



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for Coliprotec F4/F18 (EMA/V/C/004225/0000)

Common name: Porcine post-weaning diarrhoea vaccine (live)

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



Introduction

On 18 December 2015 the applicant Prevtect Microbia GmbH submitted an application for a marketing authorisation to the European Medicines Agency (the Agency) for Coliprotec F4/F18, through the centralised procedure falling within Article 3(2) (a) of Regulation (EC) No 726/2004 (new active substance).

The eligibility to the centralised procedure was agreed upon by the Committee for Medicinal Products for Veterinary Use (CVMP) on 7 May 2015 as Coliprotec F4/F18 contains a new combination of two existing active substances, live non-pathogenic *Escherichia coli* (*E. coli*) O8:K87 and *E. coli* O141:K94, which is not yet authorised as a veterinary medicinal product in the Community.

The rapporteur appointed is Noemi Garcia del Blanco and the co-rapporteur is Ewa Augustynowicz.

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

The applicant is registered as an SME pursuant the definition set out in Commission Recommendation 2003/361/EC.

Coliprotec F4/F18 is a lyophilised vaccine for oral use in pigs. The active substance is a combination of two live non-pathogenic *E. coli* O8:K87 and *E. coli* O141:K94. Each dose contains 1.3×10^8 to 9×10^8 colony forming units (CFU) of live non-pathogenic *E. coli* O8:K87 and 2.8×10^8 to 3×10^9 CFU of live non-pathogenic *E. coli* O141:K94.

The vaccine is intended for active immunisation of pigs from 18 days of age against enterotoxigenic F4-positive and F18-positive *E. coli* in order to reduce the incidence of moderate to severe post-weaning *E. coli* diarrhoea (PWD) in infected pigs and to reduce the faecal shedding of enterotoxigenic F4-positive and F18-positive *E. coli* from infected pigs. The route of administration is for oral use.

Coliprotec F4/F18 is presented in cardboard boxes containing 1 or 4 glass vials of 50 doses or 1 glass vial of 200 doses.

On 10 November 2016 CVMP adopted an opinion and CVMP assessment report.

On 9 January 2017 the European Commission adopted a Commission Decision granting the marketing authorisation for Coliprotec F4/F18.

Scientific advice

Not applicable.

MUMS limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

Documents that set out a detailed description of the system of pharmacovigilance (version 2 dated 20/08/2014) have been provided. An appropriate statement has been provided indicating that 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 are in place.

Manufacturing authorisations and inspection status

Coliprotec F4/F18 is manufactured in the EU by CZ VETERINARIA, S.A., Porriño/Pontevedra, Spain. A satisfactory certificate of Good Manufacturing Practices (GMP) compliance referring to inspection on 22 April 2015 has been provided and no further inspection is required. A qualified person's statement of GMP compliance that refers to *E. coli* strains O8:K87 and O141:K94 has also been provided.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system and the GMP certification of the manufacturing sites were considered to be in line with legal requirements.

Part 2 – Quality

Composition

Coliprotec F4/F18 is a vaccine containing live *E. coli* bacteria, strain O8:K87, 1.3×10^8 to 9×10^8 CFU/dose, and strain O141:K94, 2.8×10^8 to 3.0×10^9 CFU/dose. It is presented as a lyophilisate for reconstitution in water. Sucrose, monosodium glutamate and dextran 40,000 are included as excipients. The pharmaceutical form is an oral suspension.

Container

The vaccine is filled into 11 ml type I glass vials or 50 ml type II glass vials (in accordance with European Pharmacopoeia (Ph. Eur. chapter 3.2.1) containing 50 and 200 doses respectively. These are closed with chlorobutyl rubber stoppers (in accordance with Ph. Eur. chapter 3.2.9) and aluminium seals. Specifications and certificates demonstrating Ph. Eur. compliance were provided for the glass vials and stoppers.

Development pharmaceuticals

E. coli is an important cause of post-weaning diarrhoea (PWD) in piglets. PWD due to *E. coli* is caused primarily by enterotoxigenic *E. coli* (ETEC), a pathotype that is characterized by expression of fimbriae that mediate bacterial adherence to the intestine and enterotoxins that cause diarrhoea. Fimbriae-designated F18 and F4 (K88) are the types that are commonly found in ETEC from PWD in pigs. F4 fimbriae are flexible fimbriae occurring as F4ab, F4ac or F4ad variants, with F4ac being the most common type. F4 fimbriae mediate bacterial adherence to the intestinal epithelium throughout most of the small intestine of pigs of all ages (i.e., neonatal, post-weaning and finisher pigs). F18 fimbriae are long flexible appendages that occur as two antigenic variants, F18ab and F18ac. F4-positive and F18-positive ETEC strains produce a combination of heat-stable enterotoxins STa and STb and heat-labile enterotoxin (LT). F18-positive ETEC strains may also produce Stx2e. F18 fimbriae mediate adherence to the intestinal epithelium of pigs older than 10 days of age, the expression of the receptor then increasing gradually with age in the suckling phase and is maintained in older pigs (3 - 23 weeks old). The F4 receptor and F18 receptor on enterocytes play a crucial role in the infectious process by mediating the binding of F4 or F18 fimbriated bacteria to the intestinal epithelium, leading to

colonisation of the gut and subsequent secretion of enterotoxins. Pigs that lack the receptor for F4 and/or F18 are therefore resistant to colonisation by F4- and/or F18-positive ETEC.

A vaccine (Coliprotec F4) containing *E. coli* O8:K87 strain (F4 component of Coliprotec F4/F18) has already been approved via the EU centralised procedure for marketing authorisation. The proposed multi-dose oral presentation containing both F4 and F18 antigens provides advantages in that it requires the preparation of only one vaccine solution which is subsequently administered by drenching or via drinking water to the herd. Coliprotec F4/F18 vaccine was originally developed in Canada. Presently, the vaccine is manufactured by CZ Veterinaria A.S., Spain.

The *E. coli* O8:K87 strain in Coliprotec F4/F18 expresses F4ac fimbriae and is toxin-negative. It was isolated from a healthy pig in 1999. The strain is non-pathogenic for animals and humans since it is negative for the virulence associated genes and virulence factors which are associated with intestinal and extra-intestinal *E. coli* diseases.

The *E. coli* O141:K94 strain in Coliprotec F4/F18 expresses F18ac fimbriae and is toxin-negative. It was isolated from a pig in 1996. The strain is non-pathogenic for animals and humans since it is negative for the virulence associated genes and virulence factors which are associated with intestinal and extra-intestinal *E. coli* diseases.

Both strains were found to be only resistant to antimicrobials for which high occurrence of resistance is also found for commensal and pathogenic *E. coli* isolates from pigs.

An explanation and justification for the composition and presentation of the vaccine were provided.

Method of manufacture

The procedure for production of the *E. coli* antigens is fairly conventional for a live bacterial vaccine and is basically similar for the two strains, although there are minor differences associated with the different growth characteristics. After fermentation, each bacteria culture is concentrated and diluted with a stabilizer before being stored at recommended temperature until their blending before filling.

The minimal and maximal potencies for the vaccine strains (1.3 to 9.0×10^8 CFU per dose for O8:K87 (F4ac) and 2.8×10^8 to 3.0×10^9 CFU per dose for O141:K94 (F18ac)) were established taking into consideration the conclusions drawn from the safety laboratory trials and the efficacy laboratory trials, as well as the minimum potency to be expected at the end of the stability evaluation. The vaccine is formulated at a target CFU of 9×10^8 per dose for the O8:K87 (F4ac) and 3×10^9 per dose for O141:K94 (F18ac).

Final containers are aseptically filled and stoppered with vacuum closing chlorobutyl rubber stoppers that are only partially inserted. After lyophilisation, vials are fully closed under vacuum. Rubber stoppers are held firmly in place by an aluminium seal which is crimped on after vacuum stoppering.

Control of starting materials

Active substances

The F4-positive, toxin-negative *E. coli* O8:K87 (F4ac) strain was isolated from faeces of a healthy pig. The master seeds (MS) and working seeds (WS) have been produced according to the Seed Lot System. Results demonstrated that the MS strain was pure and was identified as O8:K87 strain, negative for toxin-related virulence factors and positive for F4. Information on the antibiotic sensitivity of the strain was provided.

The F18-positive, toxin-negative *E. coli* O141:K84 (F18ac) strain was isolated from the faeces of a pig in 1996. The MS and WS have been produced according to the Seed Lot System. Results demonstrated that the MS strain was pure, negative for toxin-related virulence factors and positive for F18. Information on the antibiotic sensitivity of the strain was provided.

The current F4 -WS bank was prepared from the F4 MS by CZ Veterinaria, Pontevedra, Spain. The current F18 WS bank was prepared from the F18 MS by CZ Veterinaria. All future WS stocks are confirmed to be produced and tested as described for the current WS banks.

Excipients

No animal derived materials are used in the manufacturing process. Dextran 40,000 powder is part of the stabiliser in the vaccine. Since Coliprotec F4/F18 is intended for oral use, Ph. Eur. monograph 0999 (dextran 40 for injection) does not apply and the application of internal specifications is justified.

Other excipients used are sucrose (in accordance with Ph. Eur. monograph 0204), monosodium glutamate (in accordance with USP 32) and purified water (in accordance with Ph. Eur. monograph 0008).

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

A comprehensive TSE risk assessment has been provided in compliance with the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3) and Commission Directive 1999/104/EEC.

The risk of transmitting TSE infection by use of this vaccine is considered negligible.

Control tests during production

The following control tests are carried out at various stages during production of the *E. coli* antigens: purity, viable count, optical density, identification by agglutination (for F4 antigen only) and by genotypic methods (for F4 and F18 antigens) and concentration factor.

The following tests are carried out on the final bulk vaccine: purity, viable count, identification and fill volume.

Tables summarising the manufacturing data and test results for three consecutive batches of *E. coli* antigens were provided. All results met the required specifications, thereby demonstrating that the *E. coli* antigens are manufactured consistently.

Control tests on the finished product

The description of the following methods used for the control of the finished product and the specifications was provided: appearance, vacuum, presentation, identity by PCR and agglutination (for F4 antigen only), potency (titre), purity and residual humidity.

Tables summarising the manufacturing data and test results for three consecutive batches of each *E. coli* antigen were provided. All results met the required specifications, thereby demonstrating that the vaccine is manufactured consistently.

Stability

Stability data demonstrating that the F4 antigen can be stored at 2-8°C for up to 14 days or at -78°C for up to 6 months and that the F18 antigen can also be stored at -78°C for up to 6 months were provided. Three consistency batches have been produced which have been formulated with aged antigens and these have been included in the stability programme. Interim stability data for these batches have been provided for up to 9 or 12 months, however further data will be required as a post authorisation recommendation to support a 15-month shelf-life for the aged antigen.

Stability data for three research batches and the three consistency batches of Coliprotec F4/F18 produced under GMP are currently available. Stability data for one research batch stored for 21 months, two further research batches stored for 18 months, and three consistency batches (commercial scale) stored for only 12 or 15 months have been provided. For all currently available time points, the tested batches complied with the specifications set and there is no obvious decline in potency. The data are sufficient to support a 15-month shelf-life. A commitment to complete the stability testing programme on these batches up to 27 months to support an eventual proposed shelf-life of 24 months has been provided.

Satisfactory data were submitted to support the stability of reconstituted product for up to 6 hours after reconstitution in purified water (reverse osmosis [RO]) or tap water. The vaccine organisms are however sensitive to free chlorine that might be present in drinking water and data have been provided to demonstrate that, in such cases, it can be protected by 5 g/litre skimmed milk (stated in the SPC). The applicant's recommended maximum in-use shelf-life of 4 hours is therefore adequately supported.

Overall conclusions on quality

Coliprotec F4/F18 is a vaccine containing live *E. coli* bacteria, strains O8:K87 and O141:K94 which is presented as a lyophilisate for reconstitution in water. Sucrose, monosodium glutamate and dextran 40,000 are included as excipients. The product is filled into 11 ml type I glass and 50 ml type II glass vials (in accordance with Ph. Eur. chapter 3.2.1) containing 50 and 200 doses, respectively. These are closed with chlorobutyl rubber stoppers (in accordance with Ph. Eur. chapter 3.2.9) and aluminium seals. Satisfactory information on the vials and stoppers was provided.

The manufacturing process for the vaccine is well described with sufficient details. A table indicating the blending details of a typical batch of vaccine is provided. No specific validation studies were presented but consistency data for three batches of antigens and three batches of finished product in support of validation of the production process have been presented. Satisfactory data were provided to support the applicant's proposed storage of the F4 bulk antigen mixed with stabiliser at 2 °C-8 °C for up to 14 days or at -78 °C for up to 6 months and also for the F18 antigen mixed with stabiliser stored for up to 6 months at -78 °C.

Starting materials listed in a pharmacopoeia are of satisfactory quality.

Production and testing of the *E. coli* MS and WS are clearly described. Confirmation that all future WS stocks will be produced and tested as described for the current WS banks has been provided. Other starting materials of biological origin are acceptable and the risk of TSE contamination is considered negligible.

Starting materials of non-biological origin are acceptable. In-house preparation of media is detailed satisfactorily.

In-process tests are described satisfactorily. Control tests on the finished product are described in satisfactory detail. Full details of the production of the F4 antiserum, including the material used to immunise the rabbits and the tests carried out on to demonstrate that it is specific for the vaccine strain have been provided. No agglutination test for the identity of F18 antigen is used as this method is unreliable for F18 strains however genotypic methods (e.g. PCR) are preferred. Assurance has been provided that the F18 antigen is monitored/controlled during the manufacturing process to ensure quality is not compromised. The methods used, namely viable bacterial count method and genotypic method confirm the presence of the F18 coding gene and identity of the *E. coli* O141:K94 vaccine strain.

Data provided indicate satisfactory batch-to-batch consistency.

Stability data for three research batches and the three consistency batches of Coliprotec F4/F18 produced under GMP are available to support the proposed shelf-life. Stability data for one research batch stored for 21 months, two further research batches stored for 18 months, and three consistency batches stored for only 12 or 15 months have been provided. For all currently available timepoints, the tested batches complied with the specifications set and there is no obvious decline in potency. A 15-month shelf-life could therefore be accepted on the basis of the data available to date and taking into account the already approved shelf-life for Coliprotec F4. Three new consistency batches have been produced which have been formulated with aged antigen and these have been included in the stability programme. Interim stability data for these batches have been provided for up to 9 or 12 months.

Satisfactory data have been submitted to support a maximum recommended in-use shelf life of four hours after reconstitution in RO or tap water. The vaccine organisms are however sensitive to free chlorine that might be present in tap water and data have been provided to demonstrate that, in such cases, it can be protected by 5 g/litre skimmed milk. This is indicated in the SPC.

Overall it is concluded that the vaccine is manufactured to a satisfactory quality.

In addition, the applicant is recommended to provide the following information post-authorisation:

The applicant is recommended to provide further stability data on vaccine formulated with aged antigen to support a 15-month shelf-life of the product.

Part 3 – Safety

Safety documentation

The dossier includes laboratory safety studies, including specific studies for live vaccines as summarised below. The safety documentation also includes the assessment of the potential risks that may result from the exposure of human beings or of the environment to the immunological product. A field study conducted in Europe has also been carried out to further investigate the safety profile for this vaccine.

Laboratory tests

Four laboratory safety studies for Coliprotec F4/F18 are presented, two of which were carried out in Canada and two in Germany.

Safety of the administration of one dose

A study was conducted in compliance with the principles of Good Laboratory Practice (GLP) with the exception of the laboratory investigations. The laboratory responsible for faecal testing is experienced and accredited to ISO/IEC 17025:2005 and it is acceptable.

The 36 animals enrolled in the study, crossbred weaned pigs, were 15 to 19 days old on day 0, which meets the requirements for minimum age. Animals were divided into four groups; group I controls, n=12, group II single dose group, n=8, Group III overdose, n=12 and group IV sentinels, n=4. For safety of the administration of one dose groups I and II were compared. All study animals were confirmed F4 receptor positive (F4R+) and F18 receptor positive (F18R+). The methods plus validation of the methods for F4 and F18 receptor status have been provided and are satisfactory. In addition, justification of the methodology used for testing of F18 receptor presence as a marker of susceptibility in animals has been provided.

The PCR methods used to test for DNA extracted from faecal samples have been provided including validation, which is satisfactory thus the test is fit for purpose.

Vaccine was administered orally using a disposable syringe directly into the mouth on the back of the tongue. The SPC suggests administration via drench or in the drinking water. The data presented are acceptable to support both routes.

Regarding body temperature, significant differences were observed between groups on day 4 post vaccination although mean rectal temperatures were within the normal expected range. Given that there were elevated temperatures in both groups an element of handling could potentially be associated with the elevated temperatures recorded however with vaccination all animals will be handled for vaccination and therefore elevation in temperature may be observed. In addition rectal temperatures are compared to a baseline which involves the handling of animals and should take into account handling associated stress. Nevertheless, individual data indicated that only one vaccinated animal (day 1) and one control animal (day 2) had rectal temperatures >40 °C and therefore, no SPC warning following single vaccination is required.

No statistically significant differences were observed between the vaccinated groups and the control group with regard to abnormal clinical signs, total clinical score, the consistency of faeces and body weight/weight gain. Significantly more control pigs were observed with abnormal faecal colour compared to the vaccinated pigs.

Based on the data presented, it can be concluded that, when Coliprotec F4/F18 was administered as a single dose, no safety concerns were observed in weaned pigs at the target age of 18 days.

Safety of one administration of an overdose

Safety of one administration of an overdose was conducted as described above in one study. None of the pigs died during the study.

Regarding body temperature, significantly higher mean rectal temperature was noted in the vaccinated groups compared to the control group on day 4. Individual data indicated that two vaccinated animals and one control animal had rectal temperatures >40°C during the study. The elevation in rectal

temperature following overdose vaccination is more than what was observed following single dose vaccination and therefore the potential for elevated temperature following vaccination with an overdose has been reflected as an SPC warning in section 4.10.

No statistically significant differences were observed between the vaccinated group and the control group with regard to abnormal clinical signs, total clinical score, the colour or consistency of faeces and body weight/weight gain.

Based on the data presented, it can be concluded that no known safety concerns were observed when Coliprotec F4/F18 was administered as an overdose, in weaned pigs at the target age of 18 days.

Safety of the repeated administration of one dose

A study to assess repeated administration of one dose is not needed because the administration schedule is a single vaccination and no booster is recommended. This is acceptable.

Examination of reproductive performance

No study on examination of reproductive performance has been provided. An appropriate warning is included in the product information (section 4.7): 'The use is not recommended during pregnancy'. This is acceptable.

Examination of immunological functions

No specific tests on immunological functions were carried out since the vaccine strain is non-pathogenic for the target species. It is reasonable to anticipate that the constituents of this product would not have a negative effect on immunological function of the pigs.

Special requirements for live vaccines

Spread of the vaccine strain

A target animal safety study to evaluate the safety and the potential shedding of the vaccines strains from pigs vaccinated orally with Coliprotec F4/F18 was conducted as described above.

All study animals were confirmed to be positive for F4R (F4R+) and for F18R (F18R+). Even though the number of sentinel animals is low, spreading of the vaccine strains to these animals was evident. The low number of animals included can therefore be accepted.

Both the F4 and F18 vaccine strains were shed in the faeces from vaccinated animals for at least 14 days after vaccination. It can be agreed that the spread of the vaccine strains to unvaccinated sentinel animals did not have a clinical effect as assessed by clinical signs, faecal examination, rectal temperature, body weight or mortality.

In conclusion, shedding vaccine strains occur in vaccinated animals and spreads to unvaccinated animals. The risk is managed via information provided in section 4.5 of the SPC which contains appropriate safety warnings and therefore this is acceptable.

Dissemination in the vaccinated animal

Dissemination of the F4 component of Coliprotec F4/F18 (*E. coli* O8:K87 strain) in vaccinated animals was investigated in study previously submitted for Coliprotec F4. Dissemination of the F18 component of Coliprotec F4/F18 (*E. coli* O141:K94 strain) in vaccinated animals was investigated in another study.

From the data provided regarding the F4 component of Coliprotec F4/F18, dissemination within the predilection sites for replication of the organism was evident on day 3 post inoculation, however dissemination of the F4 strain at this time to other organs was not observed. The timing of sample collection in terms of 3 days post inoculation being optimal for vaccine strain recovery has been justified.

From the data provided regarding the F18 component of Coliprotec F4/F18, dissemination within the predilection sites for replication of the organism (ileum, and mesenteric lymph nodes) was evident on day 3 but not day 7 post inoculation, however dissemination of the F18 strain at this time to extra-intestinal tissues, the liver and longissimus muscle was not observed on days 3 or 7 post inoculation.

Reversion to virulence of attenuated vaccines

A reversion to virulence study using F4 MS was submitted to support the safety of Coliprotec F4/F18.

Validation of the PCR-RFLP method used to confirm the status of F4 receptor in pigs and the period and conditions of storage of the samples used for the testing has been provided. Although no evidence of an increase in virulence following successive passages in piglets was observed, the interval between inoculation and necropsy was relatively short. However, it has been demonstrated that the time between inoculation and necropsy was adequate for macroscopic intestinal lesions to appear. Finally, it is not clear from the information provided whether the bacteria used to infect the next group of animals were always isolated from F4R+ animals, although it is not likely to be the case as the receptor status of the animals was analysed retrospectively. Although the most severe infections occurred in F4R+ animals, organisms were re-isolated and some diarrhoea did occur in F4 receptor negative (F4R-) pigs. The Committee accepted that the diarrhoea was not associated with the presence of F4-positive ETEC strains but with other non-F4 ETEC strains originating from the pig supplier which were enriched and concentrated through subsequent passages hence the more frequent and severe diarrhoea apparent in animals in the 5th passage. The reversion to virulence study does not demonstrate different selection pressures in F4R-negative pigs compared to F4R-positive animals.

After ingestion of the F4 vaccine strain of the Coliprotec F4 vaccine or the Coliprotec F4/F18 vaccine, the organism enters the same porcine intestinal environment for both F4R-negative and positive pigs. In F4R-positive pigs, the vaccine organisms adhere to the intestines, colonise and considerably replicate. Vaccine organisms are then found in the intestinal lumen, transported by the peristalsis along the intestines and shed in faeces. For F4R-negative animals, vaccine organisms are transported by the peristalsis along the intestines and shed in faeces without adhesion and replication. Vaccine organisms shed into the direct environment of the pigs may be ingested again by the pigs, as shown by detection of the vaccine strain in F4R-negative pigs commingled with F4R-positive pigs.

A laboratory study was performed to examine the potential for increased virulence of the F18 MS of the Coliprotec F4/F18 vaccine in pigs. Overall, this study complied with the VICH GL 41 on absence of reversion to virulence and the general monograph on safety 5.2.6. Pigs were 18 to 24 days old on day 0 which is slightly older than the minimum recommended age and were all F18R+. A proportion of the pigs enrolled in each passage were also F4R+.

Five groups were evaluated as follows: groups I, III, IV, VII and VIII corresponding to passages 1 to 5 respectively. Following inoculation, rectal temperatures were significantly different between groups III, IV, VII and VIII in comparison to group I however this difference was not considered biologically relevant and not passage related. Pyrexia was observed in all groups in the study (maximum 42.4 °C in group IV on day 11 and 40.3 °C on days 11 and 15 in group I) however maximum temperatures in

group IV animals was found to be associated with a lethal concomitant *S. suis* infection and not related to the inoculum.

Faecal samples tested prior to inoculation were negative for presence of F18-ETEC (haemolytic *E. coli* by culture and F18 by PCR analysis) with the exception of one animal in group VII, which was subsequently excluded from the study. Signs of diarrhoea were observed in all groups but they were not significantly different. Faecal samples were tested by PCR analysis for identification of the vaccine strain. The F18 vaccine strain was confirmed in all groups although it was noted that on day 21 of passage 5, the vaccine strain could not be identified from individual faecal samples from animals in group VIII. Passages 2 and 5 did not differ significantly from passage 1 in relation to faecal colour and number of animals with at least one specific abnormal finding post inoculation. There were no differences between groups for faecal consistency.

In summary, although there are some differences between groups, it is demonstrated that there is no evidence of an increase in virulence indicative of reversion to virulence during the observation period.

Biological properties of the vaccine strains

A robust argument based on publications has been provided regarding the lack of capability of the vaccine strains to colonise other tissues apart from intestinal cells in F4 and F18 susceptible pigs and in different species, including humans and in particular immunosuppressed humans. The applicant has discussed the importance of the F4 receptor and F18 receptor in order for pigs to be susceptible to challenge and as a consequence, to get disease. Furthermore, the argument that non-target species which do not have the receptors are not affected has been strengthened by providing scientifically strong argumentation.

In summary, justification has been provided that the vaccine strains will only colonise the intestines of F4/F18 receptor positive animals, not other tissues in the target species or non-target species and thus there are no additional risks for other species.

In addition, the F18 vaccine strain is haemolytic and the applicant has adequately justified that there is no additional risk for the vaccine to produce other effects in the gut.

Recombination or genomic reassortment of the strains

On the basis that multiple passages did not enhance the ability of the vaccine strains to colonise the intestine, it is concluded that the vaccine strains are genetically stable and easily eliminated by animals. The presence of strains harbouring ETEC-associated toxins simultaneously with the F4 and the F18 vaccine strains in animals demonstrated that the vaccine strains do not acquire exogenous genetic material, as after five passages the vaccine strains remained genetically stable despite the presence of strains harbouring ETEC associated toxins.

Study of residues

No studies on residues are required. The active substances being principles of biological origin intended to produce active immunity are not within the scope of Regulation (EC) No 470/2009. The excipients are either allowed substances for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates a "No MRL required" classification or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

The withdrawal period is set at zero days.

Interactions

No interaction studies have been carried out and a statement has been included in 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. In addition, since the vaccine strains are live bacteria, simultaneous use of any antimicrobials which are effective against *E. coli* should be avoided.

Field studies

Two field studies conducted in Europe have also been carried out to further investigate the safety profile for this vaccine.

Both studies were performed in Germany. Both studies were conducted to Good Clinical Practice (GCP) standard and the studies, which evaluated both safety and efficacy, were well designed. The farms selected had a history of PWD caused by F4-positive and F18-positive enterotoxigenic *E. coli* during the pre-fattening period.

The 488 pigs enrolled in the study were slightly older in the field study and the age ranged from 21 to 29 days. The status of the animals enrolled in the study regarding the presence of F4 and F18 receptors was investigated and the incidence within piglets was 1.6% for F4R+/F18R+, 85.8% for F4R-/F18R+ and 10% for F4R-/F18R- (F18 receptor negative). The number of animals with the F4R was very low which was unexpected. Whilst this may not represent a typical farm, all groups were represented in the safety subgroup.

The batch protocol for the vaccine batch used in the study was provided. All results from in-process and finished product tests were within specifications.

Adverse events were observed in both groups, however the proportion observed in the control group was significantly higher than in the vaccinated group and was linked to an outbreak of *E. coli* and *Clostridium perfringens*, which occurred early in the study before the 7 day onset of immunity (OOI) for the vaccine. Higher mortality levels were observed in the control group compared to the vaccinated group.

The proportion of serious adverse reactions in the control group was higher than the vaccinated group although this was not significant. Most of these were deaths due to *E. coli* infection. The maximum temperature observed in the control group was 40.5 °C, whilst it was 41.9 °C in the vaccine group. Regarding temperature, no difference was seen between vaccinated and control groups post vaccination except on day 6 with means higher in the vaccinated group.

Overall, no adverse events or abnormal clinical signs attributable to vaccination were observed. Body temperatures higher than 39.9 °C were observed during the study and with the early onset of *E. coli* infection it cannot be concluded whether the elevated temperatures were associated with the vaccination or *E. coli* infection. However from the laboratory studies some elevation in temperatures is known to occur. The rapporteur considers that the results from the field trial support the results from the laboratory studies and concludes that administration of the vaccine in drinking water does not cause any additional safety concerns when administered to pigs of the youngest age recommended.

User safety

A user safety risk assessment was provided in accordance with the CVMP Guideline for user safety for immunological veterinary medicinal products (EMA/CVMP/IWP/54533/2006). The main risk considerations for the user are as follows:

- The vaccine contains non-pathogenic live *E. coli* strains that are specific for pigs, as they are not able to colonise and therefore produce disease in non-target species, including humans and immunosuppressed humans. However, as vaccine organisms are transported by peristalsis along the intestines and shed in faeces, some clinical signs in other species might occur.
- Although the F18 vaccine strain is haemolytic, information has been provided to confirm that this is not a risk factor for the user or the product itself.
- The excipients used in the vaccine are commonly used excipients and do not constitute a user safety concern.
- The main exposure route would be spillage and general precautions such as hygiene measures and the use of protective gloves and glasses will be sufficient to mitigate this risk. Appropriate warnings have been included in the SPC, section 4.5.

The CVMP concluded that the user safety for this product is acceptable when used as recommended in the SPC.

Environmental risk assessment

An environmental risk assessment was provided in compliance with the Note for guidance on environmental risk assessment for immunological veterinary medicinal products (EMA/CVMP/074/95).

Coliprotec F4/F18 consists of a live avirulent non-pathogenic *E. coli* F4 strain (*E. coli* O8:K87) and a live non-pathogenic *E. coli* F18 strain (O141:K94). Only the target animal pig is susceptible to these strains, as ETEC fimbriae confer the species-specificity of the pathogen. The transmission of the strains to non-target species is theoretically possible; however, colonisation of the strains in the intestine of such animals important for the development of disease will not occur. The excipients do not constitute a risk to the environment.

The likelihood of environmental exposure is not higher than for any other intestinal *E. coli*, including commensal *E. coli* and pathogenic *E. coli* such as F4-ETEC.

In case of exposure, no harmful consequences are envisaged.

The level of risk is considered negligible and the overall risk to the environment is considered to be effectively zero.

Safety studies were performed using F4R+ and F18R+ pigs and in the efficacy studies some pigs were F18R+ but F4R- prior to challenge with F18 challenge strain. Even though no studies have been conducted including F18R- animals, acceptable argumentation has been provided to support that other species not harbouring the F18 receptor would not develop clinical signs after exposure to the vaccine, as colonisation would not be possible and thus subsequent disease would not occur.

The CVMP also agrees that due to their nature, the excipients used in the formulation do not represent a concern with regard to risk to the environment.

Therefore a phase II assessment is not considered necessary. Coliprotec F4/F18 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 Coliprotec F4/F18 was investigated in four laboratory safety studies. Two of these were carried out in accordance with GLP in Europe using either finished product manufactured at CZV or F18 MS and the other two studies were performed in Canada using either F4 or F18 MS.

The safety of a single dose (n=8) and an overdose (n=12) were investigated in 15-19 day old piglets in a single pivotal GLP study. A control group of 12 unvaccinated pigs was also included as well as 4 sentinels to evaluate spread. All groups included only animals that were both F4R-positive and F18R-positive and justification of the F18 receptor presence as a marker of susceptibility in animals has been provided. Additional methodology and its validation for determination of F18 status in animals have been provided. In both single and tenfold overdose groups, the doses of vaccine administered exceeded the maximum specified. Rectal temperatures were significantly different 4 days post vaccination in the single dose group compared to the control group and occasional peaks of fever was observed in both groups on isolated days. No statistically significant differences were observed between the vaccinated groups and control group with regard abnormal clinical signs, total clinical score, body weight, mortality or abnormal faecal findings. However, there were significantly higher abnormal findings in the control group compared to the single dose vaccinated group for faecal colour.

The safety of repeated administration of a single dose was not investigated because the vaccine is intended to be administered only once and this is acceptable.

The ability of the F4 and F18 vaccine strains to spread to unvaccinated pigs was also investigated and it was shown that both vaccine strains are excreted from vaccinated animals for at least 14 days and the F4 vaccine strain was detected in faecal material of in-contact sentinel animals. An appropriate warning is included in the SPC.

No studies to investigate the potential effect on reproductive performance have been submitted and this is acceptable since the vaccine is not intended to be administered to animals intended for breeding.

The lack of studies including F18R- pigs has been justified by the argument that the receptor is required for colonisation and subsequent disease and hence F18R- pigs are not susceptible to *E. coli* F18 challenge. This is also significant for other species that do not have the F4/F18 receptors that may come into contact with the vaccine i.e. the receptors are required for colonisation and subsequent disease. It is agreeable that there is no risk to other species that do not have the F4/F18 receptors.

The potential for the vaccine strains to spread or acquire virulence characteristics during *in vivo* passage was investigated in one study carried out in Canada (F4 MS) and one study carried out in Germany (F18 MS). Although the Canadian study was not conducted in accordance with GLP, it was carried out using the vaccine MS and reported to an adequate standard. This study was nevertheless considered relevant for the product proposed to be manufactured in Europe and therefore considered acceptable, taking into account 3R principles to avoid repetition of such study. The F4 vaccine strain was propagated through five passages in pigs and did not acquire any virulence factors during these passages. Both F4R+ and F4R- pigs were included in each passage group. The German study was conducted in accordance with GLP. The F18 vaccine strain was propagated through five passages in pigs and did not acquire any virulence factors during these passages although some fluctuations in rectal temperature were observed. Nevertheless, these were not considered passage related.

Dissemination of the F4 vaccine strain was only within the predilection sites and not any other organs

investigated. The relevance of these data regarding the timing for collection for the F4 vaccine strain has been justified. Dissemination of the F18 vaccine strain was only within the predilection sites and no other organs were investigated.

The safety of the vaccine was also investigated in two field studies carried out in Germany in accordance with GCP. Observations were generally in concordance with those seen in the laboratory studies.

It can be concluded that there are no known safety concerns for the target species. Information on a potential increase in rectal temperature following an overdose of the vaccine has been included in the SPC.

No specific residue studies are required. The withdrawal period is set at zero days.

The user safety has been adequately addressed. The user safety for this product is acceptable when used as recommended in the SPC.

Based on the data provided the ERA can stop at Phase I. Coliprotec F4/F18 is not expected to pose a risk to the environment when used according to the SPC.

Part 4 – Efficacy

Coliprotec F4/F18 is a live non-pathogenic vaccine recommended for the oral vaccination of healthy piglets of at least 18 days of age.

The vaccine is intended for active immunisation of pigs from 18 days of age against enterotoxigenic F4-positive and F18-positive *E. coli* in order to:

- reduce the incidence of moderate to severe post-weaning *E. coli* diarrhoea (PWD) in infected pigs
- reduce the faecal shedding of enterotoxigenic F4-positive and F18-positive *E. coli* from infected pigs

OOI: 7 days after vaccination

DOI: 21 days after vaccination.

Introduction and general requirements

There is no specific Ph. Eur. monograph for vaccination of piglets post-weaning with a live colibacillosis vaccine. Laboratory efficacy studies were conducted according to European and national regulatory requirements and in compliance with the following documents:

- German Epidemic Law (Tierseuchengesetz)
- German Animal Vaccination Regulation (Tierimpfstoffverordnung)
- Directive 2001/82/EC
- VICH GL9 (Good Clinical Practice, June 2000)
- EMEA/CVMP/852/99-Final: Note for Guidance: Field Trials with Veterinary Vaccines
- Guideline on Requirements for the production and Control of Immunological Veterinary Medicinal Products" EMA/CVMP/IWP/206555/2010.

Laboratory trials

In total four laboratory studies have been conducted to evaluate the efficacy of Coliprotec F4/F18; one to assess the proof of concept for F18R+ animals and three others to establish the onset of immunity (OOI) and duration of immunity (DOI). In addition, one field study has been performed in Europe.

A proof of concept study has been provided in which F18R+ pigs were challenged using a F18+ ETEC PWD challenge model. The applicant has indicated that the challenge strain has been fully characterised and the referenced publication has been provided which is acceptable.

Acceptable information has been received on the results from the faecal and serum samples collected and the method of production of the vaccine used as well as information regarding the ability of the vaccine to confer immunity against different variant subtypes. Overall, the study demonstrated the relevance of the challenge model used to demonstrate the claims proposed, following vaccination and challenge with an F18 strain.

Onset of immunity

A study was submitted to support OOI for Coliprotec F4/F18 in which animals were vaccinated with Coliprotec F4 and challenged with a F4-ETEC strain. This study was considered supportive only.

Another Study was conducted to evaluate OOI of Coliprotec F4/F18 administered once orally to reduce clinical signs of post-weaning diarrhoea after challenge with *E. coli* F4-ETEC strain in piglets. The animals were at minimum age i.e. 17-18 days of age at vaccination and F4R+/F18R+.

The vaccine was administered in drinking water (via bowls) on day 0 and was below minimum release titre for the F4 strain (5.8×10^7 CFU/dose) and the F18 strain (1.1×10^8 CFU/dose). This study supports the minimum release titre for the F4 strain within the vaccine.

The incidence of pigs with moderate to severe diarrhoea (score ≥ 3) and the duration of diarrhoea (score ≥ 3), were all significantly lower in the vaccinates compared to the controls. The maximum severity of diarrhoea (mean faecal sum scores) associated with *E. coli* (score ≥ 2) was not significantly different between vaccinates and controls.

Body weights were not statistically different between treatment groups.

Faecal shedding was significantly reduced in the vaccinated group compared to the control group from day 2, 3 and 4 post challenge. There was a significant increase in IgM and IgA levels following vaccination compared to the control group.

In conclusion, following vaccination with Coliprotec F4/F18 vaccine and challenge with F4 strain, an OOI of 7 days has been demonstrated in terms of a reduction in the incidence of moderate to severe diarrhoea, duration of diarrhoea and reduction in faecal shedding.

Another study was conducted to evaluate OOI of Coliprotec F4/F18 administered once orally to reduce clinical signs of post-weaning diarrhoea after challenge with *E. coli* F18-ETEC strain in piglets. The animals were at minimum age i.e. 17-19 days of age at vaccination and the study was conducted to a suitable standard (GCP).

The applicant discussed the impact of no F4 receptor positive pigs included in this OOI study, in particular in relation to potential interference between both strains regarding the onset of efficacy and concluded that the immune response elicited, with similar increases in anti-F18 antibodies IgM detected for both F4R+/F18R+ and F4R-/F18R+ vaccinates compared to controls, supports the lack of interference of the F4 strain in the OOI of the F18 component. This conclusion is acceptable.

The vaccine was administered in drinking water (via bowls) on day 0 and was below minimum release titre for the F18 strain: 2.8×10^8 (F18). However, the titre of the F4 strain in the vaccine used in the study, although close to minimum (1.3×10^8), was higher than the minimum titre proposed: 1.6×10^8 . As a valid OOI study has been provided for the F4 strain, the minimum release titre (1.3×10^8 CFU/dose) is supported based on the vaccine used in study: 5.8×10^8 CFU/dose.

The incidence of pigs with mild to severe diarrhoea (score ≥ 2), moderate to severe diarrhoea (score ≥ 3), the maximum severity of diarrhoea (mean faecal sum scores) associated with *E. coli* and the duration of diarrhoea (score ≥ 2), were all significantly lower in the vaccinates compared to the controls.

Body weights were statistically different between groups on day 7 and the average daily weight gain was significantly higher for the vaccinated group compared to the control group throughout the study.

Efficacy criteria were compared between groups for superiority of the vaccine over the control and overall, there was a superiority of the vaccine group. Faecal shedding was significantly reduced in the vaccinated group compared to the control group on days 3, 5, 6 and 7 post challenge. There was a significant increase in IgM and IgA levels following vaccination compared to the control group.

In conclusion, following vaccination with Coliprotec F4/F18 vaccine and challenge with F18 strain, an OOI of 7 days has been demonstrated in terms of a reduction in the incidence of mild to severe diarrhoea, the incidence of moderate to severe diarrhoea, severity of diarrhoea, duration of diarrhoea, reduction in weight loss and reduction in faecal shedding.

Duration of immunity

A study was submitted to support DOI for Coliprotec F4/F18 in which animals were vaccinated with Coliprotec F4 and challenged with a F4-ETEC strain. This study was considered supportive only.

A study was conducted to evaluate DOI of Coliprotec F4/F18 administered once orally to reduce clinical signs of post-weaning diarrhoea after challenge with *E. coli* F4-ETEC strain in piglets. The animals were at minimum age i.e. 17-18 days of age at vaccination and F4R+/F18R+.

The incidence of pigs with moderate to severe diarrhoea (score ≥ 3) and the duration of diarrhoea (score ≥ 2) were not significantly different between groups. However, only animals in the control group had diarrhoea whereas none of the vaccinated animals experienced diarrhoea. The severity of diarrhoea (mean faecal sum scores) was significantly lower in the vaccinated group compared to the control group post challenge.

Body weights were not statistically different between groups.

Faecal shedding was significantly reduced in the vaccinated group compared to the control group from days 1, 2, 3 and 4 post challenge. There was an increase in IgM and IgA levels following vaccination compared to the control group.

In conclusion, following vaccination with Coliprotec F4/F18 vaccine and challenge with F4 strain, DOI of 21 days has been demonstrated in terms of severity of diarrhoea and reduction in faecal shedding. The incidence of moderate to severe diarrhoea was lower but not significantly different in vaccinates compared to the controls.

A Study was conducted to evaluate DOI of Coliprotec F4/F18 administered once orally to reduce clinical signs of post-weaning diarrhoea after challenge with *E. coli* F18-ETEC strain in piglets.

Thirteen pigs in the study were both F4R+ and F18R+. The applicant indicated that the low number of F4R+ animals in the study had no impact in terms of potential interference between strains and

concluded that the similar immune response elicited, with increases in anti-F18 antibodies IgM detected for both F4R+/F18R+ and F4R-/F18R+ vaccinates compared to controls, supports the lack of interference of the F4 strain in the DOI of the F18 component. This conclusion is acceptable.

Prior to vaccination both treatment groups were comparable in terms of body weight, gender and physical condition. Rectal temperatures were in the normal range for pigs of this age.

The incidence of pigs with mild to severe diarrhoea (score ≥ 2), moderate to severe diarrhoea (score ≥ 3), the maximum severity of diarrhoea (mean faecal sum scores) associated with *E. coli* (score ≥ 2) and the duration of diarrhoea (score ≥ 2), were all significantly lower in the vaccinates compared to the controls.

Body weights were not statistically different between groups however the average daily weight gain was significantly higher for the vaccinated group compared to the control group following challenge.

Faecal shedding was significantly reduced in the vaccinated group compared to the control group from days 2 to 7 post challenge. Efficacy criteria were compared between groups for superiority of the vaccine over the control and overall there was a superiority of the vaccine group. There was a significant increase in anti-F4 and anti-F18 IgM and IgA levels following vaccination compared to the control group. There was a significant increase in anti-F18 IgG levels in the vaccinated group compared to the controls at the end of the study, day 28 whereas levels for anti-F4 IgG were significantly higher on day 10 in the vaccinated group compared to the controls.

In conclusion, following vaccination with Coliprotec F4/F18 and challenge with F18 strain, DOI of 21 days has been demonstrated in terms of a reduction in the incidence of mild to severe diarrhoea, the incidence of moderate to severe diarrhoea, severity of diarrhoea, duration of diarrhoea and reduction in faecal shedding.

The influence of maternal antibodies on the efficacy of the vaccine

No study evaluating the influence of maternally derived antibodies has been provided however the lack of interference from maternal antibodies has been adequately justified and this is considered acceptable.

Field trials

The study was regarded to be GCP-compliant, evaluated both safety and efficacy, and was regarded as well designed. The farm selected had a history of PWD caused by F4-positive and F18-positive enterotoxigenic *E. coli* during the pre-fattening period.

The pigs in the study were slightly older in the field study and the age ranged from 21 to 29 days. The status of the animals enrolled in the study regarding the presence of F4 and F18 receptors was investigated and the incidence within piglets was 1.6% for F4R+/F18R+, 85.8% for F4R-/F18R+ and 10% for F4R-/F18R-. The number of animals with the F4R was very low which was unexpected. Whilst this may not represent a typical farm, all groups were represented in the safety subgroup.

The batch protocol for the vaccine batch used in the study was provided. All results from in-process and finished product tests were within specifications.

The evaluation of efficacy based on the reduction of incidence and severity of diarrhoea was inconclusive due to natural *E. coli* infection prior to the 7-day OOI of the vaccine and concomitant disease. Serologic levels of anti-F18 IgM were significantly higher in vaccinated animals on day 7 post vaccination compared with the control animals. For anti-F18 IgA, levels were significantly higher in the

vaccinated group compared to the controls on day 21 post vaccination. As for anti-F18 IgG antibody levels, there was no significant difference between treatment groups post vaccination. Serological levels of anti-F4 IgM were not significantly different between groups. For anti-F4 IgA levels, this was significantly higher in the vaccinated group compared to the controls on day 7 post vaccination. As for anti-F4 IgG antibody levels, this was not significantly different between treatment groups post vaccination.

Although the study plan included evaluation of all efficacy parameters included in the laboratory studies, an *E. coli* infection occurred prior to the 7 day OOI, thus efficacy could not be adequately demonstrated in the field. However, this study supports the findings from the laboratory studies and therefore the claims proposed.

Overall conclusion on efficacy

Four laboratory efficacy studies were submitted.

One study was a proof of concept study which was conducted to GCP and used *E. coli* F18 vaccine (Canadian product). The piglets were F18R+ however the F4 receptor status is unknown. The other three studies which were also conducted to GCP were well designed to establish the OOI and DOI and used EU produced product. One study involved vaccination with Coliprotec F4 only and included piglets with and without the F4 receptor gene however the F18 receptor status is unknown. Animals in the other two studies were vaccinated with the bivalent Coliprotec F4/F18 vaccine and included piglets with F18R+/F4R+ (limited number in DOI only) or F18R+/F4R-. The low number of F4R+ animals in the studies was considered not to have any impact in terms of potential interference between strains. Comparing the OOI/DOI with F4 challenge (F4R-/F18R+ or F4R+/F18R+ [few animals in DOI]) and OOI/DOI (F4R+/F18R+) with F18 challenge, the immune response (with similar increases in anti-F18 IgM detected for both F4R+/F18R+ and F4R-/F18R+ vaccinates compared to controls) supports the lack of interference. In addition one field efficacy study was conducted.

An efficacy study was carried out to establish the OOI and DOI following vaccination with Coliprotec F4 and challenge with F4-ETEC. The vaccine was administered at 1.3×10^8 CFU/pig in drinking water to piglets from 18 days of age. In addition, OOI and DOI studies for the F4 vaccine strain were performed following vaccination with Coliprotec F4/F18.

In a pivotal study carried out to establish the OOI and DOI following vaccination with Coliprotec F4/F18 and challenge with F4-ETEC, the vaccine was administered at 5.8×10^7 CFU/pig (F4) and 1.1×10^8 CFU/pig (F18) (thus this supports the minimum efficacious titre for both strains) in drinking water to piglets from 18 days of age. The main efficacy parameters evaluated were the incidence, severity and duration of diarrhoea and faecal shedding. The efficacy parameters supported for both OOI and DOI are a reduction in the incidence of diarrhoea, as well as a reduction in shedding of enterotoxigenic F4-positive *E. coli*. OOI of seven days post-vaccination and DOI of 21 days are supported for F4.

In the pivotal study carried out to establish the OOI and DOI following vaccination with Coliprotec F4/F18 and challenge with F18-ETEC, vaccine was administered at 1.6×10^8 CFU/pig (F4) and 2.8×10^8 CFU/pig (F18) in drinking water to piglets from 17 days of age. The main efficacy parameters evaluated were the incidence, severity and duration of diarrhoea and faecal shedding. The efficacy parameters supported for both OOI and DOI the reduction in the incidence, severity and duration of diarrhoea, as well as a reduction in shedding of enterotoxigenic F18-positive *E. coli*. OOI of seven days post-vaccination and DOI of 21 days are supported for F18.

In both the laboratory and field efficacy studies an assessment of average daily weight gain has been made however the applicant has not made a claim for reduction in weight loss and therefore this parameter will not be considered further.

An OOI and DOI following vaccination with Coliprotec F4 can only be considered supportive, however the anti-*E. coli* F4 IgG were measured and found to be high at the start of the study. The piglets were sourced from pregnant dams which were vaccinated against *E. coli*, hence the presence of maternal antibodies which had not declined at the point of vaccination in study piglets. The maternally derived antibodies present in the piglets did not interfere with the efficacy of the vaccine and this supports the finding of an earlier study.

The route of administration for the OOI and DOI study was in drinking water and therefore the actual volume, and hence dose, that each piglet received could vary and indeed some piglets may not actually receive the minimal intended dose. Nevertheless, as vaccine was administered by drench in the safety studies and the OOI and DOI study did confirm efficacy following vaccination with Coliprotec F18, the recommendation to use of the vaccine as both an oral drench and in drinking water is considered acceptable.

Field studies support the laboratory efficacy studies.

The results from laboratory and field trials show that the product is effective for active immunisation of pigs from 18 days of age against enterotoxigenic F4-positive and F18-positive *E. coli* in order to reduce the incidence of moderate and severe PWD in infected pigs and to reduce faecal shedding of enterotoxigenic F4-positive and F18-positive *E. coli* from infected pigs.

OOI: 7 days after vaccination

DOI: 21 days after vaccination.

Part 5 – Benefit-risk assessment

Introduction

Coliprotec F4/F18 is a live vaccine for active immunisation of pigs against PWD caused by F4-positive and F18-positive *E. coli*. It is presented as a lyophilisate that can be reconstituted in water for administration by drench or in drinking water.

The *E. coli* O8:K87 strain in Coliprotec F4/F18 expresses F4ac fimbriae and is toxin-negative. The strain is non-pathogenic for animals and humans since it is negative for toxins and virulence associated genes associated with intestinal and extra-intestinal *E. coli* diseases.

The *E. coli* O141:K94 strain in Coliprotec F4/F18 expresses F18ac fimbriae and is toxin-negative. The strain is non-pathogenic for animals and humans since it is negative for the toxins, and virulence associated genes, associated with intestinal and extra-intestinal *E. coli* diseases.

The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC.

Benefit assessment

Direct therapeutic benefit

Coliprotec F4/F18 is of value in the treatment of PWD due to *E. coli* which is caused primarily by ETEC, a pathotype that is characterised by production of adhesins that mediate bacterial adherence to the

intestine and enterotoxins that cause diarrhoea. The types of *E. coli* associated with PWD usually have either F4 or F18 fimbrial adhesins that mediate their attachment to intestinal cells.

Coliprotec F4/F18 has been shown to reduce the incidence of moderate to severe post-weaning *E. coli* diarrhoea and faecal shedding of enterotoxigenic F4-positive and F18-positive *E. coli* in pigs vaccinated from 18 days of age.

OOI was demonstrated from 7 days post-vaccination

DOI was demonstrated from 21 days post-vaccination.

Additional benefits

Vaccination of young pigs against PWD caused by *E. coli* may lead to a reduction in the use of therapeutic antibiotics.

The vaccine is administered by drench or in drinking water and is therefore easy to apply. Moreover, the multi-dose presentation and the combination of the two vaccine strains (bivalent vaccine), instead of having two monovalent vaccines administered at the same period, has the advantage of reducing the handling of multiple vials during the preparation of the single vaccine solution by the user.

The vaccine reduces the shedding of F4-positive *E. coli* and F18-positive *E. coli* and may therefore reduce the field contamination by this organism.

Risk assessment

Main potential risks:

Quality:

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

Safety:

For the target animal:

Administration of Coliprotec F4/F18 in accordance with SPC recommendations is generally well tolerated in the target animal.

The administration of an overdose of F4 and F18 did not cause significant clinical signs, however, there were isolated peaks of body temperature which were more severe following an overdose of vaccine and this is reflected in section 4.10 of the SPC.

The vaccine consists of live organisms which spread, and therefore has the potential to infect non vaccinated animals. However, the vaccine strains lack the genes for a range of virulence factors and are therefore not pathogenic. Serial passage through pigs did not indicate any risk that the organisms might increase in virulence or acquire virulence factors from environmental bacteria. Infection of non-vaccinated in-contact animals might occur, however this is without adhesion and replication in species which do not have the F4/F18 receptors.

For the user:

The vaccine strains are specific for pigs and hence the strains are not able to colonise and therefore reproduce disease in non-target species, including humans and immunosuppressed humans. As vaccine organisms are transported by peristalsis along the intestines and shed in faeces of pigs, infection might occur in other animal species, however this is without adhesion and replication in species which do not have the F4/F18 receptors. The F18 vaccine strain is haemolytic and information has been provided to confirm that this is not a risk factor for the user. The CVMP concluded that the user safety for the product is acceptable when used as recommended and taking into account the safety advice in the SPC.

For the environment:

Environmental exposure is likely however this would not be higher than for any other intestinal *E. coli*, including commensal *E. coli* and pathogenic *E. coli* such as F4-ETEC.

No studies have been conducted using F18R- animals therefore it might be possible for other species that do not have the F18 receptor to also develop some clinical signs after exposure to the vaccine. Vaccine organisms are transported by peristalsis along the intestines and shed in faeces which may result in some clinical signs, however without the F4/F18 receptors there is no adhesion and replication, hence the safety risk for other species can be considered negligible. Coliprotec F4/F18 is not expected to pose a risk for the environment when used according to the SPC.

For the consumer:

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

Risk management or mitigation measures

Appropriate information has been included in the SPC to inform on the potential risks of this product relevant to the target animal, user, consumer and the environment and to provide advice on how to prevent or reduce these risks.

Evaluation of the benefit-risk balance

The vaccine is efficacious in reducing the incidence of moderate and severe post-weaning *E. coli* diarrhoea and faecal shedding of enterotoxigenic F4-positive and F18-positive *E. coli* in pigs vaccinated from 18 days of age.

Information on development, manufacture and control of the active substances and finished product has been presented and lead to the conclusion that the product should have satisfactory and uniform performance in clinical use. It is well tolerated by the target animals (pigs) and presents an acceptable risk for users and the environment, when used as recommended.

Appropriate precautionary measures, including withdrawal period, have been included in the SPC and other product information.

The overall benefit-risk evaluation for the product is positive.

Conclusion on benefit-risk balance

Based on the CVMP review of the data on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for Coliprotec F4/F18 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.