EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Gumbohatch (EMEA/V/C/004967/0000)

Vaccine common name: Avian infectious bursal disease vaccine (live)

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

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Introduction

The applicant LABORATORIOS HIPRA, S.A. submitted on 24 April 2018 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Gumbohatch, through the centralised procedure under Article 3(2)b of Regulation (EC) No 726/2004 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 18 January 2018 as the applicant showed that the product would provide a significant technical innovation.

The applicant applied for the following indications: For active immunisation of chicken to reduce mortality, clinical signs, loss of weight and lesions of bursa of Fabricius (BF) caused by avian infectious bursal disease viruses.

The indication which was considered acceptable by CVMP is: For active immunisation of 1-day-old broiler chicks and embryonated broiler chicken eggs to reduce clinical signs and lesions of the bursa of Fabricius caused by very virulent avian infectious bursal disease virus infection.

The active substance is a live attenuated avian infectious bursal disease virus (IBDV), strain 1052. In the vaccine formulation this attenuated virus is present as an immune complex with an IBDV-specific antibody. As the vaccine virus is trapped in the immune complex if the vaccine is administered when the maternally derived antibody (MDA) concentration is high the vaccine virus is prevented from reaching the bursa. Once the MDA decline to a low level, the vaccine virus can reach the bursa and replicate.

Gumbohatch is presented as a lyophilisate and solvent for suspension for in ovo injection into broiler chicken eggs at day 18 of incubation, or by subcutaneous (s.c.) injection into 1-day-old broiler chicks in the hatchery. The lyophilisate is contained in a type I glass vial of 10 ml and the solvent is presented in polypropylene bags of 250 ml, 500 ml or 1,000 ml.

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

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

On 12 September 2019, the CVMP adopted an opinion and CVMP assessment report.

On 12 November 2019, the European Commission adopted a Commission Decision granting the marketing authorisation for Gumbohatch.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system (dated 14/01/2019) which fulfils the

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

Manufacturing authorisations and inspection status

Appropriate and up to date certificates of Good Manufacturing Practice (GMP) compliance are provided for:

(a) the site responsible for manufacture of the active substance and the lyophilised vaccine: LABORATORIOS HIPRA, S.A., Carretera C-63, Km 48.300 Poligono Industrial El Rieral, 17170 Amer, Spain: (dated 30/04/2019).

(b) the site responsible for manufacture of the solvent, secondary packaging and batch release: LABORATORIOS HIPRA, S.A., Avda. La Selva, 135, 17170 Amer, Spain: (dated 08/05/2019)

The certificates are issued by the Spanish competent authority (Agencia Española de Medicamentos y Productos Sanitarios).

The Qualified Person's (QP) declaration states that the manufacture of the active substance at LABORATORIOS HIPRA, S.A., Carretera C-63, Km 48.300 Poligono Industrial El rieral, Amer, 17170 Amer, Spain (audited 08/11/2017) is in accordance with the detailed guideline on good manufacturing practice for active substances used as starting materials as required by Article 46(f) of Directive 2001/83/EC and Article 50(f) of Directive 2001/82/EC.

Overall conclusions on administrative particulars

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

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

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

Qualitative and quantitative particulars

The vaccine Gumbohatch is presented as a lyophilisate and solvent for suspension for in ovo or subcutaneous administration to chickens. Gumbohatch contains an attenuated IBDV serotype 1, strain 1052, as active substance at a titre of $10^{1.48}$ –$10^{2.63}$ potency units (PU) per 0.05 ml in ovo dose /0.2 ml subcutaneous dose. The vaccine does not contain an adjuvant.

The excipients in the lyophilisate are IBDV-specific IgY, sucrose, glycine, L-histidine, potassium chloride, disodium phosphate dodecahydrate, potassium dihydrogen phosphate and sodium chloride. The solvent is phosphate buffered saline (PBS) which is used to reconstitute the lyophilisate and this

contains disodium phosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride, potassium chloride and water for injections (WFI).

Container and closure

The colourless 10 ml type I glass vials (European Pharmacopoeia (Ph. Eur.) chapter 3.2.1) and the type I rubber stopper (Ph. Eur. chapter 3.2.9) used for the lyophilisate are in compliance with the pharmacopoeial requirements and their sterilisation is adequate. The aluminium cap on the vials has no product contact.

Similarly, the polypropylene bags (Ph. Eur. chapters 3.1.6 and 3.1.3) used to fill the PBS solvent are in compliance with the pharmacopoeial requirements. The PBS solvent is available in polypropylene bags of 250 ml (containing 200 ml solvent), 500 ml (containing 400 ml solvent) and 1,000 ml (containing 800 ml or 1,000 ml of solvent). Satisfactory details were given on the sealing of the solvent bags including information on the integrity of the seal. Each bag is in a plastic overpouch made of polyester and polypropylene. The overpouch has no product contact; the function of the overpouch is to provide the bags with a further physical protection to prevent them from breaking during storage and transport. The overpouch was used during stability evaluation of the PBS solvent batches as proposed for the commercial product.

The PBS solvent is terminally sterilised in accordance with Ph. Eur. chapter 5.1.1 conditions, sterilisation is performed after the solvent bag is overpouched with the external bag. Validation of the terminal sterilisation of the PBS bags with the overpouch is provided.

Product development

A satisfactory overview of infectious bursal disease (IBD) (also known as Gumboro disease) and the clinical and sub-clinical effects is given. Briefly, the causative agent is a double-stranded RNA virus of the genus Avibirnavirus (family Birnaviridae) which targets immature B lymphocytes of the BF, a primary lymphoid organ in avian species. This results in B-cell depletion in bursal follicles which may cause significant depression of humoral antibody responses thus promoting secondary infections. While the virus infects a number of avian species, clinical disease occurs solely in chickens. Chickens younger than 10 weeks are usually clinically affected while older chickens do not show clinical signs. Severe acute disease usually in 3 to 6 week-old birds is associated with high mortality but less acute or subclinical infections are common earlier in life. Two IBDV serotypes (designated 1 and 2) are recognised - clinical disease is associated with serotype 1 however there are no reports of clinical disease due to serotype 2 infection justifying the use of the serotype 1 strain 1052 in Gumbohatch.

The applicant classifies the Gumbohatch vaccine strain 1052 as an 'intermediate-plus' strain based on a bursal index value. It is also outlined that the Gumbohatch vaccine strain 1052 is derived from the W2512 strain. Considering the W2512 strain is contained in a number of currently authorised EU vaccines the relevance of the Gumbohatch vaccine strain to protect against current circulating EU IBDV strains is supported.

Gumbohatch has been developed as an immune complex (Icx) vaccine where the vaccine virus strain 1052 is complexed with a solution containing IBDV-specific IgY antibodies extracted from the eggs from hens of healthy flocks (Ph. Eur. chapter 5.2.13). After administration Icx vaccines are considered to interact with follicular dendritic cells trapping the vaccine virus such that when the MDA concentration is high the vaccine virus is prevented from reaching the bursa. Once the MDAs decline to a low level, the vaccine virus can reach the bursa and replicate. Icx vaccines therefore can be given at a young age because they are not blocked by MDA. Literature publications supporting this mode of

action are provided and Icx vaccines are a recommended vaccine model in chapter 2-3-12 of the O.I.E. Manual on infectious bursal disease.

In the manufacture of Gumbohatch the vaccine virus is handled in a seed lot system and in compliance with Ph. Eur. chapter 0062. All passages are on embryonated eggs from specific-pathogen-free (SPF) flocks (Ph. Eur. chapter 5.2.2). Gentamicin and ampicillin are used during virus growth in embryonated eggs in accordance with Ph. Eur. chapter 0062 which restricts the use of antibiotics to certain processes including egg inocula. It has been demonstrated that only trace amounts remain in the vaccine which is acceptable.

The amount of viral harvest and IBDV-specific IgY solution required for blending is based on the virus neutralisation units (VNU) of the batch of IBDV-specific IgY solution so that the IBDV-specific IgY solution ensures complete neutralisation of the vaccine strain. Tests for unbound IgY and virus neutralisation are performed as finished product tests to check for complete neutralisation.

The freeze-drying excipient (GHS) is composed of sucrose, glycine, L-histidine, disodium phosphate dodecahydrate, potassium dihydrogen phosphate, potassium chloride and sodium chloride and is used to stabilise the immune complex during lyophilisation. It is sterilised by filtration. The production of the finished product involves an aseptic manufacturing process under GMP conditions using sterilised ingredients which have been tested for sterility by suitable methods.

The PBS solvent used to reconstitute the lyophilisate for parenteral administration contains the standard components for saline buffers.

Description of the manufacturing method

The manufacturing process involves a number of steps as follows: (a) production of the live IBDV strain 1052 viral harvest (b) production of the freeze-drying GHS excipient (c) production of the IBD-specific IgY solution (d) production of the lyophilisate and (e) production of the PBS vaccine solvent. Satisfactory flow charts for each step are provided.

(a) Production of the live IBDV strain 1052 viral harvest

SPF eggs are inoculated via the chorioallantoic route with Working Seed Virus (WSV) dissolved in a phosphate buffered saline solution containing gentamicin and ampicillin. After incubation the eggs are cooled to 2 – 8 °C and the chorioallantoic fluid and embryos are collected. A solution of gentamicin and ampicillin is added to the chorioallantoic fluid. The embryos are homogenised and gentamicin and ampicillin are added. The chorioallantoic fluid and embryo fractions are then mixed according to a defined range of v/v ratios. The mixture is then clarified and directly used for further processing without any intermediate storage. The range used for mixing the embryo and chorioallantoic fluid fractions is justified.

(b) Production of the freeze-drying GHS excipient

Formulation of the freeze-drying GHS excipient is satisfactorily described and involves dissolution of the individual components in WFI. The pH is adjusted and the solution is pre-filtered (0.2µm filter), a sample is taken for bioburden after which the solution is sterile filtered (0.2 µm filter) into sterile plastic containers which are stored at 15 – 25 ºC for a maximum of 8 months. The integrity of the sterilising filter is checked before and after the filtration step. Data supporting the 8 months storage time at 15 – 25 ºC are discussed later in this report under 'Stability'. Appropriate data on the validation of the filter sterilisation has been provided.

(c) Production of the IBDV-specific IgY solution

The egg content is extracted from eggs from healthy chicken flocks, previously immunised with IBDV vaccine, in a sequence of steps including an inactivation treatment and a further neutralization of the inactivating agent. The resulting IgY solution is then sterilised by filtration involving a pre-filtration step and a final 0.2 µm sterilising filtration. The sterilised IgY solution is collected into sterile plastic containers and stored at 2 – 8 ºC for ≤ 6 months. Stability data for this storage condition are discussed later in this report under 'Stability'.

The integrity of the sterilising filter is checked before and after the filtration step and acceptable validation data for the sterilising filtration are provided. Data are also provided to support the inactivation of relevant extraneous agents during the inactivation treatment step by a minimum of 6-log reduction. Sufficient details have been provided.

The applicant states the eggs used as starting material for the IBDV-specific IgY are from healthy chicken flocks meeting the requirements of Ph. Eur. chapter 5.2.13. It is confirmed that the immunisation strategy applied in these flocks is fixed and is always identical in all flock batches. To produce IBDV specific IgY the flocks must be immunised with IBDV and the same vaccine strain is used to immunise the flocks.

The applicant has provided sufficient information that IgY have no impact on routine vaccination programmes.

(d) Production of the lyophilisate

Each vaccine vial before lyophilisation contains the combination of viral harvest and IBD-specific IgY solution and the freeze-drying excipient. The amount of IgY solution required depends on the VNU of the IgY batch which is determined by inoculating embryonated eggs with serial dilutions of the IBDV-specific IgY solution and a standard amount of a reference stock of virus.

There is no stop in the manufacturing process for testing therefore the blending of the final vaccine will be performed assuming a fixed virus concentration. The volume of IgY added guarantees complete neutralisation of the virus. For the production of consistent batches, an in-process control of EID_{50}/ml has been established for each viral harvest with a specification range to be complied with.

In addition, the amount of viral harvest used for vaccine blending has also been fixed, resulting in the infective titre in the final vaccine (measured in PUs) being consistent. The required lyophilisation excipient is also fixed and this implies that only the IgY solution volume to neutralise the virus can vary depending on its VNU titre. Moreover, depending on the VNU titre of the used IgY solution batch, it may be necessary to adjust this solution with WFI up to the target volume, or not.

During the vial filling process, the blend is aseptically filled into the vials, a stopper is automatically placed over each vial and the vials are lyophilised according to a validated cycle. At the end of the cycle the stoppers are fully inserted into the vials and the vials are unloaded, capped and stored at 2 - 8°C for up to 21 months.

(e) Production of the PBS vaccine solvent

The formulation of the PBS solvent is satisfactorily described and involves dissolution of the components in WFI followed by 0.2 µm filtration of the solution into a sterilised tank. The filtered solution can be held in this tank for a maximum of 5 days at ≤ 25 °C prior to filling into polypropylene bags which are then terminally sterilised in an autoclave at ≥ 121 ºC for 30 minutes after which they are stored at a temperature of ≤ 25 °C. The external overpouch bag is applied before terminal sterilisation.

Production and control of starting materials

Starting materials listed in pharmacopoeias

Representative certificates of analysis (CoA) for all starting materials of pharmacopoeial origin are provided. For the SPF embryonated eggs, the certificates from each supplier confirm the flocks have been tested for the agents listed in Ph. Eur. chapter 5.2.2, in some cases an alternative test to that specified in the monograph is used however the suitability of the alternative method is provided. For the eggs from healthy flocks the representative certificate confirms compliance with Ph. Eur. chapter 5.2.13 and as required by this monograph a risk assessment in accordance with Ph. Eur. chapter 5.2.5 has been performed which supports a minimal risk for extraneous agent contamination due to the use of these eggs.

As mentioned earlier in the section 'Description of the manufacturing method' as IBDV-specific IgY is extracted from these eggs the flocks must be immunised with IBDV. Adequate details are provided on how these flocks are immunised and managed to be adequate sources of IBD-specific IgY.

For all other materials the documentation provided supports compliance with pharmacopoeial requirements.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

The only non-pharmacopoeial biological origin starting materials used for production are the IBDV strain 1052 master seed virus (MSV) and working seed virus (WSV).

The passage history of the MSV is satisfactorily explained i.e. the W2512 IBDV strain originally isolated from broilers in the US was received by LABORATORIOS HIPRA S.A. from the US Department of Agriculture. The Original Virus (OV) stock was produced at LABORATORIOS HIPRA S.A. The OV was dispensed into vials and stored at ≤ -70 °C.

The MSV was prepared using SPF embryonated eggs. At the end of the incubation, the chorioallantoic fluid and the embryos were collected, mixed and homogenized. After clarification glycerol was added and the suspension was dispensed into vials and stored frozen.

The MSV has been tested in accordance with Ph. Eur. chapter 0062 and Ph. Eur. chapter 2.6.24 with test procedures and validation data provided where relevant. Identity testing was done using a validated PCR test and sterility and mycoplasma testing were done according to Ph. Eur. chapter 2.6.1 and Ph. Eur. chapter 2.6.7, respectively. The MSV was tested for all of the extraneous agents listed in Ph. Eur. chapter 2.6.24 and all test procedures are provided. Overall the testing is acceptable. Acceptable validation data for the nucleic acid amplification techniques (NAT) used for the detection of avian leucosis virus (ALV), avian reticuloendotheliosis virus (REV) and chick anaemia virus (CAV) are provided.

The WSV is prepared using SPF embryonated eggs. At the end of the incubation the chorioallantoic fluid and the embryos are collected, mixed and homogenized. After clarification glycerol is added and the suspension is dispensed into vials and stored frozen. The WSV is tested for sterility (Ph. Eur. chapter 2.6.1) and viral titre by a validated method.

The vaccine virus strain originates from a strain isolated from broilers and all subsequent passages including those used to produce the vaccine virus are carried out on SPF embryonated eggs. Similarly, the IBDV-IgY solution is extracted from eggs from healthy chicken flocks. As birds are considered a non-TSE relevant species according to the TSE Note for Guidance EMA/410/01 and no other animal origin materials are used for production, the risk of transmitting TSE infection by the vaccine is negligible.

The applicant states the risk of extraneous agent contamination by the vaccine is negligible as the MSV has been tested for freedom for relevant agents according to Ph. Eur. chapter 0062 and Ph. Eur. chapter 2.6.24, and all passages from the MSV to vaccine virus are done using SPF embryonated eggs meeting Ph. Eur. chapter 5.2.2 requirements.

Starting materials of non-biological origin

Suitable certificates of analysis have been provided for the starting materials of non-biological origin.

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

The details given on the qualitative and quantitative composition of the media used during production, their treatment processes and storage conditions are acceptable.

Control tests during the manufacturing process

Appropriate flow charts for the production processes for live IBDV strain 1052, the GHS and the excipient of IBD specific IgY solution were provided including where control testing takes place. The frequency of testing, the function and the limits of acceptance were provided, including adequate descriptions of the various methods. Control testing includes sterility of the viral harvest, bioburden and filter integrity during the production process of the freeze-drying excipient, and appearance, sterility and pH on the freeze-drying excipient. Controls performed during the production process of IBD-specific solution of IgY include bioburden and filter integrity and on each batch of IBD-specific IgY solution testing for appearance, sterility, determination of VNU and complete neutralisation of inactivating agent.

Sterility testing is performed in line with Ph. Eur. chapter 2.6.1. Validation of the sterility control by membrane filtration of the IBD-specific IgY solution is provided, parameters tested passed the acceptance criteria outlined. A satisfactory description for measuring pH is provided however a method for appearance testing is requested. Bioburden is carried out on each batch of freeze-drying excipient and IgY solution before filtration with an acceptance limit of not more than 10 cfu/100 ml in accordance with GMP requirements. The filter integrity method is adequately described and the filters are tested before and after each use and the acceptance criteria for the filter integrity test is provided. The results of the control tests carried out on three batches of freeze-drying excipient met the required in process specifications.

A method for the determination of the VNU of the IgY solution batches used to formulate Gumbohatch is provided and overall acceptable details of the production and source of the IgY solution as well as on the immunisation strategy of the flocks are given. The VNU is determined for each batch of solution to quantify the virus neutralisation activity of the batch of IgY as each batch of Gumbohatch is formulated by combining the attenuated IBDV virus and the appropriate concentration of IgY solution containing

specific antibodies against the virus (VN_{50} titre). The results for the batches of IgY solution provided met the required in process specifications.

An appropriate method for the determination of complete neutralisation of the inactivating agent was provided including an acceptable in-house validation of the method. A CoA for the neutralising agent was provided and specifications were met.

Volume control for the antigenic fraction of the vaccine and solvent is measured on each batch during the filling process at the beginning, in the middle and at the end of the process and the results of 3 consecutive batches of vaccine and solvent were presented in Part 2F for batch-to-batch consistency and the required specifications were met.

Control tests on the finished product

The controls described for the finished product (lyophilised fraction) and specifications were provided for appearance, solubility, residual humidity, potency, identity, unbound IgY serum neutralisation test, virus neutralisation test, sterility, mycoplasma, extraneous agents testing (using cell culture and embryonated eggs), test for egg drop syndrome virus (EDSV), Marek's disease virus, turkey rhinotracheitis (TRT) virus and CAV. Control tests on the solvent included pH, sterility, appearance and volume control. The identity testing by PCR showed results for accuracy and precision which are acceptable. Overall the identity testing and validation of the method are considered acceptable.

The potency test is described and a validation report is provided. A justification for choosing the proposed method is provided. The proposed test is a RT-PCR titration for IBDV on each batch of reconstituted vaccine with an internal control for the quantification of 'viable' virus in the vaccine. From the data provided, the level of variation within the validation of the potency test can be considered acceptable. An in-process control of EID_{50}/ml has been established for each viral harvest. In addition, the amount of viral harvest used for vaccine blending has been fixed, resulting in the infective titre in the final vaccine (measured in PUs) being consistent. Therefore, a link exists between the amount of infectious virus in the viral harvest and the potency unit result in the finished product. Overall the potency test is considered adequate.

The validation of the potency test can be considered satisfactory.

An appropriate protocol for replacement of the internal control for the potency test is provided.

In addition, the applicant has confirmed detecting only live virus by testing an inactivated "Gumbohatch" test vaccine using the described batch potency test.

A description and validation of the unbound IgY serum neutralisation titre of the vaccine is provided. Based on the results the range for the serum neutralisation units (SNU)/vial has been established. All batches tested met the acceptance criteria. The applicant has included the upper limit for unbound IgY as a release criterion. There are two different test methods to determine the IgY titre used. A serum neutralisation test with titration in embryonated chicken eggs and a serum neutralisation test with detection of cytopathic effect (CPE) in chicken fibroblast cell test.

In the context of animal welfare the applicant has committed to change the method of devitalisation of the embryonated chicken eggs to a short time at freezing conditions.

A satisfactory description of the virus neutralisation test and validation of the test method is provided. Based on the validation of the test method, the specification for unbound IgY is given. The applicant has provided a protocol for how the IBDV control will eventually be replaced.

Sterility testing and validation are performed according to Ph. Eur. chapter 2.6.1. The mycoplasma test and validation are performed in line with Ph. Eur. chapter 2.6.7.

The residual moisture testing and validation are adequately described and a new specification is set.

Tests for extraneous agents using cell culture and embryonated eggs were appropriately described and are in accordance with Ph. Eur. chapter 2.6.25. The methods and acceptable validation results for the tests for EDSV, Marek's Disease virus, TRT virus and CAV have been provided. The control tests for extraneous agents will be performed for routine testing as the possibility remains that eggs containing extraneous agents may be used in vaccine production.

Control of the solvent by pH, sterility (in line with Ph. Eur. chapter 2.6.1), appearance and volume control (according to Ph. Eur. chapter 2.9.17) are considered appropriate.

Batch-to-batch consistency

Three consecutive batches of vaccine were tested for consistency of manufacture using the control tests described in Part 2D and 2E. Three consecutive batches of solvent were also tested with each of the vaccine batches respectively. Each batch tested met the required specifications.

The potency values for the consistency testing met the proposed specification.

For unbound IgY solution an appropriate range of SNU/vial is given. An upper limit in the finished product specifications to control the upper limit of unbound IgY to ensure consistency of manufacture has been introduced.

Results show the consistency batches to be sterile and free from mycoplasma as per the Ph. Eur.

The extraneous agents testing showed no contamination for each batch tested using embryonated hen's eggs and chicken embryo fibroblast cells in line with Ph. Eur. chapter 2.6.25. EDSV, Marek's disease virus, TRT virus and CAV were tested for on the consistency batches in line with Ph. Eur. chapter 2.6.25 and were found to be absent. The applicant has requested to waive the routine testing for extraneous agents given the data presented in line with requirements for Vaccines for Veterinary Use Ph. Eur. However, this request cannot be granted as Gumbohatch is a live vaccine, manufactured in SPF eggs, and therefore it is considered that the potential for contamination is present in each batch and therefore the routine extraneous agents testing for all batches will be performed by the applicant in line with Ph. Eur. 2.6.25.

Stability

For active substance:

Results from two experimental batches manufactured after storage of the bulk antigen for 4 or 8 days at +2 to +8 °C are available at T0 and meet the required specifications however no further data will be presented as the process is continuous and therefore there will be no further bulk storage data requested.

For finished product:

Based on the real time testing for the finished product up to 24 months, Gumbohatch can be assigned a 21 month shelf life in glass vials stored at 2-8 °C. All of the results presented in the stability studies for the finished product met the required specifications.

In stability update results for a vaccine batch frozen at -20 ºC for 12 months and then tested at 12 months when subsequently stored at 2-8 °C was provided. All of the parameters tested met the required specifications for appearance, solubility, residual moisture, potency, unbound IgY serum neutralisation test and virus neutralisation test at 12 months. Testing is planned to continue to 27 months to demonstrate that the vaccine can be stored frozen for a 12 month period before being stored at 2-8 °C over 24 month shelf life however the data provided is only supportive data at this stage, results to 27 months are pending for the batch tested, therefore no conclusions on the storage of the vaccine frozen prior to storage at the recommended shelf life can be made. A pre-storage at -20 °C up to 12 months of the lyophilisate fraction is acceptable.

For freeze-drying excipient:

The results for three batches of freeze-drying excipient met the required specifications at the different time points to 8 months.

For the IgY solution:

The stability of the lyophilisate fraction using an IgY solution batch previously stored for 6 months at +2 – 8 ºC was studied to determine the maximum storage period for the IgY solution before being used for blending. The results at T0 met the required specifications, results at T27 are pending. The study validates the storage of the IgY solution to 6 months at 2 – 8 °C.

For solvent:

The stability results to 36 months for the solvent (PBS) are pending. However, as the presented results for a period of up to 24 months for 3 consecutive batches tested met the required specifications, and as this is a common salt, 36-month shelf life can be assigned.

For reconstituted product:

A vaccine batch was reconstituted in solvent, tested at immediately after reconstitution (T0) and at 2 hours after reconstitution (T2). The results met the required specifications and therefore, the reconstitution of the vaccine for use up to a maximum of 2 hours could be considered acceptable.

Overall conclusions on quality

The containers and closures meet the necessary Ph. Eur. standards and are appropriately sterilised.

The qualitative composition of the vaccine is satisfactorily described. The amount of virus is expressed as $10^{1.48}$ – $10^{2.63}$ PU / dose.

The use of a serotype 1 IBDV strain in the vaccine is satisfactorily explained and the virulence of the vaccine strain is satisfactorily resolved.

The development of Gumbohatch as an Icx vaccine is satisfactorily justified.

Acceptable flowcharts are provided for each stage of the production process. Overall the production of the viral harvest is satisfactorily described.

The GHS excipient is formulated by dissolving all of the component ingredients in WFI followed by filter sterilisation (0.2 µm).

The IBDV-specific IgY solution is extracted from eggs sourced from healthy flocks, previously immunised with IBDV vaccine; adequate details on the immunisation strategy are provided.

The production of the PBS solvent is satisfactorily described.

Acceptable certificates of analysis are provided for each of the starting materials listed in the pharmacopoeia. The suitability of the alternative tests to those specified in Ph. Eur. 5.2.2 for extraneous agent testing of the SPF flocks have been demonstrated.

The MSV and WSV of the IBDV viral strain 1052 are manufactured and handled in a seed lot system in line with Ph. Eur. chapter 0062. The MSV has been tested according to Ph. Eur. chapter 2.6.24 with validated NAT used for the detection of ALV, REV and CAV. The testing of the MSV and WSV is satisfactory and in line with Ph. Eur. chapters 0062 and 2.6.24.

Overall, the TSE risk for the materials used in the manufacture of Gumbohatch is considered as negligible. Appropriate certificates of analysis are provided for starting materials of non-biological origin. The information regarding the qualitative and quantitative composition and treatment of the media solutions is acceptable; details on the storage time for each solution are provided.

Appropriate control tests during the manufacturing process were described including validation for the production processes for live IBDV strain 1052, the freeze-drying excipient (GHS) and the IBD specific IgY solution. A method for the determination of the VNU of the IgY solution batches used to formulate Gumbohatch is provided.

An appropriate method for determination of the complete neutralisation of the inactivating agent was provided including an acceptable in-house validation. The in-process testing results of 3 consecutive batches of vaccine and solvent were presented in Part 2F for batch-to-batch consistency and met the required specifications. The results of the control tests were carried out on three batches of freeze-drying excipient and IgY solution and batches met the required in-process specifications. An upper limit for VNU/ml is provided.

Overall, appropriate descriptions and validations of the control tests on the finished product were provided. A validation and description of replacement of the virus control of the virus neutralisation test are provided. The sterility and mycoplasma testing are performed in line with the Ph. Eur. The finished product specification includes a test and justified limits for residual moisture.

Tests for extraneous agents using cell culture and embryonated eggs were adequately described and are in accordance with Ph. Eur. 2.6.25. Methods and acceptable validation for the tests for EDSV, Marek's Disease virus, TRT virus and CAV have been adequately described. The applicant has requested to waive the routine testing for extraneous agents given the data presented in line with requirements for Vaccines for Veterinary Use Ph. Eur. chapter 0062 however, considering this is a live vaccine, manufactured in SPF eggs, this cannot be accepted and the extraneous agents testing will be retained for routine testing of all batches as per Ph. Eur. 2.6.25. Control of the solvent is adequately described and testing is in line with Ph. Eur. where relevant.

The proposed potency test is a quantitation by RT-PCR titration of 'viable' IBDV virus in each batch of reconstituted vaccine. The applicant has described a link between the proposed potency test and a quantifiable live infectious vaccine. Overall the validation of the potency test is considered satisfactory.

The potency values for the consistency testing met the proposed specifications. The extraneous agents testing showed no contamination and omission of this testing is not considered acceptable. Overall, the consistency batches (x 3) met the required specifications and therefore the consistency of manufacture is considered acceptable.

Stability results for the active ingredient manufactured after storage of the bulk antigen for 4 or 8 days met the required specifications at T0. However, the process is now considered continuous and no further data is necessary. Based on the real time testing for the finished product (24 months thus far met the required specifications) Gumbohatch can be assigned a 21 month shelf life at 2-8 °C in glass vials, however data to 27 months is pending. Results for three batches of freeze-drying excipient are

validated for storage of the freeze drying excipient to 8 months. A study to determine the maximum storage period for the IgY solution before being used for blending validated the storage of the IgY solution to 6 months.

The stability results to 36 months for the solvent (PBS) are pending however, as initial results for 24 months for 3 consecutive batches tested met the required specifications, a 36 month shelf life could be assigned.

The results for a reconstituted vaccine batch tested immediately after reconstitution (T0) and at 2 hours after reconstitution (T2) met the required specifications, and therefore, the reconstitution of the vaccine for use up to a maximum of 2 hours could be considered acceptable.

Part 3 – Safety

Introduction and general requirements

Gumbohatch is a vaccine containing live attenuated IBDV, strain 1052, intended for the active immunisation of chickens to reduce clinical signs and lesions of the BF caused by very virulent avian infectious bursal disease virus infection. A single dose of vaccine is proposed to be administered by either the in ovo route to chicken embryos on the 18th day of incubation or by the subcutaneous route for 1-day-old broiler chickens. The proposed range of active substance included in each dose is $10^{1.48}$ – $10^{2.63}$ PU, with a dose volume of 0.05 ml for the in ovo route and 0.2 ml for the subcutaneous route. In Gumbohatch, the live attenuated IBDV is contained forming an immune-complex with IBD-specific immunoglobulins. The vaccine virus is an 'intermediate-plus' virulence IBDV vaccine strain.

A full safety file in accordance with Article 12(3) has been provided. The safety of the immunological veterinary medicinal product has been investigated in accordance with the requirements of Directive 2001/82/EC, as amended. In addition, Ph. Eur. chapter 5.2.6 'Evaluation of safety of veterinary vaccines and immunosera', and the specific requirements outlined in Ph. Eur. monograph 0587 'Avian Infectious Bursal Disease Vaccine (live)' have been taken into account in order to demonstrate the safety of the vaccine. While Ph. Eur. 0587 applies to vaccines containing strains of low virulence, but not to those containing strains of higher virulence, Gumbohatch is not within the scope of the monograph, however it is considered appropriate that the requirements of the monograph have been used as a basis for the evaluation of safety.

Safety documentation

Eight laboratory trials and three multi-centre field trials were carried out to assess the safety of Gumbohatch. The laboratory and the field studies were conducted according to Good Laboratory Practice (GLP) standards and Good Clinical Practice (GCP) guidelines, respectively.

Safety tests have been carried out for each recommended route and method of administration. The tests outlined in Ph. Eur. 0587 specify the evaluation of the safety of the vaccine using SPF chicks. Therefore, laboratory studies have been performed using SPF chicks; however, the safety in the intended category of target species (broiler chickens) was assessed in a laboratory study conducted to assess safety and immunosuppression. Most of the studies have been performed using both of the proposed routes of administration. However, in cases where the least safe route is required to be investigated or where studies were not mandatory (e.g. investigation of bursal damage caused by one dose), the in ovo route was chosen, considering that using this route, animals are inoculated at the youngest age.

The batches used in the safety trials were stated to have been manufactured in accordance with the manufacturing process described in Part 2.B of the dossier and at the same manufacturing facilities that will be used for future production batches. Two batches of vaccine have been used in the laboratory safety studies, and one batch used in the three field studies. The titre of the active substance used in the laboratory safety studies was $10^{2.80}$ PU/dose and $10^{2.90}$ PU/dose, which is above the maximum proposed range for the active substance ($10^{2.63}$ PU/dose). A standard batch was used for the combined safety and efficacy field studies ($10^{2.05}$ PU/dose). The requirement for the investigation of safety using vaccine virus at the least attenuated passage level that will be present in a batch of vaccine does not apply to this vaccine, as the passage level from the MSV for production is fixed.

It is noted that in addition to the titre of the vaccine virus, the amount of IgY bound to the vaccine virus (forming the 'immune complex' vaccine /'Icx' vaccine) is also an important concept for this vaccine. In order to ensure that the vaccine virus is completely neutralised by IgY in each batch of vaccine, the vaccine virus is complexed with IgY (assured by an excess of unbound IgY in each vial), with a proposed specification range for unbound IgY, corresponding to an upper limit which is lower than that demonstrated to be safe in the safety studies (SNUs per dose; higher due to administration of an overdose of vaccine). It is accepted that higher levels of unbound IgY would not have any adverse implications regarding efficacy, given that higher levels of unbound IgY would likely make a negligible contribution to the total MDA in the animal. Studies applicable to live vaccines were conducted to investigate the dissemination of a single dose of the vaccine strain, the spread from vaccinated animals to non-vaccinated contacts and reversion to virulence.

Study title	***Potency of batch used***
Evaluation of the safety of one dose of Gumbohatch live vaccine in SPF chickens regarding bursal damage (in ovo administration)	Potency $10^{4.78}$ PU/ml, SNU: 19.61 SNU/vial Dose (0.05 ml): $10^{2.80}$ PU/egg
Evaluation of safety of the administration of an overdose of Gumbohatch live vaccine in SPF chickens regarding clinical signs	Potency $10^{4.78}$ PU/ml, SNU: 19.61 SNU/vial Dose (0.2 ml): $10^{3.80}$ PU per egg or chick
Evaluation of damage to the BF after the administration of an overdose of Gumbohatch live vaccine in SPF chickens (in ovo administration)	Potency $10^{4.78}$ PU/ml, SNU: 19.61 SNU/vial Dose (0.2 ml): $10^{3.80}$ PU/egg
Evaluation of damage to the BF after the administration of an overdose of Gumbohatch live vaccine in SPF chickens (subcutaneous route)	Potency $10^{4.78}$ PU/ml, SNU: 19.61 SNU/vial Dose: (0.2 ml): $10^{3.80}$ PU/chick
Study of the immunosuppression of the Gumbohatch IBDV vaccine in broiler chickens	Potency $10^{4.32}$ PU/ml, SNU: 19.64 SNU/vial Dose (0.05 ml): $10^{2.90}$ PU/egg
Dissemination and spread of the Infectious Bursal Disease Virus Gumbohatch strain in vaccinated animals	MSV (bound to IBD-specific antibodies) Dose (0.1 ml): $\geq 10^{5.2}$ EID_{50}/ egg or chick) (1x max dose)
Study of the increase in virulence of Gumbohatch IBDV vaccine strain: Sequential passages of the vaccine virus through 5 groups of chickens via natural spreading	MSV (bound to IBD-specific antibodies) Dose (0.1 ml): $\geq 10^{5.2}$ EID_{50}/ egg) (1x max dose)
Study of the increase in or reversion to virulence of Gumbohatch vaccine: Comparison of the unpassaged virus strain to the virus strain obtained after being passaged through 5 groups of chicks	MSV (bound to IBD-specific antibodies) Dose (0.1 ml): $\geq 10^{5.2}$ EID_{50}/ egg) (1x max dose)
Evaluation of the safety and the efficacy under field conditions of Gumbohatch vaccine against Infectious Bursal Disease (IBD) administered in ovo	Potency: $10^{2.05}$ PU/dose, SNU: 19.32 SNU/vial
Evaluation of the safety and the efficacy under field conditions of Gumbohatch vaccine against Infectious Bursal Disease (IBD) administered in ovo	

Study title	Potency of batch used
Evaluation of the safety and the efficacy under field conditions of Gumbohatch vaccine against Infectious Bursal Disease (IBD) administered by subcutaneous route	

Laboratory tests

Eight GLP laboratory studies have been conducted for the assessment of safety of Gumbohatch. SPF, IBD-free chicks were used in the safety laboratory studies. Within the laboratory safety studies, vaccinated animals underwent individual clinical monitoring, with assessment of general or local reactions, clinical signs of Gumboro disease (depression, diarrhoea, ruffled or fluffed feathers, body condition, abnormal behaviour), euthanasia due to end-point criteria, mortality, lesions in the BF, body weight, BF weight, spleen weight, ratio of BF to body weight (BF:BW ratio) and ratio of spleen to body weight. Macroscopic lesions of the BF were categorised as absent (score 0), slight or subtle (score 1), moderate (score 2) or severe (score 3). The following lesions were monitored: loss of structure, necrosis, haemorrhages, oedema, exudate, petechiae, fibrin and congestion.

The degree of bursal damage following histological assessment of lesions was scored using the scale proposed in the Ph. Eur. 0587; for each individual bursal sample, one hundred follicles were randomly selected and examined.

Safety of the administration of one dose

The safety of the administration of a 1X in ovo dose was investigated in 18-day-old embryonated SPF chicken eggs without IBDV antibodies. In this study, 35 eggs were vaccinated by the in ovo route and 35 eggs were mock-vaccinated with PBS. Following hatching on day 0 of the study, birds were observed until day 35, with daily clinical observations and sequential necropsy of 6 chicks/group on a weekly basis for evaluation of bursal lesions (macroscopic and histological), body, bursa and spleen weight and serological response to vaccination.

Results showed that the degree of bursal damage induced by the vaccine was severe, with the maximum mean BF lesion score observed at 7 days of age, decreasing subsequently on days 14, 21, 28 and 35. Thus, while repopulation of the bursae by lymphocytes was evident, the mean BF lesion score was still notably high at 35 days of age according to the Ph. Eur. 0587 scale. Consistent with the lesions observed, atrophy of the bursa was evident as indicated by statistically significantly lower bursa weights in the vaccinated group and statistically significantly lower BF:BW ratios in the vaccinated group, on each of the days evaluated. However, no clinical signs, mortalities or differences between groups in body weight, spleen weight and spleen to body weight ratio were observed.

On the basis that severe bursal damage was observed following vaccination of 18-day-old embryonated eggs without MDAs, in addition to the fact that immunosuppression has been investigated only in MDA-positive birds (see: examination of immunological functions), a contraindication against use in flocks without MDA to IBDV is included in the product information. While similar findings for vaccine-induced bursal damage in the intended category of target species (seropositive flocks) were also raised as a concern, justification was provided based on detailed histopathological analyses of bursae from vaccinated non-challenged versus non-vaccinated control birds in the efficacy study, to support that the nature of vaccine-induced changes was different to that induced by very virulent IBD (vvIBDV) infection. In addition, while bursal lesions scores were high post-vaccination, there was no corresponding loss of bursal function as demonstrated by the

immunosuppression study, even though it is noted that lymphoid depletion was not as high in this study as observed in efficacy studies (see: 'examination of immunological functions').

Safety of one administration of an overdose

The safety of the administration of a 10X overdose by the in ovo route and by the subcutaneous route was investigated in seronegative 18-day-old embryonated SPF chicken eggs and 1-day-old SPF chickens, respectively. Ten eggs were vaccinated by the in ovo route, 10 chicks were vaccinated by the subcutaneous route and ten eggs were included in the control mock-vaccinated group. Following hatching on day 0, birds were observed daily for clinical signs until necropsy on day 14 for evaluation of bursal lesions (macroscopic only), body, bursa and spleen weight, injection site reactions in the subcutaneously vaccinated group, and serological response to vaccination.

The results showed that there were no mortalities, clinical signs, adverse effects on body weight, or local reactions following vaccination. Slight macroscopic alterations of the bursa (congestion and mild exudate) were observed in the vaccinated groups, in variable numbers of birds on each of the days, but were absent in the mock-vaccinated control group. Thus, the higher frequency of these findings in the test group suggests a test-article related effect and consequently, these lesions are described in the summary of product characteristics (SPC) section 4.10. While the degree of bursal damage was not evaluated in this study, there was a statistically significantly lower BF weight and BF:BW ratio in both of the two vaccinated groups at 14 days of age compared to the control group, suggesting that administration of a 10X overdose results in a significant degree of bursal damage in vaccinated animals. The safety of the administration of a 10X overdose by the in ovo route in 18-day-old embryonated SPF seronegative chicken eggs was investigated in another study, which included the histological evaluation of bursal damage. In this study, 35 eggs were vaccinated by the in ovo route and 35 eggs were mock-vaccinated with PBS. Following hatching on day 0 of the study, birds were observed until day 35, with daily clinical observations and sequential necropsy of 6 chicks/group on a weekly basis, for evaluation of bursal lesions (macroscopic and histological), body, bursa and spleen weight and serological response to vaccination.

The results demonstrated that there were no mortalities, abnormal clinical signs or significant differences in body weight compared to the mock-vaccinated group. Severe lesions in the BF were observed in the vaccinated group. Statistically significantly lower BF:BW ratios were observed in the test group compared to the control group on each of the days of necropsy. While it was noted that the maximum mean lesion score was marginally lower in this 10X overdose study compared to the 1X in ovo study, it was accepted that this was due to biological variability and that bursal damage was relatively similar following the administration of a single dose or a 10X overdose. Macroscopic alterations of the bursa were observed in the vaccinated group (exudate, congestion, petechiae, haemorrhage) and in the control group to a lesser extent (exudate and congestion). As noted for the previous study, the higher frequency of the macroscopic lesions in the test group suggest a test-article related effect, thus, these lesions are described in the SPC section 4.10.

The safety of the administration of a 10X overdose by the subcutaneous route in 1-day-old SPF seronegative chicks was investigated in a different study which included the histological evaluation of bursal damage. In this study, 35 1-day-old chicks were vaccinated by the in ovo route and 35 1-day-old chicks were mock-vaccinated with PBS on day 0. Birds were observed until day 35, with daily clinical observations and sequential necropsy of 6 chicks/group on a weekly basis, for evaluation of bursal lesions (macroscopic and histological), body, bursa and spleen weight and serological response to vaccination.

Results showed that there were no test-article related mortalities or clinical signs observed in study animals or significant differences in body weight between groups. Severe lesions in the BF were observed in the vaccinated group. While the mean BF lesion score of 2.83 at day 28 would indicate a slow repopulation of the bursae by lymphocytes, it is noted that at day 35, 3/6 birds had a BF lesion score of 4, indicating persisting severe lymphocyte depletion in these birds. As discussed, a contraindication against use in seronegative birds is included in the product information (see 'safety of the administration of one dose'). Consistent with previous studies, macroscopic alterations of the bursa were observed in the vaccinated group at a higher frequency than the control group (exudate, congestion).

Safety of the repeated administration of one dose

The safety of the repeated administration of one dose has not been investigated; this is acceptable considering that the vaccination schedule consists of the administration of a single dose only.

Examination of reproductive performance

No reproductive studies were provided for Gumbohatch, this is considered acceptable considering that there are no data to suggest that the starting material is a risk factor and that the vaccine is not recommended for use in animal categories intended for laying or reproduction. The applicant has included a suitable warning in section 4.7 of the SPC. ('Laying birds: The safety of the veterinary medicinal product has not been established during lay. Do not use in birds in lay or breeding birds, or within 4 weeks before the start of the laying period.')

Examination of immunological functions

The immunosuppressive effect of vaccination with Gumbohatch was evaluated in a study in accordance with the study design outlined in Ph. Eur. 0587, however, since it was expected that immunosuppression would occur in MDA-negative birds, the test was carried out in 18-day-old eggs from a commercial broiler flock with low levels of MDAs, considered to be representative of 'worst case scenario' conditions under field conditions. This approach was considered acceptable.

The study involved two parts; a sub-study to determine the time point of maximal bursal damage in vaccinated birds in order to determine the most appropriate time to investigate the potential for immunosuppression. The immunosuppression study consisted of vaccination of 18-day-old embryonated eggs with a 1X dose of Gumbohatch and the response to Newcastle Disease (ND) vaccination (strain Hitchner B1 (live), administered on day 18 of age, when maximum bursal damage was observed) was compared with a group vaccinated against ND vaccine only at 18 days of age. Following virulent challenge with NDV, strain Herts, at 21 days after ND vaccination, the protection in both groups was compared. An appropriate non-challenged control group was included.

Results demonstrated that there was no evidence of adverse effects on immunological function following the administration of a 1X dose in ovo (i.e., use of the product as recommended), as evaluated by the response to ND vaccination; 100% of animals in each vaccinated group were protected (no clinical signs or mortality), while 80% of control group animals died or were euthanised according to end-point criteria, with clinical signs compatible with NDV challenge in the remaining control group birds. Evaluation of the serological response to ND vaccination showed comparable haemagglutination inhibition titres in the Gumbohatch + ND vaccine group and the ND vaccine group.

It is noted that this study also provides useful information in terms of bursal damage following the administration of a 1X in ovo dose under field conditions, in flocks with low levels of MDA; in which it was observed that the maximum mean bursal lesion score following in ovo vaccination was 3.8 and occurred at 18 days of age after hatching under the conditions of this study and that the mean BF lesion score was 1.8 at day 35. Overall, the CVMP can accept that the vaccine does not cause immunosuppression in vaccinated birds (with MDA levels of mean ELISA titres of 2701 and 3962 as evaluated by the CIVTEST AVI IBD test and the IDEXX ELISA kits, respectively), despite the damage and lymphocyte depletion observed in the BF following virus replication. However, as immunosuppression in the presented study has been examined in animals with lower levels of lymphoid depletion than observed in efficacy studies after administration of a minimum dose of vaccine, it was questioned if the risk of immunosuppression under field conditions could be fully excluded. It was clarified that at time of maximum bursal damage in the immunosuppression study, at which time birds were administered Newcastle Disease vaccine, 4 of 5 birds had bursal lesion scores of 4. Considering that there were no indications of adverse effect of immune function when 80% of birds had bursal lesion scores, together with supportive histopathological data generated in the efficacy studies that vaccine-induced bursal damage was markedly distinct from that induced by wild type very virulent IBDV, it was accepted that the risk of immunosuppression associated with Gumbohatch vaccination could be excluded according to the requirements of Ph. Eur 0587.

Special requirements for live vaccines

Spread of the vaccine strain

Dissemination and spread of the vaccine strain in seronegative animals were evaluated in one study.

The spread of the vaccine strain from vaccinated to unvaccinated animals was investigated by placing 10 non-vaccinated chicks in contact with 35 in ovo vaccinated chicks on day of hatching, or by placing 10 non-vaccinated chicks with 35 subcutaneously vaccinated chicks on day of hatching (vaccination with master seed virus, at higher virus titre than would be expected under conditions of vaccination with a 1X maximum dose). The duration of contact was 28 days (with sequential removal of 5 birds from the vaccinated group at study time points to evaluate the dissemination of the vaccine virus, refer to following section). Clinical signs, macroscopic lesions in the BG, body weight, BF weight, spleen weight, BF:BW ratio and spleen to body weight ratio were evaluated in the in-contact group. No statistical analyses of these parameters were undertaken. IBDV antibodies were evaluated to monitor the serological response to spread of the vaccine strain.

Results demonstrated that the vaccine virus readily spreads to in-contact unvaccinated chicks following both routes of administration and resulted in seroconversion in both groups of in-contact animals. Similar outputs for mean body weight, mean BF:BW ratios and mean spleen to body weight ratios were observed in the vaccinated and non-vaccinated groups, and were stated to be within normal limits expected in vaccinated SPF birds. It is considered a shortcoming of the study that there was no histological evaluation of BF lesions, given that the findings from previous studies suggest that histological evidence of severe bursal damage may exist when macroscopic lesions of the bursa are present only at a relatively low incidence in both vaccinated and mock-vaccinated birds and consist mainly of congestion or slight exudate following the administration of a 1X dose. However, overall, the data presented in this study are considered sufficient to exclude a risk for in-contact animals. However, it is considered prudent to include a warning to limit the use of the product to sites where vvIBD has been isolated or is epidemiologically relevant.

The investigation of spread to non-target species has not been investigated, this is considered acceptable on the basis that while serotype 1 IBDV and antibodies against this serotype are found in avian species other than chickens (e.g. turkeys, ducks, guinea fowl and ostriches), it causes clinical disease solely in young chickens.

Dissemination in the vaccinated animal

Refer to previous section. Dissemination of the vaccine strain in vaccinated animals was also investigated.

Dissemination in the target species, after in ovo and subcutaneous vaccination (1X dose, MSV, bound to IBD-specific antibodies) of 18-day-old SPF eggs (n=35) and 1-day-old chicks (n=35), respectively, was investigated by necropsy of 5 animals/group at defined time points until day 28 of life. Spread of the vaccine strain in internal organs and in secretions was evaluated by testing for presence of vaccine virus by PCR.

The results demonstrated that the vaccine strain widely disseminates throughout the organs of vaccinated birds and is shed by vaccinated animals via oral and faecal routes for up to 21 days of life after in ovo and subcutaneous vaccination of seronegative animals, resulting in spread to in-contact animals as discussed in the previous section. However, in the absence of monitoring of viral presence in organs beyond day 28, it is unknown for how long virus is detectable in vaccinated animals. Furthermore, in view of the potential for spread a warning to limit the use of the product to sites where vvIBD has been isolated or is epidemiologically relevant was considered necessary.

The following warning was considered appropriate for inclusion in the SPC section 4.5:

'This product should only be used after it has been demonstrated that very virulent IBDV strains are epidemiologically relevant in the area of vaccination.

Vaccinated birds may excrete the vaccine strain up to 3 weeks following vaccine take. During this time, contact between the vaccinated chickens and any immunosuppressed or unvaccinated birds should be avoided. Appropriate veterinary and husbandry measures should be taken to avoid spread of the vaccine virus to susceptible wild and domestic birds.

It is recommended to vaccinate all chickens on a site at the same time.'

Reversion to virulence of attenuated vaccines

The reversion to virulence of the vaccine strain was investigated in two studies. The studies were based on the requirements of Ph. Eur. 5.2.6 and Ph. Eur. 0587.

In the first study, the reversion to virulence following passage through 5 groups of chickens by natural spreading after initial vaccination by the in ovo route (MSV, 1X maximum dose) (group A) was evaluated. SPF chicks of 1 – 21 days of age were placed in contact with the vaccinated chicks when it was expected that viral shedding would occur (group B). This step was repeated for groups C, D and E, with larger numbers included in group E consisting only of 1-day-old chicks (group E.1 used for recovery of virus, group E.2 used for evaluation of BF damage) to permit necropsy of 5 birds/group at intervals for 35 days after group E had been placed in-contact with group D. The safety of the 5 times passaged virus strain was tested by evaluating damage to the BF in group E.2, and the results of group E.2 were compared with results obtained in the safety of the administration of one dose study 1X in ovo dose in 18-day-old embryonated SPF chicken eggs without IBDV antibodies. Thus, the safety

of the material used for the 1st passage and the virus at the final passage was not directly compared in this study.

The results demonstrated that IBDV was detected in the BF pools of each passage. No clinical signs attributed to IBDV were observed in the last group of chicks infected by natural spreading. Mild macroscopic lesions in the BFs were observed in group E.2 (exudate, congestion) observed in a maximum of 3/5 birds at day 21 after infection. One bird at day 35 showed moderate presence of exudate in the BF (score 2). The mean bursal lesion score in group E.2 was 4.60, 4.20, 3.40, 3.40 and 2.40 on days 7, 14, 21, 28 and 35. However, it was raised as a concern that the results of the test for damage to the BF in the 5th passage of animals were compared with results from a separate study, safety of administration of 1X in ovo dose, rather than testing the material from the 1st passage within the same study. Therefore, given that this was not a like-for-like comparison; the applicant conducted an additional study, in which MSV+5 virus strain which was obtained at the end of the first study or MSV (at the same EID_{50} titre) was administered to 1 day-old chicks by the subcutaneous route on Day 0 of the study.

At day 0, five groups of 30 SPF seronegative birds were randomly formed. Two groups of 30 animals were inoculated with MSV, two groups with MSV+5, and one control group with PBS. Clinical signs were monitored daily. Damage to the BF (relative weights and histopathologic study) was studied at days 7, 14, 21, 28 and 35 of the study. Each group included 25 animals that were used to perform the necropsies and 5 extra animals.

The results showed that no clinical signs or mortality due to treatment administration were observed. No significant differences in mean body weight were observed between groups. There were no significant differences between the MSV+5 and the MSV group for the sum of macroscopic lesions of the BF, for the BF: BW ratio or for the spleen: BW ratio on days 7, 14, 21, 28 or 35. Histopathological analysis of bursas from each group indicated severe bursal damage at 7 days post-inoculation (BF histopathological score of 5 for all bursas of each group), which decreased to mean scores of 3.9 and 3.6 in the MSV and MSV+5 group at day 35, respectively. However, there were no significant differences between the MSV+5 and the MSV group for the mean histopathological score of BF at necropsy at days 7, 14, 21, 28 or 35.

While the severe lymphocyte depletion at 7 days after inoculation with MSV or MSV+5 observed in this study is noted, SPF seronegative 1 day-old chicks are a more sensitive category than the intended category of target species (MDA positive flocks) for Gumbohatch, and use of the vaccine is contraindicated in MDA negative flocks.

Overall, the absence of reversion to virulence is considered to have been satisfactorily demonstrated, in accordance with requirements.

Biological properties of the vaccine strain

No specific studies have been conducted to determine the intrinsic biological properties of the vaccine strain. The biological properties of the vaccine strain have been briefly described; in the studies conducted, the vaccine strain did not cause clinical signs or immunosuppression in the target species, while it is known to spread to non-vaccinated animals and causes damage to the BF, as expected, for an intermediate-plus strain.

Recombination or genomic reassortment of the strains

No specific trials regarding the genomic reassortment or recombination/redistribution of the vaccine strain with other different strains of IBDV have been performed. While the absence of such studies is considered acceptable, it is noted that the applicant has only poorly addressed the requirement to consider the potential for recombination or genomic reassortment of strains, with the provision of one (old) supporting literature reference. Preferably, a more detailed summary, supported by up-to-date literature references and peer-reviewed articles, should have been provided. However, since the vaccine strain 1052 is derived from the Winterfield 2512 strain, which is included in at least one other Gumboro vaccine authorised for use in the EU, the CVMP can accept that the potential for genomic reassortment and the emergence of reassorted strains will not be any greater than the risk presented by other vaccines currently available in the EU.

User safety

The applicant has presented a user safety risk assessment which has been conducted in accordance with CVMP guideline EMEA/CVMP/IWP/54533/2006.

The main potential routes of accidental contact with the product have been considered and it was concluded that the most likely exposure routes are those of accidental self-injection and dermal or ocular exposure arising from improper use or breakage of the containers. In addition, since the vaccine antigen is excreted from vaccinated animals, there is also a risk of exposure to the vaccine virus in persons assisting/attending the animals or the facilities. However, since IBDV is not pathogenic for humans, no specific hazard is identified for the user. The other components of the vaccine are claimed to be commonly used in other vaccines and do not pose a risk for the user.

The CVMP accepts that the components of the vaccine (active substance and the excipients) are not expected to pose any risk to the person handling the product or the person who is in contact with vaccinated animals. The other components of the product are the excipient and solvent, composed of IBDV-specific IgY, sucrose, glycine, L-histidine, potassium chloride, disodium phosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride and water for injections. However, it is noted that not all excipients included in the vaccine are common excipients as claimed by the applicant; IBDV-specific IgY is not considered to be a commonly used 'excipient', and the applicant has not discussed any potential risk to the user presented by this component. It can be accepted that there is no risk presented to the user arising from the inclusion of this component (IgY is a not a human class of immunoglobulins, so it is not expected that there is any risk of immunological reactions any greater than would be expected following accidental exposure to vaccine-related proteins). Further, it is acknowledged that other IBD vaccines are already authorised in the EU that constitute a similar immune-complex vaccine containing poultry antibodies against bursal disease.

The CVMP agrees that the main route of potential exposure is accidental self-injection, but also that exposure may also arise from exposure to vaccinated animals shedding virus and to premises in which vaccinated chickens are housed. However, the risk of exposure is considered negligible given that the vaccine virus strain included in Gumbohatch is not a zoonotic agent.

As there are no identified risks posed by the use of Gumbohatch for the end user, the proposed statement for inclusion in section 4.5 'In case adverse reactions developed following accidental self-injection, seek medical advice immediately and show the package leaflet or the label to the physician.' was considered appropriate with minor modification (i.e. 'In case of adverse reactions following accidental self-injection…'), and the advice to wash and disinfect hands and equipment after use was considered appropriate. In addition, it was considered appropriate to include the following advice:

'Wash and disinfect hands after handling vaccinated birds or their litter because the virus is excreted by vaccinated birds for up to 3 weeks.'

Overall, based on the above risk assessment, it can be accepted that the product will not present an unacceptable risk to the user when stored, handled and administered in accordance with the recommendations included in the SPC.

Study of residues

No studies on residues have been performed.

MRLs

The active substance being a principle of biological origin intended to produce active immunity is not within the scope of Regulation (EC) No 470/2009. All other components of the vaccine are either allowed substances with 'no MRL required' classification according to Table 1 of Regulation (EU) No. 37/2010, or are substances considered as not falling within the scope of Regulation (EC) No. 470/2009.

Consequently, it is considered that there is no need to perform residue studies for Gumbohatch and a withdrawal period of zero days is accepted.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

Other than the study to investigate the potential for immunosuppression referred to in 'Examination of immunological functions', which demonstrated no adverse effect of Gumbohatch vaccination on the response to ND vaccination, no specific studies to investigate the interactions of this product with other veterinary immunological products have been carried out. Animals included in the field trials were stated to have been vaccinated in accordance with the established vaccination programs on each farm.

The applicant proposes to include a statement in Section 4.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis.' The CVMP considers that in the absence of any additional studies to investigate potential interactions with other veterinary medicinal products, the conventional text to reflect the absence of such studies is acceptable. The main concern is the potential for vaccine-related immunosuppression, however this has been satisfactorily investigated under laboratory conditions and it was demonstrated that the use of Gumbohatch does not lead to immunosuppression, as evaluated by the response to Newcastle Disease vaccination.

Field studies

Three multicentre, randomised, blinded, positively-controlled clinical field trials, conducted in accordance with GCP guidelines, were carried out to assess both the safety and efficacy of Gumbohatch under field conditions. In the field studies, animals received either the test product or a positive control (an authorised vaccine in many EU member states, containing live attenuated IBD

virus, strain Winterfield, together with bursal disease antibody). The vaccine virus strain in that vaccine is classified as an 'intermediate-plus' strain.

The in ovo route of vaccination was investigated in an EU trial conducted at one member state) and a trial conducted outside the EU. In the EU trial, while the production phase was conducted at 3 different sites, all animals were supplied by the same hatchery. The applicant justified that the husbandry practices at the hatchery and the broiler production at the 3 sites included in the EU trial are representative of widespread EU production practices. In addition, the relevance of the trial conducted outside the EU was justified with respect to EU practices, given that the system used for in ovo vaccination at hatcheries is claimed to be identical to that used in Europe. Other than the longer mean broiler life span (approximately 40 days), bird genetics, management and housing conditions are claimed to be comparable to those used in standard broiler production systems in Europe. The subcutaneous route of vaccination was investigated in an EU trial conducted at two EU member states stated to be representative of EU production practices.

In all three field studies presented, hens had been vaccinated against Gumboro disease. Although it is not stated when eggs used in the studies had been laid (e.g. at the start or the end of the laying period), MDA titres in study animals (evaluated in both cases using the applicant's own commercial ELISA kit) appeared to be high overall, with mean titres of 5,000 or higher in all but one site. However, it was also noted that there was variability in the MDA titres on each field study site at the start of the study, and it was accepted that safety has been investigated under sufficiently diverse levels of MDAs (i.e. in farms with different mean MDA levels, from low to high).

The results of the field studies demonstrated the absence of adverse events related to test or control article administration, and supported that there were no relevant differences between the test and positive control groups for hatching rates and body weight after hatching (where applicable), mortality rates and European Production Efficiency Factor (EPEF). Macroscopic evaluation of the bursa and calculation of BF:BW ratio, assessed in 15 animals per group on days 21, 28, 35 and before slaughter, demonstrated similar BF:BW ratios in study groups.

Overall, the data presented were considered to support the safety of in ovo and subcutaneous vaccination under field conditions of use.

Environmental risk assessment

An environmental risk assessment, conducted in accordance with the CVMP Note for Guidance EMEA/CVMP/074/95, was provided. The likelihood of the active ingredient to cause hazards to the environment can be considered negligible. It is accepted that the risk for the environment when using the vaccine Gumbohatch can be considered as effectively nil. As the use of Gumbohatch does not pose an environmental risk, no specific control measures are needed apart from the general management and disinfection recommendations of the poultry farms and the precautions included in the package leaflet concerning the handling and disposal of unused veterinary medicinal product or waste materials derived from the use of such product.

The CVMP accepts that the standard disposal statements proposed for inclusion in the SPC and package leaflet are appropriate. Furthermore, it is noted that precautionary statements are included in the SPC which highlight the ability of the vaccine to spread to unvaccinated chickens, that virus is detectable in the environment for up to 3 weeks and to recommend that appropriate veterinary and husbandry measures should be taken to avoid cross contamination of flocks.

Overall conclusions on the safety documentation

The applicant has provided eight laboratory studies and three combined safety and efficacy field studies in support of the safety of Gumbohatch, conducted in 18-day-old embryonated SPF eggs or in 1-day-old chicks seronegative for IBDV antibodies, vaccinated in ovo or subcutaneously, respectively, and in chicks/eggs from hens at the end of the laying period with MDAs.

Overall, following the administration of a 1X or a 10X dose by the in ovo route or following a 10X dose by the subcutaneous route in seronegative animals, severe bursal damage occurred, with maximum mean lesion scores of 4.67 and 4.17 following in ovo and subcutaneous use respectively. Generally, by 35 days of age, the mean lesion scores decreased, indicative of repopulation of the bursae by lymphocytes, however still at this stage many of the vaccinated birds had bursal lesion scores of 4. Given the severe bursal damage observed following vaccination by the in ovo route in flocks without MDA, and, since the degree of bursal damage following the administration of a 1X dose by the subcutaneous route is unknown, it was concluded that the safety of administration of the vaccine to flocks without MDAs has not been adequately supported. In addition, the degree of immunosuppression which may occur in seronegative birds is unknown, therefore it was considered necessary to contraindicate use of the vaccine in flocks without MDA against IBDV.

Whilst seronegative, SPF, 18-day-old embryonated eggs are accepted as the most sensitive category of target species, it was raised as a concern that severe bursal damage was also observed following both in ovo use and subcutaneous use in flocks with MDA following the administration of a single dose of vaccine at minimum titre in the laboratory efficacy studies (e.g. maximum mean lesion scores of 4.0 and 4.2 following in ovo and subcutaneous use, respectively in one study and maximum mean lesion scores 4.6 following both in ovo and subcutaneous use in another study). The applicant presented detailed supplementary histopathological analyses of bursae from chickens included in the efficacy studies, in which it was demonstrated that the bursal damage induced by the vaccine strain is notably distinct from the bursal damage induced by vvIBDV pathogenic field strains. It was highlighted that at the same time as vaccine-induced lymphoid depletion is most severe, functional immunoreaction of the bursa is maintained, as demonstrated in the study to investigate the potential for immunosuppression. The vaccine-induced lymphoid depletion is not associated with other characteristic macro- and microscopic changes associated with severe acute stages of vvIBDV infection, such as clear signs of oedema and lymphoid necrosis. Therefore, it was concluded that the safety of the vaccine for the target species (i.e. eggs or 1-day-old chicks from commercial broiler flocks with MDA against IBDV) was adequately supported.

The degree of bursal damage observed in the studies following the administration of a single dose is included in the SPC section 4.6. In addition, regarding the macroscopic lesions observed in the vaccine groups throughout the overdose studies, given that a test-article related effect cannot be excluded, the macroscopic lesions observed are described in the SPC section 4.10. Consistent with bursal damage, a lower BF:BW ratio was observed in vaccinated birds compared to their control counterparts.

The safety of the repeated administration of one dose has not been investigated; this is acceptable considering that the vaccination schedule consists of the administration of a single dose only.

No studies on reproductive performance have been presented; on the basis that the vaccine is indicated for use in broiler chicks. This is considered acceptable.

The product is expected to adversely affect the immune response of MDA-negative target animals, and therefore a suitable test on the immunological function in target animals with MDA (intended target species) was carried out, and the results demonstrated that vaccination did not result in immunosuppression when examined in accordance with requirements. As this is a live vaccine, the applicant also conducted studies to establish the potential for spread and dissemination of the vaccine

strain, which demonstrated that the vaccine virus disseminates widely throughout the vaccinated bird and is present in secretions for up to 21 days following vaccine take. In addition, spread to in-contact non-vaccinated birds was demonstrated to rapidly occur, however there were no clinical signs observed in the in-contact birds.

Reversion to virulence was investigated in accordance with requirements. It was concluded that the data provided supported the absence of the potential for reversion to virulence.

The biological properties of the vaccine strain were adequately described. Gumbohatch contains a vaccine strain classified as an 'intermediate-plus' virulence strain. A warning is included in the product information to use the vaccine only after it has been demonstrated that very virulent IBDV strains are epidemiologically relevant in the area of vaccination.

A user safety assessment conducted in line with the relevant guidance document has been presented. Based on the assessment presented, the product does not pose an unacceptable risk to the user when used in accordance with the SPC. Appropriate warnings for the user have been included in the product literature and are considered acceptable.

All components of the product are either allowed substances with 'no MRL required' classification according to Table 1 of Regulation (EU) No. 37/2010, or are substances considered as not falling within the scope of Regulation (EC) No. 470/2009. Consequently, a withdrawal period of zero days is established.

An appropriate environmental risk assessment was provided. It was concluded that Gumbohatch is not expected to pose a risk for the environment when used in accordance with recommendations.

Part 4 – Efficacy

Introduction and general requirements

The vaccine is intended to reduce clinical signs and lesions of the BF caused by avian infectious bursal disease virus infection, when administered to chickens with MDA against IBDV, by the in ovo route to 18-day-old embryonated eggs, or by the subcutaneous route to 1-day-old chicks. The onset of immunity depends on the initial MDA level of the broiler chickens/chicken eggs and in practice occurs from approximately 24 days of age. The duration of immunity is claimed as 43 days of age. The vaccine contains live attenuated IBDV, strain 1052, forming an immune-complex (Icx) with IBD-specific immunoglobulins. The vaccine virus is an 'intermediate-plus' virulence vaccine strain.

Efficacy was demonstrated in compliance with the European Directive 2001/82/EC (as amended by 2004/28/EC and Directive 2009/9/EC), and the European Pharmacopoeia (Ph. Eur.) chapter 5.2.7. In addition, the specific monograph Ph. Eur. 0587 'Avian Infectious Bursal Disease vaccine (live)' has been used as a guide, even though Gumbohatch is not within the scope of this monograph which applies to vaccine strains of low virulence.

The hypothesis of the immunological mechanism of action of the IBDV-immune complex vaccine is claimed to be related to its specific cellular interaction with follicular dendritic cells in spleen and bursa (Jeurissen et al, 1998), although the exact mechanism of action is not fully known. However, as stated by the applicant '*as long as MDAs concentration are high, the antigen-antibody complexes are prevented from reaching the bursa. When MDAs have reached a low level, vaccine virus can reach the bursa and replicate.*' It is accepted by CVMP that the use of such vaccines for immunisation against Gumboro disease in MDA-positive broiler chickens is an accepted practice and that there are at least two other 'immune complex' IBDV vaccines authorised within the EU by decentralised procedures.

Thus, it is not considered that there is a need to question the immunological mechanism of action of Gumbohatch, provided that the efficacy claims can be supported. It is also noted that according to a review by Muller et al, 2012, it is stated that 'at challenge, the experimental efficacy of the Icx vaccines was identical to or better than that induced by vaccination with live IBDV vaccines' and that, referring to Jeurissen et al, 1998, 'most remarkable was the low level of depletion of bursal and splenic B lymphocytes in chickens vaccinated experimentally with IBDV-Icx.' However, as described in Part 3, the level of depletion of bursal B lymphocytes was severe following vaccination with Gumbohatch.

Challenge model

The challenge strain used was a vvIBDV strain, VG-248, heterologous to the vaccine strain, and was isolated in Girona, Spain, by the applicant. This strain was digested by the TaqI, SspI and StyI enzymes, and the sequences obtained place it closely related to other European very virulent strains, such as UK661, CS89, 74-89A, Brown and Tula 94 (Majó, N., 2002). The oculo-nasal route was used for challenge as recommended in Ph. Eur. 0587.

The challenge model resulted in severe manifestations of disease in seronegative SPF chicks (mortality and clinical signs). In seropositive animals, the primary efficacy parameter was related to differences between groups for clinical signs and the damage to the BF given that mortality was not manifested in commercial seropositive broiler chicks. The applicant justified that, while the challenge was less severe in broiler chicks, effects of the infection at BF level are evident. During the acute phase of infection, non-protected chickens inoculated with vvIBDV show swollen bursae, a thin layer of gelatinous bursal oedema covering the serous surface and splenomegaly (Aricibasi et al., 2010, Ingrao et al., 2013).

Efficacy parameters and tests

The efficacy parameters as provided in Ph. Eur. 0587 were used as a basis for the investigation of efficacy; histological examination for lesions of the BF and scoring of the degree of bursal damage in accordance with the scale specified in Ph. Eur. 0587 2-4-2. In addition, clinical signs of Gumboro disease, examination of the bursae for the presence of external oedema and for characteristic macroscopic lesions, measurement of body weight, bursa weight, spleen weight and calculation of BF:BW ratio and spleen: BW ratio were investigated in the efficacy studies. Refer to Part 3 for the description of the scoring systems for clinical signs and macroscopic lesions. In addition, the presence of vaccine virus in BF (to confirm vaccine virus replication, or vaccine virus 'take') was evaluated by PCR, and the level of antibodies against IBDV was evaluated by ELISA (using the applicant's commercially available CIVTEST AVI IBD ELISA kit, in addition to the IDEXX ELISA, another commercially available kit, in two of the studies). The parameters chosen are considered appropriate for evaluating the efficacy of a live IBDV vaccine.

Efficacy documentation

Eight studies were conducted to investigate the efficacy of the product and included 5 laboratory studies and 3 combined safety and efficacy field trials.

Study title	Dose administered
Study of the efficacy of the vaccine Gumbohatch (live vaccine against Infectious Bursal Disease) in SPF chicks	1x $10^{4.48}$ PU/ml, adjusted to a titre of $10^{1.48}$ PU/dose (0.05 ml for in ovo dose, 0.2 ml for subcutaneous dose
Study of the onset and duration of protection of the vaccine Gumbohatch (live vaccine against Infectious Bursal Disease) in broiler chicken with low maternally-derived antibodies.	

Study title	Dose administered
Study of the efficacy of the vaccine Gumbohatch (live vaccine against Infectious Bursal Disease) in broiler chicken with high maternally derived antibodies	
Study of the efficacy of Gumbohatch vaccine in broiler chicken (OOI at 24 days of age after in ovo vaccination, higher challenge dose)	$10^{1.48}$ PU/dose
Study of the efficacy of Gumbohatch vaccine in broiler chicken (OO1 at 28 days of age, DOI at 43 days of age, after in ovo or subcutaneous vaccination, higher challenge dose)	$10^{1.48}$ PU/dose
Evaluation of the safety and the efficacy under field conditions of Gumbohatch vaccine against Infectious Bursal Disease (IBD) administered in ovo	Potency: $10^{2.05}$ PU/dose, SNU: 19.32 SNU/vial
Evaluation of the safety and the efficacy under field conditions of Gumbohatch vaccine against Infectious Bursal Disease (IBD) administered in ovo	
Evaluation of the safety and the efficacy under field conditions of Gumbohatch vaccine against Infectious Bursal Disease (IBD) administered by subcutaneous route	

Laboratory trials

Laboratory studies were well documented and carried out in target animals of the minimum age recommended for vaccination, using batches manufactured according to the method proposed in Part 2 of the file. The dose of Gumbohatch administered in the efficacy laboratory trials was the amount to be recommended for use and was stated to contain the minimum titre to be included in one dose of vaccine, $10^{1.48}$ PU/dose.

Dose determination

The proposed dose of $10^{1.48}$– $10^{2.80}$ PU per dose (0.05 ml for in ovo dose or 0.2 ml for subcutaneous dose) was proposed in the absence of a specific dose determination study. This is acceptable considering that efficacy of the vaccine was stated to have been investigated at the proposed minimum dose, $10^{1.48}$ PU/dose.

Onset of immunity (OOI)

Five studies were carried out in the target species of minimum age to investigate the onset of protection, by each of the recommended administration routes. The first study was conducted in SPF, seronegative animals, while four studies were carried out in seropositive animals, and are discussed under the section 'Maternally derived antibodies (MDA)'.

The OOI at 14 days of age following in ovo vaccination and subcutaneous vaccination was investigated in one study in 18-day-old embryonated SPF seronegative chicken eggs and 1-day-old SPF seronegative chicks. Four groups of 10 animals were used, two vaccinated (either in ovo, group A, or subcutaneous, group B), one control mock-vaccinated group (group C), and one mock-vaccinated non-challenged sentinel group (group D). At 14 days of age, groups A, B and C were challenged with a vvIBDV strain. Chicks were observed daily for 11 days after challenge, with monitoring of clinical signs, mortality, serological response and body weight (BW) of animals. BFs and spleens were weighed and examined macroscopically at the end of the study at necropsy. The primary variable of this study was

the proportion of clinically affected animals (mortality and clinical signs). The secondary variables included relative weight of the BF and growth rate of animals.

Results: After challenge, 0/10 birds in group A and 0/10 birds in group B were affected by IBDV; no clinical signs or mortality were observed in birds of groups A or B, while in group C, 10/10 birds were affected by IBDV; 5/10 birds died on day 3 or 4 post-challenge, and the remaining 5/10 birds displayed clinical signs compatible with Gumboro disease (depression, ruffled feathers in all 5 birds). Only one of the affected animals in group C recovered before the end of the observation period. The differences between the study groups for the proportion of animals affected by IBDV and for the proportion of mortality were statistically significantly different ($p<0.001$, Chi-square test).

All animals in group C showed macroscopic lesions of bursae with scores that ranged from slight to severe. These lesions included loss of structure, necrosis, haemorrhages, oedema, exudate, petechiae, fibrin, congestion and external oedema. In the vaccinated groups, mild exudate (considered to be due to vaccine-virus replication) was observed in 2/10 and 1/10 animals, in groups A and B, respectively. There were no statistically significant differences (SSDs) between the challenged groups A, B and C for BF:BW ratio. A statistically significantly higher spleen: BW ratio, indicative of splenomegaly in the control group, was observed in group C compared to the remaining study groups ($p<0.001$, ANOVA), with no differences observed between the remaining groups. A statistically significantly lower growth rate was observed in the control group C compared to group A ($p<0.05$) and group B ($p<0.05$) between day 14 and 25 of the study.

Overall, it is concluded that this study supports the claim for a reduction in mortality, clinical signs and loss of weight caused by IBDV, in the target species without MDA against IBDV. A claim for a reduction of lesions of BF is not supported by the data presented; only macroscopic evaluation of the bursae was conducted (BF samples were collected and stored but not analysed histologically, since the primary objective of the study was to evaluate mortality and clinical signs). The comparison of the BF:BW ratios in this study did not reveal any differences between the vaccinated groups and the control group C, this is not entirely unexpected given that it is known that vaccine virus replication leads to a lower BF:BW ratio, this parameter therefore is not considered informative for evaluating challenge-related effects on BF:BW ratios.

Nevertheless, results of this study are not regarded applicable to substantiate any efficacy claims, given that in accordance with the working mechanism of the immune complex vaccine, Gumbohatch is intended to be used in the presence of MDA, with vaccine virus take occurring when MDA decline to a level that allows the vaccine virus to replicate in the BF. Therefore, results are considered as informative only (as probable worst case scenario for challenge/validation of challenge). Accordingly, onset of immunity cannot be stated based on this study.

Duration of immunity

The duration of immunity was investigated in the target species with presence of MDA; refer to following section 'Maternally derived antibodies (MDA)'

Maternally derived antibodies (MDA)

The immunity at 21 days of age, 28 days of age and 63 days of age, in support of the onset and duration of immunity, was investigated in one study conducted in seropositive ('low' MDAs) commercial broiler eggs/chicks, following in ovo vaccination of 18-day-old embryonated eggs, and following subcutaneous vaccination of 1-day-old chicks, in accordance with recommendations with a minimum titre dose of vaccine. Three groups of animals were used, two vaccinated (either in ovo, group A, or

subcutaneous, group B) and one control group (mock-vaccinated). Additional birds were included in each group that were not challenged in order to evaluate changes in the BF to confirm vaccine virus take and to follow longer-term changes in birds that were vaccinated but not challenged.

Birds included in the study were obtained from IBDV-vaccinated hens at the end of the laying period in order to include eggs/chicks with low levels of MDAs. Mean MDA titres in batch control birds were 4468 ELISA units (EU) with the CIVTEST and 4022 EU with the IDEXX ELISA test. The applicant was requested to justify that these levels are representative of 'low' MDA levels. In their response, the applicant acknowledged that it cannot be considered as a clear representation of low levels of MDAs in the EU commercial farms, but is representative of possible MDA levels found in farms and illustrates the heterogeneity of MDAs, while the mean level of MDA was 4468 EU, the range was 1555 to 6151 EU. Nevertheless, it is argued that since vaccine virus replication in each individual chick is dependent on the MDA level of the particular animal (i.e. in accordance with the principle of immune-complex vaccines), there is no need to demonstrate efficacy with different MDA levels, however it was considered appropriate to perform trials at different MDA conditions in order to better support the working mechanism of the vaccine. Vaccine virus replication occurred marginally earlier in the in ovo vaccinated birds, with vaccine virus present in the BF in 80% of group A birds and 20% of group B birds on day 19, with all birds positive for vaccine virus in the BF by day 28. The profiles for seroconversion and vaccine-induced bursal damage were also consistent with the detection of virus; on day 21, 32% and 28% of animals were seropositive in group A and B, respectively, with percentages increasing in each group until 100% seroconversion was reported at day 34 in each group. The maximum bursal damage was observed on day 28 in both groups.

At 21, 28 and 63 days of age, 10, 15 and 10 birds, respectively, in each group were challenged with a vvIBDV strain (groups A2, B2 and C2). During the 6 day follow-up period after each challenge, clinical signs, mortality, serological response and weights of animals were monitored. At necropsy, 6 days after each challenge, body, bursa and spleen weights were recorded. BF lesions were evaluated at necropsy and the degree of histopathological damage scored according to Ph. Eur., in addition to macroscopic lesions and presence of external oedema. The presence of IBDV (vaccine virus strain) in BFs was evaluated by PCR. The primary efficacy variable of this study was the histopathological score for bursal damage.

Results: No clinical signs or mortalities were observed following challenge of commercial broiler chicks with MDAs (as expected). For each of the challenges, it is clear that a valid challenge was established in the control group, as evidenced by mean BF lesion scores in the control group, and a higher spleen:BW ratio in the control challenged birds.

Histological evaluation of bursal damage demonstrated that after the day 21 challenge, the mean BF lesion scores in groups A2, B2 and C2 were 4.2, 3.8 and 4.6, respectively. After the day 28 challenge, the mean BF lesion scores in groups A2, B2 and C2 were 3.7, 3.8 and 4.9, respectively. After the day 63 challenge, the mean BF lesion scores in groups A2, B2 and C2 were 2.2, 1.9 and 5.0, respectively. In the response to questions raised, a re-analysis of histopathological data of this study was presented in which the appropriate statistical comparisons were conducted (which had not been conducted in the original study report); i.e. comparisons of the in ovo vaccinated challenged group versus the mock-vaccinated challenged group, and comparisons of the s.c. vaccinated challenged group versus the mock-vaccinated challenged group. The rapporteur notes that these analyses have been conducted post-hoc as they were not pre-specified in the study protocol and therefore the results must be approached with caution. That said, the rapporteur is of the opinion that these analyses are more relevant for determining efficacy of the product given that more appropriate comparisons are made. These analyses demonstrate the following:

- A statistically significant difference in the histopathological mean bursal lesion score was not demonstrated 6 days after challenge at day 21 of life in the group vaccinated by the in ovo route (p=0.083), however a statistically significant difference was demonstrated 6 days after challenge at day 21 of life in the group vaccinated by the s.c. route at 1 day of age (p=0.049).
- However, a statistically significant difference in the histopathological mean bursal lesion score was demonstrated 6 days after challenge at day 28 of life in the group vaccinated by the in ovo route (p<0.001) and by the s.c. route (p<0.001).
- A statistically significant difference in the histopathological mean bursal lesion score was demonstrated 6 days after challenge at day 63 of life in the group vaccinated by the in ovo route (p<0.001) and by the s.c. route (p<0.001).

In addition, the applicant has conducted statistical analyses of the mean scores for macroscopic external oedema between vaccinated and control groups, and for macroscopic lesions between vaccinated and control groups at 6 days after challenge at day 21, day 28 and day 63 of life. At all time points, statistically significant differences between the respective vaccine group and the control group were demonstrated. The applicant provided extensive histopathological analyses to support that there was a notable distinction between vaccine-induced bursal changes compared to changes induced by very virulent IBDV challenge. Even if no studies were presented to clearly support the clinical relevance of the found differences, it can be assumed based on bibliographic references.

Overall, the reduction in lesions of BF is considered to have been adequately supported by the data presented.

The results demonstrate a reduction in loss of weight caused by IBDV after the day 28 challenge only (the mean growth rate from day 27 to 34 was statistically significantly higher both in the in ovo vaccinated group (p<0.05) and in the subcutaneously vaccinated group (p<0.05) compared to the control group**.** There was no positive effect of vaccination observed at day 21 or at day 63 on the reduction in loss of weight caused by IBDV.

The immunity at 28 days and at 35 days of age was investigated in a second study conducted in seropositive ('high' MDAs) commercial broiler eggs/chicks, following in ovo vaccination of 18-day-old embryonated eggs, and following subcutaneous vaccination of 1-day-old chicks, in accordance with recommendations with a minimum titre dose of vaccine. Three groups of animals were used, two vaccinated (either in ovo, group A, or subcutaneous, group B) and one control group (mock-vaccinated). As for the previous study, additional birds were included in each group that were not challenged in order to evaluate changes in the BF.

Birds included in the study were obtained from IBDV-vaccinated hens at the start of the laying period in order to achieve a situation in which high levels of MDAs would be present in vaccinated eggs/chicks. Mean MDA titres in batch control birds were 5447 EU with the CIVTEST and 4803 EU with the IDEXX ELISA test. Similar to the 'low' MDA study, it was questioned if these titres are representative of high MDA levels amongst EU production systems. It was noted that while the range of titres demonstrated a high degree of heterogeneity (mean; 5447 EU, range; 2840 – 8454 EU), the titres were not as high as had been anticipated but are still representative of MDA levels that may be encountered under field conditions.

It is unclear precisely when vaccine virus take occurred in the vaccinated groups since evaluation of efficacy parameters commenced on day 26; however, 100% of vaccinated birds in both groups were positive for the presence of the vaccine virus in the BF on day 26. In addition, it is noted that the bursal damage was severe in the in ovo (non-challenged) group on day 26, whereas the bursal

damage in the subcutaneously vaccinated (non-challenged) group on day 26 was 2.4, but then increased to 3.8 and 4.6 on days 28 and 34, respectively.

At 28 and 35 days of age, 10 birds in each group were challenged with a vvIBDV strain (groups A2, B2 and C2). The efficacy parameters evaluated during the 6 day follow-up period after each challenge was the same as for the previous study.

Results: No clinical signs or mortalities were observed following challenge of commercial broiler chicks with 'high' levels of MDAs (as expected). However, it is clear that a valid challenge was established in the control group at each time point, as evidenced by BF lesion scores in the control group. In addition, a higher spleen:BW ratio was also observed in the challenged control birds compared to the non-challenged control birds. However, the BF:BW ratio was not different in control challenged versus non-challenged birds, thus as highlighted for the previous study, it is considered that this parameter is unsuitable for evaluation of protection against challenge.

Histological evaluation of bursal damage demonstrated that after the day 28 challenge, the mean BF lesion scores in groups A2, B2 and C2 were 3.9, 2.9 and 4.4, respectively. After the day 35 challenge, the mean BF lesion scores in groups A2, B2 and C2 were 3.3, 3.3 and 4.9, respectively. In response to questions raised, the applicant presented a re-analysis of histopathological data of this study, in which the appropriate statistical comparisons were conducted (which had not been conducted in the original study report); i.e. comparisons of the in ovo vaccinated challenged group versus the mock-vaccinated challenged group, and comparisons of the s.c. vaccinated challenged group versus the mock-vaccinated challenged group. As commented for the previous study, while it is noted that these analyses were conducted post-hoc, it can be considered that these analyses are more relevant for determining efficacy of the product given that more appropriate comparisons are made. These analyses demonstrate the following:

- A statistically significant difference in the histopathological mean bursal lesion score was not demonstrated 6 days after challenge at day 28 of life in the group vaccinated by the in ovo route compared to the control group (p=0.252), however a statistically significant difference was demonstrated 6 days after challenge at day 28 of life in the group vaccinated by the s.c. route at 1 day of age compared to the control group (p=0.007).
- However, a statistically significant difference between groups for the presence of macroscopic external oedema was demonstrated 6 days after challenge at day 28 of life in the group vaccinated by the in ovo route compared to the control group (p=<0.001), and a statistically significant difference was demonstrated 6 days after challenge at day 28 of life in the group vaccinated by the s.c. route at 1 day of age compared to the control group (p=<0.001).
- In addition, a statistically significant difference between groups for the presence of macroscopic lesions was demonstrated 6 days after challenge at day 28 of life in the group vaccinated by the in ovo route compared to the control group (p=<0.001), and a statistically significant difference was demonstrated 6 days after challenge at day 28 of life in the group vaccinated by the s.c. route at 1 day of age compared to the control group (p=<0.001).
- Statistically significant differences between the in ovo vaccinated group (p<0.001) and in the s.c. vaccinated group (p<0.001) compared to the mock-vaccinated control group in the histopathological mean bursal lesion score was demonstrated 6 days after challenge at day 35 of life, in addition to statistically significant differences in each group compared to the control group for both macroscopic external oedema and for macroscopic lesions.

Overall, it is concluded that a reduction in lesions of BF is adequately supported by the data presented.

The data provided in this study do not support a reduction in loss of weight caused by IBDV, as there were no statistically significant differences between groups for the mean growth rate between after the challenge conducted at day 28 or at day 35.

In another study, efficacy was investigated after challenge with the same vvIBDV challenge strain but with a higher challenge dose than used in previous studies. The OOI at 24 days of age following in ovo vaccination was investigated in 18-day-old embryonated seropositive chicken eggs. The average MDA level was 4471 ELISA units at hatch (eggs from breeders at the end of the laying period; low MDA expected). Two groups of 10 animals were used, one vaccinated in ovo (group A) and one control mock-vaccinated group (group B). The vaccine was administered at minimum titre ($10^{1.48}$ PU). At 24 days of age, groups A and B were challenged with a vvIBDV strain, VG-248, $10^{5.0}$ EID_{50}/chick. Chicks were observed daily for 6 days after challenge, with monitoring of clinical signs, mortality, serological response and weight of animals. At 6 days post-challenge, when the acute phase of the infection was expected, animals were necropsied. BFs and spleens were weighed and examined macroscopically. Bursae were examined macroscopically for presence of external oedema, followed by histopathological analysis. The primary variables of this study were the histopathological scores for bursal damage and clinical signs after challenge. Secondary variables were macroscopic lesions on the BF, presence of external oedema in the bursae, the relative weight of the BF, spleen and growth rate of animals.

Results: After challenge, no clinical signs or mortality were observed in group A. No mortality occurred in group B, however 4/10 birds displayed mild clinical signs compatible with Gumboro disease. The relevance of the mild clinical signs in group B was demonstrated by a corresponding statistically significant difference in growth rate between day 23 to day 30 of the study. The differences between the study groups for the proportion of clinical signs were statistically significantly different (p=0.030, P-value of the Mantel-Haenszel test comparing Kaplan-Meier estimates of survival). External oedema of the bursa was observed in 9/10 bursae of group B and in 0/10 bursae of group A (p<0.001, Fisher exact test).

There was a statistically significant difference observed between groups for the sum of macroscopic BF lesions at day 30 (p<0.001, Mann-Whitney test).

Histopathological examination of bursal lesions demonstrated the following:

- There was no difference in the mean score for lymphoid depletion between the groups (p=0.087).
- Bursae from animals of group B presented a moderate to severe mixed, diffuse, inflammatory infiltrate in the plica (including lymphoid necrosis) accompanied by severe oedema that affected the full thickness of the bursae. In contrast, bursae from the vaccinated group presented a significantly lower inflammatory infiltrate and a total absence of oedema.
- Statistically significant differences between groups were observed for heterophil infiltration mononuclear infiltration, plical oedema, oedema of the muscular wall, serosal oedema, and lymphoid necrosis, with higher values in the control group for each parameter.
- Statistically significant differences between groups were observed for cystic degeneration, plical atrophy and early lymphoid repopulation, with higher values in the vaccinated group for each parameter.
- The summary score for 'acute histological functional lesions score', obtained with a sum of individual scores for heterophil and mononuclear infiltration, haemorrhage, luminal exudate, plical oedema, oedema of the muscular wall, serosal oedema, necrotic cysts and lymphoid necrosis, which are the features indicating acute lesions, demonstrated a statistically significant difference

between group A compared to group B, p<0.001, Mann-Whitney test. A statistically significant difference between groups A and B for BF:BW ratio was observed (p=0.006, t-test). A statistically significantly higher spleen: BW ratio, indicative of splenomegaly in group B was observed compared to group A (p<0.001, t-test).

Overall, it is concluded that this study supports the claim for a reduction in clinical signs and lesions of the BF caused by very virulent IBDV, following in ovo vaccination in the target species with MDA against IBDV. While a statistically significant difference in the Ph. Eur. bursal lymphoid depletion score was not demonstrated between groups, this deviation from requirements is accepted given that a markedly more comprehensive evaluation than that required by Ph. Eur. 0587 has been performed, which supports a statistically significant reduction in histopathological lesions of the bursa due to very virulent IBDV challenge.

In another study, efficacy of in ovo and s.c. use was investigated using the increased challenge dose of vvIBDV strain, VG-248. The immunity at 28 days of age and 43 days of age was investigated in this study, conducted in seropositive ('low' MDA) commercial broiler eggs/chicks, following in ovo vaccination of 18-day-old embryonated eggs, and following subcutaneous vaccination of 1-day-old chicks, in accordance with recommendations with a minimum titre dose of vaccine. The average MDA level was 5086 ELISA units at hatch (eggs from breeders at the end of the laying period; low MDA expected). Four groups of animals were used, two vaccinated (either in ovo, group A, or subcutaneous, group B) and one control group (mock-vaccinated, group C). In addition, a group of seronegative SPF 14 day-old chicks were included in order to validate the challenges (group D). Additional birds were included in groups A and B that were not challenged in order to evaluate changes in the BF.

At 28 days of age, a subgroup of birds from each of the groups A, B and C were challenged with a vvIBDV strain, VG-248, $10^{5.0}$ EID_{50}/chick. Chicks were observed daily after challenge, with monitoring of clinical signs, mortality, serological response and weight of animals. At 6 days post-challenge, when the acute phase of the infection was expected, five animals of each group were necropsied to assess acute lesions in the bursa, while the remaining birds of each group were necropsied at 11 days post-challenge (n=20/group). BFs and spleens were weighed and examined macroscopically. Bursae were examined macroscopically for presence of external oedema, followed by histopathological analysis. At 43 days of age, the second challenge took place with similar study design, except necropsy for the majority of the birds took place at 10 days post-challenge (n=20/group). The primary variables of this study were the histopathological scores for bursal damage and clinical signs after challenge. Secondary variables were macroscopic lesions on the BF, presence of external oedema in the bursae, the relative weight of the BF, spleen and growth rate of animals.

Challenge at day 28

Results: After challenge at day 28, no clinical signs or mortality were observed in group A or group B. No mortality occurred in group C, however 8/25 (32%) birds displayed mild clinical signs compatible with Gumboro disease. The differences between group A and group C, and between group B and group C for the proportion of clinical signs was statistically significantly different (p=0.002, P-value of the Mantel-Haenszel test comparing Kaplan-Meier estimates of survival). There were no notable differences in mean growth rate between groups A, B or C. In group D (SPF seronegative control birds), 10/10 (100%) birds presented with clinical signs compatible with Gumboro disease and 3/10 (30%) died, indicating that the challenge conducted can be considered severe.

At day 6 post-challenge, external oedema of the bursa was observed in 5/5 bursae of group C and in 0/7 bursae of group A (p=0.001, Fisher exact test) and 0/5 bursae of group B (p=0.008, Fisher exact test). By day 11 post-challenge, the acute phase of challenge infection had passed and external oedema was not observed in the control group (or vaccinated groups).

There was a statistically significant difference observed between group A versus group C and between group B versus group C (i.e. for each of the vaccinated groups versus the control group) for the mean sum of macroscopic lesions of BF at 6 days after challenge, and at 11 days after challenge ($p<0.05$, Mann-Whitney test).

Histopathological examination of bursal lesions demonstrated the following:

- At day 6 post-challenge (necropsy of 5 birds from group A and group C), there was a statistically significant difference (higher) bursal lesion score in the control group compared to the in ovo vaccinated group A p=0.012, Mann-Whitney test, in addition to SSDs between groups for other histopathological scores associated with acute lesions, whereby scores were higher in the control group. There was no statistically significant difference in the bursal lesion score in the control group compared to the s.c. vaccinated group B, however, there were SSDs between groups for other histopathological scores associated with acute lesions, whereby scores were higher in the control group.
- At day 11 post-challenge (necropsy of 20 birds from group A and group C), there was a statistically significant (higher) bursal lesion score in the control group compared to the in ovo vaccinated group A ($p<0.001$, Mann-Whitney test), in addition to SSDs between groups for other histopathological scores associated with acute lesions, whereby scores were higher in the control group. There was a statistically significant difference in the bursal lesion score in the control group compared to the s.c. vaccinated group B ($p<0.001$), in addition to SSDs between groups for other histopathological scores associated with acute lesions, whereby scores were higher in the control group.

Challenge at day 43

Results: After challenge at day 43, no clinical signs or mortality were observed in group A or group B. No mortality occurred in group C, however 5/26 (19.2%) birds displayed mild clinical signs compatible with Gumboro disease. The differences between group A and group C, and between group B and group C for the proportion of clinical signs was statistically significantly different (p=0.020, P-value of the Mantel-Haenszel test comparing Kaplan-Meier estimates of survival). There were no differences in mean growth rate between groups A, B or C. In group D (SPF seronegative control birds), 10/10 (100%) birds presented with clinical signs compatible with Gumboro disease (depression, ruffled feathers) and 4/10 (40%) died, indicating that the challenge conducted can be considered severe.

At day 6 post-challenge, external oedema of the bursa was observed in 5/6 bursae of group C and in 0/7 bursae of group A (p=0.005, Fisher exact test) and 0/5 bursae of group B (p=0.015, Fisher exact test). By day 10 post-challenge, the acute phase of challenge infection had passed and external oedema was not observed in the control group (or vaccinated groups).

There was a statistically significant difference observed between group A versus group C and between group B versus group C (i.e. for each of the vaccinated groups versus the control group) for the mean sum of macroscopic lesions of BF at 6 days after challenge, and at 10 days after challenge ($p<0.05$, Mann-Whitney test).

Histopathological examination of bursal lesions demonstrated the following:

- At day 6 post-challenge (necropsy of 7 birds from group A and 6 birds from group C), there was a statistically significant difference (higher) bursal lesion score in the control group compared to the in ovo vaccinated group A (p=0.006, Mann-Whitney test), in addition to SSDs between groups for other histopathological scores associated with acute lesions, whereby scores were higher in the control group. There was no statistically significant difference in the bursal lesion score in the control group compared to the s.c. vaccinated group B, however, there were SSDs between groups

for other histopathological scores associated with acute lesions, whereby scores were higher in the control group.

- At day 10 post-challenge (necropsy of 20 birds from group A and group C), there was a statistically significant (higher) bursal lesion score in the control group compared to the in ovo vaccinated group A $p<0.001$, Mann-Whitney test, in addition to SSDs between groups for other histopathological scores associated with acute lesions, whereby scores were higher in the control group. There was a statistically significant difference in the bursal lesion score in the control group compared to the s.c. vaccinated group B $p<0.001$, in addition to SSDs between groups for other histopathological scores associated with acute lesions, whereby scores were higher in the control group.

Overall, it is concluded that this study supports the claim for a reduction in clinical signs and lesions of the BF caused by very virulent IBDV, following in ovo vaccination or subcutaneous vaccination in the target species with MDA against IBDV at day 28 of age and at 43 days of age.

Field trials

As noted in Part 3, three GCP multicentre, randomised, blinded, positively-controlled clinical field trials were carried out to assess both the safety and efficacy of Gumbohatch under field conditions. Refer to Part 3. The efficacy variables were the same in each of the field trials, and consisted of the evaluation of the serological response of groups (primary efficacy variable) determined by ELISA (CIVTEST AVI IBD). Other variables had been planned to be evaluated in the event of an outbreak, which did not occur. The field studies are considered as supportive only in terms of efficacy, given the absence of outbreaks of Gumboro disease. This is not unexpected considering the high level of vaccination against IBDV practised in commercial broiler production systems to reduce outbreaks of disease.

Based on the data provided, it can be accepted that similar patterns of serological response and replication of the vaccine virus strain in the BF was observed for Gumbohatch vaccinated birds compared to the control group vaccinated with an authorised IBDV 'immune complex' vaccine, and demonstrates successful vaccine virus take following the decline of MDA levels. It may be concluded that production parameters feed conversion ratio (FCR) and EPEF, and mortality levels, were similar between test and control groups throughout the field trials.

Overall conclusion on efficacy

The originally proposed claim for a reduction of mortality is considered to have been adequately demonstrated only in MDA-negative 18-day-old embryonated eggs and 1-day-old chicks. However, since MDA-negative birds are not the intended target species, and that the claim for a reduction of mortality has not been supported in MDA-positive embryonated eggs and 1-day-old chicks, it is the opinion of the CVMP that this indication has been inadequately supported.

The claim for a reduction of weight loss due to IBDV infection is not considered to have been adequately supported; a statistically significantly higher growth rate was observed in both of the vaccinated groups relative to the control group only following the day 28 challenge in MDA 'low' birds, but there were no statistically significant differences in favour of the vaccinated groups compared to the control groups following any of the other vvIBDV challenges, in the 'high' or 'low' MDA studies.

It is concluded that the data provided for Gumbohatch supports the claim for a reduction in clinical signs and lesions of the BF caused by very virulent avian infectious bursal disease virus infection, following in ovo vaccination or subcutaneous vaccination in the target species with MDA against IBDV.

The onset of immunity in the two challenge studies conducted with a higher virulent challenge dose demonstrate onset of immunity from day 24 of age for in ovo vaccination, and at day 28 following in ovo or subcutaneous vaccination. It is noted that the claimed onset of immunity is slightly arbitrary since it will depend on biological variability of decline of initial MDA levels and vaccine virus take will occur at different times in individual animals, which is reflected in the SPC.

The demonstrated duration of immunity is 43 days of age.

As only broilers were used in relevant efficacy studies, the use of Gumbohatch in day-old chicks as well as embryonated eggs is restricted to broilers and this is clearly reflected in the SPC (section 4.2 and 4.9).

Overall, it is considered that the efficacy of Gumbohatch has been demonstrated for a reduction of clinical signs and lesions of BF due to very virulent avian infectious bursal disease virus infection in chickens with MDA against IBDV.

Part 5 – Benefit-risk assessment

Introduction

Gumbohatch is a vaccine containing live attenuated IBDV, strain 1052, intended for the active immunisation of chickens with MDA against IBDV, to reduce clinical signs and lesions of BF caused by virulent strains of avian IBD viruses. A single dose of vaccine is proposed to be administered by either the in ovo route for chicken embryos on the 18th day of incubation or by the subcutaneous route for 1-day-old broiler chickens.

In the vaccine formulation an attenuated virus is present as an immune complex with an IBDV-specific antibody.

Gumbohatch is presented as a lyophilisate and solvent for suspension for chickens.

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

Benefit assessment

Direct therapeutic benefit

The benefit of Gumbohatch is its efficacy in the treatment of chickens against Gumboro disease to reduce clinical signs and lesions of the BF due to very virulent avian infectious bursal disease virus infection. Efficacy was shown in a number of laboratory studies which demonstrated that the product is efficacious and that the following SPC claims are supported: For active immunisation of 1-day-old broiler chicks and embryonated broiler chicken eggs to reduce clinical signs and lesions of the bursa of Fabricius caused by very virulent avian infectious bursal disease virus infection. Onset of immunity is from 24 days of age and duration of immunity is up to 43 days of age.

Additional benefits

Gumbohatch would increase the range of available vaccines for the control of IBDV infection and, due to the formulation of the vaccine as an 'immune complex vaccine', would facilitate vaccination of broiler chicken flocks without the requirement for the measurement of MDA against Gumboro disease

and calculation of the optimum time for vaccination based on decline of MDA levels.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Safety:

Risks for the target animal:

Administration of the product in accordance with label recommendations is generally well tolerated in the target animals. No adverse reactions were observed after vaccination under recommended conditions of use or following the administration of a tenfold overdose of Gumbohatch, although severe damage to the BF was observed in vaccinated birds (lymphoid depletion). However, the lymphoid depletion did not correlate with a loss of function of the bursa of vaccinated birds, and did not result in immunosuppression. In addition, the histopathological damage induced by the vaccine virus following replication in the BF was notably distinct from the histopathological damage caused by very virulent IBDV field infection. In addition, the absence of the potential risk of reversion to virulence has been demonstrated. The vaccine virus strain is capable of spreading to non-vaccinated in-contact birds via oral and faecal secretions. A suitable warning is included in the product information. Safety in birds without MDA cannot be assured and use in this category of birds is contraindicated.

Risk for the user:

The use of Gumbohatch is not expected to pose a risk to the user, when used in accordance with recommendations.

Risk for the consumer:

There are no risks identified for consumers of animals vaccinated with Gumbohatch. All components included in the product are either allowed substances with 'no MRL required' classification according to Table 1 of Regulation (EU) No. 37/2010, or are substances considered as not falling within the scope of Regulation (EC) No. 470/2009, therefore a withdrawal period of zero days is considered acceptable.

Risk for the environment:

The use of Gumbohatch is not expected to pose a risk to the environment, when used in accordance with recommendations.

Risk management or mitigation measures

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

Evaluation of the benefit-risk balance

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, the environment and consumers, when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information.

Conclusion

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

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

Divergent position on a CVMP opinion on the granting of a marketing authorisation for Gumbohatch

(EMEA/V/C/4967/0000)

Gumbohatch is a new immunocomplex vaccine (Icx) which combines an old IBDV strain isolated in the 60s (W2512) and already largely used alone as in marketed vaccines, with an anti-IBDV serum (BDA). When administered to chickens with a high titre of anti-IBDV antibodies, Gumbohatch is intended to circumvent their high passive immunity.

The BDA used to be combined/complexed with W2512 strain has no specific characteristics claimed in the dossier and is simply produced by immunisation of hens with the same vaccine strain. In the dossier, the combination of W2512 with BDA is supported by literature only (2 publications of Whitfill et al., 1995 and Haddad et al., 1997) without product-specific experimental or clinical evidence.

The applicant suggests that this preformed complex would be trapped by the follicular dendritic cells in the spleen and the bursa and hidden in to be safeguarded from the clearance by the maternally derived antibodies (MDA) until their decrease, while such mechanism does not occur when the complex occurs in-situ where the injected W2512 strain is complexed by the same kind of antibodies provided by the hen (MDA).

In his study, Whitfill showed that administered at 1 day of age, an Icx vaccine allows for delayed lesions of the bursa until after days 6-8 of age in SPF chicks. By applying the Occam's razor principle, there is no need to ascribe hiding properties to the BDA and all these results can be explained by clearance of the Icx vaccine which may decrease the amount of noxious viral vaccine particles making lesions in the bursa. This interpretation is corroborated by results from Haddad where conventional chickens were protected alike whether the vaccine strain was combined with BDA or not.

Consequently whether the W2512 strain is complexed before (in Icx vaccine) or further to vaccination (by in-vivo MDA) does not change the outcome of vaccination. Therefore BDA have no added value for the chicken population intended to be vaccinated with this vaccine, which is contraindicated in flocks free of anti-IBDV MDAs.

Moreover, the addition of a biological component (BDA) which is unnecessary to the administration of the vaccine strain, raises ethical concerns and does not meet the 3R principles since BDA were drawn from flocks specifically bred and immunised in compliance with Pharmacopoeia requirements.

The undersigned considers that the absence of added value of BDA component and the unnecessary use of laboratory animals should prevent the marketing authorisation of such a product.

Amsterdam, 10 September 2019

Frédéric Klein