SCIENTIFIC DISCUSSION

Invented name:	Equilis Prequenza
Active substance/INN:	A/equine-1/Praque/1/56 A/equine-2/Newmarket/1/93 A/equine-2/Newmarket/2/93
Target species:	Horses
Therapeutic indication:	Active immunisation of horses from 6 months of age against equine influenza to reduce clinical signs and virus excretion after infection.
Withdrawal period:	Zero days
Pharmaceutical form:	Suspension for injection
ATCvet code:	QI05AA01
Pharmaco-therapeutic group:	Equine Influenza vaccine
Marketing Authorisation Holder:	Intervet International B.V. Wim de Körverstraat 35 NL-5831 AN Boxmeer The Netherlands

I. SUMMARY OF THE DOSSIER

Equilis Prequenza is presented in a 1 ml glass vial or a pre-filled glass syringe. The vaccine contains purified haemagglutin subunits (HA) from three different equine influenza virus strains. The product is indicated for active immunisation of horses from 6 months of age against equine influenza to reduce clinical signs and virus excretion after infection. The route of administration is intramuscular.

2. QUALITY ASSESSMENT

Composition

The vaccine contains purified haemagglutin subunits (HA) from three different equine influenza virus strains; A/equine-1/Prague/1/56 100 AU(antigenic units)/ml ; A/equine-2/Newmarket/1/93 50 AU/ml ; A/equine-2/Newmarket/2/93 50 AU/ml. Standard excipients used as stabilisers or buffer components all comply with the Ph.Eur. Per dose the vaccine contains traces of thiomersal as a remnant of starting materials.

Container

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 choice of the 3 strains of equine influenza virus included in the vaccine was justified based on current outbreaks of influenza. The development of a subunit vaccine was justified. The subunit vaccine also reduces potential adverse effects by means of the production process.

The vaccine contains purified haemagglutinin and neuraminidase glycoproteins, however the neuraminidase content of the vaccine is not measured.

The influenza potency is determined *in-vivo* (guinea pigs Ph.Eur. 0249). The initially proposed release limits for the three influenza components (Prague/56, Newmarket-1 and Newmarket-2 respectively), were revised and higher limits with revised pass criteria agreed.

The adjuvant for this vaccine 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. Thus, the iscom-matrix, possesses no or drastically reduced haemolytic activity. Induction of high levels of serum antibodies against equine influenza which persisted for an unexpectedly long time were seen in studies and the matrix has an excellent safety profile in the target species. The amount of purified saponin in the vaccine has been set at 375 μg/ dose.

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.

Method of manufacture

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

Production of the equine influenza components
The production and purification of the monovalent influenza antigens is presented and includes information on the type of control tests performed. A detailed description of the manufacturing process is given. Each antigen is purified by affinity chromatography. After the purification process samples are collected for quality control tests including the determination of the amount of HA protein. The sterilised material itself is stored at 5 ± 3°C pending further processing.

Preparation of the finished product
The required amount of the three influenza antigens is prepared for production and the volume of this trivalent mixture is determined. As the antigen mixture is contained in a buffer containing part of the excipients, the extra amount of these excipients required to formulate a batch in which their concentration is standard, is calculated. The excipients are dissolved in Water for Injections, filtered and added to the bulk mixture. A sample is taken for the test on inactivation.

The product is produced in different batches sizes. The suspension is stored at 2-8 °C until filling. The final production steps are filling, using an automatic filling line and capping followed by quality control.

Validation data on the inactivation kinetics study of EIV Prague/1/56, Newmarket/2/93 and Newmarket/1/93, antigen-capture ELISA for influenza antigen qualification, potency test for the EIV-components, endotoxin test, inactivation control test on final product, HI antibody test, sterility test on final product were presented and considered satisfactory. Validation data presented demonstrate consistency in the manufacturing process.

Control of Starting Materials

Active substances
Production strains of equine influenza
All influenza master seeds were produced on SPF eggs, the required documentation of the SPF eggs used for the production for the NM-1 and NM-2 seed were provided, for the Prague seed (1986) these are not available. The eggs for the NM-1 and NM-2 seed were from SPF flocks that were controlled as specified by the Ph.Eur. at the time of testing. Data showing that all three influenza MSVs have been tested free of avian extraneous agents were provided.

A/equine-1/Prague/56 The original source of this stain was nasal washings from a horse in the CSSR in 1956 as mentioned in the ATCC form. After passages in 9-11 day old embryonated eggs the master seed virus (MSV) was established. The MSV is stored at –20° C. Control tests on the MSV for identity, freedom from bacterial and fungal contamination, exclusion of extraneous agents were conducted. The sterility of the MSV is tested according to the Ph.Eur. requirements. The MSV is found to be free from bacterial, fungal and mycoplasma contamination. An overview presenting the results for the absence of pestiviruses and the equine extraneous agents as listed in the Note for guidance III/3427/93 was provided. Further details of test performances of the specific tests and their validity concerning n-cpe pestiviruses, rabies virus, EAV, EIA virus and equine herpes virus was provided and deemed satisfactory.

A/equine-2/Newmarket/1/93 (US-Type) The virus was isolated from nasopharyngeal swabs from horses during an influenza outbreak in 1993. After one passage in embryonated SPF hen's eggs, the MSV stock was prepared by multiplying this first passage on 10 days preincubated embryonated SPF hen's eggs. The MSV is stored at –20° C. Control tests on the MSV for identity, freedom from bacterial and fungal contamination, exclusion of extraneous agents were conducted. The sterility of the MSV is tested according to the Ph.Eur. requirements 2.6.1. and 2.6.7. The MSV is found to be free from bacterial, fungal and mycoplasma contamination. General tests of the MSV including the observation of any cytopathic effect or visible abnormality by Giemsa staining, haemadsorption and haemagglutinating activity were performed after inoculation of several cell lines with virus seed. Specific tests for the exclusion of non-cytophogenic pestiviruses and rabies virus have been performed with satisfying results. Tests to exclude any contamination with equine arteritis virus (EAV) and equine herpes viruses types 1, 2, 3 and 4 (EHV) were conducted.

A/equine-2/Newmarket/2/93 (EU-Type) The virus was isolated from nasopharyngeal swabs from horses during an influenza outbreak in 1993. The master seed stock was prepared after one passage on 11 days pre-incubated embryonated SPF hen's eggs. The harvested amnio-allantoic fluid was lyophilised and then stored at – 20° C. Control tests on the MSV for identity, freedom from bacterial and fungal contamination, exclusion of extraneous agents were conducted. The sterility of the MSV is tested according to the Ph.Eur. requirements 2.6.1. and 2.6.7. The MSV is found to be free from bacterial, fungal and mycoplasma contamination. General tests of the MSV including the observation of any cytopathic effect or visible abnormality by Giemsa staining, haemadsorption and haemagglutinating activity were performed after inoculation of several cell lines with virus seed. Specific tests for the exclusion of non-cytophogenic pestiviruses and rabies virus have been performed with satisfying results. Tests to exclude any contamination with equine arteritis virus (EAV) and equine herpes viruses types 1, 2, 3 and 4 (EHV) were conducted.

Justification for freedom from some viruses has been based on freedom of the country of origin from these infections. Confirmation was provided that these viruses were not used in any laboratories where the virus stocks were handled.

Preparation and description of controls and tests carried out on Working Seed Virus (WSV)
Working seed virus stocks of EIV-Prague/1/56, EIV-Newmarket/1/93 and EIV-Newmarket/2/93 were produced on embryonated SPF chicken eggs up to passage level MSV+4 and freeze-dried. The vials are stored at –20° C. The infectivity titre of the WSV was determined by SPF egg titration. After collection of amnio-allantoic fluid the haemagglutination activity was determined and the titre calculated as EID_{50} or log 10 EID_{50} per ml. The WSV is tested for sterility according to Ph.Eur. 2.6.1 and for the absence of any mycoplasma contamination according to Ph.Eur. 2.6.7. All starting materials of non-biological origin used during the production processes for the equine influenza antigens are used before purification of the antigens by affinity chromatography, and are not contained in the final product. Certificates of analysis for all materials used were presented showing compliance with the specifications set. Different buffers are used during the purification processes for the equine influenza antigens. Detailed information on the composition of the individual buffers is given in the dossier.

Eggs
Embryonated eggs which derive from healthy non-SPF flocks are routinely used for the production of EIV-antigens for the final product. The fertilised hen's eggs are subjected to a thorough control system that guarantees the absence of embryo-derived extraneous agents in the product and the flocks are certified to be free from pathogenic poultry diseases, including avian leucosis and reticuloendotheliosis.

Adjuvant
The adjuvant for this vaccine is based on iscom-matrix technology and consists of cholesterol, phosphatidyl choline and 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 of one batch of 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

Equine influenza components
The main in-process controls (IPC) conducted are; Inactivation control on at least 10 vaccine doses; Determination of antigen content; Endotoxin test; Sterility test. Results of in-process control tests on three consecutive production batches are provided and met the specifications. 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 influenza components, sterility and purity tests, inactivation control tests for EIV) 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.

Validation of the influenza batch potency test was performed in guinea pigs and horses. The final product test is also intended to check for the presence of a sufficient antigen amount and appropriate release limits were set. The setting of appropriate criteria provides additional assurance that a sub-standard batch will not be released.

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. Confirmation was provided that the horses used for the final product batch safety test would have no equine influenza virus antibodies or, at most, a low level, and have not been vaccinated against equine influenza.

An inactivation control test is performed to check the bulk of trivalent equine influenza antigens for absence of possible live virus. The inactivation control test was performed according to the guidelines of the Ph.Eur. monograph 0249. A live viral titration will be performed on each harvest to demonstrate that titres are below those shown in the inactivation kinetic study.

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 antigens

Satisfactory stability data has been provided to justify a shelf life for the influenza bulk antigens.

Stability of the finished product

Stability studies have been conducted using batches of Equilis Prequenza stored at +2°C to +8°C. Based on the data provided, transportation need not be carried out under refrigerated conditions (+2°C to +8°C). The proposed shelf life of 24 months for the finished product is justified.

OVERALL CONCLUSION ON PART II

The analytical part of the dossier is well described. The production of the influenza antigens, purification and manufacture of the finished product was described in detail.

A detailed description of the manufacturing process for the adjuvant, the quantitative composition, the shelf life and the quality control was given. The risk of contamination with extraneous agents was assessed and deemed satisfactory. The method of manufacture was well described, validated and the main in-process controls detailed in full. Data showing that all three imfluenza master seed viruses are tested as free of avian extraneous agents was provided.

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.

Validation of the influenza virus batch potency test was addressed in detail and appropriate pass criteria defined. A satisfactory test for complete inactivation of influenza virus in the finished product was presented. Control tests on the finished product including the batch potency test provide confirmation that batches of consistent quality and potency are produced. Safety tests are carried out on each batch.

Based on the stability data provided a shelf life for the influenza bulk antigens and for the finished product of 24 months respectively was justified.

3. SAFETY ASSESSMENT

Equilis Prequenza is an inactivated adjuvanted vaccine indicated for the active immunisation of horses against the effects of an infection with wild type equine influenza virus of the subtypes A/equine-1 or A/equine-2. The basic vaccination scheme consists of two 1 ml intramuscular injections, each a single dose, with an interval of 4 weeks. The minimum age for vaccination is 6 months. The safety studies were based on the relevant Ph.Eur. monographs and guidelines.

Five laboratory studies and 6 field studies have been conducted. All studies have been performed with Equilis Prequenza Te, an inactivated adjuvanted vaccine containing the same three influenza strains as Equilis Prequenza and tetanus toxoid (40 Flocculation units /ml) as the active components. Equilis Prequenza Te contains standard antigen content for all components. 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 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 influenza antibody titres HI $< 2^4$ and not previously vaccinated against influenza or tetanus. On the day of vaccination half of the foals were seronegative ($< 2^4$) for equine influenza strains Prague/56, Newmarket-1/93, Newmarket-2/93. The other half showed low ($\leq 2^4$ or 2^5) antibody responses to one of the equine influenza strains. 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 a further 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. 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 several 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. Vaccine remnants, abscess formation, haemorrhages, calcification or necrosis of the tissue were not observed. The muscle tissue itself did not show any macroscopic abnormalities. Vaccine remnants, abscessation, haemorrhages, calcification or necrosis of the tissue were not observed in either 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. 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 six 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 Prequenza is a vaccine containing inactivated viral protein. Replication of vaccine virus or bacteria in any cell involved 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 Prequenza on immunological functions.

Study of residues

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

Interactions

No specific studies on interactions were presented. Therefore the following is stated in the SPC: "No information is available on the safety and efficacy from the concurrent use of this vaccine with any other vaccine. It is therefore recommended that no other vaccines should be administered shortly before or after vaccination with the product."

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 reflected in the 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 Teor 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 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 2 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 a competitor vaccine 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 control product 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 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 4 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 vaccine. 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 12 months ago, the horses received a basic vaccination course consisting of 2 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 inactivated viral antigens as active components together with lactose and buffer salts. 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 Prequenza 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 Prequenza 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 3 days. Other systemic reactions were not induced by the vaccine. That means that 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. An assessment of the ecotoxicity risks showed that the overall risk of the vaccine to the environment, humans and other animals is effectively zero.

As the adverse reactions following vaccination with a single dose, an overdose and a repeated single dose to foals and older horses as well as to pregnant mares were minor and transient in all studies, the vaccine may be used safely in competition horses. Vaccination of horses just before or just after competition should be not performed. Additionally, any stress after vaccination, e.g. by training should be minimised.

4. EFFICACY ASSESSMENT

Equine influenza is an infectious respiratory disease caused by a virus belonging to the orthomyxovirus group. It is one of the most severe equine respiratory diseases and rapid spreading of the infection is typical. In an unprotected population morbidity rates of more than 80 % may occur. The disease is characterised by high fever and persistent severe cough. Infections caused by influenza virus often predispose to bacterial superinfection of the respiratory tract. This leads to a much more severe course of the disease than influenza infection itself. Strains belonging to the influenza subtypes A/Equi-1 and A/Equi-2 are responsible for the disease in horses. During recent years no A/Equi-1 field strains appeared. Strains belonging to subtype A/Equi-2 continue to circulate and cause large epidemics throughout horses world-wide (except for Australia and New Zealand). Vaccination is one of the accepted methods to prevent the disease. The protective antigenic proteins of the influenza virus correspond to the haemagglutinin and neuramidase contained in the membrane part of the viral envelope.

Requirements for demonstrating the efficacy of the vaccine are specified in the "Requirements for immunological veterinary medicinal products" (Directive 2001/82/EC), Ph.Eur. monograph 0062 "Vaccines for veterinary use" and CVMP guideline "Specific requirements for the production and control of live and inactivated vaccines for horses". The requirements with regard to efficacy of the "Note for guidance: Duration of protection achieved by veterinary vaccines" (EMEA/CVMP/682/99-FINAL) and the Ph.Eur. general text 5.2.7 were fulfilled.

Laboratory trials
Establishment of a challenge model

Equine Influenza
The influenza challenges were performed in accordance with Ph.Eur. monograph 0249 in the target animal. The parameter used to assess the efficacy of Equilis Prequenza (Te) against equine influenza was the level of serum antibodies against the three influenza vaccine strains induced after vaccination. This was determined by means of a haemagglutination inhibition test (HI). The HI titre was identified in a twofold dilution series and specified as HI $\log_2$ titre. The vaccination was considered successful if a HI titre of ≥ 6 $\log_2$ was induced and a titre increase of at least 2 $\log_2$ could be detected. The detected antibody levels in the trials against the H7N7 strain was satisfactory.

The second parameter was the comparison of the clinical signs and virus excretion induced by a challenge infection with strain A-equine/Kentucky/9/95 or A/Equine-2/South Africa/04/03 in a vaccinated group and a control group of animals. The vaccine was considered to be sufficiently effective if significant differences in the quantity of virus excreted and quantity and severity of the symptoms observed after 14 days could be identified in favour of the vaccinates.

Determination of the vaccine dose

No separate dose-response trials have been performed with regard to the EIV-components. Reviewing the data from the batch potency validation test performed in guinea pigs and the target animal a dose-dependant immunoresponse measured in HI antibody titres was demonstrated.

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. Information regarding the challenge strain was provided. The relevance of the strain for the current European field strain situation was supported. After challenge horses were monitored for clinical signs of influenza, rectal temperatures, virus

excretion and serology. Clinical scores, total days of virus excretion and virus reisolation data from vaccinated and nonvaccinated animals were statistically compared.

Vaccination with Equilis Prequenza Te in foals, according to the recommended vaccination scheme, strongly reduces clinical signs and virus excretion when challenged with equine influenza virus strain A/equine-2/Kentucky/95. The challenge was performed by the oronasal route and the challenge model used in the studies described in the dossier is the only model described so far that enables a fairly accurate calculation of the challenge dose per horse.

EIV-Serology
The HI antibody titres against the three influenza strains were listed separately for each horse. At the beginning of the trial, all foals were seronegative against all three influenza strains. No anamnestic seroresponse was detected in the vaccinates one week after V 1. The vaccinates responded to the first immunisation with a slight development of antibody titres against all three strains. After the second immunisation a fast increase of the level could be detected. The titre in all controls remained negative until the day of challenge. The challenge clearly induced the development of antibodies against Newmarket/1/93. For Newmarket/2/93 the antibody titres increased to low levels 14 days after challenge. No control foal seroconverted against the Prague strain.

All observed parameters of the challenge were calculated for significant differences between the vaccinates and controls. After the basic vaccination course, the vaccinates developed high levels of HI antibody titres against all three influenza strains and high ToBI titres as well. After challenge with Kentucky/9/95 4 weeks after the primary vaccination the vaccinates were not fully protected, but a significant difference in favour of the vaccinates could be demonstrated for all observed parameters. The clearest differences between the two groups could be observed in virus shedding, occurrence of coughing and development of fever.

The Influence of Maternal Antibody on the Efficacy of the Vaccine

The interference of maternal antibodies on the efficacy of inactivated vaccines against equine influenza and tetanus is well known. The data from the field trials presented in the dossier indicate that the humoral response of Equilis Prequenza Te may be impaired by interference with maternal antibodies against influenza. Field data and laboratory data indicate that young animals at the age of 6 months that are without maternal antibody against influenza 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 equine influenza 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 with Equilis Prequenza Te during the last 2 months of gestation should not be vaccinated before the age of 6 months. This has been taken into account in section 5.10 of the SPC.

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). At the day of challenge, all vaccinates had a HI serum titre above 6.0 $\log_2$ against all influenza strains of the vaccine.

After challenge all horses were observed for a period of 16 days for clinical signs of influenza and virus excretion. The clinical signs of the vaccinates were significantly lower ($p<0.05$) compared to the

findings of the control group. The duration and amount of virus excretion was significantly lower ($p<0.05$) in the vaccinates compared to the controls.

At the beginning of the trial, all foals were seronegative against all three influenza strains. No anamnestic seroresponse was detected in the vaccinates one week after V1. After the basic vaccination course the vaccinates developed high levels of HI antibody titres against all three influenza strains. After the second immunisation, a fast increase of the serum titre could be detected. From that level a continuous small decrease of the antibody levels could be detected over the following weeks. Five months after V2, antibody titres indicate sufficient protection against influenza. After challenge all control animals developed clear clinical signs of influenza. Responding to the challenge a strong increase of HI serum titres for the Newmarket strain could be observed. Concerning the controls the challenge induced the development of antibodies against Newmarket/1/93 and for Newmarket/2/93, whereas the controls did not seroconvert against the Prague strain. Since sufficient protection against an influenza challenge five months after the primary vaccination course with Equilis Prequenza Te could be demonstrated, a first revaccination 5 months after the basic immunisation can be supported.

For the observed parameters after challenge a significant difference in favour of the vaccinates could be demonstrated. The clearest differences between the two groups could be observed in virus shedding, occurrence of coughing and development of fever.

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 influenza after the first revaccination in horses. 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 vaccine Equilis Prequenza. At 12 months after the third vaccination all of the foals of the vaccination group were challenged together with unvaccinated foals serving as control group with a recent field strain of Equine influenza A of subtype H3N8 (Kentucky/9/95, “American” lineage). At the day of challenge, all vaccinates had a HI serum titre above 6.0 $\log_2$ against all influenza strains of the vaccine.

Equine influenza

The HI antibody titres against the three influenza strains are listed separately for each horse. The means of the group titres transformed in curve diagrams were presented. At the beginning of the trial, all foals were seronegative against all three influenza strains. No anamnestic seroresponse was detected in the vaccinates one week after V1. The titre in all controls remained negative until the day of challenge. A fast and strong increase of antibodies against all strains could be observed after the revaccination at week 26 with the influenza vaccine. These titres decreased about 2 $\log_2$ ranges during the next two months. Then, the titre decrease was continuous but slower and plateaued nearly half a year later.

At the day of challenge (12 months after V3) the group mean of the serum titres were identical with the titres obtained after bleeding 4 months earlier. Compared to the levels obtained at week 26 (V3), these titres are identical for the Prague strain and 1 $\log_2$ range higher for the two Newmarket strains.

After challenge the clinical signs of the vaccinates were significantly lower compared to the findings of the control group. The duration and amount of virus excretion was significantly reduced in the vaccinates compared to the controls. A sufficient protection against an influenza challenge 12 months after the first revaccination with Equilis Prequenza in foals, which have had a basic vaccination course with Equilis Prequenza Te, could be demonstrated. A yearly booster vaccination beginning with the second revaccination was supported.

In the *vaccination* group, nasal discharge was the only clinical sign which occurred after challenge. All foals developed serous nasal discharge at day 2. During the next days mild nasal discharge was observed in the majority of the *vaccinates*. Only one foal developed a marked mucopurulent discharge

for 2 days. From day 8 until the end of the observation period this clinical sign was observed in very rare cases in the vaccinates. On day 14 all vaccinates seemed to have recovered completely.

Reduced appetite was observed in the *control group* on several days. One or 2 animals additionally developed malaise and depression. Nearly all controls were affected by nasal discharge from day 2 to 7, resulting sometimes in a mucopurulent quality. Beginning with day 9 the observed cases decreased. On day 14 no clinical signs could be found in the *control* animals. All parameters observed during challenge were calculated for significant differences between the vaccinates and controls.

Vaccination with Equilis Prequenza Te led to the induction of high antibody titres against all equine influenza antigens and tetanus toxoid included in the vaccine. These antibody titres remained high throughout the study. Based on the results of this study, it is justified to conclude that the vaccine offers protection against equine influenza for at least one year after the third vaccination.

Efficacy of Equilis Prequenza Te in horses against a challenge with influenza A/Equine-2/South Africa/04/03 after the basic vaccination course

The objective of the study was to test the efficacy of Equilis Prequenza Te against the A/Equine-2/South Africa/04/03 strain. Influenza- and tetanus-seronegative yearlings were included in the study. Horses were either vaccinated with two doses of Equilis Prequenza Te 4 weeks apart or served as untreated control animals. Three weeks after the administration of the second vaccine dose, all yearlings were challenged by aerosol with strain A/Equine-2/South Africa/04/03.

At the day of challenge, the average titres in the vaccination group were similar to those seen in other studies. The group antibody titre against this strain was quite high at challenge day. The control yearlings remained seronegative against influenza up to the day of challenge. In the vaccination group, only very mild signs of disease occurred after challenge. Within the group of controls all animals developed clear signs of influenza. Virus isolation from nasal swabs was successful over a period of one week with high titres within the controls. In contrast, in the vaccination group 70 % of vaccinates shed virus for one day only with low titres.

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.

Foals of group A developed high levels of antibody titres against all 3 influenza strains of the vaccine. The foals with maternal antibody titres against all influenza strains at the beginning of the trial did not reach the antibody levels of the seronegative foals where an antibody increase was found comparable to the induced antibody levels of the foals used for the laboratory trials. When comparing group A and B the antibody levels reached against the Prague strain were nearly identical. Regarding the antibody levels against the 2 Newmarket strains the antibody increase was higher in Group A compared to group B but could be related to the different strains which are incorporated in the vaccines used. Descriptive statistics were used to summarise the data.

In the Equilis Prequenza Te group 63 % animals responded to vaccination; in the positive control group 43 % responded. All foals with an Influenza HI titre < 4.0 showed a response to vaccination. However, in some foals with high levels of maternal antibodies at the moment of the first vaccination an increase in HI influenza titre did not occur. In some cases foals without detectable maternally

derived antibodies at the first immunisation responded poorly to the vaccination. The results obtained from the positive control group showed the same tendencies so the problems are not product-related.

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

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

The objective of the 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 trial was conducted in compliance with GCP guidelines. 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 with one dose of Equilis Prequenza Te, one group was vaccinated with one dose of the control product and the third group was not vaccinated. The horses were vaccinated i.m. in the neck on days 0 and 29 and were monitored for local and systemic reaction up to day 57.

HI titres of 4 and below were considered as negative. It is concluded from the data that Equilis Prequenza Te is an efficacious vaccine against equine influenza. The titre in the Equilis Prequenza Te group was higher compared to that detected in the control group. At the end of the basic immunisation course, the young foals of the trial showed a higher antibody titre against the influenza vaccine strains compared to the older animals used in 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 received 2 vaccinations with Equilis Prequenza at an interval of 28 days. Group 2 were administered 2 vaccinations with Equilis Prequenza Te at an interval of 28 days. Group 3 served as controls. The horses were sero-negative for equine influenza. The serological response was assessed from blood samples taken on day 7, 28, 42, and 56 after V 1.

The serological response was measured against the influenza strains incorporated into the vaccines. At day 42, all vaccinated horses reached HI titres above the protection level of HI titre 6.0 $\log_2$. At day 56, for two horses of the Equilis Prequenza Te group and one horse of the Equilis Prequenza group the level of protection for all strains was lower than seen in other studies. The three horses were adults, two of them > 10 years of age. For these horses it must be expected that the titre will decrease below the protection level within the next weeks and possibly a gap of protection will occur for one of the strains at the time before V3. For strains Prague/56 and Newmarket/1/93 the induced antibody titres of the Equilis Prequenza group were higher compared to the Equilis Prequenza Te group. It is concluded from the data provided that both vaccines are efficacious against equine influenza and that the titres induced by Equilis Prequenza are higher in comparison with 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 a competitor product. The animal phase of the study was not blinded, but determination of antibody titres was performed under blinded conditions. Clinically healthy horses, of 14 breeds, and aged between 1.2 – 25 years, were separated into 3 groups at 4 different sites. Per site, two 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 product.

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. 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. Descriptive statistics were used to summarise the data. At admission, the majority of horses had moderate to high titres against equine influenza and tetanus.

All horses that underwent the basic immunisation course responded to all influenza strains with protective antibody titres up to day 56. A significant difference between the two test vaccines could not be detected. Both vaccines induced protective levels of influenza antibodies in horses.

The horses that received one booster vaccination responded with a mean influenza titre increase between 1.4 to 2.0 $\log_2$ against all tested strains 14 days after the vaccination. A sufficient booster effect could be detected in adult influenza seropositive horses. A significant difference between the two test vaccines did not occur.

Both vaccines induced protective levels of influenza antibodies in horses. A sufficient booster effect could be detected in adult influenza seropositive horses. If the vaccine is administered to horses with high antibody titres, a further titre increase is induced. None of the horses in this trial had been vaccinated with a single influenza vaccine within 12 months preceding the booster vaccination.

A Positive Controlled Safety and Efficacy Trial of Equilis Prequenza Te in Pregnant Mares

The aim of the study was to determine the efficacy of the vaccine against influenza in pregnant mares as well as the assessment of negative influences of vaccination on the ongoing pregnancy and foal delivery. Sufficient increase and the time of persistence of maternally derived influenza antibodies of the offspring was to be investigated under field conditions. Mares of different breeds and ages housed on different farms and pregnant for 3-9 months were randomly assigned to two groups; vaccinated with Equilis Prequenza Te or vaccinated with the positive control product. The mares which showed HI influenza antibody titres > 6 $\log_2$ at the beginning of the trial received a single vaccination dose. All mares with lower titres were given two vaccination doses 4 weeks apart. All mares received an additional vaccination dose 4 to 5 weeks before the expected foaling date. The level of maternally derived antibodies in the foals were high. The decrease was very slow and a level of insufficient protection was reached from 5 months of age onwards.

Descriptive statistics were used to summarise the data. Differences in qualitative data (i.e. proportion of healthy newborn foals, incidence, nature and severity of local and systemic reactions) between the treatments were evaluated by non-parametric methods (Chi-square or Fisher exact test). Differences in antibody titres were evaluated by parametric methods (ANOVA).

The majority of the mares had a titre of < 6 against one or more strains at admission, and received a basic vaccination (i.e. two vaccinations with a 4 weeks interval). Within the Equilis Prequenza Te group, this was 72 % of the mares and 73 % within the control group. The remaining mares received a single vaccination.

HI-influenza antibody titres before first vaccination were significantly higher in the group of mares that received a single vaccination as compared to the mares that received a basic vaccination. After the pre-foaling vaccination the mares were analysed per treatment group because – within one treatment group - the HI influenza antibody titres in the single and basic vaccination groups were at the same level.

<u>Single vaccination</u>
Twenty-eight days after the first vaccination the mares of the Equilis Prequenza Te group showed significantly increased titres against all 3 strains compared to the titres on day 0. The mares of the control group showed a significant increase for Newmarket/1/93 on day 28 compared to the titre on

day 0, but not for strains Prague/56 and Newmarket/2/93. The HI-influenza antibody titres did not differ significantly between the Equilis Prequenza Te group and the positive control group.

Primary vaccination
Four weeks after the first vaccination (day 28) and 4 weeks after the second vaccination (day 42) the mares of both test groups showed a significant increase of titres against all 3 strains compared to the titres prior to vaccination on days 0 and 28. The HI-influenza antibody titres differed significantly between the Equilis Prequenza Te group and the positive control group.

Two weeks after the pre-foaling vaccination the HI-influenza antibody titres of the mares of both test groups were significantly increased for all 3 strains compared to the titres prior to the pre-foaling vaccination.The HI-influenza antibody titres before and after the pre-foaling vaccination did not differ significantly between the Equilis Prequenza Te group and the positive control group. The influenza HI titres of the foals in both treatment groups gradually declined with increasing age. The level of the HI-influenza antibody titres indicates that the mares were protected against influenza field infections during the whole trial period. It can be concluded that Equilis Prequenza Te is well tolerated and induces high HI-influenza antibody titres in pregnant mares.

The foals had high levels of maternally derived antibodies against equine influenza. At week 24 after birth, the age of first vaccination, in most of the foals the titres were still at a level that a sufficient active immunisation cannot be expected.

The main objective of the trial was the safety for the unborn foal. Therefore, the highest number of possible vaccinations was chosen. The information that a booster vaccination is administered 4 weeks before the expected foaling date, is relevant for the expected duration of maternally derived antibodies in the offspring of the dams which were treated in this manner.

The persistence of higher levels of maternal antibody that were found in 50-75% of the foals at the age of 5 months is directly related to the vaccination schedule applied. However, vaccination should not start earlier than 6 months of age unless levels of maternal antibody in foals have been determined. This is reflected in the SPC.

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 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 (375 µg) in one dose of 1 ml. The full combination product Equilis Prequenza Te which also contains tetanus toxoid in addition to inactivated antigen of three different equine influenza strains, has been used in all efficacy and safety studies. According to the Note for Guidance ”Requirements for combined Veterinary Vaccines” (CVMP/IWP/52/97-Final), this approach is acceptable. If the full combination product has been demonstrated to be safe, these results can also be regarded as indicative for the mono-component vaccines. Equivalence of the HI titres induced was demonstrated. The results confirm that for Equilis Prequenza Te and Equilis Prequenza the same level of protection can be expected.

The influenza vaccine strains of Equilis Prequenza are in accordance with the actual recommendation of the OIE. The presented studies are undertaken in accordance with the Ph.Eur. monograph 0249. The test batches of the vaccine used in the efficacy trials were produced as for batches for the market. So the amount of antigen and adjuvant was not adjusted to minimum level. The use of batches with standard amounts of antigen and adjuvant is acceptable for the efficacy trials.

The development of antibodies and the outcome of the challenges undertaken demonstrate good efficacy of the vaccine. If any signs occur after infection they are very mild and only a small amount of virus shedding is expected. Complete recovery of the horse will only take a few days. This is in accordance with the SPC. The chosen immunisation scheme for the vaccine is two vaccinations

4 weeks apart followed by a third vaccination 5 months later with yearly booster vaccinations afterwards. The possibility of alternating revaccination with Equilis Prequenza Te and Equilis Prequenza is supported as indicated in the SPC section 5.8. According to the findings of the field trial undertaken in pregnant mares and their offspring the vaccine is fully efficacious in pregnant mares. Should the vaccine product be changed, no renewed basic immunisation is necessary.

Foals born to mares with high influenza antibody titres after an immunisation in the late stage of pregnancy developed a high level of maternally derived antibodies. This provides an excellent protection for the first months of life. However, antibody levels can persist up to an age of 5 months, therefore the age of the first vaccination was fixed at 6 months. The presence of maternal antibodies against equine influenza will influence vaccine efficacy. In general, the majority of foals from 6 months of age will be free of maternal antibodies and therefore vaccination at an earlier stage is not recommended. The minimum age of vaccination is described in the SPC. Because of possible interference by maternally derived antibodies, foals should not be vaccinated before the age of 6 months, especially when born to mares that were revaccinated in the last two months of gestation. If vaccination is intended before 6 months of age, foals should be at least 4 months of age. Vaccination before 6 months of age should always be followed by a full basic vaccination course from 6 months of age onwards. No data are presented that support the sufficient basic immunisation starting at this young age therefore a full basic vaccination course from the age of 6 months has to follow this early vaccination.

In one field trial, several foals with a maternally derived antibody level did not respond to the vaccinations sufficiently. Therefore, it was agreed to fix the age for first immunisation to 6 months.

A strain exchange related to the A-Equi-2 American lineage was discussed and at the current time (April 2005), a strain update of the vaccine is not considered necessary.

The safety data have shown that Equilis Prequenza Te can be administered to foals aged 2-4 months old. The laboratory efficacy trials and the field trials have shown that foals when free or having low levels of antibodies against equine influenza, from the age of 3 months can develop the same humoral immune response as e.g. adult horses.

5. RISK-BENEFIT

Equilis Prequenza is indicated for active immunisation of horses from 6 months of age against equine influenza to reduce clinical signs and virus excretion after infection. Equilis Prequenza is an aqueous vaccine containing purified haemagglutinin subunits (A/equine-1/Prague/1/56 = 100 AU, A/equine-2/Newmarket/1/93 = 50 AU, A/equine-2/Newmarket/2/93 = 50 AU) and 375 µg of purified saponin adjuvant per dose of 1 ml. The 3 influenza strains represent 2 subtypes of influenza A virus. The efficacy of vaccines against Equine influenza is monitored closely and surveillance programmes to detect new subtypes of Equine influenza have been implemented.

The hemagglutinin subunits are 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, the production of the influenza antigens, purification and manufacture of the finished product was described in detail. Validation of the influenza virus batch potency test was addressed in detail and appropriate pass criteria defined. The starting materials of animal origin used in the production of the final product comply with the current TSE Note for Guidance (EMEA/410/01-Rev.2) and Commission Directive 1999/104/EEC.

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 was studied. The vaccine may induce local reactions. These local reactions are characterised by soft or sometimes hard swellings mostly with a diameter less 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 normally they disappeared 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 vaccine will be well tolerated by horses of different ages. Each vaccine batch is tested for safety in the target animal before batch release. Appropriate warnings are indicated in sections 5.4, 5.9 and 5.12 of the SPC.

The development of antibodies and the outcome of the challenges undertaken demonstrate a good efficacy of the vaccine. A complete protection against influenza at every time against field infections cannot be achieved by this vaccine. However, if any symptoms occur after infection they are very mild and only a small amount of virus shedding is expected. Complete recovery of the horse will only take a few days. The chosen immunisation scheme for the vaccine with two vaccinations 4 weeks apart followed by a third vaccination 5 months later with yearly booster vaccinations afterwards is effective. The possibility of alternating revaccination with Equilis Prequenza Te and Equilis Prequenza is supported. According to the findings of the field trial undertaken in pregnant mares and their offspring the vaccine is fully efficacious in pregnant mares. 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 interference of maternal antibodies on the efficacy of inactivated vaccines against equine influenza well known. The majority of foals from 6 months of age will be free of maternal antibodies and therefore earlier vaccination is not recommended. Because of possible interference by maternally derived antibodies, foals should not be vaccinated before the age of 6 months, especially when born to mares that were revaccinated in the last two months of gestation. If vaccination is intended before 6 months of age, foals should be at least 4 months of age. Vaccination before 6 months of age should always be followed by a full basic vaccination course from 6 months of age onwards.

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

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 Prequenza was considered to be in accordance with the requirements of Council Directive 2001/82/EC.