15 June 2017
EMA/377181/2017
Veterinary Medicines Division

## Committee for Medicinal Products for Veterinary Use

# CVMP assessment report for VEPURED (EMEA/V/C/004364/0000)

Common name: *E. coli* verotoxoid vaccine (inactivated recombinant)

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

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# Introduction

On 20 May 2016 the applicant, Laboratorios HIPRA, S.A. submitted an application for a marketing authorisation to the European Medicines Agency (the Agency) for VEPURED, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 10 December 2015 as VEPURED is developed by means of a biotechnological process (the recombinant Stx2e is produced using recombinant technology).

VEPURED contains the active ingredient recombinant verotoxin 2e (VT2e) as a suspension for injection for pigs and is adjuvanted with aluminium hydroxide and DEAE-Dextran.

The indication is for the active immunisation of piglets from 2 days of age to prevent mortality and reduce clinical signs of oedema disease (caused by verotoxin 2e produced by *Escherichia coli* (*E. coli*) and to reduce the loss of daily weight gain during the finishing period in the face of infections with verotoxin 2e producing *E. coli* until slaughter.

The product is intended for administration by a single intramuscular injection of 1 ml. VEPURED is presented in packs containing vials of 10, 50, 100 and 250 ml or in packs containing 10 vials of 10 ml.

The rapporteur appointed is Ellen-Margrethe Vestergaard and the co-rapporteur is Gerrit Johan Schefferlie.

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

On 15 June 2017 the CVMP adopted an opinion and CVMP assessment report.

On 17 August 2017 the European Commission adopted a Commission Decision granting the marketing authorisation for VEPURED.

## Marketing authorisation under exceptional circumstances

Not applicable.

## *Scientific advice*

Not applicable.

## MUMS/limited market status

Not applicable.

# Part 1 - Administrative particulars

## *Detailed description of the pharmacovigilance system*

A detailed description of the pharmacovigilance system (dated 02/03/2012) which fulfils the requirements of Directive 2001/82/EC was provided. 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 reaction occurring either in the Community or in a third country.

### *Manufacturing authorisations and inspection status*

VEPURED is manufactured in the European Union. The active substance of VEPURED is manufactured in CIAMER, the antigen production plant of Laboratorios HIPRA, located in C-63 Km 48.3 de Polígon el Rieral, Amer, 17170 Gerona, Spain and Laboratorios HIPRA site located in Avda. La Selva 135, Amer, 17170 Gerona, Spain. A manufacturing authorisation issued by the Spanish Agency for Medicines and Healthcare Products of the Ministry of Health and Consumer Affairs is dated 4 October 2016.

A Good Manufacturing Practices (GMP) declaration for the active substance manufacturing sites was provided from the Qualified Person (QP) at the EU batch release site (Laboratorios HIPRA located in Avda. La Selva 135, Amer, 17170 Gerona, Spain) dated 3 June 2016. The declaration was based on an on-site audit by HIPRA's annual internal corporate audit, which has taken into consideration the GMP certificate available for the active substance manufacturing site issued by the Spanish Agency for Medicines and Healthcare Products of the Ministry of Health and Consumer Affairs. A GMP certificate which confirms the date of the last inspection 6 November 2015 is valid for three years and shows that the site is authorised for the manufacture of the active substance.

The manufacture of the vaccine (blending, filling and labelling) is carried out in the EU as well as the in-process control tests and the control tests on the finished product. The site has a manufacturing authorisation from the corresponding EU Competent Authority. A GMP certificate which confirms the date of the last inspection (6 November 2015) is valid for three years and shows that the site is authorised for the manufacture and batch release of such veterinary dosage forms, has been provided.

### *Overall conclusions on administrative particulars*

The detailed description of the pharmacovigilance system was considered in accordance with legal requirements.

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

# Part 2 - Quality

### *Chemical, pharmaceutical and biological/microbiological information (quality)*

### Qualitative and quantitative particulars

VEPURED is a recombinant inactivated vaccine, in which the pharmaceutical form is a suspension for parenteral administration to pigs. It contains a genetically modified recombinant Shiga toxin (rStx2e) together with an aluminium hydroxide gel and DEAE-dextran as adjuvant. Disodium phosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride and potassium chloride are included as excipients, as well as simethicone and sodium hydroxide. A preservative is not included in the vaccine. The vaccine is available in multidose presentations.

An in-use stability study has been performed and the results are acceptable.

The vaccine is intended for intramuscular administration to induce active immunity of piglets from 2 days of age against *E.coli* strains expressing Stx2e toxin, responsible for oedema disease. Oedema disease is caused by certain strains of *E. coli* bacteria that express the Stx2e toxin. The toxin enters the blood stream and damages vessel walls resulting in oedema in the targeted tissues. Most notably, cerebral oedema leads to the predominant nervous signs that are characteristic of the disease.

Recombinant Stx2e toxin is added at formulation of the vaccine on the fixed content 600 ELISA Units of Antigenic Mass (UEMA) per dose (1 millilitre). The finished product potency test is based on the quantification of protective antigen in the final vaccine. This *in vitro* potency test for the vaccine is a ELISA (Enzyme-Linked Immunosorbent Assay). The quantification of the antigen is based on the calculation of the relative potency of the tested batch in comparison to a reference vaccine (RP≥1.5) which has demonstrated to be efficacious in the pivotal efficacy trials.

## *Container and closure*

VEPURED is presented in plastic (PET) multidose airtight vials, stoppered with rubber stoppers, sealed with aluminium caps. These materials comply with the European Pharmacopoeia (Ph. Eur.) monographs (3.1.15, 3.2.2. and 3.2.9.) and the irradiation process is in accordance with the Ph. Eur. 5.1.1 monograph.

The presentations available are 10, 50, 100 and 250 ml.

Stoppers for PET vials are bromobutyl rubbers. The capsules are made of anodised aluminium and their function is to ensure the correct closure of the stoppers.

## *Product development*

Shiga toxin (Stx2e) is composed of an enzymatically active A subunit surrounded by a pentamer of B subunits that recognize specific glycolipid receptors. The A subunit enters the host cell and mediates cell death by the inhibition of protein synthesis at the ribosomal level. Oedema disease is a frequently fatal disease in newly weaned piglets that involves neurological impairment.

The development of the product is well described. Adequate information has been provided on the choice of antigen, adjuvant, excipients, container-closure system as well as the information about development of the genetic construct and about vaccine production. Stx2e toxin is obtained using a host-vector system, with the host strain a genetically modified strain of *E. coli* harbouring a plasmid vector that encodes for the 2 subunits of the non-toxic derivative STx2e that includes mutations in the active site of the subunit A component.

The final formulation of VEPURED has been set in view of the results on safety and efficacy trials obtained during the assessment of different formulations. The choice of a suitable adjuvant fraction and the dose of the genetically modified recombinant Stx2e antigen (rStx2e) have been the most important targets during the pharmaceutical development of the vaccine.

Several preliminary studies were carried out and focused on the study of the immunogenicity of the vaccine and the adjuvant fraction. These studies were performed in piglets in order to find a suspension able to generate appropriate immune response without significant local and systemic reactions. Multiple formulations administered by the intramuscular route were tested and serum conversion against Stx2e was observed for some of them.

Different formulations and different administration routes were also studied.

The parameters recorded after vaccination were temperature, clinical signs and serological results.

A challenge with Stx2e wild type was carried out in piglets vaccinated with each formulation. After the challenge, the same parameters were monitored.

Hence, the formulation with a fixed antigen content 600 UEMA per dose (1 millilitre) was selected as the final vaccine composition for VEPURED and dose-response studies were carried out in order to confirm the appropriateness of the basic formulation. Moreover, these dose response trials were used to adjust the final antigen dose among four different concentrations of the genetically modified recombinant Stx2e antigen. The above were acceptable and these studies were summarised and included in the Efficacy Part of the dossier.

## *Description of the manufacturing method*

The production of the vaccine is performed in two phases: firstly, the production and purification of the antigen and, secondly, the vaccine formulation to obtain the final product.

The recombinant Stx2e antigen is produced from a recombinant *E. coli* strain harbouring a plasmid vector, which encodes for the two subunits of the non-toxic Stx2e derivative. The process starts with one vial of the working seed bank (WSB). The working seed is expanded to produce a main fermentation culture in a fermenter. The duration and temperature of each passage is defined. The main fermentation is conducted in two phases; firstly, bacteria are cultivated up to a defined turbidity value, after which the culture is induced to express recombinant Stx2e protein. The downstream processing of the main fermentation culture first involves separation of the biomass (containing the recombinant Stx2e antigen) from the supernatant. Then, cellular fragmentation is induced. The active ingredient is purified. Finally, a diafiltration and sterilising filtration is carried out to obtain the antigen. The maximum storage period and storage conditions of the antigen are defined. The final antigen is tested for rStx2e content by HPLC and then it is blended with adjuvants (Aluminium hydroxide and DEAE-dextran) and excipients. The antigen content is set per dose (1 ml), aluminium concentration is 2.117 mg/ml and DEAE-Dextran content is 10 mg/ml. The pH is adjusted. The maximum storage period of the bulk vaccine before filling has been established. Filling and packaging completes the production process. The standard industrial batch size and volume of final bulk have been defined.

The purification step performed is considered adequate regarding removal of impurities. Concerning the control and validation of the sterile filtration of the active substance the validations were provided for two filters and for process filtration.

The second filtration step was included prior to final sterile filtration and acceptable bioburden limit has been implemented prior to final sterile filtration of Stx2e.

For manufacture of the final vaccine, the validations of sterilisation steps were provided. The overall manufacturing process has been validated by the manufacture of three consecutive batches.

Consistency of production is supported by data from 3 vaccine batches and including batches filled in each of the proposed presentations (10 ml, 50 ml, 100 ml and 250 ml PET). A summary of data from bulk testing and finished product testing has been provided. All batches comply with the proposed product testing specifications. Batch release protocols for all batches used have been included.

## *Production and control of starting materials*

### Starting materials listed in pharmacopoeias

The following excipients are listed in the Ph. Eur.: aluminium hydroxide, sodium hydroxide, disodium phosphate dodecahydrate, potassium chloride, potassium dihydrogen phosphate, sodium chloride and water for injections. Other starting materials listed in the Ph. Eur. are used during manufacture. The applicant provided estimates of removal when applicable and maximum possible concentrations at various stages of the production.

Simethicone (excipient) and another starting material used during production comply with the respective USP requirements.

### Starting materials not listed in pharmacopoeias

### Starting materials of biological origin

Starting materials of biological origin, which are not listed in the Ph. Eur. include the genetically modified recombinant Stx2e antigen (in addition, bovine-derived casein amino acids are used as media component).

The genetically detoxified Shiga-like toxin (Stx2e) is obtained using a host-vector system which is composed of a genetically modified strain of *E. coli* (host strain) and the plasmidic vector of recombinant DNA which codes for the 2 subunits of the non-toxic derivative STx2e. Detailed description of the host vector system was provided including controls carried out on master and working seed bacteria.

Control tests for the purified Stx2e antigen are described in the control tests during manufacturing process. The purified antigen is tested for purity (quantitative SDS-PAGE), antigen content (HPLC that also demonstrates identity by retention time), pH and sterility. The SDS-PAGEis a quantitative method that allows determining the antigen, the degraded antigen and the residual host cell proteins by means of a densitometric analysis. The performance of this analytical method is deemed acceptable for the intended purpose.

The proposed test panel is in line with the Ph. Eur. 0784.

The stability programme and submitted stability data for the bulk antigen are considered adequate to support the proposed claim of 12 months shelf life at 2–8 °C.

Overall, the host-vector system and its characteristics are adequately described. A master seed bank (MSB) and WSB have been established and tested for identity, purity, viability, titre, fungal sterility, and genetic stability.

### Starting materials of non-biological origin

There are starting materials of non-biological origin which are not listed in the Ph. Eur., DEAE-dextran amongst others.

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

The in-house preparation of media and other reagents are well described. Certificates for all media components have been provided. In addition, implementation of sterility testing for media and solutions added to the bacterial culture is provided.

The confirmation of compliance with the Ph. Eur. 5.2.5. has been provided for starting materials of animal origin (gelatine, casein amino acids).

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

The original strain of *E. coli* used in the production of the recombinant inactivated Stx2e toxin was purchased from a third party.

There is no ingredient of ruminant origin in the culture medium used to obtain the master seed bacteria from the original bacteria. There is no ingredient of ruminant origin in the culture medium used to obtain the working seed bacteria from the master seed bacteria. The risk that the bacterial master/ working seed might be contaminated with TSE agent is negligible.

Gelatine is obtained from countries of origin are considered to be free of TSE infection. Therefore, There are no concerns about transmission of Spongiform Encephalopathies for gelatine of bovine origin, as confirmed by the presented EDQM Certificate of suitability.

The casein is obtained from bovine milk from healthy animals under identical conditions as milk intended for human consumption and no other materials of ruminant origin are used in the preparation of these derivatives. Therefore, it is considered that this lactic derivative is in accordance with the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01-Rev.3); therefore, it does not present any risk of TSEs.

The starting materials of animal origin used in the production of the final product comply with the current regulatory requirements related to the Ph. Eur. monograph 5.2.8 "Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" and TSE Note for Guidance (EMEA/410/01-Rev.3)".

## *Control tests during the manufacturing process*

The list of the tests conducted at various stages during the production of the antigen (Gram stain, viability, purity, turbidity, viable bacteria count, pH, bacterial and fungal sterility, antigen quantification (HPLC), purity (SDS-PAGE), bioburden) have been provided. The proposed analytical methods are adequate and provide reasonable control of most steps of the production process.

A process description of the chromatography step has been provided.

The purity data provided indicates that the chromatography step effectively removes protein impurities present after the clarification step. In addition, comparable purity and similar chromatographs of the HPLC analysis were obtained for different antigen batches and support the conclusion that it performs consistently. Filter validation reports have been submitted for two filter types used for sterile filtration of the purified Stx2e antigen. The submitted documentation demonstrates adequate validation of the sterile filtration.

An additional 0.2 µm filtration is performed and an acceptable bioburden limit has been implemented prior to final sterile filtration of Stx2e.

The SDS-PAGE method enables a quantitative analysis of intact antigen, degraded antigen and residual host cell proteins. The analytical test acceptance criteria are deemed adequate to control performance of the SDS-PAGE method. The method is described in sufficient detail and the analytical test acceptance criteria seem to be sufficient to control performance of the SDS-PAGE method.

In accordance with VICH GL1 and GL2 parameters accuracy, precision (repeatability, intermediate precision and reproducibility), specificity, limit of quantitation, linearity, range and robustness of the procedure have been validated. Overall, the performance of the analytical method is deemed acceptable for the intended purpose.

The results provided demonstrate consistent removal of DNA impurities have been provided. Thus, routine testing for residual DNA is not considered necessary. Taking into account that just a single dose of vaccine is administered and polymyxin B is not well absorbed via the oral route the proposal to omit testing for residual polymyxin B is considered acceptable.

The complete results of the in-process controls carried out on three consecutive batches are included in the manufacturer batch protocols enclosed and are acceptable.

## *Control tests on the finished product*

Finished product controls performed on the bulk vaccine are: appearance, pH, concentration of aluminium hydroxide, concentration of DEAE-Dextran, potency test of Stx2e and identification of Stx2e or on the filled product: appearance, pH, sterility, volume control.

The proposed controls to be carried out on the bulk have been performed both in the bulk and in filled product for 3 pilot batches. The results obtained demonstrated that the filling process has no impact on the result, so that for routine testing, the concentration of the adjuvants and the potency of the antigens will only be tested before the filling process on the bulk. The specifications proposed are appropriate to control the quality of the finished product. The finished product potency test is based on the quantification of protective antigen in the final vaccine. This *in vitro* model used to test the potency of the vaccine is an ELISA (Enzyme-Linked Immunosorbent Assay). The quantification of the antigen is based on the calculation of the relative potency of the tested batch in comparison to a Reference vaccine (RP 1) and has been demonstrated to be efficacious in the efficacy trials. RP limit for production batches 600 UEMA/ml is set at ≥ 1.5 RP. An overdose study was performed with 1200 UEMA/ml antigen content and demonstrated the innocuousness of the administration of a vaccine, the upper limit is not therefore required.

The monoclonal antibody used in the potency assay was sufficiently characterised. Potency test is able to detect an alteration of the integrity of the antigen and is capable to detect a decline in its immunogenic properties.

Validation reports have been provided for sterility control by direct inoculation, for batch potency test and for determination of DEAE-Dextran and aluminium hydroxide. It is acceptable.

It is proposed to omit the control for bacterial endotoxins as routine test on each batch. In the light of the results submitted in the file, the proposal is to waive this control with the agreement of the regulatory authorities. Validation of the endotoxin test has been provided which is in accordance with Ph. Eur. The omission of this test is acceptable.

## Batch-to-batch consistency

The results of the analysis of three consecutive production runs were presented and they comply with the required specifications.

## *Stability*

Three batches of the finished product were placed on stability trial. The vaccine is filled in different container sizes (10 ml, 50 ml, 100 and 250 ml) but these containers are made of the same type of plastic (PET) and with the same type of closures, so bracketing can be applied. For this purpose, three consecutive batches of the vaccine filled in the smallest (10 ml equivalent to 10 doses) and the biggest (250 ml equivalent to 250 doses) presentation were used. The specifications of the controlled parameters are identical to those established for the set of control tests on the finished product for batch release.

Stability data have been provided for the finished product for three batches until 18 months. These batches were tested at 0, 3, 6, 9, 12, 15 and 18 months. The results showed that the product keeps its quality specifications within the established limits for at least 18 months. Therefore, a shelf-life of 15 months is supported by the data presented.

The in-use stability study has been performed and the results confirmed stability for 10 hours after first opening the immediate packaging. It has been confirmed that the batches used were at the end of the shelf life.

It is suggested that the bulk antigen may be stored up to 12 months. The maximum storage period of 12 months was verified by stability data performed on three antigen batches. The proposed analytical programme (pH, sterility, antigen quantification by HPLC and purity and identity by SDS-PAGE at release and 12-month time point) appears sufficient to detect changes in purity and potency of the product.

For bulk product, the maximum storage period of 120 hours before filling was verified by stability data for one batch. The data provided for one batch can be considered acceptable. However, since data from only one batch may not be representative, the data for two other batches will be collected and analysed.

## *Overall conclusions on quality*

The applicant produces vaccine on the fixed Stx2e content. The RP limit for production batches of 600 UEMA/ml is set at ≥ 1.5 RP. The overdose study was performed with 1200 UEMA/ml antigen content and demonstrated the innocuousness of the administration of a vaccine.

The potency test is able to detect an alteration of the integrity of the antigen and is capable to detect a decline in its immunogenic properties.

Validation of the endotoxin test has been provided which is in accordance with the Ph. Eur. requirements. The omission of this test as routine test on each batch is acceptable.

Stability data have been provided for the finished product for three batches until 18 months.

It is proposed that the bulk antigen may be stored up to 12 months. The maximum storage period 12 months was verified by stability data performed on three antigen batches. The proposed analytical programme appears sufficient to detect changes in purity and potency of the product.

Information regarding the qualitative and quantitative composition, the starting materials, production method, quality controls and stability are provided and are acceptable. Test results of three consecutive batches of VEPURED were presented in order to demonstrate batch-to-batch consistency.

The production process is described in detail.

All starting materials are defined and comply with the provisions of the Ph. Eur., where applicable.

The main risks concerning TSE are considered negligible.

The in-process and finished product controls performed ensure a consistent production of VEPURED

In conclusion, the production and quality control of VEPURED are adequately described and comply with the respective legal requirements including the TSE risk assessment.

### *Recommendation for further quality development*

The stability data provided for one batch can be considered acceptable. However, since data from only one batch may not be representative, the data for two other batches with storage periods close to 120 h should be collected and analysed.

## Part 3 – Safety

### *Safety documentation*

Evaluation of the safety of VEPURED was made according to the Council Directive 2009/09/EC amending Directive 2001/82/EC for immunological products. The principles described in the Ph. Eur. monograph no. 50206 "Evaluation of safety of veterinary vaccines and immunosera" and the Guideline on target animal safety for veterinary live and inactivated vaccines (VICH GL 44) were both taken into account to demonstrate the safety of this vaccine. No specific monograph exists for inactivated oedema disease vaccines. The laboratory safety trial was carried out according to the principles of GLP, and the multicentre field study according to GCP principles, both studies using batches of vaccine above 1.5 times the minimum relative potency for batches to be produced in the future product. These batches were accepted as appropriate for the safety trials as they were near the maximum dose.

### *Laboratory tests*

### *Safety of the administration of one dose*

The safety of a single dose administration study was performed with 26 piglets, both males and females (13 vaccinates and 13 controls) at the youngest age of administration (2–3 days old). The study was randomised and blinded; piglets were ranked from the largest to the smallest piglet in terms of pre-vaccination live weight (at D-1) and then piglets were sorted by the sow. Piglet body weights were standardised between vaccinates and controls.
One ml of VEPURED (RP≥ 1.5) was administered to vaccinates by the intramuscular route in the right side of the neck; phosphate-buffered saline (PBS) was administered to controls in the same manner. The safety parameters evaluated were live weight at D-1 and D21; general clinical signs and/or adverse reactions D-1, D0, D0+4h, daily on D1-21; local reactions measured as inflammation on a scale from 0–3 and nodules on an absence/presence scale; rectal temperature: D-1, D0, D0+4h, D1-4; blood sampling at D0 and D21. Necropsy was carried out at D21 and macroscopic inspection of

injection sites (histology if adverse events (AEs) identified).

The statistical analysis was made as follows: rectal temperature data were analysed by descriptive statistics, t-test and ANOVA (repeated analysis) for body temperature evolution by treatment over time. Body weight and results from serology were analysed by t-test for comparison of groups. A significance level $p<0.05$ was used for all variables.

Results showed no significant rise in post administration rectal temperatures in vaccinates over temperatures in controls at any time point from D0+4h until D4. The maximum temperature rise in individuals post administration was 1.4 °C in a piglet from the control group and 1.2 °C in a vaccinated piglet. The mean temperature rise of vaccinates did not exceed 0.5 °C as detected four hours post administration. The SPC, Section 4.6 has been amended as standard information to veterinarians.

General clinical signs observed as mild depression were registered in six vaccinates and in one control animal. The applicant was asked also to insert this AE with the correct frequency into the SPC, Section 4.6. Local reactions were recorded in two vaccinates consisting of slight inflammation (<3 cm in diameter) and moderate inflammation (3–5 cm in diameter), respectively. These reactions resolved spontaneously within two days. The proposed SPC already included these AEs correctly.

All piglets were seronegative for Stx2e antibodies at D0, and 10 out of 13 vaccinates were seropositive at D21, while controls remained negative. Piglet live weight was not affected by administration of VEPURED, as the live weight was the same for both groups of piglets at D21 before necropsy.

On the basis of the above data, the safety of the administration of one dose of the vaccine was considered acceptable.

## *Safety of one administration of an overdose*

According to the Commission Directive 2009/9/EC amending Directive 2001/82/EC, the Notice to Applicants Volume 6B and the Ph. Eur. monograph no. 50206 the safety of one overdose only needs to be assessed for live vaccines, therefore no overdose studies were included for VEPURED as it is an inactivated vaccine. This was considered acceptable.

## *Safety of the repeated administration of one dose*

The vaccine schedule for VEPURED consists of a single lifetime dose to piglets from two days of age. The VICH GL44, Commission Directive 2009/9/EC amending Directive 2001/82/EC, and Notice to Applicants Vol. 6B all state that the safety of a repeated administration is not required for this type of product. Hence, no repeated dose studies were included in the dossier for this vaccine. This approach was therefore considered acceptable.

## *Examination of reproductive performance*

According to Directive 2009/9/EC amending Directive 2001/82/EC, and Notice to Applicants Vol. 6B reproductive performance shall be considered and investigated in the safety tests, when data suggest that the starting material from which the product is derived may be a risk factor or when the vaccine is recommended for use in pregnant animals. VEPURED is an inactivated recombinant vaccine intended for use only from 2 days in piglets; therefore, examination of the reproductive performance was not required to be included in the dossier. This was considered acceptable.

### *Examination of immunological functions*

According to Directive 2001/82/EC, where the product might adversely affect the immune response of the animal to which the product is administered or of its progeny, suitable tests on the immunological functions have to be carried out. VEPURED is not recommended for use in pregnant animals and the product is inactivated. Therefore, examination of immunological functions as required by the Directive 2009/9/EC amending Directive 2001/82/EC were not performed, and this was accepted.

### *Special requirements for live vaccines*

Not applicable.

### *User safety*

A user safety assessment was made according to the CVMP Guideline on user safety for immunological veterinary medicinal products (EMEA/CVMP/IWP/54533/2006) including hazard identification and characterisation, exposure, and risks. The active substance is an inactivated protein and is not infectious. The excipients including adjuvants are commonly used in other vaccines and do not constitute a user safety concern. There were no specific risks identified from use of this product.

### *Study of residues*

Residues studies were not performed as this is not required.

### *MRLs*

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

The excipients, including adjuvants listed in section 6.1 of the SPC are either allowed substances for which table 1 of the Annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

## Withdrawal period

A withdrawal period of zero days is established.

### *Interactions*

During the procedure, new data from a laboratory trial were provided in order to study the possible impact on safety and efficacy when VEPURED and an iron dextran product were administered concurrently (at the same time but at separate administration sites). The iron dextran product has been authorised in several Member States as a solution for injection containing 200 mg/ml of iron as a gleptoferron complex. The product is intended for prevention of iron deficiency anaemia in piglets.

Briefly, twelve 2-day-old piglets received one dose of the VEPURED vaccine according to the

recommended vaccination schedule in the right side of the neck and another intramuscular administration concurrently of iron dextran product in the left side of the neck.

Safety aspects were evaluated based on the parameter body temperatures recorded for all animals on the day before vaccination, at the time of vaccination, 4 and 6 hours after vaccination and during the following 3 days. Furthermore, general signs and local reactions were observed daily until day 22 after administration and the body weights of the piglets were measured at the day before vaccination and at day 28 post-administration. The data generated were compared with those observed in the preclinical studies submitted in the dossier. However, these dossier data were not provided.

The efficacy was assessed by means of serology (seroneutralising antibodies) as a marker parameter of efficacy. In general, toxin neutralising antibodies play a fundamental role in the protective effect of this kind of vaccine. The vaccination with VEPURED activates the piglets' immune system, generating high levels of neutralising antibodies against VT2e. The correlation between neutralising antibodies and efficacy of the VEPURED vaccine was demonstrated, where all available serological data from 5 vaccination-challenge laboratory studies (128 vaccinated and 64 control animals) were assessed. The statistical analysis confirms the correlation between SN antibodies and mortality ($p < 0.05$) and the correlation between SN antibodies and clinical signs ($p < 0.05$).

Taking into consideration the presence of seroneutralising antibodies in vaccinated piglets obtained in the study, the stated comparable protection of VEPURED if administered concurrently with the iron dextran preparation might be considered acceptable. This might also apply to the demonstration that the OOI is not negatively affected.

Nevertheless, no information was provided regarding the possible impact of vaccination on the efficacy of the iron dextran complex. Even though this impact is not likely, the evaluation of the efficacy of the iron dextran product based on blood samples taken (e.g. biochemical tests, hematopoietic parameters) is still expected.

Although the efforts to generate data in order to reduce stress caused by handling of little piglets are acknowledged, the abbreviated study report does not allow a final conclusion because some information is missing.

The CVMP concluded that further information is needed for the Committee to accept the concurrent use of both products. Appropriate warnings are included in section 4.8 of the SPC.

## *Field studies*

One multicentre, randomised, double blinded, parallel negative control group clinical field trial was performed to evaluate the safety and efficacy of VEPURED against oedema disease caused by Stx2e toxin produced by *E. coli*. The study was carried out in six farms with historical records of oedema disease. The study included six farms in total, 4 in France and 2 in Belgium. Two batches of vaccine were used, both above 1.5 times the minimum relative potency for batches to be produced in the future (RP≥1.5). In total 2221 piglets were enrolled hereof 1173 vaccinates and 1048 controls. The piglets were 2-3 days old commercial hybrids with a live weight at D0 ≥ 1 kg, and they were individually identified with ear tags before vaccination. VEPURED was administered to the 1173 vaccinates according to the proposed SPC when 2-3 days of age. The safety parameters evaluated were as follows: live weight was evaluated in 120 animals per group in each farm at D-1 and D28; general clinical signs and adverse reactions D0, D0+4h, D1, D2; local reactions in 30 animals per group in five out of the six farms and recorded were signs of inflammation; rectal temperature in 30 animals per group in five out of six farms at D-1, D0, D0+4h, D1, D2; adverse events intensively from D0-D7

and thereafter daily throughout the follow-up period.

Statistical analysis was made per individual, as the experimental unit was the piglet.

Results showed that local reactions were mild and did not exceed 1.5 cm in any animals at any time-point. At D2 post administration 8% of vaccinates and 3% of controls still showed a mild degree of inflammation at the injection site, and spontaneously resolved without treatment. Rectal temperatures showed statistical significant increase in vaccinated piglets as measured four hours after administration of VEPURED. None of the vaccinates showed a temperature rise higher than 1.1 °C. The temperature rise was within the physiological range and returned to normal values after 24 hours. The mean and maximum rise in temperature are included into the SPC, Section 4.6. Piglet body weight was not influenced by vaccination when measured at D28 prior to euthanasia.

The results supported an acceptable level of safety of the product when the product is used according to the SPC.

## *Environmental risk assessment*

A risk assessment has been provided in compliance with the CVMP Guideline for environmental risk assessment for immunological veterinary medicinal products (EMEA/CVMP/074/95-FINAL).

In case of improper use of VEPURED, none of the components in the formulation are known to cause environmental problems. The product contains inactivated protein as antigen (genetically modified recombinant Stx2e toxoid), and there is no possibility to release any kind of toxin into the environment; the manufacturing process includes a purification step, which assures that no residual DNA is present in the final antigen. Such dissemination of microorganisms or related DNA into the environment is not possible.

Since VEPURED does not contain live organisms or agents capable of replicating within the host, then the probability of causing negative impacts is negligible. The product is administered via the intramuscular route to individual piglets, therefore the possible release and exposure to the environment can be considered effectively zero. A Phase 1 hazard identification and assessment was provided and the overall risk to the environment was regarded effectively zero, therefore no Phase 2 studies were needed. This decision was agreed upon by the CVMP.

Based on the data provided the ERA can stop at Phase I. VEPURED 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*

Vaccine contains only pure recombinant protein without presence of the genetically modified organism or its part (DNA and other proteins from *E. coli*). The purity of the antigen is controlled during manufacture process, which eliminates the possibility of viable GMO being present.

## *Overall conclusions on the safety documentation*

The safety of VEPURED was investigated in two studies; a laboratory safety study (single dose) and a multicentre field study including piglets from six farms with batches of appropriate antigen content and potency for such studies. Both batches used in the safety documentation had a potency above 1.5 times the minimum relative potency for batches to be produced in the future product and thus

close to the maximum potency. These safety batches were accepted as appropriate.

Piglets from the youngest target age (2 - 3 days old) were vaccinated intramuscularly according to recommendations in the SPC. The animals were observed daily for clinical signs of general and local reactions. Other measurements included body temperature, blood sampling and weight performance post administration until D21.

The results showed that the product was safe when administered to the youngest target age of piglets. Adverse reactions are correctly reflected in the product literature.

No overdose nor repeated dose administration were carried out as this product is inactivated and only consists of a single lifetime dose to piglets at two days of age. No examination of reproductive performance nor of immunological functions were investigated as the product is not intended for breeders. This was accepted.

A user safety assessment in line with the relevant guidance document has been presented. The user safety for this product is acceptable when used in accordance with the SPC.

VEPURED is not expected to pose a risk for the environment when used according to the SPC.

Residues studies are not required.

The withdrawal period is set at zero days.

# Part 4 – Efficacy

## *Introduction and general requirements*

Certain *E. coli*, known as Oedema Disease *E. coli* (EDEC), can colonise the small intestines of pigs and can cause oedema disease. These *E. coli* bacteria proliferate and produce a Shiga toxin, which is also known as verotoxin (VT), VT2 or Stx2e. The toxin enters the blood stream and damages vessel walls resulting in oedema in the targeted tissues. Most notably, cerebral oedema leads to the predominant nervous signs that are characteristic of the disease. The genome sequence of the Stx2e is common for all strains of EDEC; therefore, no geographical difference exists with respect to the toxin. The Stx2e induces a dose-dependent disease indistinguishable from oedema disease when administered intravenously to pigs.

VEPURED is a recombinant inactivated vaccine intended for "active immunisation of piglets from 2 days of age to prevent mortality and reduce clinical signs of oedema disease (caused by verotoxin 2e produced by *E. coli*) and to reduce the loss of daily weight gain during the finishing period in the face of infections with verotoxin 2e producing *E. coli* until slaughter from 164 days of age. Onset of immunity is 21 days after vaccination; duration of immunity is 112 days after vaccination in pre-clinical studies (challenge).

The efficacy of VEPURED was demonstrated according to requirements laid down in the Commission Directive 2009/9/EC amending Directive 2001/82/EC. As there is no specific monograph for oedema disease, the efficacy studies were carried out in accordance with the general principles and requirements of the general Ph. Eur. monograph No. 50207 (Evaluation of efficacy of veterinary vaccines and immunosera). Demonstration of a possible impact of maternally derived antibodies on vaccine efficacy in young animals was carried out according to the Reflection paper on the demonstration of a possible impact of maternally derived antibodies on vaccine efficacy in young animals (EMA/CVMP/IWP/439467/2007).

## *Laboratory trials*

Establishment of challenge model

Two challenge studies were performed in 4 weeks old pigs as well as in 3–4 months old pigs. *E. coli* strain CP-640 was used to prepare Stx2e toxin, and the challenge dose was 4.7 x $10^4$ $CD_{50}$/kg body weight administered -intravenously. In total 14 pigs were included in this study. Five animals in each age group were challenged with the toxin while two animals in each age group served as PBS controls. The clinical symptoms and mortality associated with the disease were used as primary variables for evaluating the differences between infected and non-infected groups.

The results from this study demonstrated that the challenge model was valid in 4-week old pigs after receiving a dose of toxin with 4.7 x $10^4$ $CD_{50}$/kg body weight. The same challenge model observed in 3–4 months old animals appears to be too aggressive and acute, and therefore a further study was made to optimise the challenge model in this target age group of pigs. The challenge dose in 4-weeks old pigs was accepted.

A new challenge study was made to reduce the dose of toxin administered to 3-4 months old pigs in order to reduce aggressiveness of the challenge model. In total 10 pigs were included and they were 15 weeks of age at D0 with similar weight and free of antibodies to Stx2e. *E. coli* strain CP-640 was used to prepare Stx2e toxin and the challenge dose was either 9 x $10^3$ $CD_{50}$/kg body weight or 6 x $10^3$ $CD_{50}$/kg body weight administered intravenously. Five animals were challenged intravenously with the toxin at the higher dose and five animals were inoculated with the lower dose.

The results demonstrated that the challenge model still appeared too aggressive and acute in 3-4 months old pigs after receiving a dose of toxin on 9 x $10^3$ $CD_{50}$/kg body weight. This dose resulted in 100% death of pigs before 24-hours post inoculation. On the contrary, the model obtained after administration of another toxin dose of 6 x $10^3$ $CD_{50}$/kg body weight seemed more suitable for testing of vaccine candidates, because mortality and morbidity was not as acute, although also reaching 100%. The lowest challenge dose in 3–4 months old pigs was accepted.

Dose finding studies

Two dose-finding studies were performed the first applying either 300 UEMA/ml; 150 UEMA/ml or 75 UEMA/ml to piglets when 28 days old. *E. coli* strain CP-640 was used to prepare the Stx2e toxin and the challenge dose was 4.7 x $10^4$ $CD_{50}$/kg body weight administered intravenously. In total 65 commercial hybrid piglets were included at 2 days of age, both males and females being free of antibodies to Stx2e. The piglets were divided into four groups of 15 animals (Group A, B, C, and D) and 5 piglets served as non-vaccinated, non-challenged sentinels. Vaccinates in the four groups were administered 1 ml of VEPURED by the intramuscular route in the right side of the neck (A, B, C) and PBS controls (D) was administered in the same way.

Results showed that the two formulations with the highest concentrations of the Stx2e antigen A (300 UEMA/ml) and B (150 UEMA/ml) were efficacious against oedema disease after an experimental challenge by intravenous injection of the toxin.

The two formulations with the highest antigen content (A and B) could not be separated with respect to their efficacy regarding survival and weight gain after challenge. The weight gain from the three vaccinated groups was at the same level as for the sentinels, such vaccination helped to control the growth retardation normally associated with oedema disease in non-vaccinated piglets.

The second dose-finding study investigated either 600 UEMA/ml; 300 UEMA/ml or 150 UEMA/ml to

piglets when 28 days old. *E. coli* strain CP-640 was used to prepare the Stx2e toxin and the challenge dose was 4.7 x $10^4$ $CD_{50}$/kg body weight administered intravenously. In total 65 commercial hybrid piglets were included at 2 days of age, both males and females being free of antibodies to Stx2e. The piglets were divided into four groups of 15 animals (Group A, B, C, and D) and 5 piglets served as non-vaccinated, non-challenged sentinels. Vaccinates in the four groups were administered 1 ml of VEPURED by the intramuscular route in the right side of the neck (A, B, C) and PBS controls (D) was administered in the same way.

Results showed that all three formulations of the Stx2e antigen A (600 UEMA/ml), B (300 UEMA/ml), and C (150 UEMA/ml) were efficacious against oedema disease after an experimental challenge by intravenous injection of the toxin. The three formulations showed a difference with respect to their efficacy regarding piglet survival as all pigs from group A survived the challenge while group B and C showed a reduction in mortality only. The weight gain in the three vaccinated groups was at the same level as for the sentinels, as such vaccination helped to control growth retardation associated with oedema disease. The potency of VEPURED's Stx2e antigen A was therefore set at 600 UEMA/ml, which was shown to prevent mortality of piglets after challenge. This antigen level was found very relevant, as the main clinical efficacy response from use of this product is to avoid mortality in piglets. The experimental design of the field efficacy studies were statistically sufficiently powered in order to show a significant reduction in mortality of piglets due to oedema disease. In power calculations it is only possible to use one primary efficacy parameter, and reduction in mortality is the most difficult to use, because variation in mortality is very large. Therefore, there was a need to include more than 2000 piglets in their field trials. By reducing mortality also, reduction of all secondary efficacy parameters would be covered with respect to statistical power to show a real efficacy effect from this product.

Onset of immunity

Onset of protection was demonstrated in a randomised and blinded study. A vaccine batch was used which was acceptable as it was close to the minimum dose. In total 35 commercial hybrid piglets were included 2 days of age at D0, both males and females, and free of antibodies to Stx2e. Fifteen piglets were vaccinated according to the recommendations in the proposed SPC with 1 ml of VEPURED by the intramuscular route in the right side of the neck at D0, and 15 PBS controls were administered in the same way. Five non-vaccinated and non-challenged piglets served as sentinels. *E. coli* strain CP-640 used to prepare Stx2e toxin, and the challenge dose at D21 was 4.7 x $10^4$ $CD_{50}$/kg body weight administered intravenously.

Results showed that the severity of clinical signs after challenge (total clinical score) as well as the number of piglets affected in the non-vaccinated control group were significantly higher than in the vaccinated group. All controls showed varying degree of oedema disease and related clinical signs at least one day after the challenge. Clinical signs observed in vaccinates were milder than in controls and fewer animals expressed these symptoms as well. Thus, the results from this study confirmed that vaccination with VEPURED reduced the clinical signs of oedema disease and prevented mortality caused by oedema disease after an experimental infection. An onset of immunity on 21 days was accepted.

Maternally derived antibodies (MDA)

Influence of maternally derived antibodies on the efficacy of the vaccine was demonstrated in a randomised, blinded study. An acceptable vaccine batch close to the minimum dose was used. In total 33 commercial hybrid piglets were included 2 days of age at D0, both males and females. Sixteen of the piglets were free of specific antibodies to Stx2e and 17 piglets showed varying titres of MDA to Stx2e. The piglets were from a farm also included in the field trial for this product, because this farm

had a historical persistent problem with oedema disease. In total eight piglets with MDA (group A) were vaccinated with VEPURED according to recommendations from the SPC, and nine piglets with MDA (group B) were administered PBS and served as positive MDA controls. Eight piglets without MDA were vaccinated with VEPURED (group C) and eight piglets served as non-vaccinated and non-challenged sentinels. Groups A, B, and C were challenged at D62 with an *E. coli* strain CP-640 used to prepare Stx2e toxin, and the challenge dose was 1.6 x $10^4$ $CD_{50}$/kg body weight administered intravenously. Post challenge from D62 to D69 all pigs were observed and general clinical signs were recorded including depression, diarrhoea, dyspnoea. Oedema and neurological signs were recorded occurring as palpebral, throat, ataxia, tremors, rigidity, paralysis, and opisthotonus. Moribund animals with severe dyspnoea were euthanised. All animals were observed twice per day and pigs with paralysis or opisthotonus during two consecutive observations were euthanised. Serology and piglet weight were also recorded during the experiment (blood sampling at D-1, D42, D62, D69 and weight at D62 and D69).

Results showed that VEPURED induced seroconversion of seronegative vaccinated animals, detected at D42 and D62. Most of the seropositive vaccinated animals remained so during the study period. All animals from the non-vaccinated MDA+ group (B) were seronegative at D62 when the challenge took place in groups A, B, and C.

The severity of clinical signs after challenge (total clinical score) as well as the number of piglets affected in the non-vaccinated group were significantly higher than in the vaccinated groups. All the non-vaccinated piglets showed clinical signs related to oedema disease at least one day post challenge. On the contrary, only a few animals from the vaccinated groups showed clinical signs of oedema disease. Moreover, the clinical signs recorded in vaccinates were milder than the signs recorded from non-vaccinated animals. Most of the piglets in the non-vaccinated group died after challenge (7/9) while all vaccinates survived (16/16). Thus, vaccination prevented mortality after experimental infection even in the presence of MDA levels representative for the field situation in a herd infected by oedema disease. The MDA levels from piglets included in this study were compared to the levels from piglets under field situations and they were found representative for the field situation.

It was accepted that VEPURED could prevent the risk of piglets dying as well as reduce the clinical signs after challenge despite the presence of MDA at levels representing the field situation in a chronically affected oedema disease infected herd.

Duration of immunity

Duration of immunity was established in a randomised and blinded study using a vaccine batch close to the minimum dose. Fifty-seven commercial hybrid piglets were included, 2 days at D0 both males and females, free of antibodies to Stx2e. The piglets were divided into three groups, 17 piglets were vaccinated with VEPURED intamuscular according to recommendations; 17 piglets were administered PBS and served as controls, and 5 piglets served as non-administered sentinels. These sentinels were included in order to detect any possible spread of disease during the experiment and to measure optimal growth performance in non-injected, non-stressed animals. *E. coli* strain CP-640 was used to prepare Stx2e toxin, and the challenge dose at D112 was 6 x $10^3$ $CD_{50}$/kg body weight administered intravenously.

Post challenge from D112 to D119 all pigs were observed and general clinical signs were recorded including depression, diarrhoea, dyspnoea. Oedema and neurological signs were recorded occurring as palpebral, throat, ataxia, tremors, rigidity, paralysis, and opisthotonus. Moribund animals with severe dyspnoea were euthanised. All animals were observed twice per day and pigs with paralysis or opisthotonus during two consecutive observations were euthanised. Serology and piglet weight were

also recorded during the experiment (blood sampling at D0, D28, D56, D84, D98, D105, D112, D119 and weight at D0, D112 and D119).

Results showed that all vaccinates raised seroneutralizing antibodies against Stx2e toxin post vaccination, while no controls nor sentinels developed antibodies at any time point during this study.

The severity of clinical signs after challenge (total clinical score) as well as the number of piglets affected in the non-vaccinated control group were significantly higher than in the vaccinated group. Most controls (13/16) showed varying degree of oedema disease and related clinical signs at least one day after challenge. Only one vaccinated pig showed mild clinical signs related to oedema disease after challenge. Clinical signs observed in vaccinates were milder than in controls and fewer animals expressed these symptoms as well. Mortality was 68.7% in the non-vaccinated control group while all vaccinated pigs survived.

With respect to body weight, it was shown that vaccinates had a similar performance post-challenge as sentinels, which did not receive the challenge. Therefore, it was demonstrated that vaccination could also assist to control growth retardation associated with oedema disease.

Thus, results from this study confirmed that vaccination with VEPURED reduced both the incidence and severity of oedema disease and prevented mortality caused by oedema disease after an experimental challenge infection. A duration of immunity on 112 days based on challenge at day 112 was accepted.

## *Field trials*

One safety and efficacy multisite field study was presented using two acceptable vaccine batches in different farms. In total 2221 piglets were included (vaccinates =1173; controls =1048), 2–3 days old at D0, commercial hybrids with a live weight ≥ 1 kg being individually identified with ear tags. The experimental design was multi-centre, randomised, double blinded, with a parallel negative control group. One ml of VEPURED was administered to vaccinates by the intramuscular route and PBS controls were administered in the same way. All efficacy parameters were considered acceptable. Statistical power calculation was performed using mortality as primary efficacy parameter. This is accepted, as only one parameter can be included into a statistical power calculation. Difference in mortality rate is the most difficult clinical parameter to use in a field experimental design, therefore more than 2200 piglets were included. All other clinical signs (dyspnoea, oedema, and neurological signs) would be expected also to be correctly powered, as these clinical signs would occur more often than death of the animals.

Efficacy parameters evaluated were:

**Primary efficacy parameters**

- Mortality as recorded in both groups of animals until the end of the fattening period. The cause of death was described and whenever possible, necropsies of dead animals were performed or biological samples were sent to the diagnostic laboratory for detection of Shiga-toxin *producing E. coli* to confirm the cause of death. Mortality was evaluated from D0 to D115 both in general and specifically with relation to oedema disease.
- Clinical signs of oedema disease were recorded as general clinical signs; dyspnoea; oedema; neurological signs.

**Secondary efficacy parameters**

- Body weight (average daily gain, ADG) was evaluated in a minimum of 120 animals per group in

each farm at D-1, D42, D115 as well as at the end of the fattening period a few days before slaughter.

- Serology was planned to be performed in 30 animals per group in each farm on D0, D28, and D42 for determination of serological status with respect to Stx2 antibodies.
- Concomitant treatments the number of treatments administered in both groups was performed by farmers during the whole follow-up period.

The experimental unit was the piglet, as all variables were collected and evaluated per individual.

Justification on the sample size needed to evaluate differences in mortality rates at a significance level of 5% (α) and a power of 80% (β) was used. To detect significant differences assuming that the mortality in vaccinates was 1% and 6% in controls the number of pigs per group would be approximately 257 (as a sum of all farms).

Justification on sample size for body weight was made assuming a relevant superiority of 0.5 kg in vaccinates and applying the same levels of statistical significance and power as above (α and β) the number of pigs than should be weighed per group were approximately 112.

Results showed that mortality due to oedema disease is the relevant primary clinical parameter to look at in order to evaluate the efficacy of the vaccine. In four out of five farms (KER, LON, TIN, MAE) a statistical significant reduction in mortality was observed. In one farm (KRI) mortality due to oedema disease was not observed in any enrolled vaccinates nor controls. In addition to the fact that the incidence of oedema disease was lower in vaccinates than in controls the severity of the clinical symptoms related to oedema disease was much higher in controls than in vaccinates.

In four out of five farms a statistical significant improvement in ADG was recorded on D115, in one farm (KER), a numerical improvement of 2 kg in vaccinates was recorded although not statistically significant. When the mean weights of pigs from all farms was included at D115, then vaccinates weighed approximately 2.9 kg more than controls, and this was statistically significant. Before animals were sent for slaughter the mean weight difference between vaccinates and controls was around 4 kg, and this was highly statistical significant ($p<0.001$). The ADG in all farms, as measured from birth to slaughter, increased from 575 g/day in controls to 599 g/day in vaccinates.

Results showed that no individual concomitant treatments were made in farm KRI, where animals were treated against *E. coli*, *S. suis* and pneumonia. When all farms were analysed together vaccinates showed a lower number of treated animals than controls, but no statistical significant difference was revealed. The number of animals treated due to oedema disease was lower in vaccinates than controls in three out of five farms (statistical significant in two farms). In two farms no registrations of treatments due to oedema disease were performed.

Serological results showed that on days 28 and 42 after vaccination, the majority of vaccinates were seropositive (86.4% and 91.6%, respectively). On the contrary, only a small percentage of non-vaccinated animals were positive on these days (3.5% and 1.4% respectively). On day 115, blood samples were taken only in two farms. At this time point a mean of 87.1% of vaccinates remained seropositive whereas controls were seronegative.

At the end of the fattening period, blood samples were taken only in three farms. At slaughter 84% of vaccinates were seropositive whereas 5% of animals in the control group were seropositive. The difference between percentages of vaccinates and controls being seropositive was statistically significant.

It was accepted, that this field study showed a prevention of mortality caused by oedema disease

(99.8%), as well as a significant reduction of the incidence of clinical signs caused by oedema disease. The product also significantly reduced the loss of daily weight gain during the finishing period in the face of infections with verotoxin 2e producing *E. coli* until slaughter, conferred persistent seroneutralizing antibodies in vaccinates until the end of the fattening period, and significantly reduced the percentage of animals treated due to oedema disease.

The efficacy results obtained at field level were consistent with the findings observed at laboratory level. The applied "prevention of mortality" claim can be supported as protection was 99.8% under field conditions.

## *Overall conclusion on efficacy*

The efficacy of VEPURED was demonstrated according to requirements laid down in the Commission Directive 2009/9/EC amending Directive 2001/82/EC. As there is no specific monograph for oedema disease, the efficacy studies were carried out in accordance with the general principles and requirements of the general Ph. Eur. monograph No. 50207 Evaluation of efficacy of veterinary vaccines and immunosera. Demonstration of a possible impact of maternally derived antibodies on vaccine efficacy in young animals was carried out according to the Reflection paper on the demonstration of a possible impact of maternally derived antibodies on vaccine efficacy in young animals (EMA/CVMP/IWP/439467/2007). The laboratory and field efficacy studies were performed using a batch which is acceptable, as it is close to the minimum potency for future batches to be produced for this product (RP≥ 1.5).

Challenge studies were performed in 4 weeks old pigs as well as in 3–4 months old pigs. The results demonstrated that the challenge model was valid in 4-week old pigs after receiving a dose of toxin with $4.7x\ 10^4$ $CD_{50}$/kg body weight. The challenge dose in 4-weeks old pigs was accepted.

In the second study the dose of toxin administered to 3–4 months old pigs was reduced in order to reduce aggressiveness for this target age of animals. The model obtained after administration of a toxin dose of $6 \times 10^3$ $CD_{50}$/kg body weight was suitable because mortality and morbidity was not as acute as when higher challenge doses were used, although also reaching 100%. This challenge dose in 3–4 months old pigs was accepted.

Two dose-finding studies were presented, the first applying either 300 UEMA/ml; 150 UEMA/ml or 75 UEMA/ml and the second applying either 600 UEMA/ml; 300 UEMA/ml or 150 UEMA/ml to piglets when 28 days old. *E. coli* strain CP-640 was used to prepare the Stx2e toxin and the challenge dose was $4.7 \times 10^4$ $CD_{50}$/kg body weight administered intravenously. All pigs from the highest antigen group survived the challenge while pigs from the lower antigen groups only showed a reduction in mortality. The potency of VEPURED's antigen was therefore set at 600 UEMA/ml.

Onset of protection was demonstrated in a randomised and blinded study. Pigs were challenged 21 days post administration. Results showed that the severity of clinical signs after challenge (total clinical score) as well as the number of piglets affected in the non-vaccinated control group were significantly higher than in the vaccinated group. An onset of immunity on 21 days was accepted.

Influence of maternally derived antibodies on the efficacy of the vaccine was demonstrated in a randomised and blinded study.

Results showed that the severity of clinical signs after challenge (total clinical score) as well as the number of piglets affected in the non-vaccinated group were significantly higher than in the vaccinated groups. Vaccination prevented mortality after experimental infection even in the presence of MDA levels representative for the field situation in a herd infected by oedema disease. It was accepted that

VEPURED under laboratory conditions could protect piglets from dying as well as reduced the clinical symptoms after challenge despite the presence of MDA at levels representing the field situation in a chronical oedema disease infected herd.

Duration of immunity was established in a randomised and blinded study. The severity of clinical signs after challenge (total clinical score) as well as the number of piglets affected in the non-vaccinated control group were significantly higher than in the vaccinated group. Approximately 70% of pigs from the non-vaccinated control group died after challenge while all vaccinated survived.

With respect to body weight, vaccinates had a similar performance post challenge as sentinels, which did not receive challenge. Therefore, it was confirmed that vaccination could also help to control growth retardation associated with oedema disease. A duration of immunity on 112 days based on challenge was accepted.

One safety and efficacy multisite field study was performed using two vaccine batches with relative potency RP≥ 1.5. In total 2221 piglets were included (vaccinates = 1173; controls =1048), 2–3 days old at D0. The experimental design was a multi-centre, randomised, double blinded, with a parallel negative control group.

The efficacy results obtained at field level were in line with the findings observed at laboratory level and confirmed that the VEPURED vaccine could:

- Prevent the mortality caused by oedema disease.
- Reduce the incidence of clinical signs caused by oedema disease.
- Reduce the loss of daily weight gain during the finishing period in the face of infections with verotoxin 2e producing *E. coli* until slaughter from 164 days of age.
- Vaccinated animals are able to neutralise the VT2e toxin. The applied "prevention of mortality" claim can be supported based on 100% protection at laboratory level and on 99.8% level in the field situation.

# Part 5 – Benefit-risk assessment

## *Introduction*

VEPURED is an immunological veterinary medicinal product that is developed by means of a biotechnological process. The product is a recombinant inactivated vaccine and the pharmaceutical form is a suspension for parenteral administration. It contains a genetically modified recombinant Shiga toxin (Stx2e) together with an aluminium hydroxide gel and DEAE-dextran as adjuvant.

The product is intended for active immunisation of piglets from 2 days of age to prevent mortality and reduce clinical signs of oedema disease caused by verotoxin 2e produced by *E. coli* and to improve their weight performance until slaughter. The vaccination scheme is a single intramuscular injection of 1 ml from 2 days of age.

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

## *Benefit assessment*

### Direct therapeutic benefit

VEPURED is intended for induction of active immunity of piglets from 2 days of age to protect against *E. coli* strains expressing Stx2e toxin, responsible for oedema disease. The onset of immunity was documented at 21 days post administration, and the duration of immunity was proven at 112 days after vaccination by challenge. The vaccine has been documented to prevent mortality and reduce clinical signs of oedema disease until slaughter from 164 days of age. Well-designed laboratory studies in accordance with GLP and clinical trials in accordance with GCP demonstrated that the product is efficacious in intended indications

### Additional benefits

VEPURED reduces the loss of daily weight gain during the finishing period in the face of infections with verotoxin 2e producing *E. coli* until slaughter from 164 days of age. VEPURED should reduce the future need of antimicrobials that are considered critically important to human medicine (e.g. Colistin, which currently is used to treat the clinical symptoms coursed by oedema disease). It will do this by reducing the incidence of clinical disease, thus providing confidence to avoid using antimicrobials both for treatment and metaphylaxis.

## *Risk assessment*

Quality:

Information on development, manufacture and control of the active substance and finished product have generally been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Safety:

Measures to manage the risks identified below are included in the risk management section.

*Risks for the target animal:*

The adverse reactions observed in the target animals are relatively mild with post-administration rise in temperatures well below 2 °C and mild depression on the day of vaccination and local mild inflammation at the injection site. These adverse reactions spontaneously resolved within a few days. Therefore, administration of VEPURED is generally well tolerated.

*Risk for the user:*

The CVMP concluded that the user safety for the product is acceptable when used according to the SPC recommendations.

*Risk for the environment:*

VEPURED is not expected to pose a risk for the environment when used according to the SPC.

*Risk for the consumer*:

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

### *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.

## Evaluation of the benefit-risk balance

The product has been shown to be efficacious for active immunisation of piglets from 2 days of age to prevent mortality and reduce clinical signs of oedema disease (caused by verotoxin 2e produced by *E. coli*) and to reduce the loss of daily weight gain during the finishing period in the face of infections with verotoxin 2e producing *E. coli* until slaughter from 164 days of age.

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animal and presents an acceptable risk for users, the environment and consumers, when used as recommended. Appropriate precautionary measures, including withdrawal period, have been included in the SPC and other product information.

## Conclusion

Based on the data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for VEPURED is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with 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 VEPURED.