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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for type II variation for Equip WNV (EMEA/V/C/000137/II/0012)

Common name: Inactivated West Nile virus, strain VM-2

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

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1. Background information on the variation

1.1. Submission of the variation application

In accordance with Article 16 of Commission Regulation (EC) No. 1234/2008, the marketing authorisation holder, Zoetis Belgium SA (former name Pfizer Limited) (the applicant), submitted to the European Medicines Agency (the Agency) on 8 April 2013 an application for a type II variation for Equip WNV.

The CVMP agreed that the data requirements specified in the appropriate CVMP guidelines on "Minor-Use-Minor-Species" (MUMS) are applicable when assessing the application.

The rapporteur appointed was J-C. Rouby.

1.2. Scope of the variation

The scope of the variation was to add an indication against West Nile virus lineage 2 strains.

Current	Proposed
SPC/Labelling/Package leaflet Section 4.2./Section 6/Section 4 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.	**SPC/Labelling/Package leaflet** Section 4.2./Section 6/Section 4 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. **Prevention of mortality and viraemia and reduction of duration and severity of clinical signs has been demonstrated following WNV (lineage 2) challenge.**

2. Scientific discussion

2.1. Assessment

The vaccine contains a West Nile virus (WNV) lineage 1 strain. In the original dossier (marketing authorisation was granted in 2008) the efficacy was demonstrated against the WNV lineage 1 strains.

Outbreaks in Europe were initially caused by lineage 1 strains but WNV lineage 2 strains were also responsible for clinical disease identified in Europe in 2011–2012. Therefore, the efficacy against lineage 2 was tested to extend the claim of Equip WNV to a protection against lineage 2 strains.

The CVMP noted that evidence of implication of lineage 2 in recent outbreaks was confirmed by literature (i.e. Savini et al., 2012[1]).

The applicant proposed to modify the indication as it stated in the table of section 1.2 above. To support the proposed claim, the applicant provided results of an efficacy trial using Equip WNV against a WNV lineage 2 strain.

Study design. Twenty crossbred, mixed sex horses aged 15 months and sero-negative to WNV lineages 1 and 2 were used in the blinded trial (10 vaccinated animals and 10 controls). An Equip WNV batch with

[1] Savini G, Capelli G, Monaco F, Polci A, Russo F, Di Gennaro A, Marini V, Teodori L, Montarsi F, Pinoni C, Pisciella M, Terregino C, Marangon S, Capua I, Lelli R. Evidence of WNV lineage 2 circulation in Northern Italy. Veterinary Microbiology, 2012, Vol. 158(3-4), P. 267–273.

standard formulation (RP = 1.96/dose) was used for vaccination intramuscularly: 1 dose of 1 mL on day 0 and 1 dose of 1 mL on day 21. Control horses were not vaccinated. On day 42, all horses were challenged by the intrathecal route, using $1.1x10^5$ PFU/horse.

Challenge model.

A challenge strain of lineage 2 (Isolate Nea Santa 2010 from a human blood sample from Greece, 7 passages in Vero cells) was used.

Clinical monitoring.

The animals were monitored on the basis of primary and secondary parameters as follows:

Primary efficacy parameters:

- Death / euthanasia (mortality).
- Clinical observation (temperature and clinical signs).
- Viraemia (isolation by plaque assay).

Secondary efficacy parameters:

- Serological response every week against lineage 2 WNV from day 0 (1st vaccination) until day 63 (21 days after challenge) by plaque reduction neutralization test (PRNT).
- Histopathology: record at post-mortem of the lesions of the cervical spinal cord, medulla and pons; scoring.
- Examination for gross lesion at necropsy.

Statistical analysis was based on death/euthanasia (2-tailed p-value), clinical signs (on frequency of distribution and percentage of day with clinical observation), rectal temperature, virus isolation and serological titre.

Study results

Primary efficacy parameters:

- Significant reduction in the number of euthanised horses (6/10 controls, vs 0/10 vaccinated animals, p=0.01); the horses were euthanised 9 to 19 days after challenge.
- Significant reduction in the frequency and duration of clinical signs (anxiety-depression-aggression, muscle fasciculation-head tremor, mild paresis) of vaccinated animals when compared to controls.
- Significant reduction of hyperthermia in the vaccinated animals when compared to controls observed between 8 and 12 days after challenge.
- Significant reduction of the virus isolation in vaccinated animals when compared to controls for 4 days after challenge. Viraemia was detected only once in a single vaccinated animal (15 PFU/mL), vs several isolation in each of 8 out of the 10 controls.

Secondary efficacy parameters:

- Lineages 1 and 2 strains: all horses were confirmed sero-negative for both strains before vaccination.
- Lineage 2 strain: There was a detectable serological response in vaccinated animals from 14 days after the 1st injection, with a booster effect of the 2nd injection and a plateau after challenge. The controls seroconverted after challenge.

- Histopathology: the scores of the lesions recorded in the vaccinated animals ranged between 0 or 1, whereas the controls displayed more severe lesions and their lesions were scored between 2 and 5.
- No gross lesion detected, either in vaccinated animals, or in controls.

Literature was also provided to support the statements on the West Nile disease in horses (epidemiology, clinical signs, etc.)(one source), to justify the intrathecal challenge model (2 sources) and to support the methods for serology and virology assays (2 sources).

The CVMP noted that the literature was only informative. **Assessment and conclusions**

Study design. The specification for batch potency is RP = 1.0–2.2; therefore, the batch chosen was close to the maximum potency and not one of minimum potency as expected in such efficacy trials. The applicant justified the use of a standard batch.

Moreover the horses were not of the minimum age recommended for vaccination. However, efficacy in horses of the minimum age was demonstrated in studies presented in the initial dossier, and use of older horses in this new study was considered acceptable. The SPC contains a warning with regard to possible interference of maternally derived antibodies for correct use of the vaccine.

Challenge model. Justification was provided as to how a claim regarding prevention/reduction of viraemia (relevant after a natural infection) could be made given that the intrathecal route which was chosen is not a natural route for challenge, because the virus is directly injected into the central nervous system. Although different challenge models (mosquito feeding and needle inoculation) are available to study the efficacy of WNV vaccines and have been successfully used in the past (investigation of viraemia) neither of these models is capable of inducing significant clinical signs of WNV in horses (Seino[2] et al., 2007). By contrast, natural infection, specifically with lineage 2 strains is known to induce severe and sometimes fatal signs in horses. Therefore the applicant sought a model that, although not the natural route of infection, was capable of inducing the type of clinical signs seen in the field.

Furthermore several groups have reported viraemia in 70% to 100% of non-vaccinated horses post intrathecal challenge (Long[3] et al., 2007; Minke[4] et al., 2011; Seino[1] et al., 2007) which suggested that viraemia is characteristic for this model as well. In the current study 80% of the non-vaccinated horses showed viraemia within 5 days post challenge. This transient character and time-frame are similar to the 4–6 day time-frame observed with the subcutaneous challenge model and following the use of a mosquito challenge model (Davis[5] et al., 2001). The highest virus titre detected in the current intrathecal challenge study was 700 PFU/mL while the highest titre detected in the mosquito challenge model was approximately 250 PFU/mL. This indicated that the intrathecal challenge model could result into more severe viraemia than a more natural model of infection and thus was considered acceptable.

Viraemia. It was noted that, viraemia was detected in one vaccinate which did not allow a claim for prevention. On day 46 (4th day post challenge) one of the vaccinated horses had positive virus isolation in the morning. However, the virus titre was considered to be very low (15 PFU/mL) and the blood sample collected during the afternoon of the same day (10 hours later) was found to be negative. Therefore, this single point of virus isolation was not considered as true viraemia. Nonetheless, the applicant accepted

[2] Seino KK, Long MT, Gibbs EP, Bowen RA, Beachboard SE, Humphrey PP, Dixon MA, Bourgeois MA. Comparative efficacies of three commercially available vaccines against West Nile virus (WNV) in a short-duration challenge trial involving an equine WNV encephalitis model. Clin Vaccine Immunol. 2007 Nov; 14(11): 1465–1471.
[3] Long MT, Gibbs EP, Mellencamp MW, Bowen RA, Seino KK, Zhang S, Beachboard SE, Humphrey PP. Efficacy, duration, and onset of immunogenicity of a West Nile virus vaccine, live Flavivirus chimera, in horses with a clinical disease challenge model. Equine Vet J. 2007 Nov; 39(6): 491-497.
[4] Minke JM, Siger L, Cupillard L, Powers B, Bakonyi T, Boyum S, Nowotny N, Bowen R. Protection provided by a recombinant ALVAC(®)-WNV vaccine expressing the prM/E genes of a lineage 1 strain of WNV against a virulent challenge with a lineage 2 strain. Vaccine 2011 Jun 20; 29(28): 4608-4612.
[5] Davis BS, Chang GJ, Cropp B, Roehrig JT, Martin DA, Mitchell CJ, Bowen R, Bunning ML. West Nile virus recombinant DNA vaccine protects mouse and horse from virus challenge and expresses in vitro a noninfectious recombinant antigen that can be used in enzyme-linked immunosorbent assays. J Virol. 2001 May; 75(9): 4040-4047.

that these data do not fully prove prevention of the virus entering the blood and that therefore a claim of "reduction of viraemia" in this case would be appropriate.

Mortality. Humane end-points were defined by Colorado State University, in agreement with the IACUC (Institutional Animal Care and User Committee) group taking into account clinical signs. The CVMP acknowledged the criteria for euthanasia and the blinding of the study.

It was clarified that the mortality claim was considered to be relevant by the applicant because WNV lineage 2 has been indicated to cause fatal neurologic disease in horses (Venter[6] et al., 2009) and the model used was designed to try and mimic this type of disease course. Also from the established Clinical Scoring system it could be anticipated that, without treatment, the majority of euthanised horses would likely have progressed to a moribund status very shortly after withdrawal. Considering animal welfare the applicant felt that it was not justified to have allowed the horses enter a moribund state.

The CVMP agreed that West Nile Virus lineage 2 can cause fatal neurologic disease (as confirmed by the article of Venter[5] et al., 2009). However the CVMP still questioned that vaccination can prevent/reduce mortality. The CVMP acknowledged that the intrathecal challenge induces clinical signs such that euthanasia may be necessary for animal welfare reason. However, it also recognises that due to euthanasia it becomes impossible to assess and claim any effect on mortality that may be caused by the challenge.

Therefore, the CVMP considered that the claim on mortality could not be acceptable.

Onset of immunity against West Nile Virus lineage 2 strains. Regarding the onset of immunity, the current claim was not questioned as the challenge of the present trial was carried out at the time of onset of immunity (3 weeks after the 2nd injection of the primary course).

Duration of immunity (DOI) against West Nile Virus lineage 2 strains. The CVMP agreed that the claim of DOI for 12 months was not supported for a lineage 2 strain challenge. As this was a heterologous challenge (the vaccine contains a lineage 1 virus), the duration of cross-protection to a lineage 2 challenge of a vaccine containing a lineage 1 virus may be reduced compared to what has been established for lineage 1 challenges. In such a case, the DOI has to be split into 1 year for lineage 1, not established for lineage 2.

On the basis of all of the information presented the CVMP accepted the indication:

"For active immunisation of horses of 6 months of age or older against West Nile virus disease to reduce the number of viraemic horses after infection with WNV lineage 1 or 2 strains and to reduce duration and severity of clinical signs against WNV of lineage 2 strains.

Onset of immunity: 3 weeks after the primary vaccination course.

Duration of immunity: 12 months after primary vaccination course for WNV lineage 1 strains. For WNV lineage 2 strains the duration of immunity has not been established."

2.2. Summary and conclusions

The efficacy against a WNV lineage 2 challenge was clearly established in the data provided in support of this variation (reduction of viraemia, severity and duration of clinical signs). However, this demonstration of efficacy by an intrathecal challenge inducing a disease was not consistent with the existing dossier, where the efficacy against lineage 1 virus was established by demonstrating the ability of the vaccine to reduce the number of viraemic horses after a subcutaneous challenge. Therefore, it was not possible to

[6] Venter M, Human S, Zaayman D, Gerdes GH, Williams J, Steyl J, Leman PA, Paweska JT, Setzkorn H, Rous G, Murray S, Parker R, Donnellan C, Swanepoel R. Lineage 2 West Nile virus as cause of fatal neurologic disease in horses, South Africa. Emerg Infect Dis. 2009 June; 15(6): 877–884.

correlate the existing efficacy data for lineage 1 with the new efficacy trial for lineage 2.

The CVMP accepted the claim against WNV lineage 2 strains and taking into account the presented data the CVMP proposed the following modification in the SPC:

"4.2 Indications for use, specifying the target species

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 after infection with WNV lineage 1 or 2 strains and to reduce duration and severity of clinical signs against WNV of lineage 2 strains.

Onset of immunity: 3 weeks after primary vaccination course.

Duration of immunity: 12 months after primary vaccination course for WNV lineage 1 strains. For WNV lineage 2 strains the duration of immunity has not been established."

3. Benefit-risk assessment

The scope of the following benefit-risk assessment for Equip WNV relates to the addition of a new indication regarding the efficacy of the vaccine against a WNV lineage 2 strain infection.

3.1. Benefit assessment

Direct therapeutic benefit:

The benefit of Equip WNV following this variation is the production of sufficient immunity after active immunisation of horses against WNV to reduce the number of viraemic horses after infection with WNV lineage 1 or 2 strains and to reduce duration and severity of clinical signs against WNV lineage 2 strains.

A controlled laboratory trial demonstrated that the product is efficacious against a WNV lineage 2 strain.

Additional benefit:

As a consequence of the reduction of viraemic horses due to the lineage 2 strains, the incidence of clinical outbreaks due to these strains is reduced.

3.2. Risk assessment

This variation does not modify the existing properties of the vaccine and therefore no additional risk to the user, the environment or the consumer is expected than those stated in the assessment of the original dossier.

As the demonstration of the efficacy against lineage 2 differs from what was shown for lineage 1, appropriate wording has been included in the SPC to inform of the differences concerning duration and severity of clinical signs and duration of immunity in the target animals.

3.3. Evaluation of the benefit-risk balance

The product has been shown to be efficacious for the amended indication to reduce the number of viraemic horses after infection with WNV lineage 1 or 2 strains and to reduce duration and severity of clinical signs against WNV lineage 2 strains.

Appropriate wording has been included in the product literature.

No change to the impact on the environment is envisaged.

The benefit-risk balance remains unchanged.

4. Overall conclusions of the evaluation and recommendations

The CVMP considers that this variation, accompanied by the submitted documentation which demonstrates that the conditions laid down in Commission Regulation (EC) No. 1234/2008 for the requested variation are met, is approvable.

It is also recommended to update the product information in accordance with the proposed text in the section 2.2.

4.1. Changes to the community marketing authorisation

Changes are required in the following annexes of the Community marketing authorisation:

- I, IIIA and IIIB.

On 11 October 2013, the European Commission adopted a Commission Decision for this application.