EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Prevexxion RN+HVT+IBD (EMEA/V/C/005057/0000)

Vaccine common name: Infectious bursal disease and Marek's disease vaccine (live recombinant)

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

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Introduction

The applicant Merial submitted on 29 November 2018 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Prevexxion RN+HVT+IBD, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope). The name of the applicant was subsequently changed during the procedure to Boehringer Ingelheim Vetmedica GmbH.

The eligibility to the centralised procedure was agreed upon by the CVMP on 25 May 2018 as Prevexxion RN+HVT+IBD has been developed by recombinant DNA technology.

The applicant applied for the following indications:

For active immunisation of one-day-old chicks:

- To prevent mortality and clinical signs and reduce lesions caused by Marek's disease (MD) virus (including very virulent MD virus),
- To prevent mortality, clinical signs and lesions caused by infectious bursal disease (IBD) virus.

PREVEXXION RN+HVT+IBD contains two active substances:

- live recombinant avian herpesvirus (Marek's disease virus), RN 1250 strain, genetically modified to contain genomic parts of three different serotype 1 MD virus strains.
- live recombinant turkey herpesvirus, vHVT013-69 strain, expressing the viral protein 2 (VP2) coding sequence of the infectious bursal disease (IBD) virus.

The target species is chickens. The product is intended for administration by subcutaneous (SC) route.

Prevexxion RN+HVT+IBD consists of a frozen viral suspension (concentrate) to be diluted in an aqueous solvent to obtain the final suspension for injection. Each dose of vaccine (0.2 ml) contains 2.9 to 3.9 $\log_{10}$ plaque forming units (PFU) of live recombinant Marek's disease virus, serotype 1, strain RN1250 and 3.6 to 4.4 $\log_{10}$ PFU of live recombinant turkey herpesvirus, strain vHVT013-69.

The vaccine is presented in glass ampoules containing 1,000, 2,000 or 4,000 doses in packs sizes of 5 ampoules per carrier (1,000-dose and 2,000-dose presentations) or 4 ampoules per carrier (4,000-dose presentation). The solvent is presented in plastic bags containing 200 ml, 400 ml, 600 ml, 800 ml, 1,000 ml, 1,200 ml, 1,600 ml, 1,800 ml or 2,400 ml.

The rapporteur appointed is Frédéric Klein and the co-rapporteur is Esther Werner

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

Marketing authorisation under exceptional circumstances

Not applicable.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system which fulfils the requirements of Directive 2001/82/EC was provided. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Manufacturer of the active substance:

Boehringer Ingelheim Animal Health France SCS

Laboratoire Porte des Alpes
99, Rue de l'Aviation
69800 Saint Priest
FRANCE

Manufacturer of the solvent:

Laboratoire BIOLUZ
Zone Industrielle de JALDAY
64500 SAINT JEAN DE LUZ
FRANCE

Manufacturer responsible for batch release:

Boehringer Ingelheim Animal Health France SCS

Laboratoire Porte des Alpes
99, Rue de l'Aviation
69800 Saint Priest
FRANCE

General comments on compliance with GMP, GLP, GCP:

All manufacturing sites have been recently inspected by the French competent authorities and were found to be GMP compliant with regard to the applicable manufacturing activities. GMP certificates are available in EudraGMP database.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system was considered in line with legal requirements.

The GMP status of the active substance(s) and of the finished product manufacturing sites has been satisfactorily established and is in line with legal requirements.

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

Prevexxion RN+HVT+IBD consists of a frozen cell-associated viral suspension containing two active ingredients, a live recombinant Marek's disease virus serotype 1 (RN1250 component) and a live recombinant turkey herpesvirus expressing the VP2 gene of infectious bursal disease virus (vHVT013-69 component), to be diluted in an aqueous solvent used for the suspension preparation of Boehringer Ingelheim Vetmedica GmbH (formerly MERIAL) frozen vaccines against Marek's and Gumboro diseases. The solvent does not contain any active ingredient.

The excipients of the frozen cell-associated viral suspension are dimethylsulfoxide, 199 Earle medium, sodium hydrogen carbonate, hydrochloric acid and water for injections. The excipients of the solvent are sucrose, casein hydrolysate, dipotassium phosphate, potassium dihydrogen phosphate, phenol red, sodium hydroxide or hydrochloric acid water for injections.

Container and closure

Frozen cell suspension:

The vaccine is filled into Type I glass ampoules of 2-ml and 5-ml (compliant with current European Pharmacopoeia (Ph. Eur.)), which are sealed using a flame.

Solvent for dilution:

The solvent is filled in heat-sealed bags with two connecting tubes, one for filling and one fitted with a septum for puncture. The bags are made of polyvinylchloride (250-ml, 500-ml, 1,000-ml, 2,000-ml and 3,000-ml volumes) complying with the current Ph. Eur. edition and can be filled with different volumes depending on the presentations:

Presentation (nominal volume)	Bag size
200 ml	250-ml bag
400 ml	500-ml bag
600 ml	1,000-ml bag
800 ml	1,000-ml bag
1,000ml	1,000-ml bag
1,200 ml	2,000-ml bag
1,600 ml	2,000-ml bag
1,800 ml	2,000-ml bag
2,400 ml	3,000-ml bag

Each bag is then placed in a protective overpouch that is heat-sealed.

Product development

An explanation and justification for the composition and presentation of the vaccine has been provided.

Frozen cell suspension:

Prevexxion RN+HVT+IBD is a live vaccine intended for vaccination against Marek's disease (MD) and infectious bursal disease (IBD or Gumboro disease) by subcutaneous administration to one-day-old chicks.

Boehringer Ingelheim Vetmedica GmbH has already registered a vaccine against MD, CRYOMAREX RISPENS, which is currently used worldwide for years (first registrations in Europe more than 20 years ago, extended in 2014 through a mutual recognition procedure). Its active substance is the attenuated live Marek's disease virus (MDV), serotype 1, Rispens strain (CVI988). Boehringer Ingelheim Vetmedica GmbH has now developed Prevexxion RN+HVT+IBD, containing both serotype 1, RN1250 strain and the vHVT013-69 strain, a recombinant turkey herpesvirus (HVT) expressing the VP2 coding sequence of infectious bursal disease virus (IBDV) already registered in the Vaxxitek HVT+IBD vaccine (vaccine authorised in EU through a centralised procedure since 2002).

Development of the vaccine started in the USA, leading to a full market authorisation in 2017 for the monovalent RN1250 vaccine and in 2018 for the bivalent RN1250+vHVT013-69 vaccine.

Choice of the vaccine strains

The MD vaccine strain, RN1250 was originally generated and tested at the USDA ARS, Avian Disease Oncology Laboratory (ADOL) in the USA. RN1250 strain has been constructed by genetic engineering to generate a Marek's disease virus, serotype 1 (MDV1), strain CVI988 containing two copies of a long terminal repeat (LTR) sequence of a reticuloendotheliosis virus (REV). The CVI988 Rispens vaccine strain is used in licensed vaccines worldwide and more particularly in Europe since the 1970s.

Thus, RN1250 strain includes in particular genomic parts:

- from the currently most efficacious and safe vaccine MDV strain, the CVI988 Rispens strain,
- from the MDV RM1 strain, derived from a virulent MDV strain (JM/102W) in the genome of which two copies of REV LTR were inserted.
- from the very virulent MDV Md5 strain, in which a fragment of MDV RM1 strain containing the REV LTRs was inserted.

The vHVT013-69 strain is an HVT vaccinal strain expressing the VP2 coding sequence of IBDV, which is the same as the one used in the Vaxxitek HVT+IBD already authorised in EU. This strain was constructed by genetic engineering to generate, from the parental strain HVT FC-126, a recombinant virus containing the IBDV capsid (VP2) gene. The VP2 protein is to date the only known protein of IBDV that induces protection against Gumboro disease.

Choice of the antigen manufacturing process and active substance quantification method

The manufacturing process chosen for the RN1250 and vHVT013-69 antigens is classical of this type of vaccine. The antigen is a suspension of SPF chicken embryo fibroblasts infected with RN1250 or vHVT013-69. The virus used for the inoculation of the production culture is amplified by passages in specific pathogen-free (SPF) chicken embryo cells . The infected cells are harvested using trypsin and centrifuged. At the last passage, the cell pellet is suspended in dilution medium and calfserum. The cell suspension is then sieved and constitutes a batch of active ingredient.. Both RN1250 and vHVT013-69 active ingredients are titrated in the finished product according to the induction of a cytopathic effect revealed in the form of foci (called PFU or plaque forming units) after inoculation on chicken embryo

fibroblasts, as classically done for Marek's vaccines. PFU are revealed by indirect immunofluorescence using specific monoclonal antibodies..

Finished product presentation

The vaccine is presented in the form of a sealed glass ampoule containing the frozen antigen suspension to be diluted in the solvent used for the preparation of Boehringer Ingelheim Vetmedica GmbH cell-associated poultry vaccines, presented in PVC bags. Both RN1250 and vHVT013-69 strains are cell-associated viruses. Three presentations are proposed:

- a 1,000-dose presentation corresponding to 2 mL antigen suspension to be diluted in 200 mL solvent.
- a 2,000-dose presentation corresponding to 2 mL antigen suspension to be diluted in 400 mL solvent.
- a 4,000-dose presentation corresponding to 4 mL antigen suspension to be diluted in 800 mL solvent.

Choice of excipients

The vaccine excipient dimethyl sulfoxide (DMSO) was selected for its capacity to protect cells infected by the vaccine virus during freezing.

Choice of containers

The container constituents for the vaccine suspension (type I glass ampoule) were selected for their pharmaceutical quality and their compliance with the Ph. Eur. requirements. The containers are also appropriate for liquid nitrogen storage.

Definition of the specifications

For RN1250: The minimum protective dose was set at 2.9 log10 PFU/dose, according to the results of the efficacy studies. The minimum release titre was set to 0.3 log10 above the minimum protective dose, i.e. 3.2 log10 PFU/dose. The maximum release dose was set to 3.9 log10 PFU/dose, based on the results of the safety studies.

For vHVT013-69: The minimum protective dose was set at 3.6 log10 PFU/dose, according to the results of the efficacy studies. The minimum release titre was set to 0.2 log10 above the minimum protective dose, i.e. 3.8 log10 PFU/dose. The maximum release dose was set to 4.4 log10 PFU/dose, based on the results of the safety studies.

The MDV1 and HVT strains have been shown in the studies presented as well as in published literature (Gimeno, 2019) to influence each other. For protection against MD, the viruses appear to complement each other. However, MDV1 at high dose may have a negative effect on HVT replication, which consequently may delay the immune response induced by the foreign protein (such as IBD protein VP2 in this case) expressed by HVT vector. Studies showed that IBD protection was slightly delayed in birds with maternally-derived antibodies when the RN1250:vHVT013 titre ratio was exceeding a defined threshold. Based on the available results and information, the current specifications are not believed to result in any biologically significant interference and are thus deemed acceptable.

Solvent for dilution:

The solvent is a saline solution supplemented with different components which act as a nutritive component (sucrose), protective agent during the reconstitution of the vaccine (casein hydrolysate), osmolarity agents (sucrose, potassium dihydrogen phosphate, dipotassium phosphate), or are used to adjust the pH (hydrochloric acid, sodium hydroxide). The phenol red allows an additional control that

the pH is within acceptable limits. Casein hydrolysate is the only starting material of animal origin.This starting material is treated by gamma irradiation.

Choice of containers

The container constituent (polyvinylchloride bag) was chosen for its pharmaceutical quality and compliance with the Ph. Eur. requirements. Furthermore, this material, being heat-resistant, is appropriate for terminal sterilisation. After sterilisation step, each PVC bag is placed in a overpouch which protects the bag from possible water losses.

Description of the manufacturing method

RN1250 component

The active ingredient is a suspension of MDV SR-1 RN1250, multiplied in SPF chicken embryo cells. A seed lot system is used for the preparation of active ingredients. Batches of active ingredient consist of the 5th passage at most in SPF chicken embryo cells, from the master seed virus (MSV). After incubation of chicken embryo cells with virus, when the cytopathic effect caused by the virus is optimum, the cells are harvested, centrifuged, diluted in a medium with calf serum and sieved. The cell suspension constitutes a batch of active ingredient.

vHVT013-69 component

The active ingredient is a suspension of vHVT013-69 virus, multiplied in SPF chicken embryo cells. A seed lot system is used for the preparation of active ingredients. Batches of active ingredient consist of the 5th passage at most in SPF chicken embryo cells, from the MSV. After incubation, when the cytopathic effect caused by the virus is optimum, the cells are harvested, centrifuged, diluted in a medium with calf serum and sieved. The cell suspension constitutes a batch of active ingredient.

Frozen cell suspension

Formulation is based on volume. The active ingredients, RN1250 and vHVT013-69, are mixed and stirred. DMSO is mixed with dilution mediumand added to the active ingredients under stirring. The bulk product is filled into sterilised ampoules. Ampoules are subsequently sealed using a flame. Filled ampules are frozen in a controlled manner and stored in liquid nitrogen.

Solvent for suspension for injection

The different constituents of the diluent (sucrose, casein hydrolysate, dipotassium phosphate, potassium dihydrogen phosphate) are blended and stirred. After the addition of phenol red and of water, the pH is adjusted to between 6.9 and 7.3 using NaOH or HCl. Then, water is added to reach the final volume. The bulk obtained is maintained under stirring until filling. The bulk product is filled into containers that are already labelled. A terminal sterilisation process is used after the filling step. Bags are then stored at room temperature. Then, each bag is placed in a protective overpouch. Immediately after addition of the overpouch, the secondary packaging is carried out. After secondary packaging, the diluent is stored at room temperature.

All steps of the manufacturing process have been validated. It has been demonstrated that the manufacturing process is capable of producing finished product of the intended quality in a reproducible and consistent manner. The in-process controls are adequate for this type of manufacturing process.

Production and control of starting materials

Detailed information and certificates of analysis were provided for all starting materials listed in Ph. Eur. demonstrating compliance with the relevant monographs.

Starting materials no listed in a pharmacopoeia were described in detail. Adequate information was provided on the culture media composition and components.

RN1250 master seed virus

RN1250 is an engineered Marek's disease virus based on the MDV CVI988 parental vaccine virus that contains two copies of reticuloendotheliosis virus (REV) long terminal repeats (LTR) from MDV RM1 strain inserted in its genome. The RN1250 recombinant virus was generated by in vitro homologous recombination between CVI988 and a cosmid containing a genomic fragment of RM1 and of Md5 MDV1 strains. The sequence analysis confirmed that the RN1250 selected clone is a virus containing MDV genomic segment from three different MDV viruses: CVI988, RM1 and Md5.

The MSV was qualified using the following tests: bacterial and fungal sterility, mycoplasma, identity, virus titre and viral purity (as described in and in compliance with Ph. Eur. 2.6.24: Avian viral vaccines, tests for extraneous agents in seed lots). The working seed virus (WSV) is obtained from the MSV by carrying out passages in SPF chicken embryo cells. . The WSV was qualified using the following tests: bacterial and fungal sterility, mycoplasma, identity and virus titre. Both the MSV and WSV are stored frozen in liquid nitrogen.

The genetic stability of RN1250 was evaluated by comparing the genome structure of the RN1250 master seed virus (MSV) with that of RN1250 passaged in CEF cell cultures or in chickens. Results of different molecular biology techniques showed no difference between the in vitro and in vivo passaged viruses and the MSV, indicating RN1250 genetic stability. A deep sequencing of two monovalent RN1250 vaccine batches showed a very high sequence homology between those two RN1250 batches and MSV.

vHVT013-69 master seed virus

The vHVT013-69 virus was constructed by genetic engineering. The gene coding for VP2 protein was inserted in the HVT FC-126 vector. The sequence coding for the VP2 protein of IBDV was cloned from the IBDV 52/70 Faragher strain. An expression cassette was constructed that contained a promoter followed by the IBDV or Gumboro disease virus VP2 coding sequence (= VP2 "gene"), followed by a polyadenylation signal (polyA). The recombinant virus vHVT013-69 was obtained by homologous recombination.

The MSV was qualified using the following tests: bacterial and fungal sterility, mycoplasma, identity, virus titre and viral purity (as described in and in compliance with Ph. Eur. 2.6.24: Avian viral vaccines, tests for extraneous agents in seed lots). The WSV is obtained from the MSV by carrying out passages in SPF chicken embryo cells. The WSV was qualified using the following tests: bacterial and fungal sterility, mycoplasma, identity and virus titre. Both the MSV and WSV are stored frozen in liquid nitrogen.

In vitro genetic stability of recombinant virus vHVT013-69 has been assessed after *in vitro* passages on chicken embryo primary fibroblasts (CEF) using different molecular biology techniques. No differences were observed between the initial and the in vitro passaged viral populations. The stability of expression of the IBDV VP2 gene inserted into the recombinant virus vHVT013-69 was studied after several passages in vitro on CEF. 100% of the vHVT013-69 viral population expressed the IBDV VP2 protein after in vitro passages. In vivo genetic stability of recombinant virus vHVT013-69 has been

assessed after several successive passages in chickens using different molecular biology techniques. No differences were observed between the initial and in vivo passaged viral populations.

Solvent

The only starting material of biological origin for the production of the solvent that is not listed in a Pharmacopoeia is: casein hydrolysate. The casein used for casein hydrolysate preparation is made from bovine milk that is sourced from healthy animals originating from bovine spongiform encephalopathy (BSE)-free regions of the USA, New Zealand, Australia and Canada in accordance with the requirements of the European Note for Guidance on "Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products". The casein hydrolysate is irradiated (≥ 35 kGy).

Starting materials of non-biological origin

Detailed information and certificates of analysis were provided for all starting materials listed in Ph. Eur. demonstrating compliance with the relevant monographs.

Starting materials no listed in a pharmacopoeia were described in detail. Adequate information was provided on the culture media composition and components.

Control tests during the manufacturing process

Frozen cell suspension

Cultures and harvests are visually inspected..

Time is recorded during mixing and homogenising of active ingredients and excipients in the final formulation. Volume is measured during filling of finished product in vials. Filled vials are checked for appearance. During freezing of vials, time and temperature are recorded.

Solvent

Time is monitored during blending, sterilisation and drying. Osmolality and pH are measured at the level of the final bulk. Volume is measured during filling in bags. Terminal sterilisation is monitored. Temperature is recorded. The finished product is checked for appearance.

Control tests on the finished product

The description of the methods used for the control of the frozen cells suspension (RN1250 and vHVT013 identity, RN1250 and vHVT013 titration, visual appearance, pH, volume, bacterial and fungal sterility, mycoplasma and viral purity) and of the solvent (visual appearance, pH, volume, cryoscopic depression and bacterial and fungal sterility) were provided. The specifications proposed at release and at the end of shelf life are appropriate to control the quality of the finished product.

Batch-to-batch consistency

The applicant presented final product data for the manufacture of at least three consecutive final product batches (both frozen cell suspension and solvent). The results were compliant with the specifications as mentioned above.

Stability

No stability data were provided for the active ingredients, which is acceptable as they are not stored but immediately processed into finished product.

Frozen cell suspension

Long-term stability of the finished product was followed on 6 batches of Prevexxion RN+HVT+IBD (frozen fraction) manufactured on Boehringer Ingelheim Animal Health - Porte des Alpes manufacturing site (France) and stored in liquid nitrogen.

For these batches data up to 21 months are available. In conclusion, the stability of the vaccine over a period of 21 months has been confirmed and thus, a shelf life of 18 months storage in liquid nitrogen can be accepted.

The in-use stability of the reconstituted vaccine was evaluated after reconstitution of the thawed viral suspension in the solvent. Titration results show that the vaccine must be used within 2 hours at room temperature after reconstitution.

Solvent

The solvent has been historically used to reconstitute all Boehringer Ingelheim Animal Health cell-associated Marek's disease vaccines.. This solvent was granted a 36-month shelf life based on the historical stability study carried out on six batches of solvent bags. Except for the extractable volume and pH, the long-term stability results of the solvent do not show any change after 36 months of storage under long-term test conditions. The decrease in pH and volume over time were taken into account in the product specifications.

Very recently, a new stability study was launched on six additional batches. The stability results obtained under long-term test conditions do not show significant change after 30 months of storage for all the tested parameters, except for pH and extractable volume. As expected, a slight decrease in pH results is observed for all formats but all results remain within the specifications. A decrease of extractable volume is observed for all presentations but volumes remain within the approved specifications. The proposed shelf life of 36 months for the solvent is acceptable, but should be completed by the 36 months results of the recent study. The applicant has committed to provide these data when available.

Overall conclusions on quality

The applicant has provided a description of the composition of the vaccine, which consists of a frozen component with the active substances (RN1250 and vHVT013-69) and a solvent. Information was provided on the different containers for both active substance and solvent. The product development section describes the choice of the antigens, excipients and containers, as well as the virus titre specification limits. The proposed specifications for the vaccine strains are acceptable.

The manufacturing process of the active substances (RN1250 and vHVT013-69) and the final vaccine were sufficiently described.

All starting materials were listed and certificates of analysis were provided (where applicable). As regards to starting materials of biological origin, a detailed description was provided of the two vaccine virus strains (RN1250 and vHVT013-69). Virus seeds of both strains were properly qualified and shown to be genetically stable (for at least 5 passages).

An overview was provided of the control tests performed during manufacturing and of the control tests performed on the finished product.

Batch data were provided for 3 consecutive batches of the frozen cell suspension component and the solvent, confirming the validated status of the manufacturing processes. Also, homogeneity during formulation and filling was properly validated.

To support the proposed shelf life of 18 months, the applicant provided stability data of 6 vaccine batches (data up to 21 months). The results complied with the specifications. Accordingly, 18-month shelf life can be granted for the vaccine. The proposed shelf life of 36 months for the solvent is acceptable, but should be completed as commited by the applicant.

In conclusion, based on the review of data on quality, the manufacture and control of Prevexxion RN+HVT+IBD can be considered acceptable.

Part 3 – Safety

Introduction and general requirements

Prevexxion RN+HVT+IBD is a bivalent vaccine containing two cell-associated live GMO vaccine strains, an engineered Marek's disease virus (MDV-1) serotype 1, named RN1250 strain, in combination with an already authorised recombinant turkey herpesvirus (HVT) expressing the VP2 coding sequence of infectious bursal disease Virus (IBDV), named vHVT013-69. The vaccine is intended for a single administration to one-day old chicks by the SC route.

Safety documentation

The vHVT013-69 strain is already included in a marketed vaccine (Vaxxitek HVT+IBD); therefore 18 studies already presented to support the Vaxxitek HVT+IBD have been resubmitted in this application.

Prevexxion RN+HVT+IBD has been developed both in the US and EU. A total of 8 studies were carried out in the US where Prevexxion RN is on market since 2017. The similarity between the RN1250 component manufactured in the US and the one manufactured in the EU has been proven by appropriate documents.

The safety profile of Prevexxion RN+HVT+IBD is supported by 32 laboratory studies and 3 field studies. Requirements listed by Ph. Eur. monograph 0589 for MD (live) were fulfilled as well as general requirements of the Ph. Eur. general chapter 5.2.6 Evaluation of safety of veterinary vaccines and immunosera and in Directive 2001/82/EC. According to special requirements for live vaccines, studies were provided on the spread of the vaccine strains from vaccinated chickens to contact target species and non-target species, as well as the spread from non-target species to non-target species. Studies were provided also on dissemination in the vaccinated animals, on reversion to virulence, on biological properties and recombination or genomic re-assortment of the vaccine strains.

Laboratory tests

Safety studies were reported in compliance with Good Laboratory Practice (GLP) standards except for the US studies which complied to US regulation.

Safety of one administration of one dose and of an overdose

A first study investigated the vaccine at 1 and 10 times its highest titre. Day-old SPF chickens, the most susceptible birds, were vaccinated at the lowest age recommended in the SPC. Mortality and morbidity were monitored over 21 days and all dead birds were carefully necropsied.

Birds were properly vaccinated since RN1250 vaccine strain was only detected in the spleen of all the vaccinates tested on day 7 and anti-IBDV antibodies were detected in all tested blood from vaccinates on day 21.

One unspecific early mortality was observed in the control group and in the group vaccinated with the vaccine at 1 time its highest titre . No local reactions were detected. No MD-related signs or gross lesions were noticed. A mean 5% body weight growth retardation (11.8 g) was found out at the end of the observation period (day 21) with the overdose vaccination.

As the study is not compliant with Ph. Eur. 0589 requirements for residual pathogenicity, because the monitoring was shorter and fewer birds were included, another overdose study with the combined product was performed.

One-day old SPF chickens were vaccinated with a 10X overdose of the vaccine and monitored over 120 days; their body was weighted on D0 then at three occasions until D120. The chicken breed was fully susceptible to Marek virus since 89% get the disease after a challenge with a very virulent MDV strain. Birds were properly vaccinated since RN1250 vaccine strain was only detected in the spleen of all tested vaccinates on day 6 and anti-IBDV antibodies were detected in serum of all tested vaccinated birds on D42. Neither clinical signs nor mortality was observed while a transient (day 8 only) and slight (5%) growth slowdown was reported. This transient adverse event has been included in section 4.10 of the SPC.

Safety of the repeated administration of one dose

The repeated administration of the vaccine was not studied because it is intended to be administered once to each bird.

Examination of reproductive performance

The safety on the reproductive performance of layers has been addressed in a field study where both RN1250 (associated with a commercial live HVT vaccine) and RN1250+ vHVT013-69 vaccines were compared to an authorised vaccine with the same combination of valences. Two different breeds of pullets were monitored up to 77 or 85 weeks of age (whole production period).

The take of the vaccine was confirmed as the vaccine strain RN1250 was amplified by PCR overall in 67% of the tested spleens of the vaccinated groups sampled on day 8-9.

No difference between vaccines on the quality and the number of eggs was reported in pullets vaccinated according to the proposed SPC long before laying.

In conclusion, the vaccine can be administered safely to future layers and a proper warning include in the SPC reminds that the vaccine is intended to be administered to one-day old chickens.

Examination of immunological functions

Examination of vaccination on immunological functions was evaluated in three studies.

In a first study, the repercussion of RN1250 vaccine strain on the immune system was compared to

the one of a Rispens US licensed vaccine in a USDA compliant study. The anatomy of 3 organs of the immune system, the thymus, the bursa of Fabricius and the spleen was investigated at 4 time points up to 49 days after vaccination in vaccinates, in contact control birds or in controls reared without contact with vaccinates. The birds included in the study were SPF and vaccinated at one day of age, the lowest age of vaccination. RN1250 was administered at the highest passage (x5) and dosed just below the maximum release titre (3.7 instead of 3.9 PFU/dose) and Rismavac was at an equivalent titre (3.8 log10 PFU/dose).

No MDV-like gross lesions were reported. The immune organs/body weight ratios did not differ between RN1250 vaccinates and both control groups. On day 7 a mild to moderate lymphocyte depletion in the thymus (mean score of 2.3) was reported in both vaccines. An increase of germinal centres of the spleen was reported 4 weeks after vaccination in both vaccinate groups as well as RN1250 contact control group, which was probably caused by antigen stimulation of a subclinical infection.

Morphologically, RN1250 near its maximum titre had a slight and transient impact on the thymus.

In another study, the impact of vHVT013-69 vaccine strain at the highest passage (x5) and dosed above the maximum release titre (5.0 instead of 4.4 log10 PFU/dose) on the protection afforded by Newcastle vaccination was studied in a GLP study. The protection of Newcastle vaccination of SPF chickens after vaccination with vHVT013-69 was challenged 2 weeks further by IM injection of 10^5 LD50 of a virulent Newcastle disease virus strain. The Newcastle live vaccine was administrated at the minimal dose.

No anti-NDV antibodies were detected in controls conversely to all vaccinates. All the control birds died while all Newcastle vaccinated birds survived regardless of whether they were beforehand vaccinated with vHVT013-69 or not. The magnitude of ND seroconversion was similar in both NDV vaccinate groups. Therefore, vHVT013-69 did not impair the protection provided by Newcastle vaccination and showed no immunosuppressive activity.

To check whether the transient histological lymphocyte depletion of the thymus at D7 could portend a functional impairment of the immune system, the efficacy of NDV vaccination after Prevexxion RN+HVT+IBD or Prevexxion RN vaccination was challenged in a last study. Both Prevexxion vaccines were at least at their maximal titres and NDV vaccine was at minimal one used according to its label. Chickens were challenged 2 weeks later by a virulent NDV strain. While 100% of NDV non-vaccinated birds died within 2 days, 100% of the vaccinates were protected regardless of whether they were beforehand vaccinated with Prevexxion RN+HVT+IBD, Prevexxion RN or not. In conclusion, the vaccine does not impair the functioning of immune system as checked throughout its response to NDV vaccination.

In conclusion, despite a moderate histological lymphocyte depletion in the thymus transient at day 7, the immune system was able to mount a protective response to Newcastle disease virus demonstrated by a challenge 2 weeks later, suggesting that no functional immunosuppression is caused by vaccination with Prevexxion.

Special requirements for live vaccines

Dissemination in the vaccinated animal

RN1250

Four studies were performed to evaluate the dissemination of the vaccine strain in the vaccinated

animals.

In a first study, the dissemination of RN1250 vaccine at a 10-time overdose and MSV+5 passage in one-day-old SPF chickens was compared with that of a commercial vaccine) composed of a Rispens strain. In addition, its spreading potential was investigated by allowing contact of the vaccinates with hatch mates (contacts) in a ratio of 3 to 1. Birds were investigated at 4 time points up to 7 weeks after vaccination and monitored for clinical signs of MD daily. Tracheal and cloacal swabs were taken from 30/30 birds per group while feather follicles of 2/30 birds per group were collected. The spleen of 5 contact and 5 vaccinates was drawn 3 and 7 weeks after vaccination.

No vaccine strains were isolated from contact birds. The spleen of the vaccinates was not investigated. Rispens MD strain was isolated from the feather follicles of 2/2 bird 21 days after vaccination only, conversely to RN1250 which was never isolated. Neither Rispens nor RN1250 MDV strains were isolated from tracheal or cloacal swabs. Finally, MDV was never isolated from the spleen of birds in contact with Rispens or RN1250 MDV strains nor was it amplified by SR-1 MD PCR at the completion of the study (7 weeks) suggesting that there was no spread to contacts.

In a second study, the distribution of RN1250 in the spleen, liver, lungs, kidneys, and gonads was compared to the one of a commercial Rispens vaccine, in day-old SPF chickens. RN1250 was injected SC at the highest recommended dose and passage (3.9 log10 PFU/dose; MSV+5). One and 4 weeks after vaccination, spleen, liver, lungs, kidneys, and gonads where sampled from 4 to 5 vaccinates.

RN1250 was isolated from 1/4 spleens at day 28 while Rispens strain was isolated from 4/5 spleens both at day 7 and 28 as well as from few lung, kidney, liver samples and even one gonad sample.

In a third study, day-old SPF chickens were subcutaneously vaccinated in the neck on D0 with 4.3 log10 PFU, a vaccine amount above the maximum recommended dose and at MSV+2 passage. Another group of hatch mates which were left unvaccinated was put in contact with the vaccinated birds (equal number of birds for each group) in the same unit of cages rapidly after vaccination (day-old) and until the end of the study.

The dissemination of RN1250 vaccine strain in blood, spleen and feathers was checked weekly over 6 weeks after vaccination, in 5 vaccinated chickens and 5 contact chickens at each time point. RN1250 specific real-time PCR was carried out in blood, spleen and feathers samples which are known to be the most susceptible organs/tissues to MDV infection. In addition, the presence of RN1250 vaccine strain was investigated in the environment using the same technique carried out on dust samples taken on D21 and D42.
No clinical signs were observed except pecking in birds in day 35 which were preferentially chosen for sampling and no mortality was recorded. RN1250 vaccine strain DNA was amplified in the blood 7 days after vaccination, in the feather follicles up to day 21 and in the spleen up to the completion of the study (day 42). It was also amplified in the dust from the air filter on day 21 in coherence with feather results. On the other hand, none of the organ/tissues of contact birds was positive. .

This study showed that RN1250 vaccine strain DNA can be found in the skin and in dander in the dust aerosol but without biological significance for contact birds.

In conclusion, RN1250 strain was not isolated by cell culture from feathers in any of the studies (see also the first study described in the section "Biological properties of the vaccines strains – Replication particularities"). However, RN1250 DNA was detected in feathers up to 3 weeks after vaccination which was corroborated by environmental dust samples of the same study. Conversely, Rispens strain was isolated from 2/2 feather samples 3 weeks after vaccination. RN1250 virus was not isolated from cloaca and trachea swabs . In internal organs, spleen was the organ where RN1250 strain was detected the longest time (DNA still detected 7 weeks after vaccination) and conversely to the serotype 1 Rispens vaccine strain, RN1250 was not isolated from gonad, kidney, liver lung 1 or 4 weeks after

vaccination .

vHVT013-69 strain

The dissemination of vHVT013-69 strain was compared to its parental strain (HVT FC-126) by monitoring the bursa of Fabricius of SPF day-old chickens, the feathers and the tracheal and cloacal mucosae. Further to subcutaneous administration at D1 with vHVT013-69 (MVS+2) at a dose higher than the maximal dose (5.0 log10 PFU/dose) or HVT FC-126 at 5.1 log10 PFU/dose, or the diluent, chickens were monitored over 28 days.

To check the susceptibility of the SPF chicken to MD, a group of birds was challenged with RB1B strain and 5/10 birds died until the completion of the study.

Anti-HVT antibodies were detected in all vaccinates.

Both vaccine strains were found in feathers 14, 21 and 28 days after their injection and were not in the tracheal and cloacal mucosae as determined by cell culture.

In conclusion, the distribution of vHVT013-69 is similar to its parental strain and the strain was not found in tracheal and cloacal mucosae.

Spread of the vaccine strain

RN1250

In the first study described in section "Dissemination in the vaccinated animals" , where 10 birds were put in contact with 30 vaccinates with an overdose of RN1250 or a commercial Rispens vaccine, no MDV strain was isolated from the spleen of contacts nor was it amplified by SR-1 MD PCR at the completion of the study (7 weeks) suggesting a lack of live virus spreading to contacts.

The third study described in section "Dissemination in the vaccinated animals" corroborated the lack of spreading reported in the first study of the same section. The spread of the vaccine strain to contact hatch mates was monitored with a RN1250 specific real-time PCR on the spleen of contact birds sampled weekly until 42 days after vaccination at one-day old. RN1250 was detected in none of these spleens

In the first reversion to virulence study, 5 naïve birds were put in contact with 15 vaccinates at each of the 6 passages of the vaccine strain. The vaccine strain was never isolated from the blood of the contacts while it was from the blood of vaccinates.

In conclusion, the spreading of RN1250 strain has been examined in 3 studies. RN1250 vaccine strain was neither isolated nor amplified in the spleen of birds in contact with birds vaccinated with the highest recommended dose of vaccine or above.

vHVT013-69 strain

The spreading of vHVT013-69 strain has been examined in 3 studies.

In a first study, the spreading capability of vHVT013-69 vaccine strain to contact birds was compared to the one of its parental strain in SPF day-old chickens. This spread was investigated by both virus isolation and serology after a contact of 56 days between vaccinates (6 birds/group) with naïve birds (4 birds/group).

After SC administration at a titre above the maximum recommended dose (5.0 log10 PFU), vHVT013-69 and its parental strain did multiply in the birds since they were isolated both at 29 and 56 days

after vaccination while no virus was isolated from the contact birds.

All the birds vaccinated with vHVT013-69 seroconverted to IBDV while contact birds didn't. Each bird vaccinated either with vHVT013-69 or HVT FC-126 became MD seropositive both on D42 and D56 while their contacts did not.

In conclusion neither the parental strain nor vHVT013-69 were shown to spread to contact birds by direct or indirect assays.

However, the spread of vHVT013-69 to contact chickens was detected by serology in an efficacy study, described in sections "Onset of immunity" and "Maternally derived antibodies (MDA)", where chickens were challenged 5 days after vaccination. One out of 10 tested birds vaccinated with RN1250 and reared in contact with RN1250 + vHVT013-69 vaccinated birds, seroconverted to IBDV.

In conclusion, the spread of vHVT013-69, which was not detected in the study originally provided to support the authorisation, was incidentally detected in a new larger study intended to support efficacy of the RN1250+vHVT013-69 combination. The MD challenge undertaken in this study would have enhanced vHVT013-69 excretion 5 days after its administration in accordance with Islam's article (2007).

In a third study, the spread from vaccinated chickens to non-vaccinated turkeys was investigated. Turkeys were put in contact during 4 weeks with one-day-old chickens vaccinated either with vHVT013-69 at a titre above the maximum recommended dose (5.0 log10 PFU/dose) or parental HVT FC-126 strain at the same titre. The contact turkeys were kept for 2 weeks more and then the spread of the vaccine strain was investigated. Both vHVT013-69 and HVT FC-126 were found in turkeys 4 and 6 weeks after contact with vaccinated chickens and all turkeys in contact with vHVT013-069 vaccinates had already IBDV seroconverted upon 4 weeks. Anti-MDV antibodies in contact turkeys rose between 4 and 6 weeks.

Reversion to virulence of attenuated vaccines

RN1250

The reversion to virulence of RN1250 strain has been examined in 2 studies.

In a first study, reversion to virulence was monitored over 6 sequential passages of the master seed virus RN1250 in 15 one-day old chickens administered IP and the sequential passage 6 was compared to the initial MSV in 20 one-day old chickens, in a last study step as recommended in Ph. Eur. 0589. The initial birds were administered with 3.8 log10 PFU and then a suspension of white blood cells was injected to birds at each passage. The white blood cells were obtained from the birds of the previous *in-vivo* passage.

While RN1250 vaccine strain was passed successfully over the 6 passages, it was isolated from vaccinates' white blood cells from passages 2, 3, 5 and 6 but not from passages 1 and 4. The absence of growing RN1250 virus in the white blood cells of the first passage was corroborated by PCR at the last study step when it was compared to the passage 5 strain.

Contact birds were put together with vaccinates at each passage and RN1250 vaccine strain was never isolated from them.

At each passage, the clinical surveillance of 10 vaccinates (or 20 for the last step) lasted 49 days and none showed clinical signs or lesions associated with MD.

Another reversion to virulence study was performed, which complies with Ph. Eur. 0589 requirements. The passages were performed in SPF birds which were inoculated *in ovo* with RN1250 (MSV+2) with a

titre of 4.3 log10 PFU above the highest recommended titre (3.9 log10 PFU/dose). Then in-vivo passages were done every 7 days by IP administration with white blood cells sampled from all the birds of the previous in-vivo passage. The vaccine strain was passed over 5 in-vivo passages.

No impact on the hatchability was observed at first inoculation. Neither clinical signs nor MD lesions were reported over the 7 days of surveillance. The vaccine strain was isolated by cell sub-culture at first in-vivo passage (G1). It was necessary to have also a second in-vitro passage to clearly see the cytopathic effect at the second in-vivo passage (G2). From the 3rd in-vivo passage onwards, the vaccine strain virus was isolated upon first cell culture passage suggesting an increase of fitness of RN1250. The investigation of any reversion to virulence of passage 5 was then undertaken in the residual pathogenicity study presented below. While a slight reduction of growth (around 6%) was reported transiently for RN1250 P5, 8 and 61 days after administration, its intrinsic pathogenicity was not increased since no MD signs or lesions were reported when 45 vaccinates were monitored over 121 days.

In conclusion, while the RN1250 fitness was increased by 5 in-vivo passages, neither an increase of virulence according to Ph. Eur. criteria nor a spreading to contact birds were reported. These results have been corroborated by pharmacovigilance from non-EU countries where this vaccine is already marketed.

vHVT013-69 strain

The reversion to virulence of vHVT013-69 was monitored in SPF birds which were initially inoculated IM with vHVT013-69 (MSV+2) at a titre of 5 log10 PFU above the highest recommended titre (4.4 log10 PFU/dose). Then 9 in-vivo passages were done every 7 days by IP administration of 10 birds with a suspension of white blood cells . The white blood cells were obtained from the birds of the previous in-vivo passage.

The vaccine strain was passed over the 9 in-vivo passages and the vaccine strain was isolated in cell culture at each passage. Neither clinical signs nor mortality were noted within the 7 days of surveillance. The residual pathogenicity was then investigated in another study (see section "Biological properties of the vaccine strain – Residual pathogenicity" for the description of this study).

Biological properties of the vaccine strain – Residual pathogenicity

RN1250 strain

The pathogenicity of RN1250 strain at passage 5 (P5) embedded in white blood cell (around 3.5 PFU/chick) (obtained from the reversion to virulence study) was compared to that of the initial RN1250 strain (MSV+2) at more than 10 time overdose (5.3 log10 PFU MSV+2 RN1250) and to that of vvMDV strain (RB1B) and to control birds. One-day old chickens (at least 50) were administered with these strains and monitored over a 121-day period except those injected with RB1B strain. Indeed 19 birds injected with RB1B strain died or were euthanised until day 70 and 22/26 remaining birds had MD-related lesions. Birds included into this study were thus fully susceptible to MDV since RB1B strain caused MD in 86.7% of the birds.

The adequate take of the vaccine was confirmed since the vaccine strains were detected by PCR in the spleen of 5/5 and 3/5 birds injected with RN1250 (MSV+2) and RN1250 P5, respectively. Viremia was detected by virus isolation on cell culture when RN1250 P5 was injected and was not with RN1250 (MSV+2).
No growth retardation was noticed 121 days after vaccination by comparison to controls. On D8, significant differences in body weight between G1 (received MSV+2) and G2 (received passage 5) as

well as differences in virus isolation in the blood were found.

Results of body weight were not interpretable at D8, because several chickens lost their wing tags in groups GA, G1 and G2 after weighing at D8, which could have biased the statistical analysis.

Several birds were sick in vaccinated groups. However, MD suspicions were ruled out due to the absence of specific macro and microscopic lesions (5 birds).

In conclusion, in this study which is compliant to Ph. Eur. 0589, the master seed RN1250 virus at passage 2 (MSV+2) exhibited no residual pathogenicity nor was different from the strain collected further 5 in-vivo passages in birds.

vHVT013-69 strain

A study was undertaken to examine any modification of vHVT013-69 vaccine strain safety profile after 9 passages in SPF chickens in compliance with Ph. Eur. 0589 in force at the time of the study (1997); it is worth noting that vHVT013-69 vaccine strain was derived from the non-pathogenic MD strain of turkey by addition of the Gumboro VP2 gene and such a test is not required by Ph. Eur. 0589 for the parental strain. Groups of 40 birds were administered either with vHVT013-69 P9 (vaccine strain after 9 passages in SPF chickens) or with an overdose (10X) of vHVT013-69 (MVS+2) vaccine strain or with an overdose (10X) of vHVT013-69 (MVS+5) vaccine strain or with vHVT013-69 P9 amplified by 1 passage on chicken embryo cell culture.

The susceptibility of the chicks to MD was verified because 97.5% of another group of 40 birds died or showed macroscopic lesions of MD within 69 days further administration of a vvMDV strain (RB1B). Furthermore, the chicks were free of MDV since birds of an unchallenged control group were found having neither macroscopic/microscopic lesions of MD nor antibodies against Marek's disease virus over the 120-day monitoring period.

While the mortality in the group injected with vHVT013-69 P9 was around the lower Ph. Eur. 0589 threshold for study validation, none of these deaths were associated with MD macro/microscopic lesions nor were such lesions reported in birds necropsied at the completion of the study 120 day after administration. However, 1/5 bird of this group seroconverted to IBDV and none to MDV and the applicant justified that the viral load of vHVT013-69 P9 dose would have been very low, which was brought out in another study.

Besides, the vHVT013-69 P9 which was further passaged on chicken embryo cell, immunised 5/5 serologically tested chickens and did not cause any MD macro/microscopic lesions as well. This group met Ph. Eur. 0589 validation criteria.

Finally, the initial vHVT013-69 (MSV+2) and vHVT013-69 (MSV+5) strains, before any in-vivo passage, did not elicit any MD macro/microscopic lesions as well, while they immunised all tested birds of this group.

In conclusion, this study did not well address the virulence of vHVT013-69 after 9 in-vivo passage in birds since the dose administered to the susceptible birds would have been very low . But, the risk of increased virulence of vHVT013-69 strain has been shown negligible by the pharmacovigilance since this vaccine strain is on the market since nearly 15 years in the product Vaxxitek HVT+IBD.

Biological properties of the vaccine strains – Replication particularities

RN1250 strain

In a study, RN1250 close to its highest titre (3.8 log10 PFU/dose) was administered to one-day old SPF chickens and its dissemination at three time points up to day 49 was compared to the one of a commercial Rispens vaccine, at the same titre.

The chickens were susceptible to MD infection since Rispens vaccine strain was isolated or amplified over the 49 days of monitoring both in internal organs and feathers in line with the biology of this strain; viral particles were still isolated from the spleen of 2/5 birds, blood of 1/5 birds and lung of 1/5 birds, 49 days after administration.

RN1250 was isolated from 1 kidney, 1 liver, 2 blood and 2 spleen samples until day 14 while it was not in the feather; All samples at D49 were negative for RN1250 virus isolation. RN1250 was amplified by a specific RN1250 PCR, from 6/6 spleen samples, 5/6 blood samples, 2/5 kidney samples, 2/4 liver samples and 2/4 lung samples until day 14. This study confirms that RN1250 caused viraemia which lasted at least 2 but less than 7 weeks and that spleen is the organ where MDV strains were the most frequently detected.

The results from this study should be reconciled with those of the dissemination studies where RN1250 was compared with another MD serotype 1 strain, Rispens strain and where it turned out that RN1250 was less frequently detected. All these results suggest that RN1250 has less replicative properties in-vivo than other MD serotype 1 vaccine strains.

vHVT013-69 strain

The viremia of the vHVT013-69 vaccine strain in SPF chickens was compared to that of its parental strain (HVT). A very low load of virus - below the lowest dose of the vHVT013-69 (3 log10 PFU/dose) – was administered SC to 1-day old SPF chickens. Viremia was monitored by virus isolation on cell culture on white blood cells pooled by 2 birds. Blood samples were taken at 3 time points from day 28to 61 days after injection.

Viremia was detected in most of the samples of vHVT013-16 vaccinates on D28 (4/5 positive pools), and in all of them afterwards, while all the samples of HVT parental strain vaccinates were positive at each date of sampling. At D61, the applicant quantified the virus load grown in the cell culture and found that vHVT013-69 viremia was 4 times lower than HVT parental strain viremia (respectively 0.16 and 0.66 PFU/10e6 leukocytes).

In corroboration of this viremia, all birds had seroneutralising antibodies to IBDV from 3 weeks onwards after vHVT013-69 administration while control birds didn’t.

In conclusion, vHVT013-69 as well as its parental strain caused a viremia longer than 8 weeks but its load in white blood cell after 61 days was 4 times lower than the parental strain.

Finally, direct evidence of possible interference between RN1250 and vHVT013-69 (rHVT) is missing. However, negative interference of RN1250 on HVT replication has been suspected because the level of IBD protection in conventional chickens with maternally–derived antibodies decreased when RN1250/HVT ratio increased from 1:4 to 2:1 . The final composition has been adapted accordingly. However, the possible reverse interference does not appear to be biologically significant because both strains contribute to Marek’s disease protection and while their specific contribution remains non-dissected, an analysis of this risk has concluded that this is negligible.

Safety for various species

RN1250

In a study, RN1250 vaccine strain at its highest titre in the finished product (4.7 log10 PFU/dose) and its highest passage (MSV+5) was administered subcutaneously to SPF chicken, quail, turkey, duck, pheasant and pigeon avian species.

Clinical signs were monitored over a period of 46-47 days and MD gross lesions were investigated in dead or euthanised birds. MD seroconversion was assessed 46-47 days after vaccination.

Neither clinical signs nor lesions were observed. No birds did MD seroconvert over this period which is not abnormal as MD serology in general is not a proper tool to monitor vaccine exposure. RN1250 was found to be harmless to these species directly by administration as the wild MDV strains in ducks and pigeons in the literature (Schat and Nair, 2013).

To rule out that RN1250 was able to replicate into mammalian tissues, 21 female SPF mice, 6-7 weeks old, were administered both subcutaneously and intradermally with 4.9 log10 PFU. Five mice were investigated between 3 and 14 days further the administration of an overdose of the vaccine (around 10 time) by both routes. No virus was isolated from their trachea, brain, spleen, heart, liver, lungs, kidneys, and their skin tissue at the 2 sites of inoculation.

vHVT013-69 strain

The safety of RN1250 strain on various species has been examined in 10 studies.

In a first study, vHVT013-69 (MSV+5) was administered subcutaneously to 7 days old pheasants above its maximum dose (5.0 log10 PFU/dose). Neither clinical signs nor macroscopic lesions were observed after 42 days while the replication of vHVT013-69 was noted but at a low level compared to chickens (IBDV seroconversion).

In a second study, vHVT013-69 (MSV+5) was administered subcutaneously to 7 days old Pekin ducks above its maximum dose (5.0 log10 PFU/dose). No clinical signs or macroscopic lesions were observed in vaccinated ducks after 42 days. The vaccine can thus be considered as safe in ducks. A minor vHVT013-69 replication was noted (IBDV seroconversion).

In a third studyvHVT013-69 (MSV+5) was administered subcutaneously to 7 days old partridges above its maximum dose (5.0 log10 PFU/dose). No clinical signs or macroscopic lesions were observed in vaccinated partridges after 42 days. A vHVT013-69 replication (IBDV seroconversion) was noted in partridges but lower compared to chickens.

In a fourth study, vHVT013-69 (MSV+5) was administered subcutaneously to 6 weeks old conventional pigeons above its maximum dose (5.0 log10 PFU/dose). No clinical signs or macroscopic lesions attributable to MDV or IBDV were observed in vaccinated pigeons after 42 days. Given the high dose administered and the low levels of antibodies measured, it is thus likely that there is no multiplication of the virus in pigeons. The vaccine can thus be considered as safe in pigeons.

In a fifth study, vHVT013-69 (MSV+5) was administered subcutaneously to 7 days old conventional quails above its maximum dose (5.0 log10 PFU/dose). No clinical signs or macroscopic lesions were observed in vaccinated quails after 42 days. A vHVT013-69 replication (IBDV seroconversion) was noted in large majority of the quails. Immunofluorescence (IF) results were positive for inoculated chickens only (serotype III). No anti-Marek's disease antibodies were found in inoculated quails. This can be explained by a lack of sensitivity of the technique used in quails (the goat anti-chicken

conjugate may not have detected quail antibodies).

A sixth study was performed in quails where vHVT013-69 at different titres was compared to its parental strain. vHVT013-69 (MSV+5) was administered to 2 weeks old conventional quails either subcutaneously at 3 different doses (1, 3, 5 log10 PFU) or by the oropharyngeal route (50 log10 PFU) and was compared to HVTFC-126 administered at 5 log10 PFU either subcutaneously or by oropharyngeal route. The positive virus isolation results show that both the vHVT013-69 virus as well as HVT virus replicate in quails. The level of replication seems to be lower after inoculation by oropharyngeal route compared to subcutaneous administration. However, neither MD gross nor microscopic lesions were observed in quails after 42 days. It can be concluded that vHVT013-69 and the HVT parental strain are safe for quails.

In a seventh study, vHVT013-69 (MSV+5) was administered subcutaneously to 11 days old SPF turkeys above its maximum dose (5.0 log10 PFU/dose) and compared to its HVT parental strain. While all vaccinated turkeys seroconverted, no MD adverse clinical or pathological events were observed after 42 days.

The previous study was carried out under laboratory conditions, with SPF and highly susceptible turkeys. To complete the knowledge about the spread in turkeys in more realistic conditions, another trial was carried out to test the spread of the vaccine strain in a context of a HVT infection.

A complex study design was devised to assess spread of vHVT013-69 strain and its HVT parental strain from day-old conventional turkeys to HVT-infected or non-HVT-infected turkeys over 1 or 2 passages. To this end, conventional turkeys which were put in contact with vaccinated turkeys over a 28-day period were put again in contact (2nd passage) with new day-old conventional turkeys over a successive 29-day period.

The viral isolation results showed that the conventional day-old turkeys were not naturally infected with HVT virus but they did have maternally derived MDV antibodies. Vaccination with HVT or vHVT013-69 brought about a MD seroconversion in few birds after 28 days (2/10) which was complete within 7 weeks. vHVT013-69 triggered IBDV seroconversion in almost all vaccinates within 28 days.

Serology demonstrated that HVT spread from inoculated to non-vaccinated turkeys while vHVT013-69 spread was also confirmed, although it was less marked than the HVT spread. At the first passage, the spread of vHVT013-69 to HVT-vaccinated turkeys seemed to be unlikely. Moreover, even if this spread was possible, it did not occur at the second passage. And in presence of HVT-vaccinated turkeys, vHVT013-69 was unable to spread to non-vaccinated contact turkeys. In this study, vHVT013-69 did not spread from turkeys to turkeys in the context of a HVT infection.

In a ninth study and in order to assess the general safety of vHVT013-69 in mice, SPF mice (17-21g), were administered subcutaneously with 4.9 log10 PFU either with vHVT013-69 (MSV+2) or the HVT parental strain. Neither abnormal mortality nor morbidity were reported over the 7-day monitoring and no statistical difference was evidenced between groups regarding body weight gain. There was no evidence of an abnormal toxicity in mouse of vHVT013-69 and the parental strain.

In a last study, SPF guinea pigs (about 271-303g), were administered subcutaneously with 4.9 log10 PFU either with vHVT013-69 (MSV+2) or the HVT parental strain. Neither abnormal mortality nor morbidity were reported over the 7-day monitoring .

Recombination or genomic reassortment of the strains

RN1250 strain

The safety of the co-administration of RN1250 with the HVT vaccine strain or with the vMDV GA 22 was

studied in 1-day old SPF chickens. RN1250 was administered above its highest recommended dose (4.0-4.1 log10 PFU/dose) while vMDV GA22 titre was 3.7-3.6 log10 PFU/dose and HVT titre was 3.9 log10 PFU/dose. The body weight gain, MD associated morbidity mortality and gross lesions in sciatic nerve, liver, spleen, kidneys, gonads, bursa, and skin with feather follicles were monitored over a 35-day timespan and viraemic strain and splenic viral loads were explored at day 21 and 35 respectively.

As expected, birds administered with vMDV GA 22 alone or HVT alone respectively did and didn't have MD; MD lesions were found in 15/49 vMDV GA 22 administered birds. vMDV GA 22 and HVT strains were isolated from 5/5 and 8/10 spleen samples respectively 35 days after administration. The co-administration of these strains with RN1250 decreased the course of MD caused by vMDV GA 22 while no change was reported for HVT.

In co-administration with vMDV GA 22, RN1250 decreased MD lesions upon the completion of the study (2/49 instead of 15/49 in groups RN1250+vMDV GA 22 and vMDV GA 22 respectively). However, there were no statistical differences in average body weight between the 2 groups. And only vMDV GA 22 strain was isolated and amplified in the spleen (D35) or in the blood (D21) of the co-administered chickens by a qualitative PCR.

The co-administration with HVT did not bring about any pathogenicity and again, only HVT strain was amplified in 10/10 spleens (D33) or isolated in the blood (D21).

In conclusion, when co-administered with two very different strains, a pathogenic and an apathogenic one, it was always the co-administered strain which was detected in the blood or in the spleen. However RN1250 did have an actual effect on vMDV GA 22 by lessening its pathogenicity over the 35 days of the study. With this MDV strain, the effect was beneficial. However, the mechanisms underlying this effect are largely unknown and it is not possible to rule out the involvement of recombination.

vHVT013-69 strain

The safety of the co-administration of vHVT013-69 with serotype 1 vaccine strain (Rispens) or mild pathogenic strain (MDV-1 GA22), or with the serotype 2 vaccine strain SB1, was studied in 1-day old SPF chickens, by comparison to each virus administered alone. vHVT013-69 was administered at an intermediate dose to the recommended one (titre 4.0 log10 PFU/dose) while the other strains were administered at a dose lower than the vHVT013-69 one, that is 2.1 log10 PFU/dose (MDV-1 GA22), 3.7 log10 PFU/dose (MDV SB1) and 3.7 log10 PFU/dose (Rispens virus). The body weight gain, MD associated morbidity, mortality and gross lesions were monitored over a 120-day period and sciatic nerves, liver, spleen kidneys of 10 birds administered with the pathogenic strain MDV GA22 were sampled upon completion of the study. Blood was drawn at D0 and D120 to substantiate seroconversion to MDV-1, MDV-2 and MDV-3.

All the sampled birds administered with a MDV strain seroconverted to MD, all serotypes taken into account, whilst none seroconverted in the control group.

Half of the birds injected with the mild pathogenic strain (MDV GA22) (25/50) died with MD macroscopic or microscopic lesions within the 120 days. One out of the 50 birds administered with the SB1 strain had paralysis and MD neurological lesion (lymphoid infiltrates). Neither of the Rispens injected birds nor the vHVT013-69 ones experienced MD.

Finally, the co-administration of vHVT013-69 did not modify the outcome of the multiplication of the other strains either on the percentage of MD sick birds or on the mean bodyweight gain of groups. MD was diagnosed in 27/50 birds co-administered with MDV GA22, 1/50 birds with SB-1 (lymphoid infiltrates) and none with Rispens strain.

However, a difference of mortality rates in the groups administered either with MDV GA22 or MDV GA22+ vHVT013-69 within the course of the disease, could portend a negative interference of vHVT013-69 on MDV GA22 induced MD.

In conclusion, in this study vHVT013-69 did not increase the pathogenicity of the co-administered mild pathogenic MDV-1, MDV-1 or MDV2 vaccine strains.

User safety

Prevexxion RN+HVT+IBD vaccine is a cell-associated live vaccine which contains the recombinant herpesvirus of turkey strain vHVT013-69 and the MDV-1 recombinant strain RN1250. In general, avian herpesviruses are not known to be a hazard to humans. Avian herpesviruses are not indicated in EU Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work. Their genetic modifications have attenuated the genuine biological properties of the strains and a study in mice has corroborated that RN1250 does not infect mammals.

Prevexxion RN+HVT +IBD contains no adjuvant, but the following excipients: DMSO and dilution medium and is diluted in a solvent including sucrose, casein hydrolysate, phenol red and salts before administration. The DMSO present in the vaccine is listed in Table 1 of Commission Regulation No 37/2010/EC and is considered to be safe and none of diluent's ingredients poses a risk for the user.

The vaccine is filled in glass ampoules stored in liquid nitrogen which might explode when removed from cold storage and thawed, leading to exposure or cuts by the glass. This risk is considered very low because these frozen ampoules are already very common in practice today, and users are professionals well trained to handle this kind of vaccine.

The risk of accidental self-injection of the vaccine to the user when administered subcutaneously to day-old chickens is low as the users are trained professionals and the volume to be injected is small (0.2 ml). The consequences of accidental self-administration are therefore negligible.

Pharmacovigilance for both RN1250 and vHVT013-69 have no reported human adverse event.

Finally, in sections 4.5 and 4.9 of the SPC the attention of the user of the vaccine is drawn on the potential risk of ampoule explosion and advices are given how to handle the administration.

Study of residues

Prevexxion RN+HVT+IBD is a bivalent vaccine which does not contain any adjuvant.
The components are either listed in table 1 of the annex of Commission Regulation No. 37/2010 as allowed substances for which no MRLs are required or considered as substances not falling within the scope of regulation (EC) No. 470/2009 (e.g. active principles of biological origin intended to produce active immunity, used in IVMPs) with regard to residues of veterinary medicinal products in foodstuffs of animal origin. The theoretical maximal concentration of gentamycin per dose of vaccine is considered negligible.

Interactions

The applicant has not provided data investigating interactions of the vaccine with other veterinary medicinal products and therefore proposed to include a statement in Section 4.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case-by-case basis.

Field studies

Safety of the Prevexxion RN+HVT+IBD (referred as to RN1250+vHVT013-69) and Prevexxion RN1250 (referred as to RN1250) was investigated in three field studies in broilers and pullets.

A first studywas performed in order to substantiate the safety and the efficacy of the monovalent (RN1250) and bivalent (RN1250+vHVT013-69) vaccines in long-living broilers throughout their production life, until slaughter at about 80 days of age.

Vaccination with RN1250 was compared to vaccination with a commercial Rispens vaccine in 2 farms (4 subgroups) while vaccination with RN1250+vHVT013-69 was compared to vaccination with a commercial Rispens+HVT vaccine associated with a classical live intermediate IBDV vaccine in 2 other farms (4 subgroups).

Birds were vaccinated subcutaneously at hatchery either with RN1250 or with RN1250 + vHVT013-69 at intermediate titre or with commercial batches of Rispens or Rispens+HVT vaccines. Then both vaccinated groups and comparator groups were raised until slaughter in 4 farms in France. Birds vaccinated with Rispens+HVT vaccine were vaccinated on day 20 and 26 with a classical live intermediate IBDV vaccine by drinking water.

Mortality and morbidity were recorded daily as well as the feed intake. Birds randomly selected each week were weighed. To check the vaccination take, spleen was taken from 10 birds of each subgroup at day 8/9 and blood from birds vaccinated with RN1250+vHVT013-69 or Rispens+HVT/live intermediate IBDV (4 subgroups) at 5 occasions between around days 20and 80. Birds were slaughtered at about 80 days of age; their weight and the number of condemned animals were recorded.

RN1250 vaccine strain was detected in 90% of the spleen samples from RN1250 and RN1250+vHVT013-69 vaccinates. Birds seroconverted to IBDV by 30 days after vaccination with RN1250+vHVT013-69 while 1 out of the 2 subgroups vaccinated with the live intermediate IBDV vaccine seroconverted at day 53.

Neither immediate reactions after vaccination nor adverse events throughout the study were reported. Mortality rate between subgroups raised in a same farm was not different and below the alert threshold of 2%. One week after vaccination, body weight growth was slightly increased in RN1250 vaccinates in one farm and inversely in the other one. RN1250 + vHVT013-69 slightly increased body weight growth in one farm and didn't in the other one. However, no difference in term of slaughtered or condemned birds, feed intake and feed conversion were reported at the time of slaughter.

In conclusion, no solid differences in term of safety issues and production parameters of long-living broilers were reported between RN1250 or RN1250+vHVT013-69 and the commercial control vaccines used.

The safety and efficacy on layers have been addressed in a field study where both the bivalent RN1250+vHVT013-69 and monovalent RN1250 (associated to an authorised HVT vaccine) vaccines were compared to an authorised Rispens+HVT vaccine with the same combination of valences. Two different breeds of pullets were monitored up to 30 weeks of age (12 weeks after they have started laying), each one in a multiple-site production system of 2 farms. At day 1 of age, 20,000 female chicks were injected subcutaneously with RN1250 (associated to an authorised HVT vaccine) or Rispens+HVT vaccine and about 60,000 with RN1250 + vHVT013-69 or Rispens+HVT vaccine followed by a classical live intermediate IBDV vaccine at day 22 and 29 in the farm. From 18 weeks of age onwards, birds were transferred to 2 laying farms each farm raising a vaccine group and its control group in the same time.

The take of the vaccine was confirmed as the vaccine strain RN1250 was amplified by PCR overall in

67% of the spleens of the vaccinated groups sampled on day 8-9. No immediate reactions after vaccination were reported as well as no MD clinical signs, but one RN1250-vaccinated pullet which showed MD paralysis 111 days after vaccination.

Overall mortality was low whatever the group and the stage of the life (1.2 to 4.2%). While mortality of birds vaccinated with RN1250+vHVT013-69 was higher than control at the pullet stage, it was the reverse at the layer stage; this observation is associated with a colibacillosis outbreak at the start of the raising. There were no differences for the group vaccinated with the monovalent vaccine.

However, no difference in body weight between vaccinates and their relative control 18 weeks after vaccination and further on, nor was the feed intake of birds reared in the same condition.

There were no differences on the quality (class 1, decommissioned and destroyed) and the number of eggs.

The applicant has provided the report of a third study which is considered valid despite chickens were vaccinated *in ovo* with MD and IBD vaccines prior to their inclusion into this study. However the applicant invalidated this study because of this previous inoculation. Regarding safety aspects, no negative impact on the outcome of the study due to prior vaccination is suggested.

Environmental risk assessment

The proposed vaccine is compliant with Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms.

The applicant has provided a detailed risk assessment of the combined vaccine containing both RN1250 and vHVT013-69 viruses compliant to NTA ENTR/F2/KK D (2006).

Hazards

Both vaccine strains were shown to infect/replicate only in susceptible birds, but not in mammals and while they were found in feathers, only vHVT013-69 was shown to be able to spread to susceptible chickens under very specific conditions.

The biological pattern of both vaccine strains was shown to be not very different from their parental strains and their genetic and phenotypic stability after serial passages in chickens was acceptable. Components, other than the vaccine strains, used to formulate the vaccine are classical components, used in many biological products, known for their absence of effect on the environment.

Taking all the risk factors into consideration, the level of risk to the environment of Prevexxion RN+HVT+IBD can be considered as negligible. Prevexxion RN+HVT+IBD is not expected to pose a risk to the environment when used according to the SPC.

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

Detailed information was provided on the possible environmental risk of the vaccine that contains two genetically modified live viruses.

The vector construction, vector elements, analytical test methods and vector characteristics including genetic stability, have been extensively described for both the RN1250 virus strain and the vHVT013 virus strain. The vectors do not contain any potentially harmful sequences. All sequences present also

occur in naturally occurring viruses. The associated risk of recombination events between circulating avian retroviruses and RN1250 containing two copies of REV LTRs was thoroughly discussed and the risk assessment amended by the applicant as requested.

Information was provided on possible release of these GMOs to the environment. The likelihood is considered very low. In case of spread, homologous recombination with other (wild type) herpesviruses cannot be excluded. However, such recombination events (if any) would result in viruses with the same characteristics as either the wild type variant or the vaccine virus; it cannot result in a more virulent strain. Besides birds, no other species are known to be infected with the vaccine viruses, which are non-pathogenic for humans. Consequently, there is no intrinsic risk for humans related to the vaccine.

HVT strains are widely used as vaccine strains against Marek's disease. Their safety in target and non-target species has been proven for many years.

In conclusion, the overall risk of the current vaccine towards humans and the environment could be considered negligible.

Overall conclusions on the safety documentation

The short-term clinical safety of 10 time the maximum release titre (overdose) and a dose at the maximum release titre of RN1250+vHVT013-69 vaccine was investigated over 21 days further injection to one-day old SPF chickens and only a slight growth retardation at the overdose occurring at the end of the study, was reported.

The long-term safety at more than 10 times the maximum release titre of RN1250 strain and vHVT013-69 strain was studied separately at 121 days after vaccination in a Ph. Eur. 0589 compliant studies and no impact on growth or MD clinical signs or lesions were observed (residual pathogenicity studies). In another Ph. Eur. 0589 compliant overdose study with the combined product a transient (day 8 only) and slight (5%) growth slowdown was reported. This transient adverse event has been included in section 4.10. of the SPC.

To rule out an impact of vaccination on reproductive performance, the applicant showed in a field study that neither RN1250 nor RN1250+vHVT013-69 have an impact on the quantity and quality of laying in laying hens.

Absence of impact on the immune system was demonstrated at the functional level for both vHVT013-69 and RN1250 (no impact on Newcastle vaccine protection) and at macroscopic level (no impact on thymus, bursa or spleen weight on body weight ratios measured at 3 time points after vaccination), while a slight and transient lymphocyte depletion of the thymus was detected 7 days further RN1250 administration.

The biology of the RN1250 strain has been determined in SPF chickens. RN1250 strain caused a 2-week viremia in the course of which it was found by culture isolation or DNA amplification in whatever organs it was searched (lung, liver, kidney and spleen). Then the strain was only isolated in the spleen at 4 weeks and its DNA detected at 7 weeks.

By comparison with Rispens vaccine strain, which is its main parental strain, RN1250 strain exhibited a weaker biology. RN1250 replicated less and for a shorter time in chickens. In spleen, which is the main organ where MDVs are replicating, RN1250 was isolated in 1/4 spleen 28 days after administration, while Rispens strain was isolated both at 7 and 28 days after administration in 4/5 spleens . However, RN1250 DNA was detected from day 7 to day42 in spleen. In other internal organs, while Rispens viral particles were isolated up to 7 weeks in lung and blood, RN1250 viral particles were isolated from few birds at D7 from liver and kidney, and only up to 2 weeks in blood only.

In feathers, RN1250 was never isolated but its DNA was able to be amplified up to 2 weeks after injection while Rispens strain was isolated 3 weeks after injection .

The biology of vHVT013-69 is similar to its parental strain (HVT FC-126). vHVT013-69 distribution was investigated in organs associated with its spread. Live vHVT013-69 was isolated from feathers 14, 21 and 28 days after its injection but not in the tracheal and cloacal mucosae and its viremia was still detected at 61 days, albeit with a viral load 4 times lower than its parental strain.

No spread of RN1250 to naïve one-day-old chickens was detected in the spleen after a 7- or 6-week contact with vaccinates in line to what was observed for the Rispens parental strain in the tested conditions.

vHVT013-69 didn't spread to naïve chickens after an 8-week contact with vaccinates. However, limited spread to RN1250 vaccinated birds (1 positive out of 10 contact birds) was detected in an efficacy study were birds were also challenged. This result shows that under special conditions and presumably very rarely, vHVT013-69 can spread from chicken to chicken. As anticipated for an HVT derived vaccine, SPF turkeys put in contact with chickens vaccinated with vHVT013-69 were all infected with the vaccine strain. In a study in conventional turkeys, vHVT013-69 could only spread to conventional in-contact turkeys in the absence of an HVT infection. An appropriate warning about potential spread of the vaccine strains is included in the SPC.

Quail, turkey, duck, pigeon and pheasant were currently shown unsusceptible (no seroconversion, no clinical or pathological findings) to SC administration of RN1250 near its maximum titre. RN1250 was not isolated from mice after vaccination.

vHVT013-69 can replicate in pheasant, Pekin duck, partridges quails and doubtfully in pigeons but neither clinical signs nor lesions were detected. An IBDV seroconversion was reported in pheasants, Pekin ducks, partridges, quails and doubtfully in pigeons. Conversely to RN1250, vHVT013-69 replicated well in turkeys but did not induce MD clinical signs or lesions. No morbidity was observed during a seven days' observation period when mice or guinea pigs, considered as a mammalian models, were vaccinated with vHVT013-69.

In one of the two different reversion to virulence studies, RN1250 load in blood increased over passages which would suggest that the viremia ability of the strain was increasing over passages. However, no MD clinical signs or gross lesions were reported in the 10 birds monitored over 49 days at each passage nor were they reported in birds administered with a 10X overdose of MSV+2 strain or with back passage 5 strain. The same kind of study was undertaken for vHVT013-69 while this was not required by Ph. Eur. 0589 for a MDV-3 derived strain, and no increase virulence was reported.

Recombination of the vaccine strains with other Mardiviruses (MDV-1 GA22 or MDV-2 SB1) was experimentally checked in 2 studies and their co-administration did not increase the clinical presentation which was similar to the one of the co-administered Mardiviruses suggesting that if recombination or genomic recombination occurred, they had no detrimental impact on the safety profile of the vaccine strains.

Besides the applicant has argued that wild MDV-1 naturally recombinant with avian retroviruses are circulating worldwide and that the risk of an increase of virulence further recombination with vaccine strains is highly unlikely. Therefore, recombination's, if any, are considered to pose negligible risk to the target species, the environment and the human beings.

Information concerning release of genetically modified organisms into the environment has been provided and the associated risks assessed. The two GMO vaccine strains have been shown to be phenotypically stable over 5 passages. The insertion of the foreign genetic information did not change their non-pathogenic properties in target species or other avian species or mammalians. Any risk emerging from the use of the Prevexxion RN+HVT+IBD is negligible for humans and has to be

considered as low for the environment.

Since the absence of replication of the vaccine strains in mammalian tissues was corroborated by 3 experimental studies, the user safety encompasses mainly the handling of vaccine ampoules frozen in liquid nitrogen and the risks of needle stick injuries. Adequate warnings have been included in sections 4.5 and 4.9 of the SPC.

Residues of the vaccine are not considered to represent a consumer safety concern; a withdrawal period of zero days would be appropriate.

The safety profile was confirmed in a field study with broilers and another one with pullets where the vaccine was compared to already marketed vaccines. Neither adverse reactions nor worsening of zootechnical parameters were reported.

The vaccine is considered to be safe for the target species and non-target species, the user, the consumer and the environment.

Part 4 – Efficacy

Introduction and general requirements

Efficacy of Prevexxion RN+HVT+IBD against Marek's and Gumboro disease was investigated. The vaccine is claimed to prevent mortality and clinical signs and reduce lesions caused by Marek's disease (MD) virus (including very virulent MD virus) and to prevent mortality, clinical signs and lesions caused by infectious bursal disease (IBD) virus further vaccination of one-day-old chicks.

Challenge model:

Studies were designed in compliance with requirements of Ph. Eur. monograph 0589 on Marek's disease vaccine (live), and Ph. Eur. monograph 0587 on Gumboro vaccine (live).

MD challenge was performed with the MDV-1 strains RB1B which is classified as very virulent according to ADOL scale and Gumboro challenge with the strains Faragher (classical strain), and 100045 or 168/980702 which were claimed to be very virulent.

Efficacy parameters and tests:

The diagnostic criteria of a Marek's disease or a Gumboro disease applied in the laboratory studies were those described in Ph. Eur. monograph 0589 and Ph. Eur. monograph 0587 respectively. When relevant, efficacy criteria set by monographs were applied.

Efficacy documentation

The efficacy of Prevexxion RN+HVT+IBD was demonstrated in 8 laboratory studies and 2 field studies associated with 3 laboratory challenges.

Laboratory trials

Dose determination

The MD protection afforded by a range of doses of RN1250 monovalent vaccine, from 2.5 up to 3.1 log10 PFU/dose, against a challenge by a vvMDV strain (RB1B) was determined 4 days after vaccination of SPF chickens at 1 day of age and compared to the protection provided by an authorised SR-3 vaccine. Groups of 35 birds were clinically monitored over a 45-day post-challenge period.

The severity of the challenge was high; 91% of control birds were MD positive. RN1250 prevented MD in more than 80% of the chickens of all vaccinated groups, meeting the minimum relative protection Ph. Eur. 0589 criteria, while the SR-3 vaccine failed protection (73%). While RN1250 met Ph. Eur. 0589 criteria for all tested doses 4 days after vaccination, the applicant chose the 2.9 log10 PFU dose as the minimum because it provided 94% of protection.

The study can be accepted as dose determination study but not as onset of immunity study since a reduction of the observation period from 70 days as requested by the Ph. Eur. monograph 0589 to 45 days is not acceptable.

Onset of immunity

The MD protection afforded by RN1250 strain only, at its minimum dose (2.9 log10 PFU) or in association with vHVT013-69 at its minimum dose (3.6 log10 PFU) was determined 9 days after vaccination of SPF chicken at 1 day of age. This study was compliant to Ph. Eur. 0589 and birds were monitored over 70 days.

The severity of the challenge (73% of MD positive birds) was above the threshold required by Ph. Eur. 0589.

The RN1250 at its lowest dose, alone or with vHVT013-69 provided a relative protection against MD caused by a challenge with a vvMDV strain (RB1B) in 91% and 100% chickens which were vaccinated 9 days beforehand. .

The protection against Gumboro disease afforded by a Prevexxion RN+HVT+IBD at the minimum dose (RN1250 strain 2.9 log10 pfu/dose; vHVT013-69 3.6 log10 pfu/dose) was determined 14 days after vaccination of SPF chicken at 1 day of age.

The correct take of the vaccine was confirmed since the RN1250 vaccine strain was detected by RT-PCR in 5/5 vaccinates and anti-IBDV antibodies were detected in 6/10 vaccinates while control birds remained negative.

The birds were challenged with 2.8 log10 EID50 of a classical pathogenic strain (Faragher) by ocular route. The severity of the challenge was higher than Ph. Eur. 0587 requirement. 100% of the control chickens showed characteristic signs of the disease (5 deaths, 3 euthanised birds, macroscopic and microscopic lesions in the 4 surviving chickens with a bursa histological lesion score of 4 or 5).

Vaccination with Prevexxion RN+HVT+IBD at the minimum virus content for each of the active substances resulted in full protection (no mortality, no clinical signs no lesions and a very higher bursa weight/bodyweight ratio of the vaccinates) 14 days after a challenge with a classical virulent IBD strain.

A 9-day onset of MD immunity may thus be granted as well as a 14-day one for Gumboro disease. However, 5 days could be acceptable for onset of MD immunity, drawn from the study presented in

section “Maternally derived antibodies (MDA)”, which is more demanding than Ph. Eur. 0589 requirements.

Duration of immunity

The immunisation by herpesvirus such MDV is lifelong and only chicks are at risk of MDV infection. Therefore, it is accepted that no study has been undertaken to back the duration of MD immunity.

The protection against Gumboro disease afforded by Prevexxion RN+HVT+IBD at low dose (RN1250 strain 3.0 log10 pfu/dose; vHVT013-69 strain 3.6 log10 pfu/dose) was determined 70 days after vaccination of conventional layer chickens at 1 day of age.

The birds had anti-MDV antibodies (measured by specific immunofluorescence), and a high level of anti-IBDV antibodies at vaccination (G0 control group) which disappeared before challenge.

The RN1250 vaccine strain was detected by RT-PCR in 3/5 birds 7 days after vaccination and high level of anti-IBDV antibodies was obtained in all the tested vaccinates 70 days after vaccination. Neither the RN1250 vaccine strain nor anti-IBDV antibodies were found in control birds.

The birds were challenged with 2.3 log10 EID50/dose of a very virulent IBDV strain (100045) by ocular route. The severity of the challenge was higher than Ph. Eur. 0587 requirement. 100% of the control chickens showed characteristic signs of the disease (1 death and 19/19 remaining chickens with macroscopic lesions of the bursa of Fabricius and a lesion score of 4).

The vaccination with Prevexxion RN+HVT+IBD at 3.6 log10 PFU/bird of vHVT013-69 in presence of 3 log10 PFU/bird of RN1250 by the subcutaneous route resulted in protection (no mortality, no clinical signs, no macroscopic lesions in the bursa of Fabricius, bursa histology lesion score <3 in all the vaccinates and a higher bursa weight/body weight ratio of the vaccinates) 70 days after a challenge with a very virulent IBDV strain.

In conclusion, a 10-week duration of Gumboro immunity met Ph. Eur. 0587 requirement while the long-life protection against Marek disease is acceptable. No re-vaccination scheme is proposed.

Maternally derived antibodies (MDA)

Marek’s disease

The protection against Marek's disease was assessed by challenge in conventional pullets 5 days after vaccination. Pullets were vaccinated at hatching (D0) either with RN1250 alone or with RN1250+vHVT013-69, both at the minimum protective dose.

MDA against MDV and IBDV were found in all the sampled birds at hatching. The vaccination was monitored at D7 andD76.

The RN1250 vaccine strain was isolated in 9/10 vaccinated chickens sampled 7 days after vaccination. Antibodies to IBDV were found in all the RN1250+vHVT013 vaccinated birds on D76.

The IP challenge with the vvMDV strain RB1B 5 days after vaccination, brought about 97% of MD specific morbidity in the control group whereas vaccination with RN1250 and RN1250+vHVT013-69 vaccine provided a relative protection of 88% and 97%, respectively. These results comply with Ph. Eur. 0589 requirements..

Although this study was performed in conventional chicken (with MDAs), an onset of immunity of 5 days for the Marek’s disease protection can be accepted based on the results obtained from this study.

Gumboro disease

The putative interference of maternally derived antibodies with protection afforded by Prevexxion RN+HVT+IBD against Gumboro disease has been investigated in broilers chicks. The primary end point was the percentage of birds which met Ph. Eur. 0587 individual criteria. Birds had high titres of maternally derived Gumboro and Marek´s disease antibodies at hatching.

Broilers were challenged 28 days after vaccination, while they still had anti-IBDV antibodies, with a very virulent IBDV strain 91-168/980702.

Unvaccinated birds did not die nor they had Gumboro clinical signs but their bursa of Fabricius was atrophied with a histological grade of 4. One death was recorded in vaccinated group on D20 and the bird had neither previous clinical signs nor gross lesions at necropsy. The protection rate of the vaccinates which was calculated according to the Ph. Eur. 0587 individual criteria that are no notable Gumboro clinical sign and no more than grade 2 lesion of the bursa of Fabricius) was 42%.

In a second study , the putative interference of maternally derived antibody with protection afforded by Prevexxion RN+HVT+IBD against Gumboro disease has been investigated in conventional broilers chicks. Birds had high titres of maternally derived Gumboro antibodies at hatching.

Broilers were challenged 28 days after vaccination, while 100% of the control birds still had anti-IBDV antibodies, with a very virulent IBDV strain 100045/170126.

Unvaccinated birds did not die nor they had Gumboro clinical signs but their bursa of Fabricius was atrophied with a histological grade of at least 3 (with a majority with a grade 4). The protection rate of the vaccinates was 65%.

In a third study , the putative interference of maternally derived antibodies with protection afforded by Prevexxion RN+HVT+IBD against Gumboro disease was investigated in conventional broilers chicks. Birds had high titres of maternally derived Gumboro antibodies at hatching.

Broilers were challenged 35 days after vaccination with a very virulent IBDV strain 100045/170126 when Gumboro MDA had merely vanished.

Unvaccinated birds did not die nor they had Gumboro clinical sign but their bursa of Fabricius was atrophied with an histological score of 4. The protection rate of the vaccinates was 100% with Prevexxion RN+HVT+IBD.

From these studies it can be concluded that vaccination of conventional broiler chickens (with MD MDAs) can achieve a full protection against Gumboro disease at 5 weeks after the vaccination and that MD MDAs may potentially delay IBD onset of immunity.

Interactions

No interaction studies have been conducted.

Field trials

The design and the methodology of the 2 field trials have been set out in part III section C.

In a first trial , the long-living broilers started to seroconverted to IBDV by 30 days after their vaccination with RN1250+vHVT013-69, earlier than those vaccinated with a commercial live Rispens+HVT vaccine at day 1, then with a classical live intermediate IBDV vaccine administered by drinking water on D20 and D26 which started to seroconvert at day 53.

Because no clinical signs suggestive of Marek's disease or Gumboro disease were anticipated to occur

in the farms during the study, some birds were taken from the hatchery before (control birds) or after their vaccination to be challenged in laboratory by very virulent MD or IBDV strains at the onset of protection.

In a second trial, two different breeds of pullets were monitored up to 77 or 85 weeks of age (whole production period) each one in a multiple-site production system of 2 farms. At day 1 of age, 20,000 female chicks were injected subcutaneously with RN1250 (associated with a commercial HVT vaccine) or commercial Rispens+HVT vaccine as control and about 60,000 female chicks with RN1250+vHVT013-69 or commercial Rispens+HVT vaccine followed by a classical live intermediate IBDV vaccine administered by drinking water on day 22 and 29 in the farm. From 18 weeks of age onwards, birds were transferred to two laying farms each farm raising a vaccine group and its control group at the same time.

Further to the detection of a pullet with MD paralysis at 16 weeks, evidence of circulation of MDV was brought out in the dust of the building housing the RN1250+vHVT013-69 group while the CVI-988 vaccine strain was circulating in the building housing its control group, located in another farm. In the RN1250+vHVT013-69 group, early colibacillosis, infectious laryngotracheitis and late coccidiosis were observed and was treated.

However, there were no conspicuous impacts on rearing parameters (body weight, egg production) and more importantly for Marek's disease, on condemned and destroyed carcasses. Thus RN1250 + vHVT013-69 did provide protection to circulating MDV under field conditions.

Anticipating an absence of MDV or IBDV circulation, some birds were taken from the hatchery after their vaccination to be challenged in laboratory by a very virulent MDV strain at the onset of protection.

From the study conducted on long-living broilers (first field trial), the applicant drew birds vaccinated either with RN1250 alone or with the complete vaccine (RN1250+vHVT013-69) and their respective controls to be challenged at day 5 by a very virulent MDV strain. The design of this study was compliant to Ph. Eur. 0589 and long-living broilers were clinically monitored over a 70-day post-challenge period.

Maternally derived IBDV antibodies at hatching were found in 20/20 chickens. The vaccination was effective since RN1250 vaccine strain was found in 5/5 and 4/5 chickens and 20/20 chickens had anti--IBDV antibodies at D76.

Less than 70% of the control chickens met Ph. Eur. 0589 criteria of MD infection. The applicant has justified this result by a genetic resistance of the long-living broiler breeds because the same challenge gave sufficient infection in study with conventional pullets and SPF layer chickens. This justification is acceptable because the genetic resistance to MDV infection and specifically broilers by comparison to laying breeds is well known (Schat, 2008).

In the group of birds vaccinated with RN1250+vHVT013-69, 14/34 birds experienced locomotor troubles; however gross pathology and histological evidence of bacteraemia were found instead of MD related lesions. Consequently, they were considered MD protected.

Vaccination of long-living broilers with an intermediate potency RN1250 vaccine alone or in combination with vHVT013-69 valence provided a relative protection around 94% against a very virulent MDV strain and corroborated the 5 day onset of protection.

From the study conducted on pullets (second trial), the applicant drew vaccinates either with RN1250 (associated with HVT vaccine) or with RN1250 + vHVT013-69 or their respective controls to be challenged at day 5 with a very virulent MDV strain . The design of this study was compliant to Ph. Eur. 0589 and layer chickens were clinically monitored over a 70-day post-challenge period.

Maternally derived anti-IBDV antibodies at hatching were found in 20/20 chickens.

The vaccination was effective since RN1250 vaccine strain was found in 4/5 (RN1250+HVT) and 5/5 (RN1250+vHVT013-69) chickens and all tested chickens had anti-IBDV vaccine strain antibodies at D76 whereas unvaccinated control birds were negative for RN1250 on D8 and negative for IBD antibodies at D76.

In control groups 25/35 (RN1250 + HVT controls) and 27/33 (RN1250 + vHVT013-69 controls) birds died during the monitoring period and 91% of the birds met Ph. Eur. 0589 criteria of MD infection. The challenge was thus Ph. Eur. 0589 compliant.

In the group of birds vaccinated with RN1250+ vHVT013-69 vaccine or its respective contol, 2/31 and 2/35 died respectively few days after MDV challenge; however, the isolation of an *E. coli* in one bird, gross pathology and histological evidence of bacteraemia were found instead of MD related lesions. Consequently, they were not considered as MD challenge-related and were excluded for the efficacy assessment.

Vaccination of layer chickens with an intermediate potency RN1250 vaccine (associated with HVT vaccine) or in combination with vHVT013-69 valence (Prevexxion RN+HVT+IBD) provided the same relative protection of 100% against a very virulent MDV strain and corroborated the 5-day onset of protection.

The applicant drew vaccinates from the study conducted on long-living broilers (first trial) which had been administered with the complete vaccine (RN1250+vHVT013-69) or its respective control to be challenged at day 29 with a very virulent IBDV strain (100045 / 170126). The design of this study was in accordance with Ph. Eur. 0587 and long-living broilers were clinically monitored over a 10-day post-challenge period.

Maternally derived anti-IBDV antibodies at hatching were found in 20/20 chickens. The vaccination was effective since RN1250 vaccine strain was found in 5/5 birds on D8 after vaccination.

The challenge was done 28 days after vaccination when anti-IBDV antibodies were still detectable in 9/10 unvaccinated birds. After challenge, the chickens in both groups showed neither clinical signs nor died. However, all control birds presented macroscopic and significant histological lesions of the bursa of Fabricius (scores of 3 or mostly 4), as well as bursal atrophy, and some additionally showed specific gross lesions of IBD on muscles. Therefore, 20/20 unvaccinated chickens were classified Gumboro positive 10 days after challenge.

In the vaccination group 12/20 chickens were classified Gumboro positive that is a protection rate of 40% and the bursa of Fabricius weight/bodyweight ratio was lower in the control chickens than in the vaccinates.

Vaccination of these long-living broilers with an intermediate potency vHVT013-69 provided a protection of 40% 28 days after vaccination against a very virulent IBDV strain.

The applicant also drew non-vaccinated long-living broiler birds from the first field trial, which were vaccinated with a classical live intermediate IBDV vaccine alone as control of the severity of the IBDV challenge performed on the study right above. They were administered by drinking water on days 20 and 26 according to the label and the protection rate was 4%.

Overall conclusion on efficacy

The efficacy of Prevexxion RN+HVT+IBD was demonstrated in 8 laboratory studies and 2 field studies (associated with 4 laboratory challenges).

The diagnostic criteria of Marek's disease or Gumboro disease applied in the laboratory studies were those described Ph. Eur. monograph 0589 and Ph. Eur. monograph 0587, respectively.

The MD protection was challenged by the MDV-1 strain RB1B which is classified as very virulent according to ADOL scale and Gumboro protection with both the classical vIBDV Faragher and a vvIBDV strain.

The minimum dose for the RN1250 component of 2.9 log 10 PFU was chosen because it provided 94% of protection against a challenge with a very virulent MDV-1 strain 4 days after vaccination. A MD onset of immunity of 5 days as demonstrated in a study conducted in conventionnal layers is regarded as supportable. The onset of protection to Gumboro disease was shown by a challenge with Faragher strain 14 days after vaccination with Prevexxion RN+HVT+IBD.

Maternally derived antibodies (MDA) did not decrease MD protection afforded by Prevexxion RN+HVT+IBD (97% protection) against a MD challenge at day 5 nor did they decrease Gumboro protection when vaccinates were challenged 35 days after vaccination (100% protection rate). However, when Gumboro challenge was performed earlier while MDA were still high, the protection rate was lower (40-65%). Therefore, a negative impact on the development of protection against Gumboro disease must be anticipated when chickens are vaccinated in the presence of high titres of MDAs against Marek's disease. A warning has been added in section 4.4 of the SPC.

The duration of MD immunity is lifelong while IBD protection until 70 days was shown after challenge of conventional laying chickens with a very virulent IBDV strain.

In field studies which were performed in long-living broilers and laying hens, RN1250 and RN1250+vHVT013-69 were compared to marketed vaccines. Broilers seroconverted against IBDV earlier with RN1250+vHVT013-69 vaccine than with Rispens and live IBD vaccines, and protection against circulating MDV similar to the comparator vaccine was reported for laying pullets vaccinated with RN1250+vHVT013-69. However, birds from these field trials were experimentally challenged in laboratory studies.

When administered to long-living broilers, RN1250 at intermediate potency alone or with vHVT013-69 provided a relative protection around 94% against a very virulent MDV strain 5 days' further vaccination and the protection was higher for layer chickens with the same study design (100%).

Prevexxion RN+HVT+IBD at intermediate potency administered at 1 day of age provided 40% of protection of conventional long-living broilers against a D29 challenge with a very virulent IBDV strain.

Part 5 – Benefit-risk assessment

Introduction

Prevexxion RN+HVT+IBD is a bivalent vaccine containing two cell-associated live GMO virus strains, an engineered Marek's disease virus (MDV-1) serotype 1, named RN1250 strain, in combination with an already authorised recombinant turkey herpesvirus (HVT) expressing the VP2 coding sequence of infectious bursal disease virus (IBDV), named vHVT013-69.

The vaccine is intended for active immunisation of one-day-old chicks to prevent mortality and clinical signs and reduce lesions caused by Marek's disease (MD) virus (including very virulent MD virus) and to prevent mortality, clinical signs and lesions caused by infectious bursal disease (IBD) virus.

The dossier was submitted in line with requirements of Article 12(3) of Directive 2001/82/EC.

Benefit assessment

Direct therapeutic benefit

In 11 laboratory and 2 field studies the vaccine was shown to be efficacious for the active immunisation of one-day-old chicks to prevent mortality and clinical signs and reduce lesions caused by Marek's disease (MD) virus (including very virulent MD virus) and to prevent mortality, clinical signs and lesions caused by infectious bursal disease (IBD) virus.

An OOI of 5 days was established against MDV infection and no data are provided for the DOI. This is acceptable as the MD virus produces a persistent infection providing a lifelong immunity.

OOI was established against IBDV infection at 14 days of age and DOI was established for 10 weeks after vaccination.

Additional benefits

Prevexxion RN+HVT+IBD was shown efficacious against IBDV strains and very virulent MDV-1.

Risk assessment

Quality:

Information on the composition, development, manufacturing process, tests performed during manufacture and on the finished product, batch-to-batch consistency and stability data have been provided. Interference between the two vaccine strains has been studied and mitigated in the final formulation.

Safety:

Risks for the target animal:

The product is generally well tolerated in the target animal. No adverse reactions were observed after administration of Prevexxion RN+HVT+IBD.

The vaccine strains, an apathogenic strain (vHVT013-69) and a MDV1 vaccine strain in which two copies of a LTR sequence from avian retroviruses were included, were shown to be safe for chickens. Spread to contact chickens of vHVT013-69 was detected once in an efficacy study and appropriate care should be taken to separate vaccinated from non-vaccinated chickens. An appropriate warning is included in the SPC.

Risk for the user:

The user safety for this product is acceptable when used as recommended and taking into account the safety advice and also the special precautions for handling nitrogen stored products listed in the SPC and package leaflet.

Risk for the environment:

The vaccine viruses are detected or shed by the feather epithelium and the infected dander can persist in the environment. However, the fitness of these vaccine strains was shown lower than their parental strains which have been used in authorised vaccines for a while. Safety studies conducted in six avian non-target species demonstrated that the vaccine strains are safe and studies in mice that they did not replicate in mammalians. Safety studies showed, that recombination (if any) would not result in more

virulent strains.

The product is not expected to pose any risk to the environment when used as recommended.

Risk for the consumer:

The withdrawal period is set at zero days.

Risk management or mitigation measures

Appropriate information has been included in the SPC to inform on the potential risks of this product relevant to the target animal, user and environment and to provide advice on how to prevent or reduce these risks.

Evaluation of the benefit-risk balance

The applicant applied for the following indication: "prevention of mortality and clinical signs and reduction of lesions caused by Marek's disease (MD) virus (including very virulent MD virus) and prevention of mortality, clinical signs and lesions caused by infectious bursal disease (IBD) virus (including very virulent IBD virus)." and the CVMP agreed to the following indication(s): prevention of mortality and clinical signs and reduction of lesions caused by Marek's disease (MD) virus (including very virulent MD virus), and prevention of mortality, clinical signs and lesions caused by infectious bursal disease (IBD) virus.

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk for users and the environment when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information. Based on the data presented, the overall benefit-risk is considered positive.

Conclusion on benefit-risk balance

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for Prevexxion RN+HVT+IBD is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned veterinary medicinal product.