



Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

El-Hage, CM;Bannai, H;Wiethoelter, AK;Firestone, SM;Heislars, CM;Allen, JL;Waller, AS;Gilkerson, JR

Title:

Serological responses of Australian horses using a commercial duplex indirect ELISA following vaccination against strangles

Date:

2019-07-01

Citation:

El-Hage, C. M., Bannai, H., Wiethoelter, A. K., Firestone, S. M., Heislars, C. M., Allen, J. L., Waller, A. S. & Gilkerson, J. R. (2019). Serological responses of Australian horses using a commercial duplex indirect ELISA following vaccination against strangles. AUSTRALIAN VETERINARY JOURNAL, 97 (7), pp.220-224. <https://doi.org/10.1111/avj.12825>.

Persistent Link:

<https://hdl.handle.net/11343/286042>

Serological responses of Australian horses using a commercial duplex indirect ELISA following vaccination against Strangles

CM El-Hage*¹, H Bannai², AK Wiethoelter¹, SM Firestone¹, CM Heislars¹,
JL Allen¹, AS Waller³ and JR Gilkerson¹

1. Faculty of Veterinary and Agricultural Sciences, The University of Melbourne Parkville Victoria 3010 Australia
2. Equine Research Institute, Japan Racing Association 1400-4 Shiba, Shimotsuke- shi, Tochigi 329-0412 Japan
3. Animal Health Trust, Kennett, Newmarket CB8 7UU, England

*Corresponding author cmeh@unimelb.du.au

Abstract

Objective

To determine the nature of serological responses in Australian horses using a commercial duplex indirect ELISA (iELISA) following vaccination against Strangles.

Design

A range of client owned horses from 5 properties were recruited to receive a primary course of a *Streptococcus equi* subspecies *equi* (*S.equi*) extract vaccine.

Serological responses were determined utilising a duplex iELISA incorporating

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1111/avj.12825](https://doi.org/10.1111/avj.12825)

S.equi-specific fragments of two cell wall proteins, SEQ2190 and the SeM protein (antigen A and C respectively).

Procedure

Nineteen horses were administered a primary Strangles vaccination course. Blood was collected immediately prior to each of the three vaccinations at two-week intervals and, additionally at 28 and 56 days following V3.

Results

Significant increases in mean antibody levels of horses following vaccination were limited only to antigen (Ag) C, which was significantly increased at T2/V3, 14 days following V2 (ratio of geometric means = 3.7; 95% CI: 1.6, 8.4; P=0.003). There was no increase in mean antibody to Ag A (ratio of geometric means = 1.4; 95% CI: 0.6, 3.2; P=0.39). Four horses (22%) exceeded the test cut-off for Ag C following vaccination.

Conclusion

Vaccination of Australian horses is unlikely to interfere greatly with detection of Strangles using the duplex iELISA. No responses would be anticipated to Ag

A following vaccination with Equivac® S/ Equivac® 2in1, and only a minority are likely to respond to Ag C. We conclude that the results of this study validate the usefulness of the duplex iELISA to assist control measures for Strangles outbreaks in Australian horse populations.

Key Words

Bacteriology, Equine diseases, Immunology Vaccines

Abbreviations

Ag A, Antigen A; Ag C, Antigen C; DIVA, Differentiate infected from vaccinated animals; dpv3, days post (third) vaccination; iELISA, indirect enzyme-linked immunosorbent assay; V1, First vaccination; V2, second vaccination; V3, third vaccination; *S.equi*, *Streptococcus equi* subspecies *equi*

Introduction

Strangles is one of the most commonly diagnosed respiratory infection of horses worldwide.^{1, 2} The causative agent *Streptococcus equi* subspecies *equi* (*S.equi*) a gram positive, β -haemolytic bacterium. Infection of susceptible equids horses leads to subsequent colonisation of the upper respiratory tract and eventual abscessation of regional lymph nodes.³ The resulting inflammation and swelling of the upper airways may lead to stertorous and extreme difficulty breathing in some horses, hence the term “Strangles”. Although complications are relatively rare following infection, and mortality considered low, some horses may develop metastatic abscessation in distant

lymph nodes termed “bastard Strangles”.^{4, 5} In rare cases, a type II immune mediated hypersensitivity reaction *purpura haemorrhagica* (PH) may occur following infection or vaccination.^{3, 5, 6}

Outbreaks of disease caused by *Streptococcus equi* subspecies *equi* (*S. equi*) occur in horses worldwide, often with serious health, welfare, logistical and financial implications.^{2, 5, 7} Despite Strangles being notifiable in several states of Australia, it is difficult to precisely determine the incidence of disease in local horse populations. A previous survey found that 27.4% of stud farms in New South Wales reported an outbreak of Strangles over a 3-year period.¹

Infected horses may subsequently shed the organism from the upper respiratory tract for extended periods, with a minority becoming persistently infected.^{5, 8, 9} Control measures are compromised due to the difficulty detecting horses that continue to shed *S. equi* after apparent recovery. Detection of these horses presents logistical challenges and may be greatly facilitated by serological testing.^{9, 10} However, it has not been considered possible to differentiate infected from vaccinated animals (DIVA) using currently available commercial assays.^{5, 9, 11}

Vaccination against Strangles is common in Australia, particularly amongst performance horses and those within agistment centres.^{1, 12} Although a range of vaccine types are available internationally, there is only one commercially available in Australia (Equivac[®]S / Equivac 2 in 1[®], Zoetis, NSW, Australia). This vaccine is derived from an acid-extracted preparation of *S. equi*, administered via intramuscular injection.

Whilst horses that have recovered from clinical disease are considered to be protected against strangles for several years, such clinical protection is not reliably conferred following administration of extract vaccines.^{9, 13, 14} Humoral responses of horses are qualitatively and quantitatively reduced, compared to those following natural infection.¹³⁻¹⁶ This reduced clinical protection and dampened humoral response occurs in (cell-extract) vaccinates despite the extraction process exposing several cell wall associated antigens including the strongly antigenic M-Protein of *S. equi* (SeM).^{11, 13-15, 17} Vaccination against Strangles has also been considered a risk factor for PH, particularly if administration occurs during current exposure to field infection, or in horses with concurrent high antibody levels to the SeM protein of *S. equi*.^{18 5, 6, 19}

Serological testing may be useful to determine exposure of horses to *S. equi*. An assay to detect antibody levels to the SeM protein has been commercially available (IDEXX Laboratories, Rydalmere, NSW, Australia) for some time. The test has been of value to detect elevated SeM titres that practitioners may take into consideration prior to vaccination against Strangles.^{5, 6, 19} Whilst this assay is relatively sensitive, it lacks specificity due to cross-reactivity to the ubiquitous *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) thus, limiting its utility as a diagnostic tool in outbreak investigations.¹⁰ More recently, an assay utilising two antigens has been developed at the Animal Health Trust (AHT, Newmarket, United Kingdom) to serologically detect horses exposed to *S. equi*. It is recommended that two samples are taken at a 10-14 day interval to enable detection of newly exposed horses.^{10, 18} This duplex indirect ELISA assay (iELISA) incorporates *S. equi*-specific fragments of the of the SeM protein specifically the mpep1 fragment, in addition to another cell wall protein, SEQ2190 (antigen A and C respectively). The combination of these antigens has demonstrated not only high sensitivity and specificity,¹⁰ but a DIVA potential for horses vaccinated with extracted components of *S. equi*.¹⁰

We hypothesised that the duplex iELISA may remain useful in detecting exposure to field infection of Australian horses despite their vaccination status using the locally licensed extract vaccine Equivac® S/2in1. Our aim was to conduct a pilot study assessing serological responses to vaccination using the duplex iELISA.

Materials and Methods

Study Population

Horses resided on five private properties were of varying ages, sex, breeds and athletic pursuits (Table 1). All enrolled horses were paddock based and exposed either directly or indirectly to horse movements on and off their property.

Vaccination

Nineteen horses were vaccinated per manufacturer's recommendations with a primary course of three vaccinations of Equivac® S (V1 - V3). Each vaccine was administered deep into the neck musculature at a minimum of 2-week intervals. Sera were collected prior to vaccination at T0, T1 and T2 and twice following V3 at 28 and 56 days post V3(28 dpv3and 56 dpv3) (Figure 1).

iELISA protocol

Briefly, ninety-six well Immulon 2HB Flat bottom microtitre plates were coated overnight at 4°C with 200 ng of antigen A or antigen C per well. Plates were washed with phosphate buffered saline containing 0.05% Tween 20 (PBST) and blocked with PBST containing 1% non-fat milk (PBSTN) for 1 h at 37°C. Plates were washed with PBST and 100 µL of test or control serum diluted 1/800 in PBSTN was added to the appropriate well for 1 hour at 37°C. Assays were run in duplicate. Plates were washed with PBST and 100 µL of peroxidase-conjugated goat anti-horse IgG (SeraCare Life Sciences, Inc. Kirkegaard & Perry Laboratories, Inc.) diluted 1/600 in PBSTN was added to each well and the plates incubated for 1 hour at 37 °C . Plates were washed with PBST, 100 µl of Sure Blue reserve peroxidase substrate 3,3',5,5'- tetramethylbenzidine (SeraCare Life Sciences, Inc. Kirkegaard & Perry Laboratories, Inc.) was added and the plate incubated for 10 min at 21oC. 100 µL of 0.18M sulphuric acid was added and the optical density at 450 nm (OD450nm) measured. An OD450nm of ≥ 0.5 was considered to be a positive result for antigen A or antigen C

iELISAs and for the combined assay, a sample was deemed positive if either assay generated a positive result.

Assay cut-offs were determined in Robinson et al., 2013 using the student's *t*- test and Receiver Operating Characteristics (ROC) in Stata12 software.¹⁰ Briefly, sensitivity and specificity estimates for all '% relative to assay positive control' breakpoints within the data were determined relative to known infectious status of 89 horses from the UK with confirmed *S. equi* infection and 139 samples from Icelandic-resident horses, which are known to be free of strangles.¹⁰

Statistical Analysis

Antibody responses of horses at T0, prior to vaccination were used as a baseline for comparisons at each time-point. Owing to an inability to make distributional assumptions, geometric means of antibody responses to each antigen at each time-point were calculated and compared as ratios of responses arising from vaccination, and for factors including sex and age (<20 years versus ≥ 20). Significance was established at $P < 0.05$

Ethics

Animal Ethics approval was obtained for this study at the University of Melbourne (permit number 1614065).

Results

Study Population

Nineteen privately owned horses from five properties representing a range of breeds and equine activities were administered a primary vaccination course (Table 1). The age of horses ranged from 1-24 years (median 12, mean 18 years).

Of the nineteen horses, twelve were geldings (63%) and 7 mares (37%).

Horses were based in a region approximately 100 km south west of Melbourne, Victoria Australia and conducted over eighteen months commencing in January 2016.

Clinical Observations

Of the 69 vaccinations administered during this study, three local adverse reactions were observed (4.3%). Two horses suffered from mild swelling and elicited a pain response if palpated over the site of injection. One horse

reacted similarly to two vaccinations (V2 and V3), these were all transient and resolved within 72 hours following local application of cold and hot packs respectively.

Serological Responses

Antibody responses of each horse to antigen A and C are indicated in Figure 1 A and B respectively. Four horses (22%) exceeded the cut-off for Ag C. All these 4 horses were geldings and no females exceeded the cut off for either antigen, however, this sex difference was not statistically significant ($P=0.53$).

The mean antibody responses to Ag A in these 4 horses was similar to the mean of the remaining 15 horses ($P=0.49$).

The (geometric) mean antibody level for Ag C was significantly increased following T2/V3 compared to at T0/V1 (ratio of geometric means = 3.7; 95% CI: 1.6, 8.4; $P=0.003$). There was no significant increase detected in the geometric mean to Ag A, the ratio of geometric means following V3 being 1.4; 95% CI: 0.6, 3.2; $P=0.39$). Not included amongst the analysis of horses exceeding the Ag A cut off was horse 15 which did so some 56 days following V3 (56 dpv3).

Mean serological responses to either antigen in horses aged greater than 20 years of age were not statistically different to that of horses aged below. Although the ratio of geometric means to Ag C was 1.7 times higher in older horses this was not statistically different ($P=0.40$).

Mean antibody responses were numerically higher in the male (gelding/castrate) horses compared to the mean of the females for each antigen at T2/V3 (Ag A: ratio of geometric means 3.0 times higher (95% CI: 0.78, 11.9; $P=0.10$, Ag C: ratio of geometric means 2.8 times higher 95% CI: 0.73, 10.7; $P=0.13$).

Figure 1a and 1b

Discussion

Effective control of Strangles outbreaks relies on movement restrictions and basic biosecurity practices. Serological screening of potentially exposed horses greatly facilitates such measures. This study provides valuable evidence that serological responses of horses vaccinated with Equivac[®]S/2in1 are unlikely to interfere greatly with detection of infected horses. Significant advantages are

possible if bacteriological sampling of horses is limited to those with evidence of serological exposure, given the costs and logistics associated with the sampling protocol to clear horses of infection.⁵

This is particularly relevant for *S.equi* sampling that may be logistically challenging, often involving multiple nasopharyngeal swabs or lavage of each guttural pouch.^{8, 9, 19, 20} Whilst the high diagnostic sensitivity (93.3%) and specificity (99.3%) of the duplex iELISA has been demonstrated,¹⁰ most commercially available Strangles vaccines worldwide are considered to interfere with serological results, ruling out any potential for a DIVA capability.^{9, 18, 21}

Vaccination with Equivac[®]S/2in1 resulted in an increase in the mean antibody response in only one of the test antigens (Ag C) in this study population. Four (of 19) horses (22%) exceeded the serological cut off for this antigen at T2. Although one horse (4) demonstrated a strong response to T1 this was not typical and likely an anamnestic response from previous exposure to the SeM antigen. A stronger response to this Ag C may have been expected since it represents the highly antigenic cell-wall associated SeM protein that is exposed during the extraction process.^{14, 15} It is possible that the antibody

response may have been primarily directed to other components of the SeM molecule not the mpep1 fragment represented in the test antigen.²² Our findings however confirm that the *S. equi* acid extracted vaccine Equivac®S/2in1 may elicit at least some antibody response to the SeM protein. Minimal antibody responses were detected to Antigen A, representing the other cell-wall associated antigen SEQ2190, in stark contrast to that reported previously in infected horses.^{10, 13, 14} The lack of detectable antibody responses in vaccinated horses to Ag A, in this and previous studies¹⁴ suggests that this antigen is not well expressed within extract vaccines. In studies determining the properties of the duplex iELISA test, serological responses of horses confirmed to be infected with *S. equi* were predominantly detected to this antigen.¹⁰

One horse, resident on a riding school/agistment property, was found to exceed the cut off to Ag A only at the final timepoint, 56 dpv3. This response was considered unlikely to be due to vaccination and associated with several resident horses suspected to have been exposed to *S. equi* during regular horse movements on and off the property.

Given the broad range of ages and inclusion of both sexes in the study population an examination of these factors was included statistical analysis. Previous studies have reported diminished immune responses to vaccination in older humans and horses amongst aged over 20 years.^{23, 24} This factor may have been significant as 7 /19 horses (37%) were at least 20 years of age in this study. However, no statistical difference was detected these groups in our limited population.

The ratio of males to females was skewed as 14 (74%) of the study population were geldings and 5 (26%) entire females. It has been well reported in human literature that response to vaccination may have a sex-effect, whereby females often elicit stronger responses possibly due to lower testosterone levels.²⁵ Few reports are available in the literature regarding differences in adult adaptive immune responses between the sexes of equids. There was no difference detected between sexes in our study however this must be interpreted carefully as all males were geldings hence testosterone levels are unlikely to have been significantly different between geldings and females. Further studies including stallions amongst the study population would provide more

information and larger populations would be required to definitively predict any differences in serological responses.

Although there have been no reports of a DIVA capability following Strangles vaccination, previous studies have identified such potential.^{10, 11, 14, 15} Velineni determined that since the SEQ2190 protein (represented in Ag A) produced strong serological responses in infected horses, and minimal in (extract) vaccinates that there may be potential to distinguish the response of vaccinated horses.¹⁴ In a retrospective analysis of over 5000 seropositive samples submitted to the AHT from 2013-16, approximately 60% were positive to Ag A only, 20% for Ag C alone and 20% to both antigens.²⁶ The authors noted that the proportion of horses responding to solely antigen C had reduced over the time period, one possible explanation for these results are that *S. equi* strains from more recent outbreaks differ in the production or antigenicity of the surface proteins they encode, leading to a reduced response to Ag C in favour of Ag A.²⁶ Although there have been too few Australian seropositive horses with confirmed *S. equi* infection at this stage, preliminary results have mirrored this predominant serologic response to Ag A (Authors JRG and CME unpublished records). The inclusion of Ag C is important to

impart greater sensitivity for the duplex iELISA assay, hence any sample exceeding the cut off level for either antigen is considered positive.^{5, 10} We suggest that the test is valuable in local horse populations, including vaccinates, and that any individual horses yielding a positive result solely to AgC be further investigated in terms of potential exposure to both *S.equi* infection and vaccination status.

The absence of detectable responses to Ag A, and limited responses to Ag C in horses vaccinated with Equivac[®]S/2in1, indicates that serological responses to the duplex iELISA are largely unlikely to interfere with the detection of field cases of strangles. Although further studies are required to analyse larger numbers of infected and vaccinated horses, the results of this pilot study provide a basis to validate the usefulness of the duplex iELISA assay to assist Strangles control measures in Australian horse populations.

^a Equivac[®] S, Equivac[®] 2 in1 (Zoetis, Rhodes, NSW, Australia).

Conflict of Interest and Funding: No external funding or in-kind support was received for this work. The Centre for Equine Infectious diseases (CEID) is part of the University of Melbourne and holds a commercial licence for the duplex iELISA. Authors JRG, JA and CME are members of the CEID.

Acknowledgements: The authors wish to thank the Parker and Stewart families and Miss Amy Waldron for generous access and care of their horses. Professor Josh Slater provided manuscript guidance, Dr John Morton and Kim Jeffers provided skilful and kind assistance of which we were most grateful.

References

1. Jorm LR. Strangles in horse studs: incidence, risk factors and effect of vaccination. *Aust Vet J* 1990;67:436-439.
2. Parkinson N, Robin C, Newton J, Slater J, Waller A. Molecular epidemiology of strangles outbreaks in the UK during 2010. *Vet Rec* 2011;168:666-666.
3. Waller AS, Sellon D, Sweeney CR et al. Streptococcal Infections In: Sellon D, Long M, editors. *Equine Infectious Diseases*. second edn. Elsevier, 2014:265.
4. Whelchel DD, Arnold CE, Chaffin MK. Subscapular lymph node abscessation as a result of metastatic *Streptococcus equi* subspecies *equi* infection: An atypical presentation of bastard strangles in a mare. *Equine Vet Educ*. 2009;21:131-134.
5. Boyle AG, Timoney JF, Newton JR et al. Streptococcus equi Infections in Horses: Guidelines for Treatment, Control, and Prevention of Strangles-Revised Consensus Statement. *J Vet Intern Med* 2018.
6. Pusterla N, Watson JL, Affolter VK et al. Purpura haemorrhagica in 53 horses. *Vet Rec* 2003;153:118-121.
7. Pusterla N, Kass PH, Mapes S et al. Surveillance programme for important equine infectious respiratory pathogens in the USA. *Vet Rec* 2011;169:12.
8. Newton J, Verheyen K, Talbot N et al. Control of strangles outbreaks by isolation of guttural pouch carriers identified using PCR and culture of *Streptococcus equi*. *Equine Vet J*. 2000;32:515-526.
9. Waller AS. New perspectives for the diagnosis, control, treatment, and prevention of strangles in horses. *Veterinary Clinics of North America: Equine Practice* 2014;30:591-607.
10. Robinson C, Steward KF, Potts N et al. Combining two serological assays optimises sensitivity and specificity for the identification of *Streptococcus equi* subsp. *equi* exposure. *Vet J* 2013;197:188-191.
11. Hobo S, Niwa H, Anzai T, Jones JH. Changes in Serum Antibody Levels after Vaccination for Strangles and after Intranasal Challenge with *Streptococcus equi* subsp. *equi* in Horses. *J Equine Sci* 2010;21:33-37.
12. Dr R. L'Estrange Personal Communication . Zoetis, NSW, Australia.
13. Timoney JF, Qin A, Muthupalani S, Artiushin S. Vaccine potential of novel surface exposed and secreted proteins of *Streptococcus equi*. *Vaccine* 2007;25:5583-5590.

14. Velineni S, DeNegri R, Artiushin SC, Timoney JF. Comparison of specificities of serum antibody responses of horses to clinical infections caused by *Streptococcus equi* or *zooepidemicus*. *Vet Microbiol* 2015;180:253-259.
15. Sheoran AS, Sponseller BT, Holmes MA, Timoney JF. Serum and mucosal antibody isotype responses to M-like protein (SeM) of *Streptococcus equi* in convalescent and vaccinated horses. *Veterinary Immunol Immunopath* 1997;59:239-251.
16. Todd TG. Strangles. *J Comp Path Therap* 1910;23:212-229.
17. Timoney JF, Artiushin SC, Boschwitz JS. Comparison of the sequences and functions of *Streptococcus equi* M-like proteins SeM and SzPSe. *Infect Immun* 1997;65:3600-3605.
18. Boyle AG, Rankin SC, Duffee L, Boston RC, Wheeler-Aceto H. *Streptococcus equi* Detection Polymerase Chain Reaction Assay for Equine Nasopharyngeal and Guttural Pouch Wash Samples. *J Vet Intern Med* 2016;30:276-281.
19. Sweeney CR, Timoney JF, Newton JR, Hines MT. *Streptococcus equi* infections in horses: guidelines for treatment, control, and prevention of strangles. *J Vet Intern Med* 2005;19:123-134.
20. Duffee LR, Stefanovski D, Boston RC, Boyle AG. Predictor variables for and complications associated with *Streptococcus equi* subsp *equi* infection in horses. *J Am Vet Med Assoc* 2015;247:1161-1168.
21. Robinson C, Frykberg L, Flock M et al. Strangvac: A recombinant fusion protein vaccine that protects against strangles, caused by *Streptococcus equi*. *Vaccine* 2018;36:1484-1490.
22. Lannergard J, Kristensen BM, Gustafsson MC et al. Sequence variability is correlated with weak immunogenicity in *Streptococcus pyogenes* M protein. *Microbiologyopen* 2015;4:774-789.
23. Horohov DW, Adams AA, Chambers TM. Immunosenescence of the equine immune system. *J Comp Pathol* 2010;142 Suppl 1:S78-84.
24. Muirhead TL, McClure JT, Wichtel JJ et al. The effect of age on the immune response of horses to vaccination. *J Comp Pathol* 2010;142 Suppl 1:S85-90.
25. Furman D, Hejblum BP, Simon N et al. Systems analysis of sex differences reveals an immunosuppressive role for testosterone in the response to influenza vaccination. *Proceedings of the National Academy of Sciences* 2014;111:869-874.
26. Newton JR, Hermes D, Hammond TA et al. What can we learn from reviewing simple Strangles diagnostic testing data In: R.Keene, editor, Montana, USA, 2017.

Table1. Study Population of horses and ponies. Age indicates years, Breeds TB Thoroughbred, SB Standardbred, QH Quarter horse, WB Warmblood, WMP welsh Mountain Pony, ASH- Australian Stock Horse. Antibody responses from Italicised horses at bottom of table were not included.

Horse	Breed	Sex	Age	Activity
-------	-------	-----	-----	----------

1	WB	MC	10	Eventing
2	TB	MC	1	Yearling
3	WMP	F	8	Pleasure
4	WMP	MC	20	Pleasure
5	WMP	MC	20	Pleasure
6	TB	MC	20	Pleasure
7	TB	MC	5	Racehorse
8	TB	MC	23	Eventing
9	WMP	MC	15	Eventing
10	TB/ASH	F	10	Eventing
11	TB	F	2	Racehorse
12	TB	MC	4	Racehorse
13	TB	MC	5	Eventing
14	TB	MC	21	Retired
15	TB	MC	24	Retired
16	TB	F	24	Retired
17	QH	F	12	Pleasure
18	SB	MC	9	Retired
19	TB	MC	18	Eventing

Figure 1A. Individual antibody responses (OD 450nm) of horses vaccinated with a primary course of Equivac® S to Antigen A (SEQ 2190 wall protein fragment) Males denoted by triangles, females circles. Red shapes and lines indicate horses 20 years of age or over and blue shapes and lines indicate those below 20 years. The median antibody titre at each timepoint is indicated by a black bar. The positive serological cut off point of the assay (OD450nm \geq 0.5) is indicated by the dashed red line. Vaccination events indicated by arrows (V1, V2 and V3).

Figure 1B. Individual antibody responses (OD 450nm) of horses vaccinated with a primary course of Equivac® S to Antigen C (SeM cell wall protein fragment mPEP1) Males denoted by triangles, females circles. Red shapes and lines indicate horses 20 years of age or over and blue shapes and lines indicate those below 20 years. The mean antibody titre at each timepoint is indicated by a black bar.

The positive serological cut off point of the assay ($OD_{450nm} \geq 0.5$) is indicated by the dashed red line. Vaccination events indicated by arrows (V1, V2 and V3).



