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Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Bovilis Nasalgen-C (EMEA/V/C/005906/0000)

Vaccine common name: Bovine coronavirus vaccine, live attenuated

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

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Introduction

The applicant Intervet International B.V. submitted on 21 October 2021 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Bovilis Nasalgen-C, 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 15 April 2021 as the applicant showed that the product would provide a significant therapeutic innovation.

At the time of submission, the applicant applied for the following indication: for the active immunisation of calves from the day of birth onwards to reduce clinical signs of respiratory disease, nasal and faecal viral shedding and lung viral load from infection with bovine coronavirus (BCV).

The approved indication is for the active immunisation of cattle from the day of birth onwards to reduce clinical signs of upper respiratory tract disease and nasal viral shedding from infection with bovine coronavirus.

The active substance of Bovilis Nasalgen-C is a live attenuated bovine coronavirus, which stimulates active immunity against bovine coronavirus. The target species is cattle. The product is intended for administration by nasal use.

Furthermore, the CVMP considers that the live attenuated BCV-CA25 is a new active substance, as claimed by Intervet International B.V.

Each dose of Bovilis Nasalgen-C (2 ml of reconstituted vaccine) contains 5.4 – 7.8 $\log_{10}$ $TCID_{50}$ (tissue culture infective dose 50%) of bovine coronavirus, strain CA25, live attenuated and is presented in packs containing 1, 5, 10, 20, or 50 doses (lyophilisate) and 2 ml, 10 ml, 20 ml, 40 ml or 100 ml Unisolve (solvent).

The rapporteur appointed is Frida Hasslung Wikström and the co-rapporteur is Minna Leppänen.

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

On 15 February 2023, the CVMP adopted an opinion and CVMP assessment report.

On 31 March 2023, the European Commission adopted a Commission Decision granting the marketing authorisation for Bovilis Nasalgen-C.

Scientific advice

The applicant received scientific advice from the CVMP on 20 May 2020, EMA/CVMP/138385/2020. The scientific advice pertained to the clinical development of the dossier. The scientific advice was followed, in principle. Concerns relating to the relevance of the used vaccine and challenge strains are discussed in this report and found overall, acceptable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (dated 11 July 2018) which fulfils the requirements of Directive 2001/82/EC. 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 Union or in a third country.

Manufacturing authorisations and inspection status

Manufacture of the active ingredient and final product takes place at Intervet International B.V. Wim de Körverstraat 35, 5831 AN Boxmeer NL. This site is also responsible for the product batch release.

The solvent is manufactured by Intervet International B.V. Boxmeer NL or Intervet International GmbH, Unterschleissheim, DE.

For the sites listed above, appropriate and valid Good Manufacturing Practice (GMP) certificates and manufacturing authorisations were presented. Specific inspections are currently not required.

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 and of the finished product manufacturing sites has been satisfactorily established and are 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

Bovilis Nasalgen-C is a freeze-dried vaccine containing attenuated live bovine coronavirus - BCV strain CA25. Unisolve is the solvent used to reconstitute the lyophilisate. Two ml of the reconstituted vaccine represents a single dose. The vaccine is presented in glass vials containing vaccine for 1, 5, 10, 20 or 50 doses.

The solvent used for this vaccine (Unisolve) is the same solvent which has been approved for use with other vaccines produced by the applicant (e.g. Bovilis IBR Marker Live, Bovilis INtranasal RSP Live, Ovilis Toxovax, Porcilis Begonia Unisolve, Nobilis AE+POX).

Container and closure

The vaccine is supplied as a freeze-dried product in a glass vial, closed with a rubber stopper and an aluminium crimp cap as described in the Summary of product characteristics (SPC).

The associated solvent to be used for reconstitution of Bovilis Nasalgen-C is Unisolve, packaging presentations: 2 ml, 10 ml, 20 ml, 40 ml and 100 ml glass vials, closed with a rubber stopper and an aluminium crimp cap as described in the dossier. Certificates of analysis (CoA) are provided.

The information provided on the material and nature of the containers and closures is satisfactory.

Regarding the sterilisation of the material, both the vaccine and the Unisolve packaging material are sterilised to achieve a sterilisation assurance level (SAL) of 10^{-6} or better, or a 3-log reduction in heat resistant endotoxin. The conditions of the heat treatment and ionising radiation of the packaging material are indicated in the file.

Product development

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

Bovilis Nasalgen-C is a freeze-dried live vaccine containing the attenuated bovine coronavirus strain BCV-CA25. The attenuated vaccine strain BCV-CA25 was selected based on its growth characteristics, and on the fact that the attenuation process has allowed the strain to grow in suspension in media that are animal component-free.

The production of the active ingredient and finished product is conventional and uses well-known processes in the production of veterinary vaccines. During the development a change in the cell line used for the BCV titration assay was implemented, in order to improve the precision and accuracy of the results of the titration assay. The intranasal vaccination with Bovilis Nasalgen-C is aimed at providing protection against respiratory disease caused by BCV in newborn animals, with a rapid onset of immunity.

The applicant has chosen the BCV-CA25 strain based on its growth characteristics, allowing the consistent production of antigen batches with a sufficiently high batch titre without the need for animal-derived components. The composition of the vaccine is described in sufficient detail in the dossier.

The CVMP considers that the live attenuated BCV-CA25 is a new active substance, as claimed by Intervet International B.V.

Due to the fact that the BCV infection in cattle mainly affects the lungs, the virus strain to be used in a live vaccine needs to be attenuated for safety reasons. This has been done by propagating the virus for multiple serial passages in cell culture at 36-37 °C followed by several passages at suboptimal temperature.

The applicant has shown that the attenuation was selected based on its growth characteristics, and on the fact that the attenuation process has allowed the strain to grow in suspension in media that are animal component-free. Also, the applicant has tested the potential of the vaccine strain to revert to virulence and no increase in virulence was shown. This would indicate that the attenuation is stable. The molecular mechanism leading to the attenuation and adaptation to growth at suboptimal temperature has not been investigated by the applicant but will not change the conclusion that the selected vaccine virus strain is suitable for its purpose.

The qualitative and quantitative composition of the vaccine including the function of the individual ingredients is presented. The amount of stabiliser is the same regardless of the number of doses, which results in different relative content of stabiliser per vaccine dose. Based on stability data submitted until now covering up to 21 months of the 50-dose presentation, there is no difference in stability between the different presentations.

Description of the manufacturing method

The description of the manufacture process of Bovilis Nasalgen-C is in essence found acceptable. The production methods for the vaccine strains are conventional and straightforward processes. Madin Darby Bovine Kidney – 42/E9 (MDBK-42/E9) cells are used for the production of the vaccine virus. These cells are propagated in suspension in bioreactors. When sufficient quantity of MDBK-M42/E9 cells has been produced, the cells are infected with BCV-CA25 and incubated. At the end of the incubation period, the BCV-CA25 virus is harvested, concentrated and subsequently stored at ≤ -40 °C.

The BCV-CA25 virus seed is added to the production cells. Since the vaccine contains live virus, the applicant indicated at which step a separation of virus from the cells is performed or how the viability of the cells is controlled. The applicant provided specifications for concentration of the BCV harvest (e.g. in cell count or virus titre). In their answer the applicant stated that the harvest is done on day 3 post infection and not based on cell viability at moment of harvest. Cells may be viable at time point of harvest. However, the antigen is frozen down at -45 °C without the use of any anti-freezing protection. As a consequence, all cells will be destroyed during the freezing and subsequent thawing of the antigen.

The applicant confirms that the cell debris is not separated from harvest and claims that the quality of the virus stock is not affected. This claim is supported by stability data of the virus stocks and that similar virus harvests (other products) have similar stability without separating cell debris from virus harvest. The argumentation is found reasonable.

For the production of the finished product, the vaccine virus is thawed and then, combined with a stabiliser and Veggie medium #18, filled in glass vials and subsequently lyophilised. Data from batches covering the complete span of proposed bulk sizes have been presented.

The details regarding the lyophilisation process were initially insufficient but have been clarified during the procedure. Temperature, duration and pressure for freezing and primary and secondary drying have been described. Validation data from single dose and multidose presentations have been submitted, verifying a consistent performance throughout the whole lyophilisation chamber.

The stabiliser selected for Bovilis Nasalgen-C is identical to the stabiliser used for Bovilis INtranasal RSP Live. Based on the high similarity of the production process of the antigens and the finished product between Bovilis Nasalgen-C and Bovilis INtranasal RSP Live, the applicant is of the opinion that it is expected that the stabilising properties for Bovilis Nasalgen-C are similar to the already approved products. This reasoning is deemed acceptable and is also confirmed by the results of stability studies of Bovilis Nasalgen-C.

The solvent used for this vaccine is Unisolve. This solvent is already in use by the applicant in a variety of licensed products (e.g. Bovilis INtranasal RSP Live, Bovilis IBR Marker Live, Ovilis Toxovax, Porcilis Begonia Unisolve, Nobilis AE+POX). For the production of the solvent a flow chart is provided for the preparation, in-process controls and finished product control steps accompanied by adequate details, which is deemed acceptable.

The consistent production of batches of virus harvests and final product is further discussed in the dossier, where results of in-process controls and finished product control tests on three batches of BCV antigen and five batches of final product are presented. No preservative is added to the vaccine or solvent. After reconstitution in Unisolve, the vaccine should be used within 24 hours.

Validation:

The applicant has presented adequate insight into the developmental process of the product. The change in cell line used for the BCV titration assay, implemented during the development and as described, is deemed sufficiently validated.

The hold time data provided support the hold time for the BCV antigen after thawing, for the bulk and for the filled vials before lyophilisation.

Production and control of starting materials

Starting materials listed in pharmacopoeias

A number of starting materials are listed in this section together with the corresponding Ph. Eur. monographs. Examples of certificates of analysis of these materials are provided in the dossier.

Certificates of analysis have been provided and all conform to specifications in the Ph. Eur.

The nature of the raw materials, controls and treatments applied guarantee sterility of the vaccine and absence of introduction of any extraneous agent.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

A risk assessment on extraneous agents according to Ph. Eur. 5.2.5. for the BCV-CA25 strain was performed in order to determine the extraneous agents for which a test for absence was required. The vaccine strain BCV-CA25 has been tested for bacterial and fungal contaminants, *Mycoplasma spp*., and specific bacterial and viral extraneous agents. None of the microorganisms tested for extraneous agents was found in the BCV-CA25 seed.

Details of the risk assessment on extraneous agents as well as the master seed protocol are included in the dossier and are found acceptable. A risk assessment on extraneous agents according to Ph. Eur. 5.2.5. for the MDBK-42/E9 seed was performed in order to determine the extraneous agents for which a test for absence was required. The MDBK-42/E9 seed lot has been tested for bacterial and fungal contaminants, *Mycoplasma spp*. and specific bacterial and viral extraneous agents and this choice of tests is deemed acceptable. None of the extraneous agents tested for were found in the MDBK-42/E9 cells. Details of the risk assessment on extraneous agents as well as the master seed protocol are included in the dossier.

It was noted that the usage of passage MCS+30 is indicated as the maximum passage allowed for production without any justification or supportive data, which is not in line with Ph. Eur. 5.2.5. According to the monograph, if cells beyond passage 20 levels are to be used for production, it shall be demonstrated that the production cell cultures are essentially similar to the master cell seed. Information to show that the MCS+30 and the MCS are equivalent was provided and has shown no difference in karyology and homology by sequencing when comparing the MSV and MSV+30.

The conditions (i.e. incubation time, temperature, point in time for harvest) used to produce the MSV and WSV, respectively, are similar to the ones used for vaccine production and the virus titres of the currently used MSV and WSV have been given. The titres are the basis for determination of the quantity (volume) of virus used for infection of the cells with the specified MOI for vaccine production.

Other materials of biological origin are used in the media to grow the MDBK-42/E9 cells to produce the BCV vaccine virus. The veggie media are biological starting materials of vegetable origin. The composition of this medium has been sufficiently described.

All components of biological origin are sterilised by validated and accepted methods. Examples of certificates of analysis of these materials are provided in the dossier. All starting materials of ruminant origin used by the company for the production of veterinary medicines are stated to comply with the EU Regulations as laid down in the "Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" (EMEA/410/01 and its revisions) and the corresponding Ph. Eur. Monograph 5.2.8. This is found acceptable.

The milk derivate (pancreatic digest of casein) is derived from bovine milk sourced from healthy animals under the same conditions as milk collected for human consumption.

The applicant has made a detailed analysis of the risk if any of the original starting materials (seed) or starting materials used during production of the vaccine may pose a risk in the transmission of TSE, and the risk is estimated by the applicant as effectively zero. Appropriate certificates of suitability (CEPs), certificates and statements are included in the dossier to support this analysis. This analysis is deemed acceptable.

A risk assessment on extraneous agents according to Ph. Eur. 5.2.5. has been performed, and the seed materials have been adequately tested. It is stated that, where applicable, starting materials comply with monographs within the European Pharmacopoeia or United States Pharmacopoeia. For the seeds and other materials not described in a pharmacopoeia, the applicant provides sufficiently detailed monographs.

It has been verified that the same specifications for the starting material will be used in case of alternative suppliers and that the same specifications including TSE-related aspects will apply for the material from alternative vendors.

Although no animal component is included in the manufacture of the trypsin, being a recombinantly produced substance, it will classify the substance as a biological starting material. The manufacture and testing of the recombinant trypsin have upon request been acceptably described, including its sterilisation.

Except for the sterilised materials gelatine and casein, the product does not contain any materials of human or animal origin.

Starting materials of non-biological origin

Certificates of analysis have been provided for excipients of non-biological origin.

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

Media or solutions used for the preparation of cell and virus production media can either be purchased or made by the applicant. The virus and cell production media are prepared before use by adding poloxamer 188, antifoam, antibiotics and, for the latter medium, trypsin to Basal Veggie medium #18. The media are used within one same day.

Control tests during the manufacturing process

The in-process control tests are carried out during the production of the active ingredient and are considered adequate for this type of process and product. They consist of BCV identity and titration testing, and sterility testing of the bulk harvests and testing of adequate filling volume of the vials.

It was noted that regardless of the number of doses in the multidose preparations (5, 10, 20 or 50), 2.5 ml is filled. This means that the virus content per ml before lyophilisation will differ. This has been explained by the company that the manufacturing process does not aim at a specific number of doses, the number of doses assigned are determined based on the outcome of the assay testing results after filling and lyophilisation.

Control tests on the finished product

The procedures for the final product tests are adequately described and deemed relevant for their use. The general characteristics of the finished product, composed of the appearance of the lyophilisate, the solubility and the presence of vacuum in the vials, are checked. These tests are standard qualitative tests and are not specifically validated for the product described in the dossier. This approach is found acceptable.

The BCV identity and batch titre test ascertain the correct identity of the active ingredient and the BCV content of the finished product. Initially the BCV titration assay was developed with the MDBK-Mb cell line. During the development of the product it became apparent that this cell line caused an interference in the assay, and a switch to a different MDBK cell line (MDBK-42/E9; the cell line also used for the production of the BCV antigen) was made. The switch to the MDBK-42/E9 cell line has shown that it increased the sensitivity and robustness of the assay. The validation of this test was done by the applicant, based on the VICH GL1 "Guideline on validation of analytical procedures: definitions and procedures" and GL2 "Guideline on validation of analytical procedures: methodology". The validation report is deemed acceptable and supports the approach chosen.

The BCV-specific monoclonal antibody, used for the BCV immunofluorescence assay (IFA), was shown to provide adequate specificity and was sufficiently validated, which is deemed acceptable. The BCV batch titre test was deemed to provide adequate sensitivity, specificity, precision and robustness, and was sufficiently validated.

No reliable results can be generated any longer with the titration method using MDBK-Mb cells. Therefore, it was not possible to perform a direct comparison between both methods. In order to compare both assays, the data generated for the titration reference standards and several antigen batches were used, as these samples were stored at ≤-60 °C.

The individual titres of the different antigen batches determined by both methods were provided. The data were statistically modelled using a mixed effects linear model for repeated measurements, allowing for different variances for the two types of cells and for which the sample was defined as the subject. For antigen batches results using MDBK-42/E9 cells were found higher than results using

MDBK-Mb cells. The difference for titration standards was not statistically significant but was numerically in line with the result found for the antigen batches.

In the process of answering the questions and setting up the new laboratory efficacy study (BCV 21-46-053) a consequential error was discovered by the company. In calculating the dose for vaccination, an error by a factor of 2 was made in the dilution calculations, which means that the actual dose is 0.3 log_{10} $TCID_{50}$ higher. Therefore, the previously reported minimal dose for the listed studies (5.1 log_{10} $TCID_{50}$) is incorrect and needs to be updated. The new study (BCV 21-46-053) was conducted at the originally intended dose of 5.1 log_{10} $TCID_{50}$**.** However, the applicant proposes to change the minimum effective dose reflected in the SPC from 5.1 to 5.4 log_{10} $TCID_{50}$ as this is used in the majority of the studies. To assure that the minimal titre 5.4 log_{10} $TCID_{50}$/dose is fulfilled throughout the shelf life, a more restrictive release limit is applied. In a conservative measure a factor of 0.5 log_{10} $TCID_{50}$/dose was added to assure that the shelf life titre of not less than 5.4 log_{10} $TCID_{50}$/dose is fulfilled.

The upper limit is based on the vaccine doses used during the safety studies (7.8 log_{10} $TCID_{50}$/dose). Regarding the differences in release and shelf life specifications for potency please refer to the stability section below.

The tests for mycoplasma and for residual moisture are performed in line with the Ph. Eur.

The sterility test performed on final product is according to the Ph. Eur. 2.6.1 and Ph. Eur. 0062. Detection by visual inspection or by use of an automated detection system (BD BACTEC) was adequately validated and documented in the attached validation reports. The vaccine is tested by methods in line with relevant Ph. Eur. monographs, and the applicant has provided enough data to validate the methods.

The change in cell line used for the BCV titration assay, implemented during the development and as described, is deemed sufficiently validated.

Batch-to-batch consistency

Results of the control tests performed on consecutive antigen and vaccine batches are presented in the dossier.

Results from testing of filling volume of the final bulk can be found in the CoAs submitted and show consistent results. Final control test results are available for four batches of Bovilis Nasalgen-C. All tests fulfil the release requirements as specified in the dossier and show good reproducibility.

The acceptance criteria for residual moisture were asked to be further justified or tightened. The applicant proposed to reduce the upper limit and referred to stability data from batches with a residual moisture which is higher than the normal batches. As there does not appear to be a difference in titre stability compared to the batches with low or medium residual moisture, it can be considered that the proposed limit can be accepted.

Stability

Real-time data to support the stability of the antigen for the intended storage temperature at -40 °C for up to 2 years have been provided. The data adequately supports the proposed storage conditions of the antigen.

For the finished product (lyophilisate), the applicant claims 2 years of shelf life at +2 to +8 °C. A decreasing content is seen during storage. Final full-time data have been submitted to assure that

the release requirement of ≥5.9 log_{10} $TCID_{50}$ /dose is sufficient to assure an end-of-shelf-life specification of ≥5.4 log_{10} $TCID_{50}$/dose.

Currently five batches of the finished product have reached 27 months or more to support the claimed 24 months, all of them being 1-dose presentation except for one 5-dose presentation. These results are within the acceptance criteria. For the other presentations (10, 20 and 50 doses) stability data covering 21 months have been submitted. Taken together with the presentation specific data submitted the proposed shelf-life of 24 months at +2 to +8 °C can be accepted.

Accelerated stability data have been presented for the lyophilised product stored for 7 days at +30 °C under which no decrease in potency was seen.

In-use shelf life of the reconstituted product of 24 hours at room temperature has been acceptably supported by data. The claimed shelf life for the solvent (3 years for the 2 ml presentation and 5 years for the 10 ml, 20 ml, 40 ml and 100 ml presentations) has also been acceptably supported by data.

Overall conclusions on quality

Information on the development, manufacture and control of the active substance and the finished product has been presented in an adequate manner. In the section on product development, information on the origin of the strains is provided. The lack of information related to the molecular basis for the attenuation was considered a major objection in the initial assessment. In their response, the applicant has shown that the attenuation was selected based on its growth characteristics, and on the fact that the attenuation process has allowed the strain to grow in suspension in media that are animal component-free. Also, the applicant has tested the potential of the vaccine strain to revert to virulence and no increase in virulence was shown. This would indicate that the attenuation is stable. The molecular mechanism leading to the attenuation and adaptation to growth at suboptimal temperature has not been investigated by the applicant but will not change the conclusion that the selected vaccine virus strain is suitable for its purpose.

The qualitative and quantitative particulars of the vaccine lyophilisate, the solvent and the containers are described. The composition of the solvent is the same as of that used for other vaccines manufactured by the applicant. The production processes of the vaccine and the solvent are described, and further information has upon request been submitted on the virus production and the lyophilisation of the product. Where applicable, the starting materials comply with the provisions of Ph. Eur., and the TSE risk assessment is adequate. The respective certificates of suitability are provided. The MDBK-42/E9 cell line is considered as a suitable production system. Appropriate acceptance criteria were proposed for most of the attributes. Further calculations by the applicant revealed that the lower titre was miscalculated by a factor of 2 and the lower shelf life limit has been raised to ≥ 5.4 log_{10} $TCID_{50}$/dose. To assure that this limit is fulfilled, a release limit of ≥5.9 log_{10} $TCID_{50}$/dose has been assigned. The acceptance criteria for residual humidity have upon request been revised and the new proposed limit can be accepted.

Stability data were provided for storage of antigen, the finished product, the in-use stability of the reconstituted product and the stability of the solvent. Two-year shelf life of the veterinary medicinal product (lyophilisate) as packaged for sale has been proposed when stored at +2 to +8°C and has been supported by real time data. The shelf life of the solvent has been initially established as 3 years for the 2 ml presentation and 5 years for the 10, 20, 40, 100 ml presentations. 24-hour shelf life after reconstitution according to directions and stability for 7 days at +30 °C of the lyophilised product are considered acceptably supported.

Based on the review of the data on quality, the manufacture and control of Bovilis Nasalgen-C, no further issues are remaining and the product can be recommended for approval from a quality point of view.

Part 3 – Safety

Introduction and general requirements

Bovilis Nasalgen-C is a freeze-dried vaccine containing live attenuated bovine coronavirus (BCV) – strain CA25. A full safety file in accordance with Article 12(3)(j) of Directive 2001/82/EC has been provided. The BCV antigen content of the vaccine is 5.4 – 7.8 $\log_{10}$ $TCID_{50}$/dose. A single dose of the vaccine is intended for intranasal use in calves from the day of birth onwards. 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. monograph 5.2.6 'Evaluation of safety of veterinary vaccines and immunosera' has been taken into account to demonstrate the safety of the vaccine.

Safety documentation

Five safety studies were conducted to investigate the safety of the product and included four laboratory studies and one field trial. The vaccine was administered by the intranasal route, as recommended using pilot production batches. Batches are considered representative of commercial product. Laboratory studies were reported to be GLP compliant and carried out in target animals.

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.

Safety on one dose, an overdose and a repeat dose, as well as safety under field conditions, were assessed in the minimum age recommended for vaccination. Dissemination, shedding, spreading and reversion to virulence were not studied in the youngest category of animals but in animals aged 6–10 days. This was accepted as the category of animals was considered relevant for the purpose, and that there are challenges with performing studies in new-born calves.

The name Bovilis INtranasal C live is used synonymously for Bovilis Nasalgen-C in some studies.

Study title	***Dose used***
Bovilis INtranasal RSP live and Bovilis INtranasal C live vaccine upon intranasal administration of an overdose followed by a repeated single dose in seronegative calves within 24h after birth.	1X (7.8 $\log_{10}$ $TCID_{50}$) and 10X (8.8 $\log_{10}$ $TCID_{50}$) maximum dose, MSV+2.
Safety study of BCV-CA25 Live after an overdose intranasal administration of BCV CA25 Live in concomitant non mixed use with Bovilis INtranasal RSP Live to one week old maternally derived antibodies (MDA)-negative bovines (calves).	7.3 $\log_{10}$ $TCID_{50}$ and 8.3 $\log_{10}$ $TCID_{50}$, MSV+1.

Study title	Dose used
Dissemination, shedding and spreading of the bovine coronavirus strain CA25 after intranasal inoculation of seronegative calves at approximately one week of age.	1X maximum dose (7.8 $\log_{10}$ $TCID_{50}$), MSV+1
Increase in virulence of live attenuated BCV vaccine strain.	7.2 $\log_{10}$ $TCID_{50}$, MSV.
A clinical study in calves in Germany to assess the safety of Bovilis INtranasal C Live in associated non-mixed use with Bovilis INtranasal RSP Live	7.3 $\log_{10}$ $TCID_{50}$, MSV+5.

Laboratory tests

Four GLP-compliant laboratory safety studies were presented. Three of these studies were conducted in seronegative calves while the calves in one study were seropositive for BCV.

Safety of the administration of one dose

Refer to 'Safety of the repeated administration of one dose'.

Safety of one administration of an overdose

Refer to 'Safety of the repeated administration of one dose'.

Safety of the repeated administration of one dose

One pivotal laboratory study was conducted to investigate the safety of the administration of a 10X overdose and the repeated administration of a 1X dose, separated by an interval of 14 days.

The vaccine was administered by the recommended route (intranasal), in the recommended species. Animals were of the minimum age, as required. The vaccine was administered at the same time (associated non-mixed use) as a 10X overdose (day 0) and a 1X dose (day 14) of the vaccine Bovilis INtranasal RSP Live, which is a vaccine for administration to calves to provide active immunity against Bovine Respiratory Syncytial Virus (BRSV) and Parainfluenza virus Type 3 (PI3). Half of the dose was administered in each nostril. This means, both products were administered intranasally in each of both nostrils at the same time, i.e. simultaneously. The applicant was asked to clarify and justify how this administration of two vaccines corresponds to non-mixed associated use, even if the vaccines are not mixed before but at the inoculation site. In response to this, the applicant stated that the two vaccines are not effectively mixed in the nostrils because most of the vaccine will be absorbed before the second vaccine is administered.

Ten calves were included in the study but two of these were excluded because BRSV or BCV were detected in nasal swabs from these animals before vaccination. No control group was included. Follow-up consisted of observations until 14 days after last vaccination, with monitoring of clinical signs and rectal temperature.

Results showed that administration of a 10X intranasal dose, followed by a 1X dose 14 days later, was well-tolerated. Mild nasal discharge was observed sporadically throughout the experiment as well as sporadically increased (>39.5°C) rectal temperature. These findings are accurately reflected

in the product information. Six of the eight calves had semi-solid to watery faeces during the second week of the experiment. Rainbow tests showed that all these calves were positive for cryptosporidiosis. Calves were colostrum-deprived and therefore particularly sensitive to infections. The signs of diarrhoea showed no clear association in time to the administration of vaccine and were considered unrelated to treatment.

On the basis of the results no safety concerns arose following the administration of a 10X overdose followed by a single dose of the recommended dose (1X) administered two weeks later, to the target species of the minimum recommended age.

One additional study was presented that also investigated the safety of a 10X overdose followed two weeks later by a single 1X dose in associated non-mixed use with Bovilis INtranasal RSP Live. Calves were 5–9 days of age at time of vaccination. The calves were found to be seropositive for BCV and the study does therefore not fulfil the requirements specified in Ph. Eur. monograph 5.2.6, stating that animals must be free from antibodies against the virus contained in the vaccine. However, this study is considered supportive, and results support that the vaccine is generally well tolerated in approximately one-week-old calves that have antibodies to coronavirus.

Examination of reproductive performance

No reproductive studies were provided as the product is intended for use in young calves. The following statement is provided in section 3.7 of SPC: 'The safety of the veterinary medicinal product has not been established during pregnancy and lactation.'

Examination of immunological functions

No specific studies were conducted to investigate the effects of the product on immunological functions but no adverse effects, except mild respiratory signs, ocular discharge, and transient rise in body temperature, were observed in the safety and efficacy studies. It is unlikely that this vaccine will have an adverse effect on immunological functions due to the nature of the product (live vaccine without any known immunosuppressive effects).

Special requirements for live vaccines

Spread of the vaccine strain

The spread of the vaccine strain from vaccinated to unvaccinated animals was investigated in a study in which eight animals of the target species of age 6–9 days were vaccinated with a dose of 7.8 $\log_{10}$ $TCID_{50}$ (maximum dose) according to the recommended vaccination schedule by the recommended route and left in contact with six unvaccinated sentinels for up to 14 days. All inoculated animals started to excrete BCV vaccine virus in nasal discharge from one day after inoculation up to nine days after inoculation. However, the animals that excreted virus on day 9 were euthanised the same day. It is therefore not possible to conclude that excretion is limited to nine days. The vaccine strain was also isolated from oral swabs from one inoculated animal. The vaccine strain was isolated from nasal discharge from two sentinels after two days of contact.

It is concluded that the vaccine virus can spread to in-contact unvaccinated animals. Studies were not carried out to investigate spreading to other non-target species. The applicant was asked to discuss the possibility of spread of the vaccine strain to other species and justify the absence of testing for spread to these species. The applicant stated that there is no evidence of other species

being highly susceptible to BCV and that literature suggests that the occurrence of spread to other species is rare. Further, the applicant considered the risk of spreading of the vaccine strain to other species low since the vaccine strain is attenuated and that the spread to calves were limited to 2/6 animals and the shedding from these two animals were transient. It was agreed that the risk of spreading to other species is low, but it cannot be excluded. Bovine corona virus can infect other species than cattle, for example wild ruminants. Absence of testing of spread to other species than cattle has been reflected in the SPC.

Dissemination in the vaccinated animal

Dissemination of the vaccine strain in vaccinated animals was investigated in a study in which spread of the vaccine was also investigated (see above). Eight animals aged 6–9 days were vaccinated.

The vaccine strain was isolated from the nasopharyngeal mucosae (n=3), trachea (n=1), tonsils (n=1), serum (n=1) and the bronchial lymph nodes (n=1), mandibular lymph nodes (n=4) and retropharyngeal lymph nodes (n=3). The highest levels were detected in the nasopharyngeal mucosae (site of administration). Hence, except from one animal where BCV was detected in serum, the dissemination appears to be restricted to tissues of the respiratory tract and associated lymph nodes. In conclusion, the virus strain does disseminate following vaccination by the recommended route at a maximum dose in animals of the target species, aged approximately one week.

Reversion to virulence of attenuated vaccines

The reversion to virulence of the vaccine strain was investigated in a study in accordance with the requirements of Ph. Eur. 5.2.6. Sequential passage of vaccine strain through five groups of animals was investigated; a single dose of test vaccine was administered to target species in the first group by the recommended route. The animals of the subsequent passages (2, 3, 4, and 5) were inoculated with 1 ml of nasal swab material from the preceding passage with the highest virus content, by the same route. Animals were 7–10 days of age at the start of the study. Each passage group consisted of three animals in passage group 1–4 and ten animals in passage group 5. The vaccine strain was recovered at all five passages.

The clinical signs of disease observed were similar at all passages except at the fifth passage where calves had watery diarrhoea and were found to be positive for rotavirus. The calves were colostrum deprived and therefore particularly sensitive to infections. The signs of disease observed were considered unrelated to vaccination. It is concluded that no signs of reversion to virulence was observed following five passages *in vivo*.

Biological properties of the vaccine strain

No specific studies have been conducted to determine the intrinsic biological properties of the vaccine strain. A description of the biological properties of the vaccine strain can be found in part 2 of the dossier. In the clinical studies conducted, the vaccine strain did not cause clinical signs except mild respiratory signs, ocular discharge, and transient rise in body temperature. On the basis of these data, the safety profile of the strain can be considered acceptable.

Recombination or genomic reassortment of the strains

No specific studies regarding genomic reassortment or recombination of the vaccine strain with other different strains of BCV have been performed. Bovine coronavirus is a non-segmented RNA virus and reassortment of genome segments is not possible for such viruses. However, coronaviruses are prone to recombination. The applicant was asked to present further discussion of the risk for recombination and propose risk mitigation measures to be included in the SPC, if appropriate. The applicant stated that there are no publications on the recombination of vaccine strains and field strains for coronaviruses.

The risk of recombination between the attenuated vaccine strain and a field strain is therefore theoretical but cannot be fully excluded. The benefit of vaccination is considered to outweigh the potential risk of recombination.

User safety

The applicant has presented a user safety risk assessment which in general 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 are those of accidental self-injection during reconstitution of the vaccine, dermal during reconstitution and administration of the vaccine, and inhalation of aerosol during administration of the vaccine.

Attenuated live BCV strain CA25 is not considered to be pathogenic for humans and therefore does not pose a risk for the user.

The excipients, including components used during production of the lyophilisate, are commonly used in other vaccines and do not pose a risk for the user.

As neither the antigen or the excipients pose a risk no warnings or advice to users in the SPC are required.

Based on the above risk assessment the CVMP concluded that the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

Study of residues

No studies of residues were performed. This is considered acceptable.

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.

Most of the excipients, including components used during production of the lyophilisate, listed in section 2 of the SPC are either allowed substances for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

The requested information regarding the amounts of the antimicrobial substances polymyxin B sulphate and neomycin sulphate used during the manufacturing process was provided. It was verified that polymyxin B sulphate and neomycin sulphate are present at low residual levels in the

finished product which are not considered to constitute a risk to the consumer. Based on the intranasal application, which indicates that the exposure to the consumer will be low, together with the overall composition, remains of Veggie medium components in the vaccine are not considered to pose a risk to the consumer.

Withdrawal period

A withdrawal period of zero days is accepted.

Interactions

The applicant has provided data investigating interactions of the vaccine with Bovilis INtranasal RSP Live, a vaccine for administration to calves to provide active immunity against Bovine Respiratory Syncytial Virus (BRSV) and Parainfluenza virus Type 3 (PI3). Data provided indicated that there is no risk of interaction when these products are used together. In one of the study, the doses were divided, and the two vaccines were administered in each nostril. In the other study, the vaccines were administered in separate nostrils. It is recommended that the full dose of each vaccine should be administered into different nostrils. This is, however, not in line with the current recommendations in the SPC for Bovilis INtranasal RSP Live. According to the applicant, the SPC of Bovilis INtranasal RSP Live will be updated in a future variation.

Field studies

One Good Clinical Practice (GCP)-compliant, multicentre, randomised, fully blind, positive-controlled study was conducted to evaluate the safety of Bovilis Nasalgen-C in associated non-mixed use with Bovilis INtranasal RSP Live, under field conditions. The study was conducted at three dairy farms in Germany. The study included 120 calves, aged 15 minutes to five days, with majority of calves being < 24 hours old at time of vaccination. Calves in the test group (n=60) were administered a single dose (dose 7.3 $\log_{10}$ $TCID_{50}$) of Bovilis Nasalgen-C in the left nostril and a single dose of Bovilis INtranasal RSP Live in the right nostril. The calves in the control group received a single dose of placebo in the left nostril and a single dose of Bovilis INtranasal RSP Live in the right nostril. Follow-up consisted of observations until 14 days after last vaccination, with monitoring of clinical signs, lung auscultation, and rectal temperature. An increase in mean rectal temperature was observed after vaccination in both groups. In the test group, the maximum observed rectal temperature was 39.9°C. Respiratory signs such as nasal discharge, cough and increased respiratory rate were observed during the study. Ocular discharge was also observed. Signs were mild and occurred at similar frequency in both groups. These signs are accurately reflected in section 3.6 of the SPC. Many of the calves had diarrhoea at some time point during the study of which majority were positive for *Cryptosporidium parvum*, which could have been a contributing factor. Diarrhoea is not uncommon in young calves and diarrhoea was observed at a similar frequency in both groups and was considered unrelated to vaccination. The study was well designed and confirms that administration of Bovilis Nasalgen-C in associated non-mixed use with Bovilis INtranasal RSP Live is generally well tolerated in seropositive calves of minimum age.

Environmental risk assessment

The environmental risk assessment provided is in general performed in accordance with the applicable guideline (EU Note for Guidance EMA/CVMP/074/95).

Considerations for the environmental risk assessment

The live attenuated strain of BCV is not considered to be pathogenic for humans. It is not recognised as a zoonotic virus strain and there was no increase in virulence of the vaccine strain after five animal-to-animal passages. The vaccine virus can spread to in-contact unvaccinated animals. The exposure to non-target species is considered limited due to the nasal route of administration and that shedding from vaccinated calves was only observed from the nose or mouth. Studies to investigate the potential spread to other non-target species were however not carried out. Based on the responses to the questions on the studies on dissemination, shedding and spreading and of reversion to virulence, it was concluded that the vaccine strain is attenuated and that the risk of spread to other species is considered low but cannot be excluded.

The risk of the contamination of the production process by extraneous agents and spread of viable contaminants to target animals, other animals and/or humans is considered negligible as all production steps are performed according to procedures adequate to obtain a sterile product. With regards to potential recombination between the attenuated vaccine strain and a field strain the risk is considered theoretical. The benefit of vaccination is considered to outweigh the potential risk of recombination.

All other components of the vaccine are not considered to pose any risk to the environment.

In conclusion, Bovilis Nasalgen-C is not expected to pose a risk for the environment when used according to the provisions in the SPC.

Overall conclusions on the safety documentation

The applicant has provided one pivotal laboratory study to investigate the safety of one dose, a 10-fold overdose, and repeated administration of one dose to target animal species of the minimum recommended age using maximum titre via the recommended route. The batch used in this study was pilot but representative of commercial product.

On the basis of the results, it was concluded that the safety of the target animals when the vaccine is administered according to the recommended schedule and via the recommended route is acceptable.

Reproduction safety was not investigated. The relevant warning in the SPC has been added accordingly.

As this is a live vaccine the applicant also conducted studies to establish the potential for spread and dissemination of the vaccine strain. It was concluded that the vaccine can spread to other target animals. Possible spread to other species was not investigated which is reflected in the SPC. Dissemination is mainly restricted to tissues of the respiratory tract and associated lymph nodes.

Reversion to virulence of the strain was also investigated and there were no signs of reversion to virulence. Recombination of the strain was not investigated, but the risk is considered theoretical. The benefit of vaccination is considered to outweigh the potential risk of recombination.

The product is not expected to adversely affect the immune response of the target animals and therefore no particular tests on the immunological functions were carried out.

A field study confirmed that the safety of Bovilis Nasalgen-C administered in associated non-mixed use with Bovilis INtranasal RSP Live is acceptable.

The data presented are considered adequate to characterise the safety profile of the vaccine as

acceptable. The safety of the targeted animals when the vaccine is administered according to the recommended schedule and via the recommended route is acceptable.

A user safety assessment which is generally in line with the relevant guidance document has been presented. Based on that assessment, the potential health risk of the product to all users is considered low and acceptable when used in accordance with the SPC. No warning statements or safety advice in the SPC are required.

The live attenuated strain of BCV and most of the other components of the reconstituted vaccine do not require MRLs and have been concluded as safe for the consumer. Furthermore, it was confirmed that polymyxin B sulphate and neomycin sulphate are present at low residual levels in the finished product which are not considered to constitute a risk to the consumer. The applicant has verified that the Veggie medium components do not pose a risk to the consumer. A withdrawal period of zero days is accepted.

The live attenuated strain of BCV is not recognised as a zoonotic virus strain and there was no increase in virulence of the vaccine strain after five animal-to-animal passages. Furthermore, based on the results of the studies on dissemination, shedding and spreading, it was concluded that the risk of spread to other species is considered low but cannot be excluded. It was concluded that Bovilis Nasalgen-C is not expected to pose a risk for the environment when used according to the provisions in the SPC.

Part 4 – Efficacy

Introduction and general requirements

Bovilis Nasalgen-C is a freeze-dried live vaccine containing the attenuated strain bovine coronavirus (BCV) CA25. The vaccine strain was originally isolated from calf samples. The isolation and choice of the vaccine strain and its relevance to the current epidemiological situation in Europe has been adequately justified by the applicant. The indication originally proposed by the applicant was to reduce clinical signs of respiratory disease, nasal and faecal viral shedding and lung viral load from infection with bovine coronavirus when administered to cattle, from the day of birth onwards. Immunity was intended to be established 5 days further to a single intranasal administration lasting for 3 months. Bovine coronavirus is commonly recognised as an aetiological agent for enteric disease in calves (neonatal diarrhoea) and adult cattle (winter dysentery). A role as pathogen within the bovine respiratory disease complex (BRD) was first proposed in 1982 and has been confirmed by multiple studies since then. Moreover, BCV has been identified as the main aetiological agent involved in field cases of pneumoenteritis in neonate and juvenile calves.

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. A specific monograph is not available for live bovine coronavirus vaccines. Two monographs for live vaccines against two other viruses within the bovine respiratory disease (BRD) complex (Ph. Eur. 1176 for bovine parainfluenza virus and Ph. Eur. 1177 for bovine respiratory syncytial virus) have therefore been considered.

Scientific advice was given concerning the possibility to waive a clinical field trial, and the CVMP agreed that this omission would be acceptable if well performed laboratory studies supporting all claims are provided in the most sensitive category of animals. In the advice the CVMP highlighted that considering the nature of the BCV (RNA virus) and the known genomic variation and evolution in

the field, it is of particular importance that the challenge strain(s) used is/are adequately characterised and a justification for its/their relevance to the field situation is provided in the dossier. Also, to support the proposed claims, both routes of viral excretion, i.e. nasal and faecal, should be evaluated. The applicant has followed the scientific advice received.

Challenge model

Two different challenge strains were used, one from the US and one European. The BCV US strain MN-1988 originated from a faecal sample from a calf with diarrhoea, and the BCV strain EU-19 from a pool of nasal swab samples collected from three animals in France. Both viruses are claimed to be heterologous to the vaccine strain. The challenge viruses were administered as aerosol using a mask. The route of challenge infection is considered appropriate and mimics the route of natural infection. The challenge induced mild upper respiratory clinical signs in the efficacy laboratory studies.

The choice of challenge strains has been adequately justified by the applicant. Published data was provided supporting the fact that all currently known BCV isolates are biologically, genetically, and antigenically similar and seem to comprise a single serotype. A virus neutralisation assay was performed demonstrating that the BCV vaccine strain has a comparable affinity for both challenge strains. The US challenge strain was chosen for most studies as this strain was shown to be slightly more virulent than the EU challenge strain. This is considered acceptable.

Study reports for the laboratory studies conducted to establish and validate the challenge model have been provided.

Efficacy parameters and tests

The efficacy parameters investigated in the efficacy studies, in line with Ph. Eur. 1176 and 1177, were clinical signs of upper respiratory tract disease (URTD), nasal and faecal shedding of virus and lung viral load. Clinical signs of URTD were recorded three times daily in all studies, and the highest value per day was used. The score included evaluation of four parameters: coughing, nasal discharge, ocular discharge, and ear abnormalities. The tests performed to evaluate viral shedding (nasal and rectal swabs) and lung viral load [bronchoalveolar lavage (BAL)] were live virus titration and/or detection of viral RNA with RT-qPCR. The use of quantitative PCR instead of virus titration is considered acceptable. Parameters evaluated to assess shedding were maximum viral load (corresponding to mean titre) and number of days positive for the virus after challenge (i.e. duration), as described in the Ph. Eur. monographs. The maximum titre of viral shedding reached for each individual animal was noted and the average maximum titre was calculated for each group (vaccinated and controls, respectively). The parameters chosen are considered appropriate for evaluating the efficacy of the product. In addition to these two parameters for evaluating viral shedding, the applicant has presented calculations of the total virus excretion expressed as the area under the curve (AUC). The AUC endpoint combines the two endpoints duration of viral shedding and viral load into one, representing the total amount of virus shedding. The applicant has justified the use of AUC as being the most clinically meaningful endpoint, since it incorporates the total amount of viral shedding, i.e. both the amount of virus excretion per day and the number of days of excretion, during the post challenge period. The AUC endpoint was a predetermined efficacy parameter according to the provided study protocols.

The applicant has also provided a meta-analysis of the five pivotal efficacy studies. While meta-analysis of combined study data may provide an informative, supporting summary, these results

can usually not be regarded as the pivotal evidence, unless this has been clearly stated and justified in advance. Further, in the meta-analysis the applicant has combined studies that are not identical e.g. by using different challenge strains, animals of different age and type (MDA negative/MDA positive) of vaccination, and variable follow-up durations. In general, the studies included in the meta-analyses were, by definition, designed to investigate different research questions and are therefore supportive to each other. Conducting a meta-analysis requires the assumption that the studies included had the same research question. Therefore, the results of the meta-analysis can be considered supportive information at most.

Efficacy documentation

Five pivotal laboratory studies were conducted to investigate the efficacy of the product. Laboratory studies were well documented and carried out in target animals using production batches containing the minimum titre according to label (except in one study where the titre used was even lower). Two of the five pivotal laboratory studies contained study animals of the proposed minimum age of calves from the day of birth. One of the studies evaluated the associated use of Bovilis Nasalgen-C with Bovilis INtranasal RSP Live.

Additional studies were a pilot dose finding study, and a laboratory study evaluating the efficacy against BRSV and PI3 after associated use.

Study title
Onset of immunity against bovine coronavirus challenge five days after intranasal vaccination with bovine coronavirus live intranasal vaccine in 1-week old MDA negative calves
Onset of immunity of bovine coronavirus (BCV) given by intranasal route to calves at day of birth without maternally derived antibodies against experimental infection with a European BCV strain five days post vaccination
Dose confirmation of bovine coronavirus live intranasal vaccine with and without concomitant application of Bovilis INtranasal RSP live vaccine in 1-week old MDA negative calves challenged with BCV 3 weeks post vaccination
Efficacy of a bovine coronavirus (BCV) live vaccine given by intranasal route to 5-7 days old MDA-positive calves against experimental infection with BCV 3 weeks post vaccination (dose confirmation study)
Duration of immunity of BCV given by intranasal route to calves at day of birth without maternally derived antibodies against experimental infection with bovine coronavirus twelve weeks post vaccination
Efficacy of two different doses of bovine coronavirus (BCV) live intranasal vaccine given by intranasal route to calves at 1 week of age without maternally derived antibodies against experimental infection with BCV three weeks post vaccination
Efficacy of Bovilis INtranasal RSP live in concomitant non-mixed use with Bovine Coronavirus live intranasal vaccine in one week old MDA negative calves tested by infection with parainfluenza type 3 one week post vaccination and infection with bovine respiratory syncytial virus three weeks post vaccination

Laboratory trials

Two studies evaluated onset of immunity (OOI) at 5 days. One OOI study included animals vaccinated at 1 week of age and the other included animals vaccinated at day of birth. Two studies evaluated duration of immunity (DOI) at 21 days, and included animals vaccinated at 1 week of age, and one study evaluated DOI at 12 weeks, and included animals vaccinated at day of birth. One DOI study included associated use of Bovilis Nasalgen-C with Bovilis INtranasal RSP Live. One DOI study included maternally derived antibody (MDA)-positive animals. The recommended intranasal administration route was used in all studies.

Dose determination

The proposed dose of 5.4–7.8 log_{10} $TCID_{50}$ for Bovilis Nasalgen-C was established based on the findings of a pilot dose determination study, considered supportive only. In this study efficacy, doses of 4.8 log_{10} $TCID_{50}$ and 5.8 log_{10} $TCID_{50}$ at passage level MSV were evaluated using animals one-week old at vaccination. Challenge was performed with a US strain on D19. The challenge caused mild respiratory disease in 5 out of 6 control animals and in 1 out of 6 of vaccinated animals in each group. It is unclear how the proposed dose was selected based on the results obtained from this dose finding study, but this is acceptable considering that efficacy of the vaccine was thereafter investigated at the proposed minimum dose at the most attenuated passage level in the pivotal laboratory efficacy studies.

Onset of immunity

Two laboratory studies were carried out to investigate the OOI. The first study included calves of 1 week of age at vaccination, and in the second study calves were vaccinated at day of birth (<24 hours old).

In study **"OOI against BCV challenge five days after intranasal vaccination with BCV live intranasal vaccine in 1-week old MDA- calves"** two groups of 6 animals age 7–9 days were used. A vaccine dose of 5.4 log_{10} $TCID_{50}$ from production scale batch, was administered to group 2 by the recommended intranasal route. Group 1 were unvaccinated. All animals were challenged with BCV US strain MN-1988 by aerosol 5 days after vaccination. Clinical signs of URTD, nasal and faecal virus excretion and lung viral load were monitored for 14 days post-challenge.

Results: All control animals shed virus nasally and rectally after challenge and showed clinical signs of upper respiratory disease.

A significant reduction in the mean duration, average maximum viral load, and AUC of nasal viral shedding was observed in vaccinated calves compared to controls. A significant reduction in the mean duration, average maximum viral load, and AUC of faecal viral shedding was observed in vaccinated calves compared to controls.

Regarding reduction in clinical signs of URTD, the total sum of scores for URTD were 6.0 for the control group and 4.2 for the vaccinated calves. A maximum value of 3 per parameter and day could be obtained. The difference between vaccinated calves compared to controls was not significant ($p=0.16$). Concerning reduction of lung viral load, the average number of RNA copies (log_{10}/µl) in BAL was 4.00 for the control group and 2.09 for the vaccinated calves. The difference between vaccinated calves compared to controls was not statistically significant ($p=0.06$).

Vaccination upregulated the mRNA transcription levels of several immunity target genes.

This study, performed on one-week-old MDA- calves, supports an OOI of 5 days for reduction of nasal and faecal shedding. Clinical signs of URTD were mild in both groups, with numerically lower scores in the vaccinated calves compared to controls, although the difference was not statistically significant. The clinical relevance of the reduction in clinical signs of URTD is considered limited. A reduction in lung viral load was not supported.

In study **"OOI of BCV given by intranasal route to MDA- calves at day of birth against experimental infection with an EU BCV strain five days post vaccination"** two groups of 5 MDA negative animals age <24 hours were used. A vaccine dose of 5.4 log_{10} $TCID_{50}$ from production scale batch, was administered to group 2 by the recommended intranasal route. Group 1 were unvaccinated. All animals were challenged with BCV EU strain 19 by aerosol 5 days after

vaccination. Clinical signs of URTD, nasal and faecal virus excretion and lung viral load were monitored for 14 days post-challenge.

Results: A significant reduction in the mean duration or average maximum viral load of nasal viral shedding was not observed in vaccinated calves compared to controls, but a significant reduction in the AUC for nasal shedding was observed. A statistically significant reduction in the mean duration and AUC of faecal viral shedding was observed in vaccinated calves compared to controls but the maximum viral load of faecal shedding was not significantly reduced. The total sum of scores for URTD were 4.8 for the control group and 3.4 for the vaccinated calves. The difference between vaccinated calves compared to controls was not significant (p=0.44). The average number of RNA copies (log_{10}/µl) in BAL was 2.80 for the control group and 1.80 for the vaccinated calves. The difference between vaccinated calves compared to controls was not significant (p=0.15).

Vaccination upregulated the mRNA transcription levels of several immunity target genes.

In conclusion, an OOI of 5 days was supported for a reduction in nasal and faecal viral shedding for MDA-negative calves vaccinated at 1 week of age, and for calves vaccinated at day of birth. According to Ph. Eur. monographs 1176 and 1177 (considered in support of this application) a "notable" reduction in clinical signs is required for the vaccine virus to comply with the immunogenicity test. No unambiguous and objective definition for the term "notable" has been found, but it is understood to mean something less than statistically significant. The scores for clinical signs of URTD in both OOI studies were numerically lower for the vaccinated calves compared to the controls. In both studies the clinical signs after challenge were mild in both groups, and the difference between groups was small. Although some trend towards better reduction of URTD signs in vaccinated group was shown, the clinical relevance of this reduction is considered limited. A significant reduction in lung viral load at OOI has not been shown. Onset of immunity has not been investigated in MDA-positive animals, but data from the MDA study demonstrates that efficacy of vaccination was not notably influenced by the presence of MDAs.

Duration of immunity

Three laboratory studies were carried out to investigate the DOI. Two of the studies included calves of 1 week of age at vaccination, one was performed with MDA-positive animals and is described below under heading MDA. The other study investigated the associated use with Bovilis INtranasal RSP Live. One DOI study was performed in MDA-negative calves <24 hours old at the time of vaccination.

In study **"Dose confirmation of BCV live intranasal vaccine with and without concomitant application of Bovilis INtranasal RSP live vaccine in 1-week old MDA- calves challenged with BCV 3 weeks post vaccination"** three groups of 6 animals age 4–9 days were used. A vaccine dose of 5.4 log_{10} $TCID_{50}$ from production scale batch, was administered to groups 2 and 3 by the recommended intranasal route. Group 3 calves were also vaccinated with Bovilis INtranasal RSP Live (BRSV-PI3 vaccine). Group 1 calves were left unvaccinated. All animals were challenged with BCV US strain MN-1988 by aerosol 21 days after vaccination. Clinical signs of URTD, nasal and faecal virus excretion and lung viral load were monitored for 14 days post-challenge.

Results: A significant reduction in the mean duration, average maximum viral load, and AUC of nasal viral shedding was observed in vaccinated calves of both groups compared to controls. The live virus titre (log_{10}/µl) in BAL was 4.7, 1.1 and 0.6 for the control, BCV and BCV/BRSV-PI3 vaccinated groups respectively. The difference between vaccinated calves compared to controls was significant for both groups (all p<0.005).

A significant reduction in the mean duration and AUC of faecal viral shedding was observed in vaccinated calves (both groups) compared to controls but the average maximum viral load of faecal viral shedding was not significantly reduced. The total sum of scores for URTD were 10.3 for the control group and 6.3 for the BCV vaccinated calves and 6.2 for the BCV/BRSV-PI3 vaccinated calves. The difference between vaccinated calves compared to controls was not significant (p=0.45 and p=0.64 for the BCV group and the BCV/BRSV-PI3 group, respectively).

Vaccination upregulated the mRNA transcription levels of several immunity target genes.

In study **"Duration of immunity of BCV given by intranasal route to calves at day of birth without maternally derived antibodies against experimental infection with bovine coronavirus twelve weeks post vaccination"** two groups of 7 animals age <24 hours were used. A vaccine dose of 5.1 $\log_{10}$ $TCID_{50}$ (slightly lower dose than the proposed minimum dose of 5.4 $\log_{10}$ $TCID_{50}$/dose) from production scale batch, was administered to group 2 by the recommended intranasal route. Group 1 calves were left unvaccinated. All animals were challenged with BCV US strain MN-1988 by aerosol 84 days (12 weeks) after vaccination. Clinical signs of URTD, nasal and faecal virus excretion and lung viral load were monitored for 14 days post-challenge.

Results: A significant reduction in the mean duration and AUC of nasal viral shedding was observed in vaccinated calves compared to controls. The average maximum nasal viral load was 6.2 and 5.6 $\log_{10}$ RNA copies/µl for the control and BCV vaccine group respectively (p=0.0530). A significant reduction in faecal shedding was not observed for either duration, average maximum titres, or AUC.

The average total sum of scores for clinical signs of URTD was 12.7 for the control group and 7.6 for the BCV vaccinated calves. The difference between groups was statistically significant (p=0.0227). The average sum of scores for LRTD was 1.9 (control group) and 4.4 (BCV vaccine group). The difference between groups was not statistically significant (0.2780).

In conclusion, a DOI of 84 days (12 weeks) was supported for a reduction in nasal viral shedding and a reduction in clinical signs of URTD for MDA-negative calves vaccinated at <24 hours or 1 week of age. The reduction in clinical signs of URTD was significantly different between vaccinated calves and the control group in study 21-46-053, however, the clinical signs in both groups were mild and the difference between groups was small. For a reduction in faecal viral shedding, a DOI of only 3 weeks has been supported and only in 1-week old calves. A significant reduction in lung viral load was seen at both 3 and 12 weeks DOI but was not shown at OOI. The claims for a reduction in faecal shedding and a reduction in lung viral load are thus not accepted.

Calves administered the BCV vaccine alone showed comparable results for all investigated parameters to calves administered the BCV vaccine in associated use with Bovilis INtranasal RSP live, indicating that the associated use does not affect the efficacy of Bovilis Nasalgen-C.

Maternally derived antibodies (MDA)

One study was carried out in MDA-positive animals to investigate the DOI.

In study **"Efficacy of a BCV live vaccine given by intranasal route to 5-7 days old MDA+ calves against experimental infection with BCV 3 weeks post vaccination"** two groups of 6 animals age 5–8 days were used. A vaccine dose of 5.4 $\log_{10}$ $TCID_{50}$ from a production scale batch, was administered to group 2 by the recommended intranasal route. Group 1 calves were left unvaccinated. All animals were challenged with BCV US strain MN-1988 by aerosol 21 days after vaccination. Clinical signs of URTD, nasal and faecal virus excretion and lung viral load were

monitored for 14 days post-challenge.

Results: A significant reduction in the mean duration, average maximum viral load, and AUC of faecal viral shedding was observed in vaccinated calves compared to controls. The average live virus titre (log_{10}/µl) in BAL was 4.0 and 2.8 for the control and BCV vaccinated groups respectively. The difference between vaccinated calves compared to controls was statistically significant (p=0.03).

A significant reduction in the mean duration and AUC of nasal viral shedding was observed in vaccinated calves compared to controls (p<0.02), but the average maximum viral load was not significantly reduced (p=0.13). The total sum of scores for URTD were 16.7 for the control group and 11.5 for the vaccinated calves. The difference between vaccinated calves compared to controls was not significant (p=0.29).

Vaccination upregulated the mRNA transcription levels of several immunity target genes.

In conclusion, a DOI of 21 days was supported for a reduction in nasal and faecal viral shedding and a reduction in lung viral load for MDA-positive calves vaccinated at 1 week of age.

The results of this study are comparable to those of other studies (type and magnitude of effects) performed in animals without maternally derived immunity. There is no concern identified that efficacy of vaccination should be notably influenced by the presence of MDAs.

Interactions

One laboratory study investigated the efficacy of Bovilis Nasalgen-C in associated use with Bovilis INtranasal RSP Live in 1-week-old calves, summarised above ("Dose confirmation of BCV live intranasal vaccine with and without concomitant application of Bovilis INtranasal RSP live vaccine in 1-week old MDA- calves challenged with BCV 3 weeks post vaccination"). Calves administered Bovilis Nasalgen-C alone showed comparable results for all investigated parameters to calves administered the BCV vaccine in associated use with Bovilis INtranasal RSP Live, indicating that the associated use does not affect the efficacy of Bovilis Nasalgen-C. The two vaccines should be administered in separate nostrils. Correct information regarding associated use is available in the SPC.

One laboratory study investigating the efficacy of Bovilis INtranasal RSP Live in associated use with Bovilis Nasalgen-C in one-week old calves was also included and will be used to support a future variation application for this product.

Field trials

The CVMP agreed in the Scientific Advice (EMA/CVMP/138385/2020) that the absence of field efficacy data could be acceptable for this vaccine provided that efficacy was supported by the results of laboratory studies with an experimental challenge mimicking disease occurring in the field and performed in animals relevant to the field situation (i.e. maternal antibodies, challenge strain). See comment in the introduction to part 4 regarding the omission of field studies.

Overall conclusion on efficacy

The dose of 5.4–7.8 log_{10} $TCID_{50}$ was established based on a pilot dose finding study and supported by the efficacy laboratory studies.

The efficacy parameters as provided in Ph. Eur. 1176 and 1177, investigated in the efficacy studies, were clinical signs of upper respiratory tract disease (URTD), nasal and faecal shedding of virus and lung viral load. Results from the five pivotal laboratory studies are summarised in the table below.

Study	**OOI**	**OOI**	**DOI + associated use**	**DOI**	**DOI**
Age/MDA status	1 week MDA-	<24 hours MDA-	1 week MDA-	1 week MDA+	<24 hours MDA-
Challenge	5 days		21 days		12 weeks
Challenge strain	BCV US	BCV EU	BCV US	BCV US	BCV US
Clinical signs URTD	6.0 controls 4.2 BCV *(p=0.1558)*	4.8 control 3.4 BCV *(p=0.4444)*	10.3 controls 6.3 BCV *(p=0.4134)* 6.2 BCV+ BRSV/PI3 *(p=0.7554)*	16.7 controls 11.5 BCV *(p=0.29)*	12.7 controls 7.6 BCV *(p=0.0227)*
Nasal viral shedding	Significant difference in average duration and average max titre and average AUC.*	No significant difference in average duration and average max titre. Significant difference in average AUC.	Significant difference in average duration and average max titre and average AUC.	Significant difference in average duration and AUC. No significant difference in average max titre	Significant difference in average duration and average AUC. No significant difference in average max titre *(p=0.053)*.
Faecal viral shedding	Significant difference in average duration and average max titre and average AUC.	Significant difference in average duration and average AUC. No significant difference in average max titre.	Significant difference in average duration and average AUC. No significant difference in average max titre.	Significant difference in average duration and average max titre and average AUC.	No significant difference in average duration or mean max titre or average AUC.
Lung viral load	Not significant *(p=0.0628)*	Not significant *(p=0.1508)*	Significant *(p<0.05)*	Significant *(p=0.03)*	Significant *(p<0.05)*

* A significant difference present or not present between vaccinated group and control group (p<0.05).

In conclusion, the claim to reduce nasal shedding is adequately supported for active immunisation of cattle from the day of birth, with an OOI of 5 days and a DOI of 12 weeks.

The claim to reduce clinical signs of URTD at OOI can be accepted for calves <24 hours of age based on the totality of data, with numerically lower scores for the vaccinated calves compared to the controls in both OOI studies. In all efficacy studies the clinical signs of URTD after challenge were mild, and the differences between groups were small. The clinical relevance of this reduction is considered limited. A statistically significant difference in the reduction of clinical signs of URTD between the vaccinated calves and the controls was present in the 12 week DOI study only. Nonetheless, based on the totality of data available, with a trend towards a lower scoring for clinical signs of URTD in the vaccinated calves consistently present in all studies, the claim to reduce clinical signs of URTD is considered acceptable.

A reduction of faecal shedding at DOI of 3 weeks only is supported and only in one-week-old calves. The claim has therefore been removed from the indication. The claim for a reduction of lung viral load has not been verified at OOI and has also been removed from the indication.

Part 5 – Benefit-risk assessment

Introduction

Bovilis Nasalgen-C is a live viral vaccine containing the attenuated strain bovine coronavirus (BCV) CA25.

The CVMP considers that the live attenuated BCV-CA25 is a new active substance, as claimed by Intervet International B.V.

The applicant initially claimed that the product was intended for use in cattle for reducing clinical signs of respiratory disease, nasal and faecal viral shedding, and lung viral load from infection with bovine coronavirus (BCV). The proposed dose is 5.4–7.8 $\log_{10}$ $TCID_{50}$, intranasal administration of one dose.

Bovilis Nasalgen-C provides a new treatment in principle because there are currently no available vaccines for respiratory bovine coronavirus infection in cattle.

The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC – full application.

Benefit assessment

Direct therapeutic benefit

The proposed benefit of Bovilis Nasalgen-C was its efficacy in reducing clinical signs of respiratory disease, nasal and faecal viral shedding, and lung viral load from infection with bovine coronavirus (BCV), which was investigated in five pivotal laboratory studies conducted to an acceptable standard. However, the claims for a reduction in lung viral load and a reduction in faecal viral shedding were not fully supported. These two claims were therefore removed from the indication.

The onset of immunity is claimed at 5 days after vaccination.

The duration of protection is claimed at 3 months after vaccination.

An OOI of 5 days and a DOI of 12 weeks have been shown for the proposed minimum age category (calves from the day of birth onwards) for a reduction in clinical signs of URTD and a reduction in nasal viral shedding.

Additional benefits

Bovilis Nasalgen-C is easy to apply and increases the range of available treatment possibilities for respiratory bovine coronavirus infection in cattle.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product has to a great extent 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 used in clinical trials have had a satisfactory and uniform performance.

Safety:

Administration of Bovilis Nasalgen-C in accordance with SPC recommendations is generally well tolerated. The reported adverse reactions include mild respiratory signs, ocular discharge, and rise in body temperature. The effects were mild and transient. The safety of the target animals when the vaccine is administered according to the recommended schedule and via the recommended route is acceptable.

Risk for the user:

The CVMP concluded that user safety for this product is acceptable when used according to the SPC. No safety advice in the SPC is required.

Risk for the environment:

Bovilis Nasalgen-C is not expected to pose a risk for the environment when used according to the provisions in the SPC.

Risk for the consumer:

Bovilis Nasalgen-C is not expected to pose a risk for the consumer. A withdrawal period of zero days is accepted.

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 and on how to prevent or reduce these risks.

The CVMP concluded that user safety is acceptable when used according to the SPC. No safety advice in the SPC is required.

Bovilis Nasalgen-C is not expected to pose a risk for the consumer. A withdrawal period of zero days is accepted.

Bovilis Nasalgen-C is not expected to pose a risk for the environment when used according to the provisions in the SPC.

Evaluation of the benefit-risk balance

At the time of submission, the applicant applied for the following indication: for the active immunisation of calves from the day of birth onwards to reduce clinical signs of respiratory disease, nasal and faecal viral shedding and lung viral load from infection with bovine coronavirus (BCV).

Onset of immunity: 5 days.

Duration of immunity: 3 weeks.

The product has been shown to be efficacious for a reduction in clinical signs of URTD and a reduction in nasal viral shedding, and the CVMP agreed to the following indications:

For the active immunisation of cattle from the day of birth onwards to reduce clinical signs of upper respiratory tract disease and nasal viral shedding from infection with bovine coronavirus.

Onset of immunity: 5 days.

Duration of immunity: 12 weeks.

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

Based on the data presented, the overall benefit-risk is considered positive.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Veterinary Medicinal Products (CVMP) concluded that the application for Bovilis Nasalgen-C 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 medicinal product.