SCIENTIFIC DISCUSSION

Invented name:	Equilis Te
Active substance/INN:	Tetanus toxoid
Target species:	Horses
Therapeutic indication:	Active immunisation of horses from 6 months of age against tetanus to prevent mortality.
Withdrawal period:	Zero days
Pharmaceutical form:	Suspension for injection
ATCvet code:	QI05AB03
Pharmaco-therapeutic group:	Tetanus vaccine
Marketing Authorisation Holder:	Intervet International B.V. Wim de Körverstraat 35 NL-5831 AN Boxmeer The Netherlands

1. SUMMARY OF THE DOSSIER

Equilis Te is presented in a 1 ml glass vial or a pre-filled glass syringe. The vaccine contains tetanus toxoid. The product is indicated for active immunisation of horses from 6 months of age against tetanus to prevent mortality. The route of administration is intramuscular.

2. QUALITY ASSESSMENT

Composition

The vaccine contains tetanus toxoid 40 LF (flocculation equivalents)/ml. Standard excipients are used as stabilisers, buffer components and all comply with the Ph.Eur. Per dose the vaccine contains traces of formaldehyde as a remnant of the inactivation process. Depending on the tetanus toxoid supplier traces of thiomersal can be found as well.

Container

Either glass vials or pre-filled glass syringes are used. The vials are closed with a halogenobutyl rubber stopper and encapsulated with a coded aluminium cap. The ending of the plunger and the tip cap closing of the syringe are of halogenobutyl rubber. Both containers are sterilised.

Development Pharmaceutics

The tetanus component of Equilis Te is a standard tetanus toxoid. The sterile fractionated tetanus toxoid concentrate is supplied in bulk for incorporation into Equilis Te, which is then blended, filled, packed and released by Intervet. It contains a fixed amount of purified tetanus toxoid (40 Lf per dose of 1 ml) determined via flocculation testing. The final product is tested in the potency test as prescribed in the Ph.Eur. monograph 0697 by using a Toxoid Binding Inhibition (ToBI) assay.

The adjuvant is based on iscom-matrix technology. The iscom-matrix used here is an adjuvant formulation closely related to iscom but consisting of particles with a patented composition whose shape and appearance are as that of the iscom except for their lack of incorporated antigen. These iscom-matrix particles are formed by a HPLC-purified fraction of Quillaja saponins, cholesterol and phosphatidyl choline. The iscom-matrix is mixed with the antigen only. The iscom-matrix, possesses no or drastically reduced haemolytic activity. Induction of high levels of serum antibodies against tetanus which persisted for an unexpectedly long time was seen in studies and the matrix has an excellent safety profile in horses. The amount of purified saponin in the vaccine has been set at 375 µg/dose. The exact mechanism how the iscom-matrix adjuvant induces such a broad immune response remains yet unclear.

Clinical trials formulations

Composition of batches used in all efficacy and safety studies was provided. Full details and complete protocols of all batches were provided, and all batches were produced according to the standard methods as described in the dossier. For all future batches a maximum shelf life of 36 months was accepted for the tetanus toxoid bulk.

Method of manufacture

All production steps are performed in accordance with GMP. Where applicable sterile equipment and liquids are used.

Production of the tetanus component
Following an extension of the marketing authorisation in 2008 an additional tetanus toxoid supplier was introduced. A flow chart of the method of preparation of the tetanus toxoid component and an in-depth description is provided for each tetanus toxoid supplier. The Tetanus Toxoid Concentrate (TTC)

or the Crude Tetanus Toxoid is purified, and the bulk product obtained is Tetanus Toxoid Concentrate Fractionated (TTF) or Purified Tetanus Toxoid, which is then further processed to the final product. The methods used by each tetanus toxoid supplier are highly similar and any differences have been adequately justified. Hence the supplier of the tetanus toxoid does not impact on the final product.

Preparation of the finished product
The required amount of sterile filtered tetanus toxoid bulk product, which is diluted with water for injections to the required concentration and adjuvant is added and the mixture is stirred until homogeneity and yields the final bulk product.

The product is produced in different batches sizes. The suspension is stored at 2-8 °C until filling. The maximum storage period of the bulk product before filling has been fixed. The last production steps are filling, using an automatic filling line and capping followed by quality control.

Validation data on the Toxoid Binding Inhibition test and the sterility test on the final product were presented and considered satisfactory. Validation data for the toxoiding process of the tetanus toxin from each supplier are presented and all batches satisfactorily passed the toxicity test thus demonstrating consistency in the manufacturing process.

Control of Starting Materials

Active substances

***Clostridium tetani* strain**
The production strain is a variant of the Harvard strain (ATCC Catalogue No. 10779). Deriving from this strain, the Primary Working Master Seed (MS) was obtained. MS and WS are tested for purity and identity. A testing protocol for the actual tetanus WS was provided. Confirmation was provided that current raw material specification and testing for the tetanus component comply with the requirements of the current edition of Ph.Eur. Up-to-date specifications and certificates of analysis were provided for all starting materials.

Adjuvant

The adjuvant for this vaccine is based on iscom-matrix technology and consists of cholesterol, phosphatidyl choline and a HPLC purified fraction of Quillaja saponins. Details regarding the manufacturing process, the quantitative composition as well as the quality control of the adjuvant allowing evaluation of the consistency of adjuvant batches produced by the supplier were provided. Stability data on the iscom-matrix before use in the final product have been provided. A detailed evaluation of the risk of contamination of the adjuvant with extraneous agents according to Note for Guidance III/3427/93 was provided showing that with the production processes used and the control measures implemented, the risk of contamination of the adjuvant with extraneous agents is negligible.

TSE-Risk assessment

The TSE-Risk assessment was conducted according to Directive 1999/104/EC and Note for Guidance EMEA/410/01-Rev. 2. Statements of the manufacturers with respect to the source of the materials and copies of EDQM Certificates of Suitability (CoS) were provided. It can be summarised that only non-ruminant material, EDQM certified ruminant derived material and bovine milk derivatives coming from milk obtained from healthy animals in the same conditions as milk collected for human consumption are used in the production of this vaccine. Transmission of TSE to equines cannot be excluded but has not occurred so far under natural or experimental conditions. Thus, in respect of the information given, the risk that animal spongiform encephalopathy agents are transmitted by the use of this vaccine is estimated as minimal/negligible.

Control tests during Production

In process controls performed during the production of the tetanus component and on the bulk product have been described in a very detailed way. Results of in-process control tests on consecutive production runs of the tetanus component were provided and deemed satisfactory. A test for filling volume is performed on each batch of finished product.

Control tests on the finished product

The description of the methods used for the control of the finished product (pH, visual appearance, potency and identity of the tetanus component, sterility and safety tests) and the specifications were provided. The specifications proposed are appropriate to control the quality of the finished product.

Validation of the Toxoid Binding Inhibition (ToBI) test was performed according to VICH GL 1 and 2. The parameters investigated were specificity, linearity, range, accuracy, precision, detection limit and robustness. A protective ToBI limit was set based on available data.

A test confirming the identity of the adjuvant in the final product is included as routine final product control test.

Safety tests are carried out on each batch. Ph.Eur. monograph 0697 for tetanus vaccines specifies a safety test in guinea pigs. Replacement of the batch safety test in guinea pigs by a batch safety test in the target species, horses was justified.

The results of the analysis of three consecutive production runs of finished product vaccine were presented which comply with the required specifications.

STABILITY

Stability of the bulk antigen

Satisfactory stability data for the tetanus toxoid bulk stored at 2-8°C were provided.

Stability of the finished product

Stability studies have been conducted using Equilis Te and Equilis Prequenza Te. Based on the stability data provided, a shelf life of 24 months for Equilis Te was accepted. Storage at 37 °C for 1 week of several batches did not adversely affect the potency of the batches. Based on the data provided, transportation need not be carried out under refrigerated conditions (+2°C to +8°C).

OVERALL CONCLUSION ON PART II

The analytical part of the dossier is well described. A satisfactory description of the production and quality control procedures was provided. The method of manufacture was well described and the main in-process controls detailed in full. Compliance of starting materials of animal origin used during production with the requirements of the Note for guidance on minimising risk of transmitting animal spongiform encephalopathy agents via human and veterinary products was shown. Following an extension of the marketing authorisation in 2008, the addition of another tetanus toxoid supplier was adequately justified.

Validation data for the ToBI test were provided and deemed satisfactory to differentiate between batches that pass or fail the batch potency test. The finished product and batch safety tests ensure a product of consistent quality is produced. Based on the stability data provided a shelf life of 24 months was justified for the finished product.

3. SAFETY ASSESSMENT

Equilis Te is an inactivated adjuvanted vaccine containing tetanus toxoid as the active component. The minimum age for vaccination is 6 months. The basic vaccination scheme consists of two 1 ml intramuscular injections, each a single dose, with an interval of 4 weeks. Each dose (1 ml) contains as active substance Tetanus toxoid (40 Flocculation units). The safety studies were based on the relevant Ph.Eur. monographs and guidelines. All studies have been performed with Equilis Prequenza Te, which is an inactivated adjuvanted vaccine containing antigen of three different influenza strains and tetanus toxoid as the active components. Five laboratory studies and 6 field studies have been conducted. For the safety studies, vaccine batches produced and tested according to the production method and the standard release requirements described in Part II of the dossier were used.

LABORATORY TESTS

Safety of the administration of one dose/ an overdose/ the repeated administration of one dose

The studies are performed in line with the requirements of Ph.Eur. monograph 0249 ("Equine Influenza vaccine"). The adverse effects seen after administration of an overdose and repeated single dose to the foals were minor, transient, and limited to a minority of the foals in all studies performed independent of the time interval between the vaccinations. The time interval of 2 or 4 weeks between vaccinations does not influence the severity and character of adverse reactions after administration of the second vaccination. Based on all safety studies performed to assess the safety of a single, double and repeated single dose using batches of standard antigen content in horses of 2 to 4 months of age and older horses, it is concluded that the vaccine will be well tolerated by horses of different ages.

Safety of Equilis Prequenza Te vaccine in foals 3 months of age - a double dose followed by a single dose

The objective of the GLP compliant study was to investigate the safety of the vaccine after intramuscular vaccination of young foals of approximately 3 months of age with an overdose and a repeated single dose.

Animals used in the study should have not previously been vaccinated against tetanus. All horses had an antitoxin titre against tetanus toxoid of < 0.04 I.U./ml. After vaccination, all animals had induced antibody titres against all antigens present in the vaccine. Unvaccinated control horses did not develop antibodies to any of the antigens present in the vaccine.

Several parameters were examined in order to assess the safety of the product (rectal temperature, local and general reactions). The adverse effects seen after administration of an overdose and repeated single dose to the foals were minor, transient, and limited to a minority of the foals. After vaccination with the double dose one foal developed a warm, diffuse and painful swelling which disappeared within 24 hours, and fever. In additional investigations no indication of an inflammatory response was found. The rectal temperature was increased in another horse 5 hours after vaccination with a double dose, and in a few horses from three days after injection of a repeated single dose onwards, lasting one to three days. There was a mild depression in a few foals on the day of vaccination with the double dose. The vaccination with a double dose followed by a single dose is safe in foals 3±1 months of age. The observed reactions are acceptable. The SPC contains appropriate statements for a description of undesirable effects after single dose and overdose vaccination.

Safety of Equilis Prequenza Te vaccine in horses - double dose followed by a single dose

The objective of this GLP compliant study was to investigate the safety of the vaccine after intramuscular vaccination of horses of one year of age with an overdose and a repeated single dose. Only healthy horses 12 to 15 months of age were included in the study. Horses were vaccinated by intramuscular injection at day 0 of the study with a double dose (2 ml) and at day 14 with one dose (1 ml). One horse remained unvaccinated to serve as a control for concurrent field infection.

The results of the antibody tests showed that the horses can be declared as being seronegative for equine influenza and tetanus at first vaccination. After vaccination, all immunised animals developed detectable levels of antibodies against tetanus toxoid and equine influenza strains present in the vaccine. The unvaccinated control horse did not develop antibodies to any of the antigens present in the vaccine.

Several parameters were examined in order to assess the safety of the product (rectal temperature, local and general reactions). The adverse effects seen in the horses after vaccination were limited and transient. After vaccination with the double dose a few horses had a local reaction at the injection site which disappeared within three days. The maximum size was 5 x 5 cm. After injection of a single dose several horses developed local reactions either as a diffuse swelling or a painful neck which disappeared after one day. The maximum size was 2 x 2 cm. All reactions disappeared within 24 to 48 hours. The rectal temperatures were increased in a few animals after vaccination with the double dose and in some horses after administration of a repeated single dose, lasting for one to two days. Systemic reactions were not observed after the two vaccinations.

The vaccination with a double dose followed by a single dose is safe in yearlings. The observed reactions are acceptable. The SPC gives a description of undesirable effects after single dose and overdose vaccination and the nature of the local reactions was added.

Safety of Equilis Prequenza Te vaccine in horses – with emphasis on macroscopical examination of the injection site

The objective of the GLP compliant study was to examine post-mortem tissues surrounding the injection site for the occurrence of macroscopic lesions after intramuscular vaccination of horses of one year of age with an overdose and a repeated single dose. This study was conducted using horses that had already received a double dose and a single dose of vaccine within the preceding 6 weeks. The macroscopic and histopathological information provided in this study relates only to reactions occurring after repeated dosing.

The macroscopic and microscopic examination of the injection site did not reveal any vaccine remnants at 24 hours, 48 hours, 7 and 14 days after vaccination. Some oedema and slight inflammation were found. Even after three vaccinations within a short time period including a double dose, the vaccine is safe for the target animal with acceptable minor adverse effects. The vaccination with a double dose followed by a single dose is safe in yearlings.

At 24 hours post vaccination, a few horses that showed a swelling with a size of 1.5 x 1.5 cm were euthanised. At post-mortem examination of the injection site, an intramuscular oedema was visible in these horses. The size was approximately 10 x 3 x 4 cm and 8 x 4.5 x 3 cm, respectively. The muscle tissue itself did not show any macroscopic abnormalities. Vaccine remnants, abscessation, haemorrhages, calcification or necrosis of the tissue were not observed in any horse.

Examination of reproductive performance

The evaluation of the safety of Equilis Prequenza Te in pregnant thoroughbred mares before and after foaling

The objective of the GLP compliant study was to investigate the safety of the vaccine after intramuscular vaccination of pregnant thoroughbred mares in the last trimester of gestation, with a double dose followed by a single dose 2 weeks later. The control mare did not develop anti-influenza antibody titres throughout the study.

Four hours after vaccination with a double dose 80% of mares, showed a minor local reaction at the injection site i.e. the maximum size was 2 x 2 cm reducing to 30 % after 24 hours. After injection of a repeated single dose 40% of mares showed a minor local reaction at the injection site. Generally, the local reactions disappeared within 24 to 48 hours. There were no systemic reactions after both

vaccinations and the rectal temperature was not increased above the normal level. No negative influence on gestation, foaling and offspring of the mares was observed. The results of a separate field trial in which pregnant horses were vaccinated during the first four months of gestation without any negative effect on the gestation, support the safety of the vaccine during the first trimester of pregnancy.

Examination of immunological functions

No specific studies were conducted. Equilis Te is a vaccine containing inactivated bacterial toxoid. Replication of bacteria in the vaccinated animals' immune system is therefore not applicable and subsequently impairment of the immune system is not to be expected. There is no reason to suspect any impact of vaccination with Equilis Te on immunological functions.

Study of residues

Studies of residues were not presented. A withdrawal period of zero days was accepted. Equilis Te is an inactivated vaccine.

Interactions

Safety and efficacy of concurrent administration of Equilis Prequenza Te vaccine and Tetanus Serum Intervet (tetanus antitoxin) in horses

The objective of the study was to investigate the safety and efficacy of the concurrent administration of the vaccines Equilis Prequenza Te and Tetanus Serum Intervet in horses.

Foals younger than one year of age obtaned from an unvaccinated herd were vaccinated with a single dose of Equilis Prequenza Te and received Tetanus serum from Intervet at the same time but at a different site, followed by a single dose of the vaccine 4 weeks later. In one of the horses, a swelling of 2 x 2 cm in size was found at the injection site after first administration, which disappeared within 24 hours. No local reactions were observed at the injection site of the Tetanus serum. Fever was observed for one day in a few horses after first vaccination. Some horses developed diarrhoea and ocular discharge after the first vaccination. The reactions were mild and transient and disappeared within a few days without treatment. After second vaccination a local reaction of 1 x 1 cm was found in one horse and a diffuse swelling of 10 x 5 cm in another horse. The reactions had disappeared the next day. No systemic reactions and no fever were recorded after the second vaccination.

The reactions observed are mild and of only short duration which indicates that concurrent use of the vaccine and Tetanus serum can be considered to be safe for horses younger than one year of age. Therefore, the statement in the SPC section 5.8 is acceptable from safety point of view.

FIELD STUDIES

Field studies were performed in compliance with the VICH guidelines of "Good Clinical Practice" (GCP) and EC guideline III/3001/93 "Specific requirements for the production and control of Equine live and inactivated viral and bacterial vaccines". A competitor product was used as positive control to enable comparison of the safety findings for Equilis Prequenza Te**.**

A positive controlled field safety and efficacy trial of Equilis Prequenza Te in foals

The objective of the study was to assess the safety of the Equilis Prequenza Te vaccine in foals kept and vaccinated under field conditions compared to a positive control product. Clinically healthy foals, were included in this multicentre study; ranging in age from 2 to 10 months old.

About one third of the horses showed a local reaction after each vaccination. The local reactions were characterised as a hard or soft swelling, mostly with a diameter of less than 2 cm and not painful. In general, the local reactions disappeared within 48 hours. From day three after vaccination onwards no

local reactions were observed. One horse showed a local reaction (> 2 cm in diameter, soft) one day after the second vaccination. An increased rectal temperature (≥ 39° C) was observed in one third of the animals after first vaccination of both vaccines. After second vaccination none of the foals had a rectal temperature ≥ 39° C. Clinical signs like dullness, diarrhoea were not reported. The majority of the foals had swollen mandibular or retropharyngeal lymph nodes before and after vaccination at different days.

The reactions observed after vaccination of young horses under field conditions with Equilis Prequenza Te are mild and transient. These are acceptable reactions and are adequately reflected in the proposed SPC.

A positive controlled field safety and efficacy trial of Equilis Prequenza Te in pregnant mares

The objective of the study was to assess the safety of the Equilis Prequenza Te vaccine in pregnant mares kept and vaccinated under field conditions compared to a positive control product and to study the level of maternal antibodies of their offspring.

Clinically healthy mares were included in this multicentre study. The age of the group ranged from 2.8 to 24.5 years. The mares were pregnant between 3 and 9 months. At admission all mares which had a HI-influenza antibody titre of > 6 for all three strains received a single vaccination. The other mares, which had a titre of ≤ 6 for one or more strains, received a basic vaccination that consisted of two vaccinations with an interval of 4 weeks. All mares received a pre-foaling vaccination 4 to 5 weeks before the expected foaling date. Mares were vaccinated with the vaccine Equilis Prequenza Te or with the positive control product. 72 % of the Equilis Prequenza Te group and 73 % of the control group received a basic vaccination, the others a single vaccination. Four to 5 weeks before the expected date of foaling a pre-foaling vaccination was given to all but one horse of the Equilis Prequenza Te group. The mares were vaccinated intramuscularly. The mares were observed from admission until foaling.

All horses, vaccinated with both vaccines, remained healthy throughout the trial. Systemic reactions after vaccination were not observed. In both test groups several mares had swollen mandibular or retropharyngeal lymph nodes before and after vaccination on different days. Occasionally, slightly red mucous membranes, increased pulse and respiration or abdominal respiration were recorded. Generally after vaccination the rectal temperatures hardly increased and remained within the normal range (< 39° C). On day 28 two horses of the Equilis Prequenza Te group had fever (39.9° C and 40.0° C respectively).

About 60 % of the Equilis Prequenza Te group and 80 % of the positive control group showed a local reaction. The local reactions after vaccination mostly had a diameter smaller than 1 cm, were of soft nature and disappeared within two days after vaccination. Sometimes the local reactions were hard of nature, painful, 1-2 cm or > 2 cm in diameter and minimally swollen. Generally, the reactions were disappeared within 24 to 48 hours. Fourteen days after vaccination no local reactions were observed in any of the animals. After the second and third vaccination no local reactions were reported for both vaccines.

In the Equilis Prequenza Te group 97 % of the mares gave birth to a healthy foal. One mare aborted two months before the expected foaling date, 129 days after second vaccination. *Post mortem* examination of the foal showed a congenital anomaly of the cardio-vascular system. No indication for an infection was found. In the control group 97 % of the mares gave birth to a healthy foal. One mare had a premature birth of twins 3 weeks before the expected foaling date, 149 days after first vaccination. In the majority of cases, the birth was finished within 15 to 30 minutes, the placenta came spontaneously within 2 hours and the foal stood and drank within 1-2 hours. The rectal temperatures of the foals were within the normal range during the first days of life.

There was no negative influence on gestation, foaling and offspring of the mares. The reactions observed after vaccination of pregnant mares were mild and transient.

A field safety and efficacy trial of Equilis Prequenza Te and competitor product in horses

The aim of the GCP study was to assess the safety and the efficacy of Equilis Prequenza Te in horses under field conditions by comparison with a positive control vaccine. Clinically healthy horses at an age of 5 - 41 months, belonging to two different farms, were randomly separated into three groups; vaccinated with one dose of Equilis Prequenza Te, one group was vaccinated with one dose of the positive control and the third group was not vaccinated.

A quarter of the horses showed a local reaction after first vaccination and one third of horses after the second vaccination with a diameter of 1 cm or less.

A field trial with Equilis Te and Equilis Prequenza Te to determine the serological response to tetanus toxoid in horses

The aim of the study was to compare the sero-response to the tetanus component of Equilis Prequenza Te and Equilis Te in horses under field conditions. The trial was conducted in compliance with GCP guidelines on a stud farm in the EU. Clinically healthy horses at an age of 4 - 13.3 years, were separated into three groups.

Two groups were vaccinated with one dose of Equilis Te, one group with one dose of Equilis Prequenza Te. The horses were vaccinated with an interval of 4 weeks i.m. in the neck.

Some minor local reactions were observed. One day after first vaccination a local reaction of approximately 3 cm was observed in one horse. One day after second vaccination local reactions were observed in several horses. In some horses the reaction was hardly visible (< 1cm). A few horses had a soft and not painful reaction of 2-3 cm. The general health was reported as being “normal”.

A positive controlled field efficacy trial of Equilis Prequenza Te in horses

The aim of the study was to assess the efficacy of Equilis Prequenza Te in horses under field conditions compared to a competitor product. The trial was conducted in compliance with GCP guidelines using horses of 14 breeds housed on four sites, clinically healthy and at an age of 1.2 – 25 years, divided into three groups.

One group (46%) was vaccinated with one dose of Equilis Prequenza Te and the other group (44%) with one dose of the competitor product. Horses that had been vaccinated against influenza within the last twelve months received a single (booster) vaccination on day 0. If the previous vaccination was more than twelve months ago, the horses received a basic vaccination course consisting of two vaccinations with an interval of 28 days. In this study no adverse events were recorded. All animals remained healthy throughout the trial.

A field trial to determine the seroresponse after vaccination with Equilis Prequenza and Equilis Prequenza Te in sero-negative horses

The objective of the study was to determine and compare the sero-responses for influenza after vaccination with Equilis Prequenza and Equilis Prequenza Te in horses. Horses of different breeds and ages were divided into 3 groups. Group 1 (46%) received 2 vaccinations with Equilis Prequenza at an interval of 28 days. Group 2 (46%) were administered 2 vaccinations with Equilis Prequenza Te at an interval of 28 days. Group 3 (8%) served as controls.

No adverse events occurred and the general health was each time scored as “normal”.

III.E. ECOTOXICITY

The product is an adjuvanted liquid vaccine containing sterile filtered purified tetanus toxoid as active components together with buffer. A phase 1 assessment was conducted and presented. On the basis of

the results of the assessment of the hazards identified and the likelihood of their occurrence is negligible, it is concluded that the level of risk associated with each of the hazards is effectively zero. Therefore the Equilis Te vaccine is judged to present no risk to the environment. On the basis of the phase 1 assessment, a phase 2 assessment to investigate the ecotoxicity of Equilis Te is not necessary.

OVERALL CONCLUSION ON PART III

Laboratory studies were conducted to assess the safety of a single, double and repeated single dose using batches of standard antigen content in horses of 2 to 4 months of age, older horses and pregnant thoroughbred mares. The vaccine may induce local reactions in the horse. These local reactions are characterised by soft or sometimes hard swellings mostly with a diameter smaller than 2 cm. In rare cases the size was up to 5 cm in diameter and the injection site was painful. The reactions were transient and they disappeared normally within 24 to 48 hours. Sometimes an increase in rectal temperature above the normal range could be observed for 24 hours, exceptionally for three days. Other systemic reactions were not induced by the vaccine. That means that the vaccine will be well tolerated by horses of different ages.

No negative influence on gestation, foaling and offspring of mares was observed after vaccination at different times during pregnancy. At the injection site, no remnants of the vaccine were found. The vaccine was administered at the same time, but at different sites with Tetanus serum. This administration was safe. An assessment of the ecotoxicity risks showed that the overall risk of the vaccine to the environment, humans and other animals is effectively zero.

4. EFFICACY ASSESSMENT

Tetanus is an acute, often fatal disease caused by the neurotoxin of *Clostridium tetani*, a slender, gram-positive, anaerobic rod that may develop terminal spores, which are widely distributed in soil and intestines of animals and humans. The disease, which usually originates from contaminated wounds, is characterised by generalised rigidity and convulsive spasms of skeletal muscles. The muscle stiffness usually involves the jaw (lockjaw) and neck and then becomes generalised. Most susceptible species to tetanus are horses and humans. Effective prophylaxis against this disease is only obtained by means of vaccination.

Almost all efficacy experiments were performed with the full combination product Equilis Prequenza Te, which besides tetanus toxoid also contains inactivated antigen of three different equine influenza strains. The amounts of tetanus toxoid (40 Lf) and adjuvant (purified saponin: 375 µg) in one dose of 1 ml of Equilis Prequenza Te and the mono-component vaccine Equilis Te are identical.

Laboratory trials
Establishment of a challenge model

For ethical reasons, no challenge experiment has been performed against tetanus. The parameter used to assess the efficacy of Equilis Te against tetanus was the level of serum antibodies against tetanus toxoid induced after vaccination, which was determined by means of a Toxoid Binding Inhibition (ToBI) test internally calibrated against the WHO tetanus antitoxin standard of the horse. Satisfactory validation data were provided for the ToBI test. A ToBI titre of 0.3 I.U./ ml has been shown to be protective in horses.

Determination of the vaccine dose

Efficacy testing has been performed with vaccine batches with a standard amount of tetanus toxoid (fixed dose of 40 Lf/ml). The amount of tetanus toxoid included in Equilis Te is based on experience with existing Intervet vaccines.

Onset of protection

Potency of Equilis Prequenza Te Vaccine in Horses against a Challenge with Influenza A/Equine-2/Kentucky/9/95

Foals (2 – 7 months, influenza and tetanus naïve) were vaccinated twice with one dose of Equilis Prequenza Te vaccine at 4 weeks interval. Some animals were left unvaccinated to serve as controls. Four weeks after the second vaccination all horses were challenged by aerosol with A/equine-2/Kentucky/9/95 (H3N8, “American type”) virus.

Regarding the onset of protection against tetanus, serum samples obtained at the time of first vaccination, one week after first vaccination, at the time of second vaccination 4 weeks after first vaccination, and 2 weeks after the second vaccination were tested for the presence of tetanus antitoxin by means of the ToBI test. Whereas all vaccinated foals were seronegative to tetanus at the time of first vaccination (< 0.05 I.U./ ml), all foals clearly showed seroconversion 2 weeks after the second vaccination. All vaccinated foals were still seronegative to tetanus one week after the first vaccination and showed only low ToBI titres at the time of second vaccination thus lacking any anamnestic immune response.

Based on the results of this study, it is justified to conclude that the onset of protection against tetanus infection is 2 weeks after the second immunisation of basic vaccination.

The Influence of Maternal Antibody on the Efficacy of the Vaccine

No data regarding the influence of maternal antibody to tetanus on the efficacy of the vaccine have been included in the dossier. All laboratory and field efficacy studies were performed with horses seronegative against tetanus at the time of (first) vaccination. Thus, the influence of specific maternal antibodies on the efficacy of Equilis Te against tetanus has not been assessed**.** The presence of specific maternal antibodies to tetanus is known to have a significant inhibitory effect on the development of active immunity against tetanus in young foals after active immunisation.

Field data and laboratory data indicate that young animals at the age of 6 months that are without maternal antibody against tetanus respond adequately to vaccination. A complete vaccination response of seronegative foals at an age below 6 months could not be clearly demonstrated. Maternal antibodies to tetanus in the foal may persist up to 4-6 months of age depending on the amount of colostrums ingested shortly after birth and the immune status of the mare. In addition, it has also been recommended that foals born from mares vaccinated during the last 2 months of gestation should not be vaccinated before the age of 6 months. This has been taken into account in SPC section 5.10.

Duration of Immunity

Duration of Protection achieved by Equilis Prequenza Te Vaccine in Horses against a Challenge with Influenza A/Equine-2/Kentucky/9/95, 6 Months after the Primary Vaccination Course

The aim of the study was to determine the duration of immunity of Equilis Prequenza Te after a basic vaccination course. Influenza and tetanus naive foals were administered twice a dose of a standard production batch of the test vaccine 4 weeks apart. Five months after the second dose the vaccinated foals were challenged together with 5 unvaccinated foals serving as control group with a recent field strain of Equine Influenza A of subtype H3N8 (Kentucky/9/95, “American” lineage). Blood samples were taken at several points throughout the study in order to control the development of the serum antibody titre against the 3 influenza strains and the tetanus component of the test vaccine.

Regarding the duration of protection against tetanus, serum samples obtained at the time of first vaccination, one week after first vaccination, at the time of second vaccination 4 weeks after first vaccination, as well as 2, 7, 12, 15 and 20 weeks after the second vaccination were tested for the presence of tetanus antitoxin by means of the ToBI test. Whereas all vaccinated foals were seronegative to tetanus at the time of first vaccination, all foals clearly showed seroconversion 2 weeks after the second vaccination. Afterwards, the serum tetanus antitoxin titres of vaccinated foals declined until 5 months after basic vaccination. All vaccinated foals were still seronegative to tetanus one week after the first vaccination and showed only low antitoxin titres at the time of second vaccination thus lacking any anamnestic immune response.

Based on the results of this study, it is justified to conclude that the vaccine offers protection against tetanus infection for at least five months after the second immunisation of basic vaccination.

Duration of Protection achieved by Equilis Prequenza Te Vaccine in Horses against a Challenge with Influenza A/Equine-2/Kentucky/9/95, 1 Year after the Third Vaccination

The aim of the study was to determine the duration of immunity of Equilis Prequenza Te against tetanus after the basic vaccination course and against influenza after the first revaccination in horses. Seven influenza and tetanus naive foals were administered twice a dose of a standard production batch of Equilis Prequenza Te 4 weeks apart. Five months after the second dose the vaccinated foals received a revaccination with one dose of a standard production batch of the influenza mono-component vaccine Equilis Prequenza.

Regarding the duration of protection against tetanus, serum samples obtained at the time of first vaccination with Equilis Prequenza Te, one week after first vaccination, at the time of second vaccination 4 weeks after first vaccination, as well as at regular until 73 weeks after the second

vaccination with Equilis Prequenza Te were tested for the presence of tetanus antitoxin by means of the ToBI test.

Whereas all vaccinated foals were seronegative to tetanus at the time of first vaccination, all foals clearly showed seroconversion 2 weeks after the second vaccination. Afterwards, the ToBI titres of the vaccinated foals decreased slowly. One and a half years after the first vaccination (at t = 77 weeks), the geometric mean of the ToBI titres was still sufficient for protection.

All vaccinated foals were still seronegative to tetanus one week after the first vaccination and showed only low ToBI titres at the time of second vaccination thus lacking any anamnestic immune response.

Based on the results of this study, it is justified to conclude that the vaccine offers protection against tetanus infection for at least 17 months after the second immunisation of basic vaccination.

Duration of Immunity against influenza and tetanus induced after vaccination with Equilis Prequenza Te and Equilis Prequenza

The aim of this laboratory study was to assess the duration of immunity against tetanus induced by Equilis Prequenza Te after the basic vaccination course and the first revaccination. Clinically healthy influenza- and tetanus-seronegative foals at an age of 5 – 9 months were included. One group (7 horses) was i.m. vaccinated with one dose of Equilis Prequenza Te, at time points = 0, 4 (second vaccination of basic vaccination course), 26 (first booster) and 129 (second booster) weeks. At time point 78 weeks, they were i.m. vaccinated with one dose of Equilis Prequenza. Two horses were left unvaccinated to serve as contact controls.

Before, 1, 2, 4 and 6 weeks after the first vaccination and at regular preset time points after the second vaccination, blood samples were taken from all animals to determine the serological response to influenza and tetanus. Antibody levels against tetanus were determined by means of the ToBI-test. Prior to first vaccination and one week after first vaccination, no antibodies against tetanus were detected. Two weeks after the basic vaccination course, ToBI titres rose to high levels. These titres dropped to ToBI levels far above the limit required for clinical protection at the time of the first booster vaccination, but increased 2 weeks after the first booster vaccination. From 2 weeks after the first revaccination, peak antibody titres against tetanus started to drop to lower levels reaching plateau values several months prior to the second booster vaccination. The plateau value found was far above the level required for clinical protection. After the second booster vaccination, antibody titres against tetanus increased again. These data imply that horses are protected against tetanus for at least 24 months after the basic vaccination and first revaccination course.

Interactions - Safety and Efficacy of Concurrent Administration of Equilis Prequenza Te Vaccine and Tetanus Serum Intervet (Tetanus Antitoxin) in Horses

The objective of the study was to investigate the safety and the efficacy of the concurrent administration of the vaccine Equilis Prequenza Te and Tetanus Serum Intervet in horses. Foals obtained from an unvaccinated herd received a single dose of the vaccine intramuscularly at one spot at the left side of the neck. At the same time the horses were injected intramuscularly with the prescribed dose of tetanus serum/kg bodyweight into the right side of the neck. Four weeks later a single dose of vaccine was administered to the horses at the left side of the neck. One horse was left unvaccinated to serve as control. All but one foal, which was 15 months old, were between 6-12 months of age.

Serum samples obtained at the time of first vaccination, 4-6 hours, 24 hours, 48 hours, 72 hours, 7 days, 10 days, 14 days, 17 days and 21 days after the first vaccination, at the time of second vaccination 4 weeks after the first vaccination as well as 2 weeks after the second vaccination were tested for the presence of tetanus antitoxin by means of the ToBI test. Serology data from vaccinated animals were used to demonstrate the ability of foals to create a humoral immune response in the face of passive immunisation against tetanus.

With regard to the efficacy after passive immunisation, as early as 4-6 hours after the concurrent administration of Equilis Prequenza Te and Tetanus-Serum Intervet, antitoxin levels against tetanus clearly exceeded the protective level required for passive protection against tetanus intoxication. These passively acquired antitoxin titres reached their maximum approximately two days after administration. Until 21 days after antiserum administration, the ToBI titres remained above the level indicative for protection for all horses in both serum groups. In 60% of horses treated with Tetanus-Serum Intervet, the antitoxin titres against tetanus were above the protective level until day 28 after treatment. With regard to the efficacy after active immunisation, the average antitoxin titres measured at the moment of the second administration of the vaccine (day 28) were equal to that found in the other laboratory efficacy studies.

It should be noted that in both groups the antitoxin titres were significantly reduced compared to results obtained in the other laboratory efficacy studies 2 weeks after the second vaccination.

Based on the results presented in this study the following conclusions can be drawn with regard to protection against tetanus intoxication: Concurrent use of Equilis Prequenza Te and Tetanus-Serum Intervet will lead to a passive protection against tetanus for at least 21 days after concurrent administration. Development of active immunity against tetanus is negatively influenced by concurrent use of Equilis Prequenza Te and Tetanus-Serum Intervet. An appropriate statement is included under section 5.8 “Posology and method of administration” of the SPC.

FIELD TRIALS

A Positive Controlled Field Safety and Efficacy Trial of Equilis Prequenza Te in Foals

The aim of the study was to determine the efficacy of Equilis Prequenza Te in foals of different breeds reared under field conditions on different farms by comparison of the test vaccine with a positive control vaccine. Foals aged 2 to 10 months were assigned to 2 groups (A+B) and received a basic immunisation course by administering one dose of vaccine i.m. into the neck on day 0 and another dose of vaccine at day 28 of the trial. Group A received the Equilis Prequenza Te and group B the positive control vaccine.

Regarding the efficacy of Equilis Prequenza Te against tetanus in the field, serum samples obtained within 15 days before admission of the study, 7 days after the first vaccination, at the time of second vaccination 4 weeks after the first vaccination as well as two and 4 weeks after the second vaccination were tested for the presence of tetanus antitoxin by means of the ToBI test. Not all serum sampleswere available. All foals were supposed to be seronegative to tetanus at the time of first vaccination; however at 7 days after the first vaccination some foals had low levels of antibodies against tetanus.

Two and 4 weeks after the second vaccination, individual ToBI titres showed a great variance, but were all far above the level indicative for protection against tetanus. The geometric mean titre reached 2 weeks after basic vaccination in the Equilis Prequenza Te group was significantly reduced compared to results obtained in the other laboratory efficacy studies 2 weeks after the second vaccination. In conclusion, Equilis Prequenza Te induces protective serum antitoxin titres to tetanus 2 weeks after basic vaccination in seronegative foals kept and vaccinated under field conditions.

Three additional field trials for the demonstration of efficacy of the tetanus component of Equilis Prequenza Te under field conditions were presented:

A Field Safety and Efficacy Trial with Equilis Prequenza Te and competitor product

The objective of the GCP study was to assess the safety and efficacy of Equilis Prequenza Te in horses under field conditions in comparison with the positive control product. The study was not blinded, but determination of antibody titres was performed under blinded conditions. Clinically healthy horses at an age of 5 - 41 months, were randomly separated into 3 groups.

One group was vaccinated i.m. with one dose of Equilis Prequenza Te, one group was vaccinated with one dose of the positive control and the third group was not vaccinated. The horses were vaccinated on days 0 and 29 and monitored for local and systemic reaction up to day 57.

Individual ToBI titres at admission in the three treatment groups varied and as most horses in each group were seropositive for tetanus, the study was not suitable for the evaluation of efficacy of Equilis Prequenza Te against tetanus in horses under field conditions.

Field Trial with Equilis Prequenza Te and Equilis Te

The aim of the GCP compliant study was to compare the sero-response to the tetanus component of Equilis Prequenza Te and Equilis Te in horses under field conditions. The animal phase of the study was not blinded, but determination of antibody titres was performed under blinded conditions. Clinically healthy horses at an age of 4 - 13.3 years were used.

Two groups were vaccinated with one dose of Equilis Te, one group with one dose of Equilis Prequenza Te. The horses were vaccinated with an interval of 4 weeks i.m. in the neck. Blood samples were taken on day 0 just before first vaccination and on day 14, 28 (prior to second vaccination), 42 and 56 after the first vaccination.

Prior to first vaccination, 57% of horses vaccinated with Equilis Te and 71% of horses vaccinated with Equilis Prequenza Te were seronegative to tetanus. Fourteen days after the first vaccination, the ToBI titres of all horses were clearly above the level indicative for protection. After second vaccination, a rapid increase in ToBI titres was observed in all animals of both test groups.

In horses that were seropositive to tetanus prior to first vaccination, Equilis Te and Equilis Prequenza Te induced comparable ToBI titres against tetanus. In horses that were seronegative to tetanus prior to first vaccination, the geometric mean ToBI titre of the horses vaccinated with Equilis Te was considerably higher compared to the geometric mean titre of the horses vaccinated with Equilis Prequenza Te. The results demonstrate that vaccination of seronegative horses with Equilis Te under field conditions will induce a sero-response that is equal or higher when compared to the sero-response after vaccination with Equilis Prequenza Te. Therefore the claimed period for the duration of immunity for Equilis Te should be at least equal but may be even longer when compared to Equilis Prequenza Te.

A positive controlled field efficacy trial of Equilis Prequenza Te in horses in The Netherlands

The aim of the GCP study was to assess the efficacy of Equilis Prequenza Te in horses under field conditions compared to the competitor product. The study was not blinded, but determination of antibody titres was performed under blinded conditions. Clinically healthy horses of 14 breeds, and at an age of 1.2 – 25 years, were separated into three groups at four different sites. Per site, the two oldest animals remained unvaccinated to detect equine influenza field infections during the trial period (negative control group). The remaining horses were randomly assigned to 2 groups. One group was vaccinated with one dose of Equilis Prequenza Te and the other group with one dose of the positive control.

Horses that had been vaccinated against influenza within the last 12 months received a single (booster) vaccination on day 0. If the previous vaccination was more than 12 months ago, the horses received a basic vaccination course consisting of 2 vaccinations with an interval of 28 days. Blood samples were taken on day 0 just before vaccination and on day 7, 14 and 28 for horses that received a single vaccination.

Animals that received the basic vaccination course were bled on the following days: day 0 (just before first vaccination), day 7, 28 (prior to second vaccination), 42 and 56 after the first vaccination. Blood samples were taken on day 0 just before vaccination and on day 7, 14 and 28 for horses that received a single vaccination.

Antibody levels against tetanus were determined by means of the ToBI test At admission, the majority of horses had moderate to high titres against equine influenza and tetanus.

Results after basic vaccination course
At admission, the ToBI titres were similar in both vaccination groups. All horses showed an increase of ToBI titres after vaccination. Day 42 and day 56 after the basic vaccination course, ToBI titres in the Equilis Prequenza Te group were significantly higher compared to the ToBI titres in the control group.

Results after single vaccination
All horses were seropositive prior to vaccination. In both vaccination groups, all but one horse showed an increase of ToBI titres after vaccination. ToBI titres in the Equilis Prequenza Te group did not differ significantly compared to the ToBI titres in the control group at all time points before and after vaccination.

It can be concluded that Equilis Prequenza Te is an effective vaccine against equine tetanus both in seronegative and seropositive horses.

Duration of immunity against tetanus under field conditions

The natural log transformed data of the average concentrations found in the laboratory trials were plotted vs time. This was to address the question, whether duration of immunity against tetanus indicated to be at least 17 months after basic vaccination, will also be obtained for animals with a low tetanus antibody titre immediately after the basic vaccination course with Equilis Prequenza Te in the field, and to get some insight into the kinetics of the tetanus serum antibody concentration after vaccination, From this, it appeared that there was a biphasic decline. The calculated half-lives allow a prediction of what for a given value at onset of immunity the final concentration at 72 weeks (i.e. point of 2nd revaccination) would be. For two serum samples from vaccinated foals with low ToBI titres after basic vaccination, the predicted decline was plotted until 76 weeks (17 months). Although both values are very low the data provide an indication that both foals are protected against tetanus until after 17 months after basic vaccination.

The prediction model was substantiated with additional ToBI titres of some horses used in field efficacy studies. Thus, the duration of immunity against tetanus after the basic vaccination course (17 months) as well as the duration of immunity after the first booster vaccination (24 months) is considered to be demonstrated after vaccination with Equilis (Prequenza) Te under field conditions.

OVERALL CONCLUSION ON PART IV

Efficacy studies have been carried out in the target species, the horse, by the recommended route of administration (intramuscular). All efficacy experiments except one were performed with batches of Equilis Prequenza Te containing standard amounts of influenza antigens (A/equine-1/Prague/1/56 = 100 AU, A/equine-2/Newmarket/1/93 and A/equine-2/Newmarket/2/93 = 50 AU each), tetanus toxoid (40 Lf) and adjuvant (saponin: 375 µg) in one dose of 1 ml. In one field trial Equilis Te was used.

It was demonstrated that basic vaccination (consisting of two vaccinations 4 weeks apart) of seronegative foals at an age of five months onwards with Equilis Prequenza Te led to serum antitoxin levels against tetanus toxoid considered to indicate protection against tetanus 2 weeks after basic vaccination until at least 17 months after basic vaccination.

All foals used in the laboratory and field efficacy studies were seronegative to tetanus at the time of first vaccination. Thus, the influence of specific maternal antibodies on the efficacy of Equilis Prequenza Te against tetanus could not be assessed. As the presence of specific maternal antibodies to tetanus is known to have a significant inhibitory effect on the development of active immunity against tetanus in young foals after active immunisation, an appropriate minimum age of vaccination was fixed at 6 months.

Regarding the concurrent use of Equilis Prequenza Te and Tetanus-Serum Intervet, a passive protection against tetanus for at least 21 days after concurrent administration has been demonstrated. Nevertheless, the development of active immunity against tetanus is negatively influenced by concurrent use of Equilis Prequenza Te and Tetanus-Serum Intervet, as antitoxin titres obtained 2 weeks after the basic vaccination were significantly reduced compared to results obtained in the other laboratory efficacy studies 2 weeks after the second vaccination. As this might have a negative influence on the duration of immunity against tetanus, it is proposed to include a third vaccination at least 4 weeks later.

Duration of immunity of Equilis Prequenza Te against tetanus after first (and further) booster immunisation was demonstrated. Data provided show that protective serum antibody titres against tetanus persisted for 24 months after the first revaccination (V3) with Equilis Prequenza Te given at 5 months after the basic vaccination course.

The results of one field trial performed with Equilis Te demonstrate that vaccination of seronegative horses with Equilis Te under field conditions will induce a sero-response that is equal or higher when compared to the sero-response after vaccination with Equilis Prequenza Te. Therefore the claimed period for the duration of immunity for Equilis Te should be at least equal but may be even longer when compared to Equilis Prequenza Te.

Thus, the duration of immunity after the basic vaccination course (17 months) as well as the duration of immunity after the first booster vaccination (24 months) can be considered to be demonstrated after vaccination with Equilis Te under laboratory and field conditions.

5. RISK-BENEFIT

Equilis Te is indicated for active immunisation of horses from 6 months of age against tetanus to prevent mortality. The vaccine contains tetanus toxoid prepared from toxin produced by *Clostridium tetani. Clostridium tetani* is a gram-positive, anaerobic, spore-forming bacillus, which produces a potent toxin that can cause spasticity and tetany of the skeletal muscle. Horses have a much higher susceptibility to the tetanus neurotoxin than other animals. In general tetanus prophylaxis should be incorporated into all equine health maintenance programmes.

The tetanus toxoid is formulated with iscom-matrix, a new innovative adjuvant. The iscom-matrix contains a purified saponin. The adjuvant has excellent immune-inducing properties and a good safety profile.

The analytical part is correctly documented, especially with regard to the production and control of the antigen and the control of the raw materials. The starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Commission Directive 1999/104/EEC.

The potential risks of the use of this inactivated adjuvanted vaccine against tetanus in horses may be that the vaccine causes abnormal local or systemic reactions in the target animal and the risk of self-injection administering the product. Both of these have been addressed and suitable warnings included in the SPC. No negative influence on gestation, foaling and offspring of mares was observed after vaccination at different times during pregnancy. The vaccine is safe in animals regarded as most sensitive. After administration of a double dose and the repeated administration of one dose no serious adverse systemic or local reactions were observed. Each vaccine batch is tested for safety in the target animal before batch release. Appropriate warnings are indicated in SPC sections 5.4, 5.9 and 5.12.

An assessment of the ecotoxicity risks showed that the overall risk of the vaccine to the environment, humans and other animals is minimal.

Vaccination with Equilis Te provides protection against tetanus starting at 2 weeks after basic vaccination. The basic vaccination course results in immunity to tetanus lasting at least 17 months. The first revaccination after the basic vaccination course results in immunity to tetanus lasting at least 24 months.

The interference of maternal antibodies on the efficacy of inactivated vaccines against tetanus is well known. Development of a humoral response of Equilis Te in the face of maternal antibodies against tetanus has not been demonstrated. Field data and laboratory data indicate that young animals at the age of 6 months that are without maternal antibody against tetanus respond adequately to vaccination. A complete vaccination response of seronegative foals at an age below six months could not be clearly demonstrated. Maternal antibodies to tetanus in the foal may persist up to 4-6 months of age depending on the amount of colostrum ingested shortly after birth and the immune status of the mare. In addition, it has also been recommended that foals born from mares vaccinated during the last two months of gestation should not be vaccinated before the age of 6 months.

Concurrent use of Equilis Te and Tetanus-Serum Intervet will lead to a passive protection against tetanus for at least 21 days. Development of active immunity against tetanus is negatively influenced by concurrent use of Equilis Prequenza Te and Tetanus-Serum Intervet. An appropriate statement has been proposed for point 5.8 of the SPC.

Based on the original and subsequent data presented, the Committee for Medicinal Products for Veterinary Use concluded by majority that the quality, safety and efficacy of Equilis Te was considered to be in accordance with the requirements of Council Directive 2001/82/EC.