Institute for State Control of Veterinary Biologicals and Medicines
Hudcova 56a
621 00 Brno
Czech Republic
(Reference Member State)

DECENTRALISED PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

BIOSUIS Entero emulsion for injection
(CZ, RO, BG, PL, HU, LT, LV, EE, SK)

BIOSUIS Rokoclos emulsion for injection
(IT)

Porvaxin Rota+Coli+Clos emulsion for injection
(ES, PT)

Rococlos vet. emulsion for injection
(FI, NO, SE)

Suigen Entero 3 emulsion for injection
(DE, FR, AT)

Rokopig Entero emulsion for injection
(IE, UK(NI))

FIXR Rota Coli Clostri emulsion for injection
(BE, NL)

MODULE 1

PRODUCT SUMMARY

EU Procedure number	CZ/V/0184/001/DC
Name, strength and pharmaceutical form	**Biosuis Entero emulsion for injection**
Applicant	Bioveta, a.s. Komenského 212 683 23 Ivanovice na Hané Česká republika
Active substance(s)	Porcine rotavirus, serogroup A, strain OSU 6, inactivated *Escherichia coli*, serotype O149:K88 (F4ac), inactivated *Escherichia coli*, serotype O101:K99 (F5 and F41), inactivated *Escherichia coli*, serotype K85:987P (F6), inactivated *Clostridium perfringens*, type C, beta toxoid
ATC Vetcode	QI09AL09
Target species	Pigs (pregnant gilts and sows)
Indication for use	For passive immunisation of newborn piglets by active immunisation of pregnant gilts and sows to reduce: - Clinical signs (neonatal diarrhoea) and mortality caused by *E. coli* strains expressing the fimbrial adhesins F4ac, F5, F6 and F41 - Clinical signs (neonatal diarrhoea, vomiting and anorexia) caused by porcine rotavirus - Clinical signs (neonatal diarrhoea, enteritis) and mortality caused by beta toxin (expressed by *Clostridium perfringens*)

MODULE 2

The Summary of Product Characteristics (SPC), the labelling and package leaflet for this veterinary medicinal product (VMP) is available in the Union Product Database (UPD).

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	Full application in accordance with Article 8 (full dossier) of Regulation (EU) 2019/6.
Date of completion of the original decentralised procedure	28/02/2024
Date product first authorised in the Reference Member State (MRP only)	NA
Concerned Member States for original procedure	RMS: CZ CMS: AT, BE, BG, DE, EE, ES, FI, FR, HU, IE, IT, LT, LV, NL, NO, PL, PT, RO, SE, SK, UK(NI)

I. SCIENTIFIC OVERVIEW

The product is manufactured 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; the slight reactions observed are 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.

II. QUALITY ASPECTS

A. Qualitative and quantitative particulars

Each dose (2 ml) contains:

Active substances:

Porcine rotavirus, serogroup A, strain OSU 6, inactivated	RP ≥ 1*
Escherichia coli, serotype O149:K88 (F4ac), inactivated	RP ≥ 1*
Escherichia coli, serotype O101:K99 (F5 and F41), inactivated	RP ≥ 1* (F5), RP ≥ 1* (F41)
Escherichia coli, serotype K85:987P (F6), inactivated	RP ≥ 1*
Clostridium perfringens, type C, beta toxoid	RP ≥ 1*, **

F = fimbrial adhesin
* RP = Relative potency (ELISA), in comparison with reference serum obtained from vaccinated mice with vaccine batch, which complied in challenge test on target species.
** minimal listed value complies with potency ≥ 20 IU required by Ph. Eur.

Adjuvant:

Montanide ISA 35 VG 0.52 ml

Excipients:
Thiomersal 0.2 mg
Formaldehyde max. 1 mg
Sodium hydrogenphosphate dodecahydrate
Potassium dihydrogenphosphate
Sodium chloride
Water for injections

Glass vials of the volume of 10 ml, 50 ml or 100 ml sealed with a rubber pierceable stopper and aluminium or flip-off caps.
Plastic vials of the volume 60 ml, 120 ml or 250 ml sealed with a rubber pierceable stopper and aluminium or flip-off caps.
The original vial is inserted into paper compressible box fitted with printing.

The choice of the adjuvant, vaccine strains, formulation, inactivating agent, presence of preservative are justified.

The inactivation process and the detection limit of the control of inactivation are correctly validated.

The product is an established pharmaceutical form and its development is adequately described in accordance with the relevant European guidelines.

B. Method of Preparation of the Product

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

Process validation data on the product have been presented in accordance with the relevant European guidelines.

C. Control of Starting Materials

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

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 Ph. Eur.

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

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:

Appearance
Content of original packing
Concentration of hydrogen ions (pH)
Viscosity [1]
Airtightness
Identification of active substance [1]
- Identification of PRV component
- Identification of E. coli component
- Identification of Clostridium perfringens (toxoid β) component

Potency [1]
- Potency test of PRV component
- Potency test of E. coli component
- Potency test of Clostridium perfringens toxoid β

Identification and assay of adjuvants
- Determination of formaldehyde [1]
- Thiomersal [1]
- Content of adjuvant [1]

Sterility
Inactivation and detoxification [2]
- Residual live virus
- Residual live bacteria - *E. coli*
- Residual live bacteria - *Clostridium perfringens*

Residual toxicity (beta toxin)
Content of alfa toxin
Bacterial endotoxins [3]

[1] Performed on bulk
[2] Performed as in-process control (IPC) immediately after the inactivation and /or detoxification
[3] The test is performed as in process control immediately after mixing the required volume of all active substances and phosphate buffer (before adding thiomersal and adjuvants). This value is calculated per dose from the determined as IPC.

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

Stability data on the active substances have been provided in accordance with applicable European guidelines, demonstrating the stability of the active substance when stored under the approved conditions.
E. coli antigens can be stored before inoculation to the fermenter at 2-8 °C for up to 72 hours, after inactivation 24 hours and after concentration and purification 12 months.
Clostridium perfringens antigen can be stored before inoculation to the fermenter at 2-8 °C for up to 48 hours, beta toxoid after inactivation and purification 48 hours or at -80 °C for up to 12 months.
Porcine rotavirus antigen can be stored after inactivation 12 months at -20 °C.

Stability data on the finished product have been provided in accordance with applicable European guidelines, demonstrating the stability of the product throughout its shelf life (2 years) when stored under the approved conditions at (2 °C – 8 °C).

The in-use shelf-life of the broached vaccine (10 hours) is supported by the data provided.

G. Other Information

Not applicable.

III. SAFETY ASSESSMENT

The vaccine is administered intramuscularly at dose 2 ml to pregnant gilts and sows.
Basic vaccination
- first administration 4 weeks before expected farrowing
- second administration 2 weeks before expected farrowing

Revaccination
- during subsequent pregnancies: administration of 1 dose 2 weeks prior expected farrowing

Safety studies have been performed with a vaccine batch with maximum antigen content produced according to the described production process.
Field studies have been performed with a representative vaccine batch produced according to the described production process.

Laboratory trials

The safety of the administration of one dose, the repeated administration of one dose and safety related to reproductive performance was performed as one controlled laboratory study on the basis of Ph. Eur. 5.2.6. which in total included 20 animals (10 vaccinated animals and 10 control animals with placebo administration).

Rectal temperatures, general health status and local reactions were observed.
The safety studies demonstrate that the administration of one dose and the repeated administration of a dose can be considered to be safe, when used in accordance with the recommended vaccination schedule. The observed reactions are reflected in the relevant SPC and package leaflet sections:

Mild increase of body temperature (maximum increase observed in individual animals of 0.7 °C, with a maximum duration of 4 days post vaccination) was very commonly observed.

Mild swelling of maximum diameter 10 mm at the injection site, which persists for a maximum of 3 days post vaccination was commonly observed.

Effects on reproductive performance were examined. No effect on the reproductive performance in vaccinated pregnant gilts and on health status of new born piglets was observed thus the following is stated in the SPC and package leaflet:

"To be used during pregnancy according to the vaccination schedule described in section 3.9."

There are 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.

The vaccine is inactivated and thus the specific tests to be performed for live vaccines are not applicable.

The adjuvant and excipients used are Montanide ISA 35 VG, formaldehyde and thiomersal. The excipient and adjuvants are included in the Appendix of the Commission Regulation (EU) No 37/2010 – the substances that are not subject to determination of residues. For this reason, the presence of the residues was not tested. Based on this information, no withdrawal period is proposed.

No specific assessment of the interaction of this product with other medicinal product was made. Therefore, an appropriate warning in the SPC is included.

Field studies

The clinical evaluation was conducted at three independent test sites. Only safety evaluation was conducted at test site A. On test site B and C safety and efficacy were tested.
At each test site, 30 animals were included in the study, which were divided in two groups (20 vaccinated and 10 control animals). All 30 animals were designated for safety evaluation from the beginning
of the study. At test sites B and C, 10 vaccinated and 10 control animals were selected, which were also included in the efficacy evaluation.
The animals were administered with tested vaccine and the control group with reference items (site A and site B) or reference item without active substances (site C), according to the vaccination schedule, i.e. with dose 2 ml in 4 weeks before expected delivery and 2 weeks later.
Piglets born to these gilts/sows were fed with the colostrum/milk from their own mothers. All gilts/sows included to the field study were administered with the third dose of vaccine and all reference items at dose 2 ml 2 weeks before second expected parturition.
The safety of the preparation was assessed based on monitoring the local and clinical reactions of the animals after vaccination, including the evaluation of the possible effect on the course of pregnancy and the vitality of newly born piglets from vaccinated mothers compared with control animals and their piglets.
The safety of the vaccine in gilts and sows in the field has been demonstrated. The negative effect of vaccination was not observed in piglets.
The results from field trials reflect those observed in laboratory trials.

Environmental Risk Assessment

The applicant provided the first phase environmental risk assessment in compliance with the relevant guideline which showed that no further assessment is required. The assessment concluded that there is a negligible risk to the environment associated with use of the vaccine.

Warnings and precautions as listed in the product literature are adequate to ensure safety to the environment when the product is used as directed.

IV. CLINICAL ASSESSMENT (EFFICACY)

The vaccine BIOSUIS Entero emulsion for injection is intended for active immunisation of pregnant gilts and sows with following protection of the offspring against disease induced by enterotoxigenic *E. coli* strains, *Clostridium perfringens* strain and rotaviral infection. The piglets are protected during the suckling period from immunised mothers by inducing of colostral and lactogenic immunity.
The challenges were performed in separately studies for porcine rotavirus, individual *E. coli* (F4, F5, F41, F6) and *Clostridium perfringens* on piglets born to the vaccinated/control dams in conformity with Ph. Eur. 0962, 0636 and 5.2.7.
For the efficacy testing the batches of the vaccine containing the minimum amount of active substances were used.

The field testing used the vaccine with standard antigens content.
All challenge strains were selected as different from vaccination strains.

Laboratory Trials

The efficacy of the product has been demonstrated in laboratory studies in accordance with the relevant requirements which show that the vaccine reduces:
- Clinical signs (neonatal diarrhoea) and mortality caused by *E. coli* strains expressing the fimbrial adhesins F4ac, F5, F6 and F41
- Clinical signs (neonatal diarrhoea, vomiting and anorexia) caused by porcine rotavirus
- Clinical signs (neonatal diarrhoea, enteritis) and mortality caused by beta toxin (expressed by *Clostridium perfringens*)

The laboratory efficacy study included the following evaluation:
Onset of immunity:
- Efficacy against E. coli strains – with challenge 12 hours after birth of piglets
- Efficacy against porcine rotavirus – with challenge 5 days after birth of piglets
- Efficacy against *Clostridium perfringens*, type C, beta toxoid – with challenge 2 days after birth of piglets

Duration of immunity:
- Demonstrated based on challenge studies: 3 weeks of age.

In each study:
A total of 8 pregnant gilts (4 vaccinated and 4 control) seronegative against antigens contained in the test vaccine were selected.
The vaccine was applied to the 4 pregnant gilts in two individual doses on D0 (4 weeks before expected parturition), D14 (2 weeks before expected parturition) by the intramuscular route with the dose of 2 ml in all preclinical studies. In the same intervals was applied the placebo (saline solution) with the same way to the control animals.
A total of 30 newborn piglets (15 from vaccinated mothers and 15 from control mothers), were administered with the challenge inoculum by the oral route to each newborn piglet.
Observation of clinical signs of all animals was performed before challenge till eight days after challenge.
The blood samples were taken for serological analysis in order to evaluate the onset of immunity to determine the protective level. The blood samples were taken from pregnant gilts on the day of farrowing, from piglet before challenge material administration and then twice a week till 28th day after challenge.

It was proven by T-test that the difference between total clinical scores of vaccinated and control animals was statistically significant as a significant reduction in clinical signs (neonatal diarrhoea) and mortality caused by *E. coli* strains expressing the fimbrial adhesins F4ac, F5, F6 and F41. The data meet the criteria given by Ph. Eur. monograph 0962 Neonatal piglets colibacillosis vaccine (inactivated).
Based on the results obtained, the prevention of clinical signs caused by rotavirus infection in piglets was demonstrated in piglets from vaccinated gilts. The efficacy was assessed in compliance with the requirements of Ph. Eur. monograph 0962 (no specific monograph for PRV). There was a statistically significant reduction in clinical score in the group of piglets from the vaccinated gilts compared to the group from the unvaccinated controls in this study.
It was proven by T-test that the difference between total clinical scores of vaccinated and control animals was statistically significant as a significant reduction in clinical signs (neonatal diarrhoea, enteritis) and mortality caused by beta toxin (expressed by *Clostridium perfringens*). The efficacy of

the vaccine was assessed in compliance with the requirements of Ph. Eur. monograph 0363 (Clostridium perfringens vaccine for veterinary use) and taking into account special article for *E. coli* for implementation of the challenge 0962 (Neonatal Piglet colibacillosis vaccine (inactivated)). Here it was used as a model for a challenge test. Vaccination of pregnant gilts before farrowing results in activation of immune system of their new born progeny and protective levels of antibodies were determined. Antibody titres were only used as supporting parameters for evaluation of results of the studies and to calculate the protective level of antibodies.

Field Trials

The results from vaccination-challenge trials conducted under laboratory conditions have been supplemented with data from field studies.
The clinical evaluation was conducted at three independent test sites. Only safety evaluation was conducted at test site A. On test site B and C safety and efficacy were tested.
At each test site, 30 animals were included in the study, which were divided in two groups (20 vaccinated and 10 control animals). All 30 animals were designated for safety evaluation from the beginning of the study. At test sites B and C, 10 vaccinated and 10 control animals were selected, which were also included in the efficacy evaluation.
The standard batch of vaccine was administered. Control animals received reference items.
The efficacy evaluation was further divided into two parts when the vaccine's effectiveness was firstly demonstrated in the first litter of piglets, which ended 28 days after the first farrowing (duration of immunity is 21 days after farrowing). After this part of the efficacy, evaluation was completed, the animals were inseminated again. The second litters of piglets were tested for efficacy with a booster dose of the test vaccine, which lasted until day 28 after the second farrowing (duration of immunity is 21 days after farrowing).
It was proven that the difference between total clinical scores of vaccinated and control animals was statistically significant as a significant reduction in clinical signs and score was observed in vaccinated group.
Serological monitoring played a supporting role in vaccinated and unvaccinated animals and their offspring. There was a statistically significant difference between the vaccinated and control groups.
Vaccination stimulates the generation of antibodies in vaccinated pregnant gilts and sows for colostral and lactogenic passive immunity of newborn piglets against colibacilosis, acute necrotic enteral clostridial infection and rotaviral disease while suckling (protection verified by challenge till 21 days of age and in clinical trial by serology till 28 days in most animals except porcine rotavirus).

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.

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 in the Union Product Database (UPD).