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Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Porcilis ColiClos (EMEA/V/C/002011)

Common name: Vaccine for the passive immunization of piglets against *E. Coli* and *C. perfringens*

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

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Introduction

An application for the granting of a Community marketing authorisation of Porcilis ColiClos has been submitted to the Agency on 28 September 2010 by Intervet International BV in accordance with article 3(1) of Regulation (EC) No. 726/2004.

The CVMP adopted an opinion and CVMP assessment report on 11 April 2012.

On 14 June 2012, the European Commission adopted a Commission Decision for this application.

Porcilis ColiClos is administered in a 2 ml dose containing *E. coli* (F4ab, F4ac, F5, F6, LT) and *C. perfringens* type C antigens and is presented in containers of 1 polyethylene terephthalate (PET)or glass vials of 20, 50, 100, 200 or 250 ml closed with a halogenobutyl rubber stopper and sealed with an aluminium cap. It is indicated for the passive immunisation of progeny by active immunisation of sows and gilts to reduce mortality and clinical signs during the first days of life, caused by those *E. coli* strains, which express the adhesins F4ab (K88ab), F4ac (K88ac), F5 (K99) or F6 (987P) and caused by *C. perfringens* type C.

The route of administration is intramuscular. The target species is pigs (sows and gilts). *Clostridium perfringens* vaccines for pigs are included in the list for minor uses/limited markets for IVMPs appended to Guideline on Data requirements for immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMEA/CVMP/IWP/123243/2006-Rev.2). The provisions of this guideline can therefore be applied for this component of the vaccine.

Part 1 - Administrative particulars

The administrative data provided are satisfactory.

Whilst the final product is formulated, filled and released at one manufacturing site only, the manufacturing of the active ingredient can take place at two alternative Intervet sites. Intervet International BV has established a pharmacovigilance system, which is considered satisfactory.

Valid manufacturing licences for all sites are available and currently valid GMP certificates for all manufacturing sites were provided.

Part 2 - Quality

Composition

Porcilis ColiClos is an inactivated subunit vaccine containing as active substances the heat-labile toxin toxoid (LT) and the fimbrial antigens F4ab, F4ac, F5 and F6 from the various types of *E. coli* as well as *C. perfringens* type C toxoid and micro Diluvac Forte, an adjuvant based on dl-α-tocopheryl acetate. The excipients are polysorbate 80, simethicone and phosphate buffered saline (PBS). The diluent is water for injections.

Container

The containers are made of PET or glass type I European Pharmacopoeia (Ph. Eur.) 3.2.1 compliant closed with a halogenobutyl rubber stopper (Ph. Eur. 3.2.9 compliant) and sealed with a γ-irradiated or heat sterilised coded aluminium cap.

Development pharmaceutics

Porcilis ColiClos has been developed for the prophylaxis of neonatal enterotoxicosis (ETEC) and *C. perfringens* type C infections in piglets by a single vaccine. Porcilis Porcoli Diluvac Forte (EU/2/96/001/003-008) is an authorised predecessor vaccine to Porcilis ColiClos. Porcilis ColiClos contains the same *E. coli* antigens present in Porcilis Porcoli Diluvac Forte apart from the amounts of LT antigen which was more than doubled. In addition Porcilis ColiClos contains *C. perfringens* type C antigen and a dl-α-tocophyeryl acetate based adjuvant. Porcilis ColiClos is administered intramuscularly with a primary injection at 6-8 weeks pre-farrowing and a second injection 4 weeks later. After primary vaccination, pigs can be re-vaccinated with a single dose during the second half of subsequent pregnancies at 2-4 weeks pre-farrowing.

Method of manufacture

The analytical part is satisfactorily documented. Validation of the production steps was provided. All the production steps are carried out by Intervet subsidiaries.

Manufacture of the vaccine antigens

The description of the manufacturing method is satisfactorily documented.

The *E. coli* antigens are produced using genetically modified *E. coli* bacteria except for the F6 antigen. The *E. coli* fimbrial antigens are removed by heat-treatment and the cells of the production strains are removed by centrifugation. The fimbrial antigens are subsequently isolated and concentrated before inactivation is carried out.

The *E. coli* F6 component will be produced using an animal component free medium (ACF). The batch protocol for a third batch manufactured using ACF F6 is still required to demonstrate consistency of production.

The LT antigen is purified and concentrated and subsequently detoxified.

The *C. perfringens* type C antigen is produced by culturing a *C. perfringens* type C strain. The toxin is harvested in the supernatant after centrifugation and detoxified.

Porcilis ColiClos contains an aqueous dl-α-tocopheryl acetate based adjuvant. The final vaccine is produced by preparing an *E. coli* and *C. perfringens* type C antigen mixture, which contains fixed amounts of all antigens sufficient for the formulation of the final product.

Control of starting materials

The nature of the raw materials, controls and treatments applied guarantee sterility of the vaccine and absence of introduction of any extraneous agent. To guarantee consistency and homogeneity of the production controls are performed on raw materials and in process parameters.

Active substance

E. coli master seeds and working seeds were prepared by the same methods. The respective *E. coli* seeds as well as the master seed for *C. perfringens* were obtained from different sources.

Excipients

Certificates of analysis of starting materials listed in Ph. Eur. were provided and are satisfactory. Up-to-date EDQM certificates and/or certificates of analysis for substances of biological origin used during

production were provided. Certificates of analysis of the starting materials of non-biological origin were provided and are satisfactory. Details of in-house preparation of media were provided.

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

The starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE Note for Guidance and Commission Directive 1999/104/EEC.

Control tests during production

Tests proposed for the *E. coli* components of the vaccine are sterility of medium, purity of inoculums and cultures, inactivation, removal of the LT production strain and determination of the antigen content for each *E. coli* component, and sterility and detoxification test for the LT component. Tests proposed for *C. perfringens* type C antigen are purity of inocula and cultures, test of detoxification, determination of toxoid content and sterility of the antigen concentrate. Validation of the toxoid content determination of the *C. perfringens* component was provided and is acceptable.

Control tests on the finished product

Tests proposed for the final vaccine are appearance, *E. coli* potency and identity test (mean $\log_2$ antibody titres obtained after vaccination of mice: F4ab:≥9.7; F4ac:≥7.8; F5:≥7.4; F6:≥7.6; LT:≥12.0), *C. perfringens* identity and potency test (≥10 IU/ml), determination dl-α-tocopheryl acetate concentration, endotoxin test, safety, sterility and second inactivation control, pH and final inspection. Tests on filling volume are carried out at regular intervals during filling. The applicant will re-evaluate the batch release titres for F5, F6 and LT based on revised calculations for the tolerance interval once a larger sample size is available. The applicant has provided an acceptable justification not to perform a final product formaldehyde test.

The description of the methods used for the control of the finished product and the specifications proposed at release and at the end of shelf-life are appropriate to control the quality of the finished product.

The results of the analysis of consecutive pilot scale production runs and production scale batches were presented which comply with the required specifications.

Stability

Data on the stability of the bulk antigen was provided with a batch that had been produced with antigens stored for 3 years. Data demonstrating stability for this batch up to 27 months support the shelf-life of 24 months.

Data to demonstrate stability of the finished product were provided for storage up to 27 months for product filled in PET and glass containers. Stability was demonstrated for the smallest and the largest PET vial presentation and the smallest vial size of glass presentation when filled from pilot batches.

Stability data on the broached vial stored for three days at 30 °C was provided and considered acceptable for the 10 hour in-use stability. It further supports that the product may be transported at room temperature.

Overall conclusions on quality

The production of the *E. coli* antigens is the same as for Porcilis Porcoli Diluvac Forte but the blending process is different. The equivalence between these two vaccines has been clarified. All information regarding the qualitative and quantitative composition, the starting materials, production method,

quality controls, and stability are described. Appropriate procedures have been implemented to ensure the absence of extraneous agents in starting materials of animal origin. A TSE risk assessment for the starting materials used is provided. The risk that the final product may transmit TSE to the target animal was estimated as zero. The production method, including appropriate in-process controls and quality control on the finished product, together with thorough control of the starting materials, ensure a consistent quality, safety and efficacy of batches of vaccine. However, the minimum pass levels for the potency testing of the *E. coli* F5, F6 and LT components still need to be further justified. The whole production process was evaluated by production of intermediates and final product. The *E. coli* F6 component will be produced using an animal component free medium (ACF). The batch protocol for a third batch manufactured using ACF F6 is still required to demonstrate consistency of production. Results of the stability tests for bulk antigen and final product support a storage period of 24 months for the final product. Stability data for the product stored at 30 °C showed that the vaccine remains stable for the claimed 10 hour in-use shelf-life and also supports transport at room-temperature.

The applicant will re-evaluate the F5, F6 and LT batch release titres based on revised calculations for the tolerance interval once a larger sample size is available.

Part 3 – Safety

Safety documentation

Porcilis ColiClos is a subunit vaccine for use in the pregnant sow and gilt, in order to provide passive protection to the piglets. The vaccine contains *E. coli* fimbrial adhesins and LT toxoid, *Clostridium perfringens* type C toxoid and a dl-α-tocopheryl acetate based adjuvant. The indications for use proposed are to reduce mortality and clinical signs caused by those *E. coli* strains which express the relevant adhesins, and that caused by *C. perfringens* type C. The antigens and adjuvant have all been used in previously approved vaccines but in somewhat different formulations. The primary course is a two dose regime, with one vaccination of 2 ml i.m. at 6-8 weeks pre-farrowing, and the second vaccination 4 weeks later approximately 2 weeks pre-farrowing. Revaccination is recommended as a single vaccination 2-4 weeks before subsequent farrowings. The current proposed SPC warnings regarding adverse reactions are:

Adverse reactions (frequency and seriousness)

An increase in body temperature up to 2 °C may be observed on the day of vaccination. Reduced activity and lack of appetite on the day of vaccination commonly occurs and/or a sometimes painful and hard swelling up to 10 cm diameter for up to 25 days may be observed at the site of injection.

Overdose

A slight local redness and/or roughness may transiently occur after a double dose vaccination. No adverse reactions other than those mentioned in the SPC section “adverse reactions” have been observed.

The overdose did not produce a greater increase in body temperature than stipulated. The duration of measured local reactions in the pregnant sow was 25 days which is adequately reflected in the SPC. Hard swellings have been observed in one study with a maximum of 7 cm having resolved after 2 days. The maximum diameter at the injection site of a single and a double dose in other studies were recorded as >6 cm. The applicant has included a maximum of 10 cm in the SPC text and this is acceptable. Pain was detected in a field study in one animal after vaccination. The major concerns regarding reproductive safety have been addressed. A follow-up study, demonstrated that

administration of a single booster dose is safe. In addition field data indicate that the repeat dose administration may not result in additional safety concerns.

Laboratory tests

Safety of the administration of one dose

There are no data presented in support of the safety of the primary schedule (two injections with a single dose) in pregnant sows, instead the sows were given a double dose and then a single dose. In this instance the double dose was given at 6 weeks pre-farrowing, and the single dose 4 weeks later, at 2 weeks pre-farrowing. In this study 67% of animals had a local reaction, with maximum size of >6 cm. Local reactions persisted in three animals past the 14 day local reaction monitoring period. Body temperatures showed only slight rises, which were well within the Ph. Eur. monograph requirements for compliance. In another study performed in pregnant sows a maximum local reaction of 7 cm (diameter) for 2 days was shown in one animal.

Two studies on the administration of one dose (single dose) in finishing pigs (not the target category) and respective histopathology results were provided with 40% animals showing transient palpable reactions with a maximum size of 2 cm in diameter for up to 10 days. Histological data were presented with samples examined at 14 and 70 days post vaccination. The histological changes were characteristic of the adjuvant, and appeared to resolve over time, although scarring may occur. These data are considered supportive only as the studies were not performed in the target category. The reactions are adequately reflected in the SPC section 4.6.

Safety of one administration of an overdose

The applicant presented initially two studies in support of the safety of an overdose. In the first study there was a death in the test group. This was due to *Staphylococcus* infection. 82% and 17% of pigs had local reactions in the two studies respectively. The maximum size of the local reactions observed was 6 cm. The reactions lasted a maximum of 25 days. Following the single dose given after a double dose in pregnant sows monitoring stopped at 14 days, when lesions were still palpable. Body temperatures showed only slight rises, which were well within the Ph. Eur. monograph requirements for compliance. In the second study none of the animals showed signs of systemic reactions. Some transient local reactions were observed (maximum diameter ≥ 6cm). The increase in body temperature was well within the Ph. Eur. requirements. A new study was performed with a double dose followed by a single dose in pregnant sows where injection site reactions observed 3 days after the first injection were resolved within 2 days. The reactions are adequately reflected in the SPC section 4.10.

Safety of the repeated administration of one dose

Revaccination is recommended at each pregnancy following that in which the primary course is given, which in commercial herds might mean approximately every 5 months. The initial studies presented a double dose followed by single dose in pregnant sows and did not include a third dose as required by the Ph. Eur. A follow-up study in which sows that had been previously vaccinated with a double dose followed by a single dose were given a third dose during a subsequent pregnancy was therefore carried out. In this study, 30% of the vaccinated animals had a local reaction of up to 2 cm diameter that disappeared by day 4. Slight temperature rises were noted following vaccination but these were similar to those seen during the primary course. It is concluded therefore that administration of a repeated single booster dose is safe.

Examination of reproductive performance

Initially, two studies were presented, both carried out in pregnant gilts. In both studies the gilts received a double dose at 6 weeks pre-parturition followed by a single dose 4 weeks later. In one study no adverse events occurred, in the second study, 75% of test gilts and 13% of control gilts had dead piglets. Also, two deformed piglets were born to test gilts, and none to controls. The percentage of dead piglets in the test group was higher than that in the control group. Further assurances regarding the safety of the vaccine during pregnancy were provided with a new study which showed no significant difference between groups. In a follow-up study, sows that had been previously vaccinated with a double dose followed by a single dose were given a third dose during a subsequent pregnancy. Farrowing data for both vaccinated and control groups were similar and close to the farm means in the same period. Therefore, it is concluded that there was no adverse effect of use of the vaccine on reproductive performance.

Examination of immunological functions

No data were presented with respect to immunological functions as no adverse effects on the immune system are known or expected with this kind of inactivated vaccine.

Special requirements for live vaccines

The vaccine is inactivated and therefore this is not applicable.

Study of residues

A zero days withdrawal period is proposed. This is based on the vaccine components, and their relative concentrations. The active substances being of biological origin intended to produce passive immunity are not within the scope of Regulation (EC) No 470/2009.

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

Interactions

No data concerning interactions with other veterinary medicines were provided and the respective standard warnings are stated in the SPC.

Field studies

Data from a total of approximately 440 test vaccinated and 300 control vaccinated pigs are presented. The local reactions observed in these studies were similar in size, nature and duration to those in the laboratory studies. General observations indicated some inappetance and suppression of normal demeanour, and this is reflected in the SPC. Satisfactory clarification with respect to adverse events such as abortions and deaths in test sows was provided; No significant difference in the outcomes of the pregnancies between the test and control groups was observed. Some discrepancies identified in these studies were addressed and were taken into account in the overall assessment.

User safety

An appropriate user safety exposure assessment was provided. Porcilis ColiClos is an inactivated sub-unit vaccine containing *E. coli* fimbrial antigens, *E. coli* LT toxoid and *C. perfringens* type C toxoid. The vaccine contains an aqueous dl-alpha-tocopheryl acetate adjuvant. Excipients included in the vaccine are polysorbate 80, dimethicone, sodium chloride, potassium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate and water for injection. The excipients and adjuvant do not pose risk to the user.

The *E. coli* fimbrial production strains are removed with centrifugation and/or filtration and finally inactivated. The *E. coli* LT production strain is removed by a combination of centrifugation and filtration. Removal of live *C. perfringens* is carried out by a combination of centrifugation, formaldehyde treatment and filtration. Inactivation and sterility are both assessed during production, and on the final product.

The user safety for this product is acceptable when used as recommended and taking into account the safety advice in the SPC.

Environmental risk assessment

Porcilis ColiClos is an inactivated subunit vaccine containing fimbrial antigens (F4ab, F4ac, F5, and F6) and LT toxoid of enterotoxinogenic *E. coli* and *C. perfringens* type C antigen, presented in an aqueous dl-alpha-tocopheryl acetate based adjuvant. The vaccine is to be administered intramuscularly to pregnant sows and gilts. Freedom from live organisms is guaranteed by the manufacturing process (GMP). The vaccine is filled into glass or PET vials, and packaging is conventional. Disposal of unused vaccine should be carried out according to local requirements. The risk of possible ecological effects of the inactivated agents, the adjuvant or excipients, is considered negligible. All hazards identified have a negligible likelihood to occur and therefore assessment of the consequences is not necessary. The level of risk is assessed as negligible, and therefore no Phase II assessment is necessary.

Overall conclusion on safety

This inactivated vaccine contains antigens, and an adjuvant, that are found in other combination vaccines. Batch release documentation is provided in Part 2.

The safety data with respect to local reactions, and body temperature rises, are unremarkable, and, where applicable, monograph compliant. During a monograph compliant study in the pregnant sows, farrowing anomalies occurred and these were addressed by performing a new study. There is a substantial amount of field data supporting safety of this product in pregnant pigs which is generally reassuring but, is set within the context of the intensive nature of pig farming enterprises and the resulting lack attention to the individual animal and the acceptance of high mortality rates in piglets. Data from a follow-up study in which sows that had been previously vaccinated with a double dose followed by a single dose were given a third dose during a subsequent pregnancy demonstrate that administration of a single booster dose is safe. Some field data are provided (with revaccination in the subsequent pregnancy at 2 weeks pre-farrowing. 12.5% of the sows of the re-vaccination group had a local reaction, whereas no control sows reacted. The maximum size recorded was 3 cm diameter, and the reaction was hard but not painful except for one animal. The longest duration of reaction was 7 - 14 days. Adequate advice on adverse reactions observed in the safety studies has been included in the SPC.

Porcilis ColiClos presents a low risk for users and the environment when used as described in the SPC.

Part 4 – Efficacy

Introduction and general requirements

Porcilis ColiClos is a vaccine for use in sows and gilts, in order to passively protect their offspring from mortality and clinical signs due to *E. coli* strains expressing the contained adhesins, and that caused by *C. perfringens* type C.

The relevant SPC claims are:

4.2 indications for use, specifying the target species

For the passive immunisation of progeny by active immunisation of sows and gilts to reduce mortality and clinical signs during the first days of life, caused by those *E. coli* strains, which express the adhesins F4ab (K88ab), F4ac (K88ac), F5 (K99) or F6 (987P) and caused by *C. perfringens* type C.

5. Immunological properties

To stimulate active immunity in order to provide passive immunity to the progeny against enterotoxicosis caused by *E. coli* expressing fimbrial adhesins F4ab (K88ab), F4ac (K88ac), F5 (K99) or F6 (987P) and LT toxin and against (necrotic) enteritis caused by *C. perfringens* type C. Vaccination results in an antibody response with neutralizing activity against LT toxin.

Primary vaccination consists of 2 intramuscular (i.m.) doses of 2 ml each, with an interval of 4 weeks. Intramuscular vaccination behind the ear is recommended, as for other vaccines in this class of animal species. The recommendations are that the first dose is to be given at 6-8 weeks pre-farrowing, and the second 4 weeks later (2-4 weeks pre-farrowing). Revaccination is recommended as a single dose of 2 ml i.m., given 2-4 weeks before each subsequent farrowing.

Ph. Eur. monographs exist for both “neonatal piglet colibacillosis vaccine (inactivated)” (E.P. 0962), and “*Clostridium perfringens* vaccine for veterinary use” (E.P. 0363).

Laboratory trials

E. coli claims

The Ph. Eur. monograph on neonatal piglet colibacillosis vaccine (01/2008:0962) is relevant.
A predecessor vaccine to Porcilis ColiClos, Porcilis Porcoli Diluvac Forte, is authorised in the EU, and data that were previously presented in support of that application were re-presented here. Porcilis ColiClos contains the same *E. coli* antigens present in Porcilis Porcoli Diluvac Forte apart from the *E. coli* LT component which is contained at a higher amount of that in Porcilis Porcoli Diluvac Forte.

The Porcilis Porcoli Diluvac Forte efficacy data are presented in 3 laboratory studies. One is a challenge study, and two serological studies. The challenge study was previously judged to be monograph compliant. It demonstrates efficacy against all four *E. coli* antigens at challenge, and the applicant calculated “protective titres” required in the colostrum.

The second Porcilis Porcoli Diluvac Forte study was serological. Experimental low-dose vaccines raised colostral antibody titres that were not statistically significantly different to those raised by the full dose vaccine.

The third study was also serological, and presents titres from pigs revaccinated with Porcilis Porcoli Diluvac Forte in a subsequent pregnancy.

New serological studies were carried out with both Porcilis ColiClos and Porcilis Porcoli Diluvac Forte. There was a statistically significant difference between Porcilis ColiClos vaccinated sows and

unvaccinated sows and the *E. coli* colostral titres were above the defined protective level. Antibody data from Porcilis ColiClos vaccinated sows in a field trial are similar to protective levels.

Usually when data from the use of a different vaccine are presented, the argument for relevance is on the argue-down principle i.e. that the data on the more complex vaccine is relevant to the less complex, which might be considered a "subset" of the antigens present. In this instance the argument rests on the establishment of a so-called "protective titre" in the Porcilis Porcoli Diluvac Forte study. As the titre is in the colostrum, and both vaccines rely on the transfer of passive immunity from colostrum to piglet, this is relatively sound providing the assay by which the protective titre is established is properly validated, reproducible and repeatable, and also, importantly, is measuring antibodies of relevance. This approach should be sustainable.

The assessment of the efficacy of Porcilis ColiClos therefore rests on the following assumptions:

- The efficacy of Porcilis Porcoli Diluvac Forte is proven by the studies presented here, and is (sufficiently) monograph compliant.
- The colostral antibody titre in one Porcilis Porcoli Diluvac Forte challenge, in which piglets were judged protected, is definitive, and can be reasonably described as a "protective titre".
- The "protective titre" as measured by the assay may be extrapolated to define the efficacy of different vaccines, and hence:
- Achieving a "protective titre" renders the efficacy of another vaccine (Porcilis ColiClos) monograph compliant by association.
- Vaccination with Porcilis ColiClos results in the generation of colostral titres at least as great as those judged protective.

Therefore the following is relevant.

- Porcilis Porcoli Diluvac Forte has a European marketing authorisation, based on the data re-supplied in this dossier, and therefore its efficacy in these studies is considered established. It is therefore considered sufficiently compliant with the monograph.
- In order to extrapolate to other vaccines, the assay employed must be measuring antibodies of relevance. Also, the assay must be adequately validated, and be reproducible and repeatable, as in this case results from recent studies are being compared with those from assays carried out previously for another vaccine.

The tests for Porcilis Porcoli Diluvac Forte and Porcilis ColiClos were all performed in the same laboratory, using the same reagents. A statistical analysis of repeatability and intermediate precision was presented by using the titres of the reference serum, which is included in every plate to monitor the performance of the test, measured in the assays performed on ten different days and the applicant demonstrated that the colostrum antibodies elicited by both vaccines are able to block the adhesion properties (haemagglutination) of pathogenic *E. coli* strains that express F4ab, F4ac, F5 or F6 fimbriae. Furthermore, it was shown that the toxicity of LT can be neutralized by colostrum from vaccinated sows. Specificity, sensitivity and relevance of the antibodies measured in the ELISA was provided.

Vaccination with Porcilis ColiClos did result in *E. coli* titres that were considered protective in the Porcoli Diluvac Forte studies.

One re-vaccination study is presented for Porcilis Porcoli Diluvac Forte, which cites titres from pigs revaccinated with Porcilis Porcoli Diluvac Forte in a subsequent pregnancy. There is no negative control group, thus, these results are of limited value.

The re-vaccination study for Porcilis ColiClos provided in the dossier was uncontrolled which made it difficult to interpret and to determine whether the re-vaccination was the cause of the higher colostrum antibody level, or whether it was due to environmental exposure. Nevertheless, the latter possibility was unlikely because of the choice of farm and the fact that the antibody levels of all components were only raised just after vaccination made it unlikely that the raise would be due to an *E. coli* infection. The data provided showed that revaccination with a single dose 2-4 weeks before each subsequent farrowing induced colostral antibody levels which were comparable with the primary vaccination or statistically higher.

Inclusion of LT toxoid in the formulation

Data demonstrating the induction of neutralising antibodies to LT toxin have been provided. In that light it is justified to describe under section 5 of the SPC that the vaccine will induce neutralising *E. coli* LT antibodies.

Clostridium perfringens type C claims:

The Clostridial component of this vaccine may be considered as a product for minor use, according to the relevant guideline EMEA/CVMP/IWP/123243/2006-Rev.2, Table 2 of that guideline lists *Clostridium perfringens*, among various other disease conditions in the pig, as minor use. Therefore, reduced data requirements in accordance with guideline EMEA/CVMP/IWP/123243/2006-Rev.2 are applicable for this component of the vaccine. In addition, the Ph. Eur. monograph 01/2008:0363, *Clostridium perfringens* vaccine for veterinary use, is relevant.

The applicant presented several laboratory studies in support of this claim.

In the first laboratory study a *C. perfringens* type C challenge was given to piglets born to vaccinated dams. Unfortunately the challenge was insufficient to allow a clear differentiation between groups. There were no significant differences in mortality or clinical scores between the vaccinated group and the unvaccinated controls. However, mean anti-beta toxin titres were significantly higher in vaccinated sows' colostrum, and in piglets in the vaccinated group, when compared to the controls. A challenge optimisation was carried out in a further study in unvaccinated animals. It was concluded that the piglets should be bottle fed on colostrum prior to challenge, to avoid wide ranges of antibody levels.

Following challenge optimisation and colostrum production, a further challenge study was undertaken. In this study neonatal piglets born to antibody negative gilts were removed from their dams and randomly assigned between two treatment groups. The test group received colostrum from sows vaccinated with Porcilis ColiClos according to schedule with a high anti-beta toxin titre. The control group received low titre colostrum. Statistically fewer piglets fed immune colostrum showed signs of disease, and clinical scores were significantly lower, compared with piglets fed control colostrum. The total number of deaths was not statistically different between groups.

A serological study was carried out on two sites. Control pigs, either vaccinated with Porcilis Porcoli Diluvac Forte or unvaccinated, depending on site, were included for serological comparison, as was a diluted Porcilis ColiClos vaccine. After the first pregnancy, and primary course, colostrum mean anti-beta toxin titres were significantly higher in the Porcilis ColiClos vaccine groups than in the control groups. There was no significant difference between the full strength and diluted Porcilis ColiClos groups. The second phase of this study involved the revaccination of the Porcilis ColiClos vaccinate group. Revaccination was carried out according to recommendations. However, no controls were

included. The mean colostral antibody titres following re-vaccination according to vaccination scheme were statistically significantly higher than those in the first pregnancy following the primary course. The applicant also tested some sera from laboratory and field trials in the mouse neutralisation test (MNT), which is an *in-vivo* challenge test described in the monograph. This is the gold standard for serological testing. Subsequently, samples have to be pooled, in order to reduce animal numbers used. The MNT results demonstrated the toxin neutralising capability in pools of colostrum and piglet serum following sow vaccination, both from the laboratory and field.

In summary, therefore, the MNT demonstrated the presence of beta toxin neutralising antibody in the colostrum of vaccinated sows, and the transfer to newborn piglets through natural suckling behaviour. Susceptible piglets bottle fed immune colostrum were afforded protection against *C. perfringens* type C challenge. Statistically significant fewer immune colostrum fed piglets showed any clinical signs following challenge, and they showed statistically significantly lower clinical scores over the observation periods, when compared to piglets fed antibody negative colostrum. It is therefore clear from the data provided that the recommended primary course stimulates antibodies in the gilt, and that these appear in the colostrum at a biologically significant level. Piglets fed the colostrum of vaccinated gilts were less likely to be affected by disease and showed a reduction in clinical signs. While, some deaths did occur in the test group, this was not significantly different from the number of deaths in control piglets.

The reduction in clinical signs caused by *C. perfringens* type C claimed for the primary course of vaccination, administered at 6 and 2 weeks pre-farrowing, is supported by the laboratory studies.

The laboratory challenge trial did not find a statistically significant difference in mortality between piglets fed immune colostrum pre-challenge, and piglets fed control colostrum pre-challenge. Data on re-vaccination provided are in a laboratory antibody study. However, in this study the re-vaccination phase was uncontrolled. Mean colostral antibody titres following re-vaccination were statistically significantly higher than those in the first pregnancy following the primary course.

There are no re-vaccination efficacy data from field. It should be noted that there are some re-vaccination safety data, from sows vaccinated approximately 6 weeks pre-farrowing, but the revaccinated animals were not bled or otherwise sampled.

Only one batch of vaccine was used in laboratory trials. All components are present in the vaccine at above the release levels. The MUMS guideline allows for derogation of minimum/maximum potency requirements, where formulation of the final product is standardised. Various batches were used in the field trials presented, which provide good evidence of a serological response to the vaccine against beta toxoid.

In conclusion, a claim for use at 6 and 2 weeks pre-farrowing, to provide reduction in clinical signs in piglets is supported whereas the claim for reduction in mortality is not supported by the laboratory studies.

Field trials

E. coli

One negative-controlled field trial using animals vaccinated with Porcilis ColiClos was provided. However a claim for use at 6-8 and 2-4 weeks pre-farrowing to provide reduction in clinical signs and mortality was dependent on the demonstration of the overall suitability and robustness of the serological assay determining protective antibody levels that is fundamental to the argument for the extrapolation from Porcilis Porcoli Diluvac Forte to Porcilis ColiClos. The suitability of the serological assay for the field trials was justified.

There are no re-vaccination efficacy data from the field studies because revaccinated animals were not bled or otherwise sampled.

C. perfringens type C

Negatively controlled field trials in approximately 400 pigs on seven farms provide evidence that a primary vaccination course with Porcilis ColiClos results in an increased anti-beta toxin titre both in colostrum and in the sera of suckling piglets.

There was no statistically significant difference in mortality in the first field trial, on 3 farms, because there was no infection pressure. On one farm the study had to be invalidated. In other two farms a statistically significant reduction in mortality was seen. There was no statistically significant impact on mortality in another farm during the first trial, but there was during the second trial.

In summary, on approximately half of the farms a statistically significant improvement on mortality was seen. On each of these farms the control group was vaccinated with Porcilis Porcoli Diluvac Forte. Therefore, the only difference in antigen status between the groups was the inclusion of beta toxoid in the test group vaccine. On one of the three farms there was a significant reduction in mortality in the group vaccinated with Porcilis ColiClos compared to the group vaccinated with Porcilis Porcoli Diluvac Forte. Clinical signs seen in this study were consistent with *C. perfringens* type C infection and *C. perfringens* type C organisms were isolated from the farm during the study. This supports a reduction in clinical signs and mortality claim against *C. perfringens* Type C. On the other two farms the applicant states that *C. perfringens* type A was diagnosed. Overall, although a reduction in mortality was seen in those farms, the mechanism of protection was inconclusive.

Overall conclusion on efficacy

Although the revaccination scheme is not supported by negatively controlled data, further justification was given that the observed increases of antibody levels in the colostrum at the second parturition were solely the result of re-vaccination.

E. coli components:

The pivotal studies were based on protective colostrum antibody titres determined with Porcilis Porcoli Diluvac Forte. A reduction in clinical signs and mortality caused by *E. coli* is supported by the laboratory and field studies. Confirmation was provided by laboratory tests (ELISA) used to measure the antibody response against the fimbrial adhesins and the LT antigen in the target animal are identical to those used in the previous studies using Porcilis Porcoli Diluvac Forte. Validation of the ELISA used in the studies for Porcilis Porcoli DF was performed at that time determining intermediate precision and repeatability of the assay but not reproducibility. In addition sensitivity and specificity have not been determined. The applicant provided confirmation that the ELISA was performed by the same laboratory as well as demonstration of sensitivity and specificity of the ELISA (antibodies detected are those biologically relevant).

Information has been provided with respect to the justification for inclusion of LT toxoid in the formulation. Although it was demonstrated that the vaccination elicits an antibody response with neutralizing activity against LT toxin, it is not clear at what extent the LT component contributes with the vaccine claims. Therefore no claim may be stated in section 4.2 of the SPC. However, it is justified to describe under section 5 of the SPC that the vaccine will induce neutralising *E. coli* LT antibodies.

C. perfringens type C component:

The reduction in clinical signs caused by *C. perfringens* type C is supported by laboratory and field studies and the reduction of mortality by field studies.

Part 5 – Benefit risk assessment

Introduction

Porcilis ColiClos is an inactivated subunit vaccine containing as active substances the LT toxoid and the fimbrial antigens F4ab, F4ac, F5 and F6 from the various types of *E. coli* as well as toxoid of *C. perfringens* type C and micro Diluvac Forte, an adjuvant based on dl-α-tocopheryl acetate.

Porcilis ColiClos was eligible for the submission of a dossier for granting of a Community marketing authorisation via the centralised procedure under Article 3(1) of Regulation (EC) No. 726/2004 as several strains are created using recombinant technology. The clostridial part of this product can be considered as a product for minor use according to the guideline on Data requirements for Immunological veterinary medicinal products intended for minor use or minor species/limited markets EMEA/CVMP/IWP/123243/2006-Rev.2.

Benefit assessment

Direct therapeutic benefit

Porcilis ColiClos with its combination of *E. coli* components and *C. perfringens* type C toxoid provides benefit by reducing mortality and clinical signs of neonatal piglet diarrhoea and necrotic enterotoxaemia. The active substances as well as the adjuvants and excipients are well-known as other products with the *E. coli* components (Porcilis Porcoli Diluvac Forte) and *C. perfringens* type C toxoid (Heptavac P Plus) have been approved previously.

Additional benefits

Porcilis ColiClos increases the range of available vaccines for the neonatal piglet diarrhoea and necrotic enterotoxaemia. It also may reduce the use of antimicrobials in porcine production units.

Risk assessment

To date the following risks have been identified:

Quality:

The batch protocol for a third batch manufactured using ACF F6 is still required to demonstrate consistency of production.

For the target animal:

- No significant risk has been identified.

For the consumer:

- No significant risk has been identified.

For the environment:

- The risk of possible ecological effects of the inactivated agents, the adjuvant or excipients, is considered negligible.

For the user:

- The user safety for this product is acceptable when used as recommended and taking into account the safety advice in the SPC.

Specific potential risks:

- The pass levels set for the potency test of the *E. coli* components need to be further justified.

Risk management or mitigation measures

Appropriate safety advice has been included in the SPC.

The marketing authorisation holder is required to complete the following post-authorisation measures:

- To demonstrate consistency of production the batch protocol for a third batch manufactured using ACF F6 is required to be provided as soon as the referred third batch is manufactured.
- The *E. coli* potency release requirements after the results of the 20 new final batches for *E. coli* components F5, F6 and LT should be recalculated and provided as soon as the results of the 20 new final batches are available.

Evaluation of the benefit-risk balance

The CVMP considers that the application is approvable considering also the post-authorisation measures as stated above. The product has been shown to have a positive benefit risk balance overall.

Conclusion on benefit-risk balance

The CVMP considers that the application is approvable with the risk mitigation measures as stated above.

Conclusion

Based on the original and complementary data presented the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the quality, safety and efficacy of Porcilis ColiClos were considered to be in accordance with the requirements of Directive 2001/82/EC and that the benefit – risk balance was favourable.