DEPARTAMENTO DE
MEDICAMENTOS
VETERINARIOS

Agencia Española de Medicamentos y Productos Sanitarios

C/Campezo 1, Edificio 8
28022 – Madrid
España
(Reference Member State)

MUTUAL RECOGNITION PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

BOPROTEC IBR MARKER LIVE LYOPHILISATE AND SOLVENT FOR SUSPENSION FOR CATTLE

CORREO ELECTRÓNICO

mresvet@aemps.es

C/ CAMPEZO, 1 – EDIFICIO 8
28022 MADRID
TEL: 91 822 54 01
FAX: 91 822 5443

Código de campo cambiado

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

MODULE 1

PRODUCT SUMMARY

EU Procedure number	ES/V/0329/001/DC
Name, strength and pharmaceutical form	BoProtec IBR marker live lyophilisate and solvent for suspension for cattle
Applicant	VETIA ANIMAL HEALTH S.A. Polígono de la Relva s/n – Torneiros 36400 Pontevedra SPAIN
Active substance(s)	Live attenuated Bovine herpesvirus type 1 (BHV-1), strain Bio-27: IBR gE - negative, $10^{5.7}$- $10^{7.5}$ $CCID_{50}$
ATC Vet code	QI02AD01
Target species	Cattle
Indication for use	For active immunisation of cattle to reduce the severity and duration of respiratory symptoms of viral infection caused by BHV-1 (IBR - infectious bovine rhinotracheitis) and to reduce the excretion of IBR virus. Onset of immunity:. The onset of immunity was demonstrated for respiratory clinical signs 7 days after intranasal vaccination, and 14 days after intramuscular vaccination of animals without maternally derived antibodies; seroconversion was demonstrated 28 days and 35 days for intrasal and intramuscular administration, respectively. Duration of immunity: In calves aged 3 months, 6 months after primary vaccination (intramuscular administration); for animals without maternally derived antibodies aged 2 weeks was demonstrated by challenge to be 10 weeks administered intranasally.

MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL
Agencia española de **medicamentos y productos sanitarios**

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the Heads of Medicines Agencies website (http://www.hma.eu).

MINISTERIO DE
SANIDAD, CONSUMO Y
BIENESTAR SOCIAL
Agencia española de
medicamentos y
productos sanitarios

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	Decentralised application in accordance with Art/icle 12(3) of Directive 2001/82/EC as amended.
Date of completion of the original mutual recognition procedure	Day 210: 03/07/2019
Date product first authorised in the Reference Member State (MRP only)	N/A
Concerned Member States for original procedure	PT

I. SCIENTIFIC OVERVIEW

The product is produced and controlled using validated methods and tests, which ensure the consistency of the product released on the market.
It has been shown that the product can be safely used in the target species; no reactions were observed along the studies as indicated in the SPC.
The product is safe for the user, the consumer of foodstuffs from treated animals and for the environment, when used as recommended. Suitable warnings and precautions are indicated in the SPC.
The efficacy of the product was demonstrated according to the claims made in the SPC.
The overall risk/benefit analysis is in favour of granting a marketing authorisation.

MINISTERIO DE SANIDAD, CONSUMO Y BIENESTAR SOCIAL
Agencia española de **medicamentos y productos sanitarios**

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

II. QUALITY ASPECTS

A. Qualitative and quantitative particulars

The product contains the active substance *Bovine herpesvirus* type 1 strain Bio-27:IBR gE-negative (titre ≥ $10^{5,7-7,5}$ $CCID_{50}$ per dose of 2 ml) in a freeze dried powder. The vaccine also contains a solvent.

The container/closure system meets the necessary Ph. Eur. Standards and are adequately sterilised. The administration device for the intranasal application is also described.

The applicant provided a brief summary in support of the product development including a justification of the strain choice. The relevance of the strain choice is based on the information described in the scientific publication about the BHV-1 biology, epidemiology, diagnosis and prophylaxis (Biswas et al 2013). The dose is supported by bibliographic information and the efficacy studies submitted in the dossier.

B. Method of Preparation of the Product

The product is manufactured fully in accordance with the principles of good manufacturing practice from a licensed manufacturing site.

The product is manufactured in accordance with the European Pharmacopoeia and relevant European guidelines.

As in many other vaccines, the manufacturing process includes the use of one antibiotic and one antifungical substance. Their content in the final product is considered negligible and far below the acceptable Daily Intake (Neomycin Summary Report, CVMP, EMEA/MRL/816/02-FINAL; Gentamicin Summary Report, CVMP, EMEA/MRL/803/01-FINAL).

C. Control of Starting Materials

The active substance is the *Bovine herpesvirus* type 1 strain Bio-27:IBR gE-negative. The active substance is manufactured in accordance with the principles of good manufacturing practice.

The active substance specification is considered adequate to control the quality of the material. Batch analytical data demonstrating compliance with this specification have been provided.

Scientific data and/or certificates of suitability issued by the EDQM have been provided and compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products has been satisfactorily demonstrated.

Starting materials of non-biological origin used in production comply with applicable pharmacopoeia monographs or in-house specifications.

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

Biological starting materials used are in compliance with the relevant Ph. Eur. Monographs and guidelines and are appropriately screened for the absence of extraneous agents according to the *PhEur 2262 Serum Bovinum, PhEur 5.2.5 Substances of animal origin for the production of immunological veterinary medicinal products, PhEur 5.2.8 Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products, PhEur 0062 and PhEur 5.2.4 Cell cultures for the production of veterinary vaccines.*

The master and working seeds have been produced according to the Seed Lot System as described in the relevant guideline.

The production and control of the diluent is adequately described. The bioburden is measured and the integrity of the filters tested.

D. Control tests during production

The tests performed during production are described and the results of 3 consecutive runs, conforming to the specifications, are provided.

E. Control Tests on the Finished Product

The tests performed on the final product conform to the relevant requirements; any deviation from these requirements is justified. The tests include in particular the appearance, identity and viral titre, bacterial and fungal sterility, extraneous agents, residual moisture and pH.

Tests for the solvent (appearance, pH, extractable volume, sterility and airthightness) and the limits applied are adequate.

The demonstration of the batch to batch consistency is based on the results of 3 batches produced according to the method described in the dossier. Other supportive data provided confirm the consistency of the production process.

F. Stability

The in-use shelf-life of the reconstituted vaccine is supported by the data provided.

G. Other Information

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

III. SAFETY ASSESSMENT

The batch employed along the safety studies was Batch no. 03 57 16 at a titre/dose of $10^{8.5}$ (antigen content at a vaccine dose, $10^{7.5}$) except for the field study in which besides the already mentioned batch a different one was used (live batch no. 02 57 16 whose antigen content at a vaccine dose was $10^{6.7}$).

Laboratory trials

Safety of the vaccine Boprotec was investigated by performing six laboratory studies; all were designed in order to comply with the relevant veterinary legislation applicable at the time they were performed; all studies were conducted according to the regulations of good laboratory practice (GLP). Animals to be included in the studies should be negative to antibodies anti-IBR, be in a normal physical condition and also lack in any clinical symptom of disease.

The safety of the administration of a single dose was not carried out, and this was deemed acceptable based on the fact that proposed vaccination schedule intended for calves always comprises a double administration of the product, and this is precisely the schedule followed in the next study (see below), but administering 10x dose/calf (safety of administration of an overdose), which it could be considered as a worst case scenario; consequently the single dose is considered covered.

Performed studies intended for supporting safety were:
Overdose administration of one dose in the target animal. Safety of Boprotec IBR marker live was studied after the intranasal administration of a tenfold overdose (10x) to calves at 2 weeks of age, and the repeated intramuscular administration at 3 months of age. The results concluded that none of the animals showed any clinical sign/systemic reactions/rectal temperature increases over 1ºC after vaccination, neither intranasally nor intramuscularly. There were no local reactions associated to intranasal or to intramuscular vaccination.
Repeated administration of one dose in the target animal. Safety of the vaccine Boprotec IBR marker live after repeated intramuscular administration (14 or 28 days later) of a first normal dose and second tenfold (10x) overdose to calves from 2-3 months of age was studied. According to results, safety was demonstrated as already established requirements were met: No systemic/local reactions were detected and rectal temperatures could be considered to remain unaltered.

In summary, laboratory studies support safety for the vaccine, as no calf showed abnormal local or systemic reactions or died from causes attributable to the vaccine virus. Points 4.6 and 4.10 of the SPC adequately reflect this conclusion.

Effects on reproductive performance were examined by means of the Study "*Safety of the vaccine Bovitex IBR marker live in pregnant cows, ability to induce abortion and passage through the placenta*", that was performed according to a plan assimilable to current Ph. Eur. 04/2013:0696. 24 pregnant cows at different gestation stages (three groups of 8 cows, each of them belonging to the first, second or last third of the gestation, respectively) received 10 times the maximum content in the vaccine at once, via intramuscular. Regardless of the gestation stage, none of the cows showed temperature increases, noticeable symptoms or local reactions. Regarding calves, they

were all normally born, being capable of sucking colostrums and without detectable IBR antibodies in blood. Consequently, the vaccine demonstrated to be safe and can be used during pregnancy and lactation (point 4.7 of the SPC).

There were no data suggesting that this product might adversely affect the immune system of the vaccinated animal or its progeny; therefore a specific study was not carried out.

Given that the vaccine is live attenuated, the Applicant carried out three studies (a to c, see below) on Boprotec IBR marker live in order to comply with applicable legislation for this specific type of vaccines:

a. Evaluation of the dissemination´ ability of the vaccine´ virus in vaccinated calves. 10 animals were employed; half of the animals was vaccinated (five were 2 weeks old, and five were 3 months old, intranasally and intramuscularly vaccinated, respectively). After the calves being vaccinated intramuscularly the virus was not isolated between day 0 (vaccination day) and day 14 in any sample from nasal and oral swabs, urine or faeces. On turn during the study of the dissemination of the vaccine IBR virus in calves vaccinated intranasally samples from nasal swabs of the vaccinated animals evidenced IBR virus from nasal swabs from Day 2 to Day 5 after vaccination; remaining samples (oral swab, faeces and urine) were negative for the observation period (along 14 days after vaccination). No other relevant findings were observed (i.e., clinical observations, temperature increases, injection sites), regardless of the vaccination route. Thus, results showed no evidence of the dissemination of the virus after intramuscular administration but dissemination by nasal discharge after intranasal administration.

b. Spread of the Virus after vaccination. The study enrolled a total number of 20 animals. Ten calves were two weeks old (5 vaccinated, intranasally as by the proposed schedule; 5 controls) and ten calves were 3 months old (5 vaccinated intramuscularly, as by the proposed schedule; 5 controls). Animals were monitored along 21 days, period where neither symptoms (local, general) nor any temperature shift over 1ºC were evidenced in the animals. In relation with the dissemination of the vaccine IBR virus nasal swabs samples as well as blood samples were taken. Virus was only isolated in nasal swabs from the intranasally vaccinated animals from day 2 to 5 after vaccination; results of serological examination showed the presence of virus neutralization antibodies to IBR on day 21 after vaccination, but no antibodies presence in controls at the same time point was evidenced.

c. Reversion of the Vaccine Strain to Virulence. The aim of the study was to demonstrate the irreversibility of the attenuation of the vaccine virus contained in the vaccine Boprotec IBR marker live, after the administration of one dose of the tested vaccine in animals vaccinated as recommended, with the vaccine batch having the maximum declared potency, and prepared from the lowest passage of the virus from MSV which can be expected in the finished product. Vaccination administered once intranasally (both nostrils) in 5 calves from 2 weeks of age (IBR delet live, $10^{8.5}$ $CCID_{50}$ 10 doses dissolved in 2 ml (MSV+1)). Successively, 4 groups of 2 calves each, received intranasally a 5 ml suspension (MSV+2, MSV+3, MSV+4, MSV+5, respectively, 2^{nd} to 5^{th} passage) obtained from nasal swabs with the demonstrated presence of IBR virus (suspensions of Days 3 and 4 after vaccination –see point b, above- were homogenized for further passaging). Lately, a group of 8 calves received

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

5 ml suspension (MSV+6, 6th passage, irreversibility of attenuation); interval between individual passages: 9-11 days (next passage was always carried out once the virus presence was demonstrated in the previous passage). The latest group (comprising eight calves) showed no post-vaccination reactions or increase in body temperature. As no calf showed signs that could be attributed to the vaccine virus and, on turn, there was no increase in the virulence of the virus obtained from the maximum passage compared with the unpassaged virus the criteria established were met; thus, the attenuation irreversibility test verified the safety of the vaccine IBR virus.

Despite the fact that attenuation showed to be irreversible, virus was isolated from nasal swabs after vaccination for a short period (maximum 5 days); accordingly SPC includes an adequate reference on potential virus´ transmission in line with obtained results (point 4.5, special precautions for use in animals).

The adjuvant and excipients used are common constituents of the vaccines; they are adequately described and documented. Only two antimicrobials are used for media, but the quantities in which they are added do not warrant the adoption of any measure and accordingly no withdrawal period is proposed.

No specific assessment/study of the interaction of this product with other medicinal product was made. Therefore, an appropriate warning (No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product) in the SPC is included (point 4.8).

Field studies

The safety of Boprotec IBR marker live was tested in field conditions. Field tests were conducted on three cattle farms in different categories of target species (calves and pregnant animals). The study aimed to evaluate safety after the scheduled administration of the vaccine dose. In parallel, the study was designed to demonstrate the efficacy in field conditions of the vaccine.

Each of the three herds contributed a total number of 40 animals (10 calves aged 2 weeks, 10 calves aged 3 months and 20 pregnant animals) which were vaccinated. Calves were vaccinated following the proposed schedule: those aged 2 weeks, intranasally at that point, revaccinated intramuscularly at the age of 3 months; calves aged 3 months vaccinated once at that age. The inclusion of pregnant cows pursued to evaluate a potential impact of vaccination on the course of pregnancy and the viability of newborn calves (pregnant animals were observed until the first day after parturition).
It was only possible forming one unvaccinated control group, because an IBR eradication programme is running there. A control group was formed of pregnant cows vaccinated with a single dose of the commercially produced vaccine Rispoval IBR Marker inactivatum. Besides, the study was designed for evaluating the effect of vaccination on milk yield on Farm 1.

Consequently, there were other additional groups on each farm (other than those vaccinated with Boprotec), as follows:
Farm 1. Pregnant cows. Control group (n=10), vaccinated with (Rispoval IBR marker

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

inactivatum); group of cows (n=10) for assessing the effect on milk production of vaccination with Boprotec; group of cows (n=10) for assessing the effect on milk production of vaccination with Rispoval IBR Marker inactivatum

Farm 2. Pregnant cows. Control group (n=10), vaccinated with (Rispoval IBR marker inactivatum).
Farm 3. Pregnant cows. Control group (n=5), vaccinated with (Rispoval IBR marker inactivatum); unvaccinated control group (n=10)

On top of local reactions and systemic reactions recordings and temperature measurement, blood samples were taken in: *Pregnant animals*: At vaccination and 3 weeks after vaccination (6 weeks later in control groups); in *calves* vaccinated IN and revaccinated IM: At vaccination, 3 weeks after vaccination, at revaccination, and 3 and 6 months after the last administration; *calves* vaccinated IM: At vaccination, 3 weeks after and vaccination and 6 months after vaccination. From sera extracted antibodies to IBR were examined by ELISA, using a commercial set BHV-1; additionally, a commercial ELISA set IDEXX IBR gE AB was used to confirm the presence of the vaccination marker.

Results showed that the administration of the tested vaccine (vaccination, revaccination) had no negative impact on the health of vaccinated calves (aged 2 weeks or 3 months, indistinctly). After the observation period no case of inadequate systemic reaction, local reaction, or body temperature increase in relation to the administered vaccine was recorded; neither was compromised safety of pregnant animals after intramuscular administration of one dose of the vaccine (2 ml) to pregnant cows or heifers at different stages of pregnancy in any of the farms as results of monitoring period evidenced. Viability of newborns was not compromised, with any abortion recorded. The administration of the tested vaccine had no negative impact on the health of pregnant cows; on turn health status of newborn calves did not reveal any negative effect of vaccination on the fertility of pregnant cows.

Environmental Assessment

The applicant provided a first phase environmental risk assessment in compliance with the relevant guideline (EMEA/CVMP/074/95) which showed that no further assessment is required, provided that the product is administered as proposed in the SPC; point 6.6 of the SPC adequately cautions on the procedure to be followed with unused product/disposal of waste products. Warnings and precautions as listed on the product literature are adequate to ensure safety to the environment when the product is used as directed.

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

IV. CLINICAL ASSESSMENT (EFFICACY)

Batches employed along the efficacy studies were No.: 01 57 16; Titre/dose: $10^{5.7}$, $CCID_{50}$ (2 ml); batch 035716, 1st passage of MSV Bio 27, 10 $^{8.5}$ $CCID_{50}$/2 ml. Batch 045119; and IBR challenge strain CHSV Bio 34:IBR $10^{7.8}$ CCID $_{50}$/1 ml.

IV.B Clinical Studies

Laboratory Trials

Nine GLP efficacy tests comprise the fulfilment of the different points to be covered in order to demonstrate efficacy of the veterinary vaccine in laboratory conditions; they were designed to meet the requirements of the applicable veterinary legislation and were performed in accordance with the recommended vaccination scheme.

Scoring system applied to assess results from the efficacy studies was based on the following scale, with slight variations: Fever. Score 0 to 3 corresponded respectively to up to 39,5ºC (score 0); 39,6-40ºC (1); 40,1-41ºC (2) and from 41,1ºC (3); Apathy. None (0); mild (1); visible (2) and severe (3); Nasal discharge: None (0); mild, serious (1); abundant, catarrhal (2) and mucopurulent (3); Eye discharge. None (0); mild, serious (1) and severe (2); Dyspnoea. Normal breathing (0); accelerated breathing (1); dyspnoea (2) and severe dyspnoea (3).

Animals to be included in the efficacy studies need to fulfil the following requirements: Free of antibodies to IBR, in a normal physical condition and without any clinical symptoms of disease. For evaluating efficacy animals were monitored for local reactions and clinical observations (after vaccination and after challenge by means of a clinical score determination system focused in fever, apathy, nasal discharge, eye discharge and dyspnea and temperature measurement, as described above) together with blood and nasal swabs sampling.

Onset of immunity
a) Onset of immunity against IBR after intranasal administration of the vaccine to calves aged 2 weeks. 5 calves were administered the vaccine once intranasally (1 ml/each nostril) on day 0. 7 days later those 5 calves and 2 control calves were challenged with IBR virus (namely, CHSV Bio-34 IBR, the same to be used in the efficacy studies), 4 ml (2 x 2 ml in each nostril) whose titre was 4 x 10^8.

The vaccine meets the test if vaccinated calves show only mild symptoms of IBR while controls show typical symptoms of the disease; if the maximum virus titre (in nasal swabs) ≤ 100 times the average maximum titre found in control calves, in at least 4-5 vaccinated calves; and if the average number of days of virus excretion in vaccinated calves is at least 3 days shorter than in the control group.

Results showed no adverse reactions, temperature´s variations remained between narrow margins after vaccination, but after challenge temperatures in unvaccinated controls were higher than in vaccinated although no statistical analysis was attempted; mean score after challenge (the next 21 days) were 5,2 and 36 in vaccinated and controls, respectively; virus excretion in nasal swabs lasted until day 18 after vaccination in controls, only up to day 12 in vaccinated animals, ie, the vaccine met the

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

tests; finally, regarding the onset of immunity the antibody response to IBR virus by VNT and ELISA was quantified on days 7 and 28 after vaccination; recorded values were similar in controls and vaccinated animals (VNT IBR) and negative on day 7 for controls, not examined in vaccinated animals.

b) Onset of immunity against IBR after the intramuscular administration of the vaccine to calves aged 2-3 months. 5 calves aged 2 weeks were administered the vaccine (batch no.: 01 57 16, $10^{5.7}$,$CCID_{50}$ (2 ml), once intramuscularly on day 0. 14 days later those 5 calves and 2 control calves were challenged with IBR virus, 4 ml (2 x 2 ml in each nostril) whose titre was 4 x 10^8. Established criteria for the vaccine to meet the test, as in the previous study (see point a), above).

After vaccination there were no adverse reactions recordings, and temperature measurements did not shift markedly; nevertheless, after challenge temperatures in unvaccinated controls were higher than in vaccinated but no statistical analysis was attempted; mean score after challenge (days 14-35, i.e. 3 weeks) were 5.2 and 41 in vaccinated and controls, respectively; virus excretion in nasal swabs lasted until day 20 after challenge in vaccinated animals (6 days), up to day 25 in controls (11 days); finally, regarding the onset of immunity the antibody response to IBR virus by VNT and ELISA was quantified on days 14 and 35 after vaccination; mean response by VNT in vaccinated animals reached 1.8 *versus* a value of 0 in controls; later, corresponding values on day 35 calculated by VNT recorded were respectively 6 and 0 (not statistically analyzed). In parallel, test by ELISA was negative on day 14 for vaccinated, positive 21 days later; besides, controls were not examined on day 14 and showed positive values on day 35.

Duration of immunity

c) Duration of immunity against IBR after intramuscular administration of calves aged 2-3 months. 5 calves not older than 3 months were vaccinated intramuscularly with Boprotec IBR marker live. 183 days after the start of the study those 5 calves and 2 control calves were challenged with IBR virus, 4 ml (2 x 2 ml in each nostril, titre 4 x 10^8). Animal requirements, monitoring and criteria for the vaccine to meet the test remain the same as in previous studies.

Neither local (monitored up to day 4) nor clinical observations (monitored up to day 14) were recorded after vaccination, and temperatures only showed slight variations from day -1 to 4. After challenge, clinical observations (days 183-204) were scored leading to an average score of 5.4 in the vaccinated group, 39 in controls; rectal temperatures after challenge were higher in the control group naked eye (days 185 to 193); regarding antibody response to IBR virus by SNT and ELISA, it was weaker than in previous studies in vaccinated animals (ranging average values of SNT IBR 1.0-2.2), always nil in controls. A clear increase was evidenced 21 days after challenge (day 204) reaching similar values for vaccinated group and controls (5 and 4.5, respectively). ELISA IDEXX IBR Ge AB showed negative values on day 14 after vaccination, but positive on day 204 while test was performed in controls only on day 204 being positive too. Virus excretion lasted in controls up to day 194 after challenge, only up to day 188 in vaccinated group. Based on the results obtained the intramuscular administration of the vaccine showed to be immunogenic for a 6 months period in calves aged 2-3 months.

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

d) Duration of immunity to IBR after the intranasal administration of a tenfold (10x) overdose at 2 weeks of age and the repeated intramuscular administration at 3 months of age and the effect of booster vaccination in calves. 5 calves were vaccinated two times, when aged 14 days (day 0 of the study, intranasally) and on day 75 (intramuscular). Vaccine was prepared dissolving 2 vials of 5 doses in 2 ml. Later, booster vaccination took part on day 255 of the study (2 ml intramuscularly, batch 035716, $10^{8.5}$ $CCID_{50}$/2 ml). No noteworthy findings after local and general symptoms monitoring, neither on rectal temperatures measurements. Antibody response to IBR virus by SNT was measured on days 0, 28, 75, 103, 255 and 283 of the study. Average values were respectively 0, 4.4, 2.8, 5, 1.8 and 5.2. Thus, the proposed schedule of vaccination allows reaching adequate antibody response, lacking any reaction, given the administered dose comprises 10 times the normal dose.

e) Duration of immunity against IBR after the intramuscular vaccination at 2-3 months of age and the effect of booster vaccination in calves. The study was performed in 7 animals aged 3 months, from which 5 received 2 doses (2 ml each) by the intramuscular route (gluteal muscle), 6 months apart. First dose, day 0 (at the age of 3 months), the second 182 days after primary vaccination; remaining 2 animals served as controls. Animal´ requirements and monitoring were as in previous studies. No noticeable signs after the schedule vaccinations. In relation with antibody response to IBR virus by SNT samples were taken on days 0, 28, 182 and 210. Results were always zero in controls, but fluctuate as follows in vaccinated animals (average values): 0, 2.4, 1.2 and 5.8. A second vaccination dose with Boprotec IBR marker live containing the minimum IBR virus titre ($10^{5.7}$ $CCID_{50}$/2 ml) made 6 months after the primary vaccination led to a rise in the level of specific antibodies to IBR virus higher than the level of antibodies obtained after the primary immunization; antibodies´ level was increased 28 days after second vaccine dose. Vaccination thus generates an increase in antibody levels that is maintained for 6 months from the initial vaccination at 3 months of age.

f) Duration of immunity against IBR after intranasal vaccination of calves aged 2 weeks. 16 calves around 2 weeks of age were splitted into two equal groups, depending on the presence (or absence) of maternally derived antibodies (MDA+/MDA-); on turn, in each group from 8 calves 6 were vaccinated and 2 served as controls. Vaccine included IBR marker live$10^{5.5}$ $CCID_{50}$/ 2 ml, batch 045119; IBR challenge strain was CHSV Bio 34:IBR $10^{7.8}$ $CCID_{50}$/1 ml; sterile phosphate buffer saline was employed for placebo/diluent. On day 0 animals were administered 2.0 ml of the tested materials by the intranasal route (1 ml/nostril), using nasal applicator. Control animals were administered with the placebo under the same conditions. On day 70 IBR challenge strain was similarly administered to all 16 animals by the same route (using a nasal applicator) but at a volume of 4 ml (2 ml/nostril) of challenge strain virus to each animal. Once more inclusion criteria, monitored items and vaccine compliance are essentially the same as in the early described studies. Nasal swabs samples (one/nostril) were collected from each animal prior to challenge strain administration on day 70 and then daily until the day 91 (end of study). Blood samples were collected on day -7 for determination of antibody status of animals, prior to test material administration on day 0, and then on days 14, 28, 42, 56, before IBR challenge strain administration (day 70) and at the end of the study (day 91).

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

Results. Rectal temperature scores records evidenced that all the MDA+ animals together with the MDA- placebo treated animals were affected; but only half of the vaccinated MDA-; mean scores were 2.8-4.5/0.5-5 (MDA+ vaccinated-placebo/MDA- vaccinated-placebo, respectively). No reaction could be observed. Total clinical scores were as follows: 22-27.5/12-38.5 (MDA+ vaccinated-placebo/MDA- vaccinated-placebo, respectively). VNT antibody titre values (geometric mean) were essentially the same in the vaccinated and placebo groups at the different sampling points in the MDA+ group; on the contrary vaccinated animals from the MDA- group always had higher values when compared to those from placebo group. Thus, duration of immunity in MDA- animals reaches 10 weeks when vaccinated with BoPrrotec IBR marker live and later challenged but vaccination was ineffective in MDA+ animals.

Influence of colostral antibodies

g) Influence of colostral antibodies on the vaccination of calves aged 3 months.15 calves were divided into three groups: Animals with maternally derived antibodies (MDA+), vaccinated (n=5); animals MDA+, not vaccinated-controls (n=5); animals MDA- vaccinated (n=5). On day 0, vaccinated animals received 2 ml intramuscularly ($10^{5.7}$ $CCID_{50}$/2 ml), and controls were administered by the same route 2 ml of diluent A; 30 days later all the animals were challenged (CHSV Bio-34 IBR challenge strain $10^{7.6}$ $CCID_{50}$/1 ml) at a dose of 2 ml (on each nostril). Animals´ inclusion criteria consisted of adequate physical condition without signs of disease, individuals not older than 3 months (+/- 7 days) and a minimum weight of 80 kg. The vaccine would meet the test if vaccinated calves show only mild signs of IBR while unvaccinated controls show typical signs of the disease; at least in 4 out of 5 vaccinated calves the maximum virus titre found in nasal swabs needs to be 100 times less than the average maximum titre found in control calves; average number of days of virus excretion in vaccinated calves should be at least three days less than in the control group; antibodies´ level after vaccination in the presence of MDAs is comparable to that of the calves without MDAs.

Follow up was covered by rectal temperatures measures (through a pre-established score in which normal temperatures were all those between 38-40ºC; records were done before vaccination and the next 4 days and from challenge up to 21 days thereafter), clinical observations (number of spontaneous coughs produced by each animal, depression/abnormal general appearance, nasal/ocular discharge and dyspnoea using a scoring system, daily along the entire study period), blood samples (on days -8, 0, 14, 21, 28, 30 and 51) and swab sampling (one sample of each nostril before challenge, on day 30 and then daily until day 51).

No reaction could be observed in any animal in the vaccination period (days 0-29). After challenge, results from temperature´ measurements showed increases over 40ºC in all unvaccinated animals (days 2-6 period) even though in 2 only was a one-day increase, while only 2/5 of each group of vaccinated animals showed similar increases on turn. Mean scores were similar in vaccinated animals (0.6 and 0.4) for MDA+ and MDA-, respectively, reaching a value of 2.0 for controls; once the animals were scored reached values were similar for vaccinated animals, regardless of the presence of maternal derived antibodies or not (MDA+: Total scores 20, median 3; MDA-: Total scores 22, median 4) while total scores and media for placebo group (MDA+) reached 132 and 25, respectively. In relation with antibody titres (IBR serology, geometric mean by VNT) an obvious difference was noted between MDA+ -vaccinated/controls- and

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

MDA- animals (91.1/90.5 vs. 2.2). The values of antibodies titres evolved over time, and on day 30, vaccinated animals had similar figures (MDA+, 78.8 and MDA-, 81.7) but values of controls were lower (30.8). Nevertheless, at the end of the study (day 51) figures were as follows: MDA+ vaccinated, 95.6; MDA+ controls, 90.5; MDA- vaccinated, 94.8). During the challenge phase all animals were shedding challenge virus and showing clinical signs; statistically analyzed vaccinates compared to control animals showed significantly reduced incidence and intensity of clinical symptoms, and a decreased magnitude and duration of IBR virus excretion: The maximum virus titre found in the nasal mucous of all 5 vaccinates (4.6 $\log_{10}$ $CCID_{50}$/Ml) was more than 100 time lower than the average of the maximum titres found in the control calves (6.9 $\log_{10}$ $CCID_{50}$/Ml); and the average number of days on which virus was excreted was more than 3 days less in vaccinated calves (6.8 days) than in the control calves (10.6 days)
In view of the results it seems that the presence of MDA could eventually interfere in the effectiveness of the vaccine.

Influence of colostral antibodies on the efficacy of vaccination
h) Influence of colostral antibodies on the efficacy of vaccination of calves aged 2 weeks. This test was not performed because, in words of the Applicant there is sufficient knowledge of the effect of maternally derived antibodies (MDAs) on the vaccination with live IBR vaccines in the literature, and thus the possibility to reduce the number of animals intended for testing of the vaccine under development. Applicant´s position is based on EMA Reflection Paper on impact of MDA´s on vaccine´efficacy on young animals (EMA/CVMP/IWP/439467/2007), on five bibliographical references (Lemaire et al., 2000; Menanteu-Horta et al., 1985; Strube et al., 1996; Patel & Shilleto, 2005; Patel, 2005) and finally is based on to the already demonstrated efficacy of a similar commercial vaccine (Bovilis IBR marker live).
Despite this approach was deemed unacceptable, it was judged that it did not interfere with final authorisation conclusions.

i) Influence of colostral antibodies on the efficacy of vaccination (with BoProtec IBR marker live) of calves aged 3 months. 12 calves were divided into three groups: Animals with maternally derived antibodies (MDA+), vaccinated (n=5); animals MDA+ not vaccinated-controls (n=2); animals MDA- vaccinated (n=5). On day 0, vaccinated animals received 2 ml intramuscularly ($10^{5.7}$ $CCID_{50}$/2 ml), and controls were administered by the same route 2 ml of diluent A; 21 days later the animals were again vaccinated under the same pattern as on day 0; finally, on day 55 all the animals were challenged (CHSV Bio-34 IBR challenge strain $10^{8.0}$ $CCID_{50}$/1 ml) at a dose of 2 ml (on each nostril) and observed during 21 days. Animals´ inclusion criteria consisted of adequate physical condition without signs of disease, individuals not older than 3 months (+/- 10 days) and not having received any immunologically active substance. The vaccine would meet the test if vaccinated calves show only mild signs of IBR while unvaccinated controls show typical signs of the disease; at least in 4 out of 5 vaccinated calves the maximum virus titre found in nasal swabs is 100 times less than the average maximum titre found in control calves; average number of days of virus excretion in vaccinated calves is at least three days less than in the control group; and if antibodies´ level after vaccination in the presence of MDAs is comparable to that of the calves without MDAs.

Study variables were: Rectal temperature (21 days after challenge); clinical examination (21 days after challenge by means of a scoring system, please refer to

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

second paragraph of section IV.B); serological examination (VNT): for that purpose blood samples are collected in vaccinated animals on days 0, 21, 35, 49, 55 and 76 of the study. From the unvaccinated calves, blood samples were collected on days 0 and 21; for excretion of IBR virus nasal swabs for the detection of the virus titre in the nasal mucus were collected from all animals on Day 55 of the study and subsequently daily until day 76 of the study.
Results. Monitoring of IBR clinical signs after challenge showed a mean score of 2.4 in group of vaccinated MDA(+), 0.8 in the group of vaccinated MDA(-), and 41.5 in unvaccinated MDA(+). Significant differences were found between the groups with (0.8) and without MDAs (2.4), and between vaccinated versus the unvaccinated control group of animals with MDAs. Rectal temperatures after challenge in the vaccinated animals (MDA+ and MDA-) fluctuated in the physiological range (38.2-39.5ºC) until the end of the study; no calf manifested temperature rise by more than 2.0°C. Rectal temperatures in the unvaccinated control animals with MDAs increased significantly between Day 3 and Day 7 after challenge. The maximum temperature was 41.5°C on Days 4 and 6 after challenge; Seroconversion in titres 1-2(log 2) was recorded in the vaccinated animals MDA- after the administration of one dose of the vaccine until day 21 of the study, when calves were revaccinated; later, on Day 35 the titre of antibodies increased to 2- 3 (log 2) and these levels of antibodies persisted until Day 55, the challenge´s day. From that point antibodies were examined until the end of the study (day 76), when the titre of antibodies was 4-5 (log2). In the unvaccinated control animals gradual disintegration of colostral antibodies can be observed until their complete disappearance on day 49 of the study. After challenge, a slight increase in the titre of antibodies is observed in two out of five vaccinated calves MDA+; 21 days after challenge, the average titre of virus neutralizing antibodies increased from 4.2 to 4.6. In the vaccinated calves MDA- the average titre of antibodies increased 21 days after challenge from 2.2 to 4.4. The unvaccinated control animals had the average titre of antibodies 3.5 21 days after challenge. In the calves MDA+ vaccinated the virus was detected from day 1 to day 5 after challenge. The maximum virus titre in a calf was $10^{3.6}$ $CCID_{50}$. The maximum titre in all vaccinated calves ranged from $10^{2.8}$ $CCID_{50}$ to $10^{3.6}$ $CCID_{50}$ from day 2 to day 4 after challenge. The unvaccinated control animals manifested of virus excretion from day 1 to day 12 after challenge with the maximum average titre $10^{8.8}$ $CCID_{50}$ on Day 4 after challenge (day 59 of the trial). Finally, in calves MDA- vaccinated the virus was detected from day 1 to day 5 after challenge. The maximum virus titre in a calf was $10^{3.6}$ $CCID_{50}$. The maximum titre in all vaccinated calves ranged from $10^{2.8}$ $CCID_{50}$ to $10^{4.6}$ $CCID_{50}$ from day 2 to day 3 after challenge.
The vaccine met the conditions of the test.
According to results the presence of MDA could eventually interfere in the effectiveness of the vaccine.

Field Trials

Please refer to point III Safety, Field trials. Both safety and efficacy were covered by the same study. Complementary information focusing on efficacy is included here.

Statistical comparison of the change in the initial and final levels of antibodies after vaccination regardless of the employed vaccine (IBR BioBos delet live or Rispoval IBR marker inactivatum) in pregnant cows on the first farm showed no significant

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

differences, neither in the two-factor analysis of variance; nevertheless, calculated p-values were close to 0.05.

After statistical evaluation of the antibody levels (response) of adult pregnant animals, in two out of three farms a statistically significant increase was associated to the vaccine administration. On turn, vaccinated calves on all the farms, except one 3-months-aged group, intramuscularly vaccinated, in one farm, showed statistically significant decrease in antibody levels when the initial state was compared with the state 6 months after vaccination.

Milk yield was monitored in connection with the performed vaccination in 10 vaccinated cows (maximum potency, batch no. 035716 – $10^{8.5}$ $CCID_{50}$ per dose) in one farm. Simultaneously, milk production was monitored in a control group of 10 unvaccinated cows. In all those 20 animals daily milk production was monitored individually from day -5 to day 9 after vaccination (first group) and between the groups. Performed vaccination showed no interference on milk production after statistical analysis (two-factor analysis of variance).

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

V . OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

The data submitted in the dossier demonstrate that when the product is used in accordance with the Summary of Product Characteristics, the risk benefit profile for the target species is favourable and the quality and safety of the product for humans and the environment is acceptable.

BOPROTEC IBR MARKER VIVA LIOFILIZADO Y DISOLVEN

MODULE 4

POST-AUTHORISATION ASSESSMENTS

The SPC and package leaflet may be updated to include new information on the quality, safety and efficacy of the veterinary medicinal product. The current SPC is available on the veterinary Heads of Agencies website (www.hma.eu).

This section contains information on significant changes which have been made after the original procedure which are important for the quality, safety or efficacy of the product.

None