07 October 2021
EMA/576296/2021
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Suiseng Diff/A (EMEA/V/C/005596/0000)

Vaccine common name: *Clostridioides difficile* and *Clostridium perfringens* vaccine (inactivated)

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

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Introduction

The applicant Laboratorios Hipra, S.A. submitted on 23 July 2020 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Suiseng Diff/A, through the centralised procedure under Article 3(2)b of Regulation (EC) No 726/2004 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 18 March 2020 as the applicant showed that the product would be in the interests of animal health at Community level.

At the time of submission, the applicant applied for the following indications:

For the passive immunisation of neonatal piglets by means of the active immunisation of breeding sows and gilts:

- to prevent mortality and reduce clinical signs and macroscopic lesions caused by *Clostridioides difficile (C. difficile).*
- to reduce mortality, clinical signs and macroscopic lesions caused by *Clostridium perfringens (C. perfringens)* type A.

The indications accepted by CVMP are:

For the passive immunisation of neonatal piglets by means of the active immunisation of breeding sows and gilts:

- to prevent mortality and reduce clinical signs and macroscopic lesions caused by *C. difficile,* toxins A and B*.*
- to reduce clinical signs and macroscopic lesions caused by *C. perfringens* type A, α-toxin.

The reduction of the occurrence of neonatal diarrhoea has been demonstrated under field conditions.

The active substances of Suiseng Diff/A are *C. difficile* toxoid A (TcdA), *C. difficile* toxoid B (TcdB) and *C. perfringens* type A α-toxoid. The target species is pigs (sows and gilts). The product is intended for administration by intramuscular use. The active immunisation of pregnant sows and gilts induces the production of neutralising antibodies against *C. difficile* toxins A and B and *C. perfringens* type A α-toxin. These antibodies are transferred via the colostrum to the piglets. The uptake of sufficient colostrum within the first hours of life results in a passive protection of piglets.

Each dose of 2 ml of Suiseng Diff/A suspension for injection contains $\geqslant$ 1.60 relative potency (RP; established by ELISA) of *C. difficile* TcdA, $\geqslant$ 1.65 RP of *C. difficile* TcdB and $\geqslant$ 1.34 RP of *C. perfringens* type A α-toxoid. Suiseng Diff/A is presented in 20 ml, 50 ml, 100 ml and 250 ml PET plastic bottles closed with rubber stoppers and aluminium caps.

The rapporteur appointed is Jeremiah Gabriel Beechinor and the co-rapporteur is Manuela Leitner.

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

On 7 October 2021, the CVMP adopted an opinion and CVMP assessment report.

On 7 December 2021, the European Commission adopted a Commission Decision granting the marketing authorisation for Suiseng Diff/A.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system (dated 14/01/2019) which fulfils the requirements of Directive 2001/82/EC was provided. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Manufacture of the final product, secondary packaging and batch release takes place within the EEA at Laboratorios Hipra, S.A., Girona, Spain. The site has a manufacturing authorisation issued on 08 of May 2019 by the Spanish Agency of Medicines and Medical Devices (AEMPS). Good manufacturing practice (GMP) certification, which confirms the date of the last inspection and shows that the site is authorised for the manufacture and batch release of such veterinary dosage forms, has been provided.

A GMP declaration for the active substances manufacturing site was provided from the Qualified Person (QP) at the EU batch release site. The declaration was based on an on-site audit by the manufacturing site responsible for batch release, which has taken into consideration the GMP certificates available for the active substance sites issued by Spanish Agency of Medicines and Medical Devices (AEMPS) following inspections.

Overall conclusions on administrative particulars

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

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

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

Qualitative and quantitative particulars

The finished product is presented as a suspension for injection consisting of *C. difficile* toxoids A and B (TcdA and TcdB) and *C. perfringens* type A α–toxoid (CpA) as active substances at potency ≥ 1.60 RP / ≥ 1.65 RP / ≥ 1.34 RP (relative potency determined by ELISA) per dose of 2 ml, respectively. The product contains aluminium hydroxide, DEAE-dextran and ginseng extract as adjuvants.

Other ingredients are simethicone, disodium phosphate dodecahydrate, potassium chloride, potassium dihydrogen phosphate, sodium chloride, sodium hydroxide and water for injections.

The vaccine is intended to be available in multidose presentations but contains no preservatives.

The product is available in 20 ml, 50 ml, 100 ml and 250 ml PET plastic bottles, closed with rubber stoppers and aluminium caps.

Container and closure

The product is filled into 20 ml, 50 ml, 100 ml PET plastic bottle containing 10, 25 and 50 doses respectively. The bottless can also be partially filled into 50 ml, 100 ml and 250 ml PET vials containing 10, 25 and 50 doses respectively to allow mixing with another designated vaccine from the same applicant. The bottles are closed with bromobutyl rubber stoppers and aluminium seals, which have no product contact. The containers and closures comply with the pharmacopoeial requirements Ph. Eur. chapter 3.2.2 and 3.2.9 and their sterilisation is adequate. The certificates of gamma irradiation for all the PET bottles comply with the requirements of Ph. Eur. 3.2.2. Satisfactory information for the single use sterile bags, which are used as container closure system for the active substances, is provided and is in line with Annex II of the Guideline on plastic immediate packaging materials (CPMP/QWP/4359/03 and EMEA/CVMP/205/04).

The pack /container sizes are consistent with the vaccination schedule and intended use.

Product development

A satisfactory background to the Clostridial disease in swine and the choice of vaccine antigens are provided. Briefly, Suiseng Diff/A was developed to be administered intramuscularly to female pigs (gilts and sows) during pregnancy to provide passive immunisation to suckling piglets against enteric diseases caused by *C. perfringens* type A and *C. difficile,* the common causing agents for the enteric disease in pigs. At the submission of the file, the applicant stated that there was currently no vaccine against *C. difficile* available.

An acceptable explanation and justification for the composition, including the strains, and presentation of the vaccine has been provided. The minimum protective dose was based on the toxoid content of the 'standard batch dose', which had been used to investigate the efficacy of both the basic vaccination scheme and the efficacy of single dose revaccination.

The product development section includes a description about the manufacturing process of the antigens and vaccine. The manufacture is based on a seed lot system, in accordance with the general Ph. Eur. monograph requirements on vaccines for veterinary use (Ph. Eur. 0062). The choice of the vaccine antigens and vaccine strains has been justified and their characteristics and controls have been described. The inactivation process and controls are described in sufficient detail. An inactivation kinetics study was conducted for each toxoid suspension to demonstrate that the time required for inactivation meets Ph. Eur. requirements.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SPC.

The formulation of batches used during clinical studies is the same as that intended for marketing.

Formaldehyde solution (35%) is the inactivant of choice for both *C. difficile* and *C. perfringens* toxins. The detoxification method demonstrated ability to detoxify *C. difficile* toxins and α-toxin of *C. perfringens* Type A. An inactivation kinetics study was conducted to demonstrate that the time required for

detoxification does not exceed 67% of the total duration of the detoxification process in accordance with Ph. Eur. 0062. A maximum pre-detoxification titre have been stated for each toxoid.

The formaldehyde is removed and the toxoids are concentrated. A method assaying the cytotoxic effect (CTE_{50}) on cell culture is used to determine the toxin titres and residual toxicity of each toxoid. Validation of the *in vitro* titration and residual toxicity assays were provided and is acceptable.

Description of the manufacturing method

The production of the vaccine is performed in two phases: the production of the antigens and the vaccine blending. Each stage of the process takes place according to GMP requirements. The manufacturing processes are satisfactorily described.

Production of the antigens follows the seed lot system as required by Ph. Eur. 0062 and preparation of the seed lots is adequately described. Phase one consists of production of the toxins and recovery of the toxoids. The detoxification of the toxins has been adequately described and validated. Stage two consists of preparation of the adjuvant solutions, preparation of the aqueous phase, blending of the antigens with the adjuvants (final suspension) and filling.

Major steps of the manufacturing process of the antigens have been validated by presenting data for three consecutive antigen batches. It has been demonstrated that the manufacturing process is capable of producing the antigens of intended quality in a reproducible and consistent manner at the proposed manufacturing scale.

The final product manufacture consists of aseptically mixing and homogenising the pre-mixed sterile filtered adjuvant solutions with the sterile bulk antigen solutions. The sterilisation of the antigen solution, sodium hydroxide and ginseng solutions have been validated satisfactorily. The homogenised bulk finished product is then filled into sterile bottles and can be stored at +2 to +8°C for 15 months. The proposed duration of storage is supported by the stability data provided up to 18 months. Overall, it has been demonstrated that the manufacturing process is capable of producing the vaccine of intended quality in a reproducible and consistent manner.

Production and control of starting materials

Starting materials listed in pharmacopoeias

Certificates of analysis are provided for all starting materials listed in the pharmacopeia and all specifications are met. Two of these ingredients do not have a Ph. Eur. monograph; however, they are tested in line with USP 30 NF25, which is acceptable.

The nature of the raw materials, controls and treatments applied guarantee sterility of the vaccine and absence of introduction of any extraneous agent. The materials are tested for sterility, bioburden and/or endotoxin in line with relevant Ph. Eur. monographs. Removal of extraneous agents from the freeze-drying excipient is validated. The sterile filtration of the Ginseng and sodium hydroxide solutions are validated. Starting materials of biological origin.

The only material of animal origin used in the production of the active substance is gelatine, used as an ingredient of the freeze-drying excipient of the seed lots. It is confirmed that the gelatine is produced from bovine bones from Spain and bovine skin from EU countries and is compliant with the Note for Guidance (NfG) on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and veterinary medicinal products (EMA/410/01 rev 3).

Valid TSE certificates of suitability were provided. Valid TSE declarations from the Technical Director and Qualified Person of Laboratorios Hipra, S.A. of the Suiseng Diff/A vaccine have been provided.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

The following materials of biological origin not listed in a Pharmacopoeia are used in the production of Suiseng Diff/A vaccine: the antigens *C. difficile* TcdA and TcdB and *C. perfringens* type A, α-toxoid; and the culture medium ingredients vegetable peptone and yeast extract. The production process does not include any materials derived from human or animal origin except the gelatine as described above.

The applicant presented satisfactory description of the selection, source and passage history of the antigens. Preparation of the master and working seeds of bacteria (MSB and WSB) for both antigens are sufficiently described. The control and tests carried out on the seeds are satisfactory.

The MSB and WSB are preserved using a sufficiently described freeze-dry method with addition of freeze excipient. In addition, the WSB can be preserved by an alternative method of cryopreservation. Both methods of cryopreservation are acceptable and supported by the data provided for process validation of consistency of manufacture.

None of the starting materials used for the active substance or the finished product are TSE risk materials as defined in the current version of Ph. Eur 5.2.8 and the NfG (EMA/410/01 rev 3), except gelatine, as described above.

All starting materials of biological origin, which do not fall within the scope of Ph. Eur. 5.2.8 are either tested for or treated to ensure that there are no contaminants or further assurance is given that there is no potential risk. Valid TSE declarations from the manufacturers of the culture medium ingredients have been provided.

The applicant provided a satisfactory risk assessment for the potential for the *C. difficile* and *C. perfringens* seed material becoming contaminated with TSE agents in the period between the initial isolation until the applicant obtained the material, and until production of the MBS. The risk of TSE transmission is considered negligible.

Specific measures concerning the prevention of the transmission of Extraneous Agents

The applicant demonstrated the purity of the seed lots of both bacterial cultures *C. difficile* and *C. perfringens*, therefore no specific testing for absence of extraneous agents has been performed, which is acceptable in line with Ph. Eur. 0062.

Starting materials of non-biological origin

Certificates of analysis have been provided for non-biological starting material. Both materials conform to in-house specifications.

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

Information regarding the qualitative and quantitative composition of all culture media, in-house solutions and freeze-drying excipient and their sterilisation treatment is provided. All components are either tested for or treated in line with requirements. The storage details for the in-house preparations

are sufficiently described.

Control tests during the manufacturing process

The applicant presented in-process data for the manufacture of three consecutive *C. difficile* antigen bulks and three consecutive *C. perfringens* bulks. During the manufacture of the antigen the following tests are carried out: turbidity, gram stain, viability, purity, toxin quantification, detoxification, bioburden, toxoid quantification, pH and sterility.

The concentration of active toxin in each harvest of *C. difficile* and *C. perfringens* is quantified by means of cytotoxic effect in a cell based assays. The same toxin quantification assays for *C. difficile* and *C. perfringens* are used to determine the detoxification of antigen post-inactivation in the respective batches. The limit of detection (LOD) concentration for the *C. difficile* and *C. perfringens* assays indicated no clinical effect in mouse lethality studies and are considered acceptable.

Toxoid quantification for *C. difficile* TcdA, TcdB, and *C. perfringens* α-toxoid is determined at the bulk stage using an in-house capture ELISA specific to each antigen. The specificity, accuracy, precision, range of quantification, LOQ and robustness ara supported and validated properly. Adequate information is provided on the replacement of the reference standard and reagents.

The pH, bioburden and sterility testing are in accordance with the Ph. Eur. The volume fill is controlled by checking the weight in order to verify the amount dispensed.

Additional in-process controls for bioburden and filter integrity are described for the control of the manufacturing solutions are acceptable.

Test descriptions and the limits of acceptance were presented as well as satisfactory results for three consecutive full-scale batches. Additional acceptance criteria for the TcdB was introduced to control for the variation observed in the ratio of TcdA to TcdB content between batches. The relevant test methods for in-process controls are satisfactorily validated. The in-process tests are deemed sufficient to control all the critical steps in the manufacturing.

Control tests on the finished product

The description of the methods used for the control of the finished product (appearance, pH, fill volume, identification and potency, concentration of aluminium hydroxide, concentration of ginseng, concentration of DEAE-dextran, residual formaldehyde and sterility) and the specifications were provided. Validation of the methods is in accordance with VICH GL1 and GL2 and considered acceptable.

The appearance test is a macroscopic observation of the vaccine final bulk and filled finished product to confirm an easily resuspendable, yellowish-white suspension. The determination of pH is carried out in accordance with the Ph. Eur. 2.2.3. It is proposed to determine the pH at the final bulk vaccine stage, and this is supported with consistency batch data.

A capture ELISA is used to determine the batch potency of each toxoid at the final bulk stage. The specificity of the antibodies to each toxoid has been demonstrated in the validation studies and is further supported by additional tests. The assay can therefore be used for the identification of the active substance.

The determination of the potency of the vaccine is based on the calculation of the Relative Potency (RP) of the tested batch compared to a reference batch that has been demonstrated to be efficacious. The specifications for the potency have now determined using the batch formulated with the standard dose, for which the efficacy of the two dose basic vaccination scheme and the single dose revaccination scheme was adequately demonstrated, and are based on a suitably justified statistical method taking into account

the variation observed in the validation of the assay and the results from the routine consistency batches. A maximum acceptance limit is not proposed for each antigen in the potency release assay as antigen content is controlled at the blending stage.
Information is provided on the parallel line analysis method used to calculate the RP and is acceptable. Information is provided regarding the stability monitoring of the reference vaccine and trigger points for replacement and is acceptable. Furthermore, details are provided on the replacement and qualification of the reference batch and critical reagents when depleted. The applicant has suitably accounted for potential drift in the RP value for the reference vaccine batch when replacing reference batches. A description of the protocols for replacement and qualification of the ELISA reagents is provided and acceptable.
A batch equivalent to the reference batch was used in the validation of the assay and is acceptable. The validation of the assay has been adequately demonstrated.

The identification and assay of adjuvants are suitably validated and includes aluminium hydroxide, ginseng and DEAE-dextran. It is proposed to test for the adjuvant components at the final bulk vaccine stage and this is supported with consistency batch data.

The determination of residual formaldehyde is in accordance with the Ph. Eur. 2.4.18. The limit has been set in accordance with the Ph. Eur. 0062.

The bacterial and fungal sterility is determined in accordance with the Ph. Eur. 2.6.1.

It is proposed to carry out the tests for pH, concentration of the adjuvant components (aluminium hydroxide, ginseng and DEAE-dextran), batch potency of each toxoid, and residual formaldehyde on the final bulk vaccine and not the filled product batches. The potency test and residual formaldehyde can be carried out on the final bulk in accordance with Ph. Eur. 0062. Batch data has been provided in part 2F for the bulk vaccine and the filled vial batches demonstrating that the remaining parameters are not altered by the filling process and testing on the bulk vaccine is acceptable.

Batch-to-batch consistency

The applicant presented final product data for the manufacture of three consecutive final product batches. Each batch was used to fill three presentations: 20 ml, 50 ml and 100 ml. Two *C. difficile* antigen batches and two *C. perfringens* antigen batches, all full manufacturing scale, were used to manufacture the three finished product batches.

Two of the finished product batches provided are representative of the lowest proposed vaccine batch size for routine batches , and one at pilot scale.

All in-process and finished product test results complied with the proposed acceptance limits for the batch data provided.

Overall, the tests to control the critical steps for manufacture of consistent batches are acceptable.

Stability

For the bulk antigen

A real-time stability study is provided for the antigens included in the composition of Suiseng/Diff A when stored in a single use sterile bag at +2 to +8 ºC. Satisfactory data are provided to support a storage period of 24 months for *C. difficile* TcdA and TcdB, and 18 months for *C. perfringens* α-toxoid. The applicant has committed to reporting any out of specification results at the end of the real time stability study. Therefore a 24 month storage period for the *C. perfringens* type A α toxoid is considered acceptable.

For the finished product

The three consistency batches of Suiseng Diff/A have been entered into a stability program. Real-time stability data at +2 to+8ºC up to 18 months are provided. The batches of Suiseng Diff/A are representative of those proposed for marketing and were packed in the proposed primary packaging. The use of a pilot batch in the study is in accordance with VICH GL17, and the applicant has provided a commitment to place the first three manufacturing scale batches into the long-term stability program after approval.

The presentations used in the study encompass the largest and smallest of those proposed: 20, 50 and 100 ml. As all of the bottles can also be presented partially filled, the stabilty of the the worst-case scenario was also assessed.

The control tests and acceptance limits proposed for stability testing are the same as those for release of routine batches. Based on the stability data provided up to 18-months, a 15-month shelf life can be assigned, including the product batches that have been produced with aged antigen (a batch with *C. difficile* antigen batch stored at +2 to +8 °C for 11 months before blending and a batch with *C. perfringens* antigen batch stored at +2 to +8 °C for 4 months before blending).

For in-use stability

An in-use stability study has been carried out in accordance with the Guideline on data requirements to support in-use stability claims for veterinary vaccines (EMA/CVMP/IWP/250147/2008). The following tests were carried out on the extracted vaccine dose: appearance, pH, concentration of DEAE dextran, aluminium hydroxide and ginseng and sterility. Stability data on the broached vial stored for 10 hours in conditions mimicking field use was provided and considered acceptable for the claimed 10-hour in-use stability.

Overall conclusions on quality

The composition of the vaccine has been adequately described and complies with the required monographs. The vaccine contains two *C. difficile* toxoids, TcdA and TcdB, and one *C. perfringens* Type A toxoid, α–toxoid. The choice of the strains is satisfactorily explained, and current *C. difficile* epidemiological status in the EU is sufficiently addressed. The efficacy of vaccination with the vaccine toxoids has been adequately supported by the laboratory and field study data presented in Part 4. The adjuvant mix is composed of aluminium hydroxide, ginseng extract and DEAE-dextran. The choice of adjuvants has been sufficiently justified.

The manufacture is standard, a seed lot system in line with Ph. Eur. is satisfactorily described. The detoxification of the toxins has been adequately validated. The identity, source and extraneous agents testing for starting materials are described in line with Directive 2001/82/EC requirements. The applicant evaluated the risk of seed material being contaminated with TSE material and has provided a QP declaration of compliance of the vaccine with NfG EMA/410/01 rev 3 and Table A: Materials of animal origin included in the scope of the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via medicinal products. A valid CEP for gelatine is also provided as discussed above. Overall it can be accepted that both *C. difficile* and *C. perfringens* type A components of the vaccine present negligible risk of transmitting TSE, and the materials meet the requirements of the Ph. Eur. 5.2.8 and NfG EMA/410/01.

The tests performed during production and for release of the vaccine generally meet the requirements of Ph. Eur. 0062: 'Vaccines for veterinary use'.

Information on the development, manufacture and control of the active substance and the finished

product has been presented in a satisfactory manner.

In process data are presented for the manufacture of three consecutive *C. difficile* antigen bulks and three consecutive *C. perfringens* bulks. The relative potency is calculated using a reference batch and the determination of the antigen content of the reference batches is sufficiently described. The description of the methods used for the control of the finished product and the specifications were provided. For the potency release assay, the relative potency is calculated using a reference batch that has been demonstrated to be efficacious. The proposed release specifications are acceptable. The in-process and finished product tests are deemed sufficient to control all the critical steps for manufacture of consistent batches.

The applicant presented final product data for the manufacture of three consecutive final product batches, using one pilot batch and 2 batches of the minimum proposed manufacturing scale. All of the in process and finished product test results provided complied with the proposed acceptance limits. The applicant is recommended to provide additional batch data for at least one batch to support the proposed maximum manufacturing scale post authorisation.

Data are provided to support a storage period for the antigens of 24 months for the *C. difficile* toxoids and *C. perfringens* α-toxoid. The three consistency batches of Suiseng Diff/A have been entered into a stability program. A commitment is provided to enter the first three manufacturing scale batches into the stability program. Based on the stability data provided for three batches, a 15-month shelf-life can be assigned. An in-use stability of 10 hours is supported by the data provided.

Based on the review of the data on quality, the manufacture and control of Suiseng Diff/A it can be concluded that in-process controls and quality controls give confidence that the manufacture will yield a consistent immunological product.

Recommendations:

The applicant is recommended to provide additional batch data for at least one batch to further support the proposed maximum manufacturing scale.

Part 3 – Safety

Introduction and general requirements

Suiseng Diff/A is a vaccine containing inactivated toxins (toxoids) of *C. difficile* (TcdA and TcdB), and *C. perfringens* type A (alpha toxoid) with an adjuvant fraction containing aluminium hydroxide gel, ginseng extract and DEAE-dextran, and is intended for the passive immunisation of neonatal piglets by the active immunisation of breeding sows and gilts to protect against mortality, clinical signs and macroscopic lesions caused by *C. difficile* and *C. perfringens* type A. The vaccine is intended for intramuscular administration to pigs (pregnant sows and gilts), at a dose of 2 ml/animal, with the following proposed vaccination scheme:

- Basic vaccination scheme: two doses; the first dose at approximately 6 weeks before farrowing and a second dose at approximately 3 weeks before farrowing (with each dose administered at alternate sides).
- Revaccination: on each subsequent gestation, administer one dose 3 weeks before the expected date of farrowing.

A full safety file in accordance with Article 12(3) has been provided. The safety of the immunological veterinary medicinal product has been investigated in accordance with the requirements of Directive 2001/82/EC, as amended. In addition, Ph. Eur. monograph 5.2.6 'Evaluation of safety of veterinary vaccines and immunosera', and VICH GL44 "Target animal safety for veterinary live and inactivated vaccines" have been taken into account in order to demonstrate the safety of the vaccine. No specific monograph exists for these *Clostridia* in the Ph. Eur.

Safety documentation

Two laboratory trials and one multicentric field trial were carried out to assess the safety of Suiseng Diff/A. The laboratory and the field studies were conducted according to good laboratory practices (GLP) standards and good clinical practices (GCP) guidelines, respectively.

Since the category of the target species for active immunisation are pregnant gilts and sows, the safety laboratory trials were performed with primiparous gilts of 8-9 months of age, which is the youngest age for vaccination within the first gestation. In the field trial, nulliparous (gilts) and multiparous (sows) pregnant females were enrolled.

Two different batches of vaccine were used in each of the laboratory safety studies and one standard batch was used in the combined safety and efficacy field study. For the first laboratory safety study, with the intention to obtain a batch that contains the higher dose, a batch containing a higher amount of each antigenic fraction was used. Thus, this batch was manufactured according to Part 2 (production of each antigenic fraction batch) but contained a higher concentration of each antigen in order to assess the safety in a worst-case scenario. This approach was accepted, however the applicant was requested to provide further support to confirm that this was the 'maximum toxoid content batch' for Suiseng Diff/A for all toxoids. In response, the applicant conducted a new study, the second laboratory safety study, using a batch formulated with the highest toxoid content that can be present in commercial batches.

Study title	***Batch used***
Evaluation of the safety of the administration of one dose and a repeated dose of Suiseng Diff/A in pregnant gilts	Batch formulated with a higher toxoid content than is permitted in commercial batches for two of the three toxoids
Evaluation of the safety of the administration of one dose and a repeated dose of Suiseng Diff/A in pregnant gilts	Batch formulated with the highest toxoid content that is permitted in commercial batches for all three toxoids
Efficacy and Safety evaluation under field conditions of Suiseng Diff/A vaccine in sows	Batch formulated with the standard toxoid content

Laboratory tests

Two laboratory trials were conducted to assess the safety of Suiseng Diff/A.

Safety of the administration of one dose

Refer to 'Safety of the repeated administration of one dose'.

Safety of one administration of an overdose

No overdose studies are required for inactivated vaccines.

Safety of the repeated administration of one dose

The first pivotal laboratory study was conducted to investigate the safety of the administration of one dose and the repeated administration of one dose, with the administration of three doses in total by the intramuscular route, separated by intervals of 14 days. In this study, 10 pregnant gilts were included in the test group and received the first dose of vaccine at 6 weeks prior to expected parturition, followed by the second and third doses at 4 and 2 weeks prior to parturition, respectively. Whilst it was accepted that the antigen content for CpA and *C. difficile* TcdA was at the maximum concentration that will be present in commercial batches, the *C. difficile* TcdB toxoid was not quantified, therefore it was unknown if this toxoid was at the maximum concentration in the batch used. Five pregnant gilts were included in the control group that were mock vaccinated with PBS. In both groups, gilts were seronegative against TcdA and TcdB toxins from *C. difficile* and were seronegative or had low titres of antibodies against α toxin from *C. perfringens* type A (CpA). Ten animals with the lowest levels were assigned to group A and 5 animals with the highest levels were assigned to group B (mock-vaccinated animals were included as controls for reproductive parameters thus their serological status was not particularly relevant for their inclusion). The timing of vaccination was based on the recommended schedule; ('the first dose at approximately 6 weeks before farrowing and a second dose at approximately 3 weeks before farrowing'), except that in order to accommodate a third dose, doses were administered at two-week intervals. Follow-up consisted of observations until 14 days after the third administration (i.e., the day of parturition) with monitoring of clinical signs, rectal temperature and local reactions after intramuscular injection and assessment of reproductive parameters of gilts.

Results showed that there were no abnormal clinical signs or systemic effects following the repeated administration of a single dose. One gilt of the vaccinated group died due to causes non-attributable to the vaccine. Local clinical reactions were absent following the administration of the 1st dose of vaccine. Mild local inflamation at the vaccination site was observed after the first or second dose of the vaccine; two days after the 2nd dose, 1/10 gilts were reported (commonly) with slight inflammation with a maximum size of 3 cm, persisting for a total of 4 days. After the 3rd dose, 3/9 gilts were reported (very commonly) with local reactions, with a maximum size of 5 cm and maximum duration of 5 days. A slight transient increase in body temperature were detected in both groups (maximum individual increase in temperature of 0.8 °C in the vaccinated group). These reactions are appropriately described in the section 4.6 of the SPC. No negative effects on reproductive parameters attributable to vaccination were observed.

Overall, it was accepted that the study demonstrated the safety of the repeated administration of a single dose of Suiseng Diff/A administered by the recommended route in the youngest category of target species (primiparous gilts, at the youngest age at time of first vaccination), and that the repeated administration of Suiseng Diff/A during pregnancy does not lead to adverse effects on reproductive parameters, with a batch at maximum toxoid content. The serological status of the animals enrolled was free or had low levels of antibodies against the toxoids contained in the vaccine and in no case, they were previously vaccinated against the disease that the vaccine was intended to prevent. The serological status of the animals was not considered to have compromised the investigation of safety. The study was conducted in accordance with the requirements of Ph. Eur. 5.2.6 for the evaluation of safety and demonstrated compliance with the requirement that no animal shows abnormal local or systemic reactions or signs of disease or dies from causes attributable to the vaccine. Safety has been investigated in an appropriate number of pregnant animals (≥8) and in the specific period of gestation recommended for use on the label, in accordance with the requirements of Ph. Eur. 5.2.6.

An additional pivotal laboratory study was provided in the responses to questions raised, conducted to investigate the safety of the administration of one dose and the repeated administration of one dose, with the administration of three doses in total by the intramuscular route, separated by intervals of 14 days. This additional study employed a similar design to the previous study, but used a batch formulated to contain the highest concentration of each antigen, to address the query to provide further support to confirm that this was the maximum concentration that may be present in commercial batches. In this study, 9 pregnant gilts were included in the test group and received the first dose of vaccine at 6 weeks prior to expected parturition, followed by the second and third doses at 4 and 2 weeks prior to parturition, respectively. Five pregnant gilts were included in the control group that were mock-vaccinated with PBS as controls for reproductive parameters. As for the previous study, the serological status of animals was considered acceptable for the investigation of safety, as gilts were either seronegative or the minority had low levels of antibodies against the toxoids contained in the vaccine.

As for the previous study, the timing of vaccination was based on the recommended schedule, except that in order to accommodate a third dose, doses were administered at two-week intervals. Follow-up consisted of observations until 14 days after the third administration (i.e. the day of parturition) with monitoring of clinical signs, rectal temperature and local reactions after intramuscular injection and assessment of reproductive parameters of gilts.

Results showed that there were no mortalities, abnormal clinical signs or other systemic effects following the repeated administration of a single dose. Animals in both groups had a slight increase in body temperature (maximum individual increase in temperature of 0.73°C in the vaccinated group). Local reactions were absent following the administration of the 1st and 2nd doses of vaccine. After the 3rd dose, a local reaction (slight inflammation) was reported in 1/9 gilts (common), with a maximum size of 1 cm and duration of 5 days. No abortions or teratogenic effects on the progeny were reported. Reproductive parameters in the vaccinated group were similar to that of the control group and thus it was accepted that Suiseng Diff/A had no effect on reproductive parameters when administered during pregnancy.

In accordance with the first safety study presented, it can be accepted that the repeated administration of Suiseng Diff/A during gestation does not lead to adverse effects on reproductive parameters. The new study demonstrated that there were no additional safety concerns when the batch used was formulated at above the maximum toxoid content for TcdA and TcdB of *C. difficile*, and at the maximum toxoid content for CpA. The study was conducted in accordance with the requirements of Ph. Eur. 5.2.6 for the evaluation of safety and demonstrated compliance with the requirement that no animal shows abnormal local or systemic reactions or signs of disease, or dies from causes attributable to the vaccine. Safety has been investigated in an appropriate number of pregnant animals (≥8) and in the specific period of gestation recommended for use on the label, in accordance with the requirements of Ph. Eur. 5.2.6.

Overall, it can be accepted that the studies presented demonstrate the safety of the repeated administration of a single dose of Suiseng Diff/A administered by the recommended route in the youngest category of target species (primiparous gilts of approximately 9 months old at time of first vaccination), and that the repeated administration of Suiseng Diff/A during pregnancy does not lead to adverse effects on reproductive parameters. The description of adverse reactions in the SPC accurately reflects the adverse reactions associated with Suiseng Diff/A; mild local inflammation at the injection site with a maximum diameter of 5 cm which subsided without treatment within 5 days occurred commonly in laboratory studies, and slight transient increases in body temperature (mean 0.27 °C, in individual pigs up to 0.95°C) which subsided without treatment, which occurred commonly in laboratory and field studies.

Examination of reproductive performance

Suiseng Diff/A is specifically intended for use during pregnancy. The evaluation of reproductive performance is a pivotal safety parameter which has been investigated in the two laboratory trials discussed in 'Safety of the repeated administration of one dose' and in the field trial. The sows included in the studies were monitored on a daily basis in order to detect any abnormal reaction (any signs of illness, heat repetition or imminent abortion) after vaccination. For each litter, data regarding the number of piglets born alive (identifying weak piglets), number of stillborn piglets, and mummified or autolytic foetuses were recorded.

It is noted that the vaccine is composed of toxoids and does not contain a live active substance which could replicate in the target species or be expected to have a detrimental effect on pregnancy or on the progeny. The data provided in the laboratory study demonstrated that there are no adverse effects on reproductive performance following the repeated administration of the vaccine (three doses) during pregnancy and data obtained in the field study (see below) has confirmed the laboratory findings. Thus, the proposed vaccination schedule in section 4.9 of the SPC, i.e. two doses to be administered during pregnancy, the first at 6 weeks prior to expected parturition and the second three weeks later, and the proposed statement 'Can be used during pregnancy' included in section 4.7 of the SPC, are considered to have been adequately supported.

Examination of immunological functions

The applicant has not carried out a specific study to examine the potential for adverse effects on immunological function. A negative influence on the immune response is not expected after vaccination and no adverse effects relating to a negative impact on immunological functions were observed in any of the safety or efficacy studies. It is therefore unlikely that this vaccine will have an adverse effect on immunological functions due to the nature of the product (vaccine containing toxoids) and the absence of specific data investigating effect on immunological function can be accepted.

User safety

The applicant has presented a user safety risk assessment which has been conducted in accordance with CVMP guideline EMEA/CVMP/IWP/54533/2006.

The main potential routes of accidental contact with the product have been considered and it was concluded that accidental self-administration is the most likely situation that could lead to exposure. However, Suiseng Diff/A is an inactivated vaccine and the risk of infection of humans does not exist. Thus, Suiseng Diff/A does not pose any risk for the person handling the product or the person who is in contact with vaccinated animals. The other components of Suiseng Diff/A are the adjuvant (aluminium hydroxide, ginseng and DEAE-dextran) and the excipients (simethicone emulsion, disodium phosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride, potassium chloride and water for injections). The adjuvant and excipients included in the vaccine are commonly used in other vaccines and do not pose a risk for the user.

Based on the above risk assessment, it is concluded that the product does not pose an unacceptable risk to the user when used in accordance with the SPC. Given that no specific risk arising from accidental self-injection has been identified, no special precautions to be taken by the person administering the veterinary medicinal product to animals are considered necessary.

Study of residues

No studies on residues have been performed. The active substance being a principle of biological origin intended to produce active immunity is not within the scope of Regulation (EC) No 470/2009.

All other components of the vaccine (adjuvant, excipients) are either allowed substances for which table 1 of the annex to Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

Consequently, it is considered that there is no need to perform residue studies for Suiseng Diff/A and a withdrawal period of zero days is accepted.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

The applicant has not proposed a compatibility claim for Suiseng Diff/A with any other veterinary medicinal product and therefore proposes to include a statement in Section 4.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis.' This is considered acceptable.

Field studies

One GCP, multicentre, randomised, double blind, placebo-controlled safety and efficacy clinical field trial was carried out to assess both the safety and efficacy of Suiseng Diff/A under field conditions. The study was conducted in three commercial farms in one EU member state stated to be representative of those used in standard breeding production among the EU. A total of 311 healthy pregnant gilts and sows were included, 155 were vaccinated in accordance with recommendations with a standard batch of Suiseng Diff/A and 156 animals were mock vaccinated with PBS. Follow-up consisted of overall safety monitoring (mortality, adverse events and reproductive parameters) and closer monitoring of a subset of 10/animals/group farm for evaluation of rectal temperature, systemic and local reactions ('post-vaccinal safety population'). Sows and their piglets were monitored until weaning (28 days after farrowing). The safety aspects of the field trial are summarised below (refer to Part 4 for assessment of efficacy).

The study was well designed and conducted and confirmed that the product is safe for vaccination of pregnant gilts and sows at 6 and 3 weeks prior to the expected farrowing date.

Results demonstrated that there were no mortalities, clinical signs, adverse reactions, or local reactions associated with test-article administration. As observed in the laboratory study, minor transient increases in temperature were observed at 4 hours post-vaccination. The temperature increases are adequately reflected in the description of adverse reactions in the SPC. No statistically significant nor clinically relevant differences were observed between the two groups. No abnormal reproductive parameters were recorded in either group. There were no statistically significant differences in mean reproductive parameters between the test and control group.

Environmental risk assessment

An environmental risk assessment (ERA), conducted in accordance with requirements, was provided.

Considerations for the environmental risk assessment

The likelihood of the active ingredient to cause hazards to the environment can be considered negligible, taking into account that Suiseng Diff/A does not contain live organisms or agents capable of replicating within the host or environment; it is a multivalent subunit inactivated vaccine composed of TcdA and TcdB toxoids of *C. difficile* and α-toxoid of *C. perfringens* type A. These antigens do not pose any hazard to the environment. Besides the active ingredients, it also contains an adjuvant fraction and the excipients, none of which are toxic or pose a risk to the environment.

Conclusions on the environmental risk assessment

Based on the data provided the ERA can stop at Phase I. Suiseng Diff/A is not expected to pose a risk for the environment when used according to the SPC. No specific control measures are needed, and the precautions included in the SPC concerning the handling and disposal of unused veterinary medicinal product or waste materials derived from the use of such product are considered appropriate.

Overall conclusions on the safety documentation

The applicant has provided two GLP compliant laboratory safety studies and one GCP compliant field safety and efficacy study in support of the safety of Suiseng Diff/A.

The vaccine is intended for intramuscular administration to pregnant gilts and sows, with a basic vaccination scheme consisting of two doses, the first dose to be administered at 6 weeks prior to the expected parturition date, and the second dose to be administered 3 weeks later. Revaccination with a single dose in each subsequent pregnancy is recommended at 3 weeks prior to the expected date of farrowing.

The first laboratory study was conducted in animals using a vaccine stated to have been manufactured to contain a higher concentration of each antigen in order to assess the safety in a worst case scenario, with the aim to obtain a vaccine batch that contains the higher dose of the range for TcdB per 2 ml dose. Further information was requested to confirm that this batch may be considered representative of the maximum toxoid content for each antigen that will be included in a commercial batch. In response, the applicant conducted an additional laboratory safety study in animals using a second vaccine batch which was manufactured to contain the highest maximum concentration of *C. perfringens* type A, α-toxoid, and greater than the maximum concentration of *C. difficile* toxoids, TcdA and TcdB. A standard batch was used in the field trial.

The applicant has thus provided two pivotal laboratory studies to investigate the safety of the repeated administration of one dose to pregnant gilts of the minimum recommended age using maximum antigen (toxoid) content via the intramuscular route. In each study, with the different batches used (see above), the data presented support that there were no adverse effects on reproductive performance in the studies. Therefore, the safety of the administration of a single dose and repeated administration of one dose of vaccine (a total of three doses administered, in support of the two-dose basic vaccination scheme and one dose revaccination) has been demonstrated to be safe.

The applicant has provided one pivotal field study to investigate the safety of vaccination under field conditions. Pregnant sows and gilts were vaccinated according to the recommended basic vaccination schedule with a standard vaccine batch via the intramuscular route. The vaccine was shown to be safe when used according to recommendations; no adverse effects on the vaccinated animals or their progeny were observed. No adverse effects on reproductive parameters were observed under field conditions.

On the basis of the results it was concluded that the safety of the target animals when the vaccine is administered according to the recommended schedule and via the recommended route is acceptable.

Reproduction safety was investigated within both the laboratory and field studies given that the product is specifically intended for use during pregnancy. The product was demonstrated to be safe when used in pregnant animals according to recommendations at 6 and 3 weeks prior to expected parturition.

A user safety assessment in line with the relevant guidance document has been presented. It is accepted that the use of Suiseng Diff/A does not pose a risk to the user, when used in accordance with recommendations. There are no specific precautions necessary for the person administering the veterinary medicinal product to animals.

An appropriate environmental risk assessment was provided. The product is not expected to pose a risk for the environment when used according to the SPC.

Part 4 – Efficacy

Introduction and general requirements

The vaccine is intended for the passive immunisation of neonatal piglets by means of the active immunisation of breeding sows and gilts to prevent mortality and reduce clinical signs and macroscopic lesions caused by *C. difficile*, and to reduce mortality, clinical signs and macroscopic lesions caused by *C. perfringens* type A, as evaluated in suckling piglets on the first day of life in challenge studies. In addition, in the indication originally applied for at the start of the procedure, the applicant claimed that the reduction of the occurrence of neonatal diarrhoea and the use of therapeutic antibiotics has been demonstrated under field conditions.

The basic vaccination scheme consists of two doses during pregnancy for protection of the offspring, and a single revaccination during each subsequent pregnancy. The period of risk for piglets is in the first week of life, and it is claimed that neutralising protective antibodies were present up to 28 days after birth.

Efficacy was demonstrated in compliance with the European Directive 2001/82/EC (as amended by 2004/28/EC and Directive 2009/9/EC), and the European Pharmacopoeia (Ph. Eur.) chapter 5.2.7.

Challenge model:

There is no specific Ph. Eur. monograph for the two clostridial species in Suiseng Diff/A, however it is generally accepted that for Ph. Eur. monographs for vaccines containing *Clostridium* species, efficacy should be demonstrated on each target species by stimulating an immune response after vaccination, i.e., the ability to induce neutralising anti-toxin antibodies in the target species. In each of the laboratory and field efficacy studies, the serological response induced after vaccination was evaluated.

It is acknowledged that the demonstration of a protective effect / 'efficacy' against clostridial diseases is typically based on demonstrating the presence of neutralising antibodies against the toxoids which are included in the vaccine, in the case of vaccines for which the only claim sought is related to the stimulation of such antibodies. However, the applicant developed a model intended to reproduce clinical signs and macroscopic lesions in newborn piglets by inoculating a suspension of each toxin, in order to support the claimed indications for use of Suiseng Diff/A.

The applicant claims that there is no evidence of any significant variability of the toxins between strains, however a heterologous strain was selected for the challenge model. For *C. difficile* the challenge strain was isolated from a field case of neonatal diarrhoea in piglets and the production of toxins A and B was

confirmed. The challenge titre was the same in each of the challenge studies. For *C. perfringens* type A, toxin from an heterologous strain was also assessed and confirmed that its parenteral inoculation induced an acute intoxication model in 100% of the piglets with clinical symptoms, macroscopic lesions and mortality.

The applicant justified the challenge timepoint at the first day of life on the basis that a suckling time of a maximum 24 hours is well accepted and recognised to be appropriate to transfer a sufficient amount of protective antibodies from the colostrum to the piglets.

Mortality and macroscopic lesions were reported in piglets from mock-vaccinated animals in the laboratory challenge studies, however the challenge was severe and resulted in mortality in the majority or all the mock-vaccinated piglets resulting in limited clinical signs manifesting prior to death. Further justification for the relevance of the severe signs observed following intraperitoneal challenge to those that may be expected under field infection conditions was requested. In response, the applicant justified that a worst-case scenario for the evaluation of efficacy was undertaken in this severe challenge model, with the worst possible outcome (i.e. death), and it was argued that the protection afforded by vaccination in terms of neutralising clinical signs due to the challenge with toxin will be effectively able to neutralise the effects observed arising from natural disease under field conditions. Moreover, the applicant justified that data from the field study demonstrated a reduction of clinical signs (neonatal diarrhoea) in piglets from vaccinated groups, confirming protection under natural occurrence of the disease under normal management conditions.

Efficacy parameters and tests:

The applicant presented a summary of porcine neonatal enteric disease due to *C. difficile* or *C. perfringens* type A. *C. difficile* affects animals 1 – 7 days of age, supported by bibliographical references. The affected animals present creamy or pasty non-haemorrhagic diarrhoea. Animals infected by *C. difficile* often experience systemic manifestations of disease, in addition to typical gastrointestinal signs. Naturally infected piglets sometimes develop respiratory distress and hydrothorax, as well as ascites. On average, 2/3 of litters in an infected farrowing facility are affected, but rates may approach 100%, although with low mortality rates, up to 16%. Weaning weights of affected pigs are 10% below the expected average weight. It is spread oro-faecally through the ingestion of the spores and opportunistically over-colonises the gut of individuals with perturbed intestine flora, where it can produce the largest clostridia exotoxins: toxin A and toxin B. The former has mild cytotoxic activity but is primarily an enteroxin causing fluid accumulation in the intestine, while the latter, is a potent cytotoxin. *C. perfringens* type A can affect neonatal and weaned pigs. The major toxin produced by *C. perfringens* type A strains is α-toxin. The disease is described as a non-haemorrhagic mucoid diarrhoea and is characterised by mucosal necrosis and villus atrophy, without attachment and invasion by the microorganisms. Lesions are usually most severe in small intestine, particularly in jejunum and ileum.

The efficacy parameters as chosen by the applicant, investigated in the efficacy studies are the level of antibodies against CpA and TcdA and TcdB, evaluated by ELISA, and the level of toxin neutralising antibody titres in piglet sera evaluated by toxin neutralisation test (seroneutralising titres), in addition to mortality, clinical signs and macroscopic lesions of disease caused by challenge with *C. difficile* or *C. perfringens* type A. Validation results were presented for the serological analysis and confirm that the tests chosen are adequately validated to provide reliable results. The parameters chosen are considered appropriate for evaluating the efficacy of the product. In addition, under field conditions, the incidence of neonatal diarrhoea, mortality, average daily weight gain, antibacterial treatment against diarrhoea and serological parameters were evaluated.

Efficacy documentation

Three studies were conducted to investigate the efficacy of the product and included two laboratory studies and one field trial. Laboratory studies were well documented and carried out in pregnant gilts of the minimum age recommended for vaccination and sows, using batches containing different levels of toxoid content for the first laboratory study (which was also a dose-finding study), a 'standard batch dose' in the second laboratory study, and a standard batch in the combined safety and efficacy field trial.

Study title	**Batch used**
Determination of the efficacious Suiseng Diff/A vaccine dose when administered to pregnant sows and the efficacy of the passive immunity transfer to their progeny	Batch 1_containing the proposed 'Standard batch dose' Batch 2_ containing less toxoids than 'standard batch dose'
Efficacy of the revaccination scheme of Suiseng Diff/A vaccine when administered to pregnant sows.	Batch 1_containing the proposed 'Standard batch dose'

Laboratory trials

Two laboratory challenge studies were performed to investigate the efficacy of Suiseng Diff/A; the first, which was also a dose-finding study, investigated the efficacy of the basic vaccination schedule, the second was conducted to investigate the efficacy of the single dose revaccination scheme.

Dose determination

The determination of the minimum protective dose was undertaken in the study conducted to determine efficacy of the basic vaccination schedule, refer to 'Onset of immunity'.

Onset of immunity

One study was carried out in pregnant gilts and sows to investigate the onset of protection, by the recommended administration route.

In this study, 4 groups of seronegative animals were used. Animals were vaccinated on day 0 (6 weeks prior to parturition) and day 21 (3 weeks prior to parturition) with a 2 ml dose of the batch 2 (group A, n=5), or the proposed standard batch dose (SBD) (group B, n=10), or 2 ml of the SBD batch mixed with 2 ml of another vaccine produced by the applicant (group C, n=5). Animals in a negative control group (group D, n=8) were mock vaccinated with PBS.

At one day of life ('DV1'), colostrum-fed piglets (3 x 65 ml colostrum within the first 24 hours of life) were selected from 5 sows in each group; 10 piglets for *C. perfringens* type A challenge, 10 piglets for *C. difficile* challenge and 5 control piglets (i.e., 5 piglets per sow per group were selected for challenge/mock-challenge) in groups A and C. In group B, 15 piglets were selected for *C. perfringens* type A challenge, 15 piglets for *C. difficile* challenge and 5 control piglets. Additional piglets in groups B and D

were blood sampled after 1, 10 and 20 days of parturition. These piglets remained with the sow at the farm for this period. The piglets selected at DV1 were inoculated intraperitoneally with 2 ml of each challenge product. Follow-up included evaluation of clinical signs in sows and piglets, antibody response in sows (serum and colostrum) and in piglets, neutralising antibody titres in piglets on DV1, and necropsy of piglets for challenge-related macroscopic lesions.

Following challenge with *C. difficile*, 10/10 (100%) of piglets in the control group died within 24 – 48 hours of challenge. In the vaccinated groups, there was a prevention of mortality, with all piglets in each vaccinated group surviving the challenge. In the mock-vaccinated groups, death was preceded by clinical signs (e.g., severe depression, unable to respond to stimuli). There was a statistically significant difference in mean clinical scores between the vaccinated groups A, B and C , compared to the control group D, with no difference reported between groups A, B and C. The applicant states that piglets in groups A, B and C recovered without treatment, however on the 5th day of life, 6/10 piglets in group A (MPD), 8/15 piglets in group B (SBD), and 7/10 piglets still had clinical signs with scores ranging from 1 – 3. Clinical signs which manifested in all three vaccinated groups (groups A, B and C) (from raw data) were mainly depression, but also loss of body condition, and neurological signs were reported (tremors, 'incurvature'). There was a statistically significant difference in macroscopic lesions in each of the vaccinated groups compared to the mock-vaccinated group, with no differences between the vaccinated groups. Lesions at necropsy in mock-vaccinated animals consisted of hydrothorax, ascites and lesions in the small and large intestine consisting of a reddened intestinal wall and/or haemorrhagic content. Based on the applicant's response to questions, additional raw data was provided for the purposes of conducting a comparison of clinical signs score between groups, with scores allocated for mortality removed, which showed that diarrhoea was reported in animals of the vaccinated groups within the post-challenge period. Following *C. difficile* challenge, up to 4/10 piglets in the MPD group and up to 6/15 piglets in the SBD group were reported with mild, moderate or profuse diarrhoea. Profuse diarrhoea (score 3) was reported in both groups. By DV5, 7/10 piglets in the MPD group and 14/15 piglets in the SBD group had normal appearance of faeces. In light of these data, it might be considered that gastrointestinal signs of disease were induced by the severe challenge model and that piglets in the vaccinated groups displayed such signs, whereas rapid mortality in the respective control groups did not allow adequate time for these symptoms to develop. It was further noted that diarrhoea was also reported in piglets from groups A, B, C and D (n=20 in total) that were mock-challenged with PBS during the post-challenge phase, indicating a baseline level of neonatal diarrhoea during the study although it is not considered that this undermined the validity of the results obtained.

Following challenge with *C. perfringens* type A, 10/10 (100%) piglets in the mock-vaccinated group died within 24 hours of challenge. Prior to death, clinical signs (severe depression) developed very rapidly after challenge. In group A (MPD batch), 1/10 (10%) piglets died and in group B (SBD batch), 2/15 piglets (13%) died on the second day post-challenge. No piglets in group C died post-challenge. Although it was stated that mild clinical signs only (mainly mild depression) were reported in the vaccinated groups; there was a statistically significant difference in mean clinical scores between the vaccinated groups A, B and C (mean clinical scores of 1.7, 1.7 and 2.5, respectively), compared to the control group (3.5), with no difference reported between groups A, B and C. However, as discussed below, additional clinical signs appear to have been reported in the vaccinated groups. There was a statistically significant difference in macroscopic lesions in each of the vaccinated groups (1.8, 1.7 and 1.8 in groups A, B and C, respectively) compared to the mock-vaccinated group (8.7), with no differences reported between the vaccinated groups. Lesions at necropsy in mock-vaccinated animals consisted of hydrothorax, ascites, and lesions in the small and large intestine consisting of a reddened intestinal wall and/or haemorrhagic content. Based on the applicant's response to questions, additional raw data was provided which showed that diarrhoea was reported in animals of the vaccinated groups within the post-challenge period. Following *C. perfringens* type A challenge, up to 4/9 piglets in the MPD group and up to 7/13 piglets in the SBD group were reported with mild, moderate or profuse diarrhoea. Again, profuse diarrhoea

(score 3) was reported in both groups, notably in 7 of 13 piglets in the SBD group on the morning of the third day post-challenge. Although on the 5th day of life when the study ended, some piglets were still reported with profuse diarrhoea, it would seem that the peak incidence of severe diarrhoea had passed. Furthermore, although severe diarrhoea was reported in both groups, by DV5, 7/9 piglets in the MPD group and 9/13 piglets in the SBD group had normal appearance of faeces. Similar to the *C. difficile* challenge, it was noted that such signs were also reported in the mock-challenged (PBS) piglets of each study group, although this was not considered to have impacted on the validity of the results obtained.

While the results reported a statistically significant reduction in clinical signs in the vaccinated groups compared to the mock-vaccinated groups, it is difficult to draw conclusions with respect to protection from typical clinical signs of disease (of which diarrhoea is the most relevant). The severe challenge resulting in 100% mortality within 24 – 48 hours in mock-vaccinated animals after both *C. difficile* and *C. perfringens* type A challenge does not allow for comparison of typical clinical signs of disease with the challenge model used. In addition, it was noted that the difference in clinical signs score will have been largely influenced by death of piglets, therefore it was considered unclear how this challenge model is capable of allowing a comparison of typical clinical signs of disease. In response to concerns raised, the applicant provided a supplementary analysis showing that, when excluding mortality from the comparison of clinical signs score between the vaccinated and control groups, there was a statistically significant difference between the mean clinical signs score for both vaccinated groups compared to the control group for the *C. difficile* challenge ($p<0.01$), and for the *C. perfringens* type A challenge ($p<0.01$). However, whilst the applicant has claimed that there was a statistically significant difference (reduction) in clinical signs (excluding mortality) between vaccinated and control animals, in the absence of control animals surviving beyond 24 – 48 hours following challenge, the additional data provided were considered inadequate to permit a definitive conclusion upon the proposed claim for reduction in clinical signs caused by *C. difficile* and *C. perfringens*. The data presented in this study were considered to support a prevention of mortality and reduction of macroscopic lesions caused by *C. difficile*, and a reduction of mortality and macroscopic lesions caused by *C. perfringens* type A under experimental challenge conditions.

However, given the demonstration of a reduction in mortality and in particular, macroscopic lesions, the CVMP accepted that as an association between macroscopic lesions and diarrhoea is assumed, then a reduction in macroscopic lesions is expected to result in a reduction of typical clinical signs (diarrhoea) and consequently, the associated claim for a reduction of clinical signs would seem reasonable. Importantly, this was supported by the findings from the field study (see below). Concerning the relevance of the challenge model to natural infection, noting that mortality does not appear to be a major consequence of the disease under field conditions, the applicant was requested to further justify how it may be considered that the prevention or reduction of mortality, due to challenge with *C. difficile* or *C. perfringens* type A, respectively, is representative of clinical signs which may be expected under field conditions. In response, the applicant provided literature references in support of the fact that mortality due to *C. difficile* and due to *C. perfringens* type A may be observed under field conditions, and that any diarrhoeic process affecting neonatal animals can result in death due to the vulnerability of the age of the animals at this low age. While it was accepted that natural *C. difficile* infection can be associated with mortality, as supported by published literature, the CVMP considered the association to be less clear for *C. perfringens* type A. *C. perfringens* type A may not be considered to present as peracute a form of disease as *C.perfringens* types B or C, and under field conditions, it was not accepted that mortality was typically associated with *C. perfringens* type A infection. Thus, the claim for protection against mortality due to *C. difficile* was considered to have been adequately supported, however the intraperitoneal model was considered to represent a poorer reflection of natural challenge for *C. perfringens* type A, given that alpha toxin is expected to have mainly local effects, and therefore the claim for a reduction of mortality due to *C. perfringens* type A, α-toxin could not be included as a claim.

It is accepted that the recommended basic vaccination of sows with Suiseng Diff/A confers passive immunisation to colostrum-fed piglets. Basic vaccination of seronegative sows induced a clear increase in antibody titres against CpA, TcdA and TcdB for most animals. The mock-vaccinated group remained seronegative for the three antigens throughout the study. The majority of animals were seropositive for CpA, TcdA and TcdB on the day of farrowing, and positive for antibodies in colostrum. Whilst a question was raised concerning the number of sows in each group that were seropositive on day of farrowing, the applicant provided further justification, which was accepted by CVMP, to support that the serological titre in sows on day of farrowing, as measured by ELISA, is not as strongly correlated with protection of piglets from challenge compared to seroneutralising antibody (SNA) titres in piglets.

In piglets' sera at DV1, SNA against CpA and *C. difficile* antigens were reported in sera pooled from sows of each of the vaccinated groups (A, B and C). There were no statistically significant differences in the mean neutralising antibody titre against CpA, with mean titres of 6.3, 4.3 and 6.5 $\log_2$ SN50% in piglets from groups A, B and C respectively, however it was noted that due to the small sample size, the statistical analyses should be interpreted with caution. Similarly, there were no statistically significant differences in the mean neutralising antibody titre against *C. difficile*, with mean titres of 5.6, 4.9 and 4.5 in piglets from groups A, B and C, respectively. The applicant proposed a serological protective criteria taking into account all the individual results obtained in both efficacy studies.

The applicant proposed a protective serological threshold, taking into account the results obtained in both laboratory efficacy studies; neutralising antibodies titres superior to 4 for CpA antigen and 1.6 for *C. difficile* antigens (log2 dilution of the sample that neutralise the toxin) in piglet sera were claimed to confer a total protection against challenge with *C. perfringens* type A and *C. difficile*, respectively. It was noted that these thresholds were based on the lowest neutralising titre for CpA and *C. difficile* obtained in pooled samples of sera from piglets of the same litter in the group of sows vaccinated with a batch containing less toxoids than will be present in commercial batches. In the case of *C. difficile*, this threshold was accepted given that a prevention of mortality was demonstrated in the respective piglets born to the sow for which the lowest SNA titres in piglets were reported. For CpA, further questions were raised because one of the piglets born to the sow was not protected from challenge. In response to questions raised, the applicant re-analysed individual piglet sera for SNA titres against each toxoid. The lowest SNA titre in piglets for *C. difficile* was 0.9, and, as previously stated, all piglets were protected from mortality, therefore this threshold level for antibody titres and corresponding protection from challenge was accepted. For CpA, data provided supported that individual titres ≥2 were associated with prevention of mortality. In addition, it was accepted that a reduction of mortality (>80%) was achieved with CpA SNA titres ≥4 in pooled serum samples of piglets from the same litter. Furthermore, it was highlighted that slight variability concerning different colostrum intake in individual piglets will result in different SNA titres of individual piglets from the same litter. Whilst this element of variability cannot be excluded, appropriate information is included in the product information to highlight the importance that protection depends on sufficient colostrum intake.

The CVMP therefore considered that the proposed SNA titres that are claimed to be correlated with protection were adequately supported.

Duration of immunity

There are no specific claims made for duration of immunity in respect of sows and gilts, since pregnant animals are intended to be vaccinated at each subsequent gestation at 3 weeks prior to expected parturition, with a single dose. The efficacy of a single dose for revaccination is investigated in the study summarised below.

However, in section 4.2 of the SPC, under 'Duration of immunity', the applicant claims that neutralising antibodies persist until 28 days of life in piglets. These data are summarised in the efficacy aspects of the field study, refer to 'Field trials'.

In the second study sows that had been previously vaccinated with the SBD batch in previous study (group B) or mock-vaccinated with PBS (group D) were included. Animals were vaccinated on day 0 (3 weeks prior to parturition) of the subsequent pregnancy with a 2 ml dose of the SBD batch (group B, n=6) or mock-vaccinated with PBS (group D, n=4).

On the first day of life ('DV1'), a total of 30 and 28 colostrum-fed piglets from sows from group B and D, respectively, were selected for challenge; 12 piglets/group for *C. perfringens* type A challenge, 12 piglets/group for *C. difficile* challenge and 6 and 4 control piglets from group B and D, respectively, for mock-challenge with PBS. The piglets selected at DV1 were inoculated intraperitoneally with 2 ml of challenge product: 12 piglets with α toxin, 12 piglets with toxins A and B (*C. difficile*) from each group of sows. Follow-up included evaluation of clinical signs in sows and piglets, antibody response in sows (serum and colostrum) and in piglets, neutralising antibody titres in piglets on DV1, and necropsy of piglets for challenge-related macroscopic lesions. One piglet from group D was excluded from the *C. perfringens* type A challenge results due to incorrect administration of challenge toxin.

Following challenge with *C. difficile*, 11/12 (92%) of piglets in the mock-vaccinated group died after challenge. In the remaining piglet in this group, the applicant states that severe clinical signs were observed (dyspnoea, digestive symptoms). In contrast, mortality was prevented in 12/12 (100%) of vaccinated piglets. Piglets from mock-vaccinated sows showed a significant ($p<0.01$) increase of the clinical signs score compared to piglets from vaccinated sows. However, as noted for the previous study, the difference in clinical signs score will have been largely attributed to the death of piglets and the study data were not considered adequate for the purposes of permitting a conclusion on the reduction of typical clinical signs of disease. It is accepted that there was a statistically significant difference in macroscopic lesions in piglets of the vaccinated group compared to the mock-vaccinated group following *C. difficile* challenge. The applicant states that lesions were observed at necropsy in the mock-vaccinated group whereas in the vaccinated group, 5/12 piglets showed mild ascites. Following the provision of all of the raw data, it was noted that in group B, following *C. difficile* challenge, digestive signs were reported in the vaccinated group; e.g. on DV3, 4/12 piglets had profuse diarrhoea, and on DV5, 7/12 piglets were reported with mild to severe diarrhoea. However, similar to the points raised for the onset of immunity study, it is noted that for the piglets obtained from sows in groups B and D, that were mock-challenged with PBS, digestive signs were also reported in these piglets which served to highlight the baseline level of diarrhoea in study animals during the trial.

Following challenge with *C. perfringens* type A, 9/11 (82%) piglets in the mock-vaccinated group died within 24 hours of challenge. Of the remaining 2/11 piglets, one piglet did not appear to be correctly challenged (incorrect injection site) and one piglet is stated to have had severe symptoms of disease. In the vaccinated group, 1/12 (8%) piglets died. There was a statistically significant increase ($p<0.01$) in the clinical signs score in piglets from mock-vaccinated sows compared to piglets from vaccinated sows. Whilst the applicant states that only mild clinical signs were reported in the vaccinated groups, it is noted that moderate to severe scores were also reported. Thus, there was a statistically significant reduction in clinical signs in the vaccinated group piglets following CpA challenge, and while it may be considered that the clinical signs reported appear to have been more consistent with that which may be expected following natural challenge, it is also noted that diarrhoea was reported in the mock-challenged piglets. However, it is also noted that at necropsy, pasty or liquid faeces were observed in the rectum in the vaccinated and mock-vaccinated groups). There was a statistically significant difference in macroscopic lesions in piglets of the vaccinated group compared to the mock-vaccinated group following *C. perfringens* type A challenge.

Overall, the data presented are considered to support a claim for a prevention of mortality and a reduction of macroscopic lesions due to *C. difficile* and a reduction of macroscopic lesions due to *C. perfringens* type A following the administration of a single revaccination at 3 weeks prior to the expected farrowing date, under experimental laboratory conditions. Although a reduction of mortality due to *C. perfringens* type A was also demonstrated in this study, as raised for the onset of immunity data, given that mortality is not considered to be associated with natural infection, a reduction of mortality claim due to *C. perfringens* type A was not considered to have been supported. Regarding the reduction of clinical signs, whilst the data do not permit a definitive conclusion on the reduction of (typical) clinical signs of disease, as commented for the onset of immunity, taking into account the high level of protection against the most severe clinical sign of disease (death) observed in these challenge models, together with the reduction of macroscopic lesions in the gastrointestinal tract, and the beneficial effect observed in the field study regarding a reduction of typical signs of disease (neonatal diarrhoea) it is considered that the claim for a reduction of clinical signs of disease has been sufficiently supported.

Due to basic vaccination during the previous pregnancy, in group B 5/6 animals were seropositive for CpA (mean titres of 107.8), whereas mean titres for TcdA and TcdB had decreased to below the threshold for positive titres (27.4 and 25.4 for TcdA and TcdB, respectively). Vaccination with a single dose induced a clear increase in antibody titres against CpA (6/6 seropositive), TcdA (6/6 seropositive) and TcdB (6/6 seropositive) for all animals. The mean antibody titres at day of parturition were higher for CpA, TcdA and TcdB than those obtained following the two dose basic vaccination scheme. Thus, taking into account that the mean antibody titres at day of parturition following the two dose basic vaccination schedule were lower , it is evident that the administration of a single dose boosts antibody levels, and that they appear to be higher than that achieved after basic vaccination (with the SBD batch antigen level). Colostrum samples from vaccinated sows (group B) on the day of parturition demonstrated high titres of antibodies against CpA, TcdA and TcdB in all animals.

As for the previous study, high antibody titres were observed for all three antigens in piglets' sera on DV1 and DV5. In group B, on the first day of life (DV1), the mean titres of antibodies against CpA, TcdA and TcdB were higher than the titres in piglets of group B sows included in the previous study. At the 5th day of life, the mean antibody titres were similar, or had increased or decreased marginally compared to the 1st day of life.

Neutralising antibodies in piglet sera at DV1 were evaluated and showed that seroneutralising antibodies were absent in group D, whilst in piglets from group B, mean titres of 4.6 for CpA and 7.6 for *C. difficile* were reported. Compared to neutralising titres in piglets from group B sows after basic vaccinationin the second study (which were 4.3 for CpA and 4.9 for *C. difficile*), the mean titres are similar for CpA and *C. difficile*, notwithstanding that these data are based on low numbers of animals.

Overall, it can be accepted that the efficacy of the proposed single dose revaccination has been satisfactorily supported.

Finally, noting that in this second study, animals that had previously been vaccinated with the 'SBD' batch (group B) were included, and these sows were again vaccinated with a single dose of the same 'SBD' batch in this study, the efficacy of revaccination with a batch containing less toxoids that the 'standard batch dose', i.e. batch 2 has not been investigated. Therefore, in the absence of information concerning the efficacy of revaccination with a single dose of batch 1, the minimum protective dose is based on the toxoid content of the 'SBD' batch, i.e., the batch of Suiseng Diff/A that has been administered to group B sows in both efficacy studies.

Maternally derived antibodies (MDA)

Suiseng Diff/A is intended to be administered to gilts or sows during their pregnancy, that is, from the age of 8–9 months onwards. As Suiseng Diff/A is not recommended in animals at an age at which maternally acquired immunity may still be present, the demonstration of efficacy of the vaccine in relation to maternally derived antibodies is not required and has not been investigated; this can be accepted.

Interactions

No claim for compatible use of Suiseng Diff/A with another veterinary medicinal product is proposed. The standard text in situations where no data are provided to investigate interactions is included in section 4.8 of the SPC and this is considered acceptable.

Field trials

One GCP, multicentre, randomised, double blind, placebo-controlled safety and efficacy clinical field trial was carried out to assess both the safety and efficacy of Suiseng Diff/A under field conditions, as discussed in Part 3. The study was conducted in three commercial farms in one EU member state (Hungary) stated to be representative of those used in standard breeding production among the EU. A total of 311 healthy pregnant gilts and sows were included, 155 were vaccinated in accordance with recommendations with a standard batch of Suiseng Diff/A and 156 animals were mock vaccinated with PBS. The primary efficacy parameters evaluated were the incidence of sows with a litter with diarrhoea, and the incidence of piglets with diarrhoea. Secondary efficacy parameters evaluated were the percentage of mortality due to diarrhoea, the average daily weight gain, the number of antibiotic treatments administered to each animal with diarrhoea and the antibody titres in sows and piglets, including neutralising antibody titres in piglets' sera at 1 and 28 days of age. Sows and their piglets were monitored until weaning (28 days after farrowing). The efficacy aspects of the field trial are summarised below.

Animals were balanced for parity amongst the test and control groups (nulliparous, multiparous), and were not previously vaccinated against the relevant vaccine components. Farms were selected according to known infection pressure with *C. difficile* and *C. perfringens* type A (animals were seropositive for *C. perfringens* type A at time of vaccination and were seronegative for antibodies against TcdA and TcdB of *C. difficile*). The applicant provided a study protocol, including a statistical analysis plan for the clinical trial and it is accepted that the conduct, reporting and analysis of results presented in the final study report is in line with what was pre-specified in the study protocol and statistical analysis plan, or introduced by study amendment. The applicant excluded data from one of the farms from the analysis of efficacy, in line with the post-inclusion criteria. This approach was consistent with what had been pre-specified and amended by way of protocol amendment.

A statistically significant difference is reported between the test and control group for the primary efficacy variable 'incidence of sows with litter with diarrhoea', based on the incidence of 45.9% (50/109) in the test group and 60.4% (61/101) in the control group ($p=0.032$). The applicant conducted additional analyses of this parameter for the 'mean percentage of piglets with diarrhoea in a litter', and a statistically significant difference is also claimed between groups ($p<0.0001$). The second primary efficacy variable, 'incidence of piglets with diarrhoea', was statistically significantly different between groups, with 15.2% (200/1316) and 23.0% (290/1259) of piglets reported with diarrhoea in the test and control group, respectively. However, it was considered that the absolute difference (7.8%) between groups was limited and the applicant was requested to provide further justification for the clinical relevance of this difference between groups. In response, the applicant argued that in terms of evaluating the efficacy of

vaccines, the relative difference and not the absolute difference of disease among vaccinated and unvaccinated animals should be taken into account. For the first of the primary efficacy variables (incidence of sows with a litter with diarrhoea with presence of *C. difficile* or/and *C. perfringens* type A), while the absolute reduction value is 15%, the relative reduction in the vaccinated group compared to the controls was 24%. For the second of the primary efficacy variables (incidence of piglets with diarrhoea with presence of *C. difficile* or/and *C. perfringens* type A), while the absolute reduction value is 7.8%, the relative reduction value is 33.9%. Given that enteric diseases are multifactorial, the applicant argues that a statistically significant reduction in these parameters with relative reduction values of this magnitude are clinically relevant and will impact positively on the health status of suckling piglets. The applicant provided further information concerning diagnostic tests for other infectious agents carried out on the farms during selection of the farm sites, and during conduct of the study (litters were positive for *C. difficile* toxin B, *C. perfringens* type A, rotavirus type A and C, and *E. coli*, and it should also be noted that farms were stated to vaccinate against coliforms, which would reduce or at least standardise the infection pressure between the test and control groups for neonatal diarrhoea due to *E. coli*). In summary, it can be accepted that, according to the study protocol, efficacy was to be demonstrated by way of statistical comparison between groups. It can be accepted that a statistically significant difference has been demonstrated for both of the two primary efficacy parameters measured, as were pre-specified in the protocol. The relative reduction in the two primary efficacy variables in the vaccinated group compared to the placebo group can be accepted as being clinically relevant, given the high level of neonatal diarrhoea on the farms and the clarification regarding the diagnostic tests for the infectious agents on the farm.

Overall, the proposed claim for a reduction of diarrhoea under field conditions is considered to have been adequately supported by the field data presented.

Regarding the secondary efficacy parameters, there were no differences in mortality rates in piglets during the weaning period, with 9.3% (122/1316) and 10.3% (130/1259) mortality in the test and control group, respectively. Of these deaths, mortality attributed to diarrhoea was 22.1% (27/122) and 25.4% (33/130) in the test and control group, respectively (p=0.622). Whilst the absence of any difference in the percentage mortality between the test and control groups under field conditions is noted, it can be accepted that protection against mortality has been supported under severe laboratory challenge conditions for *C. difficile*, given that literature references provided by the applicant indicate a mortality rate of 16% in the field, and thus, should mortality occur under field conditions, it would be expected that progeny of vaccinated animals would be protected. However, concern was raised regarding the representativeness of the experimental challenge model (with respect to protection from mortality) to the natural course of disease under field conditions for *C. perfringens* type A, given that local effects (no mortality) would be expected following field condition. It was concluded by CVMP that the claim for a reduction of mortality due to *C. perfringens* type A was not supported by the data presented. Although no specific claim is being made for daily weight gain, it is noted that there were no differences in average daily weight gain beween the test (207.77 g/day) and control group (212.88 g/day).

With respect to number of piglets treated with antibiotics against diarrhoea, the applicant originally claimed that vaccination afforded a significant reduction in antibiotic treatment in the piglets from vaccinated sows, with a difference of 3.7% of piglets treated with antibiotics in the test group compared to the control group (in the test group, 94/739 [12.7%] of piglets on farm HAJ were treated with antibiotics against diarrhoea, compared to 117/714 [16.4%] in the control group [p=0.049]). However, it was noted that the claimed reduction was based on data from one farm only, farm 'HAJ', due to the decision on farm 'DEL' not to treat animals with antibiotics during the study (even though such use was allowed). Although this parameter was evaluated in a total of 1,452 piglets, given that data has only been provided from a single site, it was unclear as to how representative the findings may be for the general target population i.e. the approximately 23% reduction reported may have arisen by chance or may have

arisen as a result of selection bias. Furthermore, and more importantly, it would generally be expected that there would be at least some benefit of vaccination in terms of reducing antibiotic usage (as for other bacterial diseases against which vaccination is used) and therefore as a point of principle, such a claim for a bacterial vaccine would not be considered appropriate by CVMP. In conclusion, the CVMP were of the view that a claim for a reduction of the use of therapeutic antibiotics under field conditions was not adequately supported and the applicant was requested to omit this claim from the indications for use, furthermore this information was not considered appropriate to include under Section 5 of the SPC either, as information in this section should be restricted to information that is relevant to the indications considered approvable in section 4.2.

Regarding the serological analyses, the claim that 'Neutralising protective antibodies were present up to 28 days after birth' was based on this study, based on analysis conducted on piglets on one of the three farms included in the field trial. At day 0, all piglets included in the vaccinated group (n=34), had neutralising titres above 4 for CpA and 1.6 for *C. difficile*, with mean titres of 7.22 ±1.50 for CpA and 5.36 ± 2.22 for *C. difficile*, decreasing at day 28 to 1.84 ± 1.61 for *C. difficile* and 4.36 ± 1.39 for *C. perfringens* type A. In response to questions raised, the applicant clarified that the percentage of piglets at day 28 for which neutralising antibody levels were at or above the individual protective threshold levels of 2.0 for CpA and 0.9 for *C. difficile* was 97% of piglets for CpA and 65% for *C. difficile*. Therefore, it is accepted that neutralising antibodies against *C. perfringens* type A are maintained until 28 days after birth in the majority of animals. However, regarding *C. difficile*, given that 35% of piglets are below the protective threshold level of 0.9 for *C. difficile* antibodies on day 28, and that 19% would be considered seronegative, it was considered that these data should be more appropriately conveyed in the SPC, by indicating that neutralising protective antibodies transferred via colostrum to the piglets were present up to 28 days after birth in the majority of piglets, i.e., not all. The applicant updated the wording in section 4.2 of the SPC accordingly.

In summary, in this field trial, farms were selected with recent confirmed cases of *C. perfringens* type A and *C. difficile*, and neonatal diarrhoea was reported during the clinical trial. A statistically significant reduction between the vaccinated and control groups with respect to the incidence of sows with a litter with diarrhoea with presence of *C. difficile* or/and *C. perfringens* type A, and the incidence of piglets with diarrhoea with presence of *C. difficile* or/and *C. perfringens* type A was reported and therefore the CVMP accepted the claimed reduction of diarrhoea under field conditions.

Overall conclusion on efficacy

For both *C. difficile* and *C. perfringens* type A, the challenge model used (intraperitoneal injection of challenge material) was severe and led to 100% mortality in piglets from mock-vaccinated animals in the first study (efficacy of the basic vaccination scheme), with prevention of mortality in piglets from vaccinated sows (C. *difficile*) and reduction of mortality in piglets from vaccinated sows (*C. perfringens* type A). Similar results were also supported in second study (efficacy of single dose revaccination).

Whilst protection from mortality was not specifically demonstrated under field conditions for *C. difficile*, it can be accepted that a severe challenge model was used under laboratory conditions and consequently, it is reasonable to conclude that protection under field conditions (less severe challenge) is expected. Therefore, the claim for a prevention of mortality due to *C. difficile* has been adequately supported under laboratory challenge conditions. However, it was considered appropriate to include further information in section 5 of the SPC to provide further information on how the indications for use were supported with respect to the use of the intraperitoneal challenge model.

In relation to the mortality claim for *C. perfringens*, while it is accepted that the *C. perfringens* type A challenge model resulted in mortality, the pathological effect of the toxins is considered to occur mainly in

the intestines and to manifest as diarrhoea, and mortality is not typically associated with field infections. Therefore, whilst the intraperitoneal model demonstrated a reduction of mortality, this indication is not considered relevant for the field situation and hence, the claim for a reduction of mortality due to *C. perfringens* type A was not considered to have been adequately supported. However, the model used is considered suitable to demonstrate toxin neutralisation.

The claim for a reduction of clinical signs due to *C. difficile* and due to *C. perfringens* type A is considered to have been adequately supported. A statistically significant reduction in the mean clinical signs score was reported in piglets from vaccinated sows compared to mock-vaccinated sows, however it was noted that the severe challenges led to rapid mortality in the control group, with the result that it was difficult to draw conclusions with respect to typical clinical signs of disease (i.e. diarrhoea) in the absence of a valid control group beyond 48 hours post-challenge. Whilst digestive signs were reported in piglets of vaccinated animals in both the onset of immunity and duration of immunity study, similar signs were also reported in mock-challenged piglets. However, given the demonstration of a reduction in mortality and in particular, macroscopic lesions under experimental challenge conditions, the CVMP accepted that as an association between macroscopic lesions and diarrhoea is assumed, then a reduction in macroscopic lesions is expected to result in a reduction of typical clinical signs (diarrhoea) and consequently, the associated claim for a reduction of clinical signs would seem reasonable. Importantly, the claim was supported by the findings from the field study. Therefore, the claim for a reduction of clinical signs of disease was considered acceptable.

The claim for a reduction of macroscopic lesions due to *C. difficile* and due to *C. perfringens* type A has been adequately supported under laboratory challenge conditions. After the basic vaccination schedule, and after the single dose revaccination, a statistically significant reduction in macroscopic lesions was reported in piglets from vaccinated animals compared to piglets from mock-vaccinated animals. Macroscopic lesions consisted of hydrothorax, ascites, reddened intestinal wall and/or haemorrhagic content, and lesions can be accepted as being representative of systemic signs of infection with *C. difficile* and *C. perfringens* type A.

Concerning the proposed minimum dose of toxoids included in Suiseng Diff/A, the first study investigated two dose levels. One batch, the proposed minimum protective dose batch and was used for vaccination of group A sows (n=5). Another batch, the proposed standard batch dose was used for vaccination of group B sows (n=10). Whilst some concerns were raised regarding antibody titres in sows on the day of farrowing following vaccination with the MPD dose, in addition to the fact that only 5 gilts had been vaccinated with this batch, these concerns were adequately addressed and it was accepted that following vaccination with the MPD batch, the claimed indications for use were supported. However, given that the efficacy of the single revaccination dose was investigated only for the SBD batch (for both basic vaccination and revaccination), it is unknown if a single dose of the MPD batch would be sufficiently protective for revaccination. Therefore, the applicant agreed to establish the 'minimum protective dose' based on the release specifications of the SBD batch.

A claim for the passive immunisation of piglets by the active immunisation of sows and gilts is considered to have been supported; vaccination of seronegative pregnant females induced an antibody response against the three toxoids, which was present in serum and colostrum on day of farrowing, and resulted in an increase in antibodies in piglet sera, which was also confirmed as seroneutralising on the first day of life in piglets' sera. Although a cut-off threshold does not need to be established for this product, (the claims are proposed to be supported by challenge data rather than relying on neutralising titres as a surrogate marker of efficacy), cut-off points above which protection from challenge were established. Noting that the passive transfer of neutralising antibodies is associated with an inherent degree of biological variability, information is included in the SPC to highlight that protection depends on piglets ingesting adequate quantities of colostrum.

One multicentric combined safety and efficacy field trial was conducted, in one EU member state on three farms with known infection pressure. During the trial, on two of the farms, sows and gilts in both the test group and placebo control group were affected by neonatal diarrhoea. On the third farm, neonatal diarrhoea was largely absent, thus the applicant excluded the data from this farm from the evaluation of efficacy parameters (apart from serological analyses). Concerning the primary efficacy variables; the incidence of sows with a litter with diarrhoea was statistically significantly lower (p=0.032) in the test group (45.9%) compared to the placebo group (60.4%), representing an absolute difference and relative reduction of 15% and 24%, respectively. The incidence of piglets with diarrhoea was statistically significantly lower (p=0.025) in the test group (15.2%) compared to the placebo group (23.0%), representing an absolute difference of 7.8%, and a relative reduction in the vaccinated group compared to the control group of 31.4%. It is accepted that these differences between groups are clinically relevant. The proposed claim that the reduction of the occurrence of neonatal diarrhoea has been demonstrated under field conditions was considered to have been adequately supported.

With respect to number of piglets treated with antibiotics against diarrhoea, the applicant originally claimed that vaccination afforded a significant reduction in antibiotic treatment in the piglets from vaccinated sows, with a difference of 3.7% of piglets treated with antibiotics in the test group compared to the control group. However, it was noted that the claimed reduction was based on data from one farm only. Although this parameter was evaluated in a total of 1,452 piglets, given that data has only been provided from a single site, it was unclear as to how representative the findings may be for the general target population i.e. the approx. 23% reduction reported may have arisen by chance or may have arisen as a result of selection bias. Furthermore, more importantly, it would generally be expected that there would be at least some benefit of vaccination in terms of reducing antibiotic usage (as for other bacterial diseases against which vaccination is used) and therefore as a point of principle, such a claim for a bacterial vaccine was not considered appropriate by CVMP. In conclusion, the CVMP were of the view that a claim for a reduction of the use of therapeutic antibiotics under field conditions was not adequately supported and the applicant was requested to omit this claim from the indications for use.

Mortality was evaluated as a secondary efficacy parameter, however no difference in overall mortality rates were reported between the test (9.3%) and control (10.3%) groups, of which mortality due to diarrhoea was responsible for 22.1% and 25.4% of the overall mortality percentage in the test and control groups, respectively. Whilst the lack of difference in percentage mortality between the test and control groups under field conditions is noted, it was accepted that protection against mortality reported under severe laboratory challenge conditions was considered to have been adequately supported and it is reasonable to conclude that protection under field conditions (less severe challenge) is expected for *C. difficile*, but not for *C. perfringens* type A, given that mortality is not accepted by CVMP as an expected outcome of *C. perfringens* type A infection.

Neutralising antibody titres were measured in piglet sera on the first and 28th day of life, and levels correlated with protection were maintained in the majority of animals at four weeks after birth.

Overall, the indications for use for a prevention of mortality and a reduction of clinical signs and macroscopic lesions caused by *C. difficile*, toxins A and B, and a reduction of clinical signs and macroscopic lesions caused by *C. perfringens* type A, α-toxin are considered to have been adequately supported by the data presented. The reduction of the occurrence of neonatal diarrhoea has been adequately demonstrated under field conditions. Regarding the onset of immunity, it is clearly stated in the SPC that protection was demonstrated in suckling piglets on the first day of life in challenge studies, and concerning the duration of immunity it has been shown that neutralising protective antibodies transferred via colostrum to the piglets were present in the majority of animals up to 28 days after birth.

Part 5 – Benefit-risk assessment

Introduction

Suiseng Diff/A is a vaccine containing inactivated toxins (toxoids) of *C. difficile* (TcdA and TcdB) toxoids, and *C. perfringens* type A (alpha toxoid), and is intended for the passive immunisation of neonatal piglets by the active immunisation of breeding sows and gilts to prevent mortality, reduce clinical signs and macroscopic lesions caused by *C. difficile* and to reduce clinical signs and macroscopic lesions caused by *C. perfringens* type A. The vaccine is presented as a suspension for injection in PET bottles containing 20 ml, 50 ml, 100 ml or 250 ml and is intended for intramuscular administration to pregnant animals, at a dose of 2 ml/animal.

Benefit assessment

Direct therapeutic benefit

Suiseng Diff/A contains a combination of clostridial toxoids that, to the best of CVMP's knowledge, are not yet available in a single vaccine. Suiseng Diff/A is claimed to be of value in the immunisation against neonatal diarrhoea in piglets caused by *C. difficile* and *C. perfringens* type A. Whilst neonatal diarrhoea in piglets is also associated with other major pathogens, e.g. *E. coli* and *C. perfringens* type C, for which subunit or toxoid-based vaccines are currently available, the applicant states that *C. difficile* and *C. perfringens* type A are more recently identified as common agents implicated in enteric disease in piglets. Infections with enteric bacterial pathogens are one of the most common causes of diarrhoea and mortality in piglets during the pre-weaning period. Thus, Suiseng Diff/A increases the range of available vaccines for the immunisation schedules against neonatal piglet diarrhoea.

Well-conducted, controlled laboratory challenge studies demonstrated that the product is efficacious in the prevention of mortality due to *C. difficile* and the reduction of macroscopic lesions caused by challenge with *C. difficile* and *C. perfringens* type A, in piglets from sows vaccinated in accordance with recommendations. Whilst the challenge model used in the studies was very severe, with the result that the worst clinical outcome (death) was induced in control animals, a statistically significant reduction in clinical signs (which included mortality) was considered to have been supported, noting the reduction of macroscopic lesions in the gastrointestinal tract and that further evidence of protection from typical clinical signs of disease was provided in the field studies. Under field conditions in farms with infection pressure from *C. difficile* and *C. perfringens* type A, a statistically significant reduction in neonatal diarrhoea was demonstrated.

The passive transfer of protective antibodies following the basic vaccination schedule (2 doses to be administered during pregnancy, the first at 6 weeks and the second at 3 weeks prior to expected farrowing) and the revaccination schedule (single dose in each subsequent pregnancy at 3 weeks prior to expected farrowing) were both supported by the laboratory challenge data. Antibody titres were measured in vaccinated animals, in sera and in colostrum samples, and in piglets' sera, and it is accepted that active immunisation of sows is associated with passive immunisation of piglets, fed colostrum within the first 24 hours after parturition. Neutralising antibodies that are above a level correlated with protection from challenge are present up to 28 days after birth, under field conditions, for protective neutralising antibody titres against *C. perfringens* type A in 97% of piglets and for protective neutralising antibodies against *C. difficile* in 65% of piglets.

Additional benefits

Suiseng Diff/A would increase the range of available vaccines for the control of neonatal piglet diarrhoea and, as a consequence, would be expected to reduce the need for antimicrobial treatment (even if this originally proposed claim was not adequately supported by the clinical trial presented in the marketing authorisation application, or that such a claim would not be considered appropriate in the SPC for a vaccine against bacterial diseases).

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product is presented in a satisfactory manner. The results of tests carried out support the consistency and uniformity of important product quality characteristics and stability.

Safety:

The safety of Suiseng Diff/A was investigated in accordance with requirements. The safety data provided for Suiseng Diff/A demonstrate that for the target animal, the adverse reactions following vaccination under recommended conditions of use consisted of slight increases in temperature and mild local reactions, which resolved spontaneously without treatment; these adverse reactions observed are described in the SPC.

The safety of Suiseng Diff/A during pregnancy was evaluated within the laboratory and field studies. It is accepted that the vaccine is safe for use during pregnancy and that there are no adverse effects on reproductive parameters or on the progeny of vaccinated animals.

The use of Suiseng Diff/A does not pose a risk to the user, when used in accordance with recommendations.

There are no risks identified for consumers of animals vaccinated with Suiseng Diff/A. All components included in the product are either allowed substances according to Table 1 of Regulation (EC) No. 37/2010, or are substances considered as not falling within the scope of Regulation (EC) No. 470/2009, therefore a withdrawal period of zero days is considered acceptable.

It is accepted that the vaccine does not pose a risk to the environment when used in accordance with recommendations.

Risk management or mitigation measures

No specific risks from use of this product were identified and consequently no specific risk management or mitigation measures are proposed.

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

Evaluation of the benefit-risk balance

The product has been shown to be efficacious for the following indications:

For the passive immunisation of neonatal piglets by means of the active immunisation of breeding sows and gilts:

- to prevent mortality and reduce clinical signs and macroscopic lesions caused by *C. difficile,* toxins A and B*.*
- to reduce clinical signs and macroscopic lesions caused by *C. perfringens* type A, α-toxin.

The reduction of the occurrence of neonatal diarrhoea has been demonstrated under field conditions.

Onset of immunity:

Protection was demonstrated in suckling piglets on the first day of life in challenge studies.

Duration of immunity:

Neutralising protective antibodies transferred via colostrum to the piglets were present up to 28 days after birth in the majority of piglets.

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

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

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Medicinal Products for Veterinary Use (CVMP) considers that the application for Suiseng Diff/A 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.