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Committee for Medicinal Products for Veterinary Use

CVMP Assessment Report for Equilis West Nile (EMEA/V/C/002241/0000)

Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.

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Introduction

The applicant Intervet International BV submitted on 20 December 2011 an application for marketing authorisation to the European Medicines Agency (the Agency) for Equilis West Nile through the centralised procedure falling within Article 3(1) of Regulation (EC) No 726/2004 (medicinal product developed by means of a biotechnological process).

The CVMP adopted an opinion and CVMP assessment report on 11 April 2013.

On 6 June 2013, the European Commission adopted a Commission Decision for this application.

Equilis West Nile contains an inactivated chimeric flavivirus strain YF-WN (Yellow Fever – West Nile) and is presented in cardboard or plastic boxes with 10 glass vials of 1 ml or 5 or 10 prefilled syringes of 1 ml. It is proposed for use for the active immunisation of horses against West Nile virus (WNV) to reduce clinical signs of disease and lesions in the brain and to reduce viraemia. The route of administration is intramuscular use. The target species is horse.

The CVMP guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets is applicable (EMEA/CVMP/IWP/123243/2006-Rev.2).

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (Version GPV-111_03) which fulfils the requirements of Directive 2001/82/EC, as amended. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Equilis West Nile is manufactured by Intervet International BV in the Netherlands in a site which has a valid manufacturing authorisation issued by the Dutch authority for veterinary medicinal products.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system and the manufacturing and batch release site are considered in line with legal requirements.

Part 2 - Quality

Composition

The vaccine Equilis West Nile represents an inactivated and adjuvanted vaccine (suspension for injection) for the active immunisation of horses against West Nile virus (WNV).

Names of substance	Quantity per dose (1 ml)	Function
Active Substance		
Inactivated chimeric flavivirus, strain YF-WN	1400 AU[1]	Induction of immunity
Adjuvant		
ISCOM-Matrix		adjuvant
Purified saponin	250 µg	adjuvant component
Cholesterol	83 µg	adjuvant component
Phosphatidyl choline	42 µg	adjuvant component
Excipients		
Phosphate-buffered saline (PBS)	ad 1 ml	diluent

[1] One dose (1 ml) is blended to 1400 antigen units (AU) corresponding to ≥492 AU in the potency test (measured in an antigen mass ELISA)

Container

The containers (vials, syringes) are made of hydrolytical class type I glass. The stoppers for the vials as well as the plunger and tip cap of the syringes are of halogenobutyl rubber. The vials are sealed with aluminium caps. The containers are in compliance with corresponding European Pharmacopoeia (Ph. Eur.) monographs.

Development pharmaceutics

The vaccine virus as active substance is a recombinant chimeric flavivirus (strain YF-WN). The vaccine virus is a flavivirus presenting the genes for the structural proteins E and prM of WNV.

For the vaccine against WNV, the genes for the structural proteins E and prM have been replaced by corresponding genes of WNV.

The resulting chimeric virus strain YF-WN has now the envelope of WNV, containing structures involved in virus-cell attachment and virus internalization, all antigenic determinants for neutralization, and epitopes for T-cell mediated immunity. The capsid protein (C), the non-structural proteins and non-translated termini (UTR) responsible for virus replication remain those of the YF-17D.

The major target for neutralizing antibodies and inducing a protective immunity is the envelope protein E. Antigenic sites involved in neutralization have been mapped to each of the three structural domains of the E protein (Oliphant and Diamond, 2007; Diamond et al., 2009). A subset of neutralizing antibodies may also recognize the prM on the virion (Diamond et al., 2009).

The vaccine virus is inactivated by using binary ethylenimine (BEI, after cyclisation of binary ethylenamine (BEA)) as inactivation agent. The use of BEI is well established in veterinary vaccine production.

The Vero cell line (epithelial kidney cell line from an African green monkey) is used as host cell system for the propagation of the vaccine virus strain YF-WN.

The adjuvant used is based on Iscom-Matrix technology (Immunostimulating Complex). The Iscom-Matrix adjuvant is a formulation closely related to classical Iscoms but consisting of particles of a patented composition (shape / appearance are of classical Iscoms but lacking of incorporated antigens). These Iscom-Matrix particles are formed by a HPLC-purified fraction of Quillaja saponins, cholesterol and phosphatidylcholine.

Method of manufacture

The production process of the active ingredient (virus strain YF-WN) as well as the finished product corresponds to a, in general, classical procedure based on classical starting materials. VERO cell cultures (as monolayer) used as host cell system and the YF-WN virus used as active ingredient (vaccine antigen) are handled in a seed-lot system using master and working seeds.

For mass cultivation, the virus is propagated in Vero cells. The virus harvests are inactivated using BEI which is neutralized by sodium thiosulphate afterwards. Thereafter, inactivated virus suspension is and subsequently concentrated. The vaccine bulks are prepared by blending with the other components (e.g. adjuvant, PBS buffer) to a homologous vaccine suspension. For preparation of the finished product, the vaccine bulk is filled into sterile vials or syringes and stored.

The production process is considered as standard manufacture for viral vaccines. In general, the production process is described in the essential parts confirming that the production process generates consistent vaccine batches.

Control of starting materials

Active substance, excipients and other starting materials

Specifications of the active ingredients (virus, adjuvant), excipients and starting materials (cells) are defined and analytical methods are provided.

The virus strain YF-WN is well characterized and considered sufficient. With regard to the controls carried out on the virus strain relevant Ph. Eur. monographs and requirements of Directive 2001/82/EC were taken into account.

The Vero cells used as the host cell system is well characterized and control testing according to the Ph. Eur. and requirements of Directive 2001/82/EC is considered sufficient.

Information concerning the adjuvant and certificates of analysis of the excipients used in the manufacture of the vaccine are provided. These materials are tested according to the Ph. Eur. or internal procedures as appropriate. In addition, information regarding the qualitative and quantitative composition of the culture media, their treatment processes and their storage conditions is provided. All starting materials are either tested for or treated to ensure that there are no contaminants or further assurance is given that there is no potential risk.

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies

In general, the starting materials of biological origin comply with the Note for Guidance on minimizing the risk of transmitting animal spongiform encephalopathies agents via human and veterinary medicinal products (EMEA/410/01 rev.3). The overall TSE risk associated with the inactivated vaccine is considered negligible.

Control tests during production

The applicant has presented in-process data for two consecutive antigen bulks and batches.

During the manufacture the following in-process control tests are carried out to assure the quality parameters:

- IPC-01 Infectious virus titration test
- IPC-02 Test for residual sodium thiosulphate
- IPC-03 Inactivation control test
- IPC-04 Sterility test
- IPC-05 Antigen mass determination
- IPC-06 Filling volume

Test descriptions and the limits of acceptance are presented. Test methods for in-process controls are satisfactorily validated.

Control tests on the finished product

The applicant has presented data for two consecutive finished product batches.

The control on the finished product is performed either on the vaccine bulk or on the filled product and is carried out to assure the quality parameters. The following tests are performed:

- FPC-01 Test for pH
- FPC-03 Potency and identity
- FPC-04 Adjuvant determination
- FPC-05 Quality and identity of adjuvant
- FPC-06 Sterility test (filled product only)
- FPC-07 Appearance (filled product only)

Test descriptions and limits of acceptance are presented. The control methods are satisfactorily validated in order to confirm that the production and control processes generate consistent vaccine batches.

Target animal batch safety tests are no longer required in accordance with Ph. Eur. monographs.

Stability

Stability data were gathered for the bulk antigen and the finished product.

Data were provided for three inactivated virus antigen bulks. These data justify a shelf life of 25 months (at ≤-18 °C) for the bulk antigen. Nevertheless, it appears that the inactivated bulk antigen can be stored at least 30 months before blending as finished product batches blended after an overall storage period of 30 months showed comparative data independently from the storage length of the antigens. Test results of these batches have met the proposed specifications.

For justification of the proposed shelf life of 12 months stability data were gathered for two batches of the finished product for up to 15 months storage at 2 °C - 8 °C. In addition, data of one production batch filled as sub-batches in vials and syringes are available. All batches passed the control tests on the finished product and met the proposed specifications. Based on these data, a shelf life of 12 months at 2 °C – 8 °C is justified for the both vaccine presentations (vials, syringes).

Overall conclusions on quality

Information regarding the qualitative and quantitative composition, the starting materials, production method, quality controls, and stability are provided in this part of the dossier. Two consecutive batches at pilot scale were provided in order to demonstrate batch-to-batch consistency.

The production methods as well as the in-process and final product quality control are appropriate to ensure the compliance with the specifications and a reproducible and consistent quality of the vaccine. The production process is described in sufficient detail to give confidence that the manufacture will yield a safe, effective and stable vaccine of consistent quality.

The seed lot system of the virus antigen has been explained. Concerning the Vero cells, the cell seed production has been described in detail. 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.

In general, in-process controls during manufacture and control tests on the finished product are appropriate to ensure the compliance with the quality specifications mentioned. Acceptance limits are properly established.

Regarding the stability, the inactivated bulk antigen is demonstrated to be stable over the proposed shelf life of 30 months (at ≤-18 °C). The shelf life of 12 months at 2 °C - 8 °C is justified for both vaccine presentations (vials, syringes).

The CVMP considers the presented analytical dossier as adequate and sufficiently detailed to give confidence that the finished product is produced according to a consistent procedure of adequate standards and including adequate controls.

Part 3 – Safety

Safety documentation

Equilis West Nile is an inactivated adjuvanted suspension for injection for the active immunization of horses against West Nile virus (WNV). It contains the inactivated chimeric flavivirus strain YF-WN as active ingredient, as previously explained. The immunogenicity of the inactivated virus strain is enhanced by the adjuvant Iscom-Matrix. Equilis West Nile is formulated to a fixed amount of antigen, i.e. 1400 AU/dose (1 ml).

Equilis West Nile is intended for horses from the age of 6 months onwards. The volume of a single dose is 1 ml to be injected by the intramuscular route. The basic vaccination schedule consists of two injections, given 3 - 5 weeks apart. For revaccination a single booster vaccination of one dose (1 ml) at an interval of 12 months is intended.

Regarding Equilis West Nile safety data are presented taking into consideration the CVMP guideline on Data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMEA/CVMP/IWP/123243/2006-Rev.2).

Laboratory and field studies, mostly combined for safety and efficacy investigation, have been performed using Equilis West Nile batches which were representative for the production process. The same batches were used for the safety and efficacy trials.

The safety studies were performed in horses of different breeds, including thoroughbred horses, which represent the most sensitive category of the target species, different ages, including animals of the youngest recommended age (6 months) as well as horses of 14 years and older and pregnant mares.

Laboratory tests

One laboratory study only is claimed to have been performed in compliance with GLP. While neither the study report nor the GLP and Quality Assurance statements are signed for this study, the applicant confirmed that the signed documents are archived.

Safety of the administration of one dose

Three combined safety-efficacy studies were presented in support of the safety of one dose. A short description of these studies is given in chapter 'Safety of the repeated administration of one dose'.

The results of these safety studies demonstrate the safety of the administration of one single dose (1 ml), followed by one single dose 4 weeks later via intramuscular injection to seronegative Fjord horses which were younger than 2 years at the beginning of the study as well as to seronegative ponies and horses of various breeds and age, 14 - 23 years at the beginning of the trial.

Only mild local reactions and occasional increase in body temperature were found. Sometimes transient fever occurred, which persisted for no longer than 48 hours. Local reactions at the injection site, such as swelling, were observed temporarily which normally disappeared within a few days. The outcomes of the studies are reflected in the wording of the SPC.

Safety of one administration of an overdose

One combined study was presented in support of the safety of an overdose.

Study 08R/0091: The objective of this study was to demonstrate the safety of an intramuscular administration of an overdose followed by the administration of two repeated single doses of the vaccine determined in susceptible thoroughbred and warm blood foals of the minimum age.

Ten seronegative thoroughbred, thoroughbred-cross and warm blood foals, 5 - 9 months old at the beginning of the study, were vaccinated, firstly, intramuscularly using 2 doses of the vaccine. After 28 and 42 days the foals were vaccinated again intramuscularly using 1 dose, respectively.

The animals were observed recording any general impression, local and systemic reactions including measurement of body temperature.

The administration of an overdose of Equilis West Nile was well tolerated. Only mild local reactions and occasional body temperature increases were found. Transient fever occurred which persisted for no longer than 48 hours. Local reactions at the injection site, such as painless swelling, were observed temporarily, which normally disappeared within a few days.

The results of this study prove the safety of the administration of one double dose (2 ml) followed by two single doses (1 ml) via intramuscular injections 4 weeks apart in each case.

The outcome of the study is reflected in the wording of the SPC.

Safety of the repeated administration of one dose

Three combined safety-efficacy studies were presented in support of the safety of the repeated administration of one dose.

Study 09R/0314: The objective of the study was to assess safety and duration of immunity of 12 months determined after the basic immunisation in horses.

Twelve seronegative horses of both genders nearly two years old were grouped. Group 1 was vaccinated twice (4 weeks apart) with one dose of the vaccine intramuscularly. Group 2 remained unvaccinated.

The animals were observed 14 days recording any general impression, local and systemic reactions including measurement of body temperature.

No elevated body temperature was observed after the first vaccination. A slight increase was measured after the second vaccination. No other systemic reactions, which can be judged as vaccine related, occurred after the vaccinations. Local reactions were not recorded in any of the horses after the two vaccinations.

The results of this study prove the safety of the repeated administration of a single dose (1 ml) via intramuscular injections 4 weeks apart.

Study 09R/0315: The objective of the study was to assess safety and efficacy of the vaccine determined in horses of at least 14 years of age.

14 seronegative horses of both genders and of different small horse breeds, all older than 14 years, were divided into two groups. Group 1 was vaccinated intramuscularly twice four weeks apart with one vaccine dose. Group 2 received an adjuvanted placebo similarly.

The animals were monitored for systemic and local reaction for 12 days after each vaccination. The body temperature was recorded for 5 days post vaccination.

No elevated body temperature was observed after the vaccinations. No systemic and local reactions, related to the vaccinations, were recorded in any of the horses after the two vaccinations.

The results of this study prove the safety of the repeated administration of a single dose (1 ml) via intramuscular injections 4 weeks apart.

Study report 10R/0361: The objective of the study was to assess safety and efficacy of the vaccine determined in horses.

18 seronegative horses of both genders and nearly two years of age were separated into three groups. Group 1 and 2 were vaccinated twice four weeks apart with one vaccine dose intramuscularly. Group 3 remained unvaccinated. In addition, a third single dose was administered 59 weeks after beginning of the study to Group 2.

The animals were observed for local and systemic reactions including measurement of the body temperature for 14 days after each vaccination.

No elevated body temperature was observed after the first vaccination. A slight increase was measured after the second vaccination. No other systemic reactions, which can be judged as vaccine related, occurred after the vaccinations. No local reactions except a mild transient swelling were recorded after the vaccinations.

The results of this study prove the safety of the repeated administration of a single dose (1 ml) via intramuscular injections 4 weeks apart and after an annual revaccination.

In conclusion, the results of the above studies obtained after repeated administration of one dose (analogous to the primary vaccination course) or an overdose followed by the administration of two single doses, respectively, to horses revealed that the adverse reactions were always limited to a transient increase of body temperature as well as a transient local reaction at the injection site. The repeated administration of Equilis West Nile to horses was well tolerated.

Examination of reproductive performance

The impact on reproductive performance was assessed in two controlled field trial including pregnant mares. Animals at different trimesters of gestation were vaccinated with Equilis West Nile and observed for teratogenic and abortifacient effects. The offspring was examined for any adverse events.

No statistically significant difference was found between vaccinated animals and controls. The percentage of mares delivering healthy foals was also similar in the two groups. The results demonstrate that Equilis West Nile is safe in pregnant mares. The outcome of the study is reflected in the wording of the SPC.

No data are available regarding the safety of the administration of Equilis West Nile during lactation. The applicant has justified the omission of those data. Therefore the use of the vaccine during the lactation is acceptable. This is reflected in the SPC.

Examination of immunological functions

No specific studies have been carried out. Due to the fact that Equilis West Nile is a vaccine containing an inactivated virus antigen replication of the vaccine virus in any cell involved in the immune system of the vaccinated animals is not applicable and subsequently impairment of the immune system is not to be expected. There is no reason to assume any impact of the vaccination with Equilis West Nile on immunological functions.

Special requirements for live vaccines

The special requirements for live vaccines are not applicable as the vaccine Equilis West Nile is inactivated.

Study of residues

The vaccine contains an inactivated antigen, a buffer solution and adjuvant. The adjuvant consists of a mixture of a purified fraction of saponin, cholesterol and phosphatidylcholine. In view of the nature of the active substance and considering that the excipients including the adjuvant are either allowed substances for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009, a zero day withdrawal period is appropriate.

Interactions

Interactions with other veterinary medicinal products have not been investigated. An appropriate statement in the SPC is given.

Field studies

The applicant presents three combined safety-efficacy field trials in support of the safety of the vaccine. These studies are fully described in Part 4.

Two field trials included pregnant mares to examine the possible impact of Equilis West Nile on reproductive performance. Mares at different trimesters of gestation were vaccinated and observed for teratogenic and abortifacient effects and healthy offspring. In addition, the vaccinated mares were monitored for the occurrence of local and systemic reactions after the vaccinations. During the study blood samples were taken on different occasions to demonstrate efficacy of the vaccine by seroconversion. Antibody titers in the serum samples of vaccinates mares were compared to mares of an unvaccinated control group.

The third field trial included horses of different breeds, genders and ages to examine the possible impact of Equilis West Nile.

The systemic as well as the local reactions observed in the three field trials were similar in nature, duration and size to those in the laboratory studies. The general observations indicated are already reflected in the SPC. There was no significant impact on and difference in the outcomes of the pregnancies. Overall, the vaccine appeared to be safe for use as recommended.

User safety

An appropriate user safety assessment was provided.

With regard to the user safety, the likelihood, the consequences and the level of risk of human exposure is expected to be negligible. Except for injuries from needles and damaged primary packages, there is no risk for the user. In case of accidental (self-) injection, no severe adverse effects are expected, except for a transient, warm, painful, local swelling that may appear at the site of injection.

Environmental risk assessment

The vaccine is administered by individual intramuscular injections; direct exposure of the environment to the product does not take place.

The vaccine does not contain any component in a concentration that poses a risk to (human) health. Residues of the vaccine in animals that may enter the food chain are not a risk to the environment. Excretion of any of the components of the vaccine or of metabolites can be excluded. Any unused product or waste material will be disposed of by the appropriate channels.

A phase I environmental risk assessment was provided outlining that the potential exposure of the environment to the product and the level of risk associated with it is considered negligible. The conclusion of this first phase is that there is no potential exposure of the environment to the product and therefore no phase II assessment is necessary.

Based on all data and information provided no update is considered necessary of the environmental risk assessment taken into account the chimeric origin of the vaccine and the zoonotic potential of the origin viruses when alive.

Equilis West Nile is not expected to pose a risk to the environment when used as recommended. An appropriate warning is included in section 6.6 of the SPC regarding the disposal of any unused product.

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

Not applicable, as the vaccine contains an inactivated chimeric flavivirus.

Overall conclusion on safety

In general, the safety profile of the product is regarded as satisfactory when administered in compliance with the SPC.

Concerning target animal safety, the presented data allows concluding that the vaccine is safe for horses. For the target species there is a risk of a slight rise in temperature (max 1.5 °C) which lasts no longer than 24 - 48 hours. Temporary swellings at the injection site (max 3.0 cm in diameter) may occur following vaccination. These swellings may last for over 5 days. The adverse reactions are adequately indicated in section 4.6 of the SPC.

The safety of the repeat dose administration to horses was demonstrated. After administration of an overdose no side-effects other than those already described in the SPC have been observed. An appropriate statement in the SPC is given in section 4.10 of the SPC.

The SPC adequately reflects in section 4.7 that safety of the vaccine administration during pregnancy and lactation is given.

Interactions with other veterinary medicinal products have not been investigated. An appropriate statement is given in section 4.8 of the SPC.

There is no reason to assume any impact of the vaccination with Equilis West Nile on immunological functions.

Concerning user safety, the risk posed by Equilis West Nile is considered low when used as recommended. For the user there is a low risk of (self-) injection or injuries from needles and damaged primary packages. However, in the absence of irritating substances such as oil adjuvants, the accidental injection of the vaccine into subcutaneous or muscular tissue does not present any specific risks when accidentally injected. This is adequately indicated in section 4.5 of the SPC.

Equilis West Nile is not expected to pose a risk to the environment when used as recommended. An appropriate warning is included in section 6.6 of the SPC regarding the disposal of any unused product and the avoidance of any risk from remains of the vaccine after use.

Concerning consumer safety, there are no components that require an MRL and no specific risks for the consumer have been identified. A zero day's withdrawal period as stated in section 4.11 of the SPC is considered appropriate.

Part 4 – Efficacy

Introduction and general requirements

Equilis West Nile is an inactivated and adjuvanted vaccine (suspension for injection) for the active immunisation of horses against West Nile virus (WNV).

Equilis West Nile contains an inactivated chimeric flavivirus strain YF-WN. The originating WNV strain is a lineage 1 strain, which is genetically closely related to strains isolated in the Middle East (Israel). Isolates from southern countries in Europe in the last years showed the same Middle East origin. Therefore the vaccine strain is suitable for a European vaccine as well.

The efficacy studies presented were performed in compliance with the VICH Guidelines of Good Laboratory Practice (GLP) or Good Clinical Practice (GCP), the requirements of Ph. Eur. General Chapter 5.2.7, and EU Note for Guidance on specific requirements for the production and control of equine live and inactivated viral and bacterial vaccines. In addition, the CVMP guideline on Data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets applies (EMEA/CVMP/IWP/123243/2006-Rev.2).

All studies have been performed under blinded conditions.

Laboratory trials

In order to demonstrate the efficacy of the Equilis West Nile vaccine, four laboratory studies, using both challenge with WNV and serological follow-up respectively, were carried out in the target animal species with unvaccinated animals as controls. In all studies, the vaccine was administered via the recommended intramuscular route, and the antigen content tested was the quantity claimed for the vaccine. Generally, the vaccinated groups received a standard batch (100% of antigen content per dose). However, in one trial for the determination of the vaccine dose two vaccinated groups received an experimental vaccine containing an antigen content of 25% and 12.5%, respectively.

Overview of the laboratory trials

Report no.	Title	Efficacy part	Batch no.	Reported in section
08R/0173	Efficacy of vaccine formulations with different concentrations of inactivated West Nile virus against	OOI of the basic vaccination course and efficacy of	061201 061201.2	4.II.B.1.

	challenge with virulent West Nile virus	different vaccine formulations	061201.3	
09R/0315	Efficacy study with an inactivated West Nile virus vaccine in horses of 14 years and older	OOI of the basic vaccination	081241	4.II.B.2.
09R/0314	Twelve months duration of immunity study with an inactivated West Nile virus vaccine in horses	DOI of the basic vaccination	091106	4.II.B.3.
10R/0361	Evaluation of the efficacy and safety of a single revaccination 12 months after the basic vaccination course for an inactivated West Nile virus vaccine	Efficacy and DOI of a single annual revaccination	091106	4.II.B.4.

Determination of the vaccine dose (also for determination of the onset of immunity)

Study 08R/0173: The aim of this study was to determine the efficacy of experimental inactivated WNV vaccines against challenge with virulent wild type WNV in order to establish the minimal effective dose in the target animal. The experimental vaccines were formulated with an antigen content of 100%, 25% and 12.5% per dose.

Three groups of six Shetland pony yearlings each were vaccinated twice (four-week interval, one dose) with experimental Equilis West Nile vaccine batches containing different concentrations of inactivated YF-WN virus and Iscom-Matrix as adjuvant (Group 1: antigen content 100%, Group 2: antigen content 25%, Group 3: antigen content 12.5%, adjuvant content equal in all three groups). Two weeks after the second vaccination an intrathecal WNV challenge was performed including a control group of six ponies.

Blood samples were taken at both vaccinations and one week after the second vaccination, at the challenge day and on Days 2, 4, 6, 8, 10, 13 and 15 post challenge. An observation period until 14 days after the challenge followed.

All controls but none of the vaccinated animals showed a temperature increase one week after the challenge. Other clinical signs, such as muscle tremor, were observed in 3 out of 23 vaccinated animals and 4 out of 6 control ponies. Viraemia was demonstrated via RT-PCR and cell culture titration method in all controls (low titres) whereas no positive result could be obtained from the vaccinated horses. A marked antibody titre increase was detected in all vaccination groups after the second vaccination. In conclusion, all vaccinated animals were protected according to the indication claims (except for the reduction of brain lesions which was not examined) after challenge performed two weeks after the second vaccination. An antigen content of 1400 AU per dose (antigen content 100 % per dose, measured in the final antigen lot) is - together with the standard adjuvant content - sufficient to induce protection against WNV infection.

Onset of protection

Study 09R/0315: The aim of this study was to demonstrate the efficacy of an inactivated WNV vaccine in horses at an age of 14 years and older.

14 horses older than 14 years were equally divided into a vaccination and control group. The vaccinated animals received a basic immunisation using Equilis West Nile. Nearly two weeks later, all horses were challenged by intrathecal route. A 14 day observation period followed. Blood samples were taken during the vaccination period and post challenge. At the end of the study, all horses were euthanized and the brains kept for histopathological examination.

One week after the challenge the body temperature increased in the control group while the values in the vaccinated horses remained within the normal range.

General and neurological clinical signs during the post challenge period were scored. Signs were observed in both groups. In total the vaccination group received clearlyclearly lower scoring points as compared to the control group.

Viraemia examined via RT-PCR and cell culture titration could not be detected in any of the samples belonging to the vaccination group (including the horses of this group which showed clear neurological signs of disease). Viraemia (low titres) could be detected in all controls via RT-PCR and in 4/7 controls via cell culture titration.

The controls remained seronegative for WNV antibodies until challenge. In the vaccinated animals a fast increase of WNV antibodies could be detected after the second vaccination.

In 2/7 vaccinated horses mild abnormalities were detected during brain examination. In contrast, all animals of the control group (6/7 were available) showed mild to severe lesions in different regions of the brain.

In conclusion, the vaccination provided significant reduction of increased body temperature and viraemia, and a reduction in neuropathological signs and histopathological lesions in the brain in an intrathecal challenge model 12 days after the second vaccination.

Duration of immunity

Study 09R/0314: The aim of this first study was to demonstrate twelve month duration of immunity with an inactivated WNV vaccine in horses.

Twelve Fjord horses of both genders nearly two years old at the start of the experiment were separated into two groups of 6 animals each. The first group was vaccinated twice (four weeks apart) with one dose of Equilis West Nile by intramuscular route. The second group served as unvaccinated control. 53 weeks after the second vaccination all horses were challenged by intrathecal route with WNV field strain NY99-4132 belonging to the WNV lineage 1. From shortly before challenge until two weeks after challenge, the body temperature was measured and observation of clinical signs was performed. Blood samples were taken at different time points during the whole study period. These samples were used to examine the antibody development and occurrence of viraemia after the challenge.

All vaccinated horses had seroconverted two weeks after basic vaccination. The titres decreased over the year, in some cases below the detection limit some weeks before challenge (the lowest antibody titre was detected in a horse, with a clinical score after challenge). The control animals remained seronegative over the whole period of one year.

A marked increase of body temperature was observed one week after the challenge in the control group in 5/6 animals. In the vaccine group this increase was marginal and did not exceed 38.7 °C.

During the observation period after challenge neurological signs and general signs of disease were given a specific score. Only one vaccinated horse received a scoring point on one day (depressed). In contrast, 5/6 control horses received scoring points for neurological signs of illness beginning with Day 9 after challenge.

After challenge viraemia could not be detected in any of the vaccinated horses. In the control group, virus could be detected in the blood samples of 2/6 horses (cell culture titration) and 5/6 controls (RT-PCR method), respectively. The virus titres were close to the detection limit.

In conclusion, vaccination with Equilis West Nile provides a significant reduction of increase in body temperature, a reduction of the occurrence of neurological signs as well as prevention of viraemia after an intrathecal challenge with virulent WNV 12 months after the primary vaccination course. Therefore, duration of immunity of one year after a primary vaccination course can be approved.

Study 10R/0361: The aim of this second study was to demonstrate efficacy and safety of a single re-vaccination 12 months after the basic vaccination course for an inactivated West Nile vaccine.

18 Fjord horses of both genders, nearly two years old at the beginning of the study, were separated into three groups of 6 animals each. Groups 1 and 2 (vaccination groups) were vaccinated twice four weeks apart with one dose of Equilis West Nile by intramuscular route into the neck. The third group served as unvaccinated control. A third single vaccination using the same vaccine batch was given to Group 2 in week 59 of the study. For Groups 1 and 3 the study was finalised after 34 weeks. In week 71, a new control group of 6 horses was included in the study (Group 4). The horses were obtained from the same stud and one year younger than the others.

A challenge was performed with Groups 2 and 4 one year after the booster vaccination had been given to Group 2.

Blood samples were taken at different time points during the whole study. These samples were used to examine the antibody development and occurrence of viraemia after the challenge.

All vaccinated horses showed seroconversion two weeks after basic vaccination. The antibody titres decreased over the year. The booster vaccination was successful in all vaccinated horses as demonstrated by clearly increased titres after booster vaccination. When followed for another year the titres then decreased again, but remained markedly above the mean titre detected at the time of booster vaccination. The controls remained seronegative over the whole two years.

A marked increase of body temperature (individual values of 40.4 °C) was observed in the control group one week after the challenge. In the vaccination group, this increase was marginal and did not exceed 38.9 °C.

During the observation period post challenge neurological signs and general signs of disease were given a specific score. Two horses of the vaccination group received scoring points for tremor for several days initiating on Day 9 after the challenge. In the control group, 4/6 horses received scoring points for muscular tremor, incoordination and ataxia, beginning with Day 9 after the challenge.

After the challenge no viraemia could be detected in the vaccinated animals. All controls were positive; however, the titres were very low with both detection methods (cell titration, RT-PCR).

In conclusion, a single dose revaccination twelve months after the primary vaccination course induces antibody responses comparable to or better than the primary vaccination course itself. However the claims reduction of clinical signs of disease could not be demonstrated sufficiently according to the duration of immunity. Therefore the following wording under SPC section 4.9 is proposed:
“Revaccination: a yearly booster injection of one dose (1ml) should be sufficient to achieve a reduction of fever, lesions in the brain and viraemia.”

The influence of maternal antibodies on the efficacy of the vaccine

No study was presented to determine the influence of maternal antibodies on the efficacy of the vaccine.

The minimum age of vaccination is 6 months. At that age, it is acceptable to neglect an influence of persistent maternal antibody titres. Young foals used in some efficacy studies were the offspring of WNV antibody naïve dams and therefore all foals were antibody negative at the start of the studies.

Field trials

In order to demonstrate the efficacy of the Equilis West Nile vaccine, three field studies were carried out in the target animal species.

Study 10R/0327: The objective of the study was to assess safety and efficacy (by serological response) of the vaccine Equilis West Nile in horses under field conditions.

173 clinically healthy horses were used with an age of ≥6 months, almost 50% Dutch warm bloods (KWPN), the other horses of any breed and gender, housed on 6 sites in The Netherlands, randomly assigned to one of two experimental groups, using site and age as classification.

86 horses were vaccinated on Day 0 and Day 28 by intramuscular route (in the neck) using Equilis West Nile. 87 horses remained as controls and received 1 ml water for injection by intramuscular route (in the neck).

Blood samples were taken from the 5 youngest and 5 oldest animals of each trial group and housing site before first administration (Day 0), before second administration (Day 28) and at Days 42 and 70. The antibody titres against WNV were determined by virus neutralisation test. Seroconversion was defined as an increase of at least 2 units (log2) in VN titre on Day 42 compared to the titre before vaccination.

At admission, none of 101tested horses had detectable antibodies against WNV. The placebo horses remained seronegative at all samplings during the study period. At Days 42 and 70, the percentage of animals that seroconverted was 92.2% (47/51) and 94.0% (47/50) respectively. The housing site had no significant effect on the seroconversion. The highest antibody titres in the vaccinated animals were found on Day 42. 9/50 (18%) of the vaccinated horses showed detectable antibody titres on Day 70 of the study.

In conclusion, following repeated administration of a single dose (1 ml) of Equilis West Nile to horses at an age of ≥6 months under field conditions, 94% of the vaccinated horses seroconverted.

Study 11R/0123: The objective of the study was to assess safety and efficacy (in terms of serological response) of Equilis West Nile in pregnant mares.

128 clinically healthy pregnant mares of various breeds were used, housed on 5 sites in Hungary, randomly assigned to one of two experimental groups, taking into account the stage of gestation (3 - 9 months) and parity.

65 horses were vaccinated on Day 0 and Day 28 by intramuscular route (in the neck) using Equilis West Nile. 63 horses remained as controls and received 1 ml water for injection by intramuscular route (in the neck).

Blood samples were taken from all animals before first administration (Day 0), before second administration (Day 28), on Days 42 and 70 as well as at foaling to determine the serological response to vaccination. The antibody titres against WNV were determined by virus neutralization test.

The number of seroconverted animals was compared between the vaccine and control group taking into account the housing site and serological status at the time of first vaccination. Seroconversion was defined as an increase of at least 2 units (log2) in VN titre on Days 42 or 70 compared to the titre before vaccination.

Before first vaccination, 20/128 mares already had an antibody titre against WNV 9 mares of the vaccinated group and 11 of the control group.

The overall percentage of animals showing seroconversion was 88.9% (56/63 – two mares did not receive the second vaccination and no further blood samples were taken) in the Equilis West Nile group. One control animal seroconverted during the study. This difference was statistically significant.

The serological status against WNV on Day 0 did not influence the response by seroconversion. The highest antibody titres in the vaccinated mares were found on Day 42.

In conclusion, following the repeated administration of a single dose (1ml) of Equilis West Nile to pregnant mares, 89% of the Equilis West Nile vaccinated mares seroconverted.

As antibody titres present after primary vaccination were low in seronegative vaccinated mares, it is questionable whether protection can be expected until the booster vaccination one year after the basic immunization course for most of the animals of this group.

Study 11R/0352: The objective of the study was to assess safety and efficacy of Equilis West Nile in pregnant Thoroughbred mares.

41 clinically healthy pregnant Thoroughbred mares were used, housed on 6 sites in Hungary, randomly assigned to one of two experimental groups, taking into account the stage of gestation (3 - 9 months) and parity.

20 horses were vaccinated on Day 0 and Day 28 intramuscularly using Equilis West Nile. 21 horses remained as controls and received 1 ml Water for Injection (WFI) by intramuscular route (in the neck).

Blood samples were taken from all animals before first administration (Day 0), before second administration (Day 28), on Days 42 and 70 as well as at foaling to determine the serological response to vaccination. The antibody titres against WNV were determined by virus neutralization test.

The number of seroconverted animals was compared between the vaccinated and control group taking into account the housing site and serological status at the time of first vaccination. Seroconversion was defined as an increase of at least 2 units (log2) in VN titre on Days 42 or 70 compared to the titre before vaccination.

Before first vaccination, 25 mares (13 vaccinates, 12 controls) had already an antibody titre against WNV.

The overall percentage of animals showing seroconversion was 95.2% in the vaccinated group. No control animal seroconverted during the study. This difference was statistically significant. The serological status against WNV on Day 0 did not influence the response by seroconversion.

In conclusion, following the repeated administration of a single dose (1 ml) of Equilis West Nile to pregnant mares, 95% of the Equilis West Nile vaccinated mares seroconverted (defined as ≥2 unit increase in titre). The serological status against WNV at vaccination did not influence the seroconversion.

Overall, three clinical studies were provided to assess safety and efficacy of Equilis West Nile in horses under field conditions. Two of these studies investigated the safety and efficacy in pregnant mares of various breeds. In all these studies trials, a serological test was included to examine the antibody titre development. In all studies a sufficient efficacy of the vaccine is demonstrated under field conditions.

However, the pregnant mares showed a good seroconversion after repeated administration of a single dose (1ml) of the inactivated WNV vaccine. In average these antibody titres did not achieve the titres of non-pregnant horses. Furthermore, as some pregnant mares showed already WNV antibody titres at beginning of the study it can be concluded that non-primed pregnant horses show a reduced serological response to the vaccination compared to primed pregnant horses.

Overall conclusion on efficacy

The efficacy of the vaccine was tested by means of four laboratory studies with intrathecal challenge and three field studies. In all efficacy trials, a serological test was included to examine the antibody titre development.

In a dose finding study it could be confirmed that batches produced according to the fixed manufacturing process with a blending target of the bulk of 1,400 AU/dose (reference is the result obtained in the antigen lot without adjuvant) and an adjuvant content of 250 µg/dose will be sufficiently efficacious with regard to the indication claims of the vaccine.

The onset of immunity 14 days after primary vaccination course could be demonstrated using the results of this dose finding study and of the onset of immunity challenge study in elderly horses and taking into account the WNV antibody development in the other laboratory tests on efficacy.

The duration of immunity of one year was demonstrated by a challenge study in young Fjord horses. It was shown that horses with only low antibody titres can be successfully boostered one year after basic vaccination. In addition, a challenge test in young Fjord horses one year after booster vaccination has been performed. However, the results of this study are considered appropriate to support an annual booster vaccination with one dose to reach a protection in line with the indication as suggested. Nevertheless, the following wording should be added in the SPC section 4.9: “Revaccination: a yearly booster injection of one dose (1ml) should be sufficient to achieve a reduction of fever, lesions in the brain and viraemia.”

At the same time, studies on titre development revealed an antibody titre decrease within one year.

However, the antibody titres induced by basic immunisation in pregnant mares and riding horses of all breeds and different ages under field conditions were comparable to those in the laboratory studies approximately 2 weeks after basic immunisation. But the values in pregnant WNV antibody naïve mares were striking. Here, only relatively low antibody titres were detected. These titres may still be regarded as sufficiently protective concerning the indication of the product and therefore annual booster vaccinations with one dose are regarded sufficient for horses at any rate. This is further supported by revaccination data from the field three years after primary vaccination course. The data were presented in the dossier of responses and showed a strong anamnestic response in all vaccinated animals including animals showing no measurable or very low neutralizing antibody titres at the time of revaccination. This would also be applicable for pregnant and sensitive horses, which are WNV antibody naive before basic immunisation and vaccinated under field conditions.

The claims refer to WNV in general; and general protection against WNV is what is expected from a WNV vaccine. WNV strains can be divided into lineage 1 and lineage 2 as well as different virus clades. Viruses from both lineages have been isolated in Europe in the past. The West Nile mother strain of the vaccine belongs to lineage 1 as well as the challenge virus used in the efficacy studies. Protection against WNV strains of lineage 1 has been demonstrated by challenge and by virus neutralisation assays using virus of lineage 1. In addition serological data are now available to assess the sufficient protection against field strains of lineage 2. In general it is indicated that a sufficient cross neutralisation is given also against strains of lineage 2. This statement is supported by the expert, who refers to his own trials in this field (independent of the vaccine applied for and unpublished data).

All challenge studies were performed with a high virus dose administered intrathecally. Thus, marked neurological clinical signs could be induced in nearly all animals of the control groups. In contrast, neurological signs in the animals of the vaccination group occurred only in isolated cases. Severity and duration were markedly and thus significantly lower in the vaccinated horses compared to the control horses. Thus, one part of the claim – ‘to reduce clinical signs’ – could be clearly demonstrated.

In the challenge studies with elderly horses, the animals were euthanized and examined histopathologically for lesions in the brains. Here as well, the vaccinated horses showed no or only slight lesions after challenge, except for one horse which showed marked alterations of the brain. In the control animals, in contrast, marked to severe lesions of the brain were found. The results of this study sufficiently demonstrate the claim 'reduce lesions in the brain'.

In order to support the viraemia claim, the presence of the virus in serum samples was tested by means of cell culture titration and RT-PCR in all studies after challenge. WNV could not be detected after intrathecal challenge in any of the vaccinated horses. A very low virus titre was demonstrated on different days in one or more samples of control animals with severe signs of disease. The differences between control and vaccination group are significant.

The validation of the PCR assay was acceptable. The use of this specific PCR is justified to appropriately investigate the claim of "reduction of viraemia".

The claim 'reduction of viraemia' is considered as demonstrated despite the fact that the challenge method chosen does not reflect the natural way of infection. The virus was applied intrathecally which is not comparable to natural infection. This means, firstly, the virus has to get over the blood-brain barrier (virus out of the brain) which is assumed to be more unlikely. Hence, it is not remarkable that virus could not be detected in the blood or only with very low titres in animals with severe signs of disease. Even in the vaccinated animals with slight clinical signs, virus could not be detected in the blood. For this specific challenge method it is imperative to reflect the natural way of infection (challenge with midges) or at least to avoid the blood-brain barrier (e.g. subcutaneous challenge). Thus, the intrathecal challenge is not a suitable method to support a 'prevention of viraemia' claim. However, the claim 'reduction of viraemia' is acceptable.

Overall, the efficacy of the vaccine was demonstrated by means of four laboratory studies with intrathecal challenge and three field studies. In all efficacy trials, a serological test was included to examine the antibody titre development.

Regarding the *indication* in section 4.2 of the SPC the active immunisation of horses against West Nile virus (WNV) to reduce clinical signs of disease and lesions in the brain and to reduce virus viraemia was consistently demonstrated throughout the efficacy studies and the claims are therefore supported.

The *onset of immunity* of 2 weeks after primary vaccination course of two injections has been demonstrated and is supported for the claims.

A *duration of immunity* of 12 months is supported for the claims. A yearly booster injection of one dose (1ml) should be sufficient to achieve a reduction of fever, lesions in the brain and viraemia.

Part 5 – Benefit-risk assessment

The topic of the following benefit-risk assessment is the application for marketing authorization of Equilis West Nile. The CVMP guideline on Data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets is applicable (EMEA/CVMP/IWP/123243/2006-Rev.2).

Equilis West Nile is a whole virus vaccine (suspension for injection) containing inactivated chimeric flavivirus (YF-WN) combined with an adjuvant. This vaccine is intended for the active immunisation of horses against infections with West Nile virus (WNV) and indicated for reduction of clinical signs of disease and lesions in the brain and reduction of viraemia.

Benefit assessment

Direct therapeutic benefit

The benefit of Equilis West Nile is the production of sufficient immunity after active immunisation of horses against West Nile virus (WNV) to reduce clinical signs of disease and lesions in the brain and to reduce virus viraemia.

Well-conducted controlled laboratory and field trials demonstrated that the product is efficacious for this indication.

Onset of immunity of 2 weeks after primary vaccination course of two injections and duration of immunity of 12 months following such primary vaccination have been demonstrated.

Additional benefits

Equilis West Nile increases the range of available vaccines (prophylaxis possibilities) for the active immunisation of horses against infections with WNV. As a consequence of the reduction of viraemic horses, the incidence of clinical outbreaks would be reduced as well.

Risk assessment

Concerning target animal safety, the presented data allows concluding that the vaccine is safe for horses. For the target species (horses) there is a risk of a slight rise in temperature (max 1.5 °C) which lasts no longer than 24 - 48 hours. Temporary swellings at the injection site (max 3.0 cm in diameter) may occur following vaccination. These swellings may last for over 5 days. Observed adverse reactions are adequately indicated in the product literature. After administration of an overdose and repeated dose no side-effects other than those already described have been observed.

The SPC adequately reflects that safety of the vaccine administration during pregnancy and lactation is given.

Interactions with other veterinary medicinal products have not been investigated. An appropriate statement in the SPC is given.

There is no reason to assume any impact of the vaccination with Equilis West Nile on immunological functions.

Concerning user safety, the risk posed by Equilis West Nile is considered low when used as recommended. To avoid any risk from remains of the vaccine after use, a respective disposal advice is given in the SPC.

Equilis West Nile is not expected to pose a risk to the environment when used as recommended. An appropriate advice is included in the SPC regarding the disposal of any unused product.

Concerning consumer safety, there are no components that require an MRL and no specific risks for the consumer have been identified. A zero days withdrawal period is considered appropriate.

Risk management or mitigation measures

Appropriate warnings and user advice have been included in the SPC to inform of potential risks to the target animals and provide advice to minimise the risk to the environment and users.

Evaluation of the benefit-risk balance

The product has been shown to have a positive benefit-risk balance overall.

The product has been shown to be efficacious for the indication to reduce clinical signs of disease and lesions in the brain and to reduce viraemia in horses.

The formulation and manufacture of Equilis West Nile is well described and the specifications set will ensure that a product of consistent quality will be produced.

The vaccine is in general well tolerated by the target animals and presents a low risk for users and the environment when used as recommended. Appropriate warnings have been included in the SPC and product literature. A sufficient withdrawal period (zero days) has been set.

Conclusion on benefit-risk balance

The overall benefit-risk evaluation is deemed positive with a sufficiently clear and complete SPC and product literature.

Conclusion

Based on the original and complementary data presented the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that, the quality, safety and efficacy of Equilis West Nile are considered to be in accordance with the requirements of Directive 2001/82/EC.