7 September 2023
EMA/428201/2023
Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Poulvac Procerta HVT-IBD (EMEA/V/C/006000/0000)

Vaccine common name: Live recombinant turkey herpes virus, strain HVT-IBD, expressing the VP2 protein of infectious bursal disease virus

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

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Introduction

The applicant Zoetis Belgium submitted on 26 July 2022 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Poulvac Procerta HVT-IBD, through the centralised procedure under Article 42(2)a of Regulation (EU) 2019/6 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 9 September 2021 as Poulvac Procerta HVT-IBD has been developed by means of a biotechnological process, i.e. using recombinant DNA technology (Article 42(2)(a)(i)).

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

For active immunisation of one-day-old chickens and 18-19 day old embryonated chicken eggs to

- reduce mortality, clinical signs and lesions caused by Marek's disease virus and

- prevent mortality and clinical signs and reduce lesions caused by infectious bursal disease virus.

Onset of immunity:	MD: 4 days of age for *in ovo* and 9 days for subcutaneous use
	IBD: 12 days of age
Duration of immunity:	MD: a single vaccination is sufficient to provide protection for the entire risk period
	IBD: 64 days of age

The vaccine is based on a live recombinant vaccine strain and is therefore a GMO product.

The active substance of Poulvac Procerta HVT-IBD is the recombinant live herpes virus of turkey Marek's disease virus [HVT, MDV serotype 3, genetically modified to express the VP2 protein of infectious bursal disease virus (IBDV)]. The target species are chicken and embryonated chicken eggs.

The vaccine consists of a deep-frozen suspension of cell-associated recombinant HVT in glass ampoules containing 2000 or 4000 doses of the vaccine. The ampoules are stored in cryopreservation containers in a cane. The solvent is a sterile, watery solution. The vaccine concentrate is mixed with the solvent prior to the subcutaneous (SC) injection in the neck or the *in ovo* injection.

The rapporteur appointed is Esther Werner and the co-rapporteur is Jeremiah Gabriel Beechinor.

The dossier has been submitted in line with the requirements for submissions under Article 8 of Regulation (EU) 2019/6 – full application.

On 7 September 2023, the CVMP adopted an opinion and CVMP assessment report.

On 26 October 2023, the European Commission adopted a Commission Decision granting the marketing authorisation for Poulvac Procerta HVT-IBD.

Part 1 - Administrative particulars

Summary of the Pharmacovigilance System Master File

The applicant provided documents that set out the summary pharmacovigilance system master file (SumPSMF) in Part 1.

A unique reference number for the PSMF is provided (PSMF-ZOETIS-BE1930-v1) and the location of the PSMF has been stated (Veterinary Medicine Research and Development, Zoetis Belgium, Mercuriusstraat 20, 1930 Zaventem, Belgium).

The name, contact details and place of operation of the qualified person responsible for pharmacovigilance (QPPV) is stated. The curriculum vitae of the QPPV is provided.

A signed and dated (21Jan2022) statement from the marketing authorisation holder and the QPPV that the applicant has a QPPV and pharmacovigilance system in place has been provided. An updated signed statement in the PSMF summary that reflects the wording required by Commission Implementing Regulation (EU) 2021/1281, Article 22(2)(b), point (i) has been provided.

The type of record management system used for adverse event reports including the name of the database (PV Works), if applicable, has been provided.

Manufacturing authorisations and inspection status

Active substance and finished product

Manufacture, in-process controls and storage of the active substance and the finished product and the primary and secondary packaging of the vaccine virus concentrate of this live recombinant turkey herpesvirus, strain HVT-IBD, expressing the VP2 protein of infectious bursal disease virus vaccine takes place outside the EEA at Zoetis Inc., 2000 Rockford Road, Charles City, IOWA, IA50616-9101, United States.

A manufacturing authorisation from the competent US authority has been provided. A certificate of GMP compliance from the competent authority in Belgium for Zoetis Inc. 2000 Rockford Road, Charles City, IA, 50616-9101, United States. (No. BE/GMP/2022/048) related to the inspection performed on 29 April 2022, issued on 24 November 2022 has been submitted.

The final product testing and the batch release is performed at Zoetis Manufacturing & Research Spain S.L Carretera De Camprodón, s/n, La Vall de Bianya, Girona, 17813, Spain.

In addition, this production site is responsible for the manufacturing, quality testing, storage and release of the solvent.

The authorisation certificate (No. 0730) has been submitted for Zoetis Manufacturing & Research Spain S.L Carretera De Camprodón, s/n, La Vall de Bianya, Girona, 17813, Spain.

The certificate was signed on 27 December 2021. All activities performed at this production plant for this vaccine are allowed according to this certificate.

A GMP certificate from 27 May 2021 from the competent authority of Spain is submitted. The certificate is based on an inspection performed on 12 March 2021.

Overall conclusions on administrative particulars

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

GMP certificates based on EU inspections are available for Zoetis Inc. 2000 Rockford Road, Charles City, IA, 50616-9101, United States and for Zoetis Manufacturing & Research Spain S.L Carretera De Camprodón, s/n, La Vall de Bianya, Girona, 17813, Spain.

Part 2 - Quality

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

Qualitative and quantitative composition

The vaccine consists of a deep-frozen suspension of cell-associated recombinant serotype 3 herpesvirus of turkey (HVT) containing the VP2 gene of infectious bursal disease virus (IBDV), at a titre of 3,580 -26,500 plaque forming units (PFU) per dose. Stabilisers (bovine serum, Dulbecco's Modified Eagle Medium (DMEM) and a cryoprotectant (DMSO) are included in the formulation.

The solvent is a sterile, watery solution, which contains sucrose, potassium dihydrogen phosphate, dipotassium phosphate, peptone (NZ Amine), a colouring agent/indicator (phenol red) and water for injections.

The vaccine concentrate is mixed with the solvent prior to use.

Container and closure system

The vaccine is filled in 2 ml heat-sealed type I sterile glass ampoules in accordance with the European Pharmacopoeia (Ph. Eur. 3.2.1). The solvent is filled in 250, 500 or 1,000 ml plastic bags made of polyvinyl chloride (PVC) for pharmaceutical use. The bags are closed with a pharmaceutical grade polycarbonate stopper with a latex-free rubber disc. The bags meet the requirements of Ph. Eur. 3.2.2.1 for plastic containers for aqueous solutions for parenteral infusion. Forming, filling, closing/sealing and terminal heat sterilisation of the bags is performed in a continuous process. Specifications and certificates demonstrating Ph. Eur. compliance for the ampoules and bags are included in the dossier. A certificate of analysis (CoA) for the stoppers translated in English has been provided.

Product development

Poulvac Procerta HVT-IBD is a frozen, cell-associated vaccine that contains the recombinant serotype 3 herpesvirus of turkeys (HVT) with genes from IBDV. The vaccine can be used to stimulate active immunity against Marek's disease (MD) and infectious bursal disease (IBD). HVT is an avirulent strain related to MDV, which has been widely used as a vaccine strain for prevention of MD. HVT has several features that make it an attractive vector for the delivery of foreign antigens. Most important is the safety profile of the vector. The virus hardly spreads, is fully apathogenic in chicken and is not infectious for any species other than avian. HVT strain FC-126 was genetically modified by inserting

the VP2 gene of IBD virus resulting in the vaccine strain HVT-IBD. The use of a recombinant vaccine has the advantage that protective immunity against both HVT and IBDV can be obtained without the use of live attenuated vaccines for IBD.

The genetically modified organism (GMO) vaccine strain is cell-associated and, therefore, needs to be stored and transported frozen in liquid nitrogen. The handling, thawing and diluting have to be performed with care and by using protective equipment.

The production system used, and the pharmaceutical form of the vaccine are identical to other MD vaccines manufactured by the applicant.

The solvent used with the vaccine is the same as for the other live MD vaccines manufactured by the applicant. An indicator (phenol red) is included to enable a check on filling of the automated vaccination equipment in the field. The absence of a virucidal effect of the solvent was investigated by many solvent batch tests performed for batches used of already licensed live MD vaccines.

The difference in dose volume (0.05 ml for *in ovo* route and 0.2 ml for subcutaneous route) is linked with the volume of sterile solvent used to reconstitute the frozen cell concentrate before administration.

Description of the manufacturing method

The manufacturing process of the **vaccine** consists of seven main steps:

In step 1, the primary chicken embryo fibroblasts (CEF) are prepared from embryos harvested from embryonated specific pathogen free (SPF) chicken eggs. The CEF suspension is seeded with a specific target of cells/ml in culture flasks. The CEF suspension is used for direct inoculation with HVT/IBD virus seed for virus seed expansion for routine production and working seed manufacture.

In step 2, CEF are infected with seed virus suspension and incubated until the characteristic cytopathogenic effect (CPE) is evident. In step 3, the infected cells are harvested by discarding growth medium and dispersal of cells and agitation. Cells are collected and pooled. The cells are centrifugated. After the supernatant is discarded, packed cells are resuspended in freeze medium for freezing down for finished product preparation. The antigen is used immediately to blend the vaccine as described in step 4.

In step 4, the bulk is prepared. The cells and debris are separated. The cell suspension is adjusted to a specific final concentration of viable cells per ml (cell input calculated in order to reach a release titre within specifications) in freeze medium. Dimethyl sulfoxide (DMSO) is added to the suspension.

The resuspended volume of cells is brought to final batch volume by slow addition of freeze medium. The mixture is slowly agitated to ensure homogeneity.

In step 5, filling is performed. The final bulk is stirred prior to and throughout the filling operations and is aseptically filled into sterile 2 ml glass ampoules. Ampoules are flame-sealed and placed in canes for storage. The filling and sealing of the ampoules are done by an automatic filling machine.

In step 6, the product is labelled and subsequently frozen in a controlled-rate freezer. The frozen product is transferred to liquid nitrogen storage.

In step 7, samples of deep-frozen ampoules are taken and sent from Charles City (USA) to Olot (Spain) for final product testing.

The complete production of the vaccine is a continuous process.

The manufacturing of the **solvent** consists of a simple mixing process. The pH is adjusted and the solution is filter sterilised. After aseptic filling into PVC bags, the product is terminally sterilised.

The solvent batches are stored at room temperature between 20 and 25 °C.

Validation data of the antigen production, blending and filling as well as of the solvent production process are provided.

Production and control of starting materials

Starting materials listed in pharmacopoeias

Example certificates of analysis have been provided and all conform to specifications in the respective Ph. Eur. monographs. A reference to the relevant Ph. Eur. monograph has been given, but for a few materials used for vaccine production, reference was given to the USP or ACS only. Only for one starting material to be used in routine production an EDQM Certificate of Suitability (CoS-CEP) was issued.

Starting materials not listed in a pharmacopoeia

Starting materials of biological origin

Master seed virus (MSV)

The testing and assessment of the MSV are based on the following legal requirements:

Ph. Eur. Monograph 5.2.5: 07/2020 Management of extraneous agents in immunological veterinary medicinal products, Guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010-Rev.1), Regulation (EC) 2019/6 as amended by Annex II, Guideline on live recombinant vector vaccines for veterinary use (EMEA/CVMP/004/04-FINAL).

Control tests performed on the MSV are sterility testing, freedom from mycoplasma and mycobacterium. Confirmation of identity was done and potency for the HVT fraction and IBDV fraction was tested. A complete extraneous agent testing was performed. The genetic stability of the recombinant strain was demonstrated. Characterisation of the MSV showed no genetic or phenotypical differences between passages. Genotypic and phenotypical stability were also confirmed.

A certificate of analysis for the MSV is provided. A transmissible spongiform encephalopathy (TSE) assessment for the MSV is submitted and confirms a negligible risk of contamination for the MSV and the following WSV lots.

Working seed virus (WSV)

WSV lots are produced according to the process described in the registration dossier.

SPF eggs for production of CEF

The eggs are sourced from suppliers that deliver routine flock certificates of analysis.

A risk assessment with considerations of the extraneous agent (EA) testing requirements as per Ph. Eur. 5.2.5 is provided the observation of the SPF embryos and the routine testing on each CEF batch at the applicant’s premises. is regarded sufficient for the exclusion of a potential contamination with

Chlamydia spp. Testing for Avian Rotavirus is performed by the suppliers.

Porcine trypsin

Porcine trypsin is manufactured from porcine pancreas from healthy animals of certified origin and contains lactose derived from bovine milk. The milk is sourced from healthy animals of certified origin under the same conditions as milk collected for human consumption.

Trypsin is gamma irradiated and the bulk material is again irradiated prior to use.

A CoA from the supplier shows compliance with the starting material specifications from Zoetis.

Calf serum (fortified bovine calf serum)

The material is sourced from the USA.

The serum is gamma irradiated before use. Extraneous agents testing must show compliance with the starting material specifications.

EDQM CEPs are provided.

A risk assessment for freedom from extraneous agents according to Ph. Eur. 5.2.5 is provided.

NZ Amine YT (peptone)

It is an enzymatic digest of casein derived from bovine milk and in compliance with the "Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products".

A TSE statement is part of the certificate and is available for every batch.

An extraneous agents risk assessment is performed and provided.

Starting materials of non-biological origin

The starting materials of non-biological origin referred in this section are used as components of media at different stages of the manufacturing of the vaccine. For use in production, certificates of analysis from supplier(s) or alternatively in-house testing must show compliance with the starting material specification. Specifications together with representative certificates of analysis are provided in starting material monographs.

In-house preparation of media and solutions consisting of several components

Detailed qualitative and quantitative composition, method of preparation and storage of the media and solutions prepared in-house are provided.

Antibiotics are used in cell growth media and solutions however, not more than minimal traces are to be expected in the final product.

Control tests during the manufacturing process

The product is manufactured in one continuous operation. The proposed control tests during the manufacturing process are considered adequate to support a consistent process.

No tests are proposed to be performed during the manufacturing of the solvent. The solvent in the

final bag is terminally sterilised.

Control tests on the finished product

The product is tested for identity, potency, sterility and absence of mycoplasmas. Reference to the relevant Ph. Eur. monographs is given, or a test standard operating procedure (SOP) has been provided. A test for the control of the fill volume is performed. These tests are suitably validated. No general tests are proposed since the finished product is stored in liquid nitrogen. Waiving of the extraneous agents testing is proposed and a risk assessment is provided. The visual appearance is checked before thawing to verify that samples were correctly frozen. The SPC description of the colour and in the visual appearance test of the ampoules is based on the thawed and not on the frozen vaccine concentrate.

Tests performed on the solvent are considered suitable to control the quality. An upper limit for the filling of the solvent bags has been fixed and internally validated.

Batch-to-batch consistency

Three consecutive production runs Poulvac Procerta HVT-IBD were manufactured in support of the batch-to-batch consistency. The results of in-process control and finished product testing indicate that the production process results in a product of consistent and appropriate quality.

Stability

No stability data for antigen batches have been generated and provided.

This is acceptable as the applicant states that the vaccine is produced via a continuous process and the antigen batches will not be stored but immediately used to formulate the final product.

Stability of the finished product

The applicant proposes a shelf life of 24 months for the finished product. A stability study with 3 batches over a period of 27 months has been submitted. No out of specification results. Therefore, the proposed shelf life of 24 months is regarded as demonstrated.

An in-use stability study could confirm the proposed shelf life for the vaccine ready for use for two hours at room temperature. The vaccine must be stored and transported in liquid nitrogen (or vapour phase) at or below -150 °C.

As the vaccine is transported overseas and inside Europe again, additional data are presented for different materials (seed material, pilot test batches, master virus material), which can confirm a longer stability at temperatures up to –100° C as proposed for this vaccine.

The proposed shelf life for the solvent stored in PVC bags at room temperature for 24 months is supported by a complete stability study over 27 months.

Overall conclusions on quality

Poulvac Procerta HVT-IBD is a live recombinant vaccine for the active immunisation of chickens against Marek's disease and infectious bursal disease. The vaccine is available in ampoules containing 2,000 or 4,000 doses and is diluted before use in solvent supplied in plastic bags (PVC) containing 200, 400, 800 or 1,000 ml.

One dose of vaccine contains 3,580 – 26,500 PFU of virus strain HVT-IBD as active ingredient. The virus is grown on CEF monolayers produced from embryos obtained from SPF chicken flocks. The manufacturing method can be considered as standard for this type of vaccine. Cells containing the virus are harvested and blended to allow storage in liquid nitrogen.

Procedures have been implemented to ensure the absence of extraneous agents in starting materials of animal origin. TSE risk assessment for the starting materials used is provided. The risk that the final product may transmit TSE to the target animal is considered negligible.

The production method, including appropriate in-process controls and quality controls on the finished product together with control of the starting materials, ensure a consistent quality of batches of vaccine. The whole production process was evaluated at production scale and shown to be consistent.

Results of the stability tests for the final product of three batches showed no loss in infectivity titre during a 27 months storage period in liquid nitrogen and a complete stability study over 27 months also supports the 24-month-shelf life of the solvent. Stability data of reconstituted product show that the vaccine remains stable at room temperature for 2 hours. In conclusion, the production process is adequately described and controls in place are appropriate to ensure the quality of the product at release and throughout the shelf life.

Part 3 – Safety documentation (safety and residues tests)

General requirements

Poulvac Procerta HVT-IBD is a bivalent, cell-associated, live recombinant virus vaccine for use in chickens and embryonated chicken eggs containing live recombinant serotype 3 turkey herpesvirus (HVT) strain FC-126 with the VP2 gene of infectious bursal disease virus (IBDV) inserted into the HVT genome. No adjuvant or preservative is included in the vaccine. The maximum antigen concentration per dose (0.05 ml *in ovo* or 0.2 ml subcutaneously) contains 26,500 PFU (Plaque Forming Units) of the recombinant HVT-IBD strain.

A full safety dossier in accordance with Article 8(1)(b) of Regulation (EU) 2019/6 has been provided. Studies to determine the safety of the vaccine were performed in accordance with Ph. Eur. monograph 0062 on vaccines for veterinary use, Ph. Eur. chapter 5.2.6 on the evaluation of safety of veterinary vaccines and immunosera, Ph. Eur. monograph 0589 "Marek's disease vaccine (live)", section IIIb of Annex II to Regulation (EU) 2019/6 and VICH GL 44. Ph. Eur. monograph 0587 "Avian infectious bursal disease vaccine (live)" was also considered.

Poulvac Procerta HVT-IBD is presented as a frozen cell suspension in flame-sealed glass ampoules stored in liquid nitrogen, which is to be diluted before use in a sterile solvent (Poulvac Solvent).

The vaccine is administered *in ovo* to 18–19-day-old embryonated chicken eggs (0.05 ml) or subcutaneously (SC) to 1-day-old chickens (0.2 ml) to stimulate active immunity against infection with Marek's disease virus (MDV) and IBDV.

Safety documentation

Seventeen (17) safety studies were conducted to investigate the safety of the product. They included 14 pre-clinical studies investigating the safety of a 10-fold overdose, immunological functions, spread to non-target species (turkeys, ducks, pheasants, quails, and mice) and examination of reversion to

virulence as well as three field trials. The vaccine was administered by the *in ovo* and SC routes in the target species of minimum age, as recommended.

Most of the laboratory studies were reported to be good laboratory practice (GLP)-compliant, with the exception of a part of the laboratory phase (back titration), one of the non-target species studies and one study on environmental survival. One production batch was used in the field trials conducted in Spain, Hungary, and Italy in compliance with the principles of good clinical practice (GCP).

Additionally, the applicant included eight non-GLP-compliant pre-clinical studies and a summary report on four clinical studies. All these studies were performed in the United States of America for registration of the product in non-European countries. In these additional only supportive studies, the US solvent (Poulvac Sterile Diluent) was used, which has a slightly different composition compared with the EU solvent. Nonetheless, the pH range and most of the ingredients are the same.

Study title/purpose
10x overdose (and reversion to virulence)
10x target animal safety
Bursal damage
Immunosuppression
Spread, shedding and dissemination
Shedding and spread
Spread from chicken to turkey
Spread from turkey to turkey
Overdose in not-target species turkeys
Non-target animal safety in turkeys
Overdose in not-target species ducks - terminated
Overdose in not-target species ducks
Overdose in not-target species pheasants
Overdose in not-target species quail
Non-target animal safety in quail
Overdose in not-target species mice
Non-target animal safety in mice
Dissemination and environmental survival
Back passage for reversion to virulence study
Back passage and reversion to virulence
Back passage and reversion to virulence - confirmation
Environmental survival
Field study in Spain
Field study in Hungary
Field study in Italy
Evaluation of field safety in the US
Evaluation of field safety in the US
Evaluation of field safety in the US
Evaluation of field safety in the US

Studies in blue colour are only supportive because they were performed for registration purposes in other parts of the world and are not GLP or GCP conform.

Pre-clinical studies

Two breeds of SPF chickens were used in the EU pre-clinical studies (GD and VALO) and one further chicken line (Charles River) in the US studies. The susceptibility of the SPF chicken breeds GD and

Charles River was shown by a challenge with a virulent MDV in two overdose studies. The susceptibility for the VALO line was not explicitly shown but is accepted as this breed was often used before. For all EU studies, SPF certificates of the parent flocks are provided.

For the assessment of the results of observations, different clinical scoring systems were used. Usage of similar scoring systems is beneficial, especially when analysing clinical signs in studies that were performed by different contractors/laboratories. However, the clinical data provided were comparable. Humane endpoints to avoid unnecessary suffering of the study animals were implemented. The inclusion of surplus healthy chickens was justified. The principles of the 3Rs should always be kept in mind while preparing new studies.

Safety of the administration of one dose

No single dose study is provided. A single dose safety testing is not required according to Ph. Eur. Monograph 0589 as an overdose study is requested here and therefore, no specific one-dose safety study was performed as suggested by Annex II to Regulation (EU) 2019/6.

Safety of one administration of an overdose

One pivotal 10-fold overdose study and one supportive overdose study were provided.

In the pivotal EU study a 10-fold maximum overdose containing 265,038 PFU/0.2 ml of Poulvac Procerta HVT-IBD was administered by the recommended routes (*in ovo* or SC) to 18-day-old embryonated SPF chicken eggs and 1-day-old SPF chicks (minimum age as required). The volume of 0.2 ml/egg was higher than the recommended volume of 0.05 ml/egg and was chosen in order to reach a sufficiently high virus titre per dose. Additionally, in this study a non-inoculated control group and two groups that were challenged with a very virulent MDV (vvMDV) strain RB1B either *in ovo* or intramuscularly (IM) were included to demonstrate susceptibility of the chicken line used to infection with MDV.

The impact of the *in ovo* vaccination on hatchability was examined. The control group and both vaccinated groups were observed for 120 days for clinical signs and mortality; the groups challenged for a maximum of 70 days. From the control group and both vaccinated groups, blood, organ samples and oropharyngeal/cloacal swabs were collected to recover the vaccine virus via cultivation on chicken embryo fibroblasts (CEFs; HVT plaque assay) and confirm via specific immunostaining (IFA). Presence of HVT-IBD antigen in feather follicles by real-time HVT-PCR was assessed as well as bursa lesions. Additionally, body weight and bursa weight were determined to calculate the bursa-to-body-weight ratio. Necropsies were performed on all chickens that died/were euthanised and at the end of the respective observation periods for examination of the presence of lesions related to MD and IBD.

No impact on hatchability was detected as hatch was over 88 % in all groups. No clinical signs or deaths or specific lesions related to MD or IBD were noted in the control group and in both vaccinated groups. 100 % and 88 % of the chicks challenged IM and *in ovo* were diagnosed with MD, respectively, indicating the susceptibility of the chicken breed used. In both vaccinated groups, virus was recovered from all organs and feather follicles on all days sampled. The swabs were positive only on some days. No virus was recovered from the control group. Mean bursa lesion scores were similar in all groups but significantly higher at day 120 (maximum 1.9) indicating changes due to the natural involution of the organ Mean body weight, mean bursa weight and mean bursa-to-body-weight ratio were comparable in all groups.

In summary, the vaccine virus is considered safe for chickens when given at a 10-fold maximum dose of 265,038 PFU to 18-day embryonated chicken eggs or 1-day-old chicks.

For the solely supportive US overdose study, Poulvac Procerta HVT-IBD was inoculated at an approximately 4-fold maximum overdose *in ovo* in 18-day-old embryonated SPF chicken eggs (114,952 PFU) and at an approximately 5-fold maximum overdose SC to 1-day-old SPF chicks (144,024 PFU). For both routes, a volume of 0.3 ml was used. A non-inoculated control group and a group challenged intraabdominally with vvMDV strain RB1B on the fifth day of life to prove susceptibility of the chicken line used to infection with MDV were also included in this study.

The impact of the *in ovo* vaccination on hatchability was examined. The control group and both vaccinated groups were observed for 120 days for clinical signs and mortality; the challenge group for a maximum of 50 days. Necropsies were performed at the end of the respective observation periods or earlier when chickens died or were euthanised. Detected tumours were examined by histology and via immunohistochemistry and PCR for confirmation. Body weights were determined.

No impact on hatchability was detected as hatch was over 95.9 % in all groups. No clinical signs or deaths or specific lesions related to MD or IBD were noted in the control group and both vaccinated groups. In the group challenged, MD lesions were present in 93.33 % of the chickens indicating the susceptibility of the chicken breed used. No differences in body weight were detected comparing groups.

In summary, the vaccine virus is considered safe when given at an approximately 4-fold maximum overdose to 18-day-old embryonated chicken eggs or 5-fold maximum overdose to 1-day-old chicks at MSV level.

Safety of the repeated administration of one dose

The vaccine is to be administered once via *in ovo* vaccination at 18-19 days of embryonation or via the subcutaneous route at one day of age. In accordance with Annex II to Regulation (EU) 2019/6, assessment of safety of repeated vaccination is therefore not required.

Examination of reproductive performance

No studies on the reproductive performance were provided as this product is not indicated for use in laying birds. In SPC section 3.7 Use during pregnancy, lactation or lay, the following adequate warning is included *'The safety of the veterinary medicinal product has not been established during lay.'*

Examination of immunological functions

Two studies were conducted to investigate the effect of the product on immunological functions. Only the MSV+3 level was used here, which was justified by the applicant.

The aim of the first EU study was to examine bursal damage after vaccination with an approximately 5-fold maximum overdose (126,080 PFU) to determine the appropriate timing for vaccination against Newcastle disease (ND) to perform the subsequent immunosuppression study. Moreover, hatchability was examined as well as clinical signs, mortality and pathological lesions.

Ninety SPF chicken eggs were incubated, 60 were *in ovo* vaccinated and after hatch, 50 SPF chickens (25 control and 25 vaccinated) were housed. Hatchability was over 93 % in all groups. No clinical signs

and mortality were observed. Considering bursa lesions scores and the development of bursas, the applicant decided to use day 14 after hatch as optimal time for vaccination with a ND vaccine.

In the subsequent EU study, 18-day-old embryonated SPF chicken eggs were vaccinated *in ovo* with the product; meanwhile another group remained naïve. Both groups were vaccinated at 14 days of life with a ND Hitchner B1 vaccine and challenged 14 days later IM with virulent NDV strain Herts to assess the influence of the vaccination with the product. Hatchability of the *in ovo* vaccinated group was only 77 %; however, in all other studies provided hatchability was over the 80 % limit. No significant difference in seroconversion after vaccination against ND or in protection rates after NDV challenge were noticed comparing the groups (the protection rate was 100 % in both groups; no clinical signs or mortality due to NDV were observed after NDV challenge, while all birds of a non-vaccinated control group succumbed to challenge).

Therefore, it is considered unlikely that this vaccine will have an adverse effect on immunological functions.

Special requirements for live vaccines

Spread of the vaccine strain

Spread in the target species

A GLP-compliant EU study examining shed, spread, and dissemination in the target species has been provided. SPF chickens vaccinated *in ovo* either with the test product (WSV) or with the parental strain were mingled in various pens each including a naïve group of SPF chickens for a maximum observation period of 42 days. Hatchability, clinical signs and mortality were observed. On days 7, 21, 28 and 42, randomly selected chickens per group and time point were bled and euthanised for pharyngeal/cloacal swabs and tissues (feather pulp, spleen, bursa, kidneys) collection. At each time point, a dust sample from each pen was taken.

No impact on hatchability was noted and no clinical signs or mortality related to the vaccination were observed. The vaccine strain and also its parental strain were found to disseminate to all tested tissues and were detectable in all tissues until the end of the observation period. The vaccine strain was shed by the pharyngeal und cloacal route until D7. However, virus was detected in dust on D28, but not on D42 anymore, indicating the possibility of the virus to shed for a maximum of 6 weeks post vaccination. This is reflected in section 3.5 of the SPC. The parental HVT strain was found in pharyngeal swabs until D7 and in cloacal swabs until D28: all dust samples were negative. No spread to in-contact birds was noticed in this study. It can be concluded that the biological properties of the vaccine strain are very similar to those of its parental strain.

In addition, some data on spread, shedding and dissemination are also included in the 10-fold overdose study.

The supportive US study investigated the spread from vaccinated chickens to naïve chickens comparing the HVT-IBD vaccine strain to its parental HVT strain. Hatchability, clinical signs and mortality were assessed as well as virus recovery from blood, spleen, oropharyngeal/cloacal swabs, and debris/dust. Shedding via feather follicles and seroconversion to IBDV was also examined. Both virus strains were shed up to 22 days (last sampling point of study) and were able to spread to naïve chickens to a limited degree indicating no change of biological properties of HVT by the VP2 insert.

Spread to the non-target species turkey (natural host of HVT)

In a first EU study (non-GLP compliant equipment used, but otherwise under GLP conditions), spread from vaccinated chickens to naïve turkeys was investigated. A second EU study examined the spread of the virus strain from vaccinated turkeys to naïve turkeys in comparison to its parental HVT strain. In both studies, it was shown that the vaccine virus is able to spread to in-contact turkeys. The pattern of spread was similar comparing the vaccine virus with its parental HVT virus.

The safety of an overdose of Poulvac Procerta HVT-IBD for turkeys was investigated in one EU study. Approximately a 5-fold maximum overdose (138,000 PFU) was applied to 1-day-old turkeys. No clinical signs or deaths or specific lesions related to MD or IBD were noted. Therefore, the vaccine is regarded as safe for turkeys, if contact with vaccinated chickens should occur.

Additionally, an US study is provided as supportive study, confirming the safety of the MSV in turkeys when given at an approximately 3-fold maximum overdose (69,228 PFU).

Spread to further non-target species

Five EU studies and two supportive US studies investigated the spread of the vaccine strain to further non-target species and foreign bird safety using an overdose of approximately 117,000 – 220,000 PFU/dose administered by the SC route. Two studies evaluated the safety of Poulvac Procerta HVT-IBD in ducks (EU studies B910N-GB-19-B06 + B914N-ES-20-C94). The first study was terminated because of unexpected deaths due to husbandry problems and had to be repeated. Five more studies were performed in pheasants (EU study B910N-GB-19-B37), quails (EU study B910N-GB-19-B36 + US study B910R-US-18-935) and in mice (EU study B990N-GB-19-182 + US study B990R-US-18-164) showing that the vaccine strain is safe in all these species and that no spreading was detected.

In conclusion, the vaccine virus strain is able to spread from chickens to chickens to a limited extent(in supportive US study only), chickens to turkeys and between turkeys. These findings are in line with the biological characteristics of the parental HVT strain, which were not changed by the insertion of the VP2 protein. Poulvac Procerta HVT-IBD was shown to be safe in turkeys, ducks, pheasants, quails, and mice.

Dissemination in the vaccinated animal

A dedicated EU study examining shed, spread, and dissemination in the target species has been provided. The vaccine virus was detected in all tested tissues during the whole observation period. No differences compared to its parental strain were noted.

Dissemination of the vaccine strain in vaccinated animals was also investigated in a supportive US study. Samples from chickens vaccinated *in ovo* with a standard dose of HVT-IBD or with the parental HVT were taken from blood, spleen, bursa, thymus, feather follicles and filter dust, and were tested for virus by cultivation of isolated white blood cells (WBC) on CEFs and confirmed via IFA. Dust samples were stored for a maximum of 7 days after the end of the study to assess survivability of the virus. Presence of HVT-IBD antigen in feather pulp was assessed by quantitative real time PCR on Flinders Technology Associates (FTA) cards.

Seroconversion of the group vaccinated with Poulvac Procerta HVT-IBD to IBDV was confirmed for 83 % of the chickens. Virus could be re-isolated from all tested organ and blood samples with a higher re-isolation rate for Poulvac Procerta HVT-IBD compared to the parental strain. Both viruses were detected in dust samples on D11 and the parental strain was also re-isolated from a stored dust

sample on D28 (stored for 7 days). Both viruses were recovered from feather pulp until D21.

It was confirmed in the supportive study that the biological characteristics of the parental HVT strain were not changed by the insertion of the VP2 protein.

Increase in virulence of attenuated vaccines

According to Ph. Eur. Monograph 0589 *"The test for increase in virulence is required for Marek's disease virus vaccine strains but not for turkey herpesvirus vaccine strains, which are naturally apathogenic."* Although the parent strain of HVT-IBD is the naturally apathogenic HVT strain FC-126 and, therefore, no test is strictly necessary, the applicant has performed two studies according to Ph. Eur. Monograph 5.2.6 to demonstrate that the genetic modification did not affect the safety profile of the HVT FC-126 strain and that the HVT-IBD strain cannot become virulent through bird to bird passages. Additionally, two solely supportive US studies are provided.

Reversion to virulence was investigated in two EU studies (back passages and comparison of MSV+2 and its 5th passage) in accordance with the requirements of Ph. Eur. 5.2.6 and Ph. Eur. 0589, respectively.

Sequential passaging of the vaccine strain through five groups of SPF chickens was performed by *in ovo* inoculation of a single maximum dose of the vaccine (28,709 PFU/dose, MSV+2) to the first group of 18-day-old embryonated SPF chicken eggs. At the following passages (2, 3, 4 and 5) 0.1 or 0.05 ml of pooled WBC suspension was prepared from blood and spleen samples and administered intraperitoneally (IP). The time between passages was seven days. Each passage group consisted of 30 animals except for the last group, which included 70 birds. The vaccine strain was recovered at all 5 passages. No clinical signs of disease were observed in any of the passage levels.

The last passage of the MSV+2 (P+5) was administered *in ovo* to 18-day-old embryonated SPF chicken eggs in the second EU study. Another group of 18-day-old embryonated SPF chicken eggs was inoculated with the unpassaged MSV+2 in order to evaluate a possible increase in virulence during the *in vivo* passages.

No abnormalities were found in the animals vaccinated with either virus material. Therefore, it is concluded that no reversion to virulence was observed following five passages *in vivo*. The highest passage level that may occur in the final vaccine is MSV+5. Based on this reversion to virulence study, it can be expected that no increase in virulence will occur even in passage MSV+7 (MSV+2 P+5). Thus, there is no need for further investigations.

Two only supportive US studies investigated also a possible increase in virulence due to *in vivo* passaging. In both studies, the MSV was used and no indication of a reversion to virulence was found. However, in the first study, a subpopulation of plaques not expressing the IBDV antigen was detected in passage 3. The proportion of this subpopulation increased over the subsequent passages. Subsequent investigation via PCR and sequencing revealed a point mutation changing the amino acid sequence and thereby hindering the expression of the VP2 protein. The insert was still present in the vaccine strain. It has to be noted that spontaneous point mutations may occur sometimes and are not predictable. The study was repeated to confirm the stability of the construct and a missing reversion to virulence, which was successful. In the final product testing, the presence of both antigens will be checked.

Biological properties of the vaccine strain

One specific *in vitro* US study was conducted to determine the intrinsic biological properties of the vaccine strain regarding survivability of the virus in the environment. The vaccine strain was compared with its parental HVT strain, and it was found that both strains remained viable after the respective suspensions were dried at room temperature and subsequently incubated at 25 °C or 30 °C for 8 hours. After that time, no viable virus could be detected anymore.

The results of all *in vivo* safety studies performed including the studies in non-target animals indicate that the biological properties of the apathogenic parental HVT strain are unaltered after insertion of the VP2 gene of IBDV and some regulatory elements except for the replication of this protein.

Based on the presented data, the safety profile of the vaccine strain can be considered acceptable.

Recombination or genomic reassortment of the strains

The chance of an *in vivo* recombination event is a theoretical possibility due to the similar replication cycle of HVT and other MDV serotypes, but to date no such events have been reported. The potential for recombination of the HVT-IBD strain with virulent MDV is not greater than that of currently authorised vaccines containing HVT. The risk of such an event is considered low.

The genome of HVT is not segmented; therefore, genomic reassortment cannot occur.

The risk of recombination with IBDV is effectively zero, since wild type IBDV is a double-stranded RNA virus replicating in the cytoplasm and, therefore, recombination with a double-stranded DNA virus, such as the HVT-IBD virus strain in Poulvac Procerta HVT-IBD, which is presumably replicating in the nucleus just like the parental HVT strain, is not possible.

It can be concluded that the event of recombination or genomic reassortment is very unlikely. This assessment was made in compliance with the respective legal requirements.

User safety

The applicant presented a user safety risk assessment, which was conducted in accordance with the CVMP "Guideline on user safety for immunological veterinary medicinal products" (EMEA/CVMP/IWP/54533/2006).

The main potential routes of accidental contact with the product were considered, and it was concluded that the most likely routes are those of accidental self-injection during SC application and dermal exposure by accidental spilling or when a glass ampoule explodes during the thawing process. Additionally, personnel involved in animal husbandry procedures may come into contact with excreted vaccine virus from feather follicles of vaccinated chickens.

In general, avian herpesviruses are not known to be a hazard to humans and HVT is not indicated as such in EU Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work. The genetic modification did not alter the biological properties of the strain as shown in the increase in virulence studies and studies on spread to non-target species.

The excipients and the constituents of the sterile solvent are commonly used in other vaccines and do not pose a risk for the user. The same applies to the traces of antibiotics present in the finished product.

None of the components of the vaccine (active ingredient or excipients) are expected to pose a risk to the user following accidental exposure. With regard to possible hazards of a vaccine stored in liquid

nitrogen (risk of an ampoule exploding) and the recommended handling of the ampoules, a warning is included in SPC section 3.5, which points out that only properly trained personnel should handle liquid nitrogen containers.

Based on the above risk assessment, it is concluded that the product does not pose an unacceptable risk to the user when used in accordance with the SPC. Additionally, the risk of liquid nitrogen burns is negligible when the product is handled as recommended in the SPC.

Study of residues

No residue depletion studies have been carried out for Poulvac Procerta HVT-IBD.

All substances included in the composition of the vaccine are listed in Table 1 of the Annex to Commission Regulation (EU) 37/2010 or in the list of substances considered as not falling within the scope of Regulation (EU) 470/2009. Phenol red sodium salt has no pharmacological activity at the concentration used in the final product. The concentrations of residual antibiotics in the final product are far below the lowest established MRL outlined in Table 1 of the Annex to Commission Regulation (EU) No 37/2010.

In conclusion, a withdrawal period of zero days is deemed appropriate for this vaccine.

Interactions

The applicant has not provided data investigating interactions of the vaccine with any other (immunological) veterinary medicinal product and therefore proposes to include the following statement in section 3.8 of the SPC: *'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.'*

Clinical studies

Three combined safety and efficacy field trials in commercial broiler chickens were performed in Spain, Hungary and Italy using a standard dose.

The following legal requirements were considered carrying out the clinical studies: VICH GL 9 (GCP) + GL 44 (target animal safety), Regulation (EU) 2019/6, EMEA/CVMP/852/99-FINAL, EMEA/CVMP/004/4-FINAL and Ph. Eur. Monograph 5.2.6.

The first field trial was performed in Spain on a commercial broiler farm in four houses (two per treatment). The performances of approximately 172,418 chickens vaccinated either *in ovo* with Poulvac Procerta HVT-IBD or *in ovo* with a comparator product were compared. Instead of 0.05 ml/egg as recommended only 0.047 ml/dose were used. However, this minor deviation is of no concern when assessing the results of this study. The study was divided in two phases, rearing until early slaughter (thinning) on D31/32 and rearing until the final processing on days 41, 42, 45.

In each of three houses (two vaccinated with the comparator product and one vaccinated with Poulvac Procerta HVT-IBD), lameness in a small group of birds was detected. Necropsies were performed on some of the concerned birds and the lesions found were suspicious for a bacterial infection and not indicative of MD or IBD infection. Bacteriology was performed and an infection with *E. coli* and *Proteus sp.* was diagnosed, which was treated accordingly. Mortality in all groups was in line with historical

mortality data at this farm.

No significant differences in body weight could be detected between the two groups and a similar zootechnical performance for the two treatment groups was calculated. Evaluation of necropsied birds on D28 showed no signs of IBD or MD lesions in gross pathology or in histopathology assessment.

The second field trial was performed in Hungary also on a commercial broiler farm in four houses (two per treatment). The performances of approximately 80,000 chickens vaccinated either SC with Poulvac Procerta HVT-IBD or SC with a comparator product were compared. There were two phases in this study, rearing until early slaughter (thinning) on D34 and rearing until the final processing on D49.

No clinical signs of IBD or MD or any other adverse events were observed. However, one bird vaccinated with Poulvac Procerta HVT-IBD was observed with a histopathological lesion that could be related to MD, but in absence of gross lesions during necropsy. Subsequent investigation thereof remained inconclusive. So, it remains unclear if this lesion was caused by MDV infection due to a breakthrough infection or if the vaccination was not performed accurately in this bird. Considering all safety studies provided it seems unlikely that this issue is a safety concern related to the vaccine virus. Evaluation of the remaining necropsied birds showed no signs of IBD or MD lesions in the gross pathology or in the histopathology assessment on D34.

Mortality was in line with historical mortality data at this farm. No significant differences in body weight could be detected between the two groups and a similar zootechnical performance for the two treatment groups was calculated.

A third field trial was performed in broiler chickens on a commercial broiler farm in four houses (two per treatment) in Italy. The performances of approximately 43,600 chickens vaccinated either SC with Poulvac Procerta HVT-IBD or SC with a comparator product were compared. As before, a commercial dose was used. In this study, females and males were separated in each house. Females were transported to the slaughterhouse on days 41/42 and males on days 49/50.

In both groups, increased mortality was noted in the first week p.v., which was more severe in the group vaccinated with Poulvac Procerta HVT-IBD. Necropsy revealed omphalitis, which was considered as not related to the vaccination. Mortality rates in all groups were in line with historical mortality data at this farm. Significant differences in body weight could be detected on D28 and D35 in favour of the group vaccinated with the test product; however, at D40 (last day of weighing at the farm) no significant difference between groups was found. Similar zootechnical performances were calculated for the two treatment groups. Evaluation of the necropsied birds revealed no signs of IBD or MD lesions in the gross pathology or in the histopathology assessment.

Additionally, the applicant provided a document summarising four field trials conducted in the US in commercial broiler chickens. In two studies, Poulvac Procerta HVT-IBD was used to vaccinate *in ovo* on day 18 or day 19 of embryonation; in two further studies, the vaccine was applied SC to 1-day-old chicks. As *in ovo* vaccination was conducted in one study in 19-day-incubated chicken eggs, the inclusion of this age group in the SPC is considered as acceptable. In all studies, a comparator vaccine was used in parallel groups from the same breeding using the same route and age of vaccination. In one case, vaccination was only performed with half of a standard dose for the comparator vaccine, which is the usual practice on this farm and had no influence on the outcome of the study.

Hatchability was acceptable in all groups. Daily observation of clinical signs and mortalities took place. Except for a case of necrotic enteritis infection, no other adverse events were noted. No clinical signs or deaths related to MD or IBD were noted. Similar mortality rates were observed in all groups. At slaughter, production data were collected and all broiler chickens performed well and the results of the

test vaccine and the comparator product were comparable.

Results of these solely supportive studies give further assurance that vaccination of commercial broiler chickens with Poulvac Procerta HVT-IBD is safe in the target species. However, these studies are of limited value as no complete study reports were provided.

It is noted that no field trials in chickens of the laying type were performed; however, as chickens of the laying type are not considered to be more sensitive as chickens of the meat type, this approach is accepted.

The field data provided show that the product is safe when used at a standard dose applied via *in ovo* to 18-19 day-old embryonated commercial chicken eggs or SC to 1-day-old commercial chicks.

Environmental risk assessment

An environmental risk assessment (ERA) according to Directive 2001/18/EC (Annex II, section D) was provided.

Based on the data provided, the ERA can stop at phase I. Poulvac Procerta HVT-IBD is not expected to pose a risk for the environment when used according to the SPC.

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

Poulvac Procerta HVT-IBD is a live vector vaccine consisting of a live recombinant herpesvirus of turkeys (HVT) strain FC-126 and the chemically synthesized gene encoding for VP2 of infectious bursal disease virus (IBDV) inserted into the HVT backbone.

Poulvac Procerta HVT-IBD falls within the scope of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms. Detailed information on the possible risks for humans and the environment, the environmental risk assessment and a description of and justification for the proposed release according to the Notice to Applicants Vol. 6C has been provided.

Poulvac Procerta HVT-IBD does not infect humans and is restricted to the infection of Galliformes birds.

The vaccine virus was shed from vaccinated animals via feather dander for a limited time span. Commingling of sentinels with vaccinated animals led to an infrequent spread to chickens and turkeys without causing disease. Accordingly, a biologically relevant spread of the vaccine viruses into the environment could be detected. However, as HVT is attenuated, it does not cause disease in in-contact turkeys and is self-limiting after spread to chickens. Upon request, the applicant provided more detailed information regarding the sensitivity and specificity of the identification technique methods of the HVT parental strain and the detection methods for the VP2 gene and protein and amended the dossier accordingly. Furthermore, all relevant information on genetic stability and factors affecting it was included in the corresponding dossier section.

Taken together, any risk emerging from the use of the attenuated vaccine is expected to be negligible for humans and low for the environment.

Overall conclusions on the safety documentation

The applicant has provided one pivotal laboratory study to investigate the safety of a 10-fold maximum

overdose in the target animal species of the minimum recommended age via the recommended routes (*in ovo* and SC). Batches used in these studies were pilot batches at MSV+2 level. A supportive study was provided performed with the MSV at a 4-5-fold maximum overdose.

Based on the results, it was concluded that the safety for the targeted animals is acceptable when the vaccine is administered according to the recommended schedule and via the recommended routes. These findings were supported by results generated in three EU field trials (and four US trials) in commercial broiler chickens.

Tests on the immunological functions were carried out. The product is not expected to adversely affect the immune response of the target animals.

Reproduction safety was not investigated as the vaccine is not intended for use in laying birds.

As this is a live vaccine, the applicant has also conducted nine EU studies to establish the potential for shed, spread and dissemination of the vaccine strain and five US studies (only supportive). Shedding of the vaccine strain from vaccinated chickens was demonstrated at least up to 22 days after the vaccination. However, it was possible to isolate the virus from environmental samples for less than 42 days. It was concluded that the vaccine virus could spread from chickens to chickens, chickens to turkeys and between turkeys. Poulvac Procerta HVT-IBD was shown to be safe in turkeys, ducks, pheasants, quails, and mice.

The biological properties of the vaccine strain were described adequately and found to be acceptable. As the vaccine strain is genetically modified, reversion to virulence and recombination or genomic reassortment of the strain were also assessed. The evaluation showed that the potential risk is very low and acceptable. The final GMO was shown to be genetically and phenotypically stable over five passages. The biological properties of the apathogenic parental HVT strain seem to be unaltered after insertion of the VP2 gene of IBDV and its regulatory elements except for the replication of this protein.

The data presented are considered adequate to characterise the safety profile of the vaccine and the active substance.

A user safety assessment in line with the relevant guidance document has been presented. Based on the presented assessment, the product does not pose an unacceptable risk to the user when used in accordance with the SPC. The worst-case scenario for user safety is considered to be risks associated with handling liquid nitrogen tanks and thawing of frozen glass vials (i.e. injuries due to exploding glass ampoules). Appropriate warnings for the user have been included in the product literature to indicate that liquid nitrogen containers and the vaccine should only be handled by properly trained personnel.

The vaccine is expected to pose a negligible risk to the environment when used as recommended. A recombination event between the vaccine virus strain and a field strain or another vaccine virus strain is unlikely.

Information concerning the release of genetically modified organisms was provided in the form of appropriate studies and through literature. Taken together, any risk emerging from the use of the attenuated vaccine is expected to be negligible for humans and low for the environment.

Poulvac Procerta HVT-IBD is considered to be safe for the target species and non-target species, the user, the consumer and the environment.

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

General requirements

Poulvac Procerta HVT-IBD is a live HVT-IBD vector vaccine against Marek's disease (MD) and infectious bursal disease (IBD). The vaccine consists of a genetically modified live herpesvirus of turkeys (HVT) strain F-126 vector with the gene encoding for the VP2 of infectious bursal disease inserted in the HVT genome. The vaccine is filled into flame-sealed ampoules, to be reconstituted before use in a sterile diluent.

The vaccine is intended

- to reduce mortality, clinical signs and lesions caused by Marek's disease virus (MDV) and
- to prevent mortality and clinical signs and reduce lesions caused by infectious bursal disease virus (IBDV)

when administered to chickens

- by *in ovo* route at 18-19 days of embryonation at a dose of 0.05 ml or
- by subcutaneous route at the day of hatch at a dose of 0.2 ml.

The vaccination scheme consists of one single injection, the minimum dose is 3,580 PFU/dose* (*Plaque Forming Units/dose).

Immunity is intended to be established

- for MD from 7 days post vaccination for *in ovo* and 9 days post vaccination for subcutaneous use,
- for IBD from 15 days post vaccination for *in ovo* and 12 days post vaccination for subcutaneous use.

A single vaccination is sufficient to provide protection for the entire risk period for MD and until 64 days of age for IBD.

Efficacy was demonstrated in compliance with Regulation (EU) 2019/6, the Ph. Eur. chapter 5.2.7 and Ph. Eur. Monographs 0587: Avian Infectious Bursal Disease Vaccine (Live) and 0589: Marek's Disease Vaccine (Live).

Challenge model

Challenge strain of Marek's disease virus

The MDV challenge strain used in the laboratory efficacy studies is a classical virulent Marek's disease serotype 1 originally isolated from an ovarian tumour from a 9-week-old bird in USA. Based on percentage of mortality and lesions caused, the virus strain was classified as a virulent Marek's disease virus (vMDV). The relationship between the challenge strain and strains collected in Europe has been described in literature (Murata et al., 2020) as there are relatively few amino acid differences in the meg sequence (virulence gene). The isolate was shown to be the US strain most closely related to the Eurasian virulent isolates (Trimpert et al., 2017; Mescolini et al., 2019). The strain has been used as challenge material for several competitor vaccines in the EU and can be found in the same cluster as the European challenge isolate Md70. It is closely related to the RB1B challenge isolate utilized in the

MD challenge models of further competitor vaccines. Therefore, the challenge strain is considered sufficiently similar to other virulent European isolates and is relevant for assessing vaccine efficacy for Europe. As the challenge strain is a MD serotype 1 isolate, it is heterologous to the MD serotype 3 HVT vaccine strain.

Two challenge model development studies demonstrate that validation criteria of Ph. Eur. Monograph 0589 are met after an appropriate dilution of stock material administered at a dose of 0.2 ml to SPF chickens and after an appropriate dilution of stock material administered at a dose of 0.2 ml to broilers and SPF chickens.

Challenge strain of infectious bursal disease virus

The IBDV challenge material used in the laboratory efficacy studies is a very virulent infectious bursal disease strain isolated from brown layer pullets in USA, which showed approximately 26 % (3,300 birds) mortality at 14 weeks of age with enlarged bursas (Jackwood et al., 2009). Based on mortality rates and histopathology results, the virus strain was classified as a very virulent infectious bursal disease virus (Jackwood et al., 2017). Molecular sequences and phylogenetic analysis confirm that the challenge strain is a very virulent IBD isolate that likely was a descendant of the vvIBDV first observed in Europe and very similar to vvIBDV isolated from EU. Therefore, the challenge strain is relevant for assessing vaccine efficacy for Europe. Following sequence analysis and phylogenetic assessment, the vvIBDV strain is considered heterologous to the IBDV strain present in the vaccine, which is a classical virulent IBD isolate from Europe and from which the VP2 insert is derived.

Three independent challenge dose calibration studies demonstrate that the validation criteria of Ph. Eur. Monograph 0587 are met after a relevant challenge dose administered at a dose of 0.1 ml to SPF chickens.

Efficacy parameters and tests

The efficacy parameters investigated in the efficacy studies are:

- Mortality, clinical signs (i.e. paralysis, severe depression) and lesions due to classical Marek's disease virus (MDV), HVT serology in the duration of immunity (DOI) and maternally derived antibodies (MDA) studies (assessed using a validated in-house immunofluorescent assay), and MD presence in feather follicles in the DOI study (assessed using a validated in-house qPCR);
- Mortality, clinical signs (i.e. severe depression) and histological bursal lesions caused by IBDV and IBD serology in the DOI and MDA studies (assessed using commercial IDEXX ELISA).

The parameters chosen are in line with the requirements of Ph. Eur. Monographs 0589 and 0587 and, therefore, considered appropriate for evaluating the efficacy of the product.

Efficacy documentation

Twenty-five studies were conducted to investigate the efficacy of the product including twenty-two pre-clinical studies and three clinical trials. Laboratory studies were well documented and carried out in SPF chickens or SPF embryonated eggs as well as in MDA-positive commercial broiler birds or embryonated broiler eggs of the minimum age recommended for vaccination.

The batches used were representative pilot batches of the production method described in Part 2.B of this dossier, at the most attenuated passage level that will be present in the vaccine (MSV+5), with a dose not higher than the minimum titre (3,580 PFU). The solvent utilized for the laboratory efficacy studies (also referred to as Poulvac Sterile Diluent in the studies) has negligible quantitative differences

in the ingredient composition. The solvent used in the field studies is the same as the one listed in the product information and referred to as Poulvac Solvent.

The laboratory efficacy tests were conducted according to previously defined protocols. The combined field safety and efficacy trials adhered to the requirements of Good Clinical Practice (GCP).

Overview of the laboratory efficacy studies:

Evaluation of efficacy against MDV of Poulvac Procerta HVT-IBD

Study reference	Study title	Vaccine dose (PFU/ds.)	Challenge dose
In ovo			
	Establishment of Minimum Infectious Dose (MID) and Onset of Immunity **(OOI)** Following HVT-IBD ***in ovo*** Vaccination Against a Virulent **Day 4 MDV** Challenge	3,302	15 PFU/dose
	Establishment of MID and **OOI** following HVT-IBD ***in ovo*** vaccination against a virulent MDV **Day 6 MDV** challenge	2,599	15 PFU/dose
	Duration of Immunity **(DOI)** of HVT-IBD by assessment of vaccine presence and IBDV serology	3,434	./.
	The efficacy of HVT-IBD against vMDV challenge at 9 days after *in ovo* vaccination in chickens with **maternal antibodies**	3,559	17 PFU/dose
Subcutaneous			
	Establishment of MID and **OOI** for HVT-IBD Vaccine Following **Subcutaneous** Vaccination Against a Virulent **Day 9 MDV** Challenge	3,655	14 PFU/dose
	Establishment of MID and **OOI** for HVT-IBD Vaccine Following **Subcutaneous** Vaccination Against a Virulent **Day 9 MDV** Challenge	3,301	23 PFU/dose
	Establishment of MID and **OOI** for HVT-IBD vaccine following **subcutaneous** vaccination against a virulent **Day 9 MDV** challenge	2,826	9 PFU/dose
	Establishment of MID and **OOI** following HVT-IBD **subcutaneous** vaccination against a virulent **Day 4 MDV** challenge	3,021	13 PFU/dose
	Establishment of MID and **OOI** following HVT-IBD **subcutaneous** vaccination against a virulent **Day 4 MDV** challenge	3,390	11 PFU/dose
	Duration of Immunity **(DOI)** of HVT-IBD by assessment of vaccine presence and IBDV serology	3,161	./.
	The efficacy of HVT-IBD against vMDV challenge at 9 days after SC vaccination in chickens with **maternal antibodies**	3,102	17 PFU/dose
	The efficacy of HVT-IBD against vMDV challenge at 9 days after SC vaccination in chickens with **maternal antibodies**	3,580	123 PFU/dose

Evaluation of efficacy against IBDV of Poulvac Procerta HVT-IBD

Study reference	Study title	Vaccine dose (PFU/ds.)	Challenge dose
In ovo			
	Establishment of MID and **OOI** for HVT-IBD vaccine following ***in ovo*** vaccination against a **vvIBDV** challenge at **Day 12**	3,445	$10^{0,4}$ EID_{50}/dose
	Establishment of MID and **OOI** for HVT-IBD vaccine following ***in ovo*** vaccination against a **vvIBDV** challenge at **D12**	3,520	$10^{1,0}$ EID_{50}/dose
	Establishment of MID and **OOI** for HVT-IBD vaccine following ***in ovo*** vaccination against a **vvIBDV** challenge at **Day 14**	3,139	$10^{2,41}$ EID_{50}/dose
	Duration of immunity **(DOI)** following *in ovo* vaccination against vvIBDV	3,264	$10^{1,28}$ EID_{50}/dose
	The efficacy of HVT-IBD against challenge at 28 days after *in ovo* or subcutaneous vaccination with vvIBDV in chickens with **maternal antibodies**	3,393	$10^{0.94}$ EID_{50}/dose
Subcutaneous			
	Establishment of MID and **OOI** for HVT-IBD vaccine following **subcutaneous** vaccination against a **vvIBDV** challenge at **Day 12**	3,088	$10^{-0,2}$ EID_{50}/dose
	Establishment of MID and **OOI** for HVT-IBD vaccine following **subcutaneous** vaccination against a **vvIBDV** challenge at **Day 12**	3,156	$10^{0.4}$ EID_{50}/dose
	Establishment of MID and **OOI** for HVT-IBD vaccine following **subcutaneous** vaccination against a **vvIBVD** challenge at **Day 14**	3,542	$10^{1,09}$ EID_{50}/dose
	Establishment of MID and **OOI** for HVT-IBD vaccine following **subcutaneous** vaccination against **vvIBDV** challenge at **Day 14**	3,126	$10^{2,26}$ EID_{50}/dose
	Establishment of MID and **OOI** for HVT-IBD vaccine following **subcutaneous** vaccination against a **vvIBDV** challenge at **Day 14**	3,372	$10^{2,62}$ EID_{50}/dose
	HVT-IBD vvIBDV Duration of Immunity **(DOI)**	3,372	$10^{1.22}$ EID_{50}/dose
	The efficacy of HVT-IBD against challenge at 28 days after in ovo or subcutaneous vaccination with vvIBDV in chickens with **maternal antibodies**	2,485	$10^{0.94}$ EID_{50}/dose

Pre-clinical studies

Dose determination

No explicit study on the determination of the vaccine dose has been performed, but all OOI studies conducted include the establishment of a Minimum Infectious Dose (MID). In summary, sufficient efficacy for both antigens was demonstrated for a minimum dose of 3,580 PFU.

Onset of immunity

MD:

Seven studies designed and validated according to requirements of Ph. Eur. Monograph 0589, 2-4-3 Immunogenicity, were performed to determine the efficacy and onset of immunity for MD in SPF chickens, two including animals vaccinated via *in ovo* and five using animals vaccinated via SC route.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose of the minimum titre or lower (i.e. ≤ 3,580 PFU/dose). Challenge was performed at 4 or 9 days of age, respectively, as required by the Ph. Eur. for both routes of vaccination with an appropriate dilution of stock solution of a vMDV challenge strain via SC route. The claimed onset of immunity (7 days post vaccination for *in ovo* and 9 days post vaccination for subcutaneous use) for the MD component corresponds to the time of challenge mentioned in the Ph. Eur. for the immunogenicity test. The animals were observed for 70 days after challenge for mortality and clinical signs and post mortem examination was performed of all dead or euthanized animals and all remaining animals after the observation period. In case of inconclusive results, histology was conducted for clarification. According to Ph. Eur. Monograph 0589, 2-4-3 Immunogenicity, the challenge is valid, if during the observation period following challenge, not less than 70 percent of the challenged control chickens died or showed severe clinical signs or macroscopic lesions of Marek's disease. The vaccine complied with the test if the relative protection percentage (RPP) was not less than 80 percent.
Hatchability was recorded for *in ovo* vaccination.

In the following studies, efficacy requirements were met:

In the first study (*in ovo* OOI against MDV [D4]), four groups of seventy 18-day-old embryonated SPF eggs were used. A vaccine dose of 3,302 PFU and 3,356 PFU was administered to groups T03 and T04, respectively, by the *in ovo* route. Groups T01 and T02 remained unvaccinated. 36 animals each of the vaccinated group T03 and of the unvaccinated group T02 were challenged with vMDV at 4 days of age. The challenge was valid as 91.7 % of the non-vaccinated/challenged control group (T02) showed characteristic signs of Marek's disease. The level of RPP after challenge was 87.9 %, which is in line with efficacy pass criteria mentioned in the Ph. Eur. Post challenge cross-contamination with the vaccine strain found in one bird of the non-vaccinated/non-challenged group was not considered to impact the validity of the study.

In a second study (SC OOI against MDV [D9]), three groups (T02-T04) of 51 and one group (T01) of 34 SPF chickens at the day of hatch were used. A vaccine dose of 3,655 PFU and 4,170 PFU was administered to groups T03 and T04, respectively, by the subcutaneous route. Groups T01 and T02 remained unvaccinated. 36 animals each of the vaccinated group T03 and of the unvaccinated group T02 were challenged with vMDV at 9 days of age. The challenge was valid as 88.6 % of the non-vaccinated/challenged control group (T02) showed characteristic signs of Marek's disease. The level of RPP after challenge was 84.3 %, which is in line with efficacy pass criteria mentioned in the Ph. Eur.. The dose was slightly above the intended minimum virus titre of 3,580 PFU/dose.

In another study (SC OOI against MDV [D9]), three groups (T02-T04) of 51 and one group (T01) of 34 SPF chickens at the day of hatch were used. A vaccine dose of 3,301 PFU and 4,628 PFU was administered to groups T03 and T04, respectively, by the subcutaneous route. Groups T01 and T02 remained unvaccinated. 36 animals each of the vaccinated group T03 and of the unvaccinated group T02 were challenged with vMDV at 9 days of age. The challenge was valid as 88.9 % of the non-vaccinated/challenged control group (T02) showed characteristic signs of Marek's disease. The level of

RPP after challenge was 87.5 %, which is in line with efficacy pass criteria mentioned in the Ph. Eur.. Post challenge cross-contamination with the vaccine strain found in two birds of the non-vaccinated/non-challenged group was not considered to impact the validity of the study.

The following studies were provided for completeness only as the dose is below the intended level for the vaccine:

In the first study (*in ovo* OOI against MDV [D6]), four groups of eighty 18-day-old embryonated SPF eggs were used. A vaccine dose of 2,228 PFU and 2,599 PFU was administered to groups T03 and T04, respectively, by the *in ovo* route. Groups T01 and T02 remained unvaccinated. 45 animals each of the vaccinated group T04 and of the unvaccinated group T02 were challenged with vMDV at 6 days of age. The challenge was valid as 93.2 % of the non-vaccinated/challenged control group (T02) showed characteristic signs of Marek's disease. The level of RPP after challenge was 69 %, which is not in line with efficacy pass criteria mentioned in the Ph. Eur..

In another study (SC OOI against MDV [D9]), three groups (T02-T04) of 60 and one group (T01) of 40 SPF chickens at the day of hatch were used. A vaccine dose of 2,770 PFU and 2,826 PFU was administered to groups T03 and T04, respectively, by the subcutaneous route. Groups T01 and T02 remained unvaccinated. 45 animals each of the vaccinated group T04 and of the unvaccinated group T02 were challenged with vMDV at 9 days of age. The challenge was valid as 81.4 % of the non-vaccinated/challenged control group (T02) showed characteristic signs of Marek's disease. The level of RPP after challenge was 72.7 %, which is not in line with efficacy pass criteria mentioned in the Ph. Eur.

The following studies are provided for completeness only as test birds were challenged at 4 days of age whereas the onset of immunity for the subcutaneous administration has been proposed as 9 days after vaccination:

In the first study (SC OOI against MDV [D4]), three groups (T02-T04) of 51 and one group (T01) of 34 SPF chickens at the day of hatch were used. A vaccine dose of 2,752 PFU and 3,390 PFU was administered to groups T03 and T04, respectively, by the subcutaneous route. Groups T01 and T02 remained unvaccinated. 36 animals each of the vaccinated group T04 and of the unvaccinated group T02 were challenged with vMDV at 4 days of age. The challenge was valid as 88.9 % of the non-vaccinated/challenged control group (T02) showed characteristic signs of Marek's disease. The level of RPP after challenge was 76.1 %, which is not in line with efficacy pass criteria mentioned in the Ph. Eur.

In another study (SC OOI against MDV [D4]), three groups (T02-T04) of 51 and one group (T01) of 34 SPF chickens at the day of hatch were used. A vaccine dose of 2,880 PFU and 3,021 PFU was administered to groups T03 and T04, respectively, by the subcutaneous route. Groups T01 and T02 remained unvaccinated. 36 animals each of the vaccinated group T04 and of the unvaccinated group T02 were challenged with vMDV at 4 days of age. The challenge was valid as 91.7 % of the non-vaccinated/challenged control group (T02) showed characteristic signs of Marek's disease. The level of RPP after challenge was 63.6 %, which is not in line with efficacy pass criteria mentioned in the Ph. Eur.

It was concluded that vaccination with a dose less than the minimum content recommended in the summary of product characteristics (SPC) was efficacious and met the efficacy requirements

- from 4 days of age (i.e. 7 days post vaccination) when administered by the *in ovo* route to 18-day-old embryonated eggs with a vaccine dose of 3,302 PFU and above;
- from 9 days of age (i.e. 9 days post vaccination) when administered by the subcutaneous route at

day old with a vaccine dose of 3,301 PFU and above.

The proposed claim "reduction of mortality, clinical signs and lesions caused by Marek's disease virus" can be supported based on the results of the presented studies.

IBD:

Eight studies designed and validated according to requirements of Ph. Eur. Monograph 0587, 2-4-5 Immunogenicity, were performed to determine the efficacy and onset of immunity for IBD in SPF chickens, three including animals vaccinated via *in ovo* and five using animals vaccinated via SC route.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose of the minimum titre or lower (i.e. ≤ 3,580 PFU/dose). Challenge was performed at 12 or 14 days of age as required by the Ph. Eur. for both routes of vaccination with an appropriate dose of a vvIBDV challenge strain via eye drop route. The time of challenge mentioned in the Ph. Eur. for the immunogenicity test is 14 days post vaccination. As the claimed onset of immunity for the IBD component is 15 days post vaccination for *in ovo* and 12 days for subcutaneous use, the time of challenge can be regarded acceptable for both routes. The animals were observed for 10 days after challenge for mortality and clinical signs. Histological examination for lesions of the bursa of Fabricius of all surviving animals was carried out at the end of the observation period with bursal damage scored as per Ph. Eur. Monograph 0587. According to Ph. Eur. Monograph 0587, 2-4-5 Immunogenicity, the challenge is valid, if during the observation period following challenge not less than 50 percent of the challenged control chickens showed characteristic signs (clinical signs or mortality) of avian infectious bursal disease and all of the surviving challenged control chickens showed ≥ degree 3 lesions of the bursa of Fabricius. The vaccine complied with the test if the dose provided ≥ 90 percent protection as assessed by notable clinical signs, mortality, and bursal lesion scores < 3. Hatchability was recorded for *in ovo* vaccination.

In the following studies, efficacy requirements were met at challenge at 12 days of age:

In the first study (*in ovo* OOI against IBDV [D12]), four groups of fifty-four 18-day-old embryonated SPF eggs were used. A vaccine dose of 2,978 PFU and 3,445 PFU was administered to groups T03 and T04, respectively, by the *in ovo* route. Groups T01 and T02 remained unvaccinated. Forty animals each of the vaccinated group T04 and of the unvaccinated group T02 were challenged with an appropriate dose of a vvIBDV strain at 12 days of age. The challenge was valid as in the non-vaccinated/challenged control group (T02) 52.5 % of the birds showed characteristic signs of IBD and 100 % of the surviving birds ≥ degree 3 lesions of the bursa of Fabricius. The level of protection after challenge was 92.5 %, which is in line with efficacy pass criteria mentioned in the Ph. Eur.. Cross-contamination with vvIBDV in one of two pens in the non-vaccinated/non-challenged group was not considered to impact the validity of the study. None of the chickens in the vaccinated group died or had clinical signs of disease, while 7.5 % of the chickens were observed to have ≥ degree 3 lesions of the bursa of Fabricius, thereby supporting the claim for a prevention of clinical signs and mortality and reduction of lesions due to IBDV.

In a second study (*in ovo* OOI against IBDV [D12]), four groups of fifty-four 18-day-old embryonated SPF eggs were used. A vaccine dose of 3,709 PFU and 3,520 PFU was administered to groups T03 and T04, respectively, by the *in ovo* route. Groups T01 and T02 remained unvaccinated. Forty animals each of the vaccinated group T04 and of the unvaccinated group T02 were challenged with an appropriate dose of a vvIBDV strain at 12 days of age. The challenge was valid as in the non-vaccinated/challenged control group (T02) 72.5 % of the birds showed characteristic signs of IBD and 100 % of the surviving birds ≥ degree 3 lesions of the bursa of Fabricius. The level of protection after challenge was 90.0 %,

which is in line with efficacy pass criteria mentioned in the Ph. Eur. The study was run to repeat study B812R-US-19-A87 due to vvIBDV cross-contamination in one pen in the non-vaccinated/non-challenged group. Again, cross-contamination with vvIBDV in one of two pens in the non-vaccinated/non-challenged group was seen but not considered to impact the validity of the study. None of the chickens in the vaccinated group died or had clinical signs of disease, while 10 % of the chickens were reported to have bursal lesion scores ≥ 3, thereby supporting the claim for a prevention of clinical signs and mortality and reduction of lesions due to IBDV.

In another study (SC OOI against IBDV [D12]), four groups of 44 SPF chickens at the day of hatch were used. A vaccine dose of 3,088 PFU and 3,670 PFU was administered to groups T03 and T04, respectively, by the subcutaneous route. Groups T01 and T02 remained unvaccinated. Forty animals each of the vaccinated group T03 and of the unvaccinated group T02 were challenged with an appropriate dose of a vvIBDV strain at 12 days of age. The challenge was valid as in the non-vaccinated/challenged control group (T02) 70 % of the birds showed characteristic signs of IBD and 100 % of the surviving birds ≥ degree 3 lesions of the bursa of Fabricius. The level of protection after challenge was 90 %, which is in line with efficacy pass criteria mentioned in the European Pharmacopoeia. None of the chickens in the vaccinated group died or had clinical signs of disease, while 10 % of the chickens were reported to have bursal lesion scores ≥ 3, thereby supporting the claim for a prevention of clinical signs and mortality and reduction of lesions due to IBDV.

The following study was provided for completeness only as the dose is below the intended level for the vaccine:

In this study (SC OOI against IBDV [D12]), four groups of 44 SPF chickens at the day of hatch were used. A vaccine dose of 3,156 PFU and 4,152 PFU was administered to groups T03 and T04, respectively, by the subcutaneous route. Groups T01 and T02 remained unvaccinated. Forty animals each of the vaccinated group T03 and of the unvaccinated group T02 were challenged with an appropriate dose of a vvIBDV strain at 12 days of age. The challenge was valid as in the non-vaccinated/challenged control group (T02) 67.5 % of the birds showed characteristic signs of IBD and 100 % of the surviving birds ≥ degree 3 lesions of the bursa of Fabricius. Again, cross-contamination with vvIBDV in one pen in the non-vaccinated/non-challenged group was seen but not considered to impact the validity of the study. As one post-challenge mortality (2.5 %) occurred in the vaccinated group T03, the claims as prevention of mortality and clinical signs could not be supported by this study but are still considered acceptable, as the study is provided for completeness only due to the very low vaccine dose. The level of protection after challenge was 87.5 %, which is not in line with efficacy pass criteria mentioned in the Ph. Eur.. Nevertheless, in study B812R-US-19-B42, with a dose of 3,088 PFU efficacy requirements were met. Such fluctuations in results are considered possible, since due to the biological variability of the test system bird (challenge) and the test system egg (back-titration), fluctuations in results are to be expected, which in the borderline range can lead to results just (one bird) below the acceptance specification when inoculating a very low dose.

The following studies are provided for completeness only as test birds were challenged at 14 days of age whereas the onset of immunity has been proposed as 12 days after vaccination:

In the first study (*in ovo* OOI against IBDV [D14]), four groups of sixty 18-day-old embryonated SPF eggs were used. A vaccine dose of 3,139 PFU and 2,649 PFU was administered to groups T03 and T04, respectively, by the *in ovo* route. Groups T01 and T02 remained unvaccinated. Four animals each of the vaccinated group T03 and of the unvaccinated group T02 were challenged with an appropriate dose of a vvIBDV strain at 14 days of age. The challenge was valid as in the non-vaccinated/challenged control group (T02) 92.5 % of the birds showed characteristic signs of IBD and 100 % of the surviving

birds ≥ degree 3 lesions of the bursa of Fabricius. The level of protection after challenge was 92.5 %, which is in line with efficacy pass criteria mentioned in the Ph. Eur.. None of the chickens in the vaccinated group died or had clinical signs of disease, while 7.5% of the chickens were reported to have bursal lesion scores ≥ 3.

In a second study (SC OOI against IBDV [D14]), four groups of 44 SPF chickens at the day of hatch were used. A vaccine dose of 3,542 PFU and 4,304 PFU was administered to groups T03 and T04, respectively, by the subcutaneous route. Groups T01 and T02 remained unvaccinated. Forty animals each of the vaccinated group T03 and of the unvaccinated group T02 were challenged with an appropriate dose of a vvIBDV strain at 14 days of age. The challenge was valid as in the non-vaccinated/challenged control group (T02) 85.0 % of the birds showed characteristic signs of IBD and 100 % of the surviving birds ≥ degree 3 lesions of the bursa of Fabricius. The level of protection after challenge was 90.0 %, which is in line with efficacy pass criteria mentioned in the Ph. Eur.. None of the chickens in the vaccinated group died or had clinical signs of disease, while 10 % of the chickens were reported to have bursal lesion scores ≥ 3.

In a third study (SC OOI against IBDV [D14]), four groups of 44 SPF chickens at the day of hatch were used. A vaccine dose of 2,783 PFU and 3,126 PFU was administered to groups T03 and T04, respectively, by the subcutaneous route. Groups T01 and T02 remained unvaccinated. Forty animals each of the vaccinated group T04 and of the unvaccinated group T02 were challenged with an appropriate dose of a vvIBDV strain at 14 days of age. The challenge was valid as in the non-vaccinated/challenged control group (T02) 82.5 % of the birds showed characteristic signs of IBD and 100 % of the surviving birds ≥ degree 3 lesions of the bursa of Fabricius. The level of protection after challenge was 82.5 %, which is not in line with efficacy pass criteria mentioned in the Ph. Eur. (the frequency of bursal lesion scores ≥3 was 17.5 %, however none of the chickens in the vaccinated group died or had clinical signs of disease). The rather high challenge dose and therefore particularly virulent challenge was considered causative for the high levels of mortality and clinical signs observed in the unvaccinated group T02 and, in combination with a very low vaccine dose, causative for not meeting the acceptance specification.

In another study (SC OOI against IBDV [D14]), four groups of 44 SPF chickens at the day of hatch were used. A vaccine dose of 3,058 PFU and 3,372 PFU was administered to groups T03 and T04, respectively, by the subcutaneous route. Groups T01 and T02 remained unvaccinated. Forty animals each of the vaccinated group T04 and of the unvaccinated group T02 were challenged with an appropriate dose of a vvIBDV strain at 14 days of age. The challenge was valid as in the non-vaccinated/challenged control group (T02) 100 % of the birds showed characteristic signs of IBD. Again, cross-contamination with vvIBDV in one pen in the non-vaccinated/non-challenged group was seen but not considered to impact the validity of the study. As one post-challenge mortality (2.5 %) occurred in the vaccinated group T04 (but no clinical signs were reported in any other chicken in this group), the claims as prevention of mortality and clinical signs could not be supported by this study but are still considered acceptable, as the study is provided for completeness only due to the high challenge dose and therefore particularly virulent challenge. The level of protection after challenge was 77.5 %, which is not in line with efficacy pass criteria mentioned in the Ph. Eur.. The rather high challenge dose and therefore particularly virulent challenge was considered causative for the high levels of mortality and clinical signs observed in the unvaccinated group T02 and causative for not meeting the acceptance specification.

It was concluded that vaccination with a dose less than the minimum content recommended in the SPC was efficacious and met the efficacy requirements,

- from 12 days of age (i.e. 15 days post vaccination) when administered by the *in ovo* route to 18-day-old embryonated eggs with a vaccine dose of 3,445 PFU and above;
- from 12 days of age (i.e. 12 days post vaccination) when administered by the subcutaneous route at day old with a vaccine dose of 3,088 PFU and above.

The proposed claim "prevention of mortality and clinical signs and reduction of lesions caused by infectious bursal disease virus" can be supported based on the results of the presented studies.

Duration of immunity

MD:

One study was performed to determine the duration of immunity for MD in SPF chickens, including animals vaccinated via *in ovo* or SC route.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose of the minimum titre or lower (i.e. ≤ 3,580 PFU/dose). The animals were observed for 63 days for mortality and abnormal clinical signs and post mortem examination was performed of all dead or euthanized animals. In case of inconclusive results, histology was conducted for clarification. Feather pulp and blood samples were taken at different time points from D21 until D63 after hatch in order to assess vaccine persistence through 63 days of age.
A group of unvaccinated SPF animals was included to validate the study. Hatchability was recorded.

In this study (*in ovo* and SC DOI for MDV), three groups of sixty 18-day-old embryonated SPF eggs were used. A vaccine dose of 3,161 PFU was administered to group T02 by the *in ovo* route. At hatch, a vaccine dose of 3,434 PFU was administered to group T03 by the subcutaneous route. Group T01 remained unvaccinated. In the feather pulp samples, the highest number of DNA copies/10,000 cells was detected on day 21. The HVT and IBD serological antibodies (geometric mean titre - GMT) for the vaccinated groups increased from D21 at each subsequent time point throughout the study until D63 with 94.4 % and 100 % positive samples, respectively. IFA titres for MDV on D21 confirmed non-vaccinated birds had not been exposed to other Marek's disease viruses.

Marek vaccines have been generally accepted to induce lifelong immunity. A DOI claim for the entire risk period utilizing a single dose is therefore justified for both administration routes against MD based on the known persistent infection of chickens with HVT and serological response of the birds in the study presented.

IBD:

Two studies basically designed according to requirements of Ph. Eur. Monograph 0587, 2-4-5 Immunogenicity, were performed to determine the duration of immunity for IBD in SPF chickens, one including animals vaccinated via *in ovo* and one using animals vaccinated via SC route.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose of the minimum titre or lower (i.e. ≤ 3,580 PFU/dose). Challenge was performed at 64 days of age, as required by the Ph. Eur. for both routes of vaccination with an appropriate dose of a vvIBDV challenge strain via eye drop route. The animals were observed for 10 days after challenge for mortality and clinical signs. Histological examination for lesions of the bursa of Fabricius of all surviving animals were carried out at the end of the observation period with bursal damage scored as per Ph. Eur. Monograph 0587. According to Ph. Eur. Monograph 0587, 2-4-5 Immunogenicity, the challenge is valid, if during the observation period following challenge not less than 50 percent of the challenged control chickens showed characteristic signs (clinical signs or

mortality) of avian infectious bursal disease and all of the surviving challenged control chickens showed ≥ degree 3 lesions of the bursa of Fabricius. The vaccine complied with the test if the dose provided ≥ 90 percent protection as assessed by notable clinical signs, mortality, and bursal lesion scores < 3. IBD serology was performed on days 14, 28, 42 (7 birds per pen culled and bled), and on days 52 and 63 (10 birds per pen) to consider seroconversion/absence of seroconversion after vaccination. Hatchability was recorded for *in ovo* vaccination.

In the first study (SC DOI against IBDV [D64]), two groups of 108 SPF chickens at the day of hatch were used. A vaccine dose of 3,372 PFU was administered to group T03 by the subcutaneous route. Group T01 remained unvaccinated. Forty-four animals of the vaccinated group T03 and 45 animals of the unvaccinated group T01 were challenged with an appropriate dose of a vvIBDV strain at 64 days of age. The challenge was valid as in the non-vaccinated/challenged control group (T01) 53.3 % of the birds showed characteristic signs of IBD and 100 % of the surviving birds ≥ degree 3 lesions of the bursa of Fabricius. The level of protection after challenge was 100.0 %, which is in line with efficacy pass criteria mentioned in the Ph. Eur. The vaccine induced the development of IBD-specific antibodies in vaccinated SPF birds within 28 days post-vaccination, which increased and maintained until challenge. The geometric mean titre at D63 was 16,734.

In the second study (*in ovo* DOI against IBDV [D64]), three groups of one hundred and thirty- five 18-day-old embryonated SPF eggs were used. A vaccine dose of 3,625 PFU and 3,264 PFU was administered to groups T02 and T03, respectively, by the *in ovo* route. Group T01 remained unvaccinated. Forty-five animals each of the vaccinated group T03 and of the unvaccinated group T01 were challenged with an appropriate dose of a vvIBDV strain at 64 days of age. The challenge was not valid according to Ph. Eur. criteria, which are set specially to demonstrate the onset of immunity, as in the non-vaccinated/challenged control group (T01) only 34.9 % of the birds showed characteristic signs of IBD. Nevertheless, 100 % of the surviving birds displayed ≥ degree 3 lesions of the bursa of Fabricius and the comparisons between the treatment groups showed a significant difference ($p=0.0018$) in characteristic signs of IBD (including mortality) between the vaccinated and non-vaccinated groups. The lower percentage of non-vaccinated chickens affected by post-challenge mortality and clinical signs is suspected to be representative of the older age of the birds (9 weeks) at a time when bursal involution has begun (from 3-6 weeks on, according to van den Berg et al., 2000). The level of protection after challenge was 100.0 %, which is in line with efficacy pass criteria mentioned in the Ph. Eur.

In these two challenge studies at 64 days of age, which is the claimed duration of immunity, efficacy requirements were met or significant difference in protection was demonstrated between vaccinated groups and controls, supporting sufficiently the proposed duration of immunity for both application routes.

In conclusion, the claimed duration of immunity for

- MD: a single vaccination is sufficient to provide protection for the entire risk period and
- IBD: 64 days of age

could be adequately supported in the studies presented.

Duration of immunity was not investigated in MDA-positive animals, which is considered acceptable as it was demonstrated that MDAs do not interfere with vaccine efficacy.

Maternally derived antibodies (MDA)

The studies were performed following the guidance of reflection paper EMA/CVMP/IWP/439467/2007:

- Three groups of animals (MDA+ non-vaccinated, MDA- vaccinated and MDA+ vaccinated at the minimal age recommended for use) were included in the studies.
- Challenge is supposed to be performed if MDA levels in control animals at the time of challenge are sufficiently low. Therefore, MD challenges were performed at 9 days of age and IBD challenges at 28 days of age, when MDA levels had waned.
- It was shown that the efficacy of the vaccine in animals vaccinated in the presence of MDAs is, notwithstanding normal biological variation, similar to that obtained in animals of the same age but vaccinated in the absence of MDAs.

MD:

Three studies basically designed and validated according to requirements of Ph. Eur. Monograph 0589, 2-4-3 Immunogenicity, were performed to determine the efficacy for MD in commercial broiler chickens with maternal antibodies, one vaccinated via *in ovo* and two vaccinated via SC route.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose of the minimum titre or lower (i.e. ≤ 3,580 PFU/dose). Challenge was performed at 9 days of age for both routes of vaccination with an appropriate dilution of stock solution of a vMDV challenge strain via intra-abdominal injection. MD and IBD serology was performed on Days 0 and 9 for all treatment groups to determine the serological status of the animals used. After challenge infection, the animals were observed for 70 days for mortality and clinical signs and post mortem examination was performed of all dead or euthanized animals and all remaining animals after the observation period. In case of inconclusive results, histology was conducted for clarification. According to Ph. Eur. Monograph 0589, 2-4-3 Immunogenicity, the challenge is valid, if during the observation period following challenge not less than 70 percent of the challenged control (T01) chickens died or showed severe clinical signs or macroscopic lesions of Marek's disease. The vaccine was considered immunogenic in the presence of MDA, if T02 (MDA+, vaccinated) percent protection, when compared to T01 (MDA+, non-vaccinated), was clinically relevant or significantly different ($p \leq 0.05$). Also, the percent susceptibility of T02 (MDA+, vaccinated) compared to T03 (SPF, vaccinated) was similar and within normal biological variation of approximately 20 % of each other. Hatchability was recorded for *in ovo* vaccination.

In the following studies, the efficacy requirements were met:

In the first study (*in ovo* MDA against MDV [D9]), three groups of seventy 18-day-old embryonated eggs, two groups with MDAs (T01 and T02) and one SPF group (T03), were used. A vaccine dose of 3,559 PFU was administered to groups T02 and T03, respectively, by the *in ovo* route. Group T01 remained unvaccinated. Thirty animals each of the vaccinated groups T02 and T03 and of the unvaccinated group T01 were challenged with vMDV at 9 days of age. The challenge was valid as 80 % of the non-vaccinated/challenged control group (T01) showed characteristic signs of Marek's disease. The percent protection of the vaccinated/challenged MDA+ chickens (T02), when compared to the non-vaccinated/ challenged MDA+ chickens (T01), was significantly different ($p = 0.0003$) . The vaccinated MDA+ (T02) group and vaccinated MDA- (T03) group showed similar protection levels with a biological variation of 3.4 %.
Regarding serology, 100 % of the broilers sampled at Day 0 had high levels of MDA against MDV (GMT 256). A decrease in group GMT was observed with a reduction in titres to 138 (non-vaccinated) and 239 (vaccinated) on D9.

In the second study (SC MDA against MDV [D9]), at the day of hatch two groups of 99 chickens with MDAs (T01 and T02) and one group of 45 SPF chickens (T03) were used. A vaccine dose of 3,580 PFU was administered to groups T02 and T03, respectively, by the subcutaneous route. Group T01 remained unvaccinated. Eighty-four animals each of the MDA+ groups T01 and T02 and 30 animals of the MDA- (SPF) group T03 were challenged with vMDV at 9 days of age. The challenge was valid as 85.3 % of the non-vaccinated/challenged control group (T01) showed characteristic signs of Marek's disease. The percent protection of the vaccinated/challenged MDA+ chickens (T02), when compared to the non-vaccinated/ challenged MDA+ chickens (T01), was significantly different ($p = 0.0001$). The vaccinated MDA+ (T02) group and vaccinated MDA- (T03) group showed similar protection levels with a biological variation of 11.7 %.
Regarding serology, 100 % of the broilers sampled at Day 0 had high levels of MDA against MDV (GMT 208). A decrease in group GMT was observed with a reduction in titres to 97 (non-vaccinated) and 158 (vaccinated) on D9.

The following study was provided for completeness only as the dose is below the intended level for the vaccine and it is considered underpowered:

In this study (SC MDA against MDV [D9]), three groups of 45 chickens at the day of hatch, two groups with MDAs (T01 and T02) and one SPF group (T03), were used. A vaccine dose of 3,102 PFU was administered to groups T02 and T03, respectively, by the subcutaneous route. Group T01 remained unvaccinated. Thirty animals each of the vaccinated groups T02 and T03 and of the unvaccinated group T01 were challenged with vMDV at 9 days of age. The challenge was not valid as only 60.0 % of the non-vaccinated/challenged control group (T01) showed characteristic signs of Marek's disease. The percent protection of the vaccinated/challenged MDA+ chickens (T02), when compared to the non-vaccinated/ challenged MDA+ chickens (T01), was not significantly different ($p = 0.1166$). The vaccinated MDA+ (T02) group and vaccinated MDA- (T03) group showed similar protection levels with a biological variation of 21.8 %. As the challenge could not be validated, a conclusion in line with EMA/CVMP/IWP/439467/2007 could not be drawn. Regarding serology, 100 % of the broilers sampled at Day 0 had high levels of MDA against MDV (GMT 256). A decrease in group GMT was observed with a reduction in titres to 194 (non-vaccinated) and 91 (vaccinated) on D9.

It was concluded that vaccination by the recommended routes with a dose less than the minimum content recommended in the SPC was efficacious and met the efficacy requirements including MDA positive animals.

The proposed claim "reduction of mortality, clinical signs and lesions caused by Marek's disease virus" can be supported in vaccinated chickens with MDA based on the results of the presented studies.

IBD:

One study basically designed and validated according to requirements of Ph. Eur. Monograph 0587, 2-4-5 Immunogenicity, were performed to determine the efficacy for IBD in commercial broiler chickens with maternal antibodies, vaccinated via the *in ovo* and via the subcutaneous route.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a dose of the minimum titre or lower (i.e. ≤ 3,580 PFU/dose). Challenge was performed at 28 days of age for both routes of vaccination with an appropriate dose of a vvIBDV challenge strain via eye drop route. IBD serology was performed on day 0 (T01/T03, T05), day 20 (T01-T03) and day 27 (T01-T05) to determine the serological status of the animals used. After challenge infection, the animals were observed for 10 days for mortality and clinical signs. Histological examination for lesions of the bursa of Fabricius of all surviving animals were carried out at the end of

the observation period with bursal damage scored as per Ph. Eur. Monograph 0587. According to Ph. Eur. Monograph 0587, 2-4-5 Immunogenicity, the challenge is valid, if during the observation period following challenge, not less than 50 percent of the challenged control (T01) chickens showed characteristic signs (clinical signs or mortality) of avian infectious bursal disease and all of the surviving challenged control chickens (T01) showed ≥ degree 3 lesions of the bursa of Fabricius. The vaccine was considered immunogenic in the presence of MDA if T02 and T03 (MDA+, vaccinated) percent protection, when compared to T01 (MDA+, non-vaccinated), was clinically relevant or significantly different ($p \leq 0.05$). Also, the percent susceptibility of T02 or T03 (MDA+, vaccinated) compared to T04 or T05 (SPF, vaccinated) was similar and within normal biological variation of approximately 20 % of each other.
Hatchability was recorded for *in ovo* vaccination.

In this study (*in ovo* and SC MDA against IBDV [D28]), five groups of sixty 18-day-old embryonated eggs, three groups with MDAs (T01, T02 and T03) and two SPF groups (T04 and T05), were used. A vaccine dose of 3,393 PFU was administered to groups T02 and T04, respectively, by the *in ovo* route. A vaccine dose of 2,485 PFU was administered at hatch to groups T03 and T05, respectively, by the subcutaneous route. Group T01 remained unvaccinated. 24 animals each of the vaccinated groups T02 - T05 and of the unvaccinated group T01 were challenged with an appropriate dose of a vvIBDV strain at 28 days of age. The challenge was not valid according to Ph. Eur. criteria, which are set specially to demonstrate the onset of immunity in SPF birds, as in the non-vaccinated/challenged control group (T01, MDA+) only 4.2 % of the birds showed characteristic signs of IBD and one bird did not show degree 3 lesions of the bursa of Fabricius. Nevertheless, the challenge is considered sufficiently validated in the absence of an unvaccinated SPF control group as both findings are considered not unexpected due to the lower susceptibility in broilers compared to SPFs (LeGros et al. 2009). The percent protection of the vaccinated/challenged MDA+ chickens (T02: 83.3 %, T03: 79.2 %), when compared to the non-vaccinated/challenged MDA+ chickens (T01: 4.2 %), was significantly different ($p < 0.0001$) for both vaccination routes. The protection percentage in the vaccinated MDA- (SPF) groups T04 and T05 was 100.0 %. The *in ovo* vaccinated groups (T02 MDA+ and T04 MDA-) showed similar protection levels with a biological variation of 16.7 %; the subcutaneously vaccinated groups (T03 MDA+ and T05 MDA-) showed similar protection levels with a biological variation of 20.8 %. The slightly higher biological variation for T03/T05 is considered due to the very low vaccine dose, which is acceptable.
Regarding serology, 100 % of the broilers sampled at Day 0 had high levels of MDA against IBDV (GMT 8,057). A decrease in group GMT was observed with a reduction in titres to 152.3 (40.8 % positive, non-vaccinated, T01) and 320.4 and 312.7 (vaccinated, T02 and T03, respectively) on D27.

It was concluded that vaccination by the recommended routes with a dose less than the minimum content recommended in the SPC was efficacious and met the efficacy requirements including MDA positive animals.

The proposed claim "prevention of mortality and clinical signs and reduction of lesions caused by infectious bursal disease virus" can be supported in vaccinated chickens with MDA based on the results of the presented study.

Interactions

No studies on interactions were performed. Therefore, the following statements are included in SPC section 3.8 and 5.1:

3.8 Interaction with other medicinal products and other forms of interaction:

"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."

5.1 Major incompatibilities

"Do not mix with any other veterinary medicinal product except solvent recommended for use with the veterinary medicinal product."

Clinical trials

No specific clinical studies to examine efficacy of MD and IBD under field conditions are presented for Poulvac Procerta HVT-IBD. The omission of such studies is justified as for both routes of administration convincing laboratory data on efficacy against challenges with either vMDV or vvIBDV, representative for the EU field situation, have been generated in SPF chickens at 4 to 64 days of age as well as in MDA+ chickens at 9 to 28 days of age with representative MDA levels. Therefore, it is acceptable to consider the pre-clinical studies sufficient to ensure that no specific clinical efficacy trials are necessary (in line with Regulation (EU) 2019/6 and the Guideline on clinical trials with immunological veterinary medicinal products EMA/CVMP/IWP/260956).

Nevertheless, three field studies, mainly performed to evaluate the safety of the vaccine under EU field conditions, were carried out in three different EU countries (Spain, Hungary, Italy) in a large number of commercial broiler chickens with representative levels of maternal antibodies using both the *in ovo* and SC routes:

Study title
Evaluation of field safety and efficacy by serology in broilers in **Spain** after vaccination via ***in ovo*** route with a herpes virus of turkey vaccine carrying a VP2 gene of IBD (Poulvac Procerta HVT-IBD)
Evaluation of field safety and efficacy by serology in broilers in **Hungary** after vaccination via **subcutaneous** route with a herpes virus of turkey vaccine carrying a VP2 gene of IBD (Poulvac Procerta HVT-IBD)
Evaluation of field safety and efficacy by serology in broilers in **Italy** after vaccination via the **subcutaneous** route with a herpes virus of turkey vaccine carrying a VP2 gene of infectious bursal disease (Poulvac Procerta HVT-IBD)

Detailed background information on the field studies is provided in the summarised study descriptions in the safety part. Serology against IBD was assessed to indirectly support the efficacy of the vaccine. The results are discussed below.

In summary, the animals were vaccinated at the minimum age, i.e. *in ovo* (18-day-old embryonated eggs) or SC at the day of hatch with a commercial dose of Poulvac Procerta HVT-IBD or a comparator vaccine. The animals were observed until slaughter for safety signs such as mortality and clinical signs, body weight and feed consumption; necropsy and histopathology of certain organs was performed for macroscopic lesions related to MD or IBD; feed conversion ratio [FCR], bursa-tobody-weight ratio, European production efficiency factor (EPEF) and percent of condemnations [CN] were calculated; hatchability was recorded. Blood samples were taken at different time points from D0 until slaughter in order to assess maternal antibody levels (HVT and IBD) and antibody development (IBD) in comparison to the comparator vaccine.

In the study carried out *in ovo* in Spain, two groups of approximately ninety thousand 18-day-old

embryonated broiler eggs were used. A vaccine dose of 5,673 PFU was administered to group T01 by the *in ovo* route. Group T02 was vaccinated *in ovo* with a commercial dose of Vaxxitek HVT+IBD. Two vaccines against infectious bronchitis virus (IBV) and coccidiae were concomitantly administered on day 0 and Amoxicillin on day 14.

The serological data on day 0 show that the broiler bird flock had maternal antibody titres fully representative for commercial birds: 84 % of the birds of T01 and 82 % of T02 were seropositive by IFA against HVT. The mean titre was 135 (T01 – Vaxxitek) and 132 (T02 – Procerta), respectively, the maximum titre was 256 for both groups. 100 % of the birds were seropositive for maternal antibody titres against IBD with a mean titre of 8,698 (T01 – Vaxxitek) and 10,688 (T02 – Procerta), respectively, and maximum titres of 13,734 and 16,347, respectively.

At day 28, maternal antibodies declined to 48 % seropositive / GMT 518 (T01 - Vaxxitek) and 40 % seropositive / GMT 614 (T02 - Procerta), respectively. By day 41, percentage seropositive again increased to 72 % / GMT 1,536 (T01 - Vaxxitek) and 66 % / GMT 913 (T02 - Procerta) in response to the vaccination, respectively.

In the study performed subcutaneously in Hungary, two groups of approximately 40,000 broiler chickens at the day of hatch were used. A vaccine dose of 5,673 PFU was administered to group T01 by the subcutaneous route. Group T02 was vaccinated subcutaneously with a commercial dose of Vaxxitek HVT+IBD. Two vaccines against IBV and Newcastle disease virus (NDV) were concomitantly administered on day 0.

The serological data on day 0 show that the broiler bird flock had maternal antibody titres fully representative for commercial birds: 83 % of the birds were seropositive by IFA against HVT. The mean titre was 170, the maximum titre was 256. 98 % of the birds were seropositive for maternal antibody titres against IBD with a mean titre of 4,869 and a maximum titre of 13,144.

At day 28, maternal antibodies declined to 12 % seropositive / GMT 197 (T01 - Vaxxitek) and 10 % seropositive / GMT 193 (T02 - Procerta), respectively. By day 34, percentage seropositive again increased to 58 % / GMT 889 (T01 - Vaxxitek) and 54 % / GMT 970 (T02 - Procerta) in response to the vaccination, respectively.

In the study performed subcutaneously in Italy, two groups of approximately 43,600 broiler chickens at the day of hatch were used. A vaccine dose of 5,673 PFU was administered to group T02 by the subcutaneous route. Group T01 was vaccinated subcutaneously with a commercial dose of Vaxxitek HVT+IBD. One vaccine against IBV and Newcastle disease virus (NDV) was concomitantly administered on day 0.

The serological data on day 0 show that the broiler bird flock had maternal antibody titres fully representative for commercial birds: 100 % of the tested birds were seropositive for maternal antibody titres against IBD with a mean titre of 13,034 and a maximum titre of 21,341. Assessing the level of HVT MDA was not possible due to US import restrictions related to the avian influenza situation in Italy.

At day 28, maternal antibodies declined to 46 % seropositive / GMT 485 (T01 - Vaxxitek) and 40 % seropositive / GMT 480 (T02 - Procerta), respectively. By day 40, percentage seropositive again increased to 86 % / GMT 1,571 (T01 - Vaxxitek) and 78 % / GMT 1,395 (T02 - Procerta) in response to the vaccination, respectively.

In conclusion, regarding the efficacy parameters, a serological response to *in ovo* (66 % on D44) and SC (54 % on D34 and 78 % on D40, respectively) vaccination was demonstrated under field conditions following the decline of maternal antibodies against IBD and was similar to the positive control vaccine

(72 %, 58 % and 86 %, respectively) in all trials.

Overall conclusion on efficacy

The applicant has adequately demonstrated the efficacy of the vaccine. The results from 22 laboratory and 3 field trials show that the product is effective for the active immunisation of one-day-old chickens and 18-19-day-old embryonated chicken eggs to

- reduce mortality, clinical signs and lesions caused by Marek's disease virus and
- prevent mortality and clinical signs and reduce lesions caused by infectious bursal disease virus,

at the proposed dose of 3,580 – 26,500 PFU.

Onset of immunity

Onset of immunity has been demonstrated

- at 7 days post vaccination for *in ovo* and 9 days for subcutaneous use for MD and
- at 15 days post vaccination for *in ovo* and 12 days for subcutaneous use for IBD.

Based on the results of the presented studies, the proposed claims concerning MD and IBD could be adequately supported.

Duration of immunity

The duration of immunity has been adequately demonstrated for

- MD: a single vaccination is sufficient to provide protection for the entire risk period and
- IBD: 64 days of age.

Maternally derived antibodies (MDA)

It has been adequately demonstrated that MDA did not interfere with vaccination.

The proposed claims could be supported in vaccinated chickens with MDA based on the results of the presented studies.

Interactions

No studies on interactions were performed. Appropriate statements are included in SPC sections 3.8 and 5.1.

Clinical trials

No specific clinical studies to examine the efficacy of MD and IBD under field conditions are presented for Poulvac Procerta HVT-IBD. The omission of such studies was sufficiently justified.

Three clinical studies, mainly performed to evaluate the safety of the vaccine under EU field conditions, were carried out in three different EU countries (Spain, Hungary, Italy). Serology against IBD indirectly supported the efficacy of the vaccine.

Part 5 – Benefit-risk assessment

Introduction

Poulvac Procerta HVT-IBD is a live recombinant vector vaccine containing a turkey herpes virus (HVT) strain with the gene encoding for the VP2 of the infectious bursal disease virus inserted into the HVT genome. This product is considered a GMO under EU legislation. The vaccine is a frozen concentrate for suspension for injection stored in liquid nitrogen in freezing medium. No adjuvant or preservative is added. This frozen concentrate is to be diluted in sterile solvent before use. The pharmaceutical form of the final vaccine is a suspension for injection. Due to the nature of the product and concentration of excipients, the proposed withdrawal period is zero days.

Poulvac Procerta HVT-IBD is innovative because the recombinant vaccine strain induces immunity against two relevant poultry pathogens, Marek's disease virus (MDV) and infectious bursal disease virus (IBDV), which are frequently isolated in poultry stocks and they have paramount importance in the poultry production industry. Furthermore, the vaccine can be applied at an early age of the birds (1-day-old) or even in embryonated chicken eggs (18-19 days-old) to provide protection against early replication of virulent MDV and IBDV in case of infection.

The vaccine is indicated for the active immunisation of chickens to reduce mortality, clinical signs and lesions caused by MDV and to prevent mortality and clinical signs and reduce lesions caused by IBDV.

The vaccine is intended for use in chickens by *in ovo* route at 18-19 days of embryonation at a dose of 0.05 ml or by subcutaneous route at day of hatch at a dose of 0.2 ml.

The dossier was submitted in line with requirements of Article 42(2)a of Regulation (EU) 2019/6.

Benefit assessment

Direct benefit

The proposed benefit of Poulvac Procerta HVT-IBD is its efficacy in active immunisation of embryonated chicken eggs or one-day-old chickens:

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

This benefit was shown in a large number of appropriately designed and well executed pre-clinical studies.

The onset of immunity against MD was established at 7 days post vaccination for *in ovo* route and 9 days for subcutaneous use; against IBD at 15 days post vaccination for *in ovo* route and 12 days for subcutaneous use. The duration of protection is adequately demonstrated for MD, where a single vaccination is sufficient to provide protection for the entire risk period and for IBD to be 64 days.

Clinical studies were mainly performed to evaluate the safety of the vaccine under EU field conditions. Serology against IBD indirectly supported the efficacy of the vaccine. The influence of maternally derived antibodies on the efficacy of the vaccine was investigated in well-designed laboratory studies, using commercial broiler chickens and eggs with confirmed MDA against MDV and IBDV.

Poulvac Procerta HVT-IBD was shown to be efficacious against MD and IBD.

Additional benefits

Poulvac Procerta HVT-IBD is easy to apply to embryonated chicken eggs by a single *in ovo* vaccination using an appropriate device/ applicator. The vaccine is also easy to apply to day-old chicks by a single subcutaneous vaccination. This limits the number of times the animals must be handled.

Poulvac Procerta HVT-IBD can be applied at an early age of the birds (1-day-old) or even in embryonated chicken eggs (18-19-day-old) at the hatchery to provide protection against early replication of virulent MDV and IBDV and thus reduces clinical signs in case of infection. Consequently, the incidence of clinical MDV and IBDV outbreaks due to field contamination is reduced.

One single *in ovo* or subcutaneous vaccination is sufficient to stimulate immunity against two relevant poultry pathogens, MDV and IBDV. The vaccine strain was shown to be fully apathogenic to other avian species, limiting the risk to the environment.

Poulvac Procerta HVT-IBD increases the range of available treatment possibilities for the active immunisation of chickens and embryonated chicken eggs against infections with MDV and IBDV.

Risk assessment

The main potential risks are identified as follows:

Quality

The formulation and manufacture of Poulvac Procerta HVT-IBD is well described and specifications set will ensure that a product of consistent quality will be produced if all conditions are fulfilled. The claimed shelf life is fully supported by the available data.

Safety

Risks for the target animal

The product is generally well tolerated in the target animal when administered in accordance with the SPC recommendations. No adverse reactions were observed after a tenfold overdose of Poulvac Procerta HVT-IBD administered by the *in ovo* or subcutaneous route.

The vaccine strain was obtained by insertion of one additional gene into a naturally apathogenic vaccine strain, which is known to be safe for chickens. The biological properties (safety, dissemination, shed, spread) of the original strain were not changed by the genetic modification. Reversion to virulence could not be demonstrated. The chance of recombination with other strains or other viruses occurring is considered to be effectively zero.

Appropriate warnings are included in section 3.5 of the SPC regarding spreading of the vaccine.

Risk for the user

The avirulent parental HVT strain is non-pathogenic for humans and infects only avian hosts without causing clinical disease. There are no indications that the genetically modified virus strain HVT-IBD behaves differently.

The vaccine is filled in glass ampoules and stored in liquid nitrogen, in exceptional cases ampoules may explode upon warming up. Appropriate precautions and warnings for safe handling of the ampoules are

included in the SPC. Only trained personnel should handle liquid nitrogen.

The user safety for this product is acceptable when used according to the SPC recommendations.

Risk for the environment

The vaccine virus is shed with feather dust and can remain infectious in the environment for some time. Spread to chickens and turkeys and between turkeys was observed. In general, HVT can infect avian species only and the vaccine strain contained in Poulvac Procerta HVT-IBD was shown to be unable to infect mice. Appropriate measures mitigating the risk of spread of the vaccine strain to turkeys are included in the SPC.

Poulvac Procerta HVT-IBD is not expected to pose a risk for the environment when used according to the SPC recommendations.

Risk for the consumer:

A residue study is not required. The withdrawal period is set at zero days.

Risk management or mitigation measures

The following measures are included in the SPC to minimise the above-mentioned risks:

- The vaccine strain can be detected in environmental samples for a maximum of six weeks.
- The vaccine strain may spread. Appropriate veterinary and husbandry measures should be taken to avoid spread of the vaccine strain to unvaccinated chickens and turkeys and also susceptible species.
- Detailed description of the handling of the vaccine ampoules stored in liquid nitrogen and a detailed description of the personal protection equipment. Liquid nitrogen should only be handled by trained personnel.
- The veterinary medicinal product is subject to a veterinary prescription.

Evaluation of the benefit-risk balance

At the time of submission, the applicant applied for the following indication:
For active immunisation of one day old chickens and 18-19 day old embryonated chicken eggs to

- reduce mortality, clinical signs and lesions caused by MDV and
- prevent mortality and clinical signs and reduce lesions caused by IBDV.

Onset of immunity:	MD: 4 days of age for *in ovo* and 9 days for subcutaneous use
	IBD: 12 days of age
Duration of immunity:	MD: a single vaccination is sufficient to provide protection for the entire risk period
	IBD: 64 days of age

The CVMP has accepted the following onset of immunity:

	MD: 7 days post vaccination for in ovo and 9 days for subcutaneous use
	IBD: 15 days post vaccination for in ovo and 12 days for subcutaneous use

Onset of immunity for MD and IBD, as detailed above, is adequately supported by data. Duration of immunity for MD is accepted to be life-long. The duration of immunity against IBD is considered to be adequately supported by data at this point. The influence of maternal antibodies on the efficacy of the vaccine against MDV was studied using commercial broiler chickens with confirmed levels of MDA against MDV and IBDV.

The formulation and manufacture of Poulvac Procerta HVT-IBD is well described and the specifications set will ensure that a product of consistent quality will be produced. Currently, the claimed shelf life is considered fully supported by the available data.

Poulvac Procerta HVT-IBD is well tolerated by the target animals and presents a low risk for users and the environment.

The product has been shown to be efficacious for these indications, and the CVMP accepted the indications as proposed by the applicant.

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

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Veterinary Medicinal Products (CVMP) concluded that the application for Poulvac Procerta HVT-IBD is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 2019/6).

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