8 October 2020
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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Enteroporc Coli AC (EMEA/V/C/005149/0000)

Vaccine common name: Neonatal piglet colibacillosis (recombinant, inactivated) and *Clostridium perfringens* vaccine (inactivated)

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

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Introduction

The eligibility to the centralised procedure was agreed upon by the CVMP on 14 September 2018 as Enteroporc Coli AC (F5 and F6 *Escherichia coli components)* has been developed by recombinant DNA technology.

The indications are:

For the passive immunisation of progeny by active immunisation of pregnant sows and gilts to reduce:

- Clinical signs (severe diarrhoea) and mortality caused by *Escherichia coli* strains expressing the fimbrial adhesins F4ab, F4ac, F5 and F6

- Clinical signs (diarrhoea during the first days of life) associated with *Clostridium perfringens* type A expressing alpha and beta2 toxins

- Clinical signs and mortality associated with haemorrhagic and necrotising enteritis caused by *Clostridium perfringens* type C expressing beta1 toxin

Onset of immunity (after uptake of colostrum):

- *E. coli* F4ab, F4ac, F5, F6: within 12 hours after birth

- *C. perfringens* type A and C: first day of life

Duration of immunity (after uptake of colostrum)

- *E. coli* F4ab, F4ac, F5 and F6: first days of life

- *C. perfringens Type A*: 14 days of life

- *C. perfringens* Type C: 21 days of life

The actives substances of Enteroporc Coli AC are inactivated *E. coli* fimbrial adhesins (F4ab, F4ac, F5 and F6) and *C. perfringens* type A/C toxoids (alpha, beta1 and beta2). The target species is pigs (pregnant sows and gilts).

Enteroporc Coli AC is a combined, multivalent inactivated vaccine for the passive immunisation of piglets by active immunisation of pregnant sows and gilts. The vaccine is intended to reduce clinical signs (severe diarrhoea) and mortality caused by *Escherichia coli* strains expressing the fimbrial adhesins F4ab, F4ac, F5 and F6, clinical signs (diarrhoea during the first days of life) associated with *Clostridium perfringens* type A (CpA) expressing alpha and beta2 toxins, and clinical signs and mortality associated with haemorrhagic and necrotising enteritis caused by *C. perfringens* type C (CpC) expressing beta1 toxin.

The product is intended for administration by intramuscular use.

Enteroporc Coli AC is presented in vials containing 10 or 25 doses.

The rapporteur appointed is Niels Christian Kyvsgaard and the co-rapporteur is Paolo Pasquali.

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

On 8 October 2020, the CVMP adopted an opinion and CVMP assessment report for an application for a marketing authorisation to the European Medicines Agency (the Agency) for Enteroporc Coli AC, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

On 9 December 2020, the European Commission adopted a Commission Decision granting the

marketing authorisation for Enteroporc Coli AC.

Marketing authorisation under exceptional circumstances

Not applicable.

Scientific advice

Not applicable.

MUMS/limited market status

The product as a whole does not classify as a MUMS given that it is intended for pigs which is a major target species. The product contains *E. coli* antigens for which the indication is considered a major use. The applicant requested EMA to classify the indication for the *C. perfringens* components contained in Enteroporc Coli AC as MUMS/Limited market. In their letter of 19 April 2018 (EMA/238010/2018) EMA informed the applicant, that Enteroporc Coli AC could be considered as intended for MUMS/Limited market with regard to its *C. perfringens* components only.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided documents that set out a detailed description of the system of pharmacovigilance. A statement signed by the applicant and the qualified person for pharmacovigilance, indicating that 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 has been provided.

The rapporteur considers that the pharmacovigilance system as described by the applicant fulfils the requirements and provides adequate evidence that the applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Enteroporc COLI AC is manufactured in the EU.

For all sites involved in the manufacture appropriate and valid manufacturing authorisation and GMP certificates were presented. Specific inspections to this vaccine are currently not required.

Manufacturers of the active substance (*Clostridium perfringens* antigens) are CZ Veterinaria S. A. and IDT Biologika GmbH.

Manufacturer of the active substance (*Escherichia coli*) and responsible for the batch release is IDT Biologika GmbH.

General comments on compliance with GMP, GLP, GCP:

No inspection issues have been identified during the assessment of this application.

Overall conclusions on administrative particulars

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

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

Qualitative and quantitative particulars

Enteroporc Coli AC is a combined, multivalent inactivated vaccine for the passive immunisation of piglets by active immunisation of pregnant sows and gilts. The vaccine is intended to reduce clinical signs (severe diarrhoea) and mortality caused by *Escherichia coli* strains expressing the fimbrial adhesins F4ab, F4ac, F5 and F6, clinical signs (diarrhoea during the first days of life) associated with *Clostridium perfringens* type A (CpA) expressing alpha and beta2 toxins, and clinical signs and mortality associated with haemorrhagic and necrotising enteritis caused by *C. perfringens* type C (CpC) expressing beta1 toxin.

The vaccine is presented as a lyophilisate component and a suspension component, each in separate containers. The lyophilisate contains alpha and beta2 toxoids of CpA and beta1 toxoid of CpC. The suspension contains *E. coli* fimbrial adhesins F4ab, F4ac, F5 and F6, and aluminium hydroxide as adjuvant. Other ingredients are sucrose and buffered saline solution as described in section 6.1 of SPC. The lyophilised *C. perfringens* type A/C fraction is resuspended prior to use with the *E. coli* suspension, for suspension for intramuscular injection. One vaccine dose corresponds to 2 ml of the resuspended product. The composition is presented in Tables 1 and 2.

The vaccine is intended to be available in multidose presentations (10 doses or 25 doses). The product is available in glass vials (lyophilisate and suspension) or PET vials (suspension) as described in section 6.5 of the SPC.

The composition of the vaccine is adequately described.

Containers and closure

The lyophilisate is filled into 10 ml glass (type I) vials (Ph. Eur. 3.2.1). The suspension is filled into 25 ml glass (type I) vials or 50 ml glass (type II) vials (Ph. Eur. 3.2.1), or in 25 ml or 50 ml PET containers (Ph. Eur. 3.2.2). Glass vials are sterilised and depyrogenated in a sterilisation tunnel. PET containers are sterilised by gamma irradiation. Justification for the minimum dose including validation reports is provided. The containers are closed with bromobutyl rubber stoppers type I (Ph. Eur. 3.2.9) and sealed with crimped caps. The stoppers are sterilised by autoclaving. Certificates of analysis have been supplied for containers and closure demonstrating compliance with the proposed specifications.

The containers and closures are in compliance with the pharmacopoeial requirements and their sterilisation is adequate.

Product development

Lyophilisate, *C. perfringens* toxoids

The lyophilisate component of Enteroporc Coli AC is based on IDT's earlier developed and approved *C. perfringens* vaccines: Clostriporc A, Enteroporc A, and Enteroporc AC. Enteroporc AC, which also contains *C. perfringens* type C beta1 toxoid and *C. perfringens* type A alpha and beta2 toxoids in a lyophilised form, was authorised in 2017 in 16 EU countries (procedure DE/V/0271/001/DC). The MUMS status was granted for all marketing authorisations.

The lyophilisate component of the new vaccine, Enteroporc Coli AC, is similar to the single *C. perfringens* vaccine Enteroporc AC. The choice of vaccine antigens and vaccine strains has been justified. The characteristics, including mode of action of the toxins, have been described as far as it is known. As compared to the authorised product Enteroporc AC, only minor optimisation of the manufacturing process has been introduced.

The product development section includes brief information about the production medium and manufacturing process. The manufacture is based on a seed lot system. The manufacturing process is a standard anaerobic fermentation in bioreactors, followed by separation of biomass and toxoidation. The manufacturing process is almost identical for the CpA and CpC toxoids. Toxoidation has been demonstrated in validation studies to completely toxoidate the antigens within the defined toxoidation conditions used for production. The demonstrated kinetics of toxoidation are considered compliant with *Ph. Eur. 0062, Vaccines for veterinary use*. A test for residual toxicity is carried out after the toxoidation step and according to *Ph. Eur. 0363, Clostridium perfringens vaccine for veterinary use*.

Suspension, *E. coli*

The suspension component of Enteroporc Coli AC is based on: *E. coli* strain for F4ab adhesin (212/078) originally isolated from a pig in Germany before 1969, and *E. coli* strain for F4ac (212/176) adhesin which was already used for the manufacture of the approved vaccines Coliporc PLUS and Clostricol. Both strains were selected because of good and stable fimbrial production. Furthermore, two recombinant strains are used: *E. coli* strain for F5 adhesin (213/220) and *E. coli* strain for F6 adhesin (212/200).

The product development section includes brief information about the production medium and manufacturing process, and the preparation of the recombinant strains is briefly described. The manufacture is based on a seed lot system. The manufacturing process is a standard *E. coli* fermentation in bioreactors. The manufacturing process is identical for the F4ab and F4ac fimbrial adhesins and almost identical for the F5 and F6 fimbrial adhesins. One focus in development was reduction of the endotoxin content inherently present when cultivating Gram negative bacteria. Inactivation of the bacteria is done with formaldehyde. The inactivation kinetics studies demonstrated that the minimum inactivation times applied during manufacturing is in accordance with *Ph. Eur. 0062*, *Vaccines for veterinary use*. An inactivation control test is performed directly after the inactivation step using a validated method. The finished product specification includes a test for free formaldehyde in accordance with Ph. Eur. 0062.

Final vaccine

The antigens of *C. perfringens* and *E. coli* are kept in two different vials until mixing because the *C. perfringens* type A/C antigens are much more stable as lyophilisate than in suspension. The lyophilisate is resuspended with the suspension containing the *E. coli* antigens and aluminium hydroxide as adjuvant. Aluminium hydroxide is a well-established adjuvant for inactivated vaccines. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. The list of excipients is included in section 6.1 of the SPC. No preservative is added as the

vaccine is intended to be used within 8 hours. The formulation of batches used during clinical studies is the same as that intended for marketing.

The advantage of this new combined vaccine is an effective protection against the most important and most frequently occurring pathogens causing neonatal diarrhea and haemorrhagic and necrotising enteritis in piglets. In addition, the combination reduces the number of injections and therefore, improves the handling and increases animal welfare. Due to the passive immunisation of the piglets by active immunisation of pregnant sows and gilts, the effort of injection administration is vastly decreased.

Part 2-1 Lyophilisate containing the C. perfringens type A/C toxoids

Description of the manufacturing method

The *C. perfringens* type A (CpA) toxoids and type C (CpC) toxoid are manufactured in two separate manufacturing processes using the strains CpA and CpC, respectively. The manufacturing process is a standard anaerobic fermentation in bioreactors, followed by separation of biomass and toxoidation. The manufacture is based on a seed lot system.

CpA toxoids are produced in bioreactors. The scale up/fermentation process consists of three steps: resuspension of working seed, scale-up pre-culture steps, and anaerobic fermentation in bioreactor. Inoculation volumes and cultivation times are defined for each step. The manufacturing process of CpC toxoid is essentially the same as for CpA toxoids. Finally, the permeate is sterile filtrated. The manufacturing process is acceptably described.

Following toxoidation residual toxicity is determined. The toxoidation validation studies demonstrated complete toxoidation within the established toxoidation conditions, and for the tested concentrations of toxins, and are generally considered in line with Ph. Eur. 0062 requirements. On request the applicant has defined relevant upper toxin limits before toxoidation for routine production and provided some further details on the toxoidation conditions.

Finally, each toxoid is concentrated. The holding times have been validated. Thereafter the toxoid is sterile filtrated.

Bulk formulation for lyophilisation is done by mixing the concentrates of CpA and CpC toxoid and sucrose is added as a stabiliser. The volume of toxoids for bulk formulation is calculated from the toxoid content in the concentrate and the minimum target concentration per vial of the respective toxoids. The applicant has justified that no upper limits are set for toxoid concentrations in the final bulk and has explained how it is ensured that the content of the three toxoids in the final vaccine does not exceed the levels tested to be safe in clinical trials. Validation of blending has been demonstrated, and a minimum mixing time for the formulated bulk is defined.

The final bulk is filled into glass vials and freeze-drying is carried out. The vials are crimped and stored at +2°C to +8°C. The freeze-drying process has been validated by four consistency batches.

Validation of the intermediate holding times was performed. A number of concerns posed on validation of the intermediate holding times were solved (refer to section on Stability).

Validation of the manufacturing process as a whole is demonstrated with the provision of results of three consecutive lyophilisate batches. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible and consistent manner. The in-process controls are considered adequate for this type of manufacturing process.

Production and control of starting materials

Starting materials listed in pharmacopoeias

The starting materials listed in a pharmacopoeia are presented together with the function of each starting material. The quality of the materials complies with Ph. Eur. with the exception of glutaraldehyde and simeticone emulsion which complies with the British Ph. and USP, respectively. This is acceptable as no Ph. Eur. monograph exists. Certificates of analyses are provided for all the listed starting materials and all conform to the relevant specifications. None of the pharmacopeial starting materials are of animal origin. The applicant has confirmed that a test for identity is performed in-house for all starting materials.

Specific materials not listed in a pharmacopoeia e.g. active ingredient, adjuvants, cell seeds and some excipients

Starting materials of biological origin

Master and working seed

C. perfringens type A and *C. perfringens* type C strains are already the basis for a number of authorised vaccines, including Enteroporc AC (DE/V/0271/001/DC). The source and history of the strains is described in sufficient detail. The manufacture, testing, and storage of the lyophilised master seed and current working seed is adequately described and documented. The tests performed on the master seeds and working seeds are in general appropriate and in accordance with Ph. Eur. 0062, Vaccines for veterinary use and with the CVMP Guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010). The applicant has confirmed that new working seeds will be manufactured as described for the current working seeds.

Other starting materials of biological origin

Components of porcine, bovine and equine origin, and yeast extract, are used for production of seed material and/or during fermentation of pre-cultures and main culture. Examples of certificates of analysis are included for all starting materials. A risk assessment has been performed demonstrating that the risk of viral contamination of the bacterial seeds, as well as biological raw materials used in the manufacture of the vaccine, can be considered negligible. The animal-based material is considered in compliance with the requirements of Ph. Eur. 5.2.5 'Substances of animal origin for the production of immunological veterinary medicinal products'.

Risk of TSE-transmission and transmission of extraneous agents (viruses and mycoplasma)

The bacterial strains were isolated from non-TSE relevant species (CpA strain), or before occurrence of BSE (CpC strain, the exact origin of which is unknown). Starting materials from TSE-relevant species comply with the TSE Note for guidance (EMA/410/01 rev.3). Furthermore, a risk assessment has been performed concluding that the risk of TSE-transmission of the animal-based starting materials can be practically excluded. Overall, the risk of TSE transmission is considered negligible.

The starting materials of biological origin are sufficiently described.

Starting materials of non-biological origin

Two defoamers of non-biological origin are used (a silicon defoamer and an alkoxylated fatty acid ester defoamer). Examples of certificates of analysis have been provided.

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

Information on the qualitative and quantitative composition, methods of preparation, sterilisation, and storage conditions of all media and solutions is provided in the dossier.

Control tests during the manufacturing process

In-process controls during manufacture of CpA and CpC toxoid concentrates include analyses of purity during fermentation, sterility and content of toxins for the sterile filtrate/diafiltrate, residual toxicity after toxoidation, and sterility and content of toxoids and residual glutaraldehyde for the final concentrates of CpA and CpC toxoids. In-process controls during bulk production and production of lyophilisate containing the *C. perfringens* type A/C toxoids includes only test of filling weight, whereas control of antigen content, pH, and sterility after bulking is performed on the final product. The applicant presented in-process data for the manufacture of four consecutive batches of CpA and CpC toxoids, respectively, and four consecutive batches of finished product lyophilisate. The analytical data and results are provided and are considered acceptable. The in-process controls are considered suitable to control consistency of production. On request the applicant has added measurement of concentration of glucose as in-process control of culture growth; furthermore, the absence of acceptance limits for the content of alpha toxin and beta2 toxin/beta1 toxin in the sterile filtrate has been adequately justified.

Descriptions of test method and their limits of acceptance are provided. The test for residual toxicity is carried out according to Ph. Eur. 0363. Further information on the mice used in the test was provided on request. Alpha toxin/toxoid, beta2 toxin/toxoid and beta1 toxin/toxoid are determined by individual ELISA assays. The method descriptions are adequate.

Non-pharmacopoeia methods are validated (purity, content of alpha toxin/toxoid and beta2 toxin/toxoid, glutaraldehyde). Sterility testing is performed according to Ph. Eur. and test of method suitability was performed. The method validation is considered adequate and in accordance with the expectations of VICH GL1 and GL2. Some concerns posed on the possible impact of beta2 toxin/toxoid on the specificity of the alpha toxin ELISA have been solved, and clarification on the method suitability of the sterile test has been provided.

Control tests on the finished product

The control tests performed on the lyophilisate containing the *C. perfringens type* A/C toxoids include analyses of general characteristics (appearance, pH, reconstitution), batch titre/potency/identification, residual moisture and sterility. Determination of residual glutaraldehyde is performed as an in-process control, this is considered acceptable. As for residues of EDTA, this component is approved for use in food-producing animals with no MRL required according to the Annex to Commission Regulation 37/2010 and is thus considered safe. No safety test is performed, which is acceptable cf. Ph. Eur. 0062. No test for endotoxin is performed, which is acceptable cf. Ph. Eur. 0363. Overall, the selection of parameters in the finished product specification is considered appropriate for the characteristics of the final product.

Batch titre/potency/identity is measured as contents of alpha toxoid, beta1 toxoid, and beta2 toxoid, expressed as relative units per ml. Lower acceptance limits at time of release and at end of shelf life are provided, based on the efficacy studies performed. The Applicant has justified the absence of tests for residual toxicity.

Descriptions of test methods and the specifications are provided for all control tests. Tests for pH, sterility, and residual moisture, are carried out according to Ph. Eur. The potency and identity test of the alpha toxoid, beta2 toxoid and beta1 toxoid are determined by individual ELISA assays. The potency test is also used as identification test, which is acceptable. Further information was requested on assay procedure, procedures for replacement of assay materials (reference, antibodies, recovery control) and origin of materials; adequate information has been provided. In addition, the correlation between antigen content and potency test result has been demonstrated on request. The potency method is considered acceptable.

Non-pharmacopoeia methods are validated. The relative potency ELISA methods for measuring the content of alpha toxoid, beta1 toxoid, and beta2 toxoid, respectively, were validated as per VICH GL1 and GL2. Sterility testing is performed according to Ph. Eur. and test for method suitability was performed. Validation of determination of residual moisture was performed on the licensed product Enteroporc A. Since the matrix and biomass are similar to the lyophilisate containing *C. perfringens type* A/C toxoids, this is considered acceptable. In general, the method validations are considered adequate and in accordance with the expectations.

Batch-to-batch consistency

The applicant presented batch data for in-process results for four consecutive consistency batches of CpA and CpC toxoid, respectively, as well as for the four corresponding batches of final lyophilisate. The batch results submitted fulfil the proposed IPC specifications and specifications for finished product and demonstrate acceptable consistency of manufacturing process and final lyophilisate product.

Batch records are provided for the consistency batches, together with batch protocols for four additional batches.

Stability

Intermediates

The stability of intermediate products at relevant process steps of the CpA and CpC toxoids, and the lyophilisate containing the *C. perfringens* type A and type C toxoids, was examined on samples taken at the particular step of a continuous process, and then kept for the respective storage period and temperature. The applicant has provided the requested stability data. The stability of intermediate products is considered demonstrated.

Active ingredients

Data from two antigen batches of CpA and one batch of CpC indicate that the antigens are stable for the proposed shelf-life of 6 months at -15 °C to -29 °C. As requested, the stability of the concentrated antigens has been demonstrated individually at manufacturing conditions and representative scaled-down conditions.

Finished product

Long-term stability data are provided for three consistency batches and two small-scale batches of the similar product Enteroporc AC, all manufactured according to the process outlined in part 2-1B. As

Enteroporc AC is considered representative for the Enteroporc Coli AC lyophilisate this is considered acceptable.

The presented data confirm that the finished product as packaged for sale is stable for the proposed shelf-life of 21 months at 2-8 °C.

In-use stability

The presented data confirm that the reconstituted final vaccine is stable for the proposed shelf-life of 8 hours at 2-8 °C.

After removal of the reconstituted final vaccine from 2-8 °C the vaccine must be used immediately.

Part 2-2 Suspension containing the E. coli fimbria adhesins

Description of the manufacturing method

The *E. coli* suspension is manufactured at IDT Biologika GmbH, Dessau-Rosslau, Germany.

The *E. coli* suspension contains four *E. coli* fimbria adhesins; F4ab, F4ac, F5 and F6, which are manufactured in four separate manufacturing processes with *E. coli* strains F4ab, F4ac, F5 and F6, respectively. The manufacturing processes are based on a seed lot system and are considered to be standard manufacturing process.

The manufacturing process is identical for the F4ab and F4ac fimbrial adhesins and almost identical for the F5 and F6 fimbrial adhesins. The F5 and F6 fimbrial adhesins are manufactured from recombinant *E. coli* strains. The manufacturing process of the suspension containing the four *E. coli* fimbria adhesins consists of bulk formulation, filling and packaging. The final bulk is filled in glass or PET bottles, closed with rubber stopper and sealed with an aluminium cap.

Storage times of intermediate products during manufacture are validated. A number of concerns posed on validation of the intermediate holding times were solved. Please refer to section on Stability.

In general, the manufacturing process is considered adequately described. Further information was provided on fermentation parameters and their validation, equipment and sterilisation conditions, and limits of in-process controls. Furthermore, a justification of omission of sterile filtration of the final bulk has been provided.

A validation study has been performed for the inactivation step for each fimbrial antigen in order to determine the minimum inactivation time and to establish the maximum titre of the antigens. The kinetic inactivation studies show that the minimum inactivation times applied during manufacturing are in accordance with Ph. Eur. 0062 'Vaccines for Veterinary use'. More information was provided with regard to the inactivation studies; the information is considered adequate. The finished product specification includes a test for free formaldehyde with a limit of ≤ 0.5 mg/ml. This is in accordance with the limit stated in the relevant Ph. Eur. monograph and removal/neutralisation of formaldehyde is not considered necessary.

The release requirements for aluminium content and for the four *E. coli* fimbrial antigens have been described. To set release limits for the potency test the loss of potency over time and the assay variation of the potency test are taken into account. Safety has been demonstrated for batch blended at the chosen (higher) target potency.

Demonstration of consistency of production of three consecutive batches and validation of the holding times is presented. Critical manufacturing steps have been defined and the process parameters has

specified by limits and validated.

Production and control of starting materials

Starting materials listed in pharmacopoeias

Starting materials listed in pharmacopoeias are presented, together with the use of each starting material. All starting materials listed comply with Ph. Eur. with the exception of simethicone emulsion which complies with USP. This is acceptable as no Ph. Eur monograph exists. Certificates of analyses are provided for all the listed starting materials. None of the pharmacopeial starting materials are of animal origin. A test for identity is performed in-house for all starting materials.

Specific materials not listed in a pharmacopoeia e.g. active ingredient, adjuvants, cell seeds and some excipients

Starting materials of biological origin

Starting materials of biological origin are presented with description of origin and function. For each bacterial strain of *E. coli,* a seed lot system is prepared. The origin, history, preparation and testing of the seed lots are described and are generally appropriate and in compliance with Ph. Eur 0062. MS and WS are also tested according to the CVMP Guideline on requirements for the production and control of immunological veterinary medicinal products (EMA/CVMP/IWP/206555/2010) *E. coli* F5 and F6 antigens are manufactured by recombinant production strains. The origin and history are described and the sequences of the final plasmids are presented. The morphology, biochemical properties, and serotype/biotype of the strains are described. The Applicant has confirmed that new working seeds will be manufactured as described for the current working seeds.

Several starting materials of animal origin are used. A certificate of analysis is presented for each starting material A risk assessment for transmission of extraneous agents during manufacturing is presented including the bacterial seed materials as well as raw materials of animal origin used for the production of the vaccine. The risk of these materials as possible source of virus contamination was evaluated to be negligible.

A TSE risk assessment of starting materials of biological origin according to EMEA/CVMP/019/01 is presented. The *E. coli* F4ab and F4ac strains are derived from swine that are not susceptible to TSE and thus will not pose any risk of transmitting TSE. For F5 and F6, the commercially available DH5 strain and K12 strain are well characterised and are considered not to pose any risk of transmitting TSE. Starting materials of bovine origin (Australia/New Zealand) used for production of the seed materials are described. All materials of animal origin used for the MS/WS or during production are derived from bovine milk in the same conditions as that for human consumption. Overall, the risk of TSE transmission is considered negligible.

The starting materials of biological origin are sufficiently described.

Starting materials of non-biological origin

Starting materials of non-biological origin are listed and example certificates of analysis are provided. The materials are steam sterilised or sterile filtered.

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

For all media and solutions, the method of preparation is described in the dossier. All media/solutions are steam sterilised or sterile filtered. The storage conditions and times are listed.

Control tests during the manufacturing process

For the manufacture of *E. coli* fimbrial adhesins the in-process controls consist of purity test, CFU count, inactivation control test, sterility test, test for aluminium content (F4b and F4ac) and test for antigen content. For the final suspension containing all four *E. coli* antigens a test for fill volume is performed. A detailed description of each test including acceptance limits is provided. In-process data for the manufacture of three consecutive batches of each antigen, and three consecutive batches of finished suspension are presented. The analytical data and results provided are considered acceptable. Overall, the in-process controls are considered suitable to control consistency of production.

Non-pharmacopoeial methods are validated (purity, CFU count, inactivation control test, test for aluminium hydroxide and determination of antigen content). Validation reports are presented in the appendices. The method validation is overall considered adequate and in accordance with the expectations of VICH GL1 and GL2. Sterility testing is performed according to Ph. Eur. and method suitability was confirmed.

Control tests on the finished product

The control tests performed on the suspension containing the *E. coli* fimbrial adhesins are listed with specification limits and reference to method. The control tests include analyses of general characteristics (appearance, pH), content of adjuvant (aluminium hydroxide), content of formaldehyde, sterility, bacterial endotoxins, and batch titre/potency. No safety test is performed, which is acceptable with reference to Ph. Eur. 0062. No test for inactivation is performed on the finished product, which is considered acceptable as a test for inactivation is performed directly after the inactivation step.

Descriptions of test method are provided for all methods. In general, the proposed control tests are considered acceptable and in line with the test requirements of Ph. Eur. The acceptance criteria established are considered sufficient to assure an acceptable and consistent quality of the product.

Batch titre/potency is measured as content of F4ab, F4ac, F5, and F6 antigen content expressed as relative units per ml (rU/ml). The ELISAs used in the batch potency tests function as tests for the identification of the active substances. It is acceptable that the potency test is also used as identification test. Further information was requested on the potency test, including assay procedure, procedures for replacement of assay materials (reference, antibodies, recovery control), origin of materials and stability of reference antigen; adequate information has been provided. In addition, the correlation between antigen content and potency test result has been demonstrated as requested. The acceptance limits for the potency test of each fimbrial adhesin is stated at time of release and at end of shelf life. No upper limit is defined and no safety test in target animals is performed. The applicant has justified the lack of upper release limits for content of antigens. Furthermore, the proposed lower release limits for antigens F4ab, F4ac, and F5 have been justified by provision of additional data and minimum potency at the end of the proposed shelf life is considered guaranteed. Non-pharmacopoeial methods are validated. Sterility testing is performed according to Ph. Eur. and test for method suitability was performed. The method validations are considered adequate and in accordance with the expectations of VICH GL1 and GL2.

Batch-to-batch consistency

Test results during production of the suspension containing the *E. coli* fimbrial adhesins are presented for three consecutive batches of F4ab, F4ac, F5 and F6 fimbrial antigens produced according to the manufacturing process described in section 2-2B at the site of IDT. All results comply with the requirements. A justification for the small batch size of the consistency batches of F5 and F6 antigens was requested; data for a full-scale batch of F6 have been presented subsequently and the applicant has committed to submit batch data for F5, once the first full scale batch has been produced (recommendation).

For the finished product, test results of five consecutive batches are presented. Upon filling, the batches were split and filled into glass and PET vials. All five consistency batches met the release specification criteria.

The results from the consistency batches demonstrate acceptable consistency of manufacturing process the *E. coli* fimbrial adhesin antigens.

Stability

Intermediates and active ingredients

The stability of intermediate products at relevant process steps of each of the *E. coli* fimbrial adhesins, and the suspension containing the adhesins, has been examined on samples taken at the particular step of a continuous process, and then kept for the respective storage period and temperature. The applicant has provided the requested missing stability data. The stability of intermediate products and active ingredients is considered demonstrated.

Finished product

Stability data for six batches manufactured according to the process outlined in part 2-2b are presented. Two batches were produced at pilot-scale, the other batches are commercial batches manufactured at production scale. The batches are stored either in PET bottles or glass vials. The shelf-life specification is presented and the limits for antigen content are in line with the limits tested in the efficacy studies.

Stability data up to 24 months are available. All batches tested complied with the specifications. For all batches a decrease in potency of F4ab, F4c, F5 and F6 was found, but the results were above the minimum limits at end of shelf life. The proposed shelf life of 21 months is therefore considered acceptable.

In-use stability

The presented data confirm that the reconstituted final vaccine is stable for the proposed shelf-life of 8 hours at 2-8 °C. After removal of the reconstituted final vaccine from 2-8 °C the vaccine must be used immediately.

Overall conclusions on quality

Enteroporc Coli AC is a combined, multivalent inactivated vaccine for the passive immunisation of piglets by active immunisation of pregnant sows and gilts. The vaccine is presented as a lyophilisate component and a suspension component each in their multidose container.

The lyophilisate contains alpha and beta2 toxoids of *C. perfringens* type A and beta1 toxoid of *C. perfringens* type C and is presented in Type I glass vials containing 10 or 25 doses.

Overall, information on the development, manufacture and control of the active substances and the finished product has been presented in a satisfactory manner.

The manufacturing is based on a seed-lot system and is performed as a standard anaerobic fermentation in bioreactors, followed by separation of biomass, toxoidation, blending, filling, lyophilisation and capping. Generally, the manufacturing process is considered adequately controlled. Based on the data from four consecutive finished product batches, acceptable batch-to-batch consistency is considered demonstrated and all results fulfilled the proposed specifications for finished product. Compliance with Ph. Eur. monographs 0062 *Vaccines for veterinary use* and 0363 *Clostridium perfringens vaccine for veterinary use* is generally considered demonstrated.

Data from stability studies for four bathes of the finished product indicate that the lyophilisate is stable for the proposed shelf-life of 36 months at 2-8 °C.

The suspension contains *E. coli* fimbrial adhesins F4ab, F4ac, F5 and F6, and aluminium hydroxide as adjuvant. Other ingredient is buffered saline solution. The suspension is presented in glass vials or PET vials containing 10 or 25 doses.

Overall, information on the development, manufacture and control of the active substances and the finished product has been presented in a satisfactory manner.

The manufacturing is based on a seed lot system. It is performed as a standard fermentation in bioreactors. The F5 and F6 fimbrial adhesins are manufactured from recombinant strains. This is followed by bulk blending and filling. Based on the data from five consecutive finished product batches, acceptable batch-to-batch consistency is considered demonstrated and all results fulfilled the proposed specifications for finished product. Compliance with Ph. Eur. monographs 0062 *Vaccines for veterinary use* and 0962 *Neonatal piglet colibacillosis vaccine (inactivated)* is generally considered demonstrated.

Data from stability studies for five batches of the *E. coli* finished suspension indicate that the suspension is stable for 24 months at 2-8 °C. The proposed shelf life of 21 months is considered acceptable.

The lyophilisate containing *C. perfringens* type A and type C toxoids is resuspended with the *E. coli* suspension prior to use. A shelf life of 8 hours after reconstitution has been demonstrated. After removal of the reconstituted vaccine from 2-8 °C the vaccine must be used immediately.

Part 3 – Safety

Introduction and general requirements

Enteroporc Coli AC is a recombinant, combined, multivalent, inactivated vaccine including alpha and beta1 toxoids of *C. perfringens* type A, beta1 toxoid of *C. perfringens* type C as well as fimbrial antigens (F4ab, F4ac, F5 and F6) of *E. coli.* The product is intended to provide passive colostral immunity to piglets by active immunisation of breeder gilts and sows. Enteroporc Coli AC is the first vaccine in the EU that contains three toxoids from *C. perfringens* together with 4 fimbrial antigens from *E. coli.*

The application has been submitted in accordance with Article 3(1) – Indent 1 – Biotech medicinal product of Regulation (EC) No 726/2004 (mandatory scope), as it is a product developed by means of a biotechnological process. A full safety file in accordance with Article 12(3)(j) has been provided.

Safety documentation

Two laboratory safety studies and three field safety/efficacy studies were conducted in order to investigate the safety of the product. One laboratory study investigated the safety of the administration of one dose and one study investigated a repeated dose. In both studies the reproductive performance was investigated. The vaccine was administered by the intramuscular route, as recommended. Laboratory studies were reported to be GLP compliant and carried out in target animals of the minimum age (gilts) recommended for vaccination, using a production batch (LM 0021017) containing the highest recommended concentrations of the product plus maximum endotoxin content.

Laboratory tests

Safety of the administration of one dose

One pivotal study was provided. The study was compliant with GLP standards. One dose (2 ml) of a production batch containing the highest recommended concentrations of the product plus maximum endotoxin content was administered by the intramuscular route which is the recommended route of administration in gilts at 5 and 2 weeks before expected farrowing. Animals were of the minimum age (gilts) being the most sensitive age as required.

The following observations and examinations for signs of systemic and local reactions were recorded in gilts. A transient increase in body temperature (mean 1.2 °C following 1st injection and 0.5 °C following 2nd, and max. up to 2 °C in individuals) was recorded very commonly. The rise in temperature reached a maximum by 6 hours post administration and returned to normal values within 24 hours. No local reactions were observed. The adverse reactions are correctly addressed in the proposed SPC, Section 4.6.

On the basis of the results no safety concerns arose following the administration of the dose to the target species of the minimum recommended age, providing therefore a valid demonstration of the safety of a single dose of the primary vaccination of the product.

Safety of one administration of an overdose

No overdose administration is required for inactivated vaccines.

Safety of the repeated administration of one dose

One pivotal laboratory study was provided using the same animals as in the previous one dose safety study. The study was compliant with GLP standards. One dose of a production batch (the same two batches as for the one dose study) was used.

One dose (2 ml) of the vaccine was administered by the intramuscular route, which is the recommended route of administration in the target species (sows) at 2 weeks before the expected farrowing. This represents one additional vaccination after the primary vaccination course. Animals were the same as used for the basic vaccination schedule as gilts, now being pregnant with their second litter as required.

The following observations and examinations for signs of systemic and local reactions were recorded in sows. A transient increase in body temperature (mean 0.7 °C and max. up to 1.1 °C in one sow) was recorded very commonly. The rise in temperature reached a maximum by 4-6 hours post

administration and values returned to normal within 24 hours. A transient small puncture of the skin (<0.5 cm) at the injection site was observed in two sows. The reactions resolved within a week post administration. The adverse reactions are correctly addressed in the proposed SPC, Section 4.6.

On the basis of the results, no safety concerns arose following the administration of the dose one additional dose of vaccine (revaccination) after the primary vaccination schedule to the target species.

Examination of reproductive performance

The safety of the reproductive performance was investigated in two studies using the same animals as in the previous "one dose and repeated one dose" safety studies.

Results showed that the reproductive performance was not affected negatively after administration of Enteroporc Coli AC to pregnant gilts or sows.

On the basis of the results no safety concerns arose following the administration of the high endotoxin content to animals of target species at 5 and 2 weeks before expected farrowing. The SPC, section 4.7 has therefore correctly been worded accordingly "Can be used during pregnancy".

Examination of immunological functions

No further studies were conducted to investigate the effects of the product on immunological functions. None of the components of the vaccine is known to have immunosuppressive effect and there are no data suggesting a negative influence on the immune response of the vaccinated animals. Since generally no adverse effects from this type of inactivated/toxoidated vaccine on the immune system are known or expected, no studies were conducted to examine immunological functions.

User safety

The applicant has presented a user safety risk assessment which has been conducted in accordance with CVMP guideline EMEA/CVMP/IWP/54533/2006 (and EMEA/CVMP/543/03-Rev.1).

The main potential routes of accidental contact with the product have been considered and it was concluded that the most likely are those of accidental self-injection and dermal and/or oral exposure. The active substances are inactivated proteins and therefore not infectious to humans, and as such do not pose a risk for the user.

The excipients including the adjuvant are commonly used in other vaccines and do not pose a risk for the user.

Based on the above risk assessment the CVMP concluded that the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

Study of residues

No study of residues has been performed and this is acceptable since no substance requiring a MRL is included.

The active substances which are of biological origin are not within the scope of the Regulation (EC) No. 470/2009.

The excipients, including the adjuvants aluminium hydroxide as well as glutaraldehyde and formaldehyde that may be contained in traces as remnants of starting materials are listed in the annex of EU Regulation No. 37/2010 (Table 1: List of allowed substances) or are considered as not falling

within the scope of Regulation (EC) No 470/20009.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

The Applicant has not provided data investigating interactions of the vaccine with other veterinary immunological products and therefore proposes to include a statement in Section 4.8 of the SPC, that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis.'

Field studies

Three field safety/efficacy studies were submitted in order to support the safety from the laboratory studies in this dossier. The studies were placebo-controlled, randomised and blinded. The studies were conducted in Germany and adhered to GCP. The batches used were of intermediate potency. Clinical investigations included observations on serious adverse reactions, systemic reactions, local reactions and pyrexia were carried out on days -1; D0, D0+6h, D1, D2 and D14 post vaccination. Reproductive performance was investigated including number of piglets per litter, stillborn piglets, underweight piglets and number of viable piglets.

The results from the three field trials were compliant with the requirements in Ph. Eur. monograph 0962, as no gilts showed any abnormal local or systemic adverse reactions or died from causes attributable to the vaccine, after the administration of two doses of vaccine 5 weeks and 2 weeks prior to expected farrowing respectively. The observed local swellings and slight colour changes at the injection site in the vaccinates resolved after maximum 7 days without further handling.

The observed body temperature rises were less than 1.5 °C on average and not higher than 2.0 °C in individuals at its maximum. Temperatures returned to normal within 24 hours. No other relevant statistically significant differences were observed for any of the analysed safety parameters. These results supported the laboratory safety studies by demonstrating the safety under field conditions in a larger number of gilts vaccinated two times representing the basic vaccination schedule.

The studies were well designed and conducted and confirmed that the product was safe under field conditions after basic vaccination with a dose of intermediate potency in gilts.

Environmental risk assessment

Considerations for the environmental risk assessment

Hazard identification

Enteroporc Coli AC consists of a suspension and a lyophilisate. The suspension contains the fimbria adhesins F4ab, F4ac, F5 and F6 of inactivated *E. coli* as the active substances and aluminium hydroxide as adjuvant. The lyophilisate contains the inactivated alpha, beta1 and beta2 toxoids from *C. perfringens* type A and C as active substances and sucrose as excipient. The lyophilisate is reconstituted with the suspension.

Enteroporc Coli AC is a vaccine which is administered intramuscularly in the neck in the area behind the ear. The vaccine is used for vaccination of gilts or sows in the last third of pregnancy. Thereby passive immunisation of piglets via colostrum is induced for protection against the clinical effects of *E. coli* strains, which express the adhesins F4ab, F4ac, F5 and F6 (serotypes K88ab, K88ac, K99 and 987p) and of *C. perfringens* alpha, beta1 and beta2 toxins.

The *C. perfringens* cells are completely removed by separation and/or microfiltration. Toxins derived from *C. perfringens* type A and C are toxoidated. The *E. coli* cells are partly removed by separation and any remaining *E. coli* bacteria are inactivated with formaldehyde. Therefore, no live micro-organisms or toxic components are present in the product.

The Phase I assessment allows the following conclusions:

Enteroporc Coli AC does not contain any live organisms, thus shedding of live organisms will not occur.

Enteroporc Coli AC is administered by intramuscular injection. If the vaccine is used according to the SPC, the potential exposure to the environment is considered negligible.

The use of the vaccine does not lead to any residues that could cause harm to the environment.

The vaccine does not contain any components of toxic or pathogenic concerns.

Since no hazards concerning the environment are indicated, no consequences need to be assessed.

No precautions need to be taken. A Phase II assessment is not deemed necessary.

Based on the data provided the ERA can stop at Phase I. Enteroporc Coli AC is not expected to pose a risk for the environment when used according to the SPC.

Overall conclusions on the safety documentation

The safety of Enteroporc Coli AC was investigated in two laboratory studies (one single dose study and one repeated dose study) and three combined safety and efficacy field studies including three commercial farms.

The single dose administration of Enteroporc Coli AC was demonstrated to be safe under a worst-case situation using a batch containing maximum endotoxin, *E. coli* antigens and *C. perfringens* toxoid contents. Pregnant gilts were used as the most sensitive age group representing the target population (gilts and sows), which is accepted. Animals of the youngest target age (gilts) were vaccinated IM 5 weeks and 2 weeks before expected farrowing according to recommendations in the proposed SPC (primary vaccination).

The results showed that the product was safe when administered to the youngest target age of pigs.

A repeated dose study with vaccination 2 weeks before expected farrowing showed no systemic reactions and only a few, mild local reactions. No impairment of reproductive performance was detected. The observed increase in rectal temperature was transient and compiled with the safety requirements of Ph. Eur. monographs 0962 and 0363. It was concluded that booster vaccination (third administration prior to second farrowing) of a single dose of Enteroporc Coli AC, containing maximum endotoxin, *E. coli* antigens and *C. perfringens* toxoid contents was safe for pregnant sows. This is accepted.

The results from the three field trials were compliant with the requirements in Ph. Eur. monograph 0962, as no gilts showed any abnormal local or systemic adverse reactions or died from causes attributable to the vaccine, after a two-time administration of a single dose 5 weeks and 2 weeks prior

to expected farrowing. The observed local swellings and slight colour changes at the injection site in vaccinates resolved after maximum 7 days without further handling. The observed body temperature rises were less than 1.5 °C on average and not higher than 2.0 °C in individual animals. Temperatures returned to normal within 24 hours. No other relevant statistically significant differences were observed for any of the analysed safety parameters. These results supported the laboratory safety studies by demonstrating the safety under field conditions in a larger number of gilts vaccinated with the basic vaccination schedule.

Based on the user safety assessment presented, the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

All substances included in the composition of this vaccine are listed in Table 1 of the Annex to Commission Regulation (EU) 37/2010 with a 'No MRL required' classification, or in the list of substances considered as not falling within the scope of Regulation (EC) No. 470/2009).

Consequently, a withdrawal period of zero days can be established.

Enteroporc Coli AC is not expected to pose a risk for the environment when used according to the SPC.

Part 4 – Efficacy

Introduction and general requirements

Enteroporc Coli AC is a combined, multivalent inactivated vaccine for passive immunisation of piglets by active immunisation of pregnant gilts and sows. Enteroporc Coli AC contains *E. coli* fimbrial antigens (F4ab, F4ac, F5 and F6), alpha and beta2 toxoids from *C. perfringens* type A and beta1 toxoid from *C. perfringens* type C.

The primary vaccination schedule is 2 ml i.m. at 5 weeks and 2 weeks before the expected date of farrowing, and revaccination is 2 ml i.m. at 2 weeks before the expected date of farrowing.

The revised indication *proposed is*:

For the passive immunisation of progeny by active immunisation of pregnant sows and gilts to reduce:

- *Clinical signs (severe diarrhoea) and mortality caused by Escherichia coli strains expressing the fimbrial adhesins F4ab, F4ac, F5 and F6*

- Clinical signs (diarrhoea during the first days of life) associated with *Clostridium perfringens* type A expressing alpha and beta2 toxins

- *Clinical signs and mortality associated with hemorrhagic and necrotising enteritis caused by Clostridium perfringens type C expressing beta1 toxin*

Onset of immunity (after uptake of colostrum):

E. coli F4ab, F4ac, F5, F6: within 12 hours after birth

C. perfringens type A and C: first day of life

Duration of immunity (after uptake of colostrum):

E. coli F4ab, F4ac, F5 and F6: first days of life

C. perfringens type A: 14 days of life

C. perfringens type C: 21 days of life

Efficacy was demonstrated in compliance with the European Directive 2001/82/EC (as amended by 2004/28/EC and Directive 2009/9/EC), and the European Pharmacopoeia (Ph. Eur.) chapter 5.2.7. as well as the following specific monographs and guidelines applicable to product:

- Ph. Eur. monograph 01/2017, 0962: "Neonatal piglet Colibacillosis vaccine, inactivated"
- Ph. Eur. monograph 04/2013, 0363: "Clostridium perfringens vaccine for veterinary use"
- Ph. Eur. monograph 04/2013, 50206: "Evaluation of safety of veterinary vaccines and immunosera"
- User safety risk assessment was conducted in accordance with EMEA/CVMP/IWP/5433/2006 – Guideline on User Safety of Immunological Veterinary Products
- Ecotoxicity was evaluated according to the CVMP guidance: "Environmental risk assessment for immunological veterinary medicinal products" (EMEA/CVMP/074/95, adopted by 26. July 1996)
- Requirements for MUMS products were evaluated in line with the "Guideline on data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets" (EMA/CVMP/IWP/123243/2006-Rev.3)

Following applicant's request, Enteroporc Coli AC was classified by EMA as MUMS/limited market with regard to the indication for *C. perfringens* only. The indication for *E. coli* is not considered MUMS/limited market. Consequently, MUMS data reductions, where applicable, can only be applied to the *C. perfringens components.*

Challenge models:

Specific challenge strains were used for the individual fimbrial adhesins and toxins in Enteroporc Coli AC. An overview of these challenge strains was provided.

With respect to the *E. coli* challenge, the challenge model was oral to piglets within 12 hours after birth. Challenge strain characteristics for F4ab was heterologous F4ab challenge strain (x-288/99) *E. coli* K88ab (F4ab) positive for STII and LT isolated in UK. Characteristics for F4ac were: heterologous F4ac challenge strain (IDT-Nr. 212/049) PCR positive for STb, LT, K88ac. For F5 the characteristics were: a heterologous F5 challenge strain (IDT-Nr. 213/009) PCR positive for STa, K99 (F5). For F6 the characteristics included a heterologous F6 challenge strain (P1667/01-3) PCR positive for estb, estap, fasA.

The oral *E. coli* challenge model was performed according to Ph. Eur. standards. The applicant developed an in-house challenge model, as the Ph. Eur. monograph 04/2013:0363 regarding *C. perfringens* Vaccine for Veterinary Use does not provide any guidance on this regard.

The clostridial challenge strains were administered by intraperitoneal (i.p.) route to 1-day old piglets. With respect to *C. perfringens* type C, the challenge strain was characterised as a sterile filtrate of a heterologous *C. perfringens* type C strain: No. MB-yellow-130 with beta1-toxin and alpha-toxin. For *C. perfringens* type A, the challenge strain was characterised as a toxin rich sterile filtrate of a heterologous *C. perfringens* type A strain: with beta2-toxin and alpha-toxin.

The intraperitoneal challenge models for *C. perfringens* were validated outside this dossier and were

questioned to be appropriate for use in the efficacy trials in order to mimic the natural conditions for infection. As a result, a major objection was raised to the applicant in order to defend their intraperitoneal in-house *C. perfringens* model, in particular with respect to *C. perfringens type A* (alpha- and beta2-toxins). A field trial using Enteroporc AC, reported the symptoms for *C. perfringens* type A as diarrhoea only. However, the applicant explained the difficulties in setting up oral challenge models. The applicant considered the i.p. challenge to be a "worst case" scenario. It is agreed that the intraperitoneal challenge model is well suited for *C. perfringens* type C challenge as the toxin has systemic effects. Intraperitoneal challenge is a poorer mirror of natural *C. perfringens* type A and beta2 effects in piglets, where the pathological effect of the toxins is considered to occur mainly in the intestines and to manifest as diarrhoea. It is, however, agreed that the intraperitoneal challenge model confirms *in-vivo* neutralisation of the toxin by specific antibodies.

Efficacy parameters and tests:

The onset and duration of immunity was established in experimental challenge tests. The establishment of protective antibody titres in colostrum against the vaccine antigens at onset and at duration of immunity were used as parameters for estimation of efficacy after booster vaccination of sows.

The serological tests performed were ELISAs, developed and validated to determine antibody titres against alpha toxin, beta1 toxin and beta2 toxin from *C. perfringens*. ELISAs for determination of antibodies against *E. coli* fimbrial antigens F4ab, F4ac, F5 and F6 in blood and colostrum were also developed and validated. For estimation of protective titres a ROC (Receiver Operating Characteristic) analysis was used. The ROC analysis is a technique that can be used to evaluate the validity of a test with a continuous outcome and provide estimates of diagnostic sensitivities and specificities at different cut-off values.

A series of major objections were posed to the applicant concerning their chosen cut-off values for the protective antibody titres against the beta1 toxin for *C. perfringens* type C, alpha and beta2 toxin for *C. perfringens* type A; and for F4ab, F4ac, F5 *and* F6 fimbrial adhesins for *E. coli.*

A direct correlation has been documented between antibody titers and colostrum of sows on one side, and on the other side biologically relevant results from experimental studies of protection afforded in piglets after uptake of colostrum from vaccinated sows. Robust documentation behind "protective titres" is a crucial point, as all the estimated colostral antibody cut-off values are based on the established correlation between level of colostral antibodies and protection against clinical symptoms in piglets.

Although there are reservations about the ROC methodology to set the presented cut-off values concerning protective antibody titers in sow serum and colostrum, it is acknowledged that the cut-off values are a reasonable parameter correlated to biological function of the antibodies and clinical protection in challenge studies after passive immunisation of piglets (colostrum uptake). Thus a significant protection (against mortality for *E. coli* fimbrial antigens and *C. perfringens* type C toxin and morbidity for *C. perfringens* type A) has been documented in piglets from gilts with colostral antibody levels above the cut-off values. The CVMP agreed that the data available are considered sufficient to support the efficacy of the vaccine.

Efficacy documentation

The efficacy of Enteroporc Coli AC was documented in seven pivotal laboratory efficacy studies and three field safety/efficacy studies. In addition, colostrum samples from two laboratory efficacy studies beta1 efficacy basic vaccination and beta1 efficacy booster vaccination were used to analyse efficacy for the alpha and beta2 toxin component as well as for the *E. coli* derived F4ab, F4ac, F5 and F6 vaccine components.

In total six reports have been presented concerning determination of biologically relevant level of protective titre for the antigens included in Enteroporc Coli AC. No report was submitted regarding protective titre for CpA-beta2 toxin, but this issue was addressed during evaluation.

Three safety/efficacy field studies were submitted in order to support results obtained in the laboratory efficacy studies. Laboratory and field studies were carried out with production batches containing minimum potency or medium potency.

An overview of the laboratory efficacy studies is presented below:

Study title
Efficacy of Enteroporc Coli AC in gilts for protection of piglets against challenge with alpha and beta2 toxins of *C. perfringens* type A
Efficacy of Enteroporc Coli AC in gilts for protection of piglets against challenge with beta1 toxin of *C. perfringens* type C
Efficacy of Enteroporc Coli AC in gilts for protection of piglets against challenge infection with *E. coli F4ab*
Efficacy of Enteroporc Coli AC in gilts for protection of piglets against challenge infection with *E. coli F4ac*
Efficacy of Enteroporc Coli AC in gilts for protection of piglets against challenge infection with *E. coli F5*
Efficacy of Enteroporc Coli AC in gilts for protection of piglets against challenge infection with *E. coli F6*
Efficacy of the *C. perfringens* type C component after booster vaccination of sows as determined by antibody titres in colostrum
Efficacy of the *C. perfringens* type A component after booster vaccination of sows as determined by antibody titres in colostrum
Efficacy of the fimbrial antigens F4ab, F4ac, F5, F6 after booster vaccination of sows as determined by antibody titres in colostrum

Dose determination

No data were provided in this dossier with respect to determination of the vaccine dose for *C. perfringens* type A and C. Antigen amounts were comparable to those of the authorised MUMS vaccine Enteroporc AC. No dose determination studies were submitted for *E. coli* fimbrial antigen F4ab. The proposed dose for E. coli F4ac was investigated in a study where two potential doses were investigated (19 rU/ml and 32 rU/ml). The minimum dose was set at 19 rU/ml.

E. coli F5 fimbrial antigen was investigated in a second study where two potential doses were investigated (13 rU/ml and 35 rU/ml). The minimum dose was set at 13 rU/ml. The proposed dose for *E. coli* F6 fimbrial antigen was investigated in third study where two potential doses were investigated (21 rU/ml and 37 rU/ml). The minimum dose was set at 37 rU/ml.

Onset of protection and duration of immunity – C. perfringens

Two studies were carried out in piglets one day of age to investigate the onset (OOI) and duration of protection (DOI) for beta1 toxin from *C. perfringens* type C and for alpha/beta2 toxins of *C. perfringens* type A.

Study for beta1 toxin from *C. perfringens* type C: 20 pregnant gilts (10 vaccinates and 10 controls), 12 months at 1st vaccination, beta-1 antibody level ≤0.02 AU/ml were vaccinated 5 and 2 weeks before expected farrowing with Enteroporc AC lyophilisate, batch VM0051116 suspended in Enteroporc COLI, batch LM0170717 (adjusted to minimum beta1-toxoid dose: 3354 rU/ml). In total 40 piglets from these gilts (2 from each gilt) were challenged i.p. at 1 day of age (OOI) and at 21 days of age at challenge 2 (DOI). Morbidity (sum of clinical score) and mortality in piglets post challenge was investigated. Results showed that a significant reduction of morbidity in piglets from vaccinated gilts was seen after OOI challenge, with a rate of 25% sick piglets per gilt in the IVP group versus 70% in the CP group. All sick piglets died or were euthanized, therefore the morbidity rate was equal to the mortality rate. For the DOI, results showed that all piglets from vaccinated gilts (10) had a reduced morbidity while none of the piglets from non-vaccinated gilts expressed reduced morbidity. The applicant stated that Enteroporc Coli AC with a minimum beta1-toxoid titre significantly reduced mortality and signs of disease related to *C. perfringens* type C beta1-toxin in piglets with an onset of immunity at 1st day of life with a duration of immunity of at least 21 days.

Study for alpha/beta2 toxins of *C. perfringens* type A: 25 pregnant gilts, 11 months at 1st vaccination, alpha and beta-2 antibody level ≤0.02 AU/ml were vaccinated 5 and 2 weeks before expected farrowing with Enteroporc AC lyophilisate, batch VM0051116 suspended in Enteroporc COLI, batch LM0170717 (adjusted to minimum beta2-toxoid dose: 125 rU/ml). In total 42 piglets from these gilts (12 vaccinated gilts and 9 controls were included) were challenged at 1 day of age (OOI) and at 22-23 days of age at challenge (DOI). Morbidity and mortality in piglets post challenge was investigated. Results showed that a significant reduction of morbidity in piglets from vaccinated gilts was seen after OOI challenge. A significant higher sum of clinical score was seen in the control (CP) group 6 hours after OOI challenge (1 day of age) compared to piglets from the vaccinated (IVP) group with means of 6,4 (CP) versus 2,2 (IVP). These numbers corresponded to signs of challenge related illness in 9 piglets out of 24 IVP piglets (37.5%) and 14 piglets out of 18 control piglets (78%).

The mean number of sick piglets per gilt after DOI challenge (22-23 days of age) showed no significant differences between piglets from vaccinated gilts and piglets from non-vaccinated controls. In the CP group 5.7 piglets per gilt showed clinical signs, compared to 3,8 sick piglets per gilt in the IVP group. The result may be clinically relevant but the difference is non-significant. In numbers, 15 out of 24 IVP piglets (63%) and 13 out of 18 CP piglets (72%) showed clinical signs 6 hour after challenge. A duration of immunity could not be established on the basis of the provided study. The applicant gave information on a new ongoing DOI study, which was presented later in the procedure.

In this study, 12 pregnant gilts were vaccinated with Enteroporc Coli AC at 5 and 2 weeks before farrowing (IVP group). The vaccine batch was adjusted to the minimum dose of 125 rU/ml of alpha toxoid. The control group (CP), also consisting of 12 gilts, received Enteroporc Coli alone. Challenge was performed at 14 days of age (in contrast to 21 days in the original experiment) in two piglets per gilt. The clinical outcome after challenge was evaluated with a clinical score on a scale from 0 to 6 per piglet.

A "sum of clinical score" was calculated for each gilt, and the gilt was considered the statistical unit. The mean clinical score in the IVP group (mean=3.6, n=12) was lower than in the CP group (mean=6.8, n=11). The difference between groups was not significant (p=0.1306). With regard to mortality, 8 out of 24 piglets (33%) of the IVP group and 13 out of 22 (59%) of the control piglets died after challenge (p=0.1294 NS).

The applicant proceeded to perform a combined analysis of the two CpA DoI studies. The report is named "Meta-analysis of morbidity and mortality of studies -ref- and -ref- to determine the duration of immunity following CpA challenge". The presented analysis showed a significant effect of vaccination on clinical scores (IVP mean 3.7, CP mean 6.3, Wilcoxon Mann-Whitney test, p=0.0342), but no significant effect on mortality (p=0.0622). The approach of merging the two datasets may be questionable due to different ages at challenge (21 vs. 14 days) and different study populations, and a question was raised to the applicant.

The applicant also performed a formal meta-analysis on the two studies, both after considering the litter as the experimental unit and after using the individual piglet as the experimental unit. Statistically significant differences could be demonstrated for morbidity at litter level (p=0.0369). For mortality, the effect did not reach statistical significance (p=0.0677). However, when using piglet as the observational unit, a higher power is obtained. In that case and a statistically significant effect of the vaccine was confirmed for morbidity (p=0.0433) and was also demonstrated for mortality (p=0.0324). The claim for duration of immunity was changed from 21 days to 14, to conform with the lower duration of the two studies.

Booster vaccination and determination of colostral antibodies – C. perfringens

Analyses of colostrum samples from 13 sows after farrowing was provided with respect to beta1 toxin and alpha toxin. Two studies were included respectively. Results were used to support a protective titre for the clostridial toxins included in Enteroporc Coli AC after booster vaccination.

With regard to *C. perfringens* type A alpha toxin, the study report included the findings of a study in gilts and in sows where colostral protective antibody titres against alpha-toxin are summarised. A significant higher percentage of vaccinated (IVP) gilts presented with protective titre ($\geqslant$ 1.53 AU/ml) compared to controls (i.e. after basis vaccination), which was also the case in the booster vaccinated sows.

The effect of booster vaccination against the *C. perfringens* type C beta1 toxin was described in a study. The percentage of sows with colostral antibody titre the protective titre was significantly higher in the IVP group than in the control group. Furthermore, the mean beta1-toxin antibody titre was found significantly higher in the IVP group compared to the control group after booster vaccination with a mean of 10.7851 AU/ml in the IVP group, which was also significantly higher than following basis vaccination (5.6015 AU/ml).

Assuming a correlation between protection and titres above the cut-off values, the findings support efficacy after booster vaccination.

Onset of protection and duration of immunity – E. coli

In total four studies were carried out in piglets less than 12 hours of age to investigate the onset of protection. No duration of protection studies were carried out.

Study for F4ab: In total 16 gilts (8 vaccinates and 8 controls) and 48 piglets (6 per gilt) were included in this OOI study. The gilts were vaccinated 5 and 2 weeks before expected farrowing with Enteroporc AC lyophilisate, batch VM0051116 suspended in Enteroporc COLI, batch LM0170717 (minimum F4ab antigen content of 23 rU/ml). The primary efficacy results showed the following:

The mean diarrhoea score sum in vaccinates (IVP) were significantly lower compared to control piglets (14.2 and 23.6 respectively) after an eight-day evaluation period post challenge.

Thirty-seven (37) out of 45 IVP piglets and all (47) control piglets were affected from soft feces or diarrhea (clinical score ≥ 1) on at least one day of the observation period. The mortality rate was significantly smaller for vaccinates (26 of 45 piglets, 58%) as compared to control piglets (100%).

The mean colostral F4ab antibody content of vaccinated gilts was 1062.5 rel. OD%, with a minimum of 486 rel. OD% in the gilt with lowest antibody content. No gilts in the control group reached an antibody content above 7 rel. OD% in colostrum on the day of farrowing, which was significantly lower compared to the vaccinates with p=0.0002.

According to the Ph. Eur. Monograph 0962, the challenge test was proven valid with a morbidity as well as a mortality rate of 100% in the control group (Ph. Eur. Requirement: at least 40% mortality and 85% morbidity). Significantly less piglets from the vaccinated gilts became sick or died as a result of the challenge. Control gilts were sero-negative before and after basis (placebo) vaccination. Vaccinated gilts were all tested sero-positive after vaccination with high content of antibodies against F4ab in serum as well as colostrum.
Based on the above results, it could be concluded that vaccination with Enteroporc Coli AC at a minimum potency of *Escherichia coli* F4ab antigen protected piglets against a severe F4ab challenge infection (worst-case scenario) after colostrum uptake and 12 hours after birth.

Study for F4ac: In total 24 gilts were included hereof 8 vaccinated with an Enteroporc Coli AC batch containing 32rU/ml and 8 gilts with 19 rU/ml F4ac antigen, respectively while 8 gilts served as non-vaccinated controls. The two batches included were IVP1: Enteroporc AC lyophilisate, batch VM0051116 suspended in Enteroporc COLI, batch LM0170717 (F4ac antigen content of 32 rU/ml) IVP2: Enteroporc AC lyophilisate, batch VM0051116 suspended in Enteroporc COLI, batch LM0180717 (F4ac antigen content of 19 rU/ml). A total of 45 piglets were included from IVP1 gilts, 36 piglets from IVP2 gilts and 39 piglets from non-vaccinated gilts. The primary efficacy results showed the following:

The diarrhoea score sum in vaccinates (IVP) were significantly lower compared to control piglets after the eight-day evaluation period post challenge, with mean scores of 17.7, 16.6 and 23.2 in the IVP1, IVP2 and CP group respectively.

A mortality rate of 100% was seen in the control group (36 of 36 piglets died), whereas a significantly smaller percentage of vaccinates died due to challenge (76% IVP1 piglets and 69% IVP2 piglets).

The mean colostral F4ac antibody content of vaccinated gilts was 649.6 and 754.8 rel. OD% in the IVP1 and IVP2 group, respectively. No gilts in the control group reached an antibody content above 31 rel. OD% in colostrum on the day of farrowing, which generated a group mean of 15.3 rel. OD%. The difference between the IVP groups and the control group was significant with p=0.0002 in both cases.

It was accepted that Enteroporc Coli AC with F4ac potencies of 19 rU/ml (IVP2) and 32 rU/ml (IVP1) significantly reduced mortality and signs of disease related to *E. coli* F4ac infection in piglets during the first days of life. As no relevant differences in efficacy results were identified between IVP1 and IVP2 the minimum dose for F4ac in Enteroporc Coli AC was set at 19 rU/ml. An appropriate onset of immunity is stated in the SPC, no duration of immunity study was submitted.

Study for F5: In total 24 gilts were included hereof 8 vaccinated with an Enteroporc Coli AC batch containing 13 rU/ml and 8 gilts with 35 rU/ml F5 antigen, respectively while 8 gilts served as non-

vaccinated controls. The two batches included were IVP1: Enteroporc AC lyophilisate, batch VM0051116 suspended in Enteroporc COLI, batch LM0170717 (F5 antigen content of 13 rU/ml) and IVP2: Enteroporc AC lyophilisate, batch VM0051116 suspended in Enteroporc COLI, batch LM0180717 (F5 antigen content of 35 rU/ml). A total of 49 piglets were included from IVP1 gilts, 42 piglets from IVP2 gilts and 47 piglets from non-vaccinated gilts. The primary efficacy results showed the following:

Significantly lower diarrhoea scores were obtained in the IVP groups (piglets from vaccinated dams) after the eight-day evaluation period post challenge, compared to the control (CP) group, with mean diarrhoea scores of 12.3, 14.7 and 20.7 in the IVP1, IVP2 and CP group respectively.

In total 39 of 44 control piglets died due to challenge (corresponding a mortality rate of 89%), whereas a significantly smaller percentage of vaccinates died due to challenge (45% IVP1 piglets and 56 % IVP2 piglets).

On the day of farrowing, the mean F5 antibody content in colostrum of vaccinated gilts was 182.5 and 227.8 rel. OD% in the IVP1 and IVP2 group. No gilts in the control group reached an antibody content above 18 rel. OD%, which generated a group mean of 8.4 rel. OD%, The difference between the IVP groups and the CP group was significant with $P \leq 0.001$ in both cases.

It was concluded that Enteroporc Coli AC with a F5 potency of 13 rU/ml (IVP1) and 35 rU/ml (IVP2) significantly reduced mortality and signs of disease related to *E. coli* F5 infection in piglets during the first days of life. As no relevant difference in efficacy was seen between IVP1 and IVP2, the minimum dose for F5 in Enteroporc Coli AC was set as 13 rU/ml.

Study for F6: In total 22 gilts were included hereof 8 vaccinated with an Enteroporc Coli AC batch containing 21 rU/ml and 7 gilts with 37 rU/ml F5 antigen, respectively while 7 gilts served as non-vaccinated controls. The two batches included were IVP1: Enteroporc AC lyophilisate, batch VM0051116 suspended in Enteroporc COLI, batch LM0170717 (F6 antigen content of 37 rU/ml) and IVP2: Enteroporc AC lyophilisate, batch VM0051116 suspended in Enteroporc COLI, batch LM0180717 (F6 antigen content of 21 rU/ml). A total of 39 piglets were included from IVP1 gilts, 27 piglets from IVP2 gilts and 23 piglets from non-vaccinated gilts. The primary efficacy results showed the following:

All piglets from the three treatment groups developed diarrhoea in varying degree within the 8 days after challenge, which resulted in a non-significant difference in diarrhoea sum score between the IVP2 and the control group. However, the difference between the IVP1 and the control group was significant with mean sum scores of 6.2 in the IVP1 group and 21.1 in the CP group.

In total 20 of 22 control piglets died during the observation period, corresponding a mortality rate of 91%, whereas a significantly smaller percentage of vaccinates died after challenge (16 % IVP1 piglets and 46% IVP2 piglets).

The mean colostral F6 antibody content of vaccinated gilts was 144.3 and 38.1 rel. OD% in the IVP1 and IVP2 group, respectively. No gilts in the control group reached an antibody content >6 rel. OD% in colostrum on the day of farrowing, which generated a group mean of 6 rel. OD%. A significant difference was found when testing the IVP groups against the control group.

It was concluded that as 100% of the control animals died, the challenge infection was considered valid. As only IVP1 reduced morbidity and mortality significantly in piglets during the first days of life Enteroporc Coli AC with the higher F6 potency (37 rU/ml (IVP1)) was selected as F6 minimum dose. No duration of immunity studies were submitted.

In line with Ph. Eur. Monograph 0962, challenge infection with respect to *E. coli* was performed within 12 hours after birth (after uptake of colostrum).

Booster vaccination and determination of colostral antibodies – E. coli

One study revealed the colostral antibody levels in sows after booster vaccination. The same 13 sows as used in the analysis for booster vaccination of sows with C. perfringens antigens were also used in detection of colostral antibodies for E. coli antigens. The results showed that booster vaccination of sows was at least as efficient as basic vaccination of gilts with respect to rising levels of colostral antibodies.

Assuming a correlation between protection and titres above the cut-off values, the findings support efficacy after booster vaccination.

Maternally derived antibodies (MDA) *E. coli* and *C. perfringens*

The vaccine is intended for administration to gilts/sows, both target groups being of an age where maternally derived antibodies are no longer present. This is supported by the fact that most gilts in the three field studies showed no detectable levels of antibodies at the time of vaccination. When comparing these seronegative animals with those gilts that had detectable levels of antibodies at the time of vaccination, no relevant difference in immune response on piglets was noted.

Field trials

An overview of the field efficacy studies is presented below.

Study title
Field study to test the safety in gilts after a basic (two-fold) administration of a single dose of Enteroporc Coli AC and the efficacy in their offspring in terms of morbidity
Field study to test the safety in gilts after two-time administration of a single dose of Enteroporc Coli AC and the efficacy in terms of antibody titre in colostrum
Field study to test the safety in gilts after two-time administration of a single dose of Enteroporc Coli AC and the efficacy in terms of antibody titre in colostrum

Three field studies were submitted in order to support both safety and efficacy laboratory studies. The vaccine batches were the same in all three farms and contained an intermediate content of the *C. perfringens* Type A toxoids (396 rU/ml alpha toxoid, 5428 rU/ml beta1 toxoid and 2515 rU/ml beta2 toxoid). The *E. coli* fimbria adhesins were blended at a fixed antigen content of 100 rU/ml for F4ab, F4ac, F6 and 120 rU/ml for F5 as used for commercial vaccine production.

Study 1: Vaccine efficacy was evaluated in terms of morbidity (diarrhoea score sum ≥2) in piglets during the first 8 days after birth (primary parameter). Blood samples (secondary parameter) were taken from all gilts on the day before first vaccination (D-1) as well as 2 days before the calculated date of farrowing (D35). Colostrum sampling (secondary parameter) was performed on D30-D42 (on day of farrowing). Clinical score in piglets was evaluated from birth until day 8 of life.

These field results showed no difference in morbidity of piglets (average 33%) originating from vaccinated or non-vaccinated gilts. Analyses of antibody titres (colostrum) showed that a median antibody content in colostrum from gilts was significantly higher in vaccinates than in controls ($p<0.0001$

for all tested antigens). In the group of vaccinated gilts, the proportion of gilts with colostral titres above the cut-off levels ranged between 52% (alpha toxin) and 98% (F4ab, F4ac). The applicant election of this farm for their field study did not seem to be a good choice, as too many other important pathogens causing neonatal infection in piglets also were present besides problems with mastitis-metritis-agalactia (MMA) and outbreak of pleuropneumonia infection in gilts at the same time.

Study 2: This farm had a good health status and efficacy parameters were evaluated in terms of percentage of gilts with a protective titre in colostrum samples equal to or above the respective protective titre against F4ab, F4ac, F5, F6, alpha toxin and beta1 toxin (primary efficacy parameter). In addition, blood samples were taken from all gilts on day -14, day -1, and day 35. All samples were analysed using ELISA for levels of antibodies against *E. coli* fimbriae F4ab, F4ac, F5 and F6 as well as against alpha, beta1 and beta2 toxins of *Clostridium perfringens* types A and C. The health status of the study animals was observed daily during the study. Pivotal results showed that the percentage of vaccinated gilts with a protective titre in colostrum was statistical significantly higher than in control gilts ($p<0.0001$ for all antigens). None of the controls expressed antibody titres equal to or above the protective titre. In vaccinates the percentage of gilts with protective antibody titres in colostrum ranged between 57% (F6) and 93% (F4ab, F4ac).

Study 3: This farm had a good health status. The efficacy was evaluated in terms of percentage of gilts with a protective titre in colostrum samples equal to or above the respective protective titre against F4ab, F4ac, F5, F6, alpha toxin and beta1 toxin (primary efficacy parameter). In addition, blood samples were taken from all gilts on day -13, -1, and day 35. All samples were analysed using ELISA for levels of antibodies against *E. coli* fimbriae F4ab, F4ac, F5 and F6 as well as against alpha, beta1 and beta2 toxins of *Clostridium perfringens* types A and C. The health status of the study animals was observed daily during the study. Pivotal results showed that the percentage of vaccinated gilts with a protective titre was statistical significantly higher in vaccinates than in the controls ($p<0.0001$ for all antigens). None of the gilts from the control group had antibody titres in colostrum equal to or above the protective titre. In vaccinates the percentage of gilts with a protective antibody titre ranged between 70% (alpha toxin) and 96% (F4ac, beta1 toxin).

The presented negatively controlled field study, **IDT15 ENTEROPORC AC FSE 01** (from a DCP Enteroporc AC dossier) supports that clinical manifestations of diarrhoea can be related to *C. perfringens* type A expressing alpha and beta2 toxins in neonatal piglets. This field study aimed to test the safety and efficacy of the repeated administration of a single dose of the vaccine, ENTEROPORC AC. This vaccine contains alpha, beta1 and beta2 toxoids of *Clostridium perfringens* types A and C as active substances, which are comparable to those of Enteroporc COLI AC of the present application, while the adjuvant system is different from that of the Enteroporc COLI AC vaccine of this application.

The study was a GCP compliant, randomised, blinded and placebo-controlled field study carried out in commercial farm with problems of neonatal diarrhoea (associated with CpA). CpC was not detected in the herd. In order to prevent the suckling piglets from diarrheal infections the sows were vaccinated against *E. coli*.

Two farrowing groups (n=35 gilts), seronegative for alpha/beta1/beta2-toxins were enrolled, Group1: IVP: 2 ml of ENTEROPORC AC (Batch-No.: VM 0010714, Antigen content: alpha toxoid: 569 rE/ml, beta2 Toxoid: 2771 rE/ml); group 2, CP: 2 ml of physiological saline 5 and 2 weeks pre-farrowing.

For evaluation of the efficacy of the vaccine serum and colostrum were analysed for antibodies against alpha-, beta1- and beta2-toxin at time of farrowing. Furthermore, all 474 included suckling piglets were continuously examined for general condition including diarrhea (+/-). Weight gain of the piglets was compared as well as losses of viable piglets during the entire suckling period.

The results showed that most cases of diarrhoea occurred until the age of 5 days. During this time there were twice as many piglets with diarrhoea from unvaccinated sows than piglets from vaccinated sows. At the age of 2 – 5 days and at the average age of 7 days, the differences between the groups were significant. During the entire suckling period a total of 38.7% piglets from vaccinated sows had diarrhoea as compared to 62.6% piglets from unvaccinated sows. This difference is highly significant ($p<0.001$). Mortality did not differ between groups and was generally low.

The overall findings in this study with a similar clostridial vaccine from the same manufacturer showed that passive colostral immunisation against *C.* perfringens type A in the field was associated with reduced clinical signs, i.e. a reduced incidence of neonatal diarrhoea.

Overall conclusion on efficacy

The efficacy of Enteroporc Coli AC was documented in seven laboratory efficacy studies and three field safety/efficacy studies. In addition, colostrum samples from two laboratory efficacy studies, beta1 efficacy, basic vaccination, and beta1 efficacy, booster vaccination, were used to analyse efficacy for the alpha and beta2 toxin component as well as the *E. coli* derived F4ab, F4ac, F5 and F6 vaccine components. An additional field study from the DCP application for Enteroporc AC (IDT15 ENTEROPORC AC FSE 01) was included in the assessment.

For *C. perfringens* derived alpha, beta1 and beta2 toxoids the findings supported protection against clinical signs and mortality caused by *C. perfringens* type C as well as clinical signs (diarrhoea) caused by *C. perfringens* type A:

- The minimum immunising doses were derived from studies for registration of the MUMS product Enteroporc AC and no new studies were provided.

The challenge models for the clostridial antigens were not carried out by oral administration as would have been preferred for this neonatal disease in piglets. While it is accepted that the model mimics the pathogenic effect of the beta1 toxin of *C. perfringens* type C as this toxin is known to exert a pronounced systemic effect, intraperitoneal challenge is a poorer mirror of natural *C. perfringens* type A and beta2 effects in piglets, where the pathological effect of the toxins is considered to occur mainly in the intestines and to manifest as diarrhoea. It is, however, agreed that the intraperitoneal challenge model confirms *in-vivo* neutralisation of the toxin by specific antibodies.

- For the alpha and beta2 toxins of *C. perfringens* type A the model is not mimicking the natural route of exposure and pathogenesis to the same degree, as these toxins are assumed to exert their effect mainly locally in the intestine. However, the difficulty in establishing a reproduceable oral model is acknowledged.
- Onset of immunity after uptake of colostrum was demonstrated for both type A and type C toxins in experimental challenge studies.
- Duration of immunity was demonstrated for type C toxin for 21 days and for type A toxins for 14 days. The latter was demonstrated in a meta-analysis of two studies.
- The effect of booster vaccination was demonstrated by determining significantly higher proportions of booster vaccinated sows with colostral titres above the cut-off values ("protective titers"). Correlation between colostral titres and mortality as well as morbidity was accepted for type C challenge. For type A the clinical signs are considered to be primarily limited to diarrhoea (confer with field study IDT 15). It is accepted that experimental type A i.p. challenge may lead to mortality, but this should be considered a demonstration of toxin neutralisation only and does not necessarily indicate protection against type A mortality in the field. Thus, the type A claim should be limited to reduction of diarrhoea.

- Field efficacy was demonstrated by use of the biological parameter of colostral antibody titres. A study from the Enteroporc AC dossier indicated that vaccination protected against neonatal diarrhoea associated with *C. perfringens* type A encoding alpha and beta2 toxins.

With regard to *E. coli* strains expressing the fimbrial adhesins F4ab, F4ac, F5 and F6 protection against clinical signs (severe diarrhoea) and mortality was supported:

- Onset of immunity was documented with respect to all *E. coli* antigens, and no duration of immunity studies were submitted for those. The claim for protection is stated as "first days of life".
- For *E. coli* derived fimbria adhesins F4ac, F5 and F6 the minimum dose was evaluated by testing two different vaccine doses for each of the respective antigens in course of the immunogenicity testing of Enteroporc Coli AC according to Ph. Eur. 0962. For F4ab only one dose was evaluated.

In total six reports were submitted calculating a level of protective titre for the antigens included in Enteroporc Coli AC (F4ab, F4ac, F5, F6, CpA-alpha and CpC-beta1). A report was submitted regarding protective titre for CpA-beta2 toxin with the response to the list of outstanding issues.

Three safety and efficacy field studies were submitted in order to support results obtained in the laboratory efficacy studies. An additional field study (IDT15 ENTEROPORC AC FSE 01) from the 2016 DCP procedure on Enteroporc AC was considered concerning neonatal diarrhoea associated with *C. perfringens* type A expressing alpha and beta2 toxins and effect of passive immunisation.

Part 5 – Benefit-risk assessment

Introduction

Enteroporc Coli AC is a recombinant, combined multivalent vaccine containing *E. coli* fimbrial antigens (F4ab, F4ac, F5 and F6) and *C. perfringens* antigens (alpha, beta1 and beta2 toxins). The vaccine is inactivated and intended to provide passive immunity to progeny by active immunisation of pregnant gilts and sows. The active substance is presented as a lyophilisate and a suspension. The route of administration is intramuscularly.

The product is intended for use in pregnant gilts as a basic vaccination (2 ml) at 5 and 2 weeks before expected farrowing. A booster vaccination in sows is scheduled at 2 weeks before expected farrowing. The proposed route of administration, and vaccination scheme has been confirmed.

Enteroporc Coli AC contains alpha and beta2 toxins of *C. perfringens* type A in addition to relevant *E. coli* fimbrial antigens as well as beta1 toxin of *C. perfringens* type C. Enteroporc Coli AC provides protection against relevant strains of *E. coli* and *C. perfringens* type A and C associated neonatal diarrhoea in piglets.

The application has been submitted in accordance with Article 3(1) – Indent 1 – Biotech medicinal product of Regulation (EC) No 726/2004 (mandatory scope), as it is a product developed by means of a biotechnological process. The dossier is submitted as a full application. The lyophilisate component of Enteroporc Coli AC, is already marketed as a separate MUMS product, (Enteroporc AC). Enteroporc COLI AC has been classified by EMA (EMA/238010/2018) as MUMS/limited market product with regard to the *C. perfringens* components only.

Benefit assessment

Direct therapeutic benefit

Enteroporc Coli AC has a potential to be of value in the treatment of neonatal piglet diarrhoea, which is of major importance worldwide. The proposed benefit of Enteroporc Coli AC is its broad range of efficacy, which was investigated in a large number of laboratory and field studies conducted mainly to an acceptable standard. Onset of immunity and duration of immunity were determined after experimental challenge, whereas the efficacy of a booster vaccination was determined indirectly through colostral titres in the gilts/sows.

A total of seven laboratory studies were conducted in accordance with GLP (2 safety studies) and three field safety/efficacy studies according to GCP. In addition, colostrum samples from two laboratory studies were used to analyse efficacy after booster vaccination of sows.

For *C. perfringens* derived alpha, beta1 and beta2 toxins, the minimum immunising dose was already determined during the pivotal studies for the MUMS product Enteroporc AC. The used in-house challenge model is administered by intraperitoneal route. The intraperitoneal challenge route is most relevant for the beta1 toxin, which has a systemic effect, but the i.p. model does not provide a direct link between experimental and clinical protection for alpha and beta2 toxins, which have mainly local effects. It is, however, accepted that the model shows toxin neutralisation, and that a suitable model for oral challenges was not available.

The onset of immunity is set at 12 hours for *E. coli* and at one day for the *C. perfringens* components, in both cases the onset is after uptake of colostrum. The duration of protection is not determined for the *E. coli* fimbrial antigens. For the beta1 toxin of *C. perfringens* type C, a duration of immunity of 21 days was demonstrated, and a duration of immunity of 14 days was demonstrated in a meta-analysis of two *C. perfringens* type A challenge studies.

Three clinical safety/efficacy trials were conducted in accordance with GCP. No safety concerns were identified. The level of reduction of clinical signs in piglets on a herd basis was demonstrated indirectly through the detection of colostral titres. For *C. perfringens* type A, a field study with the similar vaccine Enteroporc AC from the same company demonstrated that passive colostral immunisation was associated with protection against neonatal diarrhoea.

Additional benefits

Enteroporc Coli AC is easy to apply by the veterinarian/owner as it reduces the number of breeder vaccinations.

Enteroporc Coli AC potentially increases the range of available treatment possibilities for a broad range of piglet neonatal diarrhoea toxins.

Risk assessment

Quality:

Overall the quality part of the dossier is detailed and complies with relevant monographs and guidelines. Compliance with Ph. Eur monographs 0062 *Vaccines for veterinary use,* 0363 *Clostridium perfringens vaccine for veterinary use,* and 0962 *Neonatal piglet colibacillosis vaccine (inactivated)* is generally considered demonstrated.

Safety:

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

Risks for the target animal:

Administration of Enteroporc Coli AC in accordance with SPC recommendations is generally well tolerated. The main reported adverse reactions include a very common transient rise in rectal temperature (mean 0.5°C, in individual pigs up to 2°C) returning to normal within 24 hours. Transient local swellings at the injection site were also very commonly observed but they resolved without treatment within a week. A slightly depressed behaviour was commonly observed on the day of administration. The safety of Enteroporc Coli AC in gilts and sows was regarded as confirmed, and correctly reflected in the proposed SPC.

Risk for the user:

The CVMP concluded that user safety for this product is acceptable when used according to the SPC recommendations. Standard safety advice is included in the SPC.

Risk for the environment:

Enteroporc Coli AC is not expected to pose a risk for the environment when used according to the SPC recommendations. Standard advice on waste disposal is included in the SPC.

Risk for the consumer:

No concerns have been raised for related to consumer safety.

Special risks:

No special risks have been identified.

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 the following indication:

For the passive immunisation of progeny by active immunisation of pregnant sows and gilts to reduce:

- Clinical signs (severe diarrhoea) and mortality caused by *Escherichia coli* strains expressing the fimbrial adhesins F4ab, F4ac, F5 and F6

- Clinical signs (diarrhoea during the first days of life) associated with *Clostridium perfringens* type A expressing alpha and beta2 toxins

- Clinical signs and mortality associated with haemorrhagic and necrotising enteritis caused by *Clostridium perfringens* type C expressing beta1 toxin

Onset of immunity (after uptake of colostrum):

- *E. coli* F4ab, F4ac, F5, F6: within 12 hours after birth

- *C. perfringens* type A and C: First day of life

Duration of immunity (after uptake of colostrum):

- *E. coli* F4ab, F4ac, F5 and F6: first days of life

- *C. perfringens* Type A: 14 days of life

- *C. perfringens* Type C: 21 days of life

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 animals and presents an acceptable risk for users and the environment when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information.

Based on the data presented, the overall benefit-risk is considered positive.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for **Enteroporc Coli AC** 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).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above-mentioned medicinal product.