SCIENTIFIC DISCUSSION

Name of the veterinary medicinal product:	Equilis StrepE
Marketing Authorisation Holder:	Intervet International B.V Wim de Körverstraat 35 P. O. Box 31 NL-5830 AA Boxmeer The Netherlands
Active substance:	Live *Streptococcus equi* strain TW928
Target species:	Horses
Strength:	$10^{9.0}$ to $10^{9.4}$ cfu/dose
Withdrawal period:	Zero days
Therapeutic indications:	For immunisation of horses against *Streptococcus equi* to reduce clinical signs and occurrence of lymph node abscesses.

I. SUMMARY OF THE DOSSIER

Equilis StrepE is a live vaccine, containing at least $10^{9.0}$ cfu, but not more than $10^{9.4}$ cfu of *S. equi* strain TW928 per dose as active substance. The vaccine is intended for use in horses from four months of age onwards to induce immunity against *S. equi* to reduce clinical signs and occurrence of lymph node abscesses: Strangles. The vaccination schedule consists of a basic vaccination with a primary injection followed by a second injection after an interval of four weeks and a re-vaccination with one dose every three months to maintain immunity. A priming response is maintained for up to six months after basic vaccination. Therefore a single dose of vaccine is needed to restore immunity. The vaccine is administered submucosally into the inside of the upper lip, using a volume of 0.2 ml. The vaccine is presented in a lyophilised form together with the solvent, applicator and a syringe with needle.

II. OVERVIEW OF PART II OF THE DOSSIER

Composition

Equilis StrepE is a live vaccine, containing at least $10^{9.0}$ cfu, but not more than $10^{9.4}$ cfu of *S. equi* strain TW928 per dose as active substance. The vaccine contains a number of stabilisers and is presented with the solvent for reconstitution of the lyophilisate. No adjuvant or preservative is included.

Container

For both the vaccine and the solvent, vials with a nominal volume of 3 ml and of hydrolytical class type I glass are used. The vials are closed with a halogenobutyl rubber stopper and encapsulated with an aluminium cap.

Development Pharmaceutics

The product was developed in response to the economic impact of Strangles and is the first vaccine against this conditionlicensed in the EU. Due to the clonal character of *S. equi*, development of a live vaccine was favoured. The vaccine strain was isolated as a virulent field strain in The Netherlands. This field isolate was used for construction of a deletion mutant strain, named TW928. A deletion mutant strain has been selected because of environmental safety reasons. The only safety risk that theoretically may be expected is recombination with wild-type *S. equi* or other homologue species, leading only to the normal wild-type *S. equi* already present in the field. The suitability of the vaccine strain for all areas of the EU was justified based on the published literature as well as on the results of efficacy studies conducted in different regions of the European Union. *S. equi* strains are genetically stable with respect to their immunogenic properties. Further issues regarding the genetic construction of the vaccine strain as well as the question of genetic stability were addressed in the dossier.

Submucosal vaccination to the inside of the upper lip of horses was chosen as the optimal route of administration. An applicator is included to ensure that the vaccine is administered at the right depth. For production of the vaccine, routine culture conditions for a live bacterial vaccine are used.

Clinical trials formulations

Composition of batches used in the clinical trials were provided. All the vaccine batches used in the clinical trials were produced as described for the vaccine intended for the market, except for fill volume of one batch and the fermentation size for some of the batches.

Method of manufacture

The method of preparation of the active substance, the freeze-dried vaccine and the solvent was presented in detail. For production, routine culture conditions for a live bacterial vaccine (growth in culture medium, mixing with stabiliser and freeze-drying) are used. All parameters of the process are monitored carefully

to guarantee a consistent quality. Validation data of the aseptic filling of the vaccine and the lyophilisation process were presented and considered satisfactory.
The minimum and maximum number of subcultures of each seed lot prior to the production stage were specified, along with the typical minimum and maximum bulk sizes.

The solvent is filtered, filled into vials and autoclaved.

Control of Starting Materials

Active substance

The wild-type *S. equi* subspecies *equi* strain TW was isolated from a lymph node abscess of a horse with Strangles in the Netherlands in 1990. This strain was used, to construct the deletion mutant *S. equi* subspecies *equi* strain TW928. During construction of the vaccine strain the wild-type gene of strain TW was replaced by the selected gene interrupted by an antibiotic resistance gene, which was subsequently replaced by the gene with the deletion in it, which resulted in the vaccine strain TW928.

Preparation and testing of the Master Seed and Working Seed were adequately described.

Genetic stability of the vaccine strain is very high and was substantiated in the Reversion to virulence study. The risk of DNA uptake by the vaccine strain from other bacteria present in its environment is low. Wild-type *S. equi* is classified as EEC class 2 (according to EU Guideline 2000/54/EC) being only pathogenic for equidae whereas *S. equi* strain TW928 considered as non-pathogenic, is classified as EEC class 1. Strangles is not considered as a zoonosis.

Excipients

Conventional pharmaceutical excipients are used and all complied with the relevant Ph.Eur/USP monographs and Certificates of analysis were provided.

Preparation of media were described and information on starting materials used was provided.

The finished product complies with the current TSE-Risk assessment according to Commission Directive 1999/104/EC and Note for Guidance EMEA/410/01-Rev. 1.

Control tests during Production

Results of in-process control tests for the active substance, freeze-dried vaccine and solvent were provided and met the specifications.

Control Tests On The Finished Product

The description of the methods used for the control of the finished product (vacuum, final inspection, identity, purity, titre, safety, residual humidity) and the specifications were provided. The specifications proposed at release and at the end of shelf-life are appropriate to control the quality of the finished product. As Equilis StrepE is a live vaccine administered parenterally, only horses seronegative with regard to *S. equi* and with no history of Strangles or vaccination against Strangles will be used for the batch safety test. Detailed safety test results will be provided for further batches post-marketing.

Full specifications of the final product tests on the solvent were provided.

The results of the analysis of three consecutive production runs of freeze-dried vaccine and for two consecutive production batches of solvent were presented which comply with the required specifications.

Stability

Stability of the bulk antigen

The storage period of the final bulk between blending and filling at a temperature of 2-10°C under constant agitation has been validated for a maximum period of 4 days.

Stability of the finished product

Stability data to support a shelf life of 36 months for the freeze-dried vaccine has been provided. A minimum release titre of $10^{9.0}$ cfu per dose of 0.2 ml is proposed on the basis that efficacy has been demonstrated with this dose.

Stability data to support a five year shelf-life for the solvent was provided.

Stability of the reconstituted product

Stability after reconstitution for two batches reconstituted with solvent and stored at room temperature for up to 4 hours was submitted and demonstrate that the vaccine may be used up to four hours after reconstitution.

Environmental risk assessment for products containing or consisting of Genetically modified organisms

Data related to the environmental risk assessment for the product according to Directive 2001/18/EC were provided and consultation with the competent bodies under this Directive was undertaken.

The choice and number of gene deletions selected for the vaccine strain were justified. The genes responsible for the natural antibiotic resistance of the vaccine strain are neither of chromosomal or plasmidal origin. A complete antimicrobial drug resistance profile of the vaccine strain including MIC values was provided. A detection limit of the *S. equi* strain used was determined by spiking of nasal washings. The results show that *S. equi* vaccine strain TW928 can be re-isolated from nasal washings if the bacterium constitutes at least 0.028% of the bacterial flora.

The survival of the vaccine strain/wild type strains of *S. equi* in the environment (tap water and pond water) at different temperatures was investigated. It was shown that the vaccine strain cannot compete with other bacteria in its natural environment and that the vaccine strain is only able to survive in its natural environment when the general conditions for bacterial growth, including the vaccine strain, are limited.

No particular hazard was identified with the use of this product in the environment. The overall risk to humans, animals and the environment with this product was considered negligible.

OVERALL CONCLUSION ON PART II

The analytical part of the dossier is well described. Production of the vaccine is straightforward – growth of the production strain, followed by concentration of the harvest, blending with stabiliser, filling and freeze-drying. All of the critical process parameters were monitored in order to ensure a product of consistent quality. The TSE risk with this product is negligible. The quality of Equilis StrepE can be considered to be adequately demonstrated.

III. SAFETY ASSESSMENT

The vaccine is intended for use in horses from four months of age onwards to induce immunity against *S. equi* to reduce clinical signs and occurrence of lymph node abscesses. The vaccine is administered submucosally into the inside of the upper lip, using a volume of 0.2 ml. Safety studies have been carried out in the target species, the horse. Four laboratory studies and four field trials have been conducted.

Composition of batches used in the safety trials and protocols of all batches were provided. All laboratory safety studies were performed with a maximum potency batch at minimum passage level. Field safety trials were performed with batches of minimum passage level with low to intermediate potency.

Respiratory signs, including pneumonia, were seen in many horses in almost all safety trials, and even in efficacy trials. They were always considered non-specific, essentially because no *S. equi* could be isolated, contrary to *S. zooepidemicus* isolated on several occasions. In the safety studies *S. zooepidemicus* and *S. equi* were differentiated by colony structure and distinguishable from each other.

LABORATORY TESTS

One overall study was presented to address the safety of the administration of one dose, an overdose and a repeated dose of vaccine. An additional study was performed to investigate the safety of a ten-fold overdose. To fulfil the special requirements for live vaccines according to Directive 2001/82/EC part 7.C.6, several laboratory studies in the target species have been carried out (spreading and dissemination of the vaccine strain, reversion to virulence).

All control animals used in the studies had a serum antibody titre of $\leq 2^3$ cfu/ml at the moment of challenge and were susceptible to challenge. A definition of specific clinical signs of Strangles used for the assessment of the safety was provided. “Specific clinical signs of Strangles are swollen lymph nodes and strong pyrexia at the same time”. All laboratory studies were conducted in compliance with the principles of GLP.

Safety of single, repeated, overdose and spreading and dissemination of live *Streptococcus equi* strain TW928 deletion mutant vaccine in horses after submucosal vaccination

The objective of the study was to evaluate the safety of the live vaccine after submucosal vaccination of foals at an age of three to five months by investigation of local and systemic reactions. The horses were also examined for shedding of the vaccine strain, spreading of the vaccine strain to other susceptible contact horses and possible dissemination in the horses to different sites of the upper respiratory tract or draining lymph nodes.

One group of horses was vaccinated three times with one 0.2 ml dose of the vaccine submucosally in the upper lip at intervals of four weeks. Another group was vaccinated with a ten-times overdose of the vaccine submucosally in the inside of the upper lip. Unvaccinated horses served as untreated controls.

After vaccination with a single dose of vaccine, a warm and diffuse or hard swelling, which was painful upon palpation on the day of vaccination, was observed at the injection site from four hours after vaccination onwards and reached a maximum size at day 2 to 4. After two to three days the swellings discharged little exudate and then decreased rapidly in size. Some animals showed a small mucosal lesion at the injection site (day 4 to 10). All reactions disappeared within 24 days after vaccination. The reactions caused no apparent discomfort to the horses. The appetite was normal. An increase of rectal temperature up to 2.6°C occured on the day of vaccination. Occurrence of abscesses in the regional lymph nodes after vaccination with a single dose was not observed.

After vaccination with an overdose, the reactions were larger and more persistent than after single dose vaccination. The entire upper lip of all horses was diffusely swollen, warm to the touch and

painful upon palpation. This may be due to the larger volume administered. At four hours after each vaccination there was a transient increase in rectal temperature (up to 2.7°C). The temperature was normal again the next day. In the majority of horses vaccinated with a tenfold overdose, an abscess occurred in one or both of the submandibular lymph nodes, from which apurulent discharge appeared about one week later. After draining, the abscesses healed completely without any treatment.
Abscesses of the submandibular lymph nodes were seen in the horses on D14 – 24 after vaccination. The SPC was amended to reflect the risk of developing abscesses after an overdose.

Safety of a ten-times overdose of live *Streptococcus equi* strain TW928 deletion mutant in horses after submucosal vaccination

The objective of the study was to confirm the results of the ten-fold overdose. A special batch of vaccine was prepared, as it is not possible to dissolve 10 vials of the vaccine in 0.2 ml of solvent to obtain the ten-fold overdose. The mean infectivity titre was $10^{11.1}$ cfu/ml, corresponding to $10^{10.4}$ cfu per 0.2 ml dose.

Four month old, susceptible foals were used in this study. The horses were vaccinated submucosally in the inside of the upper lip with 0.2 ml of the test batch, containing $10^{10.4}$ cfu per 0.2 ml dose. All data on local and systemic reactions post vaccination as well as the results at *post-mortem* were recorded individually for each horse.

All horses developed local reactions after the overdose vaccination. A diffuse and hard swelling was observed at the injection site from four hours after vaccination onwards. From day 3 onwards, the reactions drained a little exudate and then decreased rapidly in size and subsequently disappeared within 21 days. At four hours after vaccination the rectal temperature was transiently increased (up to 2.2°C). The temperature was normal again (pre-vaccination level) the next day. After vaccination, all horses had a normal appetite and were in good condition. Transient reactive submandibular lymph nodes, i.e. slightly enlarged, were observed in five horses. Specific vaccination-related signs were noted in two horses, which developed an abscess in one of the submandibular lymph nodes, 1 to 3 weeks after vaccination. After draining, the abscesses healed completely without any treatment. The possible risk and consequences of the persistence of the vaccine strain in vaccinated horses in view of the possibility that *S. equi* can establish the carrier state was addressed. It was demonstrated that the vaccine strain does not spread to other horses by the natural route.

Examination of reproductive performance

The vaccine is not intended to be used in pregnant and lactating animals as stated in the SPC.

Examination of immunological functions

Simultaneous vaccination of Equilis StrepE with Equilis Pro-T or Equilis Equenza-T had not shown any influence on the immune response against the antigens of these vaccines. Therefore, it is concluded that Equilis StrepE does not adversely affect the immunological response in horses vaccinated with these other two Equilis vaccines.

In the literature, a rare complication after immunisation of highly-immune animals is described, purpura haemorrhagica, which causes severe vasculitis due to immune complex formation. Purpura haemorrhagica has not been observed in any of the safety studies performed during development of Equilis StrepE and this is reflected in the SPC.

Special requirements for live vaccines

Spreading and dissemination of live *Streptococcus equi* strain TW928 deletion mutant vaccine in horses after submucosal vaccination was investigated. The horses were examined for shedding of the vaccine strain, spreading of the vaccine strain to other susceptible contact horses and possible dissemination in the horses to different sites of the upper respiratory tract or draining lymph nodes.

After vaccination with a single and repeated dose *S. equi* was re-isolated from injection site swabs of the majority of horses, mainly at day 3 to 4, but never later than day 4 after each vaccination. From nasal washings, *S. equi* was not re-isolated. After vaccination with an overdose, *S. equi* was re-isolated from injection site swabs at day 2 to 7 and from nasal washings at day 2 and day 4. At *post mortem* examination *S. equi* was not isolated from any horse or tissue sampledincluding injection sites, guttural pouches and lymph nodes.

In 5 overdosed horses, submandibular "Strangles" abscesses developed, all containing *S. equi*. In none of the single or repeated dose horses or controls, were abscesses seen to develop and isolation of *S. equi* could not be made, except at the injection site D1-D4. In overdosed horses there was dissemination of the organism to the submandibular lymph nodes. The single dose and repeat dose horses developed a small suppurative inflammation locally at the injection site, whereas the in-contact horses had no signs of *S. equi* dissemination. It is reflected in the SPC, that the *S. equi* vaccine establishes a small suppurative inflammation locally at the injection site, and potentially disseminates to the regional lymph nodes. The transmission of the vaccine strain from overdosed horses with a draining abscess has not been investigated.

Carriers of the vaccine strain can be detected after three consecutive nasopharyngeal swabs with 60% success after bacteriological isolation and with 90% success with PCR-methods. The PCR method detects both viable and non-viable bacteria, but also fragments of DNA of bacteria which drained from the injection site. As for spreading and dissemination studies only viable bacteria should be detected and standard bacteriological examination methods were applied.

Reversion to virulence

Reversion to virulence was tested in two separate studies. In the first study, five passages through horses were made in total. In the second study the sixth passage in horses was performed and the safety of material from the sixth passage was compared with that of unpassaged material.

The reversion to virulence study was performed in accordance with the guidelines where the safety of material from the highest successful passage is compared with that of unpassaged material. The identity of the original vaccine strain and the vaccine strain after the last passage in horses was confirmed by PCR i.e. presence of the deletion. The choice of the intranasal challenge model used for testing of reversion to virulence was justified.

The study to perform an additional (sixth) passage in horses and to compare the safety of material from this passage with the safety of unpassaged material showed that the vaccine does not revert to virulence after six *in vivo* passages. Two weeks after intranasal inoculation the vaccine strain could not be isolated from injection site swabs/samples of the inner side of the upper lip and nasal washing samples.

One year old horses were used in this study, as when the experiment started no animals of the specified age were available. Neither the Ph.Eur. 5.2.6 nor the Directive 2001/82/EC state that reversion to virulence should be tested in animals of the minimum age.

Biological properties of the vaccine strain

Information on the biological properties of the vaccine strain and its suitability for use in a live vaccine was provided. The vaccine strain is a deletion mutant which is unable to multiply in humans or other animals.

Recombination or genomic reassortment of strains

The strain was shown to be genetically stable. The possibility of recombination or genomic reassortment of the vaccine strain with wild-type organisms was shown to be remote. *S. equi* can be considered a clonal pathogen, based on its uniform antigenicity, identical restriction fragments profiles and data from multilocus enzyme electrophoresis, indicating no or very limited horizontal gene transfer or influx of heterologous DNA.

Interactions

Some information regarding the compatibility of Equilis Pro, Equilis Pro-T, Equilis Equenza and Equilis Equenza-T has been provided. However, while there appears to be no influence on the immune response to these vaccines when Equilis StrepE is administered and in light of the limited efficacy data provided to support the concurrent claim, it is not possible to include a recommendation of concurrent vaccination.

FIELD STUDIES

Four field safety studies have been carried out. The trials were performed according to the principles of the guideline "Good clinical practice for the conduct of clinical trials for veterinary clinical products".

A field safety trial of Equilis StrepE in horses

The aim of this study was to assess the safety of Equilis StrepE under field conditions. Horses of at least 4 months of age were included in the study. Horses were vaccinated submucosally (0.2ml) into the inside of the upper lip, whereas control horses were injected with the solvent only. The horses received the first two injections at an interval of four weeks and a third injection three months after the second vaccination. The first and second vaccination were given to investigate all aspects of field safety. The third vaccination, however, was given to investigate whether the local reactions would increase after repeated vaccination.

One hour after first vaccination, no significant differences were observed between the vaccinated and the solvent group. Four hours after vaccination significantly more horses of the vaccinated group showed a local reaction compared to the solvent group. Vaccination with Equilis StrepE, resulted in a typical local reaction at the injection site, normally starting as a diffuse soft swelling. Most of the reactions were small to medium in size (<2 x 2cm - <4 x 4cm), hard in nature and sometimes painful upon palpation. The maximum size was normally reached 2 to 3 days *post vaccination*. From day 4 onwards, the reactions decreased in size, became softer in nature and sometimes led to an erosion of tissue accompanied by a discharge. The mean duration of local reactions was about seven days. On day 14, most of the reactions had disappeared.

One and four hours after second vaccination, significantly more horses of the vaccinated group showed local reactions compared to the solvent group. After the second vaccination, all swellings observed were visible from the outside of the lip. Two-thirds of the horses showed an extensive swelling with a maximum (2x2 cm to 4x4 cm, hard in nature) at day 2 and 3. This was comparable to what has been described after first vaccination.

One hour after third vaccination, no significant differences were observed between the vaccinated horses and those administered the solvent. Four hours after vaccination, significantly more horses of the vaccinated group showed a local reaction and more than half of the horses showed a large (greater than 4x4 cm) or a medium (2x2 to 4x4 cm) swelling. This is similar to that described after first vaccination. A slight and transient increase inrectal temperature after vaccination was observed as well. The average rectal temperature was 38.0±0.2°C for the vaccination group and 37.8±0.2 °C for the group administered the solvent.

On day one after first and second vaccination with Equilis StrepE, swellings of the mandibular or retropharyngeal lymph nodes were observed in the majority of vaccinated horses. Swelling of the lymph nodes had declined at the next examination time point at day 4 and reached base levels at day 14. Approximately half of the swollen lymph nodes were painful on palpation at day one after vaccination. In the group administered the solvent, lymph node swellings were also noted, but to a lesser extent and were not painful on palpation.

The SPC includes a warning that swellings at the injection site which may be "warm or painful" develop with 4 hours, and that the reaction is maximal 2-3 days post vaccination. The swelling resolves completely with 3 weeks and causes no apparent discomfort. The SPC also states 'Slight enlargements, which may be

transiently painful, of the retropharyngeal and mandibular lymph nodes may occur for a few days after vaccination. Further, an increase in rectal temperature up to 2°C may occur on the day of vaccination'.

A field safety trial of Equilis StrepE administered concurrently with Equilis Equenza-T

The objective of this study was to assess the safety of vaccination with Equilis StrepE under field conditions, when administered concurrently with Equilis Equenza-T. The Equilis Equenza-T vaccine batch used contained 100 AU subunits A/equine 1/Prague/56, 50 AU subunits of A/equine 2/Newmarket 1/93, 50 AU subunits of A/eqine 2/Newmarket 2/93, 40 Lf of purified tetanus toxoid and 125 µg saponin per dose of 1 ml.

Foals between 6 and 8 months of age were included in the study. The horses were divided into four groups.

Group A: Horses, vaccinated with **Equilis StrepE** (0.2 ml) submucosally in the upper lip, concurrently with **Equilis Equenza-T** intramuscularly in the pectoral muscle at days 0 and 28.

Group B: Horses, vaccinated with **Equilis StrepE** (0.2 ml) submucosally in the upper lip at days 0 and 28.

Group C: Horses, vaccinated with **Equilis Equenza-T** intramuscularly in the pectoral muscle at days 0 and 28.

Group D: Horses, non-vaccinated controls

All animals vaccinated with Equilis StrepE showed a local reaction at the injection site. Initially the reaction had a diffuse character. Later on the swelling changed to a hard nodule. Within a few days, the reaction discharged and then disappeared. A slight and transient increase of rectal temperature was observed.

The results show that concurrent use of Equilis StrepE and Equilis Equenza-T is safe. However, the recommendation to use such a combination has not been included on the SPC due to the limited efficacy data provided.

A field safety trial of Equilis StrepE in pregnant horses

The aim of this study was to assess the safety of vaccination with Equilis StrepE under field conditions in pregnant horses. One group of horses was vaccinated submucosally (0.2 ml) into the inside of the upper lip, whereas another group of horses was injected with the solvent only. Depending on the stage of the pregnancy, the mares received two to five injections. The horses received the first two injections at an interval of four weeks and the following injections at intervals of three months. Vaccinations were given in all stages of pregnancy. The mares were vaccinated from the day after mating up until two days before parturition.

One horse of the Equilis StrepE group gave birth twice during the study period. None of the mares aborted. Nearly all pregnancies had a normal course. Only one pregnancy in the vaccinated group had a negative outcome. A fully developed foal was stillborn together with the placenta.

This study was provided for information only, as the vaccine is not intended for use in pregnant and lactating horses. Nevertheless, the results confirm the observations made in the other safety studies included in the documentation.

ECOTOXICITY

A study was conducted to test survival of the *S. equi* strain TW928 in different environments at ambient temperature. In 0.9% salt solution at ambient temperature, the vaccine strain was inactivated within a period of three weeks. After air drying of 4.0×10^9 cfu/ml, no growth of the vaccine strain could be detected at five and six weeks after drying.

The study was performed again, but now also at +2 to +8 °C and +37 °C. In tap or pond water the vaccine strain appeared quickly inactivated and /or overgrown by other bacteria that were already present in these

waters. At higher temperatures the inactivation and overgrowing of the vaccine strain occur faster than at lower temperatures. Survivability was best in air-dried conditions at low temperatures.

Phase 1 Assessment

An assessment of risk according to EMEA/CVMP/074/95 was conducted. A Phase I ecotoxicity risk assessment was performed, and was deemed to have been adequately addressed. No Phase II risk assessment was considered necessary and was therefore not performed, in line with current guidelines.

IV. OVERVIEW OF EFFICACY TRIALS

INTRODUCTION

Efficacy studies (four laboratory studies and one field trial) have been carried out in the target species, the horse, by the recommended route of administration. The approved indication is "For immunisation of horses against *Streptococcus equi* to reduce clinical signs and occurrence of lymph node abscesses."

The following studies were performed:dose-response study; studies assessing duration of immunity and onset of immunity after basic vaccination and re-vaccination; compatibility studies; one field trial.

For laboratory efficacy studies batches of vaccine at maximum passage level (except onset of protection study: minimum passage level) with minimum or sub-minimum potency were used. The field efficacy study was performed with a medium potency batch at minimum passage level.

IV.C. LABORATORY TRIALS

For all efficacy studies the same numeric clinical scoring system has been used and the most important parameters used to assess the efficacy of the vaccine are temperature and lymph node palpation, which are specific for Strangles. Other symptoms e.g. throat swelling, stridor, anorexia and lameness (only in bastard Strangles) are only observed in very severe cases.

In all studies the rectal temperature was measured daily after challenge, but the clinical examination was performed at regular intervals during 0-4 days. Since the clinical signs in vaccinates and controls were evaluated in the same manner, advantages or disadvantages of assessing certain parameters on a limited number of days were the same for both groups. The criteria for allocation of horses to the different groups for the efficacy studies were equivalent to that for the safety studies; the horses were allotted to the different groups based on an even distribution based on age and supplier.

Although a variety of statistical tests were used to assess data resulting from studies to evaluate the efficacy of Equilis StrepE, there was little statistical planning of the experiments reported in the initial submission. Due to the lack of pre-planning the studies may have been underpowered in order to detect biologically relevant differences between the vaccinated and the control groups. However, it is acknowledged that with respect to animal welfare the number of animals used in such trials should be minimised.

The data presented were re-analysed by repeated measures ANOVA using the generalised linear model in Minitab and, where appropriate, a Dunnet's comparison with the control group. This type of analysis compares the data measured on the different days and also takes into account that the data on the different days were measured in the same animals. However, after rechecking the analysis performed, it was noted that the model used, did not account for the correlation of repeated measurements within the same animal. Therefore, a re-analysis of the data using the correct statistical model was provided and found to be acceptable.

Another point of concern with the statistical analysis as performed was the fact that only p-values were reported. Estimates of the effects seen including their pertinent confidence intervals (necessary to judge the relevance of observed statistical significances) in general were lacking.

The total clinical score used to assess the effects of vaccination was based on all clinical parameters measured including rectal temperature. The data was re-analysed focussing on the typical clinical parameters for Strangles: pyrexia (rectal temperature) and nasal discharge/lymphadenopathy.

Vaccination with Equilis StrepE results in decreases in rectal temperature: mean decreases in rectal temperature are observed of up to almost $1^{o}C$ for an 11 day period with peak decreases of up to $2.68^{o}C$.

Vaccination resulted in clinically significant decreases in mean clinical score for nasal discharge/lymphadenopathy.

The 'Split-Plot'-model used in the re-analysis of the data on temperature and nasal discharge and lymphadenopathy is appropriate.

Based on animal welfare reasons and the observation that the control animals consistently developed Strangles upon challenge, the number of control animals included in the experiments were limited and were considered to function as a check of the uptake of the challenge. A re-analysis of the cumulative abscess score data using the individual control groups and also the data of the combined control groups was provided. It can be concluded that vaccination with Equilis StrepE gives a significant reduction in the cumulative abscess scores.

Some concerns were raised regarding the pooling of controls. It was confirmed that pooling of the control groups was only performed for the analysis of the p*ost-mortem* data of the vaccination/challenge experiments and not for the analysis of the rectal temperature data or nasal discharge/lymphadenopathy data. For the latter two parameters, comparisons were only made with the corresponding control groups. For the analysis of the field trial no pooling of data of control groups was performed.

For each of the trials a large number of tests were performed without adjustment. A p-value adjustment should have been made when using multiple control groups. It is clear, that when the adjusted p-value is used in three experiments a significant difference in cumulative abscesses score is observed between vaccinated group and the pooled control group.

The three major parameters analysed to demonstrate the efficacy of Equilis StrepE were: rectal temperature; nasal discharge and lymphadenopathy; *post-mortem* analysis of the lymph nodes (abscess score). The rectal temperatures in vaccinated and control animals were determined on a large number of different days. The data were re-analysed by repeated measures ANOVA which compares the data of the vaccinated and control animals measured on the different days and also takes into account that the data measured on the different days are measured in the same animals. Therefore, multiplicity is not an issue for this parameter. The nasal discharge/lymphadenopathy data was re-analysed in the same manner as the rectal temperature data and therefore multiplicity is also not an issue for this parameter. The *post-mortem* score (abscess score) is determined at only one time point. Multiplicity is therefore not an issue for this parameter. It was noted however that not only multiple measurements over time but also multiple endpoints (as in this study) form sources for multiplicity.

Establishment of a Challenge Model

For demonstration of efficacy an intranasal challenge model in horses was used. The criteria used to diagnose Strangles were defined as "Specific clinical signs of Strangles are swollen lymph nodes caused by *S. equi* and strong pyrexia at the same time". Clinical examination was performed double blinded i.e. the veterinarian was unaware to which group, vaccinates or controls, the animal belonged. The overall condition of the individual animal at the moment of veterinary examination is decisive for determining the time point for euthanasing diseased animals.

The challenge strain was obtained from a horse with clinical Strangles and was identified as *Streptococcus equi* subspecies *equi* and was haemolytic on blood agar plates. The doses were based on preliminary studies in which Strangles was induced in unvaccinated horses. As all challenged control horses developed clinical signs, the choice of the challenge strain and method is justified. Intranasal administration of a fresh culture of the *S. equi* challenge strain containing about 10^9 cfu/ml consistently resulted in signs of Strangles within five to ten days in control animals. The serum antibody ELISA was used to verify the susceptibility of the horses to *S. equi* infections. No correlation between protection and antibody titre was shown.

Determination of the Vaccine Dose

Dose-response study in horses with Equilis StrepE administered submucosally in the lip

The objective of the study was to determine the vaccine dose of Equilis StrepE vaccine after submucosal vaccination of horses at an age of 4 to 5 months with no history of Strangles (dose-response experiment).

Horses were vaccinated twice with 10^9 cfu/0.2 ml, with 10^8 cfu/0.2 ml or with 10^7 cfu/0.2 ml at intervals of four weeks submucosally in the upper lip. Other horses were used as unvaccinated challenge controls. All horses were challenged intranasally two weeks after second vaccination.

A repeated dose of 10^8 or 10^9 cfu per dose induced a comparable protection in vaccinated horses. No difference in protection was shown between a repeated dose of 10^8 or 10^9 cfu per dose at two weeks after vaccination. The minimum protective dose was set at 10^9 cfu per dose. A dose of 10^8 cfu seemed to be the absolute minimum protective dose, but it was assumed that 10^9 cfu would induce a better duration of immunity.

Onset of protection

Efficacy of Equilis StrepE vaccine in foals vaccinated concomitantly with Nobi Equenza-T

The objective of the study was to investigate the onset of immunity after submucosal re-vaccination with one dose of Equilis StrepE at six months, after completion of the basic immunisation in horses. Additionally, the horses were simultaneously vaccinated intramuscularly with Nobi Equenza-T to test compatibility.

The Equilis StrepE vaccine batch used (at a passage level of X+6 from the MS) contained 1 x 10^9 cfu per dose. The Nobi Equenza-T vaccine batch used contained 100 µg subunits A/equine 1/Prague, 50 µg subunits of A/equine/Miami/63, 50 µg subunits of A/eqine/Fontaine-bleau/79, 40 Lf of purified tetanus toxoid and 125 µg Quil A per dose of 1 ml.

Six month old foals havingno history of Strangles, influenza and tetanus (disease or vaccination) were used in this study. The first group were vaccinated with Equilis StrepE (10^9 cfu/0.2 ml) submucosally in the upper lip and with Nobi Equenza-T (1 ml) intramuscularly. At day of first treatment all horses were seronegative or had very low titres to *S. equi*. After the second and third vaccination most of the horses showed a seroconversion. Seroconversion was also observed in the group only vaccinated with Nobi Equenza-T.

The horses were challenged with virulent *S. equi* two weeks after the last vaccination. The controls developed clear signs of Strangles, whereas no clinical signs of Strangles were observed in the vaccinated horses. A *post-mortem* investigation confirmed the clinical findings.

As limited efficacy documentation was presented for the combined vaccination any conclusions regarding any effect of vaccination with Equilis StrepE on influenza or tetanus antibody titres development are impossible. The revaccination 6 months after the second vaccination at D210, shows that the anamnestic response is present and protection is restored after one booster vaccination. A single dose after 6 months will restore immunity.

The Influence of Maternal Antibody on the Efficacy of the Vaccine

It was confirmed that maternal antibodies to *S. equi* do not have an influence on the efficacy of the vaccine. The minimum age of vaccination is 4 months and if maternal antibodies to *S. equi* are present, they should be declining or have disappeared at that time. This was confirmed by the serology results of the safety and efficacy studies.

Duration of Immunity

Duration of immunity of *S. equi* vaccine and compatibility with Equilis Equenza-T

The objective of the study was to investigate the duration of immunity after submucosal vaccination with Equilis StrepE in horses at an age of 4 to 5 months. Additionally the horses were simultaneously vaccinated intramuscularly with Equilis Equenza-T to test compatibility.

The Equilis StrepE vaccine batch (at maximum passage level) used contained 1 x 10^{10} cfu per vial. The Equilis Equenza T vaccine batch used contained 100 AU subunits A/equine 1/Prague, 50 AU subunits of A/equine 2/Newmarket 1, 50 AU subunits of A/eqine 2/Newmarket 2, 40 Lf of purified tetanus toxoid and 125 µg saponin per dose of 1 ml.

Four to five month old foals having no history of Strangles were used in this study. The horses were divided into two groups; vaccinated with Equilis StrepE 0.2 ml) submucosally in the upper lip and with Equilis Equenza-T (1 ml) intramuscularly in the neck muscle or; vaccinated with Equilis Equenza T (1 ml) intramuscularly in the neck muscle. The horses were vaccinated twice at four weekly intervals. Several horses received a third vaccination 6 months after the second. At day of first treatment all horses were seronegative or had very low titres to *S. equi*. After the second and third vaccination, most of the horses showed a seroconversion. Challenge was performed 3 months after second vaccination as well as 3 and 6 months after third vaccination.

The duration of immunity after two or three submucosal vaccinations with Equilis StrepE was examined in horses, at the youngest recommended age of vaccination. The horses were simultaneously vaccinated with Equilis Equenza-T, an influenza and tetanus vaccine, to test compatibility. The immunity against *S. equi* was tested by challenge and the efficacy of Equilis Equenza-T was evaluated serologically.

Efficacy is limited to 3 months after two injections and no protection is seen after 6 months. Therefore, re-vaccination every 3 months is required to maintain immunity.

Additionally a new study had been performed in which a group of horses received a basic vaccination of Equilis StrepE and a single booster vaccination 6 months later (group 1). At the moment of booster vaccination horses suffering from Strangles were introduced into the herd. Another group of horses (group 2) received their first vaccination at the moment that the horses suffering from Strangles were introduced into the herd and the last group of horses (group 3) served as unvaccinated controls. The objective of the study is to investigate the efficacy of Equilis StrepE in horses when vaccinated during an outbreak.

Significant differences were observed between group 1 and group 3 regarding clinical examination. The results demonstrate that most of the horses were protected against a field infection with *S. equi* about 25 weeks after a previous basic vaccination if the third vaccination and infection occurred at the same time. It was demonstrated that a single injection of Equilis StrepE after a time span of 6 months is sufficient to restore immunity at a level of protection at least equivalent to the level obtained after basic vaccination.The SPC (section 5.5) includes a sentence e.g. ”Basic vaccination performed during an outbreak is not efficacious because immunity is insufficient until basic vaccination has been completed.” Section 5.8 of the SPC included the statement: “A priming response is maintained for up to six months after basic vaccination. Therefore only a single dose of vaccine is needed to restore immunity.”

FIELD TRIALS

Field efficacy of Equilis StrepE including compatibility with Equilis Pro-T

The objective of the study was to investigate the efficacy of Equilis StrepE in young horses under field conditions, against field exposure. Additionally the horses were simultaneously vaccinated intramuscularly with Equilis Pro-T (contains three inactivated influenza antigens and tetanus) to test compatibility.

The Equilis StrepE vaccine batch used contained 1 x $10^{9.1}$ cfu per dose. Six to seven month old horses, with no history of Strangles, disease or vaccination were used in this study. The horses were divided into two groups; vaccinated with the Equilis StrepE submucosally in the upper lip and with Equilis Pro T (1 ml) intramuscularly; or vaccinated with Equilis Pro T only, (1 ml) intramuscularly. The horses were vaccinated at days 0 and 28 of the study with both vaccines.

The following parameters were significantly different between vaccinated horses and control animals ($p<0.05$):

- total clinical scores
- mean daily clinical scores
- number of horses with clinical signs of Strangles
- mean cumulative abscess score
- number of lymph nodes affected

A group, vaccinated only with Equilis StrepE, wasnot included. The Committee agreed that if horses are vaccinated with Equilis StrepE alone, the number of animals without any clinical or subclinical signs of Strangles would be greater. No recommendation for concurrent use is included in the SPC.

OVERALL CONCLUSION ON PART IV

From the results of the efficacy studies performed, it may be concluded that Equilis StrepE is efficacious at $10^{9.0}$ cfu of *S. equi* strain TW928 per dose and that reduction in clinical signs of Strangles and reduction of lymph node abscesses is obtained as early as 2 weeks after completion of the basic vaccination. The duration of immunity of Equilis StrepE is three months after completion of the basic vaccination and after each single booster vaccination.

Vaccination with Equilis StrepE seems to have a beneficial effect at $10^{9.0}$ cfu per dose leading to a reduction in specific clinical signs of Strangles (strong pyrexia and swollen lymph nodes). A justification for 10^{9} cfu chosen as minimum protective dose of vaccination was provided, as the repeated administration of 10^{8} or 10^{9} cfu per dose seemed to induce a similar protection in vaccinated horses. Statistical analysis of these data was presented. There is no difference in protection between a repeated dose of 10^{8} or 10^{9} cfu per dose at two weeks after vaccination.

RISK-BENEFIT ASSESSMENT

Strangles is a highly contagious disease of horses caused by the Gram-positive bacterium *Streptococcus equi* subspecies *equi*, a beta-haemolytic, Lancefield group C streptococcus. A high fever will normally be present, as well as coughing and difficulty in swallowing. A nasal discharge appears which begins as a clear, thick discharge but rapidly becomes yellow and contains a large amount of pus. External lymph nodes behind the back edge of the mandible, under the lower jaw, and above the eye begin to swell and become very painful. Internal lymph nodes in the head regioncan become large enough to block the airway, causing the horse to have difficulty breathing and hence the name Strangles. The lymph nodes may rupture, and dischargehighly infectious pus for 7-14 days after the onset of clinical signs.

Other horses may develop "bastard Strangles". This is a more rare, but more severe form of the disease, with an almost 100% mortality rate, that involves abscessation of internal lymph nodes, including brain, mesenteric and pulmonary lymph nodes and possible rupture of them within the body.

Strangles cannot be effectively treated with antibiotics as the latter fail to penetrate abscesses and the presence of pus inactivates most antibiotics. Strangles outbreaks are protracted and the fatality rate may reach 10%. There is currently no other vaccine licensed for Strangles in the EU. Strangles is one of the most frequently diagnosed infectious diseases of the horse. No eradication programmes exist on a European or national level against Strangles.

The main benefit for horses is that Equilis StrepE reduces the clinical signs and occurrence of lymph node abscesses after *Streptococcus equi* infection. The vaccine has to be regarded as an "orphan product" which will be administered only in horses for which a risk of *Streptococcus equi* infection has been clearly identified, due to contact with horses from areas where this pathogen is known to be present.

The analytical part of the dossier is well described.

The results of the safety trials demonstrate that Equilis StrepE is safe for horses from four months of age onwards, as the local and systemic reactions observed after single, repeated dose and overdose treatment were considered acceptable and healed completely without intervention and without residues. Furthermore, the vaccine strain did not spread to other target animals and did not revert to virulence following six passages *in- vivo*. Any risk for the operator or environment is not to be expected.

The Committee agrees, that due to the genetic nature of the vaccine strain and its clonal character, the genetic stability of the vaccine strain can be considered as high. Reversion to virulence is considered to be unlikely. Any risks for humans can be regarded as negligible, as literature data did not reveal any specific risk of zoonosis for wild-type *S. equi*. Wild type *S.equi* is only pathogenic for equidae and considered to be apathogenic for humans. Thus, this risk can be regarded as even less for a vaccine strain being a deletion mutant. The environmental risk can also be regarded as very low, as contact animals included in a laboratory safety study were not infected and survival of the vaccine strain in the environment is low.

The basic vaccination consists of 2 vaccinations 4 weeks apart. The duration of immunity is 3 months. Therefore, re-vaccination every 3 months is required to maintain immunity. A priming response is maintained for up to six month after basic vaccination. Therefore only a single dose of vaccine is needed to restore immunity. Thus the vaccine can also be used in the face of an outbreak in these conditions. Basic vaccination performed during an outbreak is not efficacious, because immunity is insufficient until basic vaccination has been completed.

CONCLUSIONS

- The mild local and systemic reactions induced by vaccination clearly outweigh the severe clinical signs after a *Streptococcus equi* infection. The risk of other side effects is considered very low after normal single dose treatment. Therefore the risk-benefit analysis is in favour of Equilis StrepE.
- The risk for veterinarians using Equilis StrepE is considered as negligible.
- The risk of the vaccine strain in the environment is also considered negligible.
- Equilis StrepE is recommended for use in horses at risk of *S. equi* infection and not as a routine vaccination.