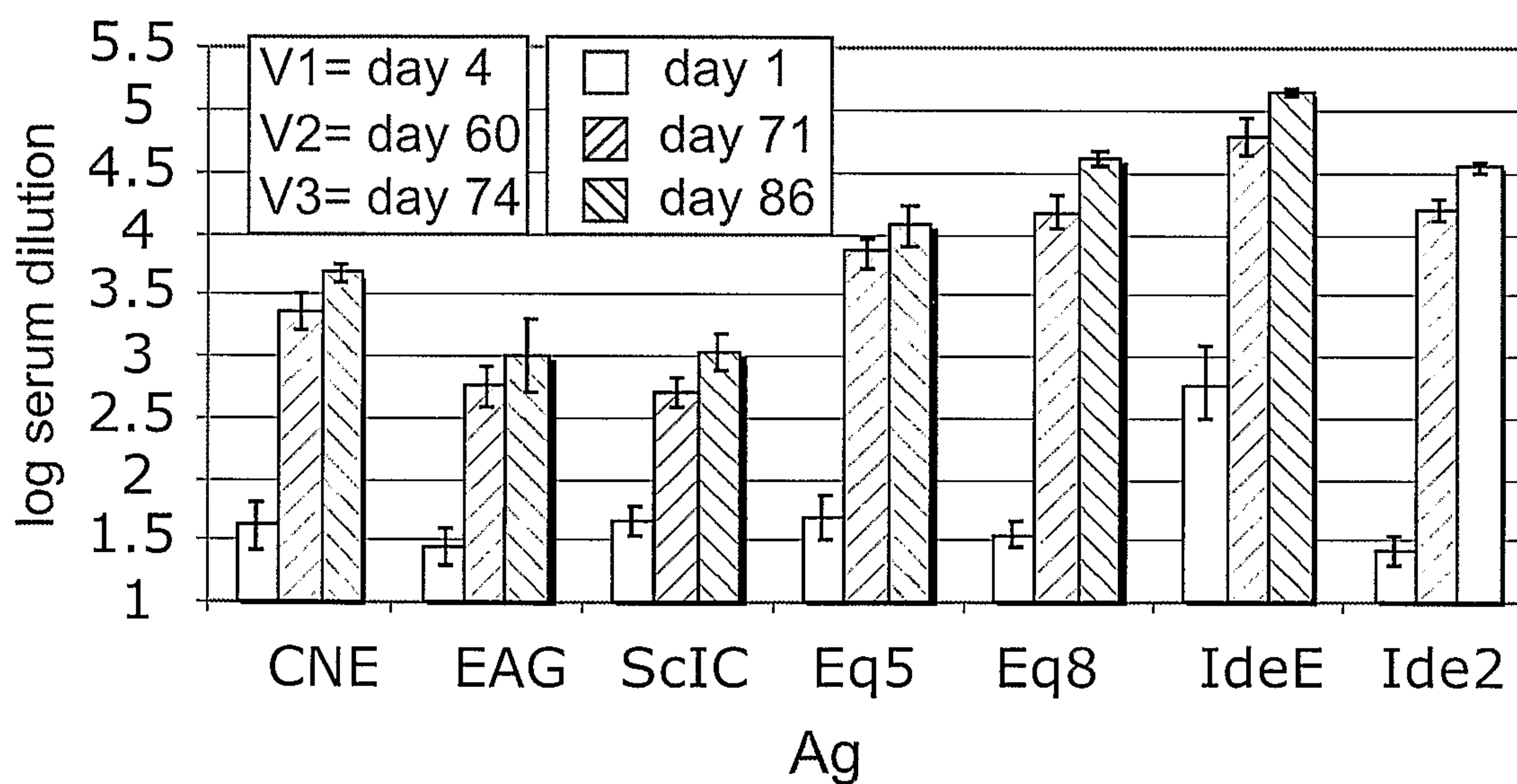


Figure 20

19/19

IgG antibodies in sera of seven immunized horses against EAG,
CNE, ScIC, Eq5 and Eq8 (Pentavac)

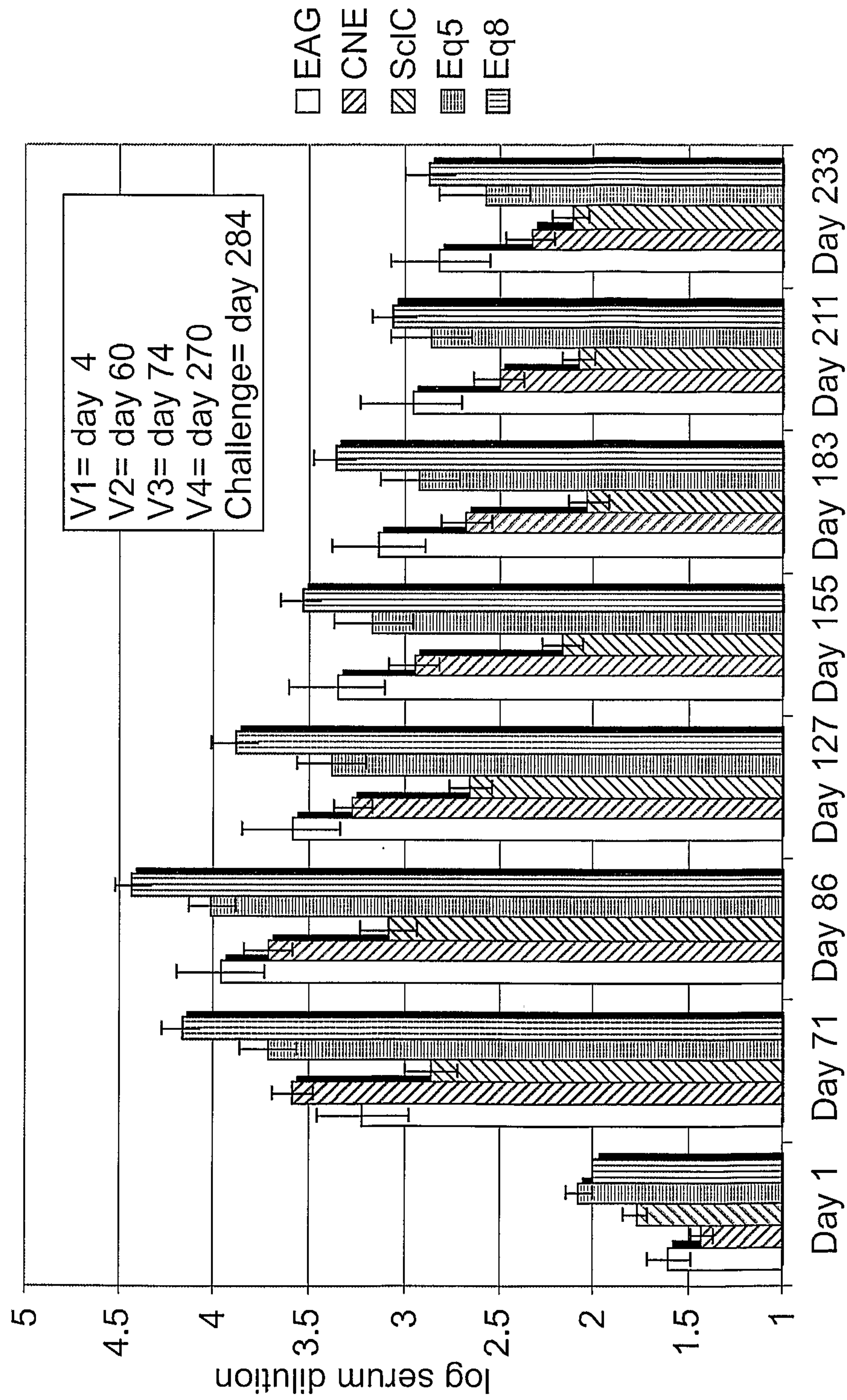


Figure 21