Institute for State Control of Veterinary Biologicals and Medicines
Ústav pro státní kontrolu veterinárních biopreparátů a léčiv

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MUTUAL RECOGNITION PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

BIOSUIS APP 2,9,11
Emulsion for injection for pigs

MODULE 1

PRODUCT SUMMARY

EU Procedure number	CZ/V/0121/001/MR
Name, strength and pharmaceutical form	**BIOSUIS APP 2,9,11** **Emulsion for injection for pigs**
Applicant	Bioveta a.s. Komenského 212 683 23 Ivanovice na Hané Czech Republic
Active substance(s)	*Actinobacillus pleuropneumoniae* serovar 2 *Actinobacillus pleuropneumoniae* serovars 9,11 toxoid APX I toxoid APX II toxoid APX
ATCvetcode	QI09AB07
Target species	Pigs
Indication for use	For active immunisation of fattening pigs to mitigate the consequences of infection caused by *Actinobacillus pleuropneumoniae* – the causative agent of pleuropneumonia in pigs. The intention of the use is to reduce typical clinical signs, typical lung lesions of the disease and to reduce infection.

MODULE 2

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

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	Mutual Recognition application in accordance with Article 31 of Directive 2001/82/EC as amended.
Date of completion of the original mutual recognition procedure	20/12/2013
Date product first authorised in the Reference Member State (MRP only)	27/04/2012
Concerned Member States for original procedure	EE, HU, LT, LV, PL, SK

I. SCIENTIFIC OVERVIEW

The vaccine Biosuis APP 2,9,11 emulsion for injection for pigs is an inactivated vaccine containing whole cell antigens of *Actinobacillus pleuropneumoniae* and toxoids, oil adjuvant, formaldehyde, thiomersal and saline. The vaccine is intended for active immunization of fattening pigs to mitigate the effects of infection with *Actinobacillus pleuropneumoniae* - a causative agent of actonobacillosis in pigs.

Active immunity starts in vaccinated piglets 21 days after revaccination and persists for at least 20 weeks.

The recommended vaccination scheme is vaccination piglets from the age of 6 weeks with two doses of 1 ml vaccine at an interval of 3 weeks.

Administration route is intramuscular, preferably to the paraauricular area.

The withdrawal period is zero days.

Stability data, which support the proposed shelf-life of 2 years, have been provided.

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; the reactions observed are indicated in the SPC.

The product is safe for the user 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. Composition

Composition:

Active substances:

Actinobacillus pleuropneumoniae serovar 2	RP ≥ 1*
Actinobacillus pleuropneumoniae serovars 9, 11	RP ≥ 1*
toxoid APX I	RP ≥ 1*
toxoid APX II	RP ≥ 1*
toxoid APX III	RP ≥ 1*

*RP = Relative potency (ELISA test) in comparison with the reference serum obtained after vaccination of mice with a vaccine batch that has successfully passed the challenge test in the target species.

List of excipients:

Oil emulsion Montanide ISA 35 VG
Formaldehyde
Thiomersal
Sodium chloride
Water for injection

The vaccine is presented:

in glass injection vials of hydrolytic class I:	10-ml vial with a content of 10 ml
in glass injection vials of hydrolytic class II:	50-ml vial with a content of 50 ml
	100-ml vial with a content of 100 ml
in plastic injection vials:	15-ml vial with a content of 10 ml
	60-ml vial with a content of 50 ml
	120-ml vial with a content of 100 ml
in plastic bottles:	250-ml bottle with a content of 250 ml

Vials or bottles are hermetically closed with a rubber stopper for perforation and an aluminium seal and placed to paper cartons or in plastic box with 10 wells.
Pack sizes: 1 x 10 ml, 10 x 10 ml, 1 x 50 ml, 1 x 100 ml, 1 x 250 ml

The particulars of the containers and controls performed are provided and conform to the regulation of monographs 3.2.1 and 3.2.9 of the European Pharmacopoeia.

The choice of the vaccine strains, of the vaccine composition, adjuvant, inactivating agent, preservative, of the dose volume and vaccination schedule 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 from a licensed manufacturing site. A corresponding manufacturing licence and GMP certificates are provided.

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

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

C. Control of Starting Materials

Starting materials of non-biological origin used in production comply with indicated pharmacopoeia monographs.

Biological starting materials used are in compliance with the relevant Ph. Eur. Monographs and guidelines and are appropriately screened and appropriately treated for the absence of extraneous agents according to the Ph. Eur monographs.

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 Europea requirements.

Specific Measures concerning the Prevention of the Transmission of Animal Spongiform Encephalopathies

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.

D. Control tests during production

The tests performed during production (purity control, density control, determination of APX I, II, III toxins in bacterial culture, inactivation control, bacterial endotoxins, sterility test, pH determination, thiomersal) are described in detail and the results of 3 consecutive runs, conforming to the specifications, are provided.

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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. Relevant validations are provided.

The tests include in particular:

- appearance
- extractable volume
- sterility
- residual toxicity
- potency and identity

- pH value
- airtightness
- thiomersal
- formaldehyde
- bacterial endotoxins
- viscosity

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.

F. Batch to batch consistency

The consistency of production has been demonstrated and the results of 3 consecutive runs, conforming to the specifications, are provided.

G. Stability

Stability data on the active substances have been provided in accordance with applicable European guidelines, demonstrating the stability of the active substances (inactivated antigens 6 months and bulk of the vaccine 1 month before filling) when stored under the approved conditions (2-8° 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 (2-8° C).

III. SAFETY ASSESSMENT

The vaccine is recommended for pigs from the age of 6 weeks for 2 doses intramuscular administration at an interval of 3 weeks.

Safety clinical findings have been based on the recommended vaccination scheme.

Laboratory trials

Safety studies have been performed with a vaccine batch (containing maximum content of all antigens) produced according the described production process.

The safety of the administration of one dose, an overdose and the repeated administration of one dose in the target animal is demonstrated in controlled laboratory studies which in total included 20 vaccinates animals (6-week-old pigs). The investigation was performed according to the recommendations of Directive 2001/82/EC as amended and the relevant guidelines.

The safety studies demonstrate that the administration of one dose, an overdose and the repeated administration of a dose can be considered to be safe, when used in accordance with the recommended vaccination schedule. Some minor, transient adverse reactions were

observed following vaccination. Appropriate warning regarding local site reactions following vaccination have been included in the SPC: Local reactions (swelling, induration) can occur with a diameter of 10 cm after the administration of the determined dose, which spontaneously subside within 3 to 14 days. Temporary elevation of body temperature by 1.0° C could occur in vaccinated animals.

Vaccine is not intended for administration to pregnant and / or lactating animals. Therefore, no specific studies were performed. The proposed text of SPC reflects this claim.

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.

Antigens are inactivated by formaldehyde, further it contains a lipoid solvent as immunity adjuvant and thiomersal as a preservative agent. Thiomersal and formaldehyde do not represent any significant risk to consumers. They are included in the List of Allowed Pharmacologically active substances in Annex to the COMMISSION REGULATION (EU) No 37/2010 without maximum residue limit. Thiomersal does not need to be determined in multiple-dose vaccines where thiomersal is used as a preservative in concentrations not exceeding 0.02%.

According Ph. Eur., where formaldehyde has been used in the preparation, the concentration of free formaldehyde is not greater than 0.5 g/l, unless a higher amount has been shown as safe. Amount of 1 mg/ml used in vaccine Biosuis APP 2,9,11 was shown as safe in safety study.

For this reason, testing of residues in tissues after application of Biosuis APP 2,9,11 was not performed. Based on this iformation, 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

Combined safety and efficacy field trial was performed on target animals.

30 piglets 6 weeks old were always used in three farms. Twenty piglets were vaccinated by the tested vaccine with the average antigen content according to the recommended vaccination scheme, ten piglets were held as the non-vaccinated control. Body temperature, clinical symptoms as shortness of breath, cough, vomiting or death in pigs was recorded regularly. At the end of fattening the animals were slaughtered and the pulmonary scope was determined in each pig in conformity with PharmEur requirements, the level of antibodies against *A. pleuropneumonie* was determined in the blood serum and re-isolation of *A. pleuropneumoniae* from the taken sample of the pulmonary tissue, tracheobronchial lymph nodes and tonsills.

The results of field studies are in compliance with Ph. Eur. Monograph 1360, no animal showed notable signs of disease or died from causes attributable to the vaccine; the average body temperature increase for all animals did not exceed 1,5 °C; and no animal showed a rise in body temperature greater than 2 °C.

The results from field trials reflect those observed in laboratory trials.

Ecotoxicity

The applicant provided a first phase environmental risk assessment in compliance with the relevant guideline which showed that no further assessment is required.

The conclusions of the environmental risk assessment as presented by the applicant, that there is a negligible risk to the environment associated with use of the vaccine, are accepted. The applicant has included the standard disposal statement for inactivated vaccines on the product literature and this is considered acceptable.

IV. EFFICACY

All trials performed to demonstrate efficacy of Biosuis APP 2,9,11 vaccine were designed to comply with the requirements of relevant European veterinary legislation including the European Directive 2001/82/EC as amended and relevant European Pharmacopoeia chapters in force. The efficacy of the product has been demonstrated in laboratory challenge studies in accordance with the Ph.Eur. monograph 1360: Porcine actinobacillosis vaccine (inactivated).

For verify the efficacy of developed vaccine in target animals were used two batches:

batch with the minimum antigen content proposed for common production batches and batch with 50% of antigen content against the consider minimum antigen content.

To induce experimental infection (challenge) Actinobacillus pleuropneumoniae s. 2 and s. 9 virulent strais of a different origin from the vaccine strain were used, producing relevant APX toxins (APX I, II and APX II, III). All three Apx groups are represented in the vaccine. The significance of toxigenic group is supported by the specific monograph (2013/1360) which states (Section 2.2.2 Immunogenicity) that “The challenge strain for the following test is chosen to ensure challenge with each Ap toxin produced by the serotypes to be stated on the label…”. In view of the fact that serotypes 9 and 11 belong to the same toxigenic group (Group 1, producing Apx I and Apx II) and Serotype 2 belongs to toxigenic group 2, producing Apx II and Apx III, then this Ph.Eur. requirement could be considered met by challenge studies with serotype 2 and 9.

Laboratory Trials

The efficacy of the product has been demonstrated in laboratory studies in accordance with the relevant requirements.

Onset of immunity

Forty-two piglets aged 6 weeks, without antibodies against antigens in the vaccine, were used.

14 piglets were vaccinated with the test vaccine with the minimum antigen content according to the recommended vaccination schedule, 14 piglets were vaccinated with the test vaccine with 50% of the minimum antigen content and 14 piglets were kept as unvaccinated controls. Twenty-one days after revaccination always 7 piglets of each group received one of two challenge strains of *A. pleuropneumoniae*: serotype 2, producing APX II and III, and serotype 9, producing APX I and II. Clinical symptoms of the disease (dyspnoea, cough, vomiting, anorexia, lethargy), or death were regularly recorded after challenge. The animals were slaughtered at the end of 7-day observation period and lung scores were determined according to the Pharm.Eur. requirements. *A. pleuropneumoniae* was re-isolated from lung tissue, tracheobronchial lymph nodes and tonsils.

Both potency variants of the test vaccine (i.e. with the lowest antigen content considered for the vaccine production and with the 50% content of this minimal variant) met the efficacy parameters required by Pharm. Eur. monograph 1360 “Vaccinum actinobacillosis inactivatum ad suem”. It was proved that the Biosuis APP 2,9,11 vaccine was able to reduce significantly

the typical clinical signs, typical lung lesions of the disease and to reduce infection three weeks after revaccination according to the proposed vaccination schedule.

Duration of immunity

Twenty-eight piglets aged 6 weeks, without antibodies against antigens included in the vaccine, were used.

Blood samples were taken before vaccination and then regularly at weekly intervals until 21 days after revaccination to determine levels of antibodies against vaccine antigens; additional sampling was performed before the experimental infection, i.e. 20 weeks after revaccination.

Fourteen piglets were vaccinated with the test vaccine with the minimum antigen content according to the recommended vaccination schedule and 14 piglets were kept as unvaccinated controls. Twenty weeks after revaccination always 7 piglets of each group received one of two challenge strains of *A. pleuropneumoniae* (producing ether APX I and II or APX II and III). Clinical symptoms of the disease (dyspnoea, cough, vomiting, anorexia, lethargy), or death were regularly recorded after challenge. The animals were slaughtered at the end of 7-day observation period and lung scores were determined according to the Pharm.Eur. requirements and *A. pleuropneumoniae* was re-isolated from lung tissue, tracheobronchial lymph nodes and tonsils.

The duration of active immunity was verified successfully by challenge test 20 weeks after revaccination, i.e. in the time covering reliably the fattening period. The duration of immunity was also supported by the persisting antibody level against individual vaccine antigens.

The levels of antibodies against individual vaccine antigens at the end of the expected duration of immunity agreed with the quantities of antibodies which were verified as protective in the subminimum vaccine batch (i.e. with 50% antigen content) in the test of the onset of immunity.

The following claimed indications for Biosuis APP 2,9,11 are considered to be supported by the laboratory studies:

For active immunisation of fattening pigs to mitigate the consequences of infection caused by Actinobacillus pleuropneumoniae – the causative agent of pleuropneumonia in pigs.

The intention of the use is to reduce typical clinical signs, typical lung lesions of the disease and to reduce infection.

Onset of immunity: 3 weeks after revaccination

Duration of immunity: 20 weeks after revaccination

Influence of Maternal antibodies on efficacy

Twenty-eight piglets aged 6 weeks, born from mothers vaccinated with the antigens contained in the test vaccine were used - the sows were vaccinated with a vaccine batch with an average content of antigens 6 weeks and 3 weeks before parturition, always with a dose of 1 ml i.m.

Blood samples were taken before the vaccination of piglets and then regularly at weekly intervals until experimental infection to determine levels of antibodies against vaccine antigens.

Fourteen piglets were vaccinated with the test vaccine with the minimum antigen content according to the recommended vaccination schedule and 14 piglets were kept as

unvaccinated controls. Twenty-one days after revaccination always 7 piglets of each group received one of two challenge strains of *A. pleuropneumoniae* (producing either APX I and II or APX II and III).

Clinical symptoms of the disease (dyspnoea, cough, vomiting, anorexia, lethargy), or death were regularly recorded after challenge. The animals were slaughtered at the end of 7-day observation period and lung scores were determined according to the Pharm.Eur. requirements and *A. pleuropneumoniae* was reisolated from lung tissue, tracheobronchial lymph nodes and tonsils.

The presence of maternally derived antibodies in piglets at the age of 6 weeks does not influence the efficacy of the vaccination and the onset of active immunity 21 days after the revaccination.

Field studies

Combined safety and efficacy field trial was performed on target animals.

In total 60 piglets from 3 farm, vaccinated at the age of 6 weeks, were used for verification of efficiency of the tested medicinal product. Piglets were examined for presence of the antibodies against A. pleuropneumonie s.2. s.9, s.11 and APX toxins. In each farm twenty piglets were vaccinated by the tested vaccine with the average antigen content according to the recommended vaccination scheme, ten piglets were held as the non-vaccinated control. Before vaccination, before revaccination and 21 days after revaccination blood samples was collected for determination of the level of antibodies against antigens in vaccine; additional sampling was conducted at the end of fattening.

By the end of fattening the animals were slaughtered and the pulmonary score was determined in each pig in conformity with Pharm.Eur. requirements, the level of antibodies against *A. pleuropneumonie* was determined in the blood serum and re-isolation of *A. pleuropneumoniae* from the taken sample of the pulmonary tissue, tracheobronchial lymph nodes and tonsillae.

Clinical symptoms as shortness of breath, coughing, vomiting or death in pigs was recorded regularly.

The results obtained in this study confirm laboratory trials findings. Administration of the vaccine at the recommended dose and by the recommended route of administration proved to be safe and effective for the target species. Significant increase of antibodies level was proved in all vaccinated animals. Suitable level of antibodies persisted for the fattening period.

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.