5 October 2023
EMA/460610/2023
Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Bovilis Cryptium (EMEA/V/C/006045/0000)

Vaccine common name: Bovine *Cryptosporidium parvum* vaccine

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 19 May 2022 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Bovilis Cryptium, through the centralised procedure under Article 42(2)a of Regulation (EU) 2019/6 (**mandatory scope**).

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

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

"Active immunisation of pregnant cows to raise antibodies in colostrum against Gp40 of *Cryptosporidium parvum*.
When calves are fed this colostrum, these antibodies have been demonstrated to reduce disease (i.e. diarrhoea, morbidity and mortality) caused by *C. parvum*."

The target species is cattle. The active substance of Bovilis Cryptium is *Cryptosporidium parvum* glycoprotein Gp40. It actively immunises pregnant cows to raise antibodies in colostrum against Gp40 of *C. parvum*. Calves that ingest such colostrum are then passively immunised against *C. parvum*; this was demonstrated to reduce disease (i.e. diarrhoea, morbidity and mortality) caused by *C. parvum*.

Bovilis Cryptium emulsion for injection contains ≥ 1 U of *Cryptosporidium parvum* glycoprotein Gp40 and is presented in packs containing 1 x 10 ml (5 doses), 1 x 100 ml (50 doses), 1 x 40 ml (20 doses) and 10 x 2 ml (10 x 1 dose).

The rapporteur appointed is Frédéric Klein and the co-rapporteur is Merete Blixenkrone-Møller.

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

On 5 October 2023, the CVMP adopted an opinion and CVMP assessment report.

On 23 November 2023, the European Commission adopted a Commission Decision granting the marketing authorisation for Bovilis Cryptium.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Summary of the Pharmacovigilance System Master File

The applicant has provided a summary of the pharmacovigilance system master file which fulfils the requirements of Article 23 of Commission Implementing Regulation (EU) 2021/1281. Based on the information provided the applicant has in place a pharmacovigilance system master file (PSMF), has the services of a qualified person responsible for pharmacovigilance, and has the necessary means to fulfil the tasks and responsibilities required by Regulation (EU) 2019/6.

Manufacturing authorisations and inspection status

The manufacturing and control testing of both the active substance and the finished product as well as batch release of the finished product is performed at Intervet International (Boxmeer, Netherlands), except for 2 quality tests which are performed by another site in the Netherlands.

Both Intervet International (Boxmeer, Netherlands) and the other site have been inspected and have valid GMP certificates.

Overall conclusions on administrative particulars

The summary of the pharmacovigilance system master file is considered to be in line with legal requirements.

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

Part 2 - Quality

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

Qualitative and quantitative composition

Composition

Bovilis Cryptium is a ready-for-use inactivated vaccine for active immunisation of pregnant heifers and cows to provide passive immunity to newborn calves against neonatal diarrhoea (scours) caused by *Cryptosporidium parvum*. The finished product is a water-in-oil emulsion with the active substance (*C. parvum* Gp40) and aluminium hydroxide in the aqueous phase and Montanide in the oil phase. The vaccine (2 ml per dose) is marketed as 1-, 5-, 20- and 50-dose presentation. The other excipients are HEPES, sodium chloride, thiomersal and water for injections.

Container and closure system

The product is filled in two types of containers. The single dose presentation is filled in glass type I containers (3 ml). The 5-dose presentation may be filled in 10 ml glass containers but also in 10 ml containers made of polyethylene terephthalate (PET). The larger presentations are only filled in PET containers (50 ml and 100 ml). Both types of containers are closed with a rubber stopper and are sealed with an aluminium cap. The rubber stoppers are not removed from the containers for use, the content of the container is withdrawn from the container by piercing the needle through the rubber stopper.

Certificates of analysis are provided for each container type. Materials used for the container are compliant with applicable Ph. Eur. requirements. Glass vials, PET vials and stoppers are sterilised by different methods.

Product development

Currently, there is no vaccine for the prevention or reduction of cryptosporidiosis. Therefore, a recombinant vaccine was developed which consists of cryptosporidium glycoprotein 40 (Gp40) derived from the most prevalent cryptosporidium related to newborn calf diarrhoea, *C. parvum*. The glycoprotein is present on the outer membrane and is shed during gliding motion of multiple infective parasite life cycle stages. It is believed to have a role in cell attachment and invasion.

The Gp40 protein was produced by recombinant DNA technology using a baculovirus expression system in a continuous insect cell line. The Gp40 antigen is purified and residual virus is inactivated. The Bovilis Cryptium vaccine contains Gp40 antigen as active ingredient and Montanide (light mineral oil) and aluminium hydroxide as adjuvants. Thiomersal is added as preservative to ensure sterility in multi-dose presentations. The vaccine will be available in 1-dose, 5-dose, 20-dose and 50-dose containers with an in-use shelf life of 28 days.

The selected light mineral oil plus aluminium hydroxide adjuvant system is the same as the one used in the applicant's product Bovilis Rotavec Corona. This adjuvant system induces good immune responses in cattle and is well tolerated by the target animal.

Choice was made for thiomersal as preservative in the final vaccine formulation. This compound is widely and successfully used as a preservative for inactivated vaccines at the specified concentration. According to EMEA/MRL/140/96 thiomersal can be safely added to multidose vaccines as a preservative at a concentration not exceeding 0.02%.

Information and antigen content results were provided for the batches used in the clinical trials.

Description of the manufacturing method

The antigen production starts with growing and upscaling of cells of an insect cell line (*Spodoptera frugiperda*), which is followed by inoculation of these cells with baculovirus that contains a gene for the Gp40 antigen. After incubation and expression of the Gp40 antigen the harvest is clarified and the Gp40 antigen is purified. After sterile filtration the Gp40 antigen is treated to inactivate any residual baculovirus. After the inactivation step the antigen can be stored at 2-8 °C.

The final vaccine production consists of the formulation of all different vaccine components: aluminium hydroxide, antigen, thiomersal, Montanide and other excipients. The final bulk is then filled in vials and stored at 2-8 °C. All steps of the production process and quality control are performed in accordance with Good Manufacturing Practice (GMP).

Process validation: The manufacturing process of the antigen and the finished product is adequately validated as demonstrated by the batch data from consecutive antigen batches and finished product batches. The results show that all control test requirements were met and that the batches are of consistent quality. The hold time of the final bulk and the inactivation of residual baculovirus were also appropriately validated (in accordance with Ph. Eur. 0062).

Production and control of starting materials

Starting materials listed in pharmacopoeias

The applicant has listed all materials used during vaccine production that are described in the European Pharmacopoeia, including the aluminium hydroxide adjuvant and the thiomersal preservative. Examples of certificates of analysis were provided for all compounds confirming compliance with Ph. Eur. requirements.

Starting materials not listed in a pharmacopoeia

Starting materials of biological origin

Cell substrate

A *Spodoptera frugiperda* (American fall armyworm) cell line is used as the substrate to propagate the recombinant baculovirus producing recombinant Gp40 antigen. Information is provided on the origin of the cell bank system, the generation of the master cell stock (MCS) and working cell stock (WCS) as well as on the testing and qualification of the MCS and WCS. It is concluded that the cell bank system is properly qualified.

Recombinant baculovirus

For recombinant production of the Gp40 protein, the Gp40 gene was cloned in a baculovirus expression vector. The genetic engineering of the recombinant baculovirus was described, as well as the generation and testing of the master seed virus (MSV) and working seed virus (WSV). Genetic stability was demonstrated. The virus seed system is properly qualified.

Medium

The medium that is used for vaccine production contains cholesterol that is derived from ovine wool grease and cod fish liver oil. Certificates are provided for both compounds. There is no risk for TSE or for contamination by extraneous agents.

Starting materials of non-biological origin

The applicant provided information on the non-biological starting materials. Certificates of analysis with the compound specifications were provided.

A risk analysis was performed with regard to TSE. It is concluded that there is no risk that TSE infectivity is present in the starting materials or could be propagated during vaccine production or that TSE infectivity could be transmitted by the vaccine.

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

Information was provided on the composition of the media used during vaccine production as well as on the media storage conditions and the method of sterilisation.

Control tests during the manufacturing process

The following tests are performed during production of the Gp40 antigen.

- Bioburden (after diafiltration and before sterilisation by filtration)
- Baculovirus titre (before inactivation)
- Inactivation control (after inactivation)
- Residual sodium thiosulphate (after inactivation)
- Sterility (after inactivation)
- Antigen content (after inactivation)
- Filling volume (during filling)

Methods for baculovirus titre, inactivation control and antigen content were appropriately validated in accordance with VICH guidelines GL1 and GL2. The purity profile of the antigen has been assessed and was shown to be consistent between batches and to remain stable during the antigen shelf life.

Control tests on the finished product

The following tests are carried out on the finished product, either on vaccine bulk or on a representative sample of the filled product.

- Appearance, conductivity and viscosity
- Identification and antigen content test by ELISA

- Aluminium content
- Montanide content test
- Thiomersal content
- Sterility

The tests for sterility and conductivity are performed in accordance with Ph. Eur. All non-compendial methods were appropriately validated.

No *in vivo* potency assay is included in the finished product specifications. This approach is acceptable, provided an appropriate *in vitro* potency assay is part of the specifications. The applicant showed that the antigen content ELISA is capable of discriminating between intact antigen and degraded antigen. The ELISA method was also adequately validated. Therefore, it seems that the ELISA to determine the Gp40 antigen content in the finished product is deemed acceptable as potency assay. In addition, the acceptance limits for potency have been sufficiently justified. The purity profile of the antigen has been assessed and was shown to be consistent between batches and to remain stable during the antigen shelf life. These purity results are also representative for the finished product.

Batch-to-batch consistency

Batch data were provided for consecutive batches of finished product, including in-process control test results (during production of antigen and finished vaccine) and results from testing on finished product. All results were compliant with the specifications and shown to be consistent.

Stability

Antigen stability

The applicant has provided stability data for finished product lots that were formulated with antigen that had been stored for 25-29 months at 2-8 °C. Stability results for finished product lots up to 21 months are all compliant with the specifications and do not show any trend. Therefore, the proposed shelf life of 24 months is deemed justified and acceptable for the antigen when stored at 2-8 °C.

Finished product stability

The applicant has initiated stability studies with pilot scale batches and commercial scale batches of the finished product. The initially proposed shelf life is 18 months at 2-8 °C. The stability study will be continued until 27 months. For the commercial scale lots, stability data up to 24-27 months are available for consecutive batches. For the pilot scale batches, data up to 27 months are available. All available results comply with the specifications. As such, a shelf life of 24 months is acceptable for the finished product.

The applicant also performed accelerated stability studies at 30 °C, showing that exposure to 30 °C for 3 days does not impact the quality of the vaccine.

Importantly, the applicant has also tested the anti-Gp40 antibody response after vaccination with a batch that was at least 2 years old. The 2-year-old vaccine induced anti-Gp40 antibody titres in colostrum that were comparable to those induced by freshly made vaccine. These findings confirm the proposed shelf life of 24 months.

In-use stability

The proposed in-use shelf life is 28 days when stored at 2-8 °C.

The applicant has performed a preservative efficacy study in accordance with Ph. Eur. 0062 and Ph. Eur. 5.1.3 on finished product at 27 months. The results from the preservative efficacy study showed acceptable preservative efficacy for Bovilis Cryptium vaccine with thiomersal 27 months after start of shelf life. These results are deemed sufficient to justify the proposed in-use shelf life of 28 days for Bovilis Cryptium vaccine when stored at 2-8 °C.

New active substance (NAS) status

Bovilis Cryptium contains *Cryptosporidium parvum* glycoprotein Gp40, expressed in recombinant baculovirus/insect cell system, as the active substance.

The applicant requested the active substance *Cryptosporidium parvum* Gp40 contained in Bovilis Cryptium to be considered a new active substance as it is novel and not hitherto authorised in a veterinary medicinal product in the European Union.

Based on the review of the data provided, the CVMP considered that the active substance *Cryptosporidium parvum* Gp40 contained in the veterinary medicinal product Bovilis Cryptium is to be qualified as a new active substance considering that this antigen has not previously been used as active substance in any registered veterinary medicinal product in the European Union.

Overall conclusions on quality

The applicant has adequately described the composition and development of the vaccine and of its active ingredient, the manufacturing process, the tests performed during manufacture and on the finished product, the batch data and the stability data. Quality control of starting materials is adequately performed; the cell bank system (used for vaccine production) and the recombinant baculovirus system were appropriately tested and qualified. The manufacturing process was shown to be properly validated. The Gp40 antigen ELISA was demonstrated to be suitable as potency assay. Validation reports of the non-compendial analytical methods confirmed their validated state. The stability data support the proposed shelf lives of the antigen and the finished product.

In conclusion, Part 2 of the registration file is considered acceptable.

Part 3 – Safety documentation (safety and residues tests)

General requirements

Bovilis Cryptium is a subunit vaccine emulsion adjuvanted with mineral oil and aluminium, devised very much like another vaccine for passive immunisation against other neonatal diseases, Bovilis Rotavec Corona. The antigen is a surface protein of *C. parvum* purified after synthesis in a baculovirus-insect cell expression system. During the primary vaccination, 2 doses of vaccines need to be injected subcutaneously to pregnant heifers 4 to 5 weeks apart, in the 12 to 3 weeks' period before calving.

Since there is no pharmacopoeia monograph dealing with *C. parvum* vaccines, the applicant followed the general requirements to document the safety.

Safety documentation

A batch dosed at twice the antigenic payload (3 AU) was used in a preclinical study and information about alternative injection sites (the ischiorectal fossa or in the dewlap) was gained with a batch dosed at 1.5 AU. Eventually, one dedicated clinical trial and two efficacy clinical trials provided complementary safety information about cows of various ages.

Study title	
Safety of Bovilis Cryptium upon repeated (3 times) subcutaneous administration in pregnant heifers in the last trimester of pregnancy	3 AU/dose
Safety of Bovilis Cryptium upon subcutaneous administration in the ischiorectal fossa or dewlap of pregnant heifers in the last trimester of pregnancy	1.5 AU/dose
A clinical study in the Netherlands to assess the safety of Bovilis Cryptium in pregnant dairy cattle	1.5 AU/dose
A clinical study in the Netherlands to assess the efficacy of Bovilis Cryptium against cryptosporidiosis in dairy calves	
A clinical study in France to assess the efficacy of Bovilis Cryptium against cryptosporidiosis in beef calves	

Pre-clinical studies

The two preclinical safety studies were performed in pregnant Holstein Frisian or cross breed heifers, which are the youngest type of pregnant cows that can be used for these studies. Three subcutaneous routes were explored at the neck, the dewlap and the ischiorectal fossa.

Safety of the administration of one dose

The safety of the administration of one dose (first dose) was investigated in two laboratory studies. These studies are further detailed below.

Safety of one administration of an overdose

The safety of an overdose is not required since Bovilis Cryptium is not a live vaccine.

Safety of the repeated administration of one dose

The primary vaccination schedule for Bovilis Cryptium consists of 2 administrations 4 - 5 weeks apart. In the two pre-clinical studies a 2-dose schedule was investigated in pregnant heifers. The safety of a third administration was also investigated in one of these laboratory study, using a formulation at the highest antigen content (3 AU) which is the double of the targeted dose (1.5 AU).

In a non-controlled study, 10 pregnant heifers were subcutaneously vaccinated with double of the targeted content of Gp40 antigen (3 AU/dose) in the side of the neck at 10, 7 and 4 weeks before the expected date of calving. The heifers were euthanised 8 weeks after calving and the local reactions were inspected by microscopical investigation; this corresponded to 12 weeks after the third and final vaccination. Local and systemic reactions, including rectal temperature, were beforehand investigated around each injection.

Temperatures increased after the second and the third injection returning to normal within a maximum of 5 days (with a maximum of 40.7 °C in two cows after the second vaccination).

Except for 1 day of watery diarrhoea 5 days after the second injection in an animal and a slight lethargy in another heifer 4 days also after the second injection, no other clinical signs were reported.

Extensive, warm and painful local reactions were observed in all vaccinated animals after every vaccination (some extended into the muscular tissue). The injection site reactions increased with the number of injections from a mean swelling of 10 cm (length or width) at the first injection up to 13 cm at the third, with a maximum estimated to 15 x 25 x 3 cm in one heifer. The warm and painful reactions after injection turned painless and cold within 2 to 4 days; however, 19 weeks after the first injection, the inflammatory reactions persisted (swelling reduced to 4-12 cm) and were still granulomatous with vaccine remnants inside. In two of the animals, granulomatous tissue extending into adjacent muscular tissue was found. Analysing the various reactions, it is showed that the swellings are large in size and decrease over the first 14 days to a stable level where they persist until end of study (day 125). What is also showed is the increase of swelling size after the second vaccination when compared to the first vaccination, but even more substantial is the increasing size of the swellings observed after the third vaccination, when compared to both the second and first vaccination.

In the second study, 18 heifers were vaccinated at the shortest time from calving according to the proposed regimen and with Bovilis Cryptium at the formulated dose (1.5 AU/dose). Two other locations of injection were investigated, the dewlap and the ischiorectal fossa; they were chosen because they are usually used in the field and to provide the vaccinator with the choice of injection site which give him the best safety according to the type of farms and rearings. A methodology similar to the previous study was followed except that no microscopical investigation was undertaken.

A slight general reaction was reported after the second injection in 2 heifers (semi-solid faeces and reduced appetite).

A slight increase in temperature was observed 1 day after the second vaccinations (below 38.6 °C or 38.7 °C for the first injection and below 38.7 °C for the second injection).

Local reaction characteristics were similar but slightly larger than in the neck, especially the height of the swellings in the dewlap was measured to a maximum of 15 cm. Pain at palpation was occasionally reported over around 2 weeks in most animals.

Regarding the local reactions, if length and width of local injection reactions after first vaccination in 10 heifers receiving the double dose of antigen (3 AU/dose) in the neck from one study are compared to the 2 x 9 heifers from the second study receiving a vaccination with the intended dose of antigen (1.5 AU/dose) in either the dewlap or the ischiorectal fossa, a tendency towards the size of local reaction in the ischiorectal fossa being smaller than in the neck is observed. Local reactions in the dewlap are, on the opposite, much bigger in some of the included heifers.

The height of the local injection site reaction was not assessed in animals receiving the vaccine in the ischiorectal fossa. This makes it difficult to evaluate and compare size of overall local injection reactions between administration in the neck and at the dewlap. As a result of the missing height of local injection reactions in the ischiorectal fossa, a comparison between local injection reactions after vaccination in the neck and the dewlap, comparing of all data for the 10 heifers included in the first study and the 9 animals in the second study receiving vaccination in the dewlap after second vaccination. Shows that the local reactions are again much bigger in size after administration in the dewlap.

Examination of reproductive performance

Since the vaccine is intended for cows in their last trimester of pregnancy, all the preclinical studies were performed in pregnant heifers.

In the first study, 1 calf out of 10 was found dead (which could have been a stillborn), in the second study, 2 out of18 heifers gave birth to premature calves (both with congenital malformations). No control groups were included.

By and large, the injections did not impact the outcome of the pregnancies.

Examination of immunological functions

Bovilis Cryptium is an inactivated subunit vaccine and is not anticipated to have adverse events on the immune system.

Special requirements for live vaccines

Not applicable.

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 (and EMEA/CVMP/543/03-Rev.1). Bovilis Cryptium is a subunit and oily adjuvanted vaccine intended to be administered by healthcare professionals. The active substance is an inactivated protein and is not infectious to humans, and the excipients are substances commonly used in other vaccines; none of these present a safety concern. Mineral oil was pointed out as the major concern and from the main potential routes of accidental contact with the vaccine the most worrying is accidental self-injection.

To minimise the risk of self-injection, 3 sites of injection have been investigated. Adequate information to address this concern has been included in section 3.5 of the SPC.

In conclusion, the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

Study of residues

The active substance being a principle of biological origin intended to produce immunity is not within the scope of Regulation (EC) No 470/2009.

The excipients, including adjuvants, 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. In that respect, it has been confirmed that thiomersal is used as preservative at a concentration not exceeding 0.02%.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

An associated non-mixed use with Bovilis Rotavec Corona was investigated in a preclinical study and in the 3 clinical trials (c.f. section 4). No modification of the safety profile was reported.

Clinical studies

In one study, the safety of the vaccination with Bovilis Cryptium in association with Bovilis Rotavec Corona was investigated in three Dutch dairy farms. On each farm, 2 groups of 22-23 cows in late pregnancy were enrolled, which were 4.5 years old on average and most of them were Holstein Friesian. One group was subcutaneously administered Bovilis Cryptium and the other saline and both groups were intramuscularly injected with Bovilis Rotavec Corona. General health and feed intake were recorded. The mean increase in rectal temperature the day after the first vaccination was 0.4 °C for both groups and 0.4 °C after the second vaccination in the test group, which does not raise any concerns. Following the first vaccination, local reactions were reported in all animals in the test group for one or more days. The mean maximum size (length or width) of the swellings was 12.5 cm and in two animals the size was more than 30 cm. One cow even had a local injection reaction reaching 40 cm. Notably, local reactions were still present in 41% of the animals 84 days post-vaccination. After the second vaccination the overall mean maximum size of the local reactions was 13.6 cm and in 57% of the animals the local reactions were between 10 and 20 cm in size. Pain was intermittently detected by palpation of the injection site in 40% and 34% of the vaccinates after first and second injection, respectively, with Bovilis Cryptium, while only 5% of the cows injected with Bovilis Rotavec Corona exhibited pain at their injection sites. The maximum period during which pain was intermittently reported between the 2 groups was similar - from few hours after injection to 53 days. However, no repercussion on the well-being and the behaviour of the vaccinates with both vaccines was detected.

Four cows from the control group and 2 from the test group gave birth to a stillborn calf and one cow from the test group aborted twins after 244 days of pregnancy. Necropsy could not relate the

deaths to the vaccine.

In another study, 295 Holstein Friesian cows from eight Dutch farms were enrolled for safety and efficacy investigations and the design of the study was identical to that of the previous one. The safety observations were similar to those in the previous studies and local reactions larger than 20 cm were reported in 3 out of 148 cows of the Bovilis Cryptium group. Two cows (1%) from the control group and nine (6%) from the test group gave birth to a stillborn calf which was considered within the normal range of stillbirths set for this study (11%).

In a third study performed in France, 300 beef cows (mostly Salers and Charolais breed) from 8 farms and between 1.8 and 15.9 years of age were vaccinated for safety and efficacy investigations, which corroborated the previous conclusions. Cows were examined for local reactions after giving birth, and no local reactions larger than 20 cm in diameter were observed. More than 2 weeks after injection, local reactions were present in approximately 10% of the vaccinated animals and the average size (among cows reacting) of reactions was approximately 5-7 cm in diameter. The number of stillborn/aborted calves in the control and test groups were comparable.

General health was recorded in the three clinical trials.

Environmental risk assessment

An environmental risk assessment in accordance with the "Note for guidance environmental risk assessment for immunological veterinary medicinal products" (EMEA/CVMP/074/95) has been provided by the applicant.

The product does not contain any live organisms and none of the product's components, at the used concentrations, are considered to have a toxic effect when directly released into the environment or in the target animal.

Based on the data provided, the ERA can stop at phase I. Bovilis Cryptium is not expected to pose a risk for the environment when used according to the SPC.

Overall conclusions on the safety documentation

The vaccination elicited extensive local injection reactions, which were warm for few a days and intermittently painful upon palpation for up to 8 weeks after injection, persisting for more than 19 weeks. All heifers from the first (n=10) and from the second pre-clinical studies (n=18) had local injection reactions after every vaccination. In the first study all reactions were at least 10 cm after all three vaccinations. In some of the clinical studies only local injection reactions > 20 cm were reported. In one of the clinical studies one cow experienced a swelling reaching 40 cm. Overall, multiple animals from both clinical and preclinical studies had local injection reactions reaching 25, 30 and 35 cm, which is considered large and overall beyond 'normal' manifestations related to injection site reactions. These are adequately reflected in the product information.

The systemic reaction reported was a slight increase of temperature mostly within the physiological range (≤ 39.5 °C) but in some animals up to 40.7 °C.

This safety profile was not modified with the concurrent injection of Bovilis Rotavec Corona by intramuscular route as it was reported in the preclinical study and the 3 clinical trials.

A comprehensive user safety has been provided by the applicant. Mineral oil was pointed out as the major concern and appropriate warning is included in the SPC.

Based on the data provided, the ERA can stop at Phase I. The product is not expected to pose a risk for the environment when used according to the SPC.

Residue studies are not required. The active substance being a principle of biological origin intended to produce immunity is not within the scope of Regulation (EC) No 470/2009. In addition, the other components of this vaccine are either allowed substances listed in Table 1 of the Annex of Commission Regulation (EU) No 37/2010 or considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

The withdrawal period is set at zero days.

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

General requirements

Bovilis Cryptium is a subunit adjuvanted vaccine intended to passively immunise calves against *C. parvum* infection and decrease the associated morbidity caused by diarrhoea. Calves are to be fed with colostrum and subsequent transition milk for 5 days, with at least 3 L colostrum within the first 6 hours after birth. The dams will have been subcutaneously injected with 2 doses of vaccine 4-5 weeks apart, in the 12 to 3 weeks' period before calving and will have been revaccinated each year with 1 dose, given in the 12 to 3 weeks' period before each subsequent calving.

Efficacy was demonstrated in compliance with the Regulation (EU) 2019/6, and the European Pharmacopoeia (Ph. Eur.) chapter 5.2.7.

Challenge model

The experimental model mimicked an acute exposure where calves were given colostrum from vaccinated cows, as recommended above, just before being challenged by *C. parvum* within the same day. They were fed with 10^3-10^4 *C. parvum* sporulated oocysts few hours after the first administration of colostrum. They were then monitored for diarrhoea and their health was scored over 2 weeks.

Efficacy parameters and tests

The applicant has chosen the main clinical sign caused by *C. parvum* infection in calves, the diarrhoea. Its characteristics (severity, duration) and its general consequences (health score and growth) were recorded. The applicant has also monitored an indirect parameter, the anti-Gp40 antibody response, in the dam and, most importantly, in its colostrum.

Efficacy documentation

The batches used in the efficacy studies had been blended at Gp40 antigen input of 1.5 antigen units (AU) per dose except one formulated at 0.4 AU per dose to test the efficacy of a subpotent batch.

Both beef and dairy cows were included in the studies and while they had never been vaccinated against *C. parvum*, they had anti-Gp40 antibody owing to natural exposure to the parasite; this is acceptable because this is a worst-case scenario and represents what is occurring in the field.

The demonstration of the efficacy of Bovilis Cryptium has been divided in 2 steps. In the first step the immune response of the pregnant cows has been characterised in 4 studies together with the compatibility with Bovilis Rotavec Corona and, in the second step, the protection afforded by the colostrum from these vaccinated cows has been monitored in 2 studies with the experimental model. Lastly, the efficacy of the vaccine was investigated in 2 field studies.

Pre-clinical studies

One preclinical study was undertaken to get colostrum from dairy heifers vaccinated with Bovilis Cryptium for experimental challenge and to measure the antibody response to 2 antigen payloads (0.4 or 1.5 AU/dose). Heifers were injected approximately 7 and 3 weeks before calving. Bovilis Rotavec Corona was also injected 1 week after the first Bovilis Cryptium injection. Anti-Gp40 antibodies were titred in sera at the times of injections and 1 week after the last injection, and in the colostrum (2 first milkings). Vaccination with 1.5 AU increased anti-Gp40 IgG titre by 7 $\log_2$ and 0.4 AU vaccine raised titres by 5 $\log_2$, i.e. lower than the one raised by 1.5 AU vaccine. The difference between the 2 dosages was less important in the colostrum (20.4 vs. 21.4 in the first milking, 18.9 vs. 20.1 $\log_2$ in the second milking).

The colostrum was stored frozen for the challenge study.

In another study, devised to monitor the safety and efficacy of alternative locations for injections (cf. section 3), anti-Gp40 IgGs were measured both in sera and colostrum. In both locations, vaccination raised the antibody titres in the blood by 6-7 $\log_2$ and their concentration in the first and second milking was respectively around 22 $\log_2$ and 20 $\log_2$.

Duration of dam's immunity

In another study, the heifers from the study described above (first pre-clinical study) were revaccinated during their second pregnancy. Both the 8 cows from the 1.5 AU group and the 10 cows from the 0.4 AU group were injected in the neck with a vaccine with an antigen payload of 1.5 AU, approximately 8 to 3 weeks before the expected date of calving, at the same time as they were vaccinated with Bovilis Rotavec Corona. A control group was left unvaccinated with Bovilis Cryptium.

The colostrum from the 2 first milkings was dosed and the mean anti-Gp40 titres after revaccination were slightly lower than those after primary vaccination (~1 $\log_2$) and the difference between the 2 groups initially vaccinated with 0.4 AU or 1.5 AU was not abolished by the 1.5 AU boost, but all these differences were not statistically significant. The volume of colostrum delivered by the cows increased by 60% during this second lactation.

Before revaccination, blood titres had only decreased by 1 $\log_2$ by comparison with the first vaccination. Revaccination increased the blood titres by 5 $\log_2$.

Protection in challenge model

In a challenge study, the colostrum collected from cows vaccinated in the first study described above and stored at ≤ -15 °C, was dispensed to newborn calves after thawing. Calves were fed with colostrum from the first milking (3 litres the first day, 1 litre on day 2 and 0.5 litre on day 3) and from the 2nd milking for calves in groups fed with 0.5 litre colostrum up to day 5.

Colostrum from cows vaccinated with Bovilis Cryptium at 1.5 AU/dose was fed for 5 days to 8 calves of group 2. Colostrum from cows vaccinated with Bovilis Cryptium at 0.4 AU/dose was fed for 5 or 3 days to calves (8 per group) of groups 1 and 3, respectively. Moreover, colostrum from cows vaccinated intramuscularly with 3 AU/dose was fed for 5 days to 4 calves of a positive control group, while a colostrum from non-vaccinated cows was fed for 5 days to 8 calves of a negative control group.

Two to four hours after the first colostrum feed, calves were challenged *per os* with 10^3-10^4 oocysts of *C. parvum*.

One calf of the 3-day-feeding of the 0.4 AU group met a humane endpoint defined by the applicant as the clinical score beyond which calves suffer unacceptable pain or distress, and this calf was euthanised. Two out of the 8 calves from the negative control group were found dead on day 7, while no fatalities were reported in the 1.5 AU group. In case of missing values due to mortality, the maximal group score was assigned to the dead calves for subsequent time points.

The protection offered by the vaccination was slightly more important in group 1.5 AU than in the two 0.4 AU groups and both duration and severity of the diarrhoea were reduced in all these groups.

The protection offered by the vaccination tended to be proportional to the antigenic payload and the duration of feeding, as anticipated. In the 1.5 AU group, the mean total diarrhoea score, the frequency of calves with diarrhoea score ≥2 (0-3 scale), as well as the duration of these episodes, were halved (the sum of observation daily averages of the diarrhoea scores was 12.4 in vaccinates and 25.3 in controls).

In a further study, 17 late pregnant beef heifers were vaccinated with Bovilis Cryptium according to the recommended schedule, with Bovilis Cryptium at 1.5 AU or with saline.

Their newborn calves (14) were orally challenged within 6 hours after calving with 10^3 oocysts after having let them suck their own dam. The consistency of the faeces, and the health of the calves was scored twice daily, their load of *C. parvum* oocysts was monitored and anti-Gp40 antibodies were titred in dam blood and colostrum.

The start, duration and severity (0-4 scale) of the diarrhoea associated with *C. parvum* challenge were decreased (the sum of observation daily averages of the diarrhoea scores was 30.9 in vaccinates and 48.6 in controls), the associated mortality abrogated (26.7% in controls) and the daily weight gain was symmetrically increased from 60 g/day in controls to 400 g/day in vaccinates.

Overall, two different challenge models have been used with respect to colostrum delivered by specified feeding regimen to dairy species of calves versus natural suckling by beef species calves.

The environment where the vaccination and challenge took place (in NL and the US) differed. Reportedly the dose of the challenge exposure with *C. parvum* differed 10-fold as well.

The pre-clinical trials in dairy and in suckling beef calves demonstrated that the transfer of maternally derived immunity via colostrum from vaccinated heifers had a certain clinical beneficial effect after experimental challenge with *C. parvum* 2-4 hours after first colostrum feeding/suckling. Colostrum administration took place for five days or suckling during the study period. The test groups showed significantly lower diarrhoea scores and a shorter duration of diarrhoea compared to the control group. No mortality occurred in the vaccinated groups of calves.

Overall, it appears that clinical outcomes with respect to efficacy are similar in the two trials. The period of investigation was 15 days in the study in beef calves and 14 days for the studies in dairy calves.

In addition, the results concerning the weight parameter add to the benefit observed in beef calves of vaccinated heifers. Statistics applied are considered appropriate.

Maternally derived antibodies (MDA)

Not applicable.

Interactions

In a study, the non-mixed use of Bovilis Cryptium with Bovilis Rotavec Corona has been studied in

dairy heifers. Bovilis Rotavec Corona was injected intramuscularly in 21 heifers at the same time as the first Bovilis Cryptium injection, i.e. approximately 7 weeks before the expected date of calving. Two control groups of 21 heifers were vaccinated either with Bovilis Cryptium alone or with Bovilis Rotavec Corona alone.

Seroconversion was measured 2 weeks after the second injection of Bovilis Cryptium and the titres of antibody against rotavirus, coronavirus, *E. coli* F5 and Gp40 were similar either with 1 or with 2 vaccines. The conclusion was identical for the titres in colostrum, especially of the first milking.

Clinical trials

In a clinical trial, the efficacy of Bovilis Cryptium in association with Bovilis Rotavec Corona was investigated in 8 Dutch dairy herds with a history of *C. parvum* infection. In this control trial, 295 pregnant Holstein Friesian cows and 293 calves were enrolled, and 148 cows were vaccinated. The general and local reactions of the vaccinates were monitored.

The calves were allowed to drink as much colostrum/transitional milk from the corresponding milking as they wanted. The intake of colostrum was checked by Gp40 serology performed between 3 to 7 days after birth. They were monitored over their first 21 days after birth for clinical health including feed intake and when diarrhoea happened a diagnostic test checking for the most prevalent causative agent was undertaken.

Diarrhoea occurred in around 71.4% of the controls and cryptosporidiosis was detected in 75% of them, *Clostridium perfringens* (>10^6 CFU/g faeces) in 37% and rotavirus in 22%.

While there was a clear increase of anti-Gp40 antibodies both in the colostrum of vaccinated cows and in the calves fed with it, the clinical results were less obvious and only the decrease of diarrhoea duration was statistically significant in passively immunised calves.

In another clinical trial, the same methodology was applied to investigate the efficacy of Bovilis Cryptium in association with Bovilis Rotavec Corona in 8 French herds with a history of *C. parvum* infection, where 152 beef cows were vaccinated and 148 were included as control.

However, diarrhoea was much less frequent in all calves (30.6% in the control group, and 28.1% in the test group) and no statistically significant results were recorded, whereas all the efficacy parameters exhibited a positive trend in the passively immunised calves except feed intake. *C. parvum* was detected in 43% of control calves and also *C. perfringens* and rotavirus were detected.

Scores were analysed for difference between treatment groups by means of a simplified marginal model (GEE) where the calf was considered as the experimental unit.

There was a clear increase of anti-Gp40 antibodies both in the colostrum of vaccinated cows and in the calves fed with it.

In summary, in the field study on dairy farms in the NL the reduction of duration of diarrhoea in calves fed colostrum of vaccinated cows was statistically significant.

Overall conclusion on efficacy

The applicant has conducted 2 challenge studies (preclinical), one in dairy calves and the other in beef calves, and 2 clinical trials in dairy and beef herds as well, to demonstrate the effectiveness of the passive immunisation. While the experimental model of the preclinical studies gave conspicuous results in dairy and beef calves, they were equivocal in field studies where diarrhoea was less

frequent than expected, especially in the French clinical trial. In the preclinical studies where calves were challenged at one day of age (onset of immunity), passive immunisation decreased the severity and the duration of diarrhoea. Differences to the negative control group were statistically significant with the 1.5 AU dosage while they were slightly less important with the 0.4 AU dosage and their statistical significance varied. The duration of passive immunisation has been shown under field conditions when monitoring for a period up to 21 days of life in beef calves and in dairy calves receiving colostrum and subsequently transition milk for the first 5 days of life.

While there is no direct correlation between the level of antibody in the blood of the dam and the protection in the calf, the applicant thus characterised the level of antibodies in the blood associated with this protection and has set down the vaccination regimen necessary to get this level of antibodies for revaccination. Heifers and cows have to get the first injection either for primary vaccination or the revaccination injection not more than 12 weeks before calving. Moreover, the primary vaccination or revaccination should be completed at least 3 weeks before calving for the onset of immunity.

The applicant has also demonstrated in preclinical studies that this level of antibodies is met, regardless of whether Bovilis Cryptium is used alone or in non-mixed use with Bovilis Rotavec Corona.

Part 5 – Benefit-risk assessment

Introduction

Bovilis Cryptium emulsion for injection for cattle contains ≥ 1 U of *Cryptosporidium parvum* glycoprotein Gp40. The target species is cattle. Bovilis Cryptium is given subcutaneously as two doses of 2 ml, 4 to 5 weeks apart, to pregnant cows, in the period 12 to 3 weeks before calving, and then a single dose is used for revaccination in the period 12 to 3 weeks before calving. Bovilis Cryptium actively immunises pregnant cows to raise antibodies in colostrum against Gp40 of *C. parvum*. The protection of calves depends on adequate ingestion of colostrum from vaccinated cows. It is recommended that all calves are fed colostrum and subsequent transition milk during the first 5 days of life. At least 3 litres of colostrum should be fed within the first 6 hours after birth; this was demonstrated to reduce clinical signs (i.e. diarrhoea) caused by *C. parvum*. The proposed withdrawal period is zero days.

Bovilis Cryptium is presented in packs containing 1 x 10 ml (5 doses), 1 x 100 ml (50 doses), 1 x 40 ml (20 doses) and 10 x 2 ml (10 x 1 dose).

The applicant has applied for a new active substance status for *Cryptosporidium parvum* glycoprotein Gp40.

This is a full application submitted in accordance with Article 42(2) of Regulation (EU) 2019/6 for a product developed by means of a biotechnological process and containing a new active substance.

Benefit assessment

Direct benefit

Bovilis Cryptium is intended to passively immunise calves against *C. parvum* and to decrease the associated morbidity caused by diarrhoea. Data on reduction of infection have not been shown.

To achieve this indication the calves need to be fed with the colostrum from their dams, which have been subcutaneously injected with 2 doses of the vaccine.

Two studies with experimental challenges have shown that calves passively immunised with colostrum of vaccinated cows had a lower morbidity upon infection, which was less severe, and had a shorter diarrhoea. These results have been corroborated by 2 clinical trials.

Additional benefits

The adequate level of anti-Gp40 antibodies is met regardless of whether the vaccine was administered alone or in association with Bovilis Rotavec Corona.

Risk assessment

Quality

The manufacturing process and quality control methods are properly validated. Starting materials are sufficiently qualified. There are no remaining risk as regards the quality of the vaccine.

Safety

Risks for the target animal

The vaccine triggers a long-lasting and initially painful local reaction, but the wellbeing and the behaviour of the cows are not significantly impacted.
The efficacy of vaccination needs a proper management of colostrum to have calves fed properly with it.

Risk for the user

The vaccine is adjuvanted by mineral oil, but the CVMP concluded that user safety for this product is acceptable when used according to the SPC recommendations where standard safety advices are included.

Risk for the environment

Bovilis Cryptium is not expected to pose a risk for the environment when used according to the SPC recommendations.

Risk for the consumer:

Residue studies are not required. The withdrawal period is set at zero days. Thus Bovilis Cryptium is not considered to pose a risk to the consumer when used according to the SPC recommendations.

Risk management or mitigation measures

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

Evaluation of the benefit-risk balance

At the time of submission, the applicant applied for the following indication: "Active immunisation of pregnant cows to raise antibodies in colostrum against Gp40 of *Cryptosporidium parvum*.
When calves are fed this colostrum, these antibodies have been demonstrated to reduce disease (i.e. diarrhoea, morbidity and mortality) caused by *C. parvum*."

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

The vaccine is intended for a significant disease of calves. The colostrum of vaccinated cows, fed to newborn calves, has been shown to decrease the severity and the duration of diarrhoea both in experimental and clinical studies.

The product has been shown to be efficacious for the reduction of clinical signs and the CVMP agreed to the following indication(s):

"For active immunisation of pregnant heifers and cows to raise antibodies in their colostrum against Gp40 of *Cryptosporidium parvum*, intended for passive immunisation of calves to reduce clinical signs (i.e. diarrhoea) caused by *C. parvum*.

Newborn calves:

Onset of immunity: Passive immunity commences from the start of colostrum feeding.
Duration of immunity: In calves that receive colostrum and transition milk as indicated and which were challenged at birth, passive immunity has been demonstrated until 2 weeks of age."

Information on development, manufacture and control of the active substance and finished product has been presented and leads 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.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Veterinary Medicinal Products (CVMP) considers that the application for Bovilis Cryptium is approvable, since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EU) 2019/6).

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

Cryptosporidium parvum glycoprotein Gp40 is to be qualified as a new active substance considering that no such antigen has yet been authorised in the EU.