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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Bovela (EMEA/V/C/003703/0000)

Common name: bovine viral diarrhoea vaccine (modified, live)

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

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Introduction

On 26 June 2013 the applicant Boehringer Ingelheim Vetmedica GmbH submitted an application for a marketing authorisation to the European Medicines Agency (the Agency) for Bovela in accordance with Regulation (EC) No 726/2004.

The product was considered eligible for the centralised procedure by the Committee for Medicinal products for Veterinary Use (CVMP) on 7 February 2013 under Article 3(1) of Regulation (EC) No 726/2004 as it is a medicinal product developed by means of a biotechnological process. The product also contains a new active substance which on the date of entry into force of the Regulation was not authorised in the European Union (EU). The product contains a genetically modified organism (GMO). The rapporteur appointed was F. Klein and co-rapporteur C. Muñoz Madero.

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

The applicant requested the following indication: For active immunisation of pregnant cattle against bovine viral diarrhoea virus (BVDV-1 and BVDV-2) to prevent transplacental infection of the foetus; for active immunisation of cattle from 3 months of age to reduce clinical signs, virus shedding and viraemia, and to minimise the reduction of leukocyte count caused by BVD virus.

Bovela contains modified live bovine viral diarrhoea virus type 1 (strain KE-9) and modified live bovine viral diarrhoea virus type 2 (strain NY-93) and is presented in packs/containers of 1 vial, 4, 6 or 10 vials containing 10 ml, 20 ml, 50 ml or 100 ml lyophilisate and 1, 4, 5 or 10 bottles containing 10 ml, 20 ml, 50 ml or 100 ml of solvent.

The proposed route of administration is intramuscular and the target species is cattle.

On 9 October 2014, the CVMP adopted an opinion and CVMP assessment report.

On 22 December 2014, the European Commission adopted a Commission Decision granting a marketing authorisation for this veterinary medicinal product.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system which fulfils the requirements of Directive 2001/82/EC, as amended.

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 event occurring either in the EU or in a third country.

Manufacturing authorisations and inspection status

The active substances and the vaccine are manufactured by Boehringer Ingelheim Vetmedica, Inc in the United States of America (USA). Manufacturer responsible for batch release is Boehringer Ingelheim Vetmedica GmbH, Germany. Valid manufacturing authorisations are available and judged to be in full compliance with legislation.

Valid good manufacturing practice (GMP) certificates are available for the active substance

manufacturing site as well as for the finished product manufacturing site and for the batch release site. Inspections were conducted and judged to be in full compliance with legislation.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system and GMP certification of the manufacturing sites was considered in line with legal requirements.

Part 2 – Quality

Composition

Bovela is a veterinary vaccine intended for the active immunisation of cattle against bovine viral diarrhoea virus type 1 and type 2 (BVDV-1, BVDV-2). The vaccine composition includes the modified live bovine viral diarrhoea virus type 1 (strain KE-9) and the modified bovine viral diarrhoea virus type 2 (strain NY-93). The vaccine is presented as a lyophilisate containing the active ingredients and a solvent for reconstitution.

The excipients of the lyophilisate are sucrose, gelatine, potassium hydroxide, L-glutamine acid, potassium dihydrogen phosphate, dipotassium phosphate, sodium chloride and water for injection.

The vaccine contains neither an adjuvant nor a preservative.

The excipients of the solvent are sodium chloride, potassium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate and water for injection.

The final product is a suspension for injection. One dose consists of a volume of 2 ml.

Container

The lyophilisate is filled in type I amber glass vials in compliance with European Pharmacopoeia (Ph. Eur.) monograph 3.2.1 on glass containers for pharmaceutical use. Vaccine vials are closed with siliconised bromobutyl rubber stoppers. The rubber stoppers and the silicone oil conform to Ph. Eur. monograph 3.2.9 on rubber closures for containers for aqueous parenteral preparations for powders and for freeze-dried powders and Ph. Eur. monograph 3.1.8 on silicone oil used as a lubricant respectively. The solvent is filled in high-density polyethylene (HDPE) vials with chlorobutyl rubber stoppers. All of the packaging components meet Ph. Eur. requirements.

Development pharmaceutics

Bovela is a modified live vaccine containing non-cytopathic BVDV-1 and BVDV-2 strains. BVDV-1 and BVDV-2 are species of the genus pestivirus within the family Flaviviridae.

The two vaccine strains were derived from wild pathogenic flavivirus strains which were attenuated. The BVDV-1 strain (field isolate KE9) was genetically related to several other European virus strains. The BVDV-2 strain (New York-93) isolated in USA, is a typical representative of the group of BVDV-2. Both vaccine virus strains contain two identical deletions: one in the N^{pro} gene prohibiting the N-terminal protease N^{pro} from being expressed and the other is in the E^{rns} gene resulting in abrogation of the ribonuclease function. Thus, the vaccine virus strains are deficient in a functional enzyme E^{rns} and N^{pro} protein. These deletions are correlated with an abolishment of the suppression of interferon induction in vivo which was shown in foetus further to intra-amniotic experimental infection of cows at 58–70 days of pregnancy (Meyers, 2007) as the field virus does. This feature was achieved by deletion of specific

genome sequences and not by insertion of any foreign deoxyribonucleic acid (DNA) fragments. The Madin-Darby bovine kidney (MDBK) cell line is used for the propagation of the attenuated BVDV-1 and BVDV-2.

The antigen production process was based on a known process which was optimized. Three consecutive lots of BVDV-1 and BVDV-2 were successfully manufactured at a pilot scale batch size. After that the manufacturing process was increased to the final batch scale. The final scale was qualified by the successful production of three further consecutive lots of BVDV-1 and BVDV-2.

The media used for the different production steps i.e. for propagation of 2006 MDBK cell cultures during the expansion of the cell cultures, for virus propagation, for the production of the master cell stock (MCS) and the working cell stock (WCS), master seed virus (MSV) and the working seed virus (WSV) preparation are adequately described and justified by intensive development trials.

Sucrose and gelatine in a phosphate buffer are used in the formulation.

Wash phosphate buffered saline (WPBS) was chosen as a diluent to rehydrate the lyophilized vaccine. WPBS contains sodium chloride, sodium phosphate, potassium chloride and potassium phosphate. The diluent does not contain any preservative as the reconstituted product is recommended for immediate use.

Method of manufacture

The production process is based on the seed-lot-system described in the Ph. Eur. general monograph 0062, which is considered the most appropriate for the manufacture of the vaccine. The manufacture and filling of the finished vaccine are carried out according to GMP, thereby establishing a process that is reproducible and appropriate for the manufacturing of the vaccine. Flow charts detailing the steps taken during the preparation of the finished product were submitted. Details of the production steps involved in the growth of the BVDV strains were provided.

The MDBK cell line is used for propagation of both BVDV-1 and BVDV-2.

BVDV-1 and BVDV-2, sucrose gelatine stabilizer (SGS) and physiological saline are blended, filled and lyophilised.

Besides the lyophilisate containing the active substances, the final product also contains a PBS diluent for reconstitution of the freeze-dried vaccine, which contains sodium chloride, potassium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate and water for injection.

The manufacturing process was adequately validated. The applicant has shown that the process is robust and yields product that complies with the product specifications stated by the applicant (the level of impurities is sufficiently low in both concentrated and non-concentrated antigens).

Control of starting materials

Active substance

Detailed descriptions were provided of the preparation and characterisation of the MSV and WSV, and of the master and working MDBK cell seeds. The recombinant BVDV-1 and BVDV-2 seeds differ from the respective wild type viruses by two identical deletions as described earlier. Both deletions together effectively lead to attenuation of the isolates. The attenuation was achieved by deletion of specific genome sequences and not by insertion of any foreign DNA fragments. Genetic stability was tested. No abnormal mutation rate was observed. Accordingly, the BVDV-1 and BVDV-2 MSV can be considered as genetically stable. Identity was demonstrated by two different methods for BVDV-1 and BVDV-2.

Stability studies were performed for the active substance (BVDV-1 and BVDV-2) at room temperature (20–25 °C), at 5 ± 3 °C, or at –40 °C and –70 °C. Potency remained within the specifications during the proposed shelf lives. Virus seeds and cell banks were also properly tested for contaminating agents (viruses, bacteria, fungi and mycoplasma) in accordance with the relevant guidelines. Based on the results adequate shelf lives were defined.

Excipients

Detailed information and certificates of analysis were provided for all starting materials listed in a Ph. Eur. of non-biological origin demonstrating compliance to respective Ph. Eur. monographs.

Starting materials not listed in a pharmacopoeia were described in detail. Adequate information was provided on the culture medium composition and components.

The excipients contained in the lyophylisate and solvent are in compliance with the relevant Ph. Eur monographs.

Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies

The starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the CVMP Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3) and Commission Directive 1999/104/EEC.

The overall transmissible spongiform encephalopathy (TSE) risk associated with this vaccine is considered negligible.

Control tests during production

For the BVDV-1 and BVDV-2, in process tests for sterility, potency and identity are performed at the level of the antigen harvest. The WPBS diluent is tested for sterility, appearance and pH. In-process test results of various vaccine virus harvests were provided and all results were in compliance with the specifications.

Control tests on the finished product

The description of the methods used for the control of the finished product (BVDV-1 and BVDV-2 identity, BVDV-1 and BVDV-2 potency, sterility, extraneous agents, mycoplasma, pH, residual humidity and visual appearance) and the specifications were provided.

The methods and specifications proposed at release and at the end of shelf life are appropriate to control the quality of the finished product.

The results of the analysis of three consecutive production runs of freeze-dried vaccine and for two consecutive production batches of solvent were presented and complied with the specifications.

Stability

Stability of the antigen

Three antigens from each virus strain were subjected to real time stability tests and their potency was tested in different conditions. It was shown that the antigens are stable at room temperature (20–25 °C), at 5 ± 3 °C, at –40 °C or at –70 °C for an adequate period.

Stability of the final product

Real time stability studies were performed on 8 batches of the finished product. During the storage of the final product at 2 °C – 8 °C all testing parameters met the requirements for all time points tested. No significant changes in appearance, residual humidity and pH were observed. Testing confirmed virus identity, sterility, mycoplasma, target animal safety and extraneous agents for all time points tested.

In view of the good stability profile of the finished product, a shelf life of 2 years is considered justified.

In-use stability:

In-use stability testing was performed to demonstrate compliance with the critical stability-indicating parameters) when the freeze-dried product is reconstituted and after 8 hours (one working day). The results obtained in the in-use stability study demonstrate that there is no change in sterility, visual appearance and pH immediately after reconstitution as well as after 8 hours storage at room temperature. Importantly, there was no significant decrease of potency after 8 hours at room temperature. In view of the results of the in-use stability study on the finished product, the proposed in-use stability of 8 hours is acceptable.

Stability of the diluent:

Satisfactory results were provided from the stability testing of the diluent justifying a shelf life of 3 years.

Overall conclusions on quality

The applicant has adequately described the composition and characteristics of the vaccine.

Detailed information has been provided on the starting materials, including the preparation and characterisation of the master and working virus seeds and the master and working MDBK cell seeds.

The manufacturing process (including development and validation) as well as control tests and specifications were described in detail. The TSE risk associated with this vaccine is considered negligible. Batch release and stability data showed compliance of the product's quality attributes with the specifications.

Part 3 – Safety

Introduction

Bovela is intended for administration in cattle from three months of age including pregnant animals. Therefore, safety studies were performed in pregnant and non-pregnant heifers and cows and in calves starting from 3 months of age.

The safety of the final product was investigated using three batches produced under GMP conditions, according to the manufacturing process described in the quality part of the application. Administration of the vaccine was performed as recommended in the summary of product characteristics (SPC). The applicant also undertook post-administration titrations of vaccine samples collected during the vaccination course. In most of these re-control titrations, the titre was found lower than expected. The titre decrease and difference is explained by a freezing/thawing step in the protocol of this post-administration survey.

The studies provided were designed to demonstrate that the vaccine is safe for each class of targeted cattle as well as for non-target animals susceptible to a BVDV infection. For the study design the applicant implemented some recommendations of the Ph. Eur. on inactivated BVDV vaccines (monograph 1952)

since there is no Ph. Eur. monograph on live BVDV vaccine. Taking into account that safety concerns about live viruses are very different from those about inactivated ones, and that flavivirus pathogenesis is very similar among species (Le Potier, 2006), Ph. Eur. monograph on live vaccines for other flaviviruses has also been considered in the assessment of the safety towards foetuses.

Some characteristics of the BVDV pathogenesis were selected as safety end-points for this vaccine including its ability to cause viraemia, to persistently infect foetuses, to induce leukopenia and thrombocytopenia, to cause digestive and pulmonary symptoms, and to cause the need for repeat breeding. Besides those safety end-points related to bovine viral diarrhoea (BVD), local and systemic reactions after vaccination were monitored. Also, the potential shedding of the vaccine virus by vaccinated cattle was investigated, except for the semen since reproductive bulls are not included in the target animals, to understand any consequence on cattle and environment.

A total of 14 safety studies were designed taking into account the requirements for live vaccines as laid down in Annex I to Directive 2001/82/EC and the Ph. Eur. monograph 5.2.6 on evaluation of safety of veterinary vaccines and immunosera.

Studies were conducted in compliance with guidelines on good laboratory practice (GLP) or good clinical practice (GCP).

Technical validations were provided for the tests performed for the safety studies i.e. BVDV isolation, seroneutralisation assay and BVDV detection by reverse transcription polymerase chain reaction (RT-PCR). Notably, BVDV detection by RT-PCR was considered at least as sensitive as virus isolation in cell culture, which was validated by ring-tests. Negative results were obtained from a persistently infected animal by RT-PCR.

Safety of the administration of one dose

No laboratory study was performed to investigate the safety of the administration of one dose. Instead, two field studies were provided. In these studies safety was monitored in vaccinated lactating cows. No local or general clinical symptoms were detected. The vaccine strain was shed into milk in both studies. Viraemia was detected in study and suspected in the other. These studies are described in more detail in the assessment of part 4.

Safety of administration of an overdose

To address the safety of a tenfold overdose of the vaccine, two laboratory studies were performed. Of these two studies, one was performed in calves and the other one in pregnant heifers. In addition, one field study in pregnant heifers was performed using a batch of the product containing virus at the lowest passage level.

Safety of a ten time overdose of a combined BVDV-1 and BVDV-2 vaccine in pregnant heifers

The safety of the administration of a ten time overdose of two vaccine batches was compared to administration of vaccine diluent in 2–3 month pregnant heifers. Thirty-three study animals were BVDV seronegative before vaccination and purchased from a seronegative herd involved since two decades in a control program.

Thirty-three study animals were randomly assigned to one of three study groups. Heifers of study group 1 received a total dose volume of 2 ml test item containing BVDV-1 with a titre analogue of $10^{6.6}$ $TCID_{50}$ and BVDV-2 of $10^{6.2}$ $TCID_{50}$. Animals of study group 2 received a total dose volume of 8 ml test item containing BVDV-1 with a higher titre analogue of $10^{7.3}$ $TCID_{50}$ and BVDV-2 of $10^{6.4}$ $TCID_{50}$. Study group 3 received 8 ml of the control item WPBS. Test and control items were administered by the intramuscular

route. The administration day was defined as day zero post-vaccination. The study animals were terminated 62 days post-vaccination and the foetuses were collected for investigation.

Safety was assessed using foetal infection rate as primary safety variable. In addition, clinical signs, rectal body temperature, injection site reactions, viraemia, leukocyte and platelet counts and seroconversion (secondary study variables) were monitored.

In one vaccinated group one foetal infection by BVDV-1 vaccine strain at 62 days and one abortion at 48 days post-vaccination occurred and in the second group, one malformation was reported which vaccine virus was not found in.

The abortion of a partially mummified foetus was observed in one heifer which showed no viraemia on the basis of testing on the day of abortion and 34 days before. BVDV was not detected in the aborted foetus or placenta by virus isolation or RT-PCR. The heifer had been submitted to stressful manipulation. In addition, this heifer was recorded to have undergone the strongest local reaction upon vaccination. No other diagnostic tests to investigate the causal relationship between BVDV and abortion were undertaken (e.g. immunohistology, foetal seroneutralisation). No investigation was undertaken to rule out involvement of other abortifacient organisms.

The BVDV-1 vaccine strain was isolated from the thymus of one collected foetus sampled 62 days after vaccination. The virus was isolated in only one of the replicates and could not be confirmed when detection was attempted by isolation from other organs or by an independent laboratory. No histology investigation was performed to substantiate any lesion. The heifer carrying this foetus showed viraemia after vaccination with a serological response higher than the mean of vaccinated animals.

In heifers, viraemia was dose dependant (3 in group 1 (lower dose) and 6 in group 2 (higher dose)) and lasted from day 4 to day 11 post-vaccination. Platelet count variations were not interpretable with more depleted animals in group 2, 4 days post-vaccination. Conversely leukopenia was maximal from day 4 to 8 after vaccination and was independent of viraemia. Mild local reactions were recorded at the injection site.

In conclusion, a dose dependent viraemia after a tenfold overdose was observed with a mild leukopenia in vaccinated groups. Moreover, the occurrence of 3 potenial transplacental infections out of 22 vaccinations in total (14%) was reported.

Safety of a tenfold administration in pregnant heifers

The safety of the administration of a ten time overdose was studied in seronegative pregnant heifers at 56-122 days and >150 days of gestation. The birth of a healthy calf was used as the primary endpoint. Additional safety parameters were monitored. No safety issues were observed. Viraemia occurred in 25% of heifers at 80–120 days of gestation. The report is further assessed as part of the examination of reproductive performance.

Safety of the repeated administration of an overdose

Evaluation of the safety of a repeated ten times overdose of a BVDV-1 and BVDV-2 vaccine in young calves

The study investigated the safety of a repeated ten time overdose. The vaccine was injected twice to a group of young calves at 3 months of age. Animals were purchased from a farm free of BVD since 1999. All study animals received two intramuscular injections 14 days apart with either 4 ml of the test item (group 2: vaccine dosed between $10^{6.7}$ and $10^{7.3}$ $TCID_{50}$) or the control item (group 1; WPBS). The test item contained ten times the maximum release titres for BVDV-1 and BVDV-2. The study was terminated 29 days after the first vaccination.

An increased body temperature was observed a few hours after vaccination and at that time there was no difference between groups. The temperature was however significantly higher in the vaccinated group five days post vaccination. Local reactions were similar clinically (swelling for 1 day) as well as histologically (investigated 15 days after the 2nd injection) in control and vaccinated animals. Mean clinical scores were more severe in vaccinated animals. No impact on platelet and leukocyte counts was observed. Three out of 11 vaccinated calves had viraemia from day 6 to 10 after the first vaccination. No viraemia was observed after the second injection when BVDV antibody levels had begun to rise. None of the two virus strains could be detected in sampled tissues 15 days after the second vaccination and no histological lesions were found in lymph nodes.

In conclusion, besides asymptomatic viraemia in some animals, no other safety concerns were raised. The antibody increase seen following the first administration of the vaccine could decrease vaccine viraemia and minimise the temperature increase in vaccinated animals 5 days after the second administration.

Examination of reproductive performance

Reproductive performance in female cattle

Reproductive performance in female cattle is very difficult to address considering that most of the reproductive impairments attributed to virulent BVDV such as infertility, embryo resorption, malformation and abortion are infrequent and difficult to diagnose. In addition, no robust experimental model is available. To compensate for these difficulties, a large number of animals were involved in the studies.

A total of 1 seronegative animal was monitored in laboratory studies and 113 seropostive with 114 seronegative animals in field studies before the critical period of gestation when persistent infection can occur (0–60 days of gestation). During the persistent infection period, i.e. 61–120 days of gestation, 38 seronegative animals were monitored in laboratory studies and 103 seropositive and 129 seronegative animals in field studies. After the persistent infection period until calving, 19 seronegative animals were monitored in laboratory studies and 353 seropositive with 381 seronegative in field studies. The seronegative females were supposed to be naïve regarding BVD immunity and representing of vaccinates to be considered.

The reproductive performance was tested at conception and in early pregnancy (field trial in dairy herds) during the critical period when the foetus may be persistently infected (two overdose studies) and after this period (one overdose study). Two field trials in dairy and beef herds also investigated the reproductive performance at difference stages of pregnancy. To be noted, the results from the two studies conducted with an overdose vaccine were anticipated to challenge the reproductive function more than those obtained in the field studies where animals were injected with an intermediate titre vaccine. Studies were conducted in accordance with GCP.

a) Infertility:

No laboratory studies were performed to study infertility. Infertility was investigated in one field trial in dairy herds where 27 BVD seronegative and 10 BVD seropositive heifers from two farms with a history of infectious bovine rhinotracheitis were included. The conception was measured by the rate of heifers that do not need more than one artificial insemination within the 56 day period after the first insemination (56-day non-return rate, 56-NRR). Heifers have a higher 56-NRR (Fouz, 2011) than cows and a more robust study result concerning this rate. In this study animals were inseminated within 14 days after vaccination. No statistically significant difference was observed between the vaccinated and the control animals. The mean 56-NRR was around 60% which is below the normal value in heifers

(70% (Fouz, 2011)). However, the study environment was not as controlled and homogeneous as would be in a laboratory study and there was also a BVDV infection pressure in the herd, which hampers any conclusion on vaccine safety. In addition, no data about the animals was provided on more independent fertility parameters (e.g. calving interval, days to first service) and to enable cross-corroboration. Additionally the dissemination of the vaccine virus into the female reproductive tract was not addressed.

There is no evidence that the vaccine has a negative impact on reproductive functions, although due to experimental design, no conclusion can be drawn about infertility.

b) Risk of a persistent infection by the vaccine virus:

Persistent infection was studied in a laboratory overdose study in pregnant heifers, in a laboratory study in pregnant heifers and in three field trials in dairy and beef herds. Only seronegative animals were considered at the end of the assessment. No persistent infection attributable to vaccination could be identified by virus isolation. These observations confirmed the rationale of attenuation and results of in vitro studies and provided literature (Meyers, 2007).

c) Risk of other pregnancy impairments further to transplacental transmission:

Unlike persistent infection, the causality link to other reproductive adverse events is seldom easily substantiated by virus detection but more often by indirect evidence such as statistical differences in frequency of abortions and abnormalities between a vaccine and a control group, or an immune reaction of the foetus to virus exposure.

Such statistical differences were not seen in field studies where the number of abortions and abnormalities was monitored in a large number of animals.

In a field study where pregnancy was monitored until completion in 37 animals seronegative prior to vaccination, only 3 stillbirths caused by aspiration of amniotic fluid were recorded.

In a laboratory study, the pregnancy of 22 heifers was interrupted 62 days post-vaccination. BVDV-1 vaccine strain was isolated from one thymus. One event of congenital abnormality (head malformation) was observed in another foetus for which the reason remains unknown. One abortion 48 days post-vaccination also remained without clear etiology. Immune reactions of the foetuses were not monitored.

In field studies 1,520 seropositive and -negative pregnant animals were exposed to the vaccine. No firm statistical conclusion could be drawn from the analysis concerning various pregnancy parameters as both vaccination and natural BVDV exposure coincided.

Other evidence listed below also show that such crossing was possible.

There was some weak evidence of a transplacental infection of virus in two studies: vaccine BVDV-1 was found in the blood of a foetus aborted at 7 months of gestation 82 days after the vaccination (field trial in dairy herds) and a BVDV-1 vaccine strain was detected in the thymus of a foetus collected 62 days after vaccination (overdose laboratory study).

In addition, vaccine viraemia was found on a number of animals in all laboratory studies (up to 25% of viraemia in one study). The viraemia was more intense in heifers in comparison to cows in early pregnancy which means that the placenta and reproductive organs of heifers are more exposed to the vaccine virus. Unfortunately the dissemination studies provided no data to assess this pressure.

More importantly, there was no determination of the pre-colostral titre of offsprings which would have been appropriate as the immune system of the foetus (which is competent from about 100 day of gestation onwards) is the most sensitive and cumulative detector of virus foetal contamination.

In conclusion, the safety of vaccination regarding prevention of persistent infection was reasonably supported by both laboratory and field studies.

The risk of transplacental transmission was not entirely ruled out.

In view of the information provided, it is considered that the vaccination of reproductive female animals should be undertaken only after a benefit-risk assessment taking into account e.g. the BVD immunological status of the animal, the time-span between vaccination and mating/insemination and the stage of pregnancy.

The vaccine is not intended for bulls.

Effects on colostrum and lactation

As Bovela is a live vaccine the potential shedding of vaccine BVDV-1 and BVDV-2 strains into milk was investigated. The investigation included two parameters: virus excretion and milk yield.

The vaccine virus excretion in milk was demonstrated in two studies where BVDV was isolated in about half of the vaccinated cows from 1 week post-vaccination. The titres were low (most below the quantification limit) and both strains were excreted. However, no vaccine virus strains were detected in a third study where the RT-PCR method was used. No correlation was found between the rate and the stage of pregnancy, the number of pregnancies carried to viable gestational age (parity) or the somatic cell count with BVDV excretion in the milk in field study in dairy herds.

The biological relevance of virus excretion to milk was investigated in a second study. In this study 2 weeks old calves fed with contaminated milk did not seroconvert for BVDV. As a result it was considered that the risk of BVDV transmission by a vaccine virus strain in milk can be deemed very low.

Milk yield was monitored in a safety field study in dairy cows where 66 animals from first to sixth months of lactation (high lactation phase) were vaccinated with a vaccine with an intermediate titre in order to evaluate a possible impact of vaccination on milk yield and compared to 66 control animals. Milk production decreased after vaccination but this was also observed in the control group and there was no statistical difference between the groups. Therefore no conclusions could be made in this study concerning an impact of vaccination on milk yield.

Examination of immunological functions

In vitro, the 2 vaccine virus strains do not block the production of type 1 interferon (Meyers et al., 2002).

In several studies including the tenfold overdose studies, a slight or moderate decrease in leukocyte counts was observed in a few vaccinated animals. Nevertheless the slight leukopenia would have no physiological impact as neither the BVDV seroconversion nor the protection to the BVDV challenge in the efficacy studies were affected in the studies.

To exclude the initiation of bovine neonatal pancytopenia (BNP), which has been observed for another BVD vaccine, bovine serum samples were processed in a separate study and were investigated for immunoglobulin IgG and IgG1 alloantibodies with the flow cytometry method. The results of this study showed no evidence for the induction or presence of BNP-related alloantibodies in the analysed serum samples from cattle vaccinated with Bovela. Some allo-reactivity was observed in a few samples from cows vaccinated with a tenfold overdose during pregnancy but these were comparable to what was observed in samples from cows vaccinated with non-BNP inducing authorised BVD vaccines. None of the samples reached serum-neutralization-ratio levels comparable to what is on average found in sera from BNP-dams.

In conclusion, apart from a mild decrease of the leukocyte counts in a minority of vaccinated study animals, there was no evidence for a negative impact on the immune system caused by a tenfold overdose vaccination of Bovela.

Special requirements for live vaccines

Spread of the vaccine virus strain

Three studies investigating spread of the vaccine strain were provided.

A pivotal dissemination and transmission study was performed with the MSV in male calves. The objective of this study was to assess the potential shedding and transmission of vaccine virus and also evaluate the dissemination of both vaccine virus types (BVDV-1 and BVDV-2) in the body of vaccinated animals and in excretions. Two-month-old male calves were selected as the study population owing to their juvenile immune system (Tizard, 2009) and the highest viral load in their nasal secretion reported in literature (Brownlie, 1990). Ten animals received intramuscularly a suspension of MSV with a titre of about $10^{5.8}$ $TCID_{50}$/ml. The calves were purchased in a BVD negative farm (periodic serological BVD monitoring) where strict control program had been undertaken since 2002. Two animals were necropsied at day 6, 9, 13, 21 and the remaining animals at day 30. Vaccine virus was searched by isolation in cell culture from sampled organs as well as in the buffy coat and nasal swabs sampled regularly in all calves at sampling points prior to termination the study animals. Five animals in a contact control group were exposed to serum free medium and serologically monitored to detect any contamination by vaccine virus.

The animal phase was claimed to be performed in compliance with GLP. Biological samples were analysed in the German reference laboratory.

Control contact calves remained seronegative for BVDV and did not exhibit any evidence of BVD infection.

The vaccine virus was first and solely isolated in blood 4 days post-vaccination in 1 of 10 vaccinated animals and then repeatedly isolated in thymus and retropharyngeal lymph node from 6 to 13 days post-vaccination. However, the virus load in these 2 organs peaked 6 days post-vaccination.

In comparison to BVDV unvaccinated controls, vaccinated calves did not exhibit significantly more BVD related symptoms nor did they show local reactions. Hyperthermia was noticed in vaccinated calves when they experienced viraemia. A great degree of variation was observed in leukocyte counts as well as in platelet counts which any conclusion is prevented to be drawn on. The large variation in leukocyte and platelet counts casts doubts on the suitability of the study to detect any impact of vaccination on these variables. A laboratory study was performed to detect BVDV in milk of 4 lactating cows after vaccination with the MSV. Cows were vaccinated 1 to 6 days after calving with a suspension of MSV to afford to each animal $10^{6.14}$ $TCID_{50}$ BVDV-1 and $10^{7.18}$ $TCID_{50}$ BVDV-2 per dose. The animals were obtained from two farms where BVDV free status was determined by periodic serological survey since 2002 in one farm and by serological test of 221 animals for the other farm.

All calves remained seronegative and no vaccine virus ribonucleic acid (RNA) was amplified in milk or milk cells. No RNA was detected in urine.

All vaccinated cows seroconverted by day 28 (ELISA – enzyme-linked immunosorbent assay) but no viraemia due to the vaccine virus was observed. Neither clinical symptoms nor leukopenia and thrombocytopenia related to BVDV infection were reported. Faecal excretion was not investigated because no analytical method was sensitive enough. No RT-PCR results were provided for nasal swabs and saliva because the transportation medium of the samples was contaminated with BVDV wild type. Investigation of nasal swabs by BVDV isolation revealed no positive results.

In conclusion, virus was not excreted into milk. Calving cows appeared not to be prone to vaccine viraemia as no vaccine virus was isolated. However such animals are not the most susceptible animals to BVDV vaccination and therefore this absence of any vaccine virus shedding via the emunctories was anticipated.

Overall, the two studies above provided no convincing information about the presence of virus in emunctories, since the design did not allow for monitoring of the excretion of the virus.

In a field study shedding into milk was observed in 75% of the vaccinated non-pregnant cows (up to 13 $TCID_{50}$/ml). More importantly, in this study contact animals which were permanently close to vaccinated animals did never seroconvert even when feeding with BVDV 13 $TCID_{50}$/ml. An epidemiologically relevant shedding of vaccine virus appears very unlikely, considering also the results provided by reversion-to-virulence studies where a productive infection was passed on twice by intranasal administration of the virus, directly sourced from infected buffy coat.

Spread to non-target animal species

Sheep

The safety of a developmental vaccine containing BVDV-1 and BVDV-2 was studied in the non-target species sheep, investigating in particular the spread of the vaccine.

Intra-nasal administration of vaccine to sheep resulted in seroconversion with high BVDV-1 and BVDV-2 titres but no clinical disease and no virus was isolated from the vaccinated animals. Neither local nor systemic reactions were observed (asymptomatic infection).

There was no indication that the virus will spread to naïve animals since the five controls contact animals remained seronegative. No clinical manifestation of BVD was observed.

Pigs

The safety of a developmental vaccine containing BVDV-1 and BVDV-2 was studied in the non-target species pig, investigating in particular the spread of the vaccine. In this study weaned piglets (n=10) were exposed to the vaccine via the intranasal route at a dose of 10^6 $TCID_{50}$/animal. PBS was administered to contact control animals (n = 5).

A serological response to BVDV-1 was observed in one vaccinate on days 28, 42 and 56. None of the other nine vaccinated animals presented a serological response to BVDV-1 at any of the sampling time points. None of the 10 vaccinated animals showed a serological response to BVDV-2 at any of the sampling time points. The virus was not isolated and no clinical manifestation of BVD or fever were observed.

The risk of vaccine spreading to pigs appears minimal since a low virus burden was detected in bovine emunctories and 1 pig out of 10 was infected when exposed intra-nasally to 10^6 $TCID_{50}$ vaccine virus.

Dissemination in the vaccinated animal

Several studies were submitted to provide information on dissemination in vaccinated animals (proof of concept study in lactating cows and milk feeding of calves: evaluation of vaccine spread in lactating cows of a BVDV type 1 and type 2 vaccine: evaluation of shedding of a BVDV-1 and BVDV-2 vaccine via milk of lactating cows in the field) in addition to a pivotal GLP study.

The pivotal GLP study on dissemination and transmission with the MSV of Bovela in calves has been assessed under "Spread of the vaccine strain" earlier in the report.

Conclusions from the provided dissemination studies were difficult to draw due to differences in the sensitivity of different methods used throughout the dossier.

No vaccine virus was detected in urine, nasal secretion or milk in any laboratory study by the RT-PCR method applied by the applicant. The method was however poorly sensitive.

Conversely, vaccine virus was detected in milk in two field studies by isolation in cell culture and in nasal secretion of colostrum deprived calves up to 7 days after nasal exposure (both described under reversion to virulence). BVDV was isolated from nasal excretion of 80% of colostrum-deprived calves following intranasal administration of the vaccine for at least 5 days.

No other emunctory besides milk was reliably investigated in these 2 studies.

Both BVDV strains were excreted in milk with a low viral burden up to 13 $TCID_{50}$/ml by 23 days.

In conclusion, the vaccine virus was excreted in milk. Its excretion cannot be ruled out in other emunctories such as urine and nasal secretion.

Since vaccine virus was isolated still at 23 days after vaccination it appears that milk or nasal secretion would have been better samples to monitor vaccine virus than blood.

Reversion to virulence of attenuated vaccines

Two studies were provided according the VICH GL41.

One study (investigated reversion to virulence by back-passage of the BVDV-1 double deletion MSV. BVDV-1 MSV was administered intranasally to colostrum-deprived calves (6–10 weeks of age). Calves were monitored until day 10 for local and general symptom manifestation, leukocyte and platelet counts, virus shedding from nasal swabs, and viraemia in buffy coats.

Back-passage 1 was carried out 3 times to get enough material (BVDV positive nasal swab and buffy coat) for the next back-passage. In back-passage 2, virus was recovered in 1 buffy coat sample of 1 animal for 1 day only. In back-passage 3, no virus was recovered. However in contradiction with VICH GL41 requirements, the back-passage 3 was not repeated in 10 more animals for welfare reasons, according to the applicant.

No manifestation typical for BVDV was observed. No vaccine virus could be recovered after the 2nd back-passage, which is a prerequisite for reversion/increase to virulence. The inoculums administered in back-passage 2 and 3 were below the limit of detection of the assay. There was no comparison of the virus recovered at the highest back-passage with either the MSV or with the wild strain of BVD vaccine of the same virus titre.

Another study investigated reversion to virulence by back-passage of the BVDV-2 double deletion MSV. In this study the BVDV-2 MSV was administered intranasally to colostrum-deprived calves (6–10 weeks of age). Animals were then monitored for 10 days for any local or general manifestation, leukocyte and platelet counts, virus shedding from nasal swabs and viraemia in buffy coats.

Back-passage 1 was carried out 3 times to get some material for the next back-passage. The titre of inoculum for back-passage 2 was below the limit of detection of the assay. In back-passage 2, no virus was recovered. In contradiction with VICH GL41 requirements, it was decided not to repeat the back-passage 2 in 10 more animals for welfare reasons. There was no comparison of the virus recovered at the highest back-passage either with the MSV or with the wild strain of BVD vaccine of the same virus titre.

In conclusion, both studies provided had deficiencies when compared to the requirements of the relevant guideline VICH GL41. However, the loss of the vaccine across the experimental passage showed that such passage is unlikely in field condition. Genotypic and phenotypic data were provided and no biologically significant changes occurred over 2 passages under the study conditions.

Taking into account the complete knowledge of this genetically modified vaccine, the two well described deletions of pivotal genes in both strains and the results of the animal studies provided, the risk of reversion to virulence is accepted as negligible, despite the deficiency in investigations.

Biological properties of the vaccine strain

BVD is characterized by clinical manifestations. Such manifestations were all investigated in studies provided to address other requirements (i.e. reproductive digestive and immune disorders, thrombopenia). Therefore no additional studies were considered necessary.

Recombination or genomic re-assortment of the strains

Information on recombination and re-assortment of the strains is provided in the environmental risk assessment for products containing or consisting of GMO.

Study of residues

Not required.

The active ingredient being a substance of biological origin intended to produce active immunity does not fall within the scope of Regulation (EC) No. 470/2009 with regard to residues of veterinary medicinal products in foodstuffs of animal origin.

In addition the other components of the vaccine are either listed in table 1 of the annex of Commission Regulation 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.

Interactions

No data were provided on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. Therefore, the standard interactions statement should be included in section 4.8 of the SPC: "No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis."

Field studies

Six field studies were completed in Germany and Hungary in accordance with the principles of GCP and using batches produced according to the manufacturing process described in the quality section. Besides the usual monitoring of any local and systemic reaction to vaccination, clinical manifestations were specifically monitored in compliance with the Ph. Eur. monograph on BVD inactivated vaccine and ascribed to infection caused by wild strain of BVDV i.e. milk yield, conception rate, foetal infection in dairy or beef cattle leukopenia and thrombocytopenia. A serological immune response for BVDV-1 and BVDV-2 was evaluated by seroneutralisation test (SNT) from blood of the vaccinated animals. Vaccine virus shedding in milk was traced in two studies.

Evaluation of the influence of vaccination with a BVDV-1 and BVDV-2 vaccine on the milk yield of lactating cows (GCP compliant)

Lactating animals (n=139) were enrolled from a farm naturally infected by BVDV. Most animals were seropositive. Half of study animals were vaccinated according to the proposed schedule using a batch with

an intermediate titre 5.30 log_{10} $TCID_{50}$/dose for BVDV-1 and 5.00 log_{10} $TCID_{50}$/dose of BVDV-2 instead of 6 log_{10} $TCID_{50}$/dose).

Milk production was not statistically different between vaccinated and control groups. An abortion occurred in the vaccinated group 6 days post-vaccination and the only result to exclude any causality relationship with vaccination is the absence of virus isolation from the placenta; however this is not a very compelling test for the diagnosis of BVDV related abortion. No other general symptoms or injection site reactions were detected.

Evaluation of shedding of a BVDV-1 and BVDV-2 vaccine via milk of lactating cows in the field (GCP compliant)

In this study, vaccine virus excretion in the milk of 40 seronegative cows in their late stage of lactation was followed over 21 days after vaccination. No control group was included. The selected farm was not involved in a BVD control program but no history of BVD was reported and no cows were BVDV seropositive when enrolled in the study. Titration of the vaccine gave a titre below the required titre for a safety study (5.90 log_{10} $TCID_{50}$/dose for BVDV-1 and 5.18 log_{10} $TCID_{50}$/dose of BVDV-2 instead of 6 log_{10} $TCID_{50}$/dose). Results from this study showed vaccine virus excretion in the milk of almost half of the cows that began 8 days and ended 18 days post-vaccination. Only four cows excreted the vaccine virus for a period longer than 1 day. A cow developed mastitis without any prior BVDV excretion. No correlation was found between rate and stage of pregnancy and parity with vaccine BVDV excretion in the milk. No local or general reactions were observed.

Evaluation of field safety and efficacy in dairy cattle after vaccination with a BVDV vaccine comprising BVDV-1 and BVDV-2 vaccine strains (GCP compliant)

The objective of this field study was to demonstrate the safety and efficacy of the vaccine in 8 dairy cattle herds with heifers and cows at all stages of pregnancy as well as non-pregnant cattle and animals that are scheduled for insemination.

The study was a randomized, blinded and negative controlled study carried out in 8 Hungarian herds, 5 of them with a history of BVD or detection of persistently infected animals at the beginning of the study. The vaccine was administered at a low titre of 10^4 $TCID_{50}$/dose. The dosing was compliant with requirements for demonstration of efficacy.

All 2,067 animals (1,097 seronegative at vaccination) but 3 animals in the vaccinated group seroconverted to both BVDV types.

Fifteen newborn calves were recorded as persistently infected. Five foetal infections were detected in the vaccine group, four in newborn by ear notches and one in an aborted foetus. The four persistently infected newborn were infected by a BVDV-1 wild-type strain. The period of infection was before vaccination for 3 of those calves or before the onset of protection for the other. One foetus was aborted 82 days post-vaccination, and the BVDV-1 vaccine strain was detected. The foetus was about 6 to 7 months of age according to pathologist and neither macerated nor mummified. In the control group, 7 newborns were detected to be BVDV-1 positive by PCR and sequencing as well as one newborn which was positive in the buffy coat, one aborted foetus and one stillborn calf. The period of infection was calculated to have occurred before vaccination (6 animals) or in the early phase of the study (4 animals, wild-type BVDV).

There were 67 abortions (34 in the control group and 33 in the vaccinated group). All but 2 foetuses (one in each group) were negative for BVDV in virus isolation testing. Vaccine BVDV was weakly detected in the vaccinated cow and wild BVDV-1 strain was isolated in the control one. If the evaluation is focused on the 4 weeks after the vaccination of seronegative cows only, 2 abortions only were recorded; BVDV was not detected nor any other abortifacient agent and their clinico-epidemiology features was non-conflicting with BVDV abortion pathogenesis.

Regarding congenital defects, 1 case of hypotrichosis was recorded in the vaccinated group and 2 cases of skull defects accompanied either by microphthalmia or hypotrichosis were reported in control group. The vaccinated dam was immunised approximately on day 180 of gestation, which is after the period that BVDV can trigger malformation (between 100 and 150 days of gestation, Duffel and Harkness, 1985; Baker, 1990).

A total of 21 clinical events, equally distributed in both treatment groups, were reported within the first 14 days after vaccination. These were predominantly changes in behaviour. No statistically significant differences between control and vaccinated cows were observed either for clinical manifestation or for fever. No injection site reaction was reported. The fertility rate was not significantly different between groups. The low titre of the vaccine used in this study also provided sufficient results on efficacy.

Evaluation of field safety and efficacy in beef cattle after vaccination with a BVDV vaccine comprising a BVDV-1 and BVDV-2 vaccine strain (GCP compliant)

In this field study, the safety and efficacy of the vaccine were evaluated in 11 Hungarian beef cattle herds with various rates of BVDV seropositive animals. The vaccine had an intermediate titre of $10^{5.05}$ $TCID_{50}$ for BVDV-1 and $10^{4.45}$ $TCID_{50}$ for BVDV-2 which was compliant for safety and efficacy purposes. Half of the animals were injected with the diluent. Control and vaccinated groups were well randomized since about 27% and 32% of the vaccinated and control cows respectively were BVDV seronegative. In the control group, more than 92% remained seronegative throughout the study, which is a hint of an absence of BVDV exposure in the course of this study. Less than 3% of the vaccinated cows remained seronegative. One newborn in both groups was BVDV-1 wild strain positive in ear notch. Four cases of abortion occurred, 3 in the vaccinated group but BVDV infection was not identified as cause of the abortion. No congenital defects were noticed in any of the newborn calves. Only three newborn calves (two from the controls, one from the vaccinated group) showed a change in behaviour at their respective day of clinical investigation.

No clinical signs related to BVDV in cows were recorded on nine out of ten farms.

Evaluation of safety of a ten time overdose of a BVDV-1 and BVDV-2 vaccine in pregnant heifers (GCP compliant)

This cross-over field study was designed to challenge the safety and efficacy of a tenfold vaccination in 37 pregnant heifers which were BVDV antibody and antigen free. While the BVDV-1 valence barely met the 10^7 $TCID_{50}$ titre/dose aimed by the applicant, the BVDV-2 valence reached only the upper titre of the vaccine. Viraemia as well as clinical symptoms were closely monitored just after vaccination. Animals were vaccinated whether during the 56–122 day period of gestation or after 150 days. The other group were injected with diluent. Reproductive parameters were recorded at birth.

No local or general symptoms related to vaccination were observed. Calves (n=33) were born healthy (no BVDV related abnormality) and no BVDV was spotted in any ear notch by RT-PCR. Stillbirths consecutive to aspiration of amniotic fluid were balanced into the 2 groups and no congenital defect was noticed. One heifer only in vaccinated group remained non-pregnant.

The BVD virus was isolated in 5/19 heifers vaccinated between 56 and 122 days of pregnancy from 4 to 10 days after vaccination while heifers vaccinated after 150 days of pregnancy didn’t experience viraemia.

No newborn was persistently infected in the vaccinated groups, or BDV related abnormalities were observed.

Evaluation of spread of BVDV-1 and BVDV-2 vaccine strains in lactating cows (not GCP compliant)

In order to determine whether the vaccine viruses can be transmitted from a vaccinated cow to a calf via milk, the milk of non-pregnant lactating cows (n=20) was given to suckling calves for 28 days; control calves were fed with commercial milk substitute.

Viraemia was detected in half of the vaccinated cows and from day 6 to 14; all cows seroconverted.

Three quarters of the cows excreted vaccine BVDV strain in milk at least one time between 6 to 23 days post-vaccination. Excretion was maximum at 11 days post-vaccination. Both BVDV strains were excreted in milk with a viral burden up to 13 $TCID_{50}$/ml by day 23.

No calves were infected by BVDV contaminated milk as no seronconversion was observed.

No local or general symptoms were identified to indicate any safety concerns in the vaccinated cows.

User safety

The applicant provided a user safety assessment compliant with the guideline for user safety for immunological veterinary medicinal product (EMEA/CVMP/IWP/54533/2006).

Bovela can be considered as presenting no particular risk for humans as:

- it does not contain any component, (including the live genetically modified component) known to be potentially harmful to humans,
- it must be administered by competent personnel, i.e. a skilled veterinarian or a trained person under supervision of a veterinarian,
- the excipients of the product do not present a risk to the user.

The user safety instructions in the SPC were considered appropriate.

The CVMP therefore concluded that the user safety for this product is acceptable when used as recommended in the SPC.

Environmental risk assessment (ERA)

The applicant provided a risk assessment in compliance with the CVMP Guideline on the environmental risk assessment of immunological veterinary medicinal products (EMEA/CVMP/074/95).

1. Hazard identification

Transmission to non-target species: The target species of the modified vaccine strains is cattle. BVDV is restricted under natural infection conditions to members of the order Artiodactyla (cloven-hoofed animals). Transmission of vaccine virus strains could not be shown between target and non-target animals. Following vaccination of cattle, pigs and sheep no evidence of transmission in those species could be observed.

Shedding of the live product organism: Dissemination within the target species and potential transmission to non-vaccinated target animals was especially investigated in dissemination study. The excretion of BVDV via nasal secretions, blood, urine and faeces were assessed to be a hazard with a low risk, especially since, no BVDV could be detected in nasal secretions which are known to be the most important source of spread of BVDV into the environment. No vaccine BVDV could be detected in any of the newborn calves of the unvaccinated animals, confirming the low risk of shedding of this vaccine and

supporting the fact that the vaccine virus is not transmitted vertically. Vaccine BVDV strains can sporadically be found in trace amounts in milk of vaccinated cows from day post-vaccination 6 to day post-vaccination 23. Shedding of the modified live vaccine BVDV strains via milk into the environment was identified as a possible hazard based on the findings of the clinical studies on milk shedding and milk shedding/feeding. The corresponding amount of the virus in the samples found positive in both studies is suggested to be very low. Importantly, none of the calves (the most susceptible category of the target species) fed with milk tested positive for BVDV was determined to become BVDV antigen positive, show any vaccine related clinical sign nor did they seroconvert.

Taken together, all the above data indicate that the shedding capacity of the vaccine BVDV in this product is to be regarded as low.

Capacity to survive, establish and disseminate: BVDV can only replicate inside a susceptible host and not in the inanimate environment. Due to its characteristics as an enveloped virus, BVDV is sensitive to environmental conditions like ultraviolet light and low humidity. Shedding of modified vaccine virus via secretions and excretions was assessed as low.

Genetic stability, reversion to virulence, and possible gene transfer: Regarding the genetic stability and the phenotypic stability of their attenuation, the vaccine strains were attenuated by double individual genomic deletions: N^{pro} codons 5 to 168 and E^{rns} codon 349. Therefore the reversion by mutation is very unlikely. The reversion of by recombination would theoretically be possible. However such event would take place during a double infection of cells with wild viruses which is very unlikely owing to superinfection exclusion mechanism and the low replication rate of the vaccine strain (attenuated phenotype).

The recombinant virus would at worst have the biological characteristics of wild parental strain, which are more or less the same than other wild BVDV strains.

The viral RNA genome is the only available genetic information and RNA cannot recombine with DNA.

Therefore, the hazard "gene transfer" from the viral RNA genome to the genomes of animals or environmental bacteria is to be regarded as negligible.

2. Exposure to hazard

The vaccine is to be administered by intramuscular injection which significantly reduces the risk for direct exposure of the environment. In addition, the vaccine will be distributed primarily as prescription only medicine (POM) to trained veterinarians. This ensures administration by professionals and a secure administration technique as well as correct disposal of unused immunological veterinary products or waste material.

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.

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

The proposed vaccine Bovela falls within the scope of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms.

The applicant has provided detailed information on the possible risks for humans and for the environment.

The pestivirus genome is a single stranded (positive) RNA. BVDV-1 and BVDV-2 differ from the respective wild type viruses only by the two deletions. The modified BVDV-1 and BVDV-2 strains from the vaccine can be distinguished by genetic analysis.

Although genetic analysis of the BVDV-1 MSV and the BVDV-2 MSV revealed some mutations as compared to the wild type, genetic stability was demonstrated in the production of the modified vaccine BVDV strains. The applicant has demonstrated genetic stability of the MSVs after a sufficient number of passages in vitro as well as in vivo. No abnormal mutation rate was observed. Accordingly, the BVDV-1 and BVDV-2 MSV can be considered as sufficiently genetically stable.

The vaccine virus strains were generated by two targeted deletions identical for BVDV-1 and BVDV-2 and do not contain any foreign sequences. The deletions minimise the risk for reversion to virulence.

Information relating to the conditions of release and the receiving environment were provided.

BVDV-1 and BVDV-2 are not known to be toxic to the infected animals and or to induce allergies in the infected animals. The data obtained in the clinical studies for target and non-target animals demonstrate that only mild local reactions occurred after administration of an overdose in the target species.

A possible recombination event between the vaccine strain and a virulent BVDV strain could lead to the creation of a recombinant BVDV strain with the same set of genes as the wild type virus or to a vaccine virus strain encoding the structural genes of the wild type virus but there is a low likelihood of this occurring.

Based on the results of the clinical studies, it can be concluded that only small amounts of the vaccine BVDV may arrive in the environment and these will not persist as BVDV can only grow in cells of an infected host organism. The vaccine virus was not shed from vaccinated animals via secretion or excretion routes except in trace amounts via milk for a limited time span. Calves fed on this milk did not develop any signs of infection or antibodies against BVDV. Similarly, mingling of sentinels with vaccinated animals did not lead to seroconversion of the sentinels. Accordingly, no biologically relevant spread of the vaccine viruses into the environment could be detected.

Infections with the vaccine viruses are restricted to Artiodactyla and spread to non-vaccinated sentinel animals does not occur.

The safety of the vaccine in the non-target species pig and sheep was evaluated. There were no adverse reactions following the direct administration of the vaccine.

The loss of N^{pro} expression is not expected to have any impact on the capability of the immune system of the host to combat the virus since N^{pro} is not an immunodominant target for the host immune response.

BVDV does not infect humans and is restricted to the infection of cloven-hoofed animals (Artiodactyla).

It is thus considered that there will be no need to monitor the presence of the vaccine viruses once a marketing authorisation has been approved. It is also considered that there will be no need for additional methods and procedures to prevent other organisms from entering the production site once a marketing authorisation has been approved.

Taken together, any risk emerging from the use of the attenuated vaccine BVDV-1 and BVDV-2 is negligible for humans and has to be considered as very low for the environment when used in accordance with the recommendations. Standard precautionary measures are considered sufficient.

Overall conclusions on the safety documentation

The vaccine virus strains with deletions are as stable as any other flavivirus and their double deletion makes their reversion to virulence theoretically very unlikely. A study conducted to corroborate the in vitro data failed to propagate the vaccine virus strains further to 2 passages between cattle because the vaccine viruses were not able to multiply enough. Consequently the in vivo reversion to virulence seems impossible in the target species.

In the target species, several safety studies were conducted at different vaccine dose to address different concerns in different categories of cattle. One study was conducted at the tenfold overdose in young calves and an increase in body temperature was observed concomitantly to the period when viraemia occurred. Results showed that viraemia was more frequent and longer in cows and specifically in naïve (seronegative) pregnant heifers. As well as in the buffy coat, live vaccine virus was found in other lymphoid organs when a sensitive assay was used (thymus, lymph nodes). Nevertheless no lesions were found in these lymphoid organs but leukopenia was observed in some studies. The protection and the BVDV antibody rise observed provided no concern for any conspicuous functional impairment of the immune system.

Regarding the reproductive function, which is a major concern for live flaviviruses, the provided studies as well as literature (Meyer, 2007) demonstrated the absence of persistent infection of foetuses.

The studies provided cannot, however, rule out other impact on fertility and transplacental transmission. Vaccine virus strain was isolated twice in foetuses from 2 different studies, even if the results were questionable and at least not indicating a persistent infection. A malformation and an abortion which could not be ascribed to any specific agent or mechanism, were observed in a laboratory study, but the investigation efforts were not optimal. In another laboratory study, a heifer supposed to be pregnant at vaccination, was found empty at calving date. Most importantly, the most sensitive marker of transplacental infection (Xue, 2009), the pre-colostral status was not provided for calves whereof the dam was vaccinated after the 4th month of gestation.

Concerning fertility, data are limited to the analysis of one reproductive parameter (persistent infection) and collected from a very few number of number of animals at risk (19) from 2 farms where the control of all agents responsible of reproductive failure was disputable. And besides the vaccine viruses were bathing organs for a long period after vaccination (long-lasting viraemia) and had a long excretion in emunctories; unfortunately no data was provided for the reproductive organs (ovaries and endometrium).

In view of the information provided, it is considered that the vaccination of reproductive females should be undertaken on a case-by-case basis as decided by the veterinarian, taking into account e.g. the BVD immunological status, the time-span between vaccination and mating/insemination and the stage of pregnancy. Vaccination of seronegative heifers, i.e. naïve female animals, would represent the higher risk for the transplacental passage of the vaccine.

No data supported the absence of risk of the semen of a recently vaccinated bull. The product is however not intended for use in bulls.

No other BVD like symptoms or immediate systematic reaction to vaccination were observed in any of the studies provided. No local reactions were seen.

The vaccine virus strain was found in emunctories (milk) when an adequately sensitive assay was used, but other excretion routes were not well investigated. The presence of the virus was longer in milk than in the blood but at low concentration.

The epidemiological consequence from the transmission of the vaccine virus was low for cattle since no contact animal seroconverted when they were included in studies. As sheep is the most sensitive non-target species, experimental exposure of sheep to high vaccine load showed neither clinical signs nor transmission to contact animals.

The spreading of the vaccine strain into the environment is highly unlikely and the environmental risk is therefore considered very low.

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.

The CVMP concluded that user safety for this product is acceptable when used as recommended in the SPC.

Residue studies are not required. The active ingredient being a substance of biological origin intended to produce active immunity does not fall within the scope of Regulation (EC) No. 470/2009 with regard to residues of veterinary medicinal products in foodstuffs of animal origin. In addition the other components of the vaccine are either listed in table 1 of the annex of Commission Regulation 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

Efficacy was investigated in accordance with the requirements of Directive 2001/82/EC and the Ph. Eur. monograph 5.2.7. Additional guidance was mainly provided by the Ph. Eur. monograph 1952 on bovine viral diarrhoea vaccine (inactivated).

Bovela is intended to protect cattle from BVDV infection and especially pregnant cows and their foetuses from persistent infection. The vaccine is intended to reduce clinical signs, viraemia and leukopenia when administered to cattle from 3 months of age onwards and prevent transplacental infection. Immunity is intended to be established 3 weeks after a single injection lasting for 12 months. When the foetus is not protected from transplacental infection, the birth of a persistently infected offspring which is the cornerstone of the disease epidemiology may occur with non-cytopathic (ncp) BVDV infection and less frequently, abortion, malformation and stillbirth may occur with both cytopathic (cp) and ncp BVDV.

The studies were carried out with batches at the minimum of potency and in some cases even lower. Field trials batches were representative of the manufacturing process and had an intermediary potency titre.

In laboratory studies, a heterologous ncp BVDV-1 or BVDV-2 strain challenge was administered according to an established model (Kovacs et al., 2003 and Charleston et al., 2001) of persistent infection of foetuses and in compliance with Ph. Eur. monograph on inactivated BVD vaccines (1952) and other relevant regulatory requirement. Pregnant cows were challenged at day 60–90 of gestation to demonstrate the protection against BVDV persistent infection of the foetus. In calves the efficacy parameters were focused on clinical manifestation of the disease, viraemia and leukopenia. The serological response to vaccination was also used as an efficacy parameter. The applicant provided documentation to demonstrate the ability to validate the measurements of the efficacy parameters. Due to low field study design and complications protection against thrombocytopenia, infertility, abortion and malformation could not be assessed conclusively.

Efficacy end points were chosen according to the Ph. Eur. monograph on inactivated BVD vaccine and were supported by literature. Reproductive primary end points were focused on the birth of persistently infected animals; therefore ear notch and blood were sampled to enable BVDV virulent strains to be isolated. Pre-colostral serology was not undertaken in field studies for practical and ethical reasons and because it covers only the part of the pregnancy when foetal immune system is operational.

Laboratory trials

Dose determination

Two studies were submitted to justify the determination of vaccine dose.

Minimum immunizing dose (MID) study (and DOI) with BVDV-1 challenge

This study was designed both to determine the titre of the vaccine necessary to the protection against BVDV-1 challenge and the duration of immunity (DOI). BVDV seronegative heifers older than 6 months were divided into 4 groups; 2 groups (26 animals) of vaccinated and challenged animals (vaccine dosed at about 10^3 and 10^4 $TCID_{50}$), one control group (9 animals) only was challenged. A strict control group (2 animals) remained which received no vaccination and no challenge in order to detect any circulation of any wild type virus.

Cows of the 3 groups were challenged by a heterologous BVDV-1 strain between 60 and 90 days of gestation and 13 months after vaccination. Foetal organs were analysed 2 months later for BVDV infection.

Foetal infection rate was the primary study variable. Any case of suspected foetal infection had to be confirmed by at least one BVDV-positive finding from tissue of respective heifers. Therefore, various foetal organ tissues (cerebellum, spleen, thymus and Peyer's Patches) from the vaccinated groups were investigated after challenge by virus isolation. None of these tissues was tested positive for BVDV. During the course of the study no wild BVDV strain circulated as foetuses of control animals were BVDV-negative and control cows (until challenge) remained seronegative.

Despite that no viraemia was detected, the challenge was effective since 8 of 9 control animals seroconverted by day 14 post-challenge and all their foetuses had organs BVDV positive. Both vaccine titres were efficient since all foetuses of vaccinated cows were BVDV negative. Cows seroconverted between day 14 and day 28 post-vaccination and there was no statistically significant difference between the vaccinated groups.

No differences in clinical manifestation were observed between groups. No biologically relevant difference of temperature between groups was noticed as well as in leukocyte and platelet counts.

All vaccinated animals showed seroconversion. In general antibody titres against BVDV-2 were lower compared to BVDV-1, but in both cases there were no differences between the two vaccinated groups. Antibody levels did not decline until challenge. By monitoring the antibody response post-re-vaccination of non-pregnant animals after 12 months, at least an equivalent antibody response compared to the first vaccination was demonstrated. There was also no significant difference in antibody titres when compared the two vaccinated groups.

Overall there were no differences in the efficacy observed in the two vaccinated groups.

Minimum immunizing dose (MID) and DOI study with BVDV-2 challenge

The study was designed similarly to the BVDV-1 MID study above. In this study a heterologous BVDV-2 challenge strain was used.

All challenged control animals (9 animals) seroconverted before day 14 and their foetuses were infected, indicating a successful challenge. Temperature was slightly increased in control 9 days post-challenge concomitantly to viraemia. Six animals out of 9 had viraemia (positive results between day 6 and 10 post-challenge). A decrease of the circulating leukocytes was observed from day 4 to 10. Vaccinated animals (29 animals challenged) seroconverted earlier for the BVDV-1 than for the BVDV-2 valence (until 2 weeks and 2 months respectively). BVDV-1 antibody titres were maximal 2 months post-vaccination and did not decline within the 12 months before challenge. Neither viraemia nor decrease of leukocyte count was detected in the two groups of vaccinated heifers. No BVDV was detected in any foetus of vaccinated heifers.

Further to revaccination, the antibody rise was similar to that after primary vaccination and did not differ between the two vaccinated groups.

No significant differences were observed in the efficacy observed with the lower dose of 10^3 $TCID_{50}$/2 ml.

In conclusion, on the basis of the two studies above, duration of immunity of 12 months was considered demonstrated for both BVDV-1 and BVDV-2 strains with regards to the protection from the birth of a persistently infected offspring.

The selection of the dose was based on the onset of immunity as the higher dose was needed in order to have a significant effect on the leukocyte count in calves.

Onset of immunity

Onset of immunity (OOI) study with BVDV-1 challenge

Three-month-old calves (n=30) were intranasally challenged with a heterologous BVDV-1a challenge strain 21 days after vaccination. One group was injected with a vaccine of an intended titre of 10^3 $TCID_{50}$/dose, and the other group 10^4 $TCID_{50}$/dose.

Calves were seropositive by 2 weeks after vaccination and BVDV-1 antibodies raised earlier than BVDV-2 ones. No distinct clinical finding was observed.

Challenge was weak. In controls, leukocytes decreased slightly and transiently on day 6 after challenge concomitantly with a viraemia detected in 2 animals. Controls had hyperthermia on day 8 after challenge; however no viral excretion was noticed.

In vaccinated calves (10 animals), the leukocyte count results were inconclusive. No viraemia was identified nor a viral excretion in the nasal swab.

Hyperthermia 8 days post-challenge was also prevented by both doses of vaccines.

In conclusion, prevention of viraemia and hyperthermia were the only end points that could be considered demonstrated in this study with an OOI at 21 days post-vaccination.

OOI study with BVDV-2 challenge

The study was designed similarly to the BVDV-1 OOI study above and challenged with a heterologous challenge strain.

All control animals had viraemia 4 days after challenge which persisted 6 days. They shed virus intermittently in their nasal secretion from day 2 to 14. No special clinical signs were noticed except from moderate increase in temperature.

Vaccinated animals (10 animals) never shed virus in their nasal secretion. There was no viraemia in all but one vaccinated animal. This animal was one of the low dose vaccinated calves and had viraemia 6 days after challenge.

Leukocyte count results were very variable, especially during the base-line period. A tendency to leukopenia after vaccination as well as prevention to leukopenia by the high dose vaccine could be noticed. Hyperthermia 8 days post-challenge was also prevented by both doses of vaccines.

In conclusion on the OOI studies presented above, an OOI at 21 days post-vaccination in three-months-old calves could be supported.

To determine the OOI in pregnant animals, the serological titres achieved in pregnant cows that protected from persistent infection of their offspring were compared to the calf titres from the onset of immunity study. Since both had comparable titres the OOI was extrapolated taking into account that there is no age-dependant difference of the serological immune response. This was considered acceptable.

Influence of maternally derived antibodies on the efficacy of the vaccine

Evaluation of the interference with maternally derived antibodies (MDA) of a BVDV-1 and BVDV-2 vaccine in calves

The MDA interference was investigated in calves (n=33) vaccinated at 3 months of age with a vaccine of an intended low titre of 10^3 $TCID_{50}$. Animals were intranasally challenged when MDA disappeared, more than 5 months later, with 12 ml of a mix of BVDV-1 and BVDV-2. Vaccination prevented viral nasal shedding, viraemia, mild leukopenia as well as the 8 day hyperthermia. Clinical signs as well thrombocytopenia and leukopenia specific to BVD failed to be induced in this mix challenge model, supporting therefore the conclusion that the MDA did not interfere with the vaccination.

In conclusion MDA did not interfere with vaccination since animals vaccinated at 3 month of age, were protected from viraemia and concomitant hyperthermia as well as leukopenia and viral nasal shedding when challenged more than 5 months later by a mix of BVDV-1 and BVDV-2.

Duration of immunity (DOI)

Three studies were submitted to support the duration of immunity and two of them have already been detailed earlier in the report. A 4th study was interrupted and only serological data were available which corroborated the serological feature of immune response to Bovela. Two studies have already been detailed earlier in the report. This study was not essential for the assessment.

Evaluation of the duration of immunity of a BVDV-1 and BVDV-2 vaccine candidate against virulent BVDV-1 challenge

A GCP compliant study was conducted to determine the duration of immunity as well as to validate the re-vaccination schedule.

BVDV seronegative heifers older than 6 months were vaccinated with a low dose vaccine (BVDV-1: $10^{4.3}$ $TCID_{50}$ and BVDV-2: $10^{3.8}$ $TCID_{50}$) and challenged 14 months later together with a control group (no mock vaccination took place).

A heterologous BVDV-2 challenge was given. Viraemia was detected in 5/6 heifers on day 8 post-challenge. The challenge decreased the number of circulating leukocytes and rose the temperature of control animals from 4 to 10 days post-challenge. All challenged control animals seroconverted before day 14 and their foetuses were infected. BVDV was isolated from all control foetuses. The above indicated a successful challenge.

All vaccinated heifers seroconverted by day 28. BVDV-1 response was faster than BVDV-2 one and antibody titres remained constant until challenge. Challenge virus was not isolated from vaccinated foetuses or from any of the buffy coats of their pregnant heifers. The temperature and leukocyte count remained unchanged.

After the booster injection, the antibody response was not different from the primo vaccination one.

In conclusion on the basis of three DOI studies, a DOI of 12 months was considered supported for both BVDV-1 and BVDV-2 strains with regards to the protection from the birth of a persistently infected offspring. The 12 month DOI was also supported with regards to clinical signs only for the BVDV-1 strain.

Re-vaccination

One year after primary vaccination the BVDV antibody level was still at plateau in the three DOI studies. Some individuals had lower titres. Since there is no protection threshold for the titre of seroneutralising

antibodies, a boost vaccination was proposed by the applicant to keep this level of antibodies higher than the one demonstrated to be protective. This approach was deemed acceptable.

Field trials

The comprehensive assessment of these studies is provided in the Part 3 on safety.

Evaluation of field safety and efficacy in dairy cattle after vaccination with a BVDV-1 and BVDV-2 vaccine

Eight dairy cattle herds with heifers and cows at all stages of pregnancy as well as non-pregnant cattle and male animals scheduled for insemination were included. In this randomized, blinded and negative controlled study, the vaccine was administered at the lowest marketing titre of about 10^4 $TCID_{50}$/dose.

Five herds had a history of BVDV circulation or a detection of persistently infected animals at the beginning of the study. Persistently infected animals were removed from the herds at the beginning of the study, lowering the risk of exposure to BVDV during the course of the study. Eight persistently infected calves were born in the control group of 2 of the 5 herds but all of their dams were at the persistent infection susceptibility period around the beginning of the study. In the control group, 1 calf with a congenitally defect supposed to be made 3 months beforehand, was found BVDV viraemic at birth and one BVDV positive foetus was calculated to be contaminated around the beginning of the study. There was no seroconversion in one of these 2 herds and only 2 out of the 8 sampled cows in the other one but just at the 1st sampling so that exposure to BVDV could have occurred before the beginning of the study. The only compelling evidence of BVDV circulation provided by serological survey was found in a herd where no persistently infected animals were born; 2 cows out of 45 seronegative ones seroconverted between the 2nd and the 3rd sampling. Besides, the infectious pressure brought by the birth of the 11 persistently infected calves was difficult to evaluate in a GCP study where biosecurity measures should be strictly implemented. In conclusion, infectious pressure appears to have been very low and mainly indirect due to the presence of persistently infected animals before the beginning of the study.

All but 4 animals in the vaccinated group seroconverted to both BVDV types further to vaccination (98%).

The foetuses of 280 vaccinated cows which were seronegative at the beginning of the study (supposed to be BVD naïve at the beginning of the study and the protection of which was only due to vaccination and not to natural immunization) had their persistent infection risk period during the course of the study. BVDV persistently infected 5 newborn calves in the vaccinated group and 10 in the control group. The dams of 4 persistently infected newborns were vaccinated after the 80–120 days of gestation period indicating that the infection occurred prior to vaccination. The 5th vaccinated cow was protected around its 70 day of pregnancy which casts doubt on the actual exposure of the foetus to BVDV before or after the persistent infection period. Beside 3 other calves born from vaccinated dams were found virus positive in the buffy coat only and a transient infection taken in calving houses or a laboratory error was deemed more plausible than a persistent infection.

In conclusion, no clear efficacy conclusion can be drawn from the study, since it was not clearly ruled out that no persistent infection occurred during the period of the study where the dams should have been protected.

Moreover, no statistical difference between vaccinated and control groups was observed regarding the other study end-points (fertility rate, abortion rate, clinical signs).

Evaluation of field safety and efficacy in beef cattle after vaccination with a BVDV-1 and BVDV-2 vaccine

In this GCP field study, the efficacy of the vaccine was evaluated in 11 Hungarian beef cattle herds with various rates of BVDV seropositive animals. The vaccine had a median titre of $10^{5.05}$ $TCID_{50}$ for BVDV-1 and $10^{4.45}$ $TCID_{50}$ for BVDV-2 per dose. The study was randomized, blinded and a control group was injected with the diluent.

In the control group, more than 92% animals remained seronegative throughout the study, indicating an absence of BVDV exposure in the course of this study. Less than 3% of the vaccinated cows remained seronegative. One newborn from each group was BVDV-1 wild strain positive in ear notch. The persistent infection susceptibility period of both calves was before the start of the study.

In conclusion there was no evidence that BVDV was circulating during the course of the study albeit it did before and therefore no conclusions can be made from this study.

Overall conclusion on efficacy

To reproduce the non-reproductive feature of the disease in laboratory trials, animals were intranasally challenged with a suspension of heterologous BVDV strains and monitored at least 2 weeks post-challenge. Unfortunately this disease model, especially the infection by the BVDV-1 strain, failed to trigger most of the characteristics of the disease. A non-cytopathic (ncp) BVDV-1a strain isolated in UK and a BVDV-2 strain isolated in Germany were used for challenging. Clinical signs except for hyperthermia as well as thrombocytopenia were never elicited. Viraemia consistently occurred when BVDV-2 was administered. The above indicate that the challenge was not particularly effective mainly and the BVDV-2 challenge appeared more successful than the BVDV-1 challenge. Nevertheless it should be noted that the most sensitive parameter which is the persistent infection of the foetus was induced by both challenge strains, regardless of the level of the viraemia detected.

The proposed indication for prevention of transplacental infection of the foetus was supported by studies designed according to the persistent infection model where cows are challenged at 60–120 days of pregnancy by a ncp strain. Other outcomes of transplacental infection were not investigated in experimental studies and cannot be supported by the two field studies. Pre-colostral serology which is a more sensitive marker of transplacental infection (Xue, 2009) was not undertaken. Consequently the proposed indication for prevention of transplacental transmission cannot be considered as fully demonstrated.

Reduction of hyperthermia, viraemia and leukopenia claims were fully supported only for the BVDV-2 challenge in naïve heifers. In the other experimental designs (calves and/or BVDV-1), some differences were detected between vaccinated and control animals but they were not statistically significant. For BVDV-1 protection was investigated successfully in both laboratory studies and a field trial but only a claim for reduction of hyperthermia and leukopenia could be supported on the basis of results. BVDV-2 protection was successfully investigated in both challenge studies under controlled laboratory conditions and by a comparison of serological titres; results supported a claim for reduction of hyperthermia, virus shedding, viraemia and minimisation of leukocyte reduction caused by BVDV-2.

Onset and duration of immunity were similar with a vaccine at 10^3 and 10^4 $TCID_{50}$/dose except for the prevention of leukopenia for which the higher dose was more effective. Results supported a primo-vaccination at 3 months of age with 10^4 $TCID_{50}$/dose. The onset of immunity was investigated in 3-months-old calves and extrapolated to pregnant cows. This was considered acceptable. The annual re-vaccination scheme was supported by a comparison of antibody increase between primary vaccination

and booster. At the time of the boosting injection the antibodies titres declined in some animals. A booster was therefore proposed by the applicant to guarantee the protection of every animal.

MDA did not interfere with vaccination since animals vaccinated at 3 month of age were protected from viraemia and concomitant hyperthermia as well as leukopenia and viral nasal shedding when challenged more than 5 months later by a mix of BVDV-1 and BVDV-2.

Two field studies were undertaken in milk and beef herds. Cows from milk herds were put at low risk of BVDV infection by culling all the persistently infected animals before the beginning of the study and it was not clearly documented that none of the persistently infected animals born during the study, had been infected in the period where the vaccine should have provided protection. No BVDV exposure happened in beef cattle. The design of these field studies did not lead to a clear-cut conclusion over the efficacy of the vaccine during pregnancy and gave no information about the other efficacy claims (hyperthermia, leukopenia, virus shedding and viraemia).

In conclusion, the product has been shown to be efficient for active immunisation of cattle from 3 months of age to reduce hyperthermia and to minimise the reduction of leukocyte count caused by bovine viral diarrhoea virus (BVDV-1 and BVDV-2), and to reduce virus shedding and viraemia caused by BVDV-2.

The product has also been shown to be effective for the active immunisation of cattle against BVDV-1 and BVDV-2, to prevent the birth of persistently infected calves caused by transplacental infection.

The product has been shown to have an onset of immunity 3 weeks (21 days) after vaccination, which was demonstrated in 3 months old calves and extrapolated to pregnant cows, and a duration of immunity of 1 year (12 months) with regards to protection from the birth of a persistently infected calf.

Part 5 – Benefit-risk assessment

Introduction

Bovela is a live vaccine against bovine viral diarrhoea (BVD) viruses that are circulating in Europe. It contains two genetically modified strains of different genotypes, BVDV-1 and BVDV-2, which are attenuated by an identical double genetic deletion (E^{rns} and N^{pro}). This is a full application submitted in accordance with Article 12(3) of Directive 2001/82/EC for a product developed by means of a biotechnological process and containing a new active substance.

Benefit assessment

Direct therapeutic benefit

Bovela is intended to prevent persistently infected newborn calves and to lower viraemia, leukopenia and hyperthermia which results from BVDV infection. The control of persistently infected calves is the cornerstone of the epidemiology of BVD and the focus of any control program.

Heterologous challenge performed in efficacy laboratory studies including calves and cows, demonstrated that Bovela is active against the strains currently circulating in Europe.

The product is effective for active immunisation of cattle from 3 months of age to reduce hyperthermia and to minimise the reduction of leukocyte count caused by bovine viral diarrhoea virus (BVDV-1 and BVDV-2), and to reduce virus shedding and viraemia caused by BVDV-2.

The product is also effective for active immunisation of cattle against BVDV-1 and BVDV-2, to prevent the birth of persistently infected calves caused by transplacental infection.

Bovela was shown to have an onset of immunity of 3 weeks (21 days) after vaccination, which was demonstrated in 3 months old calves and extrapolated to pregnant cows, and a duration of immunity of 1 year (12 months) with regards to protection from the birth of a persistently infected calf.

Prevention of transplacental infection leading to pregnancy outcomes other than persistent infection has not been fully demonstrated because important markers of such transplacental infections are missing. Nevertheless, persistent infection is considered as the pivotal hazard of the control of BVDV infection in the field and to this end Bovela gave compelling results.

Field studies were not able to definitely support these conclusions because BVDV circulation was weak or absent.

Additional benefits

Studies indicate that the vaccine may be efficient at a dose 1 $\log_{10}$ $TCID_{50}$ lower than the lowest marketing dose for reduction of shedding, hyperthermia, prevention of persistent infection in foetus, 3 weeks after one single injection.

The vaccine contains two different genotypes of BVDV and provides some protection against both after one injection.

Risk assessment

The main potential risks are addressed as follows:

Quality:

The manufacturing process was overall well documented and specifications have been set to ensure consistent quality. Genetic stability of the two deletions (N^{pro} / E^{rns}) that lead to attenuation of the vaccine viruses was demonstrated in vitro and shown to be stable in vivo in virus isolated from the reversion to virulence studies.

For the target species:

Given that a long lasting viraemia was seen in most vaccinated pregnant animals, that a vaccine BVDV-1 strain was found in two foetuses in two different studies and that no pre-colostral serology was available it cannot be excluded that a foetus infection by the vaccine strains can occur, although the likelihood is probably low.

An impact of vaccination on fertility of naïve female animals could not be completely ruled out since the assessment of the risk relied only on a weak statistical analysis of a heterogeneous cohort of 19 animals. Therefore the vaccination should be finalised 3 weeks before insemination. Otherwise a case-by-case decision should be taken concerning vaccination by the responsible veterinarian.

Vaccinated animals were viraemic and viraemia was seen for a long time in emunctories (milk). Nonetheless no spreading of the vaccine virus was substantiated in contact or milk feeding animals. However a clear warning was issued to prevent vaccination of breeding bulls.

No other BVD like symptoms or immediate systematic reaction to vaccination were observed in all the studies provided. No local reactions were observed after vaccination.

For the user:

The CVMP concluded that user safety for this product is acceptable when used as recommended and taking into account the safety advice in the SPC. BVDV does not infect humans and is restricted to the infection of cloven-hoofed animals (Artiodactyla).

For the environment:

The product is not expected to pose any risk to the environment when used as recommended.

For the consumer:

Residue studies are not required. The withdrawal period is set at zero days.

Specific potential risks:

The vaccine virus strains were shown to be genetically and phenotypically stable in study monitoring the production, with very low risk of reversion to virulence. No biologically relevant spread of the vaccine viruses to contact calves could be detected.

A recombination event between the vaccine virus strain and a virulent BVDV strain is possible however would likely lead to the creation of a recombinant virus strain with the characteristics of the parental BVDV strains used to generate the two vaccine virus strains.

The virus cannot replicate outside mammalian cells.

The safety of the vaccine in the non-target species pig and sheep was evaluated. There were no adverse reactions following the direct administration of the vaccine by the nasal route, although every sheep seroconverted.

Risk management or mitigation measures

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

Notably, vaccination should be finalised 3 weeks before insemination. Otherwise a case-by-case decision should be taken concerning vaccination by the responsible veterinarian.

In addition a special warning to avoid use in breeding bulls is considered necessary although the vaccine is not intended for this category of the species.

Evaluation of the benefit-risk balance

The product has been shown to have a positive benefit-risk balance overall.

This live vaccine contains a GMO. It is intended for use against bovine virus diarrhoea which is a significant disease of cattle subject to control programs in Europe.

This vaccine has been shown to be effective for active immunisation of cattle from 3 months of age to reduce hyperthermia and to minimise the reduction of leukocyte count caused by bovine viral diarrhoea virus (BVDV-1 and BVDV-2), and to reduce virus shedding and viraemia caused by BVDV-2.

The product is also effective for active immunisation of cattle against BVDV-1 and BVDV-2, to prevent the birth of persistently infected calves caused by transplacental infection.

The formulation and manufacture of Bovela is well described and in general specifications set will ensure that product of consistent quality will be produced.

The product is well tolerated by the target animal. An impact of vaccination on fertility of naïve female animals could not be completely ruled out, since the assessment of the risk relied only on a weak statistical analysis of a heterogeneous cohort of 19 animals. Therefore the vaccination should be finalised

3 weeks before insemination. Otherwise a case-by-case decision should be taken concerning vaccination by the responsible veterinarian.

The product presents an acceptable risk for users, for consumers and the environment when used as recommended and appropriate warnings have been included in the SPC. A sufficient withdrawal period has been set.

Conclusion on benefit-risk balance

The overall benefit-risk evaluation for the product is deemed positive with a sufficiently clear and complete product information.

Conclusion

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP concluded that the quality, safety and efficacy of Bovela were considered to be in accordance with the requirements of Directive 2001/82/EC.

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP recommends the granting of the marketing authorisation for Bovela.