EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

26 November 2020
EMA/640978/2020
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Enteroporc Coli (EMEA/V/C/005148/0000)

Vaccine common name: Neonatal piglet colibacillosis vaccine (recombinant, inactivated)

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

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Introduction

On 5 November 2020, the CVMP adopted an opinion and CVMP assessment report.

On 6 January 2021, the European Commission adopted a Commission Decision granting the marketing authorisation for Enteroporc Coli.

The applicant IDT Biologika GmbH submitted on 16 May 2019 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Enteroporc Coli, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

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

Enteroporc Coli is a fall-out formulation of the combined vaccine Enteroporc Coli AC for which a marketing authorisation has been applied for in parallel. Enteroporc Coli AC consists of the suspension Enteroporc Coli containing F4ab, F4ac, F5 and F6 antigens of *E. coli* and the lyophilisate Enteroporc AC containing alpha and beta2 toxoids of *C. perfringens* type A and beta 1 toxoid of *C. perfringens* type C. The active ingredients and the adjuvant of Enteroporc Coli are identical to those contained in Enteroporc Coli AC, only the number of active substances is decreased by deleting the lyophilisate with the toxoids of *C. perfringens* type A/C.

The applicant applied 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.

Onset of immunity: within 12 hours after birth

Duration of immunity: first days of life

The product is intended for administration by intramuscular use.

Enteroporc Coli is presented in packs containing 1 vial of 10 or 25 doses of vaccine.

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.

Marketing authorisation under exceptional circumstances

Not applicable.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

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

Manufacturer of the active substance and responsible for batch release is IDT Biologika GmbH.

For IDT Biologika GmbH manufacturing site, manufacturing authorisation was issued and a valid GMP compliance certificate was provided.

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.

The GMP status of the active substance(s) and of the finished product manufacturing sites has been satisfactorily established and are 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 is a 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.

The vaccine is presented as suspension containing *E. coli* fimbrial adhesins F4ab, F4ac, F5 and F6, and aluminium hydroxide as adjuvant. Other ingredients are buffered saline solution as described in section

6.1 of SPC. One vaccine dose corresponds to 2 ml.

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

The composition of the vaccine is adequately described.

Containers and closure

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- or 50-ml PET containers (Ph. Eur. 3.2.2). Glass vials are sterilised and depyrogenated in a sterilisation tunnel at ≥220 °C for ≥16 min. PET containers are sterilised by gamma irradiation. Justification for the minimum dose including validation reports are 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

The Enteroporc Coli suspension 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 adhesin (212/176) 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 manufacturing 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 kinetic inactivation 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.

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. No preservative is added. The formulation of batches used during clinical studies is the same as that intended for marketing.

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* fimbrial 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 were 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.

Inactivation is carried out by adding formaldehyde solution to the supernatant up to a final concentration of 0.05% for *E. coli* F4ab, F4ac and F5 and up to a final concentration of 0.1% for F6. The culture is heated up to 36 ± 2 °C for *E. coli* F4ab, F4ac and F5 and at 25 ± 3 °C for F6. The cultures are incubated under stirring for a minimum of 51 hours for F4ab, minimum of 29 hours for F4ac, minimum of 24 hours for F5 and F6. 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.

Demonstration of consistency of production of three consecutive batches and validation of the holding times is presented. Critical manufacturing steps have been defined and process parameters have been 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 material 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 overall 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 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 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 2B. 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 should 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 an acceptable consistency of the manufacturing process of the *E. coli* fimbrial adhesin antigens and the final vaccine suspension.

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

A shelf life of 21 months at 2-8 °C is proposed. Stability data for six batches manufactured according to the process outlined in part 2B are presented. Two batches were produced at pilot-scale, the other batches are commercial batches manufactured at production scale. The batches were 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. A shelf life of 21 months is considered acceptable.

In-use stability

Since no data are available to support the use shelf life under the condition during the administration the following statement is accepted: Shelf life after first opening the immediate packaging: use immediately.

Overall conclusions on quality

Enteroporc Coli is a multivalent inactivated vaccine for the passive immunisation of piglets by active immunisation of pregnant sows and gilts.

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

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 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. Shelf life after first opening the immediate packaging is "Use immediately".

Part 3 – Safety

Introduction and general requirements

Enteroporc Coli is a multivalent inactivated vaccine for the passive immunisation of piglets by active immunisation of pregnant gilts and sows. It contains relevant *E. coli* fimbrial antigens (F4ab, F4ac, F5, F6), and the product is presented as a suspension for intramuscular injection. Enteroporc Coli is a fall-out formulation of the combined vaccine Enteroporc Coli AC. Based on the nature of the antigens, no interactions of the active substances in the larger combination on the induction of protection in the vaccinated animals are to be expected. Therefore, all data referring to *E. coli* antigens which were generated for the combined vaccine Enteroporc Coli AC are considered equally acceptable for the vaccine Enteroporc Coli. This is in compliance with the CVMP guideline on requirements for combined vaccines and associations of immunological veterinary medicinal products (EMA/CVMP/594618/2010).

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 investigating 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. The studies were conducted using the larger combination product Enteroporc Coli AC.

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 concentration 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 of a production batch containing the justified multiple of the minimum release potency without no upper potency limits plus maximum endotoxin content was used. One dose (2 ml) containing the highest recommended concentrations of the product 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 increase following 1st injection 1.2°C, mean increase following 2nd vaccination 0.7°C 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 normalised 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, therefore providing 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 recommended category of 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 normalised within 24 hours. A transient small puncture of the skin (< 0.5 cm) at the injection site was observed in two sows. The swellings 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 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 and 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 to pregnant gilts or sows.

On the basis of the results no safety concerns arose following the administration of the vaccine at highest 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 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 adjuvant aluminium hydroxide as well as 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 470/2009.

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 studies were conducted using the larger combination product Enteroporc Coli AC. 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 sites 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 of gilts with a vaccine dose at an intermediate potency.

Environmental risk assessment

Considerations for the environmental risk assessment

Hazard identification

Enteroporc Coli consists of a suspension of the fimbrial adhesins F4ab, F4ac, F5 and F6 of inactivated *E. coli* as the active substances and aluminium hydroxide as adjuvant.

Enteroporc Coli 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).

The vaccine strains of *E. coli* are partly removed and any remaining *E. coli* bacteria are inactivated. Therefore, no live micro-organisms or toxic components are present in the product.

The Phase I assessment allows the following conclusions:

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

Enteroporc Coli 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 environmental risk assessment (ERA) can stop at Phase I. Enteroporc Coli 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 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 was demonstrated to be safe under a worst-case situation using a batch containing maximum endotoxin and *E. coli* antigens 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 and category 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 complied with the safety requirements of Ph. Eur. monographs 0962. It was concluded that booster vaccination (third administration prior to second farrowing) of a single dose of Enteroporc Coli, containing maximum endotoxin and *E. coli* antigen content 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 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 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 according to 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 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 is a multivalent inactivated vaccine for the passive immunisation of piglets by active immunisation of pregnant gilts and sows. It contains *E. coli* fimbrial antigens (F4ab, F4ac, F5, F6) and aluminium hydroxide as adjuvant. The product is presented as a suspension for intramuscular injection.

Enteroporc Coli is a fall-out formulation of the combined vaccine Enteroporc Coli AC. Based on the nature of the antigens, no interactions of the active substances in the larger combination on the induction of protection in the vaccinated animals are to be expected. Therefore, all data referring to *E. coli* antigens, which was generated for the combined vaccine Enteroporc Coli AC is considered equally acceptable for the vaccine Enteroporc Coli. This is in compliance with the CVMP guideline on requirements for combined vaccines and associations of immunological veterinary medicinal products (EMA/CVMP/594618/2010).

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

The indication applied for 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*

Onset of immunity: within 12 hours after birth.

Duration of immunity: *E. coli* F4ab, F4ac, F5 and F6: first 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 the product:

- Ph. Eur. monograph 01/2017, 0962: "Neonatal piglet Colibacillosis vaccine, inactivated"
- 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)

Enteroporc Coli does not classify as a MUMS product as it contains only *E. coli* antigens and the proposed indication is not considered minor use.

Challenge models:

Specific challenge strains were used for the individual fimbrial adhesins in Enteroporc Coli. An overview of these challenge strains is presented below.

With respect to the *E. coli* challenge, the challenge model was oral administration to piglets within 12 hours after birth. The challenge strain for F4ab was the heterologous strain X-288/99 *E. coli* K88ab; positive for STII and LT. The characteristics for F4ac were: heterologous F4ac challenge strain (IDT-Nr. 212/049); PCR positive for STb, LT and K88ac. For F5, the characteristics were: heterologous F5 challenge strain (IDT-Nr. 213/009); PCR positive for STa, K99 (F5). For F6, the characteristics were: heterologous F6 challenge strain (P1667/01-3); PCR positive for the estb, estap, fasA genes coding for STa and STb. The oral *E. coli* challenge model was performed according to Ph. Eur. standards.

Efficacy parameters and tests:

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

ELISA tests were developed to determine antibody titres against *E. coli* fimbrial antigens F4ab, F4ac, F5 and F6 in blood and colostrum. For validation and estimation of protective titres, a statistical ROC analysis was used. ROC analysis is a technique that can be used to evaluate the validity of a test with a continuous outcome and provide estimates similar to diagnostic sensitivities and specificities of dichotomous tests.

A series of major objections were posed to the applicant concerning their chosen cut-off values for the

protective antibody titres against the 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) 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 was documented in five 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 four reports were submitted calculating a level of protective titre for the antigens included in Enteroporc Coli (F4ab, F4ac, F5 and F6).

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 with respect to field studies.

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 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 another 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 another 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

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. The studies included were study for F4ab, study for F4ac, study for F5 and study for the F6 fimbrial antigen.

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 $\geqslant 1$) 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%).

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 got sick or died as a result of the challenge. Control gilts were sero-negative before and after basis 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 at a minimum potency of *E. coli* F4ab antigen protected piglets against severe F4ab challenge infection after colostrum uptake and 12 hours after birth.

Study for F4ac: In total 24 gilts were included hereof 8 vaccinated with an Enteroporc Coli 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 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 difference in efficacy was seen between IVP1 and IVP2 the minimum dose for F4ac in Enteroporc Coli was set at 19 rU/ml.

Based on the above results, it could be concluded that vaccination with Enteroporc Coli at a minimum potency of *E. coli* F4ac antigen protected piglets against severe F4ac challenge infection after colostrum uptake and 12 hours after birth.

Study for F5: In total 24 gilts were included hereof 8 vaccinated with an Enteroporc Coli 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 was 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).

At 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, respectively, on the day of farrowing. 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 ≤0.001 in both cases.

It was concluded that Enteroporc Coli 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 was set as 13 rU/ml.

Based on the above results, it could be concluded that vaccination with Enteroporc Coli at a minimum potency of *E. coli* F5 antigen protected piglets against severe F5 challenge infection after colostrum uptake and 12 hours after birth.

Study for F6: In total 22 gilts were included hereof 8 vaccinated with an Enteroporc Coli 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 got 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 due to 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 with the higher F6 potency (37 rU/ml (IVP1)) was selected as F6 minimum dose.

Based on the above results, it could be concluded that vaccination with Enteroporc Coli at a minimum potency of *E. coli* F6 antigen protected piglets against severe F6 challenge infection after colostrum uptake and 12 hours after birth.

Booster vaccination and determination of colostral antibodies

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)

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.

Abbreviated 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. The *E. coli* fimbrial 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.

Pivotal 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 protective levels of antibodies in colostrum ranged between 52% (alpha toxin) and 98% (F4ab, F4ac). The applicant election of this farm for their pivotal 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 an outbreak of pleuropneumonia infection in gilts at the same time.

Study 2: This farm had a good health status and efficacy parameters included were evaluated in terms of percentage of gilts with a protective titre in colostrum samples equal to or above the respective protective titres against F4ab, F4ac, F5 and F6 (primary efficacy parameters). 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. 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 titres against F4ab, F4ac, F5, and F6 (primary efficacy parameters). 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. 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).

Overall conclusion on efficacy

The efficacy of Enteroporc Coli was documented in five laboratory efficacy studies and three field safety/efficacy studies. The field studies were carried out using the product Enteroporc Coli AC and are considered representative as Enteroporc Coli is a fall-out product.

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 *E. coli* derived F4ab, F4ac, F5 and F6 vaccine components.

No dose determination studies were submitted for *E. coli* fimbrial antigen F4ab. For *E. coli* derived fimbrial adhesins F4ac, F5 and F6, the minimum dose was evaluated by testing two different vaccine doses for each of the respective antigens in the course of the immunogenicity testing of Enteroporc Coli according to Ph. Eur. 0962. In total four reports were submitted calculating a level of protective titre for the antigens included in Enteroporc Coli (F4ab, F4ac, F5 and F6).

Onset of immunity was documented with respect to all *E. coli* antigens within 12 hours after birth and shown to last for the first days of life.

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:

- 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.
- Onset of immunity was documented with respect to all *E. coli* antigens.

- No duration of immunity studies were submitted for those antigens, which is acceptable as the claim for protection is stated as "first days of life".
- Evidence for immunity after booster vaccination was provided through colostral titres

Three safety/field studies were submitted in order to support results obtained in the laboratory efficacy studies. Efficacy in field studies was also demonstrated by determination of colostral titres.

Part 5 – Benefit-risk assessment

Introduction

Enteroporc Coli is a multivalent inactivated vaccine for the passive immunisation of piglets by active immunisation of pregnant gilts and sows. It contains relevant *E. coli* fimbrial antigens (F4ab, F4ac, F5, F6) as active substances and is presented as a suspension for intramuscular injection. The vaccine is intended for passive immunisation of piglets by active immunisation of pregnant sows and gilts to reduce clinical signs (severe diarrhoea) and mortality caused by *E. coli* strains expressing the fimbrial adhesins F4ab, F4ac, F5 and F6. Enteroporc Coli provides protection against relevant strains of *E. coli* associated with neonatal diarrhoea in piglets. The basic vaccination course consists of one dose of vaccine (2 ml) administered at 5 and 2 weeks before expected farrowing. A booster vaccination in sows is scheduled at 2 weeks before each subsequent expected farrowing. The proposed route of administration, and vaccination scheme has been confirmed.

Enteroporc Coli is a fall-out formulation of the combined vaccine Enteroporc Coli AC.

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.

Benefit assessment

Direct therapeutic benefit

The proposed benefit of Enteroporc Coli is its broad range efficacy against neonatal diarrhoea caused by different *E. coli* serotypes, which was investigated in multiple laboratory and field studies conducted mainly to an acceptable standard.

A total of five laboratory studies were conducted in accordance with GLP (2 safety studies) and GCP. Three clinical safety/efficacy trials were conducted in accordance with GCP. Enteroporc Coli has a potential to be of value in the treatment of neonatal piglet diarrhoea, which is of major importance worldwide.

The onset of immunity is established (after uptake of colostrum) within 12 hours after birth for all the *E. coli* components. The duration of protection is not determined for the *E. coli* fimbrial antigens but colostrum-derived antibodies are expected to remain during the first days of life, sufficiently long to protect against *E. coli* induced neonatal diarrhoea.

Additional benefits

Enteroporc Coli is easy to apply by the veterinarian/owner.

Enteroporc Coli increases the range of available preventative possibilities for piglet neonatal diarrhoea

caused by *E. coli*.

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,* 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 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 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 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 applicant applied 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.

Onset of immunity: within 12 hours after birth.

Duration of immunity: first days of life.

The product has been shown to be efficacious for these indications, and the CVMP accepted the indications as proposed by the applicant.

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 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.