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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for type II variation for ProteqFlu-Te (EMEA/V/C/000074/II/0017)

Common name: equine influenza and tetanus vaccine

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, Merial (the applicant), submitted to the European Medicines Agency (the Agency) an application for a type II variation for ProteqFlu-Te.

The rapporteur was J.-C. Rouby and the co-rapporteur was E. Werner.

1.1.1. Scope of the variation

The type II variation No. C.II.5 is to substitute the strain vCP1533 by the strain vCP3011 in compliance with the OIE Expert Surveillance Panel on equine influenza vaccine composition.

Current	Proposed
Current vaccine composition: One dose contains: Active ingredients: Influenza A/equi-2/0hio/03 [H3N8] recombinant Canarypox virus (vCP2242) ≥ 5.3 log10 FAID50* Influenza A/equi-2/Newmarket/2/93 [H3N8] recombinant Canarypox virus (vCP1533) ≥ 5.3 log10 FAID50* * Fluorescent assay infectious dose 50 % *Clostridium tetani* toxoid: ≥ 30 IU** ** Antitoxic antibody titre induced after repeated vaccination in guinea pig sera according to Ph. Eur. Adjuvant: Carbomer 4 mg.	New vaccine composition: One dose contains: Active ingredients: Influenza A/eq/Ohio/03 [H_3N_8] recombinant canarypox virus (vCP2242) ≥ 5.3 log_{10} $FAID_{50}$* Influenza A/eq/Richmond/1/07 [H_3N_8] recombinant canarypox virus (vCP3011) ≥ 5.3 log_{10} $FAID_{50}$* * Fluorescent assay infectious dose 50% *Clostridium tetani* toxoid: ≥ 30 IU** ** Antitoxic antibody titre induced after repeated vaccination in guinea pig sera according to Ph. Eur. Adjuvant: Carbomer 4 mg.

2. Scientific discussion

2.1. Assessment

The development of this new formulation has been based on the current CVMP Note for guidance on harmonisation of requirements for equine influenza vaccines, specific requirements for substitution or addition of a strain or strains (EMA/CVMP/112/98-FINAL). No other requirements are currently available.

To be noted that the vaccine containing vCP3011, vCP2242 and *Clostridium tetani* toxoid will be called "PHN3172 vaccine" in this report, to allow differentiation with the already authorised product ProteqFlu-Te containing vCP1533, vCP2242 and *Clostridium tetani* toxoid. Once this variation approved (substitution of the vCP1533 strain by the vCP3011 strain), the vaccine will be called again ProteqFlu-Te, but will contain vCP3011 (instead of vCP1533), vCP2242 and *Clostridium tetani* toxoid.

Quality

Qualitative and quantitative particulars of the components

Composition

For a dose of 1 ml:

In order to take into account how the product is controlled, the composition indicates that the vCP content is checked by global infective titration and specific qPCR as proposed for summary of product characteristics (SPC) below:

"2. QUALITATIVE AND QUANTITATIVE COMPOSITION

One dose contains:

Active substances:

Influenza A/eq/Ohio/03 (H3N8) recombinant canarypox virus (vCP2242) ≥ 5.3 $\log_{10}$ $FAID_{50}$*

Influenza A/eq/Richmond/1/07 (H3N8) recombinant canarypox virus (vCP3011) ≥ 5.3 $\log_{10}$ $FAID_{50}$*

Clostridium tetani toxoid ≥ 30 IU**

*vCP content checked by global $FAID_{50}$ (Fluorescent assay infectious dose 50%) and qPCR ratio between vCP

**antitoxic antibody titre induced after repeated vaccination in guinea pig sera according to Ph. Eur.

Adjuvant:

Carbomer 4 mg.

For the full list of excipients, see section 6.1."

Development pharmaceutics

In 2010, the OIE Expert Surveillance Panel on Equine Influenza Vaccine Composition recommended to replace the influenza A/equi-2/Newmarket2/93 strain (Eurasian lineage) by influenza A/eq/Richmond/1/07 (American lineage, Florida sublineage, clade 2). In accordance with this new recommendation, vCP1533 is substituted by the recombinant vCP3011 construct expressing the haemagglutinin gene from influenza virus A/eq/Richmond/1/07.

The production process of vCP3011 virus is similar to the previous vCP strains.

As both constructs vCP3011 and vCP2242 are very similar, the preparation of a monoclonal antibody specific of vCP3011 HA appears impossible. As a consequence:

- at the working seed level, the vCP3011 virus is specifically identified according to Technique 200584 ("Identification by indirect IF of Canary pox vector") for the canarypox vector and according to a qPCR technique (Technique 200571) for the insert.
- at the finished product level, vCP3011 and vCP2242 are globally titrated using the same technique (Technique 200579). Moreover, vCP3011and vCP2242 are specifically identified and quantified according to the qPCR technique also used for the working seed identification (Technique 200571).

No other change is implemented with regard to the other components.

The applicant provided documentation showing that the HA protein of equine influenza virus consists of 565 amino acid residues for vCP1533 and 567 amino acid residues for vCP3011. The HA protein expressed in vCP1533 is 94% homologous to the HA protein expressed in vCP3011. Amino acid changes between the HA proteins in vCP3011 and vCP1533 affect 28 positions.

Method of preparation

The production process remains the same. The only change is the replacement of the vCP1533 component by the vCP3011 one.

The consistency of the process was demonstrated with titration results of 3 consecutive production batches.

The identification and ratio between each construct, is determined to confirm that both vCP are present and well balanced after formulation. Knowing that the qPCR Technique 200571 performs similarly in both environments with or without the tetanus toxoid component (based on reports provided by the applicant) and that both vCP are balanced at formulation in both products, the same specifications for the vCP qPCR ratio could be effectively applied to both new formulations.

Once the final stability study becomes available, the relevance of the minimum global release titre will be checked.

Validation studies

A validation report for titration and identification by $FAID_{50}$ of the active ingredients vCP2242 and vCP1533 was previously assessed and is still relevant although the technique number has been revised taking into account the replacement of vCP1533 with vCP3011 and the restriction of the scope of the technique from identification and titration to titration only. Therefore the validation report of Technique 200041 is applicable to Technique 200579.

A validation report was submitted for the control Technique 200579: quantification by $FAID_{50}$ of equine influenza canarypox virus vCP3011 (Richmond strain). The scope of the validation was to establish the linearity and precision (repeatability and intermediate precision) of Technique 200579. The principle is the haemagglutinin of strain vCP3011 grown in chicken embryo cells is quantified by an indirect immunofluorescence assay using a monoclonal antibody. The titre is expressed as the decimal logarithm of the inverse dilution giving 50% of positive wells (log_{10} $FAID_{50}$/ml), established in infected cells by linear regression method.

The linearity and the proportionality of the technique were demonstrated for titres within the following range: [4.10; 9.04] log_{10} $FAID_{50}$/ml.

An operator/session cross-effect was observed, however these differences were negligible with regard to the mean titres and the repeatability of the technique remained good.

Starting materials

Not listed in a Pharmacopoeia

Starting materials were unchanged with regard to the initial approved file (and subsequent approved variations), except for vCP3011 in place of vCP1533. Thus, only the data about vCP3011 was assessed.

Recombinant vCP3011 virus

The applicant provided information on genetic engineering with additional data on plasmid pC5 H6p Eqf.

The applicant also provided satisfactory information relating to description, control of genetic purity, control of expression and control of genetic stability for the recombinant canarypox virus vCP3011.

Controls on the master seed virus (MSV)

Relevant information regarding the monoclonal antibody EIV-617AC was provided. The applicant provided satisfactory information on identity, sterility and viral purity. Southern blot analysis was not performed because the vCP3011 construct is very similar to other canarypox recombinant vaccines with respect to

the engineering (homologous recombination), the site of insertion (C5 locus) and the promoter (H6). C5 locus is located in the inverted terminal repeats and as such, is present as two copies. A combination of assay techniques was judged sufficient to show the preservation of the insertion at the two insertion sites.

Detailed information on the ALVAC master seed virus batches used for the generation of both vCP2242 and vCP3011 constructs was provided by the applicant. The ALVAC master seed virus used for the generation of vCP2242 and vCP3011 did not change over time, and was considered acceptable.

A western blot analysis of the vCP3011 infected cell lysates was performed upon request. The results obtained show that vCP3011 produced the expected HA band similarly to vCP2242.

African horse sickness virus and Japanese B encephalitis virus were not tested because the vCP parental strains were free from these viruses.

Clarification was provided about the cells used. The cells used to amplify potential contaminants may differ from the revealing cells recognized suitable for detecting them in the purity control tests implemented.

Controls on the working seed virus (WSV)

The applicant provided information on identity, sterility and titration. In addition, certificates of analysis were provided for the Master Seed Virus and the Working Seed Virus.

Controls on the active ingredient

Satisfactory information was submitted by the applicant in relation to titration and sterility. Consistency of production is shown on 3 consecutive batches.

TSE benefit-risk assessment

The vCP3011 component is the only new component with regard to the initial file and subsequent approved variations. Thus, the risk assessment focused only on the new vCP3011 component, from the construction process to the active ingredient (the manufacturing process of the finished product remaining unchanged).

The following raw materials of biological origin are involved during the construction of vCP3011, for the production of the MSV and WSV: foetal calf serum, calf serum and tryptose phosphate broth.

Tryptose phosphate broth is a milk derivative, obtained from milk fit for human consumption. It is part of the culture media.

The risk of transmitting transmissible spongiform encephalopathy (TSE) through vCP3011 can be considered as extremely minimised, given that the influenza strain is of equine origin, that the raw materials of bovine origin are of lower or no detectable infectivity and because of the high dilution factor obtained during the manufacturing process of the finished product. Further detail was provided in relation to the suppliers and the level of use of the sera used for the establishment of the MSV vCP3011. All the sera used for the establishment of MSV vCP3011 were covered by a certificate of suitability (CEP). Copies of the CEPs of the sera used for the establishment of this MSV were also provided.

Control of finished product

The control tests remain unchanged with regard to the initially approved file (and subsequent approved variations). They were not assessed again.

“Identification of DNA insert (qPCR)” and “Identification and ratio between each construct (qPCR3011 - qPCR2242)”, with Technique 200571 was assessed and found to be satisfactory. This control test is carried out on each batch. The validation of the control technique was provided.

Viral DNA is extracted, amplified by qPCR and detected in real time by measuring the fluorescent signal emitted using two specific probes, targeting either the vCP2242 haemagglutinin sequence or the vCP3011 haemagglutinin sequence, coupled to two different fluorochromes. The signal obtained is proportional to the DNA quantity synthesized. vCP2242 and vCP3011 nucleic acid quantifications are carried out using two standard ranges specific of these two targets. The identity is checked through a positive fluorescence specific of the canarypox insert; the ratio is based on the difference in qPCR titres between vCP3011and vCP2242. It is expressed in $\log_{10}$ copies/ml.

“Global infective titration of vCP2242 and vCP3011 active ingredients” with Technique 200579 was also considered satisfactory. This control test is carried out on each batch. The validation of the control technique was also provided.

vCP2242 and vCP3011 grow in chicken embryo cells and express the haemagglutinin (HA) specific of variant A/eq/Richmond/1/07 (vCP3011) and the haemagglutinin specific of variant A/eq/Ohio/03 (vCP2242). Global HA of both vCP are detected by the monoclonal antibody currently used for the titration of vCP2242, which cross-reacts with the vCP3011 HA. Quantification of the global HA amount is achieved by the calculation of the $FAID_{50}$ titre (fluorescence assay infectious dose 50%) using the global infective titration technique.

Validation studies

A validation of the control Technique 200579 (quantification of equine influenza recombinant canarypox viruses contained in ProteqFlu vaccines by $FAID_{50}$) was submitted. The study design included linearity and precision.

The relationship between the dilution factor and the observed titre was found to be linear. The precision study yielded satisfactory standard deviations for repeatability and intermediate precision.

The validation was carried out using vaccines containing balanced active ingredients. This did not affect the robustness as different ratios of vCP2242 and vCP3011 are not expected in the finished product and should the ratio of vCP2242 and vCP3011 be unbalanced, the qPCR would detect it.

The validation of the control Technique 200571 (quantification of equine influenza recombinant canarypox virus (vCP2242 Ohio strain and vCP3011 Richmond strain) in ProteqFlu-Te vaccines by qPCR) was also assessed and found satisfactory in terms of linearity and precision.

In order to increase the precision of the method, 3 sessions of 2 titrations will be performed on a routine basis. As a consequence, the variance associated with the mean titre will be lowered.

The validation of the control Technique 200571 (identification by real-time PCR of equine influenza canarypox virus - vCP3011 Richmond strain and vCP2242 Ohio strain) was also assessed and found to be satisfactory in terms of specificity.

Stability

In compliance with the CVMP Note for guidance on harmonisation of requirements for equine influenza vaccines, specific requirements for substitution or addition of a strain or strains (EMA/CVMP/112/98-FINAL):

- the shelf life recommended for the new formulation remains identical to the current formulation (namely 3 years at 5 °C).

- stability data to confirm the current shelf life will be provided as post-approval commitment on the first production batch recently produced (and described in the dossier) and on the two first production batches to be commercialized post-approval.

To assess the stability of both vCP in the vaccine, two techniques are implemented at the same time. Technique 200583 is dedicated to stability assessment only. The validation of Technique 200583 was provided by the applicant. To estimate the loss of titre, two development batches at standard titres were monitored over a 15-month period. The titre is expressed in $\log_{10}$ $PCRID_{50}$/ml. Correlation between $PCRID_{50}$ units and $FAID_{50}$ units was also provided by the applicant.

In order to determine if the 3 titrations (global $FAID_{50}$/ml and vCP2242 and vCP3011 $PCRID_{50}$/ml) have the same behaviour over time, the models for the 3 titrations were compared. The analysis shows no significant difference between slopes (P-value Slopes > 0.05), and no significant difference between intercepts (P-value Intercepts > 0.05). The estimated loss of titre on a 15-month period can be considered similar whatever the technique. In addition, this estimated loss of titre is similar to that observed with the current vaccine. These results were considered sufficient to provide confidence in the stability of the updated vaccine knowing that the loss of titre over time is comparable to the current vaccine. They also show that the approach chosen by the applicant can be considered relevant to confirm the stability over time of three production batches as committed. The applicant confirmed that this stability study will include a modelling of the results obtained to allow quantification of any potential loss of titre with time. The applicant also committed to advise EMA immediately, if during the on-going study, results fall outside the expected ranges of values.

The stability data and the corresponding models will be reviewed once the final stability study becomes available.

Safety assessment

Introduction

The applicant followed the CVMP "Note for guidance on harmonisation of requirements for equine influenza vaccines, specific requirements for substitution or addition of a strain or strains" (EMA/CVMP/112/98-FINAL) to show safety of the new formulation.

Laboratory tests

Safety of the administration of one dose, of the administration of an overdose and of the repeated administration of one dose

The applicant submitted a report on the safety and dissemination assessment of the equine influenza vaccine containing the recombinant canarypox constructs (vCP2242 and vCP3011) formulated in tetanus and carbomer when administered as an overdose, a dose and a repeated dose in foals. The objective of the study was to assess the safety in horses of the PHN3172 vaccine (containing vCP2242, vCP3011 and tetanus), and comparison to the safety following vaccination with ProteqFlu-Te (current formulation).

Slight hyperthermia, minor and transient swellings were registered with PHN3172 vaccine. These signs are adequately reflected in the SPC.

Further clarification was provided in relation to the use of blood samples. Transitory viraemia should be considered as the only way for this ectotropic virus to spread in the body after intramuscular injection; this is the rationale for monitoring absence of virus in the blood following injection. To confirm this, several studies have already been carried out to evaluate the replication, dissemination and spread of various recombinant canarypox viruses after intra-muscular administration in horses. These studies showed that no canarypox virus was detected in blood, urine, or rectal and nasal swabs for up to 14 days. It was considered relevant to draw conclusion about the absence of Influenza virus (vCPs and standard equine influenza viruses).

The ALVAC constructs are now sufficiently well-known to conclude that the vaccine strains are safe, that the virus strains cannot replicate in mammalian cells, and hence that dissemination, spread and replication will not occur.

Special requirements for live vaccines

Spread of the vaccine strain and Dissemination in the vaccinated animal

A report on the in-vitro replication study of vCP3011 recombinant virus was assessed. The report concluded that vCP3011 did not replicate in any of the mammalian cell types tested during five successive passages, while replication was observed in primary chicken embryo cells. The results of this study are consistent with previous observations showing that canarypox viruses are replication defective in mammalian cells. The conclusions were considered to be in line with previous demonstrations that vCP constructs cannot replicate in mammalian cells, but can replicate only in avian cells.

A report on the safety and dissemination assessment of the recombinant canarypox coding HA glycoprotein of Equine flu virus strain A/eq/Richmond/07 (vCP3011) compared to the parental canarypox virus (CPpp) in canaries was also assessed. This report concluded that vCP3011 is safe and does not disseminate when administered at high titre by transcutaneous route to the canary; no difference was observed between vCP3011 and parental virus. Tropism of both viruses was similar and concerned only the skin. Histological changes were mild and conformed to expectations for this type of virus and were similar for vCP3011 and parental virus. Replication of both canarypox recombinants *in vivo* was transient. The absence of reaction and virus isolation in the sentinel canaries confirmed the absence of spread of vCP3011 and CPpp in this species.

Field trials

Justification for not providing field trial data was deemed acceptable. With regard to PHN3172 vaccine under consideration, the canarypox nature of the new construct remains unchanged; the only difference between vCP1533 and vCP3011 is the HA gene expressed and thus the HA protein induced, both proteins being 94% homologous. Apart from this, the vaccine remained strictly identical.

Environmental risk assessment

The replacement of vCP1533 by vCP3011 was not considered to impact on the ecotoxicity assessment.

GMO Assessment

The assays and trials already submitted in previous dossiers and which were already assessed and endorsed, were not reassessed again within the frame of this report.

Information relating to the interaction between the genetically modified organisms (GMOs) and the environment was considered satisfactory.

The absence of replication of vCP2242 and vCP3011 was demonstrated in cells of the target species. vCP2242 was also tested on canine cells confirming the absence of replication in mammalian cells. Therefore, in the absence of replication, vCP2242 and vCP3011 cannot disseminate within the vaccinated horse, diffuse from a vaccinated horse to a non-vaccinated contact horse or contaminate the environment. The absence of dissemination and shedding in non-avian species was also confirmed in a number of safety studies including sentinel animals and laboratory rodents (guinea-pigs and mice), ducks, chickens, pigs, dogs and horses. Data from these studies carried out with the initial strains vCP1529 and vCP1533, were confirmed, as expected since constructs are very similar, by additional studies carried out with vCP2242 and vCP3011 in horses and with vCP2242 in dogs.

Poxviruses and therefore ALVAC-based viruses are readily inactivated by chlorhexidine, benzalkonium chloride, formol and bleach. A 12 chlorometric degrees sodium hypochlorite solution and 70% ethanol

solution showed a virucidal activity against an ALVAC-based recombinant virus (vCP1533: equine influenza recombinant canarypox) and quaternary ammonium and glutaradehyde.

Because the purpose of release of vCP2242 and vCP3011 recombinant viruses is to protect horses against equine influenza H3N8, the predicted habitat of the GMO is the vaccinated horse. vCP2242 and vCP3011 do not replicate in non-avian species. Consequently, they do not persist in the horse and is not shed in the environment after vaccination.

An in vitro study was provided by the applicant demonstrating the lack of replication of vCP2242 and vCP3011 recombinant viruses in mammalian cells (of equine origin). The in vivo safety was demonstrated for vCP1529 and vCP1533 in horses, pigs, laboratory rodents, ducks, dogs, chickens and canary birds. Absence of shedding was demonstrated in horses, pigs, laboratory rodents, dogs, ducks and chickens. This was confirmed by additional studies carried out with the vCP2242 and vCP3011 in horses, canary birds and dogs. In canary birds, the safety was at least equivalent to that of CPpp parental strain at the systemic and local level.

Studies with other canarypox viruses were also submitted by the applicant. This study showed that the simulated environmental sample, mimicking horse barn and paddock, was detrimental to the survivability of both vCP2017 and CPpp, as no virus could be detected in the sample collected as soon as 30 minutes after inoculation. Another study has shown that vCP2017 could not be recovered from any horse urine samples spiked with different amounts of the canarypox virus, suggesting virucidal properties of the equine urine. These two studies demonstrated that canarypox viruses are unable to survive in the environment.

Genetic re-assortment and cross-reactivation have been observed between intact influenza viruses. However, in horses (target organism), because the GMOs cannot replicate and do not disseminate, genetic transfer capability is negligible.

Potential for genetic transfer and exchange with a virus related to the donor organism: recombination between a canarypox-based virus (DNA virus) and an influenza virus (RNA virus) is highly unlikely to happen because of the different nature of the nucleic acids and because of the different replication sites (the cytoplasm for poxvirus and the nucleus for the influenza virus).

Genetic transfer and exchange involving the GMOs with other organisms was considered highly unlikely. Therefore, genetic transfer capability of vCP2242 and vCP3011 is negligible.

The likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organism was assessed. vCP2242, vCP3011 and ALVAC-derived recombinants do not replicate in non-avian species. In permissive cells, vCP2242 and vCP3011 were shown to be genetically stable along passages. In canaries (permissive host), the wild strain becomes predominant with time and passages following a mixed infection with a wild strain of canarypox virus and an ALVAC-derived recombinant virus (document VNO16CRO). Therefore, likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organism is negligible.

Dispersal of genetic material was considered highly unlikely.

vCP2242 and vCP3011 recombinant virus do not replicate in non-avian species and so, dissemination to non-avian species including horses cannot take place. Moreover, the vaccine must be injected into horses by the intramuscular route.

In the permissive species, canary birds, wild type canarypox virus can disseminate either by contact with the skin lesions of an infected bird or, in a highly contaminated environment (aviary), by aerosol of viral particles from scabs of other birds living close. However, there is very limited dispersion for the vaccine strain. Transmission of the virus to non-avian-species, including man, has never been reported.

Although very unlikely as discussed above, the GMOs could be disseminated to canaries (the only permissive host). Potential for excessive population of the GMOs in the environment was excluded.

A competitive advantage of the GMOs in relation to the unmodified parental organism is not expected. Data on the potential for excessive population in the environment and the results of an experimental study on the mixed infection of canaries with a wild strain of canarypox virus and with an ALVAC/luciferase recombinant virus were considered. These results showed that, for any "wild strain/ALVAC" rate, no virus with higher virulence emerged from the different viral mixtures during successive passages in canaries and the wild strain became predominant with time and passages.

Anticipated mechanism and result of interaction between the released GMOs and the target organism was assessed and found to be satisfactory as persistence of vCP2242 and vCP3011 in the animal is limited to a few days at the site of injection.

The likelihood of post-release shifts in biological interactions or in host range is excluded. The non-replicative property of ALVAC vector has been extensively documented and confirmed with all ALVAC-derived constructs.

The chance that non-target organisms may be affected unwillingly is considered highly unlikely. However, should this happen, it has been demonstrated that canarypox virus host range and dissemination are extremely limited. Following a mixed infection with a wild strain of canarypox virus and an ALVAC-based recombinant virus, no virus with higher virulence emerged during successive passages and the wild strain became rapidly predominant. In the only permissive host (the canary bird), ALVAC-based recombinant viruses vCP2242 and vCP3011 are at least as safe as the CPpp parental strain derived from the vaccine strain of KANAPOX, which has an extensive safety record in the field.

Monitoring techniques, control of release and emergency response plans were reviewed and deemed to be acceptable.

Using the matrix given in the Notice to Applicant, veterinary medicinal products, "Guidance on environmental risk assessment for veterinary medicinal products consisting of or containing genetically modified organisms (GMOs) as or in products" ENTR/F2/KK D(2006), for the analysis on the consequence of hazard (judged as low or negligible) and the likelihood of hazard occurring (considered as negligible), the estimation of risk was defined as effectively zero.

The recombinant virus vCP1533 expressing the haemagglutinin gene from equine influenza virus A/equi-2/Newmarket/2/93 was substituted by the recombinant virus vCP3011 expressing the haemagglutinin gene from equine influenza virus A/eq/Richmond/1/07 in order to comply with the current epidemiological situation (last OIE/WHO recommendations).

The insert used in vCP3011 is very similar to the one used in vCP1533 (94% of homology) and the canarypox vector backbone used in both constructs is identical. Therefore, as expected, the substitution of vCP1533 by vCP3011 did not impact the environmental risk assessment for ProteqFlu-Te. All data confirmed the interest of the canarypox technology, particularly in terms of replication, spread, contamination, and dissemination into the environment. The vaccine containing vCP2242 and vCP3011 was demonstrated as safe as the current vaccine containing vCP1533 and vCP2242, both for the target species and for other species (mammalian or avian including the permissive host of canarypox virus), humans and environment.

This conclusion is mainly based on the natural property of the canarypox vector used, the absence of replication in mammalian cells. The explanation provided in the section is satisfactory.

Efficacy assessment

Introduction

The applicant followed the CVMP Note for guidance on harmonisation of requirements for equine influenza vaccines, specific requirements for substitution or addition of a strain or strains (EMA/CVMP/112/98-FINAL): to show efficacy of the new formulation.

Laboratory tests

A test report was submitted to demonstrate Bioequivalence of recombinant canarypox virus vCP3011 and vCP1533, associated to vCP2242 in a bivalent equine flu vaccine administered with tetanus in carbomer by intramuscular route in foals. The study was conducted with 22 conventional foals (10 foals of 13–14 months of age and 12 foals of 25–28 months of age), seronegative with regard to influenza and tetanus at day 0, randomly allocated to three groups, A, B and C. group A: 10 foals received an injection of 1 dose of PHN3172 vaccine at day 0, day 35 and day 189; group B: 10 foals received an injection of 1 dose of ProteqFlu-Te at day 0, day 35 and day 189 and group C: 2 foals were kept as controls. Blood sampling was conducted at day 0 (before injection), day 7, day 14, day 35 (before injection), day 49, da 70, day 98, day 126, day 154, day 189 (before injection) and day 203. Samples were tested for the presence of enzyme-linked immunosorbent assay (ELISA) antibodies against tetanus and for the presence of single radial haemolysis (SRH) influenza antibodies directed against the following strains: A/eq/South Africa/4/03 (homologous to strain A/eq/Ohio/1/03), for all groups, A/eq/Meath/1/07 (homologous to strain A/eq/Richmond/1/07, for groups A and C and A/eq-2/Newmarket/2/93, for groups B and C. Clinical examination (general condition and rectal temperature) was carried out on day 0, day 35 and day 189, each time before injection. All foals were in good general condition before each vaccination.

ELISA antibody titres to tetanus in both vaccinated groups were equivalent. A primary vaccination course of two injections with PHN3172 vaccine and ProteqFlu-Te induced high antibody levels against influenza and tetanus. All ponies from group A and B developed a strong booster response to third vaccination.

SRH antibody titres against influenza SA/4/03 in both vaccinated groups as well as SRH antibody titres against influenza virus M/1/07 and NM/2/93 in groups A and B, respectively, were equivalent. Therefore ProteqFlu-Te and PHN3172 vaccine induced similar immunogenicity against influenza and tetanus.

The study was found to be satisfactory in determining bioequivalence between the PHN3172 vaccine and the currently commercialised ProteqFlu-Te.

A test report on the serological assessment of the efficacy of the equine influenza recombinant canarypox construct vCP3011 formulated with tetanus toxoid and carbomer when administered by intramuscular route in foals was also assessed. Five foals received an injection of 1 dose of vCP3011 vaccine at day 0 and day 35, while two foals were kept as controls. Blood sampling was conducted at day 0 (before injection), day 7, day 15, day 35 (before injection), day 49 and day 70. Samples were tested for the presence of ELISA antibodies against tetanus and for the presence of SRH influenza antibodies directed against influenza strain A/equi-2/Meath/07 (homologous to strain A/eq/Richmond/1/07). All foals of group B remained seronegative throughout the monitoring period.

Immunogenicity of vCP3011 was shown at a low dose. From day 49 onwards, all vaccinated foals had protective antibody level against Flu (> 85 mm^2) and tetanus (> 0.01 UI/ml). These results were considered to be satisfactory.

2.2. Summary and conclusions

The results and conclusions obtained for PHN3172 vaccine (containing vCP2242, vCP3011 and tetanus toxoid) are satisfactory for demonstrating the safety and efficacy of the PHN3172 vaccine.

The analytical file is correctly documented.

With regard to safety, the recombinant virus vCP3011 is very similar to vCP1533: the canarypox vector backbone used in both constructs is identical, and the insert used in vCP3011 shows 94% of homology with regard to the insert of vCP1533.

The studies provided demonstrated that the PHN3172 vaccine containing vCP3011 and vCP2242 was as safe as the current ProteqFlu-Te vaccine containing vCP1533 and vCP2242. The substitution of vCP1533 by vCP3011 did not impact the safety characteristics of the vaccine. The safety profile of the PHN3172 vaccine remains unchanged in comparison to the ProteqFlu-Te vaccine, both for the target species and the permissive host of canarypox virus, for humans and for the environment.

With regard to efficacy, immunogenicity of vCP3011 has been demonstrated equivalent to vCP1533, used in the currently registered ProteqFlu-Te vaccine. It was shown through serology that vCP3011 will be able to protect against field Influenza strains of the American lineage, Florida sublineage, clade 2. Finally, it has been demonstrated by serology that this change of construct had no impact on the efficacy of the other components of the vaccine (vCP2242 and tetanus toxoid).

All these results are compliant with the requirements of the CVMP Note for guidance on harmonisation of requirements for equine influenza vaccines, specific requirements for substitution or addition of a strain or strains (EMA/CVMP/112/98-FINAL) currently in force.

It is proposed to restart the periodic safety update report (PSUR) cycle for ProteqFlu-Te. This is considered necessary in view of the substitution of one equine influenza strain. PSURs covering all authorised presentations of the product would be required at 6 monthly intervals for the next two years, followed by yearly for the subsequent two years and thereafter at three-yearly intervals.

This variation is acceptable. The SPC will be impacted in section 2 only, and will be written as follows:

"2. QUALITATIVE AND QUANTITATIVE COMPOSITION

One dose contains:

Active substances:

Influenza A/eq/Ohio/03 (H3N8) recombinant canarypox virus (vCP2242) ≥ 5.3 $\log_{10}$ $FAID_{50}$*

Influenza A/eq/Richmond/1/07 (H3N8) recombinant canarypox virus (vCP3011) ≥ 5.3 $\log_{10}$ $FAID_{50}$*

Clostridium tetani toxoid ≥ 30 IU**

*vCP content checked by global $FAID_{50}$ (Fluorescent assay infectious dose 50%) and qPCR ratio between vCP

** antitoxic antibody titre induced after repeated vaccination in guinea pig sera according to Ph. Eur.

Adjuvant**:**

Carbomer 4 mg.

For the full list of excipients, see section 6.1."

3. Benefit-risk assessment

3.1. Benefit assessment

The results and conclusions obtained for PHN3172 vaccine (containing vCP2242, vCP3011 and tetanus toxoid) are considered satisfactory.

The PHN3172 vaccine is compliant with the recommendations of the Expert Surveillance Panel on the strains to be incorporated into vaccines against equine influenza, making the new vaccine fully in line with the current epidemiological situation.

Immunogenicity of the new vCP3011 strain has been demonstrated equivalent to vCP1533, used in the ProteqFlu-Te vaccine. It was shown through serology that vCP3011 will be able to protect against field influenza strains of the American lineage, Florida sublineage, clade 2. Finally, it has been shown by serology that this change of construct had no impact on the efficacy of the other components of the vaccine, i.e. the vCP2242 construct and tetanus toxoid.

3.2. Risk assessment

The results and conclusions obtained for PHN3172 vaccine (containing vCP2242, vCP3011 and tetanus toxoid) are considered satisfactory.

The substitution of vCP1533 by vCP3011 did not impact the safety characteristics of the ProteqFlu-Te vaccine. The safety profile of the PHN3172 vaccine remains unchanged in comparison with the ProteqFlu-Te vaccine, for the target and non-target species (including the canary, which is the permissive host of canarypox virus), for humans and for the environment.

3.3. Evaluation of the benefit-risk balance

The benefit-risk balance is in favour of this vaccine (substitution of vCP1533 by vCP3011), as the PHN3172 vaccine is shown to be efficacious, as the benefit is improved because this variation will put the PHN3172 vaccine totally in adequacy with the current epidemiological situation in Europe with regard to equine influenza, and as the risk is not greater compared to the ProteqFlu-Te vaccine already registered for several years.

No change to the impact on the environment is envisaged.

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.

The product information is to be updated according to the proposal in the section 2.2.

The periodic safety update report (PSUR) cycle for ProteqFlu-Te is re-started.

5. Changes to the marketing authorisation

Changes are required in the Annexes to the marketing authorisation:

I, II, IIIA and IIIB.

On 11 July 2014, the European Commission adopted a Commission Decision for this application.