SCIENTIFIC DISCUSSION

1. SUMMARY OF THE DOSSIER

On 17 September 2008 the Committee for Medicinal Products for Veterinary Use (CVMP) adopted a positive opinion, recommending the granting of a marketing authorisation for the veterinary medicinal product Duvaxyn WNV - emulsion for injection for horses. Duvaxyn WNV contains inactivated West Nile virus strain VM-2 and is indicated for active immunisation of horses of 6 months of age or older against West Nile Virus disease by reducing the number of viraemic horses.

The Applicant for this veterinary medicinal product is Fort Dodge Animal Health.

The benefits of Duvaxyn WNV are its usefulness to reduce and prevent clinical signs in horses. The most common side effects are transient local reactions in the form of a mild, local swelling at the injection site post vaccination (maximum 1 cm in diameter) that resolve spontaneously within 1 to 2 days. In some cases hyperthermia may occur for up to 2 days.
The approved indication is:
"For the active immunisation of horses of 6 months of age or older against West Nile Virus disease by reducing the number of viraemic horses."

Onset of immunity: 3 weeks after primary vaccination course.
Duration of immunity: 12 months after primary vaccination course.

The CVMP, on the basis of quality, safety and efficacy data submitted, considered that there is a favourable benefit to risk balance for Duvaxyn WNV and therefore recommended the granting of the marketing authorisation.

Administrative particulars

The Applicant has provided a satisfactory description of the system of pharmacovigilance including a statement signed by the applicant and the qualified person for pharmacovigilance, indicating that 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.

The GMP status of all sites involved in the manufacture of this product was confirmed, as satisfactory.

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Part 2 - Quality

Composition

Ingredients	Quantity per dose (1 ml)	Function
Active ingredient West Nile Virus strain VM-2	 1.0 – 2.2 RP*	 active ingredient
Constituents of the adjuvant Metastim	 0.05 ml	 adjuvant and emulsifier
Excipients MEM medium HCl or NaOH	 QS 1.0 ml QS pH 7.0 ± 0.2	 diluent adjustment of pH

*RP = Relative Potency

Container

3 ml polypropylene syringe, with a Latex-free Type I rubber tip.

The polypropylene syringes are sterilized by irradiation (17.6-39.7 kGy).The rubber tips are sterilized by autoclaving (123°C, 30 min at least).

Development Pharmaceutics

Relevance of the WNV vaccine strain: The strain was isolated in north America and this American isolate is relevant for use in a vaccine intended for Europe. Phylogenetically, it has been shown that the WNV strains originating from the USA and Europe are all closely related. Moreover, serum cross-reactivity studies using a Plaque Reduction Neutralization Test (PRNT) showed that horses vaccinated with the vaccine strain has a similar PRNT titre compared to a french strain (PaAn001) isolated from a horse in 2000 and a Romanian strain (R097-50) isolated from a mosquito in 1996.

Inactivation: This is achieved through a two-step process: the WNV harvest stocks are firstly inactivated with 10% formalin for not less than 144 hours. This stock is further inactivated for a minimum of another 144 hours with a second addition of 10% formalin.

Potency test: it is based on an antigen sandwich ELISA assay. The monoclonal antibody used for the potency test binds to the E protein, thus preventing West Nile virus entry into host cells. The E protein is a conserved structural protein within the flaviviruses, including WNV.

Epidemiological survey programs: Duvaxyn WNV is not likely to interfere with epidemiological survey programmes for the following reasons:

- Duvaxyn WNV being an inactivated vaccine, no live viral particles would be detected by virological methods. Moreover, the amount of DNA (if not degraded by the inactivation process) is so low that it is very unlikely to be detected by PCR techniques.
- Seroprevalence surveys are commonly conducted using an IgM antibody detection assay. While natural infections consistently result in production of IgM, IgM antibody production was not detected in vaccinated horses. It is therefore possible to differentiate vaccinated horses from horses that have had recent natural infection with WNV.

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Method of manufacture

All production steps are performed in accordance with GMP. A flow chart of the method of preparation of the active substance, the vaccine suspension and the adjuvant emulsion was presented and the methods were described in detail.

All manufacturing operations are conducted in closed circuits, the connections in which are sterilised by steam (and such operations comply with the requirement of the European Pharmacopeia).

Preparation of the vaccine suspension

The manufacture of vaccine suspension, including purification of WNV antigen, is fully described and details of the conrol tests performed are provided. Following dissociation by trypsin and amplification steps performed on microcarriers in stainless steel vessels, cells are infected with the working seed virus and allowed to multiply under appropriate conditions of temperature and pH. After the culture is terminated the harvest is treated in order to rupture the cells. Culture fluids are filtered into a containment vessel. The harvest fluids are continuously stirred. A two step inactivation process is carried out under detailed conditions of temperature, pH and timing. Monitoring of pre-inactivation titres is routinely carried out and the CVMP were of the opinion that such a practice demonstrates adequate control of the production process of the virus suspension. The period of time the antigen is stored before further processing has been stated and justified.

For the preparation of final vaccine suspension, the appropriate amounts of West Nile virus inactivated stock, blending diluent and homogenized MetaStim are added to the blending vessel under continuous stirring. The pH is adjusted using HCl or NaOH.

Filling, labelling and packaging

The product, under constant agitation, is aseptically filled into sterile polypropylene syringes with an automatic filling machine. Sterile latex-free rubber tips are inserted aseptically. The presentations are then labelled and packaged.

Validations studies

Inactivation kinetics for the manufacturing process are given. Inactivation for a normal WNV production bulk (10.2 $\log_{10}$ $TCID_{50}$/ml) is achieved within 24 hours.

Control of starting materials

Active substance

Validation of Proof of Inactivation Test for West Nile virus in Antigen Stocks.

The results showed that virus was detected at a dilution of 1:3 600 000. In conclusion the sensitivity of the test was therefore found to be at least 0.8 $TCID_{50}$/ml for samples treated with the normal handling procedure.

Validation of Sterility Test for Duvaxyn WNV

The objective was to verify that the test method is able to detect a bacterial contaminant (no growth inhibition of the finished product).
In conclusion the presence of the finished product in the media under the conditions of the test did not contain bacteriostatic or fungistatic agents in sufficient amount to inhibit the growth of the 6 following micro-organisms ((*Staphylococcus aureus, Bacillus spizizenii, Pseudomonas aeruginosa, Clostridium sporogenes, Candida albicans, Aspergillus niger*)).

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Validation of sterility Testing by Direct Inoculation for Duvaxyn WNV

The objective was to verify that the test method is able to detect a bacterial contaminant (no growth inhibition of the finished product). The results showed that visible growth of the tested organisms is obtained in the spiked media.

WNV Sandwich ELISA Potency test - Establishment of new reference batches

The Applicant has developed and validated a WNV antigen ELISA method that measures the quantity of antigen present in Duvaxyn WNV relative to a reference preparation. This reference vaccine was qualified as a reference by serological testing in horses compared to the vaccine used in the duration of immunity (DOI) supportive study.

Stability of antigen stocks:

Two antigen stocks batches were tested for up to 18 months and results were satisfactory.

Excipients

Details were provided for all the starting materials listed in the European Pharmacopoeia and found to be satisfactory.

The following starting materials not listed in the European Pharmacopoeia and of biological origin were assessed and found satisfactory.

Starting material	Use
West Nile Virus, strain strain VM-2	Active ingredient
Vero cells	used for virus expansion
Foetal bovine serum	used in medium for cell expansion
Lactalbumine hydrolysate	used in preparation of cell growth medium and virus propagation medium
Bovine serum albumine	used in virus propagation medium
Porcine trypsine	used for the detachment of cell from culture vessel surface

Certificates of Analysis and details of country of origin of the animals from which the starting material listed above originated were provided.

The foetal bovine serum is irradiated under 35kGy. Lactalbumine hydrolysate, bovine serumalbumine and trypsin are irradiated at dose of 2.5 to 4.0 Mrad.

- **West Nile Virus, strain strain VM-2**

Origin and history: the virus strain was isolated from the brain tissue of a horse that died in 1999 in New York. This WNV was passaged twice in Vero cells and transferred to Fort Dodge in 2000.

Master seed virus: the WNV isolate was passaged four times in Vero cells to establish the Master Seed. Details of the indentification and control of the MSV are provided. Purity of the MSV strain is demonstrated according to Ph.Eur. monograph 0062 and the relevant EU guidelines.

Control: the MSV was neutralised using WNV antiserum of avian origin, obtained with the WNV isolate and tested for extraneous agents testing, purity and sterility. The results were satisfactory.
The extensive testing of one Working Seed, instead of the Master Seed, was considered acceptable.

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Working Seed Virus:
The level of passages used in vaccine production is at the most initial MSV + 5 passages.
Details of the WSV virus controls, storage conditions, descriptions of the different phases of production of the active ingredients and exemples of preparation of four consecutives batches are provided and are satisfactory.

- **Vero cells**

Origin and history : cells are sourced from the kidney of a normal adult African green monkey (1962). The 113th passage was submitted to the ATCC repository.

Master Cell Bank (MCB): the Vero cell line was obtained by Fort Dodge at the 127th passage and MCB was established from 2 additional passages. It is stored in liquid nitrogen.
Controls: MCB was tested for absence of cytopathic (direct or after staining) and hemadsorbent agents, karyology, tumerogenicity and identity. The results were satisfactory.
The extensive testing of one Working Seed, instead of the Master Seed, was considered acceptable.

***Working Cell Bank (WCB)* :**
The range of passages allowed for production of virus goes up to MCB+20.
The test results of one WCB for identity, sterility, purity and extraneous agents were satisfactory.

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

The following raw materials of biological origin are involved in the manufacturing process: bovine transferring, foetal bovine serum and lactalbumin hydrolysate.

The starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.1) and Commission Directive 1999/104/EEC.

Control tests during production

- on harvest material before inactivation: identity; titration.
- on harvest material after inactivation: inactivation; residual formaldehyde test, sterility (Eur. Ph. 2.6.1); potency/identity (sandwich ELISA technique).

Data of in-process control tests on 4 consecutive batches of antigen were provided in tabulated format and found to be satisfactory

Control tests on the finished product

On each batch:
- The methods used to control the WNV suspension syringe contents (visual appearance, volume, pH, and viscosity) are described and have been satisfactory validated. The specifications proposed are appropriate to control the quality of the WNV suspension syringes.

Potency and identity:

- Potency testing at batch release is conducted using an in vitro potency test method (antigen sandwich ELISA assay). Two reports detailing the qualification of the working reference, were described.. The quantitative composition is expressed in Relative Potency with regard to a reference vaccine.
  As this test is using a monoclonal antibody, it serves also to check the identity of the virus.
- specifications : $1.3 \leq RP \leq 2.2$ at release ; $1.0 \leq RP \leq 2.2$ during the shelf life of the product.

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The assay developed is immunologically relevant, specific, reproducible, and dose dependent with a linear range up to a maximum RP of 2.2. The assay was found to have an acceptable level of intermediate precision and to detect subpotent batches. Thus, this assay is acceptable for use as a potency test for the serial release of Duvaxyn WNV batches.

Identification and assay of adjuvants on each batch:
- specifications : 4.0 - 5.5 % calculated total oils

Identification and assay of excipient constituents on each batch:

- residual formaldehyde : test based on the reaction of formaldehyde with methylbenzothiazolone hydrazone hydrochloride and reading using a spectrophotometer (purified water used as a blank).
- specifications : ≤ 0.74 g/l

Safety

On each batch:
Two horses of at least 6 months of age, not previously vaccinated against West Nile disease, are vaccinated with 2 ml of vaccine and observed for 14 days. Rectal temperature is taken daily for 3 days prior to vaccination, until 14 days after vaccination.
specifications: no adverse reaction attributable to the vaccine.

Sterility and Purity

On each bulk blend and each filling lot :
Eur. Ph. sterility test 2.6.1
specifications: no growth of bacteria and/or fungi detected.

Batch to batch consistency

Batch release control tests on 3 consecutive batches of finished product from the two production sites were provided in a tabulated format.

Stability

Three batches were tested for stability, to show compliance with the acceptance criteria on finished product as described in part E.. Following tests were done at T0, T6, T7, T10, T18, T24 and T27:
- relative potency testing
- appearance, pH and viscosity
- free formaldehyde quantity (done only at T6)

The results of the stability studies supported a shelf life of 24 months at the end of the procedure.

In use stability

Not applicable, as the vaccine is presented in single-dose syringes.

Overall conclusions on quality

Overall the quality part of the dossier was satisfactory.

There was one particular aspect, linked to the reference vaccine, which was important to be resolved: reference vaccine is a keystone of the control of the finished product, and the methodology to show equivalence between old and new references batches needed to be significantly improved.

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As a result the Applicant provided detailed explanations on how the equivalency of batches of vaccine is determined by the *in-vitro* assay. Any biological system is inherently variable, especially horses or other livestock or non-laboratory species which have genetically and immunologically diverse backgrounds. Therefore, the host animal evaluation is used only to confirm that a new reference is sufficient to induce a response equal to or greater than the response induced by the original reference. The actual antigen input is determined using the *in vitro* potency assay and the serological assay is a confirmatory test.

Part 3 – Safety

Validation of Serum IgG ELISA for the Measurement of Antibody Response in Horses.
Please see also the Efficacy section.
In conclusion the capture IgG ELISA test used in the safety and efficacy studies to monitor the serological status of animals was validated. The method was shown to give a linear response and be reproducible. The intermediate precision of the test was found to be acceptable.

Laboratory tests

Examination of safety of the administration of one dose, of an overdose and of repeated administration of one dose (Safety Study in 6 Month Old Foals of Duvaxyn WNV)

A number of horses which were 6 months of age and seronegative to WNV at the beginning of the trial were divided into 3 groups. Group 1 was vaccinated intramuscularly (IM) with three 1 ml doses at three weeks apart, group 2 was vaccinated with 2 ml (2x doses) on D0, followed by another dose (3 weeks later) and group 3 served as a control group. All horses were monitored for any clinical signs and injection site reaction for 3 consecutive days prior to first injection, then once daily for 14 days after each injection. Horses were monitored for rectal temperature for 3 consecutive days prior to first injection, then on D0, D0+4h, and daily through 4 days following each injection (hyperthermia if T° > 39°C).
Overall only mild temperature increases lasting no more than 2 days were observed after vaccination with a vaccine with a maximum RP. A slight injection site reaction occurred in one horse for only 2 days following the third injection. Therefore, it is considered that the vaccine was well tolerated.

Examination of reproductive performance (Field Safety Study of Duvaxyn WNV Vaccine in Pregnant Mares)

A number of pregnant mares of 3 to 18 years of age, housed on 2 sites and seronegative to WNV at the beginning of the trials were divided into 2 groups. Group 1 was vaccinated IM with Duvaxyn WNV at 2 doses, 3-4 weeks apart and group 2 served as the control group. All reported adverse effects were mild and transient, considered as classical features of the administration of inactivated adjuvanted vaccines. Since mild pain and depression might occur, this was added in the SPC but since loss of appetite occurred on one site only in both vaccinates and controls, it was deemed not attributable to the vaccine itself, and thus not necessary to mention in the SPC.

Examination of immunological functions

There was no data submitted but this was found acceptable as there is no expected impact of this inactivated vaccine on the immunological functions.

Study of residues

No data were submitted for residues but this was found to be acceptable. In particular, Pluronic components (Poloxamers) are in Annex II of the Council regulation (EEC) 2377/90 (Reg. 2796/95); squalane is considered as “out of scope” with regard to MRL regulation (document EMEA/382454/2007-Rev.1).

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Interactions

The interaction of Duvaxyn WNV with other vaccines was not investigated. Therefore, an appropriate warning was put in the SPC.

Field studies

Report of field safety and efficacy Study of Fort Dodge Animal Health's Duvaxyn WNV.

A large group of horses in 3 sites where WNV is endemic and of variable months of age and seronegative to WNV at the beginning of the trial was divided into 2 subgroups at the beginning of the trial. Group 1 was vaccinated IM with Duvaxyn WNV at 2 doses, three to four weeks and group 2 served as controls.

Horses were monitored for rectal temperature and any clinical signs for 3 consecutive days prior to first injection, then on 0DPV1, 0DPV1+4h, 0DPV1+6h, 1-4DPV1, 7DPV1 and 14DPV1; for 3 consecutive days prior to second injection, then immediately prior to (0DPV2), then on 0DPV2+4h and 0DPV2+6h after second injection, then on 1-4DPV2, 7DPV2, 14DPV2, 22-30DPV2, 52-60DPV2, 82-90DPV2, 112-120DPV2, 142-150DPV2 and 172-180DPV2. Injection site examinations (scoring system) was undertaken at the same time than clinical observation. Serology was performed at -1DPV1 or 0DPV1, 7DPV1, 14DPV1, the day of the second injection (0DPV2), 7DPV2, 14DPV2, 22-30DPV2, 52-60DPV2, 82-90DPV2, 112-120DPV2, 142-150DPV2 and 172-180DPV2. WNV IgG ELISA testing to measure the antibody response after vaccination, and WNV IgM ELISA testing to evaluate any potential natural exposure to WNV was performed.

Results:

- the mean rectal temperature in both the vaccinated and control groups remained within the normal range. Two vaccinated horses had a febrile response immediately after the first injection, but no more later on. One other vaccinate showed hyperthermia from 3DPV1 to 5DPV1, and another vaccinate on 3DPV1 only. All these horses were otherwise normal. One control showed hyperthermia from 3DPV1 to 6DPV1 and 3 days before the second injection, probably due to an abscess. Another control showed hyperthermia 5 days after first injection and 2 days before the second injection, but otherwise normal.
- No change in appetite was observed in any of the groups.
- Depression was registered 15 times after day 0, involving 4 controls and 2 vaccinates. All except for one were located in the site of California.
- No clinical signs of neurological disease were observed.
- Twelve horses were observed with signs of strangles.
- Heat and swelling at the injection site was sometimes registered, but it remained always very moderate in size. Pain was not registered.

Antibody response against WNV measured by IgG ELISA showed that there was exposure to natural infection between 28DPV2 and 59DPV2 at California site and Iowa site (confirmed by positive IgM response in 7 horses at 59DPV2 at California site, and in 2 horses at 59DPV2 at Iowa site). No environmental exposure was seen at New Jersey site.

The use of Duvaxyn WNV vaccine did not cause any major adverse effects and it was judged satisfactory with regard to the safety aspect (please refer also to the efficacy section, for further comments about the efficacy aspect).

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User safety

No trial was deemed necessary to document user safety. As the vaccine is inactivated and administered by injection, only self-injections could be harmful for users. Section 4.5 of SPC already covers this point, by indicating that medical advice should be sought after self-injection, ingestion or spillage over the skin.

Environmental risk assessment

1 - Hazard identification

Laboratory safety and field trial studies have demonstrated that Duvaxyn WNV, an inactivated vaccine administered intramuscularly, is safe when administered to horses. In addition to being inactivated, the route of administration significantly limits the possibility of vaccine components being expelled into the environment.

The antigen is inactivated by the addition of formaldehyde, which is neutralized by sodium metabisulfite. Prior to product release, the residual formaldehyde is assayed to confirm the amount of free formaldehyde is $\leq$ 0.05%. Proof of inactivation testing is also conducted. The inactivated virus has been shown to be safe in the target species and to have no adverse effect on the environment.

The adjuvant system is SP oil, specifically containing squalane, pluronic L121 and polysorbate 80. This is a homogenised emulsion of metabolisable oil adjuvant. The squalane used is of shark origin and EP grade (EP 1630). It is not considered to be of any risk to public nor to the environment. The pluronic L121 is a poloxamer and is included in Annex II of Council regulation (EEC) No. 2377/90 (Reg. 2796/95). Similarly, polysorbate 80 is also included in Annex II of Council Regulation (EEC) No. 2377/90, and therefore both pluronic L121 and polysorbate 80 are not considered a risk to the public health or the environment.

2 - Assessment of likelihood

The probability and frequency of any hazard being manifested in the environment as a result of administration of this vaccine under normal conditions of use is negligible for the following reasons:

a. Each batch of the vaccine is proven to be inactivated by a validated test method.
b. The vaccine is categorised as a prescription only medicine and should be administered by a veterinarian or under his/her direct supervision.
c. The single dose of the vaccine makes safe use of the product easily achievable.
d. The product is recommended to be administered on a very low frequency basis per horse (two shots primarily, and then a booster every year).
e. It is recommended in the package insert that any unused product or containers are disposed of in accordance with local requirements, and therefore, are not expected to be disseminated into the environment.

3 - Assessment of the consequence of a hazard occurring

The hazards arising from use of this product are considered negligible. Similarly, the consequence of a hazard occurring with this inactivated vaccine is considered negligible.

4 - Assessment of level of risk

Since the risk from use of this product under normal conditions is considered negligible, the level of risk to the environment is considered as effectively zero.

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Overall conclusion on safety

Safety of the administration of one dose, of an overdose and of the repeated administration of one dose of Duvaxyn WNV was demonstrated on seronegative horses of 6 months of age. Safety was shown also when Duvaxyn WNV is administered to seronegative pregnant mares and on seronegative horses under field conditions. As this vaccine is inactivated, interference with antibodies (of maternal origin or present because of previous vaccinations) is not expected.
No negative impact on public health or on environment can be identified.
Therefore, Duvaxyn WNV can be considered as safe when administered in compliance with the SPC.

Part 4 – Efficacy

West Nile Virus (WNV) is an RNA virus of the genus *Flavivirus*, in the family *Flaviridae*. It is a neuropathogenic virus causing disease in birds, horses and humans. The agent circulates in nature through continuous enzootic transmission cycles between *Culicinae* mosquitoes and avian vertebrate hosts, and may be introduced into a new territory by migratory birds. Humans and large mammals are considered incidental and dead-end hosts.

Equidae appear to develop clinical signs of WNV infection, including fatal encephalitis, more readily than do other domestic mammals, with the most common clinical signs in equidae being weakness, incoordination, and ataxia. However, not all infected horses will develop clinical symptoms: only a maximum of 10% of horses infected with WNV will exhibit neurological symptoms, with a fatality rate in case of clinical disease of 25-40%. As the outbreak of clinical signs on horses is infrequent, a challenge model in laboratory conditions cannot be developed. It is also very difficult to demonstrate efficacy under laboratory conditions, as the number of horses to be included in a challenge trial would be very high.

Horses are considered as playing no role in the epidemiological cycle. Unlike WNV in various bird species, the virus does not amplify sufficiently in infected horses to infect mosquitoes and allow spread to other susceptible hosts : whereas viraemia levels in birds are in general $10^{2.2}$ to $10^{7.2}$ PFU/mL, the highest viraemia level found to date in horses was approximately $10^{2.7}$ PFU/ml. Species that had viraemia levels of $<10^5$ PFU/mL are considered to be non-infectious for a least two enzootic vectors, *Culex pipiens* and *Culex quinquefasciatus*, and hence deemed incompetent hosts. As a matter of fact, all studies involving mosquitoes that fed upon viremic horses showed that the mosquitoes were negative for the virus.

Thus, it is generally admitted that mammals infected with WNV generally develop low levels of viraemia and are incapable of sustaining a transmission cycle in nature.

Therefore, the indication of a vaccine intended for horses is limited to a reduction/absence of clinical signs. However, viraemia in such circumstances can be considered as an acceptable alternative, not in order to break down the epidemiological cycle, but because no clinical signs are expected if no, or low, viraemia occurs.

Validation of the capture IgG ELISA test

Validation of Serum IgG ELISA for the Measurement of Antibody Response in Horses.

A capture IgG ELISA was developed as an alternative to the plaque reduction neutralisation test (PRNT, highly variable). This study evaluates the linearity and reproducibility of an ELISA for the measurement of IgG antibody response to WNV. A positive control serum was generated by pooling serum samples collected from five non-vaccinated horses challenged with WNV, 21 days post-challenge; negative control serum was commercial horse serum. Additionally a positive WNV antigen was supernatant harvested from a COS-1 cell line which expresses and secretes E proteins of WNV-like particle; negative antigen was supernatant harvested from normal COS-7 cells passaged and maintained in similar conditions than the COS-1 cell line.

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The positive and negative control sera were serially diluted 1:2 from 1:25 to 1:51 200 and tested for IgG using a capture ELISA, to establish the OD linear portion. Once established, the intermediate precision (3 assays, 2 operators) in this linear range was evaluated. Linearity of the technique was checked by a linear regression of the OD values versus the logarithmic values of the dilution factors. Session and operator effects were estimated using analysis of variance.

The capture IgG ELISA test used in the safety and efficacy studies to monitor serological status of animals was validated. The method was shown to give a linear response and be reproducible. The intermediate precision of the test was found to be acceptable.

Evaluation of West Nile Virus stock Antigen Using Western Blot

Prior to the bioreactor produced West Nile antigen, roller bottle produced antigen was used to blend batches used in the twelve-month duration of immunity study described above, an onset of immunity study, and a twelve-month booster vaccination study, a comparison study needs to be done to establish bioequivalence between both production system. Three WNV antigen stocks were produced through the bioreactor process, and one through the roller bottle process. A comparison study was performed on them by Western Blot. The resultant protein bands were transferred to 2 nitrocellulose membranes, each being incubated with one primary antibody and revealed with the secondary corresponding antibody to visualize antigen-antibody reaction.
Primary antibodies used in the Western blot were either WNV monoclonal antibody, or pooled polyclonal WNV antiserum derived from non-vaccinated and WNV challenged horses at 21 days post-challenge. Secondary antibodies were phosphatase labelled goat anti-mouse IgG for the WNV monoclonal antibody, or phosphatase labelled goat anti-horse IgG for the equine sera.

A single band is detected by the monoclonal antibody, specific of the E protein. The banding pattern of all the antigen stocks detected by the polyclonal were similar. In conclusion roller bottles and bioreactor produced stocks are qualitatively equivalent.

Laboratory trials

Evaluation of the Onset of Immunity of Duvaxyn WNV

A group of horses aged 17-20 months and seronegative to WNV at the beginning of the trial was divided in 3 subgroups that were vaccinated IM with either *Fluvac Innovator Triple EFT + EHV* : experimental vaccine containing Influenza (Prague 56 and Kentucky 97), EHV1, EHV4, EEE, WEE, VEE and tetanus toxoid or *Fluvac Innovator Triple EFT + EHV + WNV* : experimental vaccine containing WNV (RP = 0.46), Influenza (Prague 56 and Kentucky 97), EHV1, EHV4, EEE, WEE, VEE and tetanus toxoid or acted as a control group.
Serology was performed at D0, D14, D21, D28, D35 and D42 – WNV IgG ELISA testing (2 testings).

The challenge was performed with WNV inoculum (North American avian isolate) by SC at D45.
Horses were then monitored for rectal temperature and any clinical signs for 3 consecutive days prior to challenge, twice daily for the first 14 days after challenge, and once daily thereafter until necropsy. Serum samples were collected on the day of challenge, twice daily until 14 days post-challenge, and weekly thereafter until 20 days post-challenge. 22 vaccinates and 10 controls were necropsied 21-22 days post-challenge: cerebrospinal fluid, spinal cord and brain tissue samples were examined for gross pathology and collected for virus isolation (in Vero cell cultures observed periodically for CPE and through indirect fluorescent antibody assay). Histopathological examination was also performed on formalin-fixed spinal cord and brain tissue samples.

The results showed that when compared with the titres obtained with a monovalent WNV batch (historical serological data), it appears that the other antigenic components may have a negative interference effect on the antibody response to WNV.
No WNV-associated neurological signs were observed in any of the challenged horses throughout the observation period. One control and two vaccinated horses had febrile response, (unlikely to be associated with WNV infection (no viraemia detected on the days around which febrile response

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occured)). 9/10 control horses and 8/20 vaccinates showed viraemia. For both groups, viraemia was transient and occurred only within the first six days after challenge. On necropsy no gross pathology was observed, except for one control animal (petechial hemorrhage in white matter and subdural hemorrage found in ther brain tissue; WNV was isolated from the brain, but not from CSF and spinal cord samples). No WNV was isolated from any of the tissue samples collected from other challenged animals.

The vaccine with a low antigen load induces a significant protection against viraemia induced by experimental WNV challenge. The onset of protection is approximately of 3 weeks after the second injection.
As the vaccine used was not Duvaxyn WNV, the results can only be considered as indicative (the vaccine used differs not only with regard to thiomersal, but also with regard to the antigens contained in the finished product, which moreover are suspected to have a negative immunological impact). Nevertheless, an onset of immunity 3 weeks after vaccination can be accepted, as this is the normal time span for an immune response to be induced, whatever the vaccine.

Evaluation of Twelve Month Duration of Immunity of Duvaxyn WNV

A group of 9-11 months old horses seronegative to WNV at the beginning of the trial was divided into 2 subgroups with 1 group vaccinated IM with 2 doses three weeks apart and the second group served as a control group. Serology was performed at D0, D14, D21, D28, D35, D42 and every 3 months thereafter until 12 months post second vaccination (D386) – WNV IgG ELISA testing.

The challenge was performed at day 386 with WNV inoculum (North American avian isolate) by subcutaneous route.

Horses were monitored for rectal temperature and any clinical signs for 2 consecutive days prior to challenge, twice daily for the first 14 days after challenge, and once daily thereafter until D21. Serum samples were collected on the day of challenge, twice daily until 14 days post-challenge, and weekly thereafter until 21 days post-challenge. Horses were necropsied 21-22 days post-challenge: cerebrospinal fluid, spinal cord and brain tissue samples were examined for gross pathology and collected for virus isolation (in Vero cell cultures observed periodically for CPE and through indirect fluorescent antibody assay). Histopathological examination was also performed on formalin-fixed spinal cord and brain tissue samples.

Antibody response against WNV was measured by IgG.

Neither WNV-associated neurological signs, nor febrile response, were observed in any of the challenged horses throughout the observation period. Nine out of 11 control horses and 1/19 vaccinates showed viraemia. For control group, viraemia occurred within the first six days after challenge, whereas for the vaccinated group, only 1 horse showed viraemia once, 2 days post-challenge. No gross pathology was observed; no WNV was isolated from any of the tissue samples collected from any of the challenged animals.

A significant protection during 12 months was induced against viraemia caused by experimental WNV challenge.

Experimental Challenge of Horses from a Field Safety and Efficacy Study with Duvaxyn WNV

A group of horses of 8-11 months of age and seronegative to WNV at the beginning of the trial were divided into 2 subgroups of which one group was vaccinated IM with Duvaxyn WNV at 2 doses, three weeks apart and the other group served as a control group.

Serology was performed at D0, D7, D14, D21, D28, D35, D49, D77, D105, D133, D168 and D196 – WNV IgG ELISA testing to measure the antibody response after vaccination, and WNV IgM ELISA testing to evaluate any potential natural exposure to WNV.

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The challenge was performed with WNV inoculum (North American avian isolate) by SC injection at D217.

Horses were monitored for rectal temperature and any clinical signs for 1 day prior to challenge, twice daily for the first 10 days after challenge. Serum samples were collected twice daily until 10 days post-challenge. Virus isolation is done on Vero cells (CPE and indirect fluorescent antibody assay).

All horses were tested negative for IgM response measured at D49 (28 DPV2) and D77 (56 DPV2).
No WNV-associated neurological signs were observed in any of the challenged horses throughout the observation period. One control had a febrile response 1 day after challenge, with viraemia detected 4 days after challenge. One vaccinate had a febrile response 4 days after challenge, but with neither viraemia nor neurological signs. 5/15 control horses and 0/13 vaccinates showed viraemia.

A significant protection was induced by Duvaxyn WNV vaccine against viraemia caused by experimental challenge at 28 weeks post vaccination.

Although this trial is considered a field trial by the applicant, it is similar to another laboratory trial as the challenge was not obtained by natural exposure, but done under laboratory conditions. This trial doesn't add more to the data already available: the number of horses involved in both groups is low and the number of viraemic animals in the control group is limited. Thus, the real level of protection cannot be precisely determined, even if reduction in viraemia after vaccination is confirmed.

Evaluation of Antibody Response Post Booster Vaccination in Horses Twelve Months after the Primary Two-Dose Vaccination with Duvaxyn WNV

A number of horses of 8-11 months of age, seronegative to WNV at the beginning of the trial was divided into 2 groups with group 1 being vaccinated IM with Duvaxyn WNV at 2 doses, three weeks apart, followed by a third injection 12 months after the second injection and group 2 serving as a control group.

Serology was performed weekly starting the day of first injection until 28 days post second injection, and then monthly until the 3rd injection. After the 3rd injection, serum samples were collected weekly until 28 days post 3rd injection, and monthly thereafter until 3 months post 3rd injection – WNV IgG ELISA testing.

Results obtained at D28 (7DPV2), D35 (14DPV2) and D393 (7DPV3) were compared to archive serum samples from the 12-month Duration-of-Immunity study.

The WNV antibody response following a booster vaccination at 12 months after the primary two-dose vaccination was significantly greater at D393 than the response at D35 of the DOI study. This induced response infers that horses given a booster vaccination 12 months after the primary vaccination will be protected for another 12 months. This trial presupposes that the dosed IgG antibody response to WNV is protective, and that the protection level is exceeded. However, it remains questionable whether a booster vaccination 12 months after the primary vaccination with a vaccine batch of RP = 1 will still protect horses for another 12 months.

As long as the relationship between IgG levels in horses and degree of protection is not established, kinetics comparison of IgG levels can only be considered as indicative, but not sufficient to validate the booster injection with a single dose. Indeed, it cannot be assumed that the total IgG antibody response, as tested through the WNV IgG ELISA test is relevant with regard to protection, as it is clearly explained that the humoral immunity responsible for early protection against West Nile infection can be attributable to the antibody binding to Domain III of the E glycoprotein of West Nile virus, whereas IgG production will be elicited towards a panel of various virus antigens.

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Moreover, the difference seen between the results obtained might be due to a different antigen load in the vaccine batches used. It was stated that the amount of antigen that an animal receives is not the critical factor in determining the magnitude of the antibody response, However, the argument implies that it is established that the magnitude of the antibody response is related to protection, whereas the above question deals precisely with the questionable relationship between the IgG amount and degree of protection, currently not considered as sufficiently linked.
- Secondly, low immunising doses trigger only high-affinity B cells, whereas high immunising doses trigger both high-affinity and low-affinity B cells. It is thus expected that antibody response is quantitatively improved when high immunising doses are used (because more B cells are involved to produce antibodies). To which extent magnitude of antibody response and protection are related is not considered solved.
- Finally, the additional argumentation to show that the amount of antigen in the booster dose is not a critical factor (post challenge antibody response in the DOI study), which is based on the serological response alone should be taken very cautiously, as immunisation with live viruses involves very different mechanisms compared to immunisation with inert antigens like inactivated viruses (MHC class I/II, CD4/8 T cells, interaction between T and B cells, memory cells preventing activation of naïve B and T cells, , …). These mechanisms are currently not completely understood, especially with regard to memory cells, their ability to be activated and the modes of activation.

However, considering that DUVAXYN WNV is a MUMS product, an appropriate warning in section 4.9 of the SPC was deemed sufficient to overcome this issue.

Field trials

Report of field Safety and efficacy Study of Fort Dodge Animal Health's Duvaxyn WNV as already described in Section "Safety – Field Studies".

This field trial was of limited value with regard to Efficacy, as the outcome of vaccination with regard to protection against WNV is not exploited (serological data alone, without correlation with protection).

However it was clarified that during the course of the field trials, 47% of the non-vaccinated control horses from the California site and 13% of the non-vaccinated control horses from the Iowa site developed a positive IgM response which is indicative of infection in these herds. No IgM responses were observed in any of the vaccinated animals. Since a sufficient level of infection that would result in an induction of an IgM response was not present in the vaccinated horses, one can infer that these horses were protected due to vaccination. This premise is supported by the outcome reported in another trial. The horses in the field trial that were housed at the New Jersey location were not exposed to West Nile virus during the study and were challenged 198 DPV2. All of the vaccinated animals were protected against the development of viraemia. Viraemia was able to be induced in several of the non-vaccinated animals indicating they were susceptible to infection. Since the horses at other 2 locations were vaccinated with the same vaccine and responded in an almost identical fashion following vaccination, the challenge of horses from the New Jersey site confirmed the benefit afforded to the horses at the other two sites.

Although none of the exposed horses at the California or Iowa sites developed neurological signs, this is not unexpected. West Nile infection in horses is not usually accompanied by presentation of clinical illness.

In addition to the data presented, the most convincing data that demonstrates the benefit of vaccination to horses in areas where WNV was endemic was provided by three other independent studies conducted in the US and Canada in 2002 and 2003 when West Nile disease was at its peak in North America.

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Since field studies evaluate the safety as well as efficacy, serials that are representative of normal batches are utilised for field studies. Efficacy was already demonstrated at the minimum antigenic dose in the laboratory trials. The field trial batches had RP's of 1.5 which is representative of normal batches. Since laboratory studies were done to assess efficacy at a minimum dose and safety at maximum dose, it would be considered unnecessary to repeat separate field studies with vaccines formulated at either the maximum or minimum antigenic content.

Serum samples from time point V2+86 were available but it was concluded that they were mishandled or mislabelled as described. This conclusion is based on the abnormal values obtained for 12 samples (6 abnormally high and 6 abnormally low). The average titre values, including the abnormal samples were reported in table 11CA but the data were included in table 10C where the individual animal responses are reported. The overall conclusion was that the data from this time point were not valid due to the mislabelling of the samples.

BIBLIOGRAPHICAL DATA

In addition to the controlled experimental studies described in this dossier, the efficacy of FDAH's West Nile-Innovator under field outbreaks was demonstrated in three independent studies conducted in the US and in Canada.

Salazar P., Traub-Dargatz JL., Morley PS., Wilmot DD., Steffen DJ., Cunningham WE., Salman MD. - Outcome of equids with clinicat signs of West Nile virus infection and factors associated with death – JAVMA, Vol.225, No. 2, July 15, 2004:267-274 :

Summary: Owners of 484 equidae with laboratory-confirmed West Nile virus infection in Nebraska and Colorado were contacted by telephone, and a questionnaire was used to obtain information on signalment, management, clinical signs, date of disease onset, duration of disease, WNV vaccination status, and health status at the time of the interview.
137 of 482 (28.4%) animals died or were euthanased. Ataxia, lethargy, muscle fasciculations, and weakness were the most common clinical signs of disease. Animals > 3 years old were more likely to die than were animals ≤ 2 years old. Unvaccinated equidae were twice as likely to die as were animals that had been vaccinated at least once prior to the onset of disease. Animals that were recumbent and unable to rise were 78 times as likely to die as were animals that never lost the ability to rise. Females were 2.9 times as likely to die as males. Two hundred seventy-one of 339 (79.9%) animals that survived recovered fully; mean duration of disease for these animals was 22.3 days.
Conclusion: Among equidae with WNV infection, age, vaccination status, an inability to rise, and sex were associated with the risk of death.

Schuler LA., Khaitsa ML., Dyer NW, Stoltenow CL. - Evaluation of an outbreak of West Nile virus infection in horses: 560 cases (2002) - JAVMA, Vol.225, No. 2, July 15,2004;225:1084-1089:

Summary: Data were obtained from veterinary laboratory records, and a questionnaire was mailed to veterinarians of affected horses (retrospective study).
Affected horses were defined as horses with typical clinical signs and seroconversion or positive results of virus isolation; affected horses were detected in 52 of the 53 counties and concentrated in the eastern and northeastern regions of the state. Among affected horses, 27% (n = 152) were vaccinated against WNV, 54% (309) were not, and 19% (108) had unknown vaccination status; 61 % (345) recovered, 22% (126) died, and 17% (98) had unknown outcome. The odds of death among non-vaccinated horses were 3 and 16 times the odds among horses that received only 1 or 2 doses of vaccine and horses that were vaccinated according to manufacturer's recommendations, respectively. Horses with recumbency, caudal paresis, and age > 5 years had higher odds of death, whereas horses with lack of co-ordination had lower odds of death, compared with affected horses without these characteristics.

Conclusion: Vaccination appears to have beneficial effects regarding infection and death caused by WNV.

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Epp TY., Waldner C., West K., Leighton FA., HGG. Townsend - Efficacy of Vaccination for West Nile Virus in Saskatchewan Horses – Medicine II, 2005, vol.51, AAEP Proceedings:180-182:

Summary: In a case-control study involving 875 horses, 527 were vaccinated, and 348 were not vaccinated. Of the vaccinated horses, nine were symptomatic; of the non-vaccinated horses, 121 were symptomatic. The crude odds of disease in unvaccinated horses were 31 times greater (95% CI - 15-61) than the odds of disease in vaccinated horses. This equates to a crude estimate of vaccine efficacy (attributable fraction) of 96.7% (95% CI = 94-98%). The odds of disease on farms that did not vaccinate any animals were more than 85 times greater than the odds of disease on farms that vaccinated all animals. Additionally, after controlling for the effect of all confounding variables at the level of the individual horse, the odds of disease in horses that were not vaccinated were more than 20 times greater than they were among horses that were vaccinated.

Conclusion: Preliminary analysis of studies conducted during the 2003 outbreak of West Nile Virus in Saskatchewan showed that vaccination was strongly associated with the prevention of clinical disease at both the individual horse and the farm level.

Overall Conclusion on bibliographic data: These publications strongly support the fact that vaccination with a vaccine named "West Nile-Innovator" reduces clinical disease in endemic areas. The significance of these data are however somewhat weakened with regard to Duvaxyn WNV, as the vaccine used is a multivalent one. Nevertheless, as West Nile disease is listed in the "Guideline on Data Requirements for Immunological Veterinary Medicinal Product Intended for minor Use or minor Species/Limited Market", and as it is very difficult to demonstrate efficacy because of the pathogenesis and epizootiology of this disease, these publications were considered as supportive data to demonstrate efficacy of Duvaxyn WNV.

Besides, the representativeness of the wild WNV which was circulating in these areas with regard to the current/recent European strains should be established, and the potential differences discussed with regard to efficacy in Europe. Phylogenetic studies on a 255-bp region of the E glycoprotein gene (genome position 1402-1656) have shown the existence of two main lineages that diverge by up to about 30% in nucleotide sequence. Lineage I includes WN strains from Africa, Europe, the Middle East, North America, India, and Australia. Lineage II comprises WN strains only from sub-Saharan Africa and Madagascar. Despite the fact that the European strains and the US strains belong to the same lineage, the antigenic differences amongst them might still be sufficient to hamper efficacy of the vaccine in Europe.

Overall Conclusion on Part IV

Considering that:

- West Nile disease is listed in the "Guideline on Data Requirements for Immunological Veterinary Medicinal Product Intended for minor Use or minor Species/Limited Market",
- horses are considered dead-end hosts, and they are not part of the WNV epidemiological cycle,
- clinical symptoms in horses are rare, but severe once present,
- WNV challenge models in laboratory conditions cannot be developed,
- efficacy under field conditions is very difficult to demonstrate, as the number of horses to be included needs to be very high,
- usefulness of a WNV vaccine in horses is to reduce/prevent clinical signs,
- absence of viraemia implies no clinical signs,
- all laboratory trials showed a reduction of viraemia in horses after vaccination,
- epidemiological field surveys strongly support the fact that vaccination reduces clinical disease in endemic areas (although another vaccine than Duvaxyn WNV was involved in these surveys),

it can be considered that the current data is sufficient to demonstrate efficacy of Duvaxyn WNV with an indication of reduction of viraemia.

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V. BENEFIT-RISK BALANCE

Safety of the administration of one dose, of an overdose and of the repeated administration of one dose of Duvaxyn WNV was demonstrated on seronegative horses of 6 months of age. Safety was shown also when Duvaxyn WNV was administered to seronegative pregnant mares and on seronegative horses under field conditions. As this vaccine is inactivated, and to be administered to animals of at least 6 months of age, interference with antibodies (of maternal origin or present because of previous vaccinations) is not expected.

As this vaccine is inactivated, and to be administered by deep intramuscular injection, no negative impact on public health or on environment is expected either.

Thus, Duvaxyn WNV can be considered as safe when administered in compliance with the SPC claims.

- With regard to Efficacy, considering that:

- West Nile disease is listed in the "Guideline on Data Requirements for Immunological Veterinary Medicinal Product Intended for minor Use or minor Species/Limited Market",
- horses are considered dead-end hosts, and they are not part of the WNV epidemiological cycle,
- clinical symptoms in horses are rare, but severe once present,
- WNV challenge models in laboratory conditions are not really representative of the disease occuring in the field,
- efficacy under field conditions is very difficult to demonstrate, as the number of horses to be included needs to be very high,
- usefulness of a WNV vaccine in horses is to reduce/prevent clinical signs,
- absence of viraemia implies no clinical signs,
- all laboratory trials showed a significant reduction of viraemia in horses after vaccination,
- epidemiological field surveys strongly support the fact that vaccination reduces clinical disease in endemic areas,

it can be considered that the current data are sufficient to demonstrate efficacy of Duvaxyn WNV when administered in compliance with the SPC claims.

Thus, the risk-benefit balance is in favour of the use of the inactivated vaccine Duvaxyn WNV. West Nile disease can become a potential threat to horses in some European regions, with only supportive chemical treatment once clinical signs appear. Prevention against West Nile disease through vaccination will greatly reduce the number of clinical outbreaks (by significantly reducing viraemia in horses), and thus reducing mortality and neurological sequelae, whilst the vaccine remains safe when used in compliance with the SPC. No environmental impact is expected as the product is inactivated and administered by individual intramuscular injections.

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 Duvaxyn WNV were considered to be in accordance with the requirements of Council Directive 2001/82/EC, as amended, and that the benefit-risk balance was favourable.

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