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Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for a variation requiring assessment for Prevexxion RN+HVT+IBD (EMEA/V/C/005057/VRA/0009)

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

Submission of the variation application

In accordance with Article 62 of Regulation (EU) 2019/6, the marketing authorisation holder, Boehringer Ingelheim Vetmedica GmbH (the applicant), submitted to the European Medicines Agency (the Agency) on 25 September 2023 an application for a variation requiring assessment for Prevexxion RN+HVT+IBD.

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Scope of the variation

Prevexxion RN+HVT+IBD is authorised for use in chickens 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.

Prevexxion RN+HVT+IBD concentrate and solvent for suspension for injection contains 2.9 to 3.9 $\log_{10}$ plaque-forming units (PFU) of cell-associated, live recombinant Marek's disease virus, serotype 1, strain RN1250 and 3.6 to 4.4 $\log_{10}$ PFU of cell-associated, live recombinant turkey herpesvirus (HVT), expressing the VP2 protein of infectious bursal disease virus, strain vHVT013-69.

This product is presented in type I glass ampoules of 1,000 doses of vaccine in 5-ampoule carrier, 2,000 doses of vaccine in 5-ampoule carrier, and 4,000 doses of vaccine in 4-ampoule carrier.

Variation(s) requested	
I.II.1.e	Changes to strength, pharmaceutical form and route of administration - Change or addition of a new route of administration

This variation is to add a new route of administration: in-ovo.

At the time of submission, the applicant applied for the following indication:

For active immunisation of one-day-old chicks or 18-day-old embryonated chicken eggs:

- to prevent mortality and clinical signs and reduce lesions caused by MD virus (including very virulent MD virus), and

- to prevent mortality and clinical signs and lesions caused by IBD (also known as Gumboro disease) virus.

Changes to the dossier held by the European Medicines Agency

This application relates to the following sections of the current dossier held by the Agency:

Part 1, Part 2, Part 3 and Part 4

Scientific advice

Not applicable.

Limited market status

Not applicable.

Part 2 - Quality

Quality documentation (physico-chemical, biological, and microbiological information)

Qualitative and quantitative composition

The composition of the vaccine remains unchanged.

Product development

Prevexxion RN+HVT+IBD is a live vaccine intended for mass vaccination against Marek's disease and infectious bursal disease (also known as Gumboro disease) (including very virulent IBDV and MDV) by subcutaneous administration to 1-day-old chicks in-ovo.

The marketing authorisation in the EU was granted in 2020.

In poultry industry, vaccination occurs more and more often in the hatchery by in-ovo route, especially for the control of Marek's, infectious bursal disease (IBD), Newcastle and avian influenza diseases. The in-ovo vaccination has the advantage to stimulate the immunity early for an optimal bird protection. Moreover, the use of an automated device for in-ovo injection ensures an accurate dose administration and is cost- and time-saving. From an animal welfare point of view, the fact that the birds' manipulation is not required reduces the stress of young chicks. All these elements make this route of administration very interesting as an effective alternative to the traditional subcutaneous one.

Finished product presentation

The vaccine is presented as a sealed glass ampoule containing the frozen antigen suspension. This needs to be diluted in the solvent used for the preparation of Boehringer Ingelheim's cell-associated poultry vaccines, which is presented in PVC bags. Three presentations are proposed for the vaccine: 1000 doses, 2000 doses and 4000 doses. Depending on the route of administration, the exact amount of vaccine ampoules and solvent bags needed should be calculated according to the table provided below as an example.

The critical element to consider in the preparation of the vaccine is the volume to administer by the new route of administration. The volume for the in-ovo route (0.05 ml/embryonated egg) is four times lower than the one used for the subcutaneous route (0.2 ml/day-old chick). In order to deliver the correct quantity of antigens per embryonated egg and also taking into account that the qualitative and quantitative composition of the finished product remains unchanged, the number of vaccine ampoules to use for a given diluent bag presentation should be four times higher compared to the number of ampoules used for the subcutaneous route of administration.

The table below describes the recommended options for the vaccine preparation and is provided in the updated quality part as well as in the product information.

Table: Examples of vaccine preparation according to the route of administration.

Solvent bag	Number of vaccine ampoules (subcutaneous route)	Number of vaccine ampoules (*in-ovo* route)
1 bag of 200 ml solvent	1 ampoule (1000 doses)	4 ampoules (1000 doses) or 2 ampoules (2000 doses) or 1 ampoule (4000 doses)
1 bag of 400 ml solvent	2 ampoules (1000 doses) or 1 ampoule (2000 doses)	8 ampoules (1000 doses) or 4 ampoules (2000 doses) or 2 ampoules (4000 doses)
1 bag of 800 ml solvent	4 ampoules (1000 doses) or 2 ampoules (2000 doses) or 1 ampoule (4000 doses)	16 ampoules (1000 doses) or 8 ampoules (2000 doses) or 4 ampoules (4000 doses)

Composition of vaccine batches used in the clinical trials

The section has been updated to include the batches used in the clinical trials provided to support the in-ovo administration. Batch release certificates have been provided for all batches. All batches comply with the specifications.

Description of the manufacturing method

This section was updated to reflect that in-use stability studies of the reconstituted product have been performed for the in-ovo route of administration. Results of these studies are presented in the Stability section.

Stability

Stability of the reconstituted product

The shelf life of the vaccine after being prepared according to instructions for an administration by the in-ovo route was evaluated. For this purpose, two in-use stability studies, with a similar design to that supporting the subcutaneous route, were carried out on the reconstituted vaccine stored at room temperature for 2 hours.

Each study was conducted in accordance with the "Guideline on data requirements to support in-use stability claims for veterinary vaccines" (EMA/CVMP/IWP/250147/2008). The criterion for evaluation of stability was the difference between the titre of the vaccine (of each vaccine component) two hours after reconstitution and its titre immediately after reconstitution (T0). The microbial sterility of the reconstituted vaccine was not tested because of the short duration of use in the field. However, indirect parameters such as pH and appearance were investigated.

According to the guideline EMA/CVMP/IWP/250147/2008, "The microbial safety of the vaccine should be demonstrated over the proposed in-use shelf-life. If microbial safety data is not considered necessary in support of the proposed in-use shelf-life, this should be justified by the applicant." For this vaccine, in-use microbial safety data are not considered necessary. In fact, Prevexxion RN+HVT+IBD vaccine is sterile at release, and it is administered by veterinary surgeons or under their direct responsibility. Therefore, a contamination of the vaccine is unlikely if prepared according to the recommendations provided in the SPC section 4.9. Furthermore, the vaccine should be used within at most 2 hours after reconstitution. A significant bacterial growth is unlikely in such

a short time and, therefore, performing a sterility test is considered to be of limited added value. Additionally, even if microbial safety data were not considered necessary in this context, it should be noted that pH and appearance tests results, which may be impacted by a bacterial contamination, were also carried out at T0 and T2. The results of these controls tests were within the range of acceptance at both time points. Titration results were stable during the monitoring period, within the specifications, with no significant titre loss observed for any antigen. The pH and appearance remained unchanged and conformed to the acceptance criteria.

Overall, these in-use stability results confirmed that the vaccine preparation for in-ovo administration did not impact the 2-hour in-use period already approved for the subcutaneous route.

Overall conclusions on quality

To qualify the new in-ovo route of administration, the applicant has updated the quality part of the registration dossier.

From a quality point of view, the new route of administration only impacts the vaccine preparation due to a lower administration volume in comparison to the one for subcutaneous route (i.e. 0.05 ml vs 0.2 ml, respectively). The production process and quality controls and the composition of the vaccine remain unchanged. Additional information regarding the vaccine preparation and the volume to be injected are documented in part 2A of the dossier and in the product information (section 4.9).

Part 2.A was also updated to include the vaccine batches used in the clinical trials performed to support the in-ovo route of administration.

To qualify the in-ovo route of administration, the applicant has also performed additional studies to demonstrate the in-use stability of the concentrated vaccine used for in-ovo administration. The study results confirmed that potency, pH and appearance remained unchanged.

Taken together, from a quality point of view, the applicant has provided all the essential information, justification and validation data to qualify the in-ovo administration as a new route of administration for Prevexxion RN+HVT+IBD vaccine.

Part 3 – Safety documentation (safety and residues tests)

General requirements

The safety of the new route of administration has been studied in 4 preclinical studies in accordance with the European Pharmacopoeia monographs for Marek's disease, for avian infectious bursal disease and the general monograph about vaccines for veterinary use. A field trial was also performed. Two supportive preclinical studies were performed in the USA in accordance with VICH GL9.

All batches comply with the European authorisation except those used for the USA studies; however, they were assessed, found comparable and therefore accepted in the original marketing authorisation procedure where they were used for subcutaneous studies.

Pre-clinical studies

Safety of the administration of one dose and an overdose

The safety of the in-ovo route was demonstrated in 2 GLP studies.

The first study was designed to comply with Ph. Eur. monograph 5.2.6. Birds were monitored over a 21-day period and then necropsied. A double volume of the vaccine (100 µl) was injected into 70 SPF embryonated White Leghorn eggs at 18-19 days of incubation either at a dose just above its maximum release (4 $\log_{10}$ of RN1250 and 4.6 $\log_{10}$ of vHVT013-69) or at a 10x overdose. Thirty-five control eggs were injected with vaccine diluent. The double volume of injection is acceptable because it is a worst case in terms of safety at the injection site.

After hatching on day 0 (D0), birds were observed on a daily basis for 21 days. Necropsy, with a particular focus on specific lesions of Marek's disease and infectious bursal disease, was carried out in any dead (including euthanised) bird and in all remaining birds on D21. Growth of the chickens was monitored regularly and was statistically analysed.
The presence of RN1250 virus was evaluated by means of a specific real-time PCR in the DNA extracted from spleen of 5 birds per group on D7. Antibodies against infectious bursal disease virus were titrated by ELISA in the sera of 10 birds per group on D21.

The eggs were correctly administered with the vaccine or solvent, since neither DNA nor antibodies to IBD were found in the birds of the control group and the contrary for the birds of the maximum dose group.

The hatchability was higher than 80% (Ph. Eur. criterion) in all groups (≥ 97%). Early mortality within the first 7 days was limited (1/35 in control group and 3/35 in the maximum dose group) and not attributable to the vaccine.

No mortality or clinical signs due to the vaccination were observed during the study. No specific gross lesions characteristic of Marek's disease or infectious bursal disease were recorded in any of the birds at necropsy; one bird of the maximum dose group had no thymus, but this was diagnosed as a malformation not related to vaccination because no other abnormalities were observed. A 10 g growth retardation was observed 23 days after vaccination in the maximum dose group compared to the controls. This was not observed in the group of vaccinated with the overdose.

In the second study, the birds were monitored over a 96-day period for the maximum dose and 120 days for the overdose in line with Ph. Eur. 5.8.9. in order to check the residual pathogenicity of Marek's disease vaccines. SPF embryonated White Leghorn eggs at 18-19 days of incubation were injected with 0.05 ml of the maximum dose (3.9 $\log_{10}$ of RN1250 and 4.4 $\log_{10}$ of vHVT013-69) of an overdose or of the vaccine diluent (controls). To confirm the susceptibility of the birds, a fourth group was injected with a very virulent Marek's disease (RB1b) virus and monitored over a 70-day period. The control and overdose groups were monitored until 120 days of age and necropsied at the end of the monitoring period to check for specific lesions of Marek's disease. The birds of the maximum dose group were included to rule out the growth retardation detected in the first study and were necropsied on D96. This monitoring time span is shorter than the one recommended by Ph. Eur. 5.8.9. but longer than the targeted vaccinates, broilers, and it is acceptable.
The white leghorn breed was fully susceptible to Marek's disease virus since all of the birds got the disease except two, which died early after hatching within 10 days without Marek's disease-related lesions.
The vaccine was well injected, since RN1250 virus DNA was detected 7 days after hatching in 100% of sampled birds in both vaccinated groups and was not detected in the 5 birds of the control group.

No IBDV antibodies were detected on day 42 in birds of the control group, in contrast to the birds of the maximum dose and the overdose groups.

Hatchability was at least 95% in all study groups.
In the control group, no signs or lesions related to Marek's or Gumboro disease were observed, as were in the maximum dose group. In the overdose group 2 birds had thymus atrophy - one was euthanised at day 21 and the other upon completion of the study, but microscopical observation was interpreted as physiological involution.

The growth curves of the vaccinated groups were similar to the ones of the control group and, especially at high dose, the vaccine had no impact on body weight at D0/D1, D7, and D61; the D21 was not tested.

Safety of the repeated administration of one dose

Prevexxion RN+HVT+IBD is intended to be injected once and, therefore, repeated administration is not applicable.

Examination of reproductive performance

Prevexxion RN+HVT+IBD is not intended for laying birds. The warning sentence already present in the current product information has been updated to add this route of administration.

Examination of immunological functions

The impact of vaccination on the immunological functions was examined for the S.C. route and a sentence was included in the European Public Assessment Report (EPAR) warning that RN1250, when administered subcutaneously near its maximum titre, had a slight and transient impact on the thymus, which did not affect the immunological functions of chickens. A new GLP study with a similar design was performed to check whether this observation could be repeated by in-ovo route.

A group of 50 SPF Leghorn chickens was vaccinated with the maximum release dose of Prevexxion RN+HVT+IBD. Seven days post-hatching, this group was vaccinated with a Newcastle disease live vaccine at its minimum dose, according to label's instructions. Two other groups, either vaccinated only with this Newcastle disease live vaccine on D7 (50 chickens) or with Marek's disease vaccine diluent on D-2 (20 chickens), were included in the study. All diluent groups and parts of the vaccinated groups were challenged with a virulent Newcastle disease virus (Herts 33 strain) on D21 and monitored over a 14-day period.

The Newcastle disease challenge was severe, since all the birds of the diluent group died within 2 days. No immunosuppression was observed after the administration of Prevexxion RN+HVT+IBD, since all the birds were protected by the Newcastle disease live vaccine, while 90% were in the group where birds were not previously vaccinated with Prevexxion RN+HVT+IBD. The 2 groups also had similar NDV antibody titres on day of challenge.

Special requirements for live vaccines

Only the special requirements for the RN1250 strain are covered in the following sections, since vHVT013-69 strain is already authorised for in-ovo use in the vaccine Vaxxitek HVT+IBD.

Spread of the vaccine strain

The MAH has argued that shedding, and thus spreading, are similar by S.C. and in-ovo route of administration, and provided bibliographical data indicating that the vaccine virus remains dormant before hatching and that the virus load was similar in the feather pulp thereafter.

However, the MAH has also included a study performed for the USA registration (GCP study), where 49 non-vaccinated birds were put in contact with 50 birds vaccinated in-ovo with 0.05 ml of RN1250 master seed virus (same MSV for EU and USA registration) at a dosage of 4.45 $\log_{10}$. Clinical and histological investigations were carried out over a 120-day period after hatching to check whether contact birds got Marek's disease. Since neither clinical signs nor macro- and microscopical lesions attributable to Marek's disease were reported, if horizontal transmission occurred, it did not induce clinical signs or lesions to contact birds.

Dissemination in the vaccinated animal

No specific in-ovo study with RN1250 strain was performed to assess dissemination. The Ph. Eur. general safety 5.2.6 monograph or the Marek's disease-specific Ph. Eur. monograph 0589 do not require to test dissemination after each route of administration and the dissemination of the strain in vaccinated animals was studied after S.C. administration.

Moreover, the sites of replication of RN1250 were similar, and the virus loads were similar or even lower than those of the parental virus with the S.C. route. Literature data showed that for the RB1B strain, the prevalence of MDV1 DNA in lung, thymus, bursa and spleen after in-ovo administration in the amniotic sac was very similar to that after post-hatch.

The absence of studies specific to the in-ovo route is thus acceptable.

Increase in virulence of attenuated vaccines

The absence of increase in virulence has been checked by the in-ovo route in the initial dossier. No reversion to virulence but an increase of fitness of RN1250 was observed when the virulence of the 5th passage was checked by the S.C. route in the residual pathogenicity study.
The in-ovo route is considered to be similar to the S.C. route because the dissemination is similar and the vaccine virus multiplication would start after hatching.

Residual pathogenicity

Two studies to support the absence of residual pathogenicity were carried out, one in the EU and the other in USA, for the in-ovo administration.

The residual pathogenicity by the in-ovo route was investigated on the MSV and a low in vitro passage of RN1250 (MSV+2), but not on the back-passaged P5 virus strain in-ovo. The justification for this, as claimed by the applicant, was for ethical reasons.

The GLP study conducted in the EU was performed with the MSV+2. However, the study was dismissed because the early mortality rate (within 7 days) was above the Ph. Eur. 5.8.9. criterion (12% instead of 10%). This early mortality was associated with unspecific lesions which were not evocative of Marek's or Gumboro disease.

A similar study was performed in the USA in compliance with 9 CFR 113.330. The RN1250 MSV was administered at around 4-fold overdose (4.45 $\log_{10}$ PFU) in 0.05 ml to seventy-five 18–19-day old SPF embryonated eggs, while the same number of eggs received Marek's disease vaccine diluent in-ovo in the same volume. After their satisfactory hatch (i.e. 93% and 95%, respectively), 50

randomly chosen vaccinated birds were observed daily during 120 days for clinical signs attributable to Marek's disease. A group of 75 non-vaccinated control birds were challenged at one day of age with vvMDV RB1B virus, demonstrating that the birds included in the study were fully susceptible to MDV, with 100% of the birds being MDV-positive within 36 days post-hatch.
None of the vaccinated birds, with either the MSV or the diluent, showed clinical signs or gross lesions attributable to Marek's disease. Moreover, the weight of MSV-vaccinated birds was slightly higher than the one of the diluent-vaccinated birds 120 days after hatching.
The dosage was 4.45 $\log_{10}$ instead of 4.9 $\log_{10}$ as required by Ph Eur 5.8.9.
In conclusion, MSV did not exhibit MD residual pathogenicity at 4-time overdose.

Biological properties of the vaccine strain

The route of administration is not expected to modify the biological properties of the strain; the vaccine virus was shown to be dormant until hatch. No specific in-ovo study with RN1250 strain was thus provided. This is acceptable.

Recombination or genomic reassortment of the strains

No specific in-ovo study with RN1250 strain was performed to assess recombination. The assessment provided for the S.C. route is adequate for the in-ovo route.

User safety

In-ovo administration of Prevexxion RN+HVT+IBD can be considered as presenting less risk for humans compared to the one set for the subcutaneous route, especially with regard to the risk of accidental self-injection since specific automated equipment is used in the hatcheries to inject the vaccine to embryonated eggs.

Warning sentences are already present in the product information and remain unchanged.

Study of residues

Since the composition of the product remains unchanged by the addition of the new route of administration, no specific study was conducted. This is considered acceptable.

Interactions

Since no compatibility is claimed for Prevexxion RN+HVT+IBD vaccine, no specific interaction studies are presented by the applicant.

Clinical studies[1]

A clinical trial compliant with GCP was performed to confirm the safety and efficacy of the bivalent vaccine Prevexxion RN+HVT+IBD representative of commercial batches (3.5 $\log_{10}$ RN1250 & 4.1 $\log_{10}$ vHVT013), in conventional embryonated eggs, from vaccination day (i.e., 18 days of incubation, noted D-3) at the hatchery to the day of slaughter at about 85 days after being reared in 2 farms. Since there is no bivalent vaccine equivalent to Prevexxion RN+HVT+IBD on the market in EU, a group vaccinated with 2 marketed products (commercial live Rispens+HVT at the hatchery and a live attenuated Gumboro vaccine at the farms) were used as a positive control group (CTRL) having the same indications as the vaccine under investigation.

[1] If relevant for safety.

The study design is summarised hereafter:

Site	Site 1		Site 2	
Group	IVP	CTRL	IVP	CTRL
Number of included chicks in eah subgroup at the farm	4145	4415	4400	4400
Vaccination date and product administered at the hatchery	D-3 with PREVEXXION RN+HVT+IBD	D-3 with commercial Rispens+HVT FC126 vaccine	D-3 with PREVEXXION RN+HVT+IBD	D-3 with commercial Rispens+HVT FC126 vaccine
Vaccination date and product administered at the farm	-	D23 and D27 with live attenuated Gumboro vaccine via drinking water	-	D22 and D28 with live attenuated Gumboro vaccine via drinking water

The antibody status of the animals enrolled in the study was assessed by serology on serum samples collected from unvaccinated animals on D1. Moreover, spleen collection and regular blood samplings were performed to assess the vaccine intake in all vaccinated groups. Hatchability, clinical monitoring (i.e. regular observations and mortality follow-up), records of production parameters (i.e. body weight, feed intake and feed conversion index) were also assessed to confirm the safety and efficacy of the vaccine.

The antibody status of the commercial birds enrolled in the study was confirmed by qualitative and quantitative detections on D1 of maternally derived antibodies specific to MDV and IBDV, respectively, in the sera of unvaccinated chicks. The vaccine intake was assessed through the detection of RN1250 by PCR and IBD serology. Despite the low detection of RN1250 in spleens of vaccinated birds (i.e. 20% and 30% in site 1 and 2, respectively), the antibody rate against IBDV demonstrated a clear serological conversion in almost all vaccinated sampled animals at week 6 (D38±2) which increased over time, indicating a good vaccine intake.

Only the results related to safety are commented below:
The hatchability was assessed with overall satisfactory rates of about 82% in the vaccinated subgroups and about 87% in the control subgroups on average. Similar hatchability between the IVP and CTRL subgroups was recorded at around 88% in the site 2. In the site 1, a lower hatching rate was observed in the vaccinated subgroup (77%) compared to the corresponding control subgroup (86%). This event was not related to Prevexxion RN+HVT+IBD according to the co-investigator, as demonstrated by the presence of higher number of non-viable eggs in the Prevexxion RN+HVT+IBD subgroup (598) before vaccination, compared to the control subgroup (369). The quality of embryonated eggs was also lower in the batch for site 1 compared to the batch for the site 2, since the hatching rate of contemporaneous egg batches from the same parent breeder flock was 82.1% for the batch corresponding to that used for the site 1 and 87.9% for the batch corresponding to that used for the site 2. Overall, the hatching data did not reveal a negative safety impact of the vaccination.
No adverse events attributable to the vaccination were recorded throughout the study in any of the subgroups.
Mortality rates were very low in all subgroups, whatever the group (Prevexxion RN+HVT+IBD or control) and below the alert threshold set at the start of the study (i.e. 1.5% up to 10 days and 10 animals a day after 10 days of age).
Production parameter records on D8±1 showed that the mean body weight was slightly higher in the CTRL subgroups in both farms compared to the Prevexxion RN+HVT+IBD subgroups, but these

differences were not statistically significant. Between week 1 and the last week of the study, the mean daily body weight gain was slightly higher in the Prevexxion RN+HVT+IBD subgroups compared to the corresponding CTRL subgroups raised in the same farm for both males and females. On the last week of the study, the mean body weight was higher in the Prevexxion RN+HVT+IBD subgroups compared the CTRL groups for both males and females and in both sites. These differences were statistically significant in the site 1 but not in the site 2.
The feed intake per animal and the economic feed conversion index were very similar between all subgroups.
Finally, the percentage of animals which were condemned at slaughter was slightly higher in the CTRL subgroups in both sites. The daily body weight gain calculated in slaughterhouse and the economic mean weight were almost identical between Prevexxion RN+HVT+IBD and CTRL subgroups raised in the same farm.
No lesions suggestive of Marek's or Gumboro diseases were observed on the day of slaughter whatever the subgroup.

To conclude, in-ovo administration of Prevexxion RN+HVT+IBD vaccine to more than 8000 chicken embryonated eggs was demonstrated to be safe under field conditions, based on the satisfactory clinical and technico-economic results obtained.

Environmental risk assessment

The risks associated with the in-ovo route are not very different from the S.C. route, since the vaccine virus remains dormant until hatching and time to S.C. vaccination, and since the biological properties of the vaccine strain remain unchanged. Thus, no modifications of the initial risk assessment are needed.

The risks of the bivalent vaccine administered by the in-ovo route to the target species, the non-target species or the environment have also been re-assessed in light of the new set of data from in-ovo pre-clinical studies and clinical trials presented above, and also from pharmacovigilance data gathered for both vaccine strains for which the in-ovo route is already granted.

Overall, after over billion of doses of vaccine administered, very rare cases of safety-related adverse events in the target species were reported from the field. No adverse events on non-target species or the environment were reported for any of the vaccines cited above.

Considering all the satisfactory safety elements presented above and the extensive pharmacovigilance data, the analysis on the consequence of hazard (judged as negligible for the S.C. route) and the likelihood of hazard occurring (considered as "negligible to low" for the S.C. route) remain unchanged. The estimation of the risk defined as negligible with the subcutaneous route remains unchanged as well.

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

The applicant provided an updated Part 3.E. Overall, there is no new information changing the initial assessment. The analysis and conclusions remain unchanged.

Overall conclusions on the safety documentation

When administered by the in-ovo route, Prevexxion RN+HVT+IBD does not impact hatchability and does not induce clinical signs or Marek's disease lesions investigated either 23 or 123 days after

vaccination in pre-clinical studies. A slight growth retardation was detected 23 days after vaccination (end of study) in one group of birds administered with the maximum dose, but this observation was not corroborated in the 10X overdose group of the same study nor in another study where birds were monitored over a period lasting at least 93 days after vaccination. This observation confirms the warning in the SPC overdose section that "a limited and transient effect on growth" might be observed in some flocks of vaccinates.
In-ovo route had also no impact on immunological functions as investigated by observing the response to Newcastle disease vaccination.

With regard to the special requirements for live vaccines, no data were provided for vHVT013-69, since this strain is already authorised for in-ovo use of the Vaxxitek HVT+IBD vaccine.
For RN1250, its absence of spread following in-ovo injection has been demonstrated in a US study where contact birds never developed a clinical disease. A residual pathogenicity by in-ovo route was also ruled out in a new study where a 4X dose of the MSV was injected. With regard to the other requirements, the data generated for S.C. route were considered relevant for the in-ovo route.

In the field trial, the vaccine had no negative impact on mortality, on final body weight, nor on economic performances.

The assessment of the environmental risks has been updated with the in-ovo route, which does not pose new risks, and with pharmacovigilance data, which have not raised concerns; thus, conclusions remain unchanged. The same update was done for the user safety risk: the conclusion is that this new route decreases the risk of self-injection. The assessment required for veterinary medicinal products containing or consisting of genetically modified organisms was updated as well. The analysis and conclusions remain unchanged.

The safety of the vaccine administered by the in-ovo route was demonstrated.

Part 4 – Efficacy documentation (pre-clinical studies and clinial trials)

To support the in-ovo route, 7 new preclinical studies, a field trial and pharmacovigilance data were provided.

Challenge model

The 2 challenge models used are drawn from Ph. Eur. 5.8.9 (Marek's disease vaccine (live)) and 5.8.7. (Avian infectious bursal disease vaccine (live)).

To assess efficacy against MD, 2 MDV challenge strains were used: RB1B and Md5. Both are classified as very virulent (vv) according to ADOL scale and are representative of strains circulating in Europe, since there are no antigenic variations between European and North American strains. Since the severity of the RB1B challenges in conventional long-life broilers was moderate in one S.C. study, only the Md5 strain was used for the in-ovo vaccine efficacy assessment in conventional broiler eggs.

To assess the efficacy against Gumboro disease, the 2 challenge strains already used for the S.C. route were injected again: the virulent Faragher reference strain and the very virulent 100045 strain, which is more representative of strains currently circulating in Europe.

Pre-clinical studies

The pre-clinical studies have been carried out with batches at the minimum titres for both vaccine strains (RN1250: 2.9 $\log_{10}$ PFU & vHVT013-69: 3.6 $\log_{10}$ PFU). Since no MD or Gumboro disease outbreaks occurred in the field, 2 efficacy studies were additionally performed at the applicant's facilities with day-old chickens from the same lot used in the field trial, which had been vaccinated with a commercial batch (RN1250: 3.5 $\log_{10}$ PFU & vHVT013-69: 4.1 $\log_{10}$ PFU).

Pre-clinical studies were conducted to comply with the applicant's internal standard - Good Scientific Practices - (GSP), with Good Laboratory Practices (GLP) or with Good Clinical Practices (GCP).

Dose determination

The dose has already been set for the S.C. route and the MAH has used the same dose for the in-ovo route. This is acceptable.

Onset of immunity

Marek's disease

In one pre-clinical study, Prevexxion RN+HVT+IBD was administered in-ovo in SPF White Leghorn layer-type embryos to check the onset of immunity. A challenge with RB1B strain was performed 9 days post-vaccination (i.e. 6 days post-hatch) in 30 vaccinated chickens as well as in 30 control chickens injected with the vaccine diluent. The MD protection level was evaluated in each group by a daily clinical monitoring for more than 70 days after challenge, followed by a necropsy and examination for specific gross lesions of MD.
The study was validated, since hatchability was at least 95%. No mortality of hatched chickens was reported until challenge. A MD-associated morbidity rate of 87% was observed in the control group: 11 birds died and 5 were euthanised for ethical reasons.
The vaccination prevented mortality and 1/30 birds showed MD-evocative clinical signs at the end of the monitoring period, without macroscopical lesions at necropsy. Thus, the relative protection rate was 96%, 6 days after hatch.

Based on this study, the OOI should be 6 days post-hatch, but a 5-day post-hatch OOI can be granted based on the two studies where the effect of maternal antibodies was investigated (see below).

Gumboro (infectious bursal) disease

Two pre-clinical studies were performed on SPF embryonated eggs of 18 days of incubation and a challenge was done with each IBDV strain.

In the first study, a virulent challenge by ocular route with Faragher strain was performed on 10 controls at 21 and 28 days after hatch, on 20 vaccinates at day 21, and on 15 at day 28. Birds (SPF broilers VLDIA254) were monitored over 10 days after challenge and then necropsied.

While serology and vaccine DNA detected in the spleen confirmed that the vaccine was well administered, the study was not valid according to Ph. Eur. 0587 criterion, as more than 10% of control or vaccinated birds (12%) were infected by *E. coli* and showed abnormal clinical signs or died from causes not attributable to the vaccine during the period between the vaccination and challenge. However, since the remaining birds responded well to the treatment, the study is provided as supportive.
The morbidity was 100% in the control birds, while vaccination protected 95% and 100% of the birds challenged 3 and 4 weeks after hatch, respectively.

A second study was thus conducted on SPF White Leghorn embryonated eggs of 18 days of incubation with a virulent challenge (Faragher strain) performed 28 days after hatch on 10 control and 20 vaccinated birds.
Serology (19/20 seropositive) and vaccine DNA in 4 spleens out of the 5 sampled confirmed that the vaccine was well administered. The study was validated, since mortality of hatched chicken before challenge was below 10%. An IBDV-associated morbidity rate of 100% was observed in the control group and 5 birds died or were euthanised before the end of the observation period.
None of the vaccinates exhibited clinical signs, and 1 bird, seronegative at D28, was not protected (grade 4 lesions of the bursa of Fabricius), which gives a 95% protection rate.

Duration of immunity

Marek's disease

No duration of immunity to Marek's disease was performed as in the original dossier.

Gumboro (infectious bursal) disease

In one study, Prevexxion RN+HVT+IBD was administered in-ovo in 35 embryos (18th day of incubation) of conventional broilers to check the duration of immunity. Vaccine diluent was administered to 50 controls. A challenge with a very virulent infectious bursal disease (vvIBDV) strain was performed 72 days after vaccination in 20 controls and 20 vaccinates.
Serology (9/10 seropositive) and vaccine DNA in 3 spleens out of the 5 sampled confirmed that the vaccine was well administered to vaccinates and demonstrated the absence of vaccination and the maternally derived antibody wash-out of the controls. Mortality of birds between hatch and D72 was below 10% and an IBDV lesions rate of 100% (histological score ≥ 3) was observed in the control group, while no clinical signs were reported throughout the 10 days of monitoring.
None of the vaccinates had bursal lesions except one, which was seronegative at D70 and had grade 4 lesions of the bursa of Fabricius; thus, the rate of protection was 95%.

The already established DOI of 10 weeks post-hatch was confirmed by the provided data for the in-ovo route and was included in the SPC.

Maternally derived antibodies (MDA)

Marek's disease – onset of protection

Two studies were conducted on conventional embryonated eggs of broiler chicken with maternal antibodies: one pre-clinical study and one efficacy study with birds from the field trial (see Clinical trials).

In the first study, Prevexxion RN+HVT+IBD was administered in-ovo in 45 embryos (18 days of incubation) to check the onset of immunity. The vaccine diluent was administered to 45 controls. A challenge with Md5 strain was performed 8 days post-vaccination (i.e. 5 days post-hatch) on 35 chickens of each group. Two other groups of 15 eggs each were used as necropsy and serological controls, and no maternally derived antibodies (MDA)-free group was included because they are not available for these conventional broilers.
The MDA against IBDV were detected in birds sampled on D0, as expected in conventional birds. No vaccine DNA was amplified in the 8 non-vaccinated chickens, while 3/7 were positive in the vaccinated group.
The MD protection level was evaluated in each group by a daily clinical monitoring for more than 70 days after challenge, followed by a necropsy and examination for specific gross lesions of MD.

The study was validated, since hatchability was at least 96%; only 1 death was reported in hatched chickens and a MD-associated morbidity rate of 91% was observed in the control group.
Vaccination prevented mortality with a relative protection rate of 81%.

As both studies demonstrated protection to a challenge in 5-day-old conventional broilers, the onset of immunity has been set at that age.

Gumboro disease

In one study, Prevexxion RN+HVT+IBD (3.6 $\log_{10}$ vHVT013 + 3.0 $\log_{10}$ RN1250 PFU/bird) was administered in-ovo to 40 conventional broiler embryos (18th day of incubation) to check the onset of immunity. Vaccine diluent was administered to 40 controls. A virulent challenge by ocular route with strain 100045 was performed either 28 or 35 days after hatch on 20 controls and 20 vaccinates. No MDA-free group was included because they are not available for these conventional broilers.
Birds were monitored over 10 days after challenge and then necropsied.
Vaccine DNA in the spleen (2/10 positive) of vaccinates as well as D28 IBDV serology confirmed that the vaccine was well administered, and IBDV serology in control birds showed that MDA level decreased over the 35-day timespan after hatch but since 50% of control still had MDA on day 35, challenges were undertaken in the presence of MDA.
The study was acceptable, since mortality of hatched chicken before challenge was below 10% and the rate of birds with atrophy of the bursa of Fabricius and IBDV lesions with a histological score ≥ 3 was 100% in the control group, while no clinical signs were reported throughout their 10 days of monitoring.
None of the vaccinates exhibited clinical signs and 6 birds out of 20 were not protected (microscopical lesions with a score ≥ 3), providing a 70% protection for challenge 4 or 5 weeks after hatch. The bursa of Fabricius weight/body weight ratio was also significantly higher in the vaccinates. In conclusion, the lower immunisation detected in vaccinates led to a lower protection.

Additional studies

Gumboro disease

In one study, any possible interference of RN1250 on Gumboro immunisation by vHVT013-69 was checked in conventional embryonated eggs of 18 days of incubation when a maximum dose of RN1250 is administered in-ovo in combination with the minimum dose of HVT013 (group 3).
In this study, a control group was inoculated with the diluent (group 1) and another one received the bivalent vaccine at minimum dose (or close to it) for each antigen (group 2). A challenge with the very virulent strain 100045 was performed 35 days post-hatch in subgroups (20 animals/subgroup). Remaining chicks (≤ 15/group) allocated to other subgroups were not challenged but their blood was sampled for IBDV serological monitoring over time until day 70.
Serology (20/20 seropositive) and vaccine DNA in 4 spleens out of the 5 sampled confirmed that the vaccine was well administered to vaccinates and demonstrated the absence of vaccination and the maternally derived antibody wash-out of the controls. However, maternally derived antibodies were still present at time of challenge.
Hatchability was above 80%, mortality of birds before challenge on day 35 was below 10% and an IBDV lesions rate of 100% (histological score ≥ 3) was observed in the control group, while no clinical signs were reported throughout the 10 days of monitoring.
Vaccinates of both group 2 and group 3 were protected (bursal lesion score of 1), except one bird of group 3 with a score of 4 and an antibody titre consistent with remaining MDA.
There was no significant difference between the IBDV titre of group 2 at the 4 tested dates and those of group 3, indicating the absence of impact of a high dose of RN1250 on the vHVT013

immunogenicity.
The absence of conspicuous interference of RN1250 on vHVT013-69 immunisation is thus confirmed by in-ovo route.

Clinical trials

One field study was undertaken using conventional 18-day-old broiler embryo chicks. No outbreak of Marek's disease or Gumboro disease was reported in the 2 farms.

However, eggs from these field trials were directly transferred to laboratory after in-ovo vaccination with a batch representative of commercial batches (3.5 $\log_{10}$ RN1250 & 4.1 $\log_{10}$ vHVT013) and challenged either with vvMDV or vvIBDV like in the preclinical studies (study design in accordance with Ph. Eur. recommendations).

Protection against Marek's disease

Healthy one-day-old chicks were transferred from the hatchery to laboratory immediately after hatching – 47 vaccinated and 47 unvaccinated chickens. Thirty-seven birds of each group were challenged 5 days later with the Md5 strain and monitored over 70 days for Marek's disease.
The study was acceptable, since 2.1% mortality unrelated to vaccination was reported before the challenge and a MD-associated morbidity rate of 100% was observed in the control group.
DNA of RN1250 vaccine strain was found in 2 spleens out of the 10 sampled 11 days after in ovo injection, and DNA of vHVT013 was found in 5 spleens; the vaccine was thus well administered. No DNA of vaccine strains was amplified in control chickens.
Vaccination prevented mortality and 1/30 birds showed MD-evocative clinical signs at the end of the monitoring period without macroscopical lesion at necropsy. Thus, the relative protection rate to a challenge 5 days after hatch was 96%.

Protection against Gumboro disease

Healthy one-day-old chicks were transferred to a laboratory after vaccination with the same Prevexxion RN+HVT+IBD commercial batch (45) or a commercial live Rispens+HVT FC126 vaccine (37) or without vaccination (35 control birds). Birds from the Rispens+HVT FC126 group were subsequently vaccinated with a live attenuated Gumboro disease vaccine on day 21 and day 27. On day 28, 10 unvaccinated birds and 20 birds of the 2 vaccinated groups were challenged by ocular route with the vvIBDV strain 100045.
Vaccination with Prevexxion RN+HVT+IBD was verified by amplification of RN1250 DNA in 4/8 (50%) and HVT DNA in 6/8 (75%) spleen samples and the lack of any amplification confirmed the absence of vaccination of the control group. Anti-IBDV MDAs were detected in the control birds on day 39; the challenge was thus undertaken before the complete wash-out of the MDAs.
The challenge triggered clinical signs in 2/10 control birds and atrophy with score 4 microscopical lesions of all the bursae of Fabricius.
Vaccination with Prevexxion RN+HVT+IBD protected 9/20 (45%) of the vaccinates (score of microscopic lesions < 3).

Overall conclusion on efficacy

The efficacy of Prevexxion RN+HVT+IBD by in-ovo vaccination was demonstrated for Marek's and Gumboro diseases in pre-clinical studies compliant with respective Ph. Eur. Monographs in SPF embryos at the onset of immunity. The vaccine was administered at its minimum release titre (2.9 $\log_{10}$ PFU RN1250 & 3.6 $\log_{10}$ PFU vHVT013) and the birds were challenged by very virulent MDV-1

or IBDV strains. Clinical signs and lesions for both infections were reduced and mortality was prevented for both viral infections.
The efficacy was also demonstrated in laboratory studies devised in accordance with the corresponding Ph. Eur. Monographs, where conventional broilers with maternally derived antibodies and vaccinated with batches at minimum titre were challenged both at the onset (Marek's and Gumboro diseases) and at the end of the claimed period of the duration of immunity (Gumboro disease). The efficacy was confirmed with a commercial batch with vaccinates from a field trial which were challenged at the onset of their immunity in similar laboratory studies.

The addition of the in-ovo route of administration is generally considered efficacious for Prevexxion RN+HVT+IBD, if used according to the information stated in the SPC.

Part 5 – Benefit-risk assessment

Introduction

Prevexxion RN+HVT+IBD is a bivalent vaccine containing two cell-associated live 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 aim of the present variation is to register a new route of administration allowing the vaccination of 18-day-old embryonated eggs by in-ovo route.

This new route of administration will increase the range of administration routes of Prevexxion RN+HVT+IBD and will decrease the risk for the user of the vaccine.

The direct benefits remain unchanged, with the same indications for this new route of administration, supported by 7 new efficacy studies. The risks listed in the original authorisation, which have been checked in 8 new safety studies, including the quality risks associated with a 4-fold concentration of vaccine viruses in the final product, also remain unchanged.

Benefit assessment

Direct benefit

The direct benefit remains unchanged by this variation.

Additional benefits

This new route of vaccination is an additional benefit of this variation.

Risk assessment

The risk assessment remains unchanged by this variation.

Risk management or mitigation measures

The risk assessment remains unchanged by this variation.

Evaluation of the benefit-risk balance

The benefit-risk balance remains unchanged by this variation.

Conclusion

Based on the original data presented on quality, safety and efficacy, the Committee for Veterinary Medicinal Products (CVMP) concluded that the application for a variation to the terms of the marketing authorisation for Prevexxion RN+HVT+IBD is approvable, since these data satisfy the requirements as set out in the legislation (Regulation (EU) 2019/6).

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