12 February 2025
EMA/64035/2025
Veterinary Medicines Division

## Committee for Veterinary Medicinal Products (CVMP)

# CVMP assessment report for a grouped variation requiring assessment for Porcilis ColiClos (EMEA/V/C/002011/VRA/0018/G)

Vaccine common name: *E. coli* and *C. perfringens* vaccine (inactivated) to provide passive immunity to pigs

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

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# Introduction

## *Submission of the variation application*

In accordance with Article 62 of Regulation (EU) 2019/6, the marketing authorisation holder, Intervet International B.V. (the applicant), submitted to the European Medicines Agency (the Agency) on 29 July 2024 an application for a group of variations requiring assessment for Porcilis ColiClos.

## *Scope of the variation*

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

This group of variations is to replace the master seed for *C. perfringens* by a new strain, to replace the current authorised site for the antigen manufacturing process and in process control tests by a new site, and to adapt the production process and in process control tests for the antigen.

| **Variation(s) requested** | |
|---|---|
| I.I.1.c | I.I.1.c - Changes to the active substance(s) - Replacement of a biological active substance with one of a slightly different molecular structure where the efficacy/safety characteristics are not significantly different, with the exception of the changes mentioned in G.I.13 and G.I.14 |
| F.I.a.1.d | F.I.a.1.d - Change in the manufacturer of a starting material/reagent/intermediate used in the manufacturing process of the active substance or change in the manufacturer (including where relevant quality control testing sites) of the active substance, where no Ph. Eur. Certificate of Suitability is part of the approved dossier - The change relates to a biological/immunological active substance or a starting material/reagent/intermediate used in the manufacture of a biological/immunological product |
| F.I.a.2.b | F.I.a.2.b - Changes in the manufacturing process of the active substance - The change refers to a biological / immunological substance or use of a different chemically derived substance in the manufacture of a biological/immunological substance, which may have a significant impact on the quality, safety and efficacy of the medicinal product and is not related to a protocol |

Moreover, the dossier has been re-structured according to the new legislation (Annex II of Regulation (EU) 2019/6).

## *Changes to the dossier held by the European Medicines Agency*

This application relates to the following sections of the current dossier held by the Agency:

Part 1 and Part 2.

# Part 1 - Administrative particulars

## *Manufacturing authorisations and inspection status*

**Active substance**

With one of the variations in this group, the applicant wishes to replace the current manufacturing site for the active substance *C. perfringens* and the associated in-process control tests, to a new manufacturing site.

For the new manufacturing site, a current valid GMP certificate has been provided. This certificate confirms adherence to the principles and guidelines of Good Manufacturing Practice (GMP).

Additionally, a declaration has been provided from the QP stating that the active substances are manufactured in compliance with EU GMP.

## *Overall conclusions on administrative particulars*

The GMP status of the active substance manufacturing site has been satisfactorily established and is in line with legal requirements.

# Part 2 - Quality

## *Quality documentation (physico-chemical, biological, and microbiological information)*

## Qualitative and quantitative composition

Currently, as active substance, each dose (2 ml) of Porcilis ColiClos suspension for injection contains:

| | |
|---|---|
| - *Escherichia coli,* fimbrial adhesin F4ab | ≥9.7 log2 Ab titre[1] |
| - *Escherichia coli,* fimbrial adhesin F4ac | ≥8.1 log2 Ab titre[1] |
| - *Escherichia coli*, fimbrial adhesin F5 | ≥8.4 log2 Ab titre[1] |
| - *Escherichia coli*, fimbrial adhesin F6 | ≥7.8 log2 Ab titre[1] |
| - *Escherichia coli*, LT toxoid | ≥10.9 log2 Ab titre[1] |
| - *Clostridium perfringens*, Type C (strain 578) beta toxoid | ≥20 IU[2] |

[1] Mean antibody titre (Ab) obtained after vaccination of mice with a 1/20 or 1/40 sow dose

[2] International units of beta antitoxin according to Ph. Eur.

Porcilis ColiClos is formulated with a fixed quantity of *Clostridium (C.) perfringens* type C beta-toxoid.

Currently, this antigen is derived from strain 578.

To improve antigen availability for the different clostridial vaccines within the company and to reduce complexity in the antigen manufacturing process/supply, the applicant would like to replace the beta-toxoid of Procilis ColiClos with the beta-toxoid strain CN 883 manufactured on a different manufacturing site, as already used for another vaccine of the same company, Bravoxin (EU license DE/V/0289/001/MR).

As the composition of the final product is not proposed to change, the product is still formulated with the same fixed quantity of beta-toxoid, i.e. 120 TCP/dose.

## *Product development*

The current *C. perfringens* type C antigen added to Porcilis ColiClos has been produced by the applicant for more than a decade as one of the components for a range of clostridial sheep vaccines.

A detailed description of the method of preparation of Porcilis ColiClos is provided in the quality part of the dossier, including the production of the antigens. The *C. perfringens* type C antigen is produced by culturing *C. perfringens* strain 578. The toxin is harvested in the supernatant after centrifugation and detoxified by addition of formaldehyde. The final vaccine is produced by preparing an *E. coli / C. perfringens* type C antigen mixture, which contains fixed amounts of all the antigens sufficient for the formulation of the final product. The antigen mixture is blended with the adjuvant and excipients followed by filling and packaging.

The beta-toxoid of Porcilis ColiClos from CperfC strain 578 has been replaced by the beta-toxoid from CperfC strain CN 883 as used in another product of the company, Bravoxin-10. The quality information regarding production of beta-toxoid from CperfC strain 578 has been moved to the annexes of the respective dossier parts. This was done to retain the information regarding quality of the beta-toxoid from CperfC strain 578 that is used in safety and efficacy laboratory and field trials.

## *Description of the manufacturing method*

For the manufacturing of the *Clostridium perfringens*, Type C beta-toxoid from strain CN 883, the applicant intends to use the same manufacturing process and site as already established for this antigen, including the starting materials and in-process controls. These production processes (steps and respective in-process controls) are very similar.

The flow-chart and the detailed description of the antigen production provided in the quality part of the dossier have been updated.

The additional changes to Part 2.B of the dossier are consequential to replacing the source strain for the beta-toxoid antigen and its production site.

All the relevant information is adequately presented and considered acceptable.

## *Production and control of starting materials*

## Starting materials not listed in a pharmacopoeia

### *Starting materials of biological origin*

#### Master seed strain CN 883 and starting materials used for the master/working seed preparation and antigen production

The master seed and working seed for producing the beta-toxoid antigen from *C. perfringens* strain CN 883 were originally established for the vaccine Bravoxin. Identical information is now included in the quality part on starting materials of biological origin of the Porcilis ColiClos dossier.

The initial source for the *C. perfringens* Type C strain CN 883 was isolated in 1940. The strain is of ovine origin and isolated in the United Kingdom.

The seed material has been adequately tested for identity and purity. The strain's origin and passage history are sufficiently presented. The *C. perfringens* Type C master seed strain CN 883 was fully characterised. Identity and purity of this strain were confirmed in line with current guidelines.

The master seed strain CN 883 complied with all relevant tests and the results are provided in the master seed protocols.

The working seed is established by inoculating the master seed into Robertsons Cooked Meat (RCM) broth and subsequent passages. Identity and purity of the working seed is also confirmed, and the test results of the working seed are provided.

Seed designation and storage conditions are described.

Additional starting materials of biological origin other than those previously listed in the Porcilis ColiClos dossier have been used to generate the seed material and are used for downstream antigen production. Hence, the TSE risk assessment and table A listing "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" were updated accordingly. Respective information is provided in the dossier. Example certificates of analysis for additional starting materials are provided in the respective dossier sections, and where applicable, a separate TSE risk assessment has been submitted. The overall conclusion on TSE risk remains unchanged. The TSE risk is negligible and the vaccine Porcilis ColiClos complies with the TSE guidelines EMEA/410/01 and EMEA/CVMP/019/01. This conclusion is supported by the CVMP.

Materials that are not used anymore during production have been moved to the historical document provided as Annex to Part 2.C of the Porcilis ColiClos dossier. This is considered acceptable.

The submitted documentation is complete and all starting materials are already approved either for Porcilis ColiClos or for Bravoxin.

To confirm the equivalence between the two C. perfringens Type C strains (578 and CN 883) the following investigation has been performed:

- **Equivalence between antigen yield test for beta-toxoid from C. perfringens strain 578 and strain CN 883:**

The amount of beta-toxoid used to blend Porcilis ColiClos is based on TCP (Total Combining Power) values. To assure that Porcilis ColiClos is formulated with the same quantity of beta-toxoid, the beta-toxoid antigen yields obtained from both origins were correlated. Therefore, samples from antigen batches produced at both sites were transferred between the two sites and allocated to test sets. The antigen contents of the test samples were determined using TCP test at both sites and the correlation between the TCP test results obtained from both sites was estimated.

A very good correlation between test results on both sites for beta-toxoid content with an R2 value of 0.9752 was found. This demonstrates the equivalence between the beta-toxoid antigen produced using these two strains and the antigen quantification assays performed at both sites. In addition, the TCP test used for antigen content determination also confirms the identity of the beta- toxoids produced at both sites as this test uses *C. perfringens* beta-antitoxin to neutralise the beta-toxoid. Subsequently, identity of the beta-toxoid antigen used for Porcilis ColiClos is again confirmed using *C. perfringens* identity and batch potency test performed on the finished product. All Porcilis ColiClos batches containing beta-toxoid from strain CN 883 that were produced to support this variation were tested for batch potency and identity using the mouse neutralisation test (MNT test), except for one batch that was tested with the current ELISA test. In the MNT test, antiserum (against beta-toxin) generated from Porcilis ColiClos vaccinated rabbits is used to neutralise the affinity purified beta-toxin that is used in this assay. The current ELISA test is performed with serum samples from rabbits immunised with beta-toxoid antigen. In summary, the beta-toxoid content determination test performed on the antigen bulk and the batch potency and identity test performed on the final product confirm the identity of the beta-toxoid from strain CN 883.

## *Control tests during the manufacturing process*

**Validation of the production process and most relevant IPC tests of beta-toxoid from strain CN 883**

The production process for the beta-toxoid from strain CN 883, in accordance with Ph. Eur. monograph 0363, is a well-established production process already used for many years for the vaccine Bravoxin.

**Validation of the *C. perfringens* type C Total Combining Power**

Validation of the C. perfringens type C Total Combining Power (TCP) assay was performed using the test method received as quality control as part of a technology transfer. Validation of this analytical test required examination of assay characteristics commonly held to be standards of assay performance. The validation data has shown the test to be specific, linear, and to have satisfactory precision and robustness.

**Validation of the inactivation of *C. perfringens* type C beta-toxoid**

The treatment of clostridial cultures with formaldehyde solution is conducted to inactivate the bacterial cells and convert the bacterial toxins to toxoids. These two processes take place simultaneously. C. perfringens type C is inactivated/detoxified by adding formalin and incubating.

The requirement for inactivation is that the time taken should be no more than 67% of the duration of the total inactivation process applied in production. The studies therefore included a

sampling regime where samples were taken at exactly (or earlier than) 67% of the total inactivation time and showed that the inactivation process rendered all organisms inactivated within 67% of the total 7 days inactivation period.

It is concluded that the inactivation regime for *C. perfringens* type C beta-toxoids is sufficient to render all organisms nonviable within 67% of the total inactivation time.

**Validation of detoxification of *C. perfringens* type C beta-toxoids**

The detoxification process using formaldehyde solution is a balance between detoxifying the toxins while retaining the immunological properties of these proteins. This requires the addition of small volumes of formaldehyde solution, testing whether the toxin is detoxified and, if necessary, further addition of formaldehyde solution and testing for the absence of toxicity.

All antigens are tested for non-toxicity after formaldehyde treatment and concentration. In addition to the non-toxicity test, the quality of the toxoids is also tested (potency and sterility), confirming that the immunological properties are always retained after treatment (and re-treatment) with formaldehyde. It is concluded that the detoxification process is a robust procedure and ensures that there will be no residual toxicity in the concentrated antigen or the final formulated vaccine and that the immunological properties of the antigens are retained. If the antigen is still toxic it must be re-treated.

The data confirm that beta-toxoid from strain CN 883 is consistently produced. Since the in-process control tests (Part 2.D of the dossier) are already approved for the same antigen contained in another EU authorised product by the same MAH, the CVMP considers the tests suitable to control beta-toxoid production for Porcilis ColiClos.

The applicant has provided an acceptable justification not to perform a final product formaldehyde test.

## *Batch-to-batch consistency*

**Quality and consistency of production of Porcilis ColiClos using beta-toxoid from strain CN 883**

To confirm that the replacement of beta-toxoid strain 578 by strain CN 883 has no impact on the finished product quality, one full scale and three small scale batches of Porcilis ColiClos were prepared and tested as described in the dossier. Although the batch safety test is no longer an official product release test, 3 of the 4 final product batches presented were tested in the batch safety test to confirm the safety of the product formulated with beta-toxoid from strain CN 883. The results of the final product control tests on these four batches of Porcilis ColiClos met all specifications.

The final vaccine batches formulated using beta-toxoid from strain CN 883 results in vaccine batches of consistent quality.

## *Stability*

Pilot scale batch was prepared with 1-year-old beta-toxoid antigen that was stored at 2-8 °C. All stability parameters of that batch remained within the specifications over a test period of 27 months supporting an antigen shelf-life for the beta-toxoid component of 12 months.

This was already shown and authorised for beta-toxoid from strain 578 as well as *C. perfringens*

Type C beta-toxoid contained in Bravoxin (refer to stability report provided in root-folder 2.B. of the dossier). Therefore, an antigen shelf-life of 1 year at 2-8°C for beta-toxoid from strain CN 883 seems appropriate for Porcilis ColiClos and is considered acceptable by the CVMP.

The proposed change in production process could potentially impact the stability of the final product. To assess the impact of this change on the vaccine stability, all four vaccine batches formulated with beta-toxoid from strain CN 883 were stored at 2-8°C and at regular interval samples were taken and tested for stability parameters. For these four vaccine batches, a full stability data set is available, and it demonstrates that the batches are stable for the full stability study period of 27 months. The dossier section Part 2.G. has been updated with the stability test results.

The full scale batch had an out of trend result (313 IU/ml instead of 120 IU/ml; batch release limit ≥10 IU/ml) for *C. perfringens* type C (CN883) batch potency test at 0-month stability time point and two batches (388 IU/ml and 338 IU/ml, both of pilot scale) at 21-month stability time point. The *C. perfringens* type C batch potency test was shown to be sensitive for the rabbits used and to ensure the consistency in future *C. perfringens* type C batch potency test results, the applicant has implemented rabbits from one specific source as the only rabbit colony for this batch potency test. Subsequent time points resulted in batch potency test results within the historical trend.

The batch potency test result for the *E. coli* LT component of the production scale batch containing *C. perfringens* type C antigen, strain CN 883 was just below the specification of ≥ 10.9 log2 at T=9 stability time point. However, the test results for the LT components were within the specification during subsequent time points. The root cause investigation pointed at the animal phase of this test. This variability induced by in-vivo part of the test is already partially captured in the possibility to remove one non-responder from the test (variation procedure number EMEA/V/C/002011/IB/0004/G) and to do a double repeat when the initial LT test is below the release specifications (10.9 - 14.1 log2 titres). After re-testing, both repeated test runs should be successful and the average of all the three tests should be within the release limits.

For the full scale batch, T=9 initial test result was 10.7 log2 titres, repeats were 12.6 and 10.7 log2 titres, resulting in an average titre of 11.3 log2, however, as 2 out of 3 test runs were just below the lower limit (10.9 log2 titres LT); hence, the T=9 LT potency test result was confirmed out of specification (OOS).

These observations confirm that the T=9 result is an outlier caused primarily by high assay (run) variability and not due to decline in LT batch potency of the vaccine batch. This conclusion is further strengthened by the fact that, the LT batch potency test results for this batch being within specification for the subsequent stability time points (T=15, T=21 and T=27).

Taken together it can be concluded that the final product formulated with beta-toxoid from strain CN 883 is stable for at least 27 months at 2-8 °C supporting the product shelf-life of 24 months.

## *Overall conclusions on quality*

The production process of beta-toxoid from *C. perfringens* strain 578 and strain CN 883 is highly similar except for small discrepancies that are not expected to affect the quality of the produced antigen. Production of CN 883 beta-toxoid is already authorised at proposed site for Bravoxin and is well validated. The flow-chart and the detailed description of the antigen production provided in the dossier have been updated. The changes to Part 2.B of the dossier are consequential to replacing the source strain for the beta-toxoid antigen and its production site. The relevant information is adequately presented and acceptable.

The master seed and working seed for producing the beta-toxoid antigen from *C. perfringens* strain CN 883 were originally established for the vaccine Bravoxin. Identical information is now included in the Porcilis ColiClos dossier. The seed material has been adequately tested for identity and purity. The strain's origin and passage history are presented. Seed designation and storage conditions are described. The TSE risk assessment and table A listing "*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*" are updated accordingly. Example certificates of analysis for additional starting materials are provided. The TSE risk is negligible.

Overall, the data from the correlation study support the applicant's conclusion that one unit of beta-toxoid from strain 578 is equivalent to one unit of beta-toxoid from strain CN 883. This also suggests that finished product blended with beta-toxoid from *C. perfringens* strain 578 is highly comparable/equal to finished product prepared with beta-toxoid from *C. perfringens* strain CN 883. This is supported by further quality, safety and efficacy data.

Validation of the Total Combining Power (TCP) assay for antigen quantification as in-process control test during antigen production was established for Bravoxin. The test method was found to be well validated, in line with Ph. Eur. monograph 0363, and adequate to quantify beta-toxoid from *C. perfringens* strain CN 883.

Validation of the inactivation of *C. perfringens* Type C at the manufacturing site was established for Bravoxin and is in line with Ph. Eur. monograph 0062. Addition of formalin and incubation was found to be effective to inactivate the bacteria cells.

Detoxification: a general SOP for testing non-toxicity or residual toxicity of vaccine components in mice is included in the dossier. No formal validation of the test has been done; however, it is considered fit-for-purpose since it has been successfully performed for *C. perfringens* beta-toxoid antigen incorporated in other products by the applicant (e.g. Bravoxin) for several years. Additionally, during inactivation/detoxification, the antigen's immunological properties should be maintained, which is evaluated by the potency test.

Batch protocols are provided for three small-scale and one production-scale batch produced with the proposed beta-toxoid of *C. perfringens* strain CN 883. All presented batch data are within the authorised specifications. The data support that vaccine batches produced with CN 883 antigen are of comparable quality as batches produced with the currently authorised antigen.

The proposed shelf-life for Porcilis ColiClos is 24 months. To support the shelf-life, the applicant prepared a new stability study over 27 months with four vaccine batches (3 pilot scale and 1 production scale) containing beta-toxoid of *C. perfringens* CN 883 produced at the site.

Overall, the stability data based on potency, biophysical characterisation (dl-α-tocopheryl acetate content, pH and appearance) and sterility so far support the shelf-life of 24 months for the finished product prepared with beta-toxoid from stain CN 883. One batch was prepared with 1-year-old beta-toxoid antigen supporting an antigen shelf-life for the beta-toxoid component of 12 months.

# Part 3 – Safety documentation (Safety and residues tests)

To support that the replacement of the beta-toxoid does not result in different safety characteristics of the vaccine, final product containing beta-toxoid from strain CN 883 was tested in one laboratory safety study.

## *Pre-clinical studies*

**Safety and efficacy of Porcilis ColiClos vaccine with beta-toxoid from strain CN 883**
**Safety study 1:** Safety of Porcilis ColiClos in pregnant gilts after repeated intramuscular vaccination

**Study design:**

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|---|---|
| **Animals + vaccination scheme** | Twenty pregnant gilts were randomised into 2 groups:<br><br>**<u>Group 1</u>**:<br><br>**D0** 10 gilts received intramuscularly a double dose (4 ml) of **Porcilis ColiClos** 6-8 weeks before farrowing.<br><br>**D28** (repeated dose): single dose **Porcilis ColiClos** (2 ml)<br><br>**<u>Group 2</u>**:<br><br>**D0** 10 gilts received intramuscularly 4 ml PBS<br><br>**D28** (repeated dose): 2 ml PBS |
| **Vaccine** | **Porcilis ColiClos: D0 + D28**<br><br>**Reference item**: PBS |
| **Administration route** | Intramuscular (i.m.)<br><br>Day 0: inoculation right side of the neck<br><br>Day 28: inoculation left side of the neck |
| **Housing** | Animals of Group 1 and Group 2 were housed in the pregnant sow unit at farm ToJa at 23 – 25 °C.<br><br>Water ad libitum. Standard feed mixture. |
| **Follow-up** | **<u>Observations:</u>**<br><br>**Clinical observation:**<br>D0 before vaccination, 2 ,4 ,6 hours after vaccination then daily until 14 days after vaccination<br><br>0 = normal<br>1 = less active<br>2 = vomiting<br>3 = depressed<br>4 = dead<br><br>**Rectal temperature:**<br>D-1, D0 before vaccination, 2, 4, 6 hours after vaccination, 1, 2, 3, 4 days after vaccination.<br><br>***Local examination:***<br>Palpation injection site: D0, daily until 14 days after vaccination.<br><br>Size (diameter or length x width in cm) Type of reaction (H = hard, S = soft, W = warm, P = painful<br><br>**Reproduction:** |

| | |
|---|---|
| | Number of live-born piglets, number of stillborn piglets, number of mummies (stillborn piglets and mummies were necropsied). |

**Results:**

**Clinical observation**

All sows were pregnant as established by ultrasound scanning on the day prior to vaccination. The veterinary control at the start of the study revealed no abnormalities; all sows were declared healthy. Clinical abnormalities were absent in all sows after the first and the second vaccination.

**Rectal temperature**

After the first (double dose) vaccination with Porcilis ColiClos, a transient temperature reaction with a mean maximum increase of 0.8 °C between 2 and 6 hours post vaccination (p.v.) was observed. The maximum individual increase was 2.2 °C. Injection with 4 ml PBS resulted in a mean maximum increase of 0.3 °C at 2 hours post vaccination and an individual maximum increase of 1.4 °C. Temperatures of all gilts had returned to the normal level on the day after vaccination. After the single dose booster vaccination with Porcilis ColiClos, the mean maximum increase was again 0.4 °C with an individual maximum of 1.1 °C. The mean maximum increase after the second PBS injection was 0.2 °C at 4 hours p.v., with an individual maximum of 0.5 °C. All animals had returned to baseline on day 1 after the second vaccination.

**Local examination**

No local reactions were observed after the double dose vaccination, with the exception of one sow (group 1) which showed small (0.5-1 cm) hard local reactions on day one and two p.v. After the second vaccination, all but one of the Porcilis ColiClos-vaccinated sows exhibited local reactions varying from 0.5 to 5 cm, and both soft and hard injection sites were observed. By day 11 p.v., the local reactions had disappeared in all but two gilts. In these two animals, the local reactions were still present at day 14 p.v. In the PBS control group, one animal showed a local reaction (1 cm) on the day after vaccination only.

**Reproduction**

All sows farrowed in term and dead piglets and/or mummies were observed in eight litters in the Porcilis ColiClos group and in seven litters in the PBS group. There were some discrepancies between the scoring of mummies on the farm and by the pathologist, but the scoring performed by the pathologist is considered to be more reliable and was used to calculate means. The mean numbers of live-born piglets, stillborn piglets and mummies in Porcilis ColiClos vaccinated sows were 13.8, 1.5 and 0.5, respectively. In sows treated with PBS the mean numbers of live-born piglets, stillborn piglets and mummies were 14.4, 1.6 and 0.7. Necropsy findings in stillborn piglets from Porcilis ColiClos vaccinated sows and in piglets from PBS treated sows were similar and only revealed complete atelectasis in most stillborn piglets. There were no clear causes for these perinatal deaths.

One litter in the Porcilis ColiClos group and five in the PBS group had mummies. Mummification had taken place approximately a month before and three days after double dose vaccination and at twenty-four days after double dose vaccination with Porcilis ColiClos. In PBS treated animals, mummification had taken place seven, thirteen, nineteen and twenty-seven days after first injection with a double dose and four and seven days after the second injection with the single dose. Foetal death as a result of treatment is very unlikely but cannot be completely excluded as

the calculated mummification dates of one of the three mummies in the Porcilis ColiClos group and four of the seven mummies in the PBS group were calculated to be around the time of first or second treatment.

### *Overall conclusions on the safety documentation*

Porcilis ColiClos formulated with the alternative *C. perfringens* type C antigen containing beta-toxoid from strain CN 883 is safe for pregnant gilts.

It was shown that vaccination with Porcilis ColiClos containing beta-toxoid from strain CN 883 resulted in a maximum transient temperature increase of 2.2 °C. Local reactions, soft and hard, were observed varying from 0.5-5 cm and disappeared in most cases within the follow-up period.

Based on these results it can be concluded that Porcilis ColiClos with beta-toxoid from strain CN 883 is safe for pregnant sows and gilts and shows a comparable safety profile as concluded from previous safety studies with Porcilis ColiClos and as reflected in section 4.6 of the SPC. In addition, 3 final product batches formulated with the beta-toxoid from strain CN 883 passed the former batch safety test, supporting the safety of the product formulated with beta- toxoid from strain CN 883. The relevant safety study has been included in the respective part of the dossier.

## Part 4 – Efficacy documentation (pre-clinical studies and clinical trials)

To support that the replacement of the beta-toxoid does not result in different efficacy characteristics of the vaccine, final product containing beta-toxoid from strain CN 883 was tested in one laboratory efficacy study.

### *Pre-clinical studies*

**Efficacy Study 1:** Efficacy study of Porcilis ColiClos prepared with different clostridial antigens in pregnant gilts after intramuscular vaccination.

**Study design:**

| | |
|---|---|
| **Animals + vaccination scheme** | Twenty-five pregnant gilts were randomised into 2 groups:<br>**Group 1**:<br>**D0** 10 gilts received intramuscularly a single dose (2 ml) of **Porcilis ColiClos strain 578** antigen 6-8 weeks before farrowing.<br>**D28** (repeated dose): single dose **Porcilis ColiClos strain 578** (2 ml)<br>**Group 2**:<br>**D0** 10 gilts received intramuscularly a single dose (2 ml) of **Porcilis ColiClos strain CN 883** antigen 6-8 weeks before farrowing.<br>**D28** (repeated dose): single dose **Porcilis ColiClos strain CN 883** (2 ml)<br>**Group3:** |

| | |
|---|---|
| | **D0** 5 gilts received intramuscularly 2 ml PBS.<br>**D28** (repeated dose): 2 ml PBS |
| **Vaccine** | **Porcilis ColiClos strain 578 Antigen: D0 + D28**<br>**Porcilis ColiClos strain CN 883 Antigen: D0 + D28**<br>**Reference item**: PBS |
| **Administration route** | Intramuscular (i.m.)<br>Day 0: inoculation right side of the neck<br>Day 28: inoculation left side of the neck |
| **Housing** | Animals of Group 1, Group 2 and Group 3 were housed in the pregnant sow unit at farm ToJa at 23 – 25 °C.<br>Water ad libitum. Standard feed mixture. |
| **Follow-up** | **Observations:**<br>**Clinical observation:** Daily<br>**Serum sampling:** At D0 and D28<br>**Colostrum sampling:** After partus. Pooling per group for *in vivo* beta-toxin neutralisation test |

**Results:**

**Clinical observation**

All sows were pregnant as established by ultrasound scanning on the day prior to vaccination. The veterinary control at the start of the study revealed no abnormalities; all sows were healthy. Gilt 2135 (Group 2, strain CN 883 antigen) aborted on day 16 post priming vaccination. This interval makes a direct correlation to vaccination highly unlikely. No follow up investigation of the aborted litter was performed.

**Serology**

At the start of the experiment, the gilts were seronegative or had low antibody titres against the *E. coli* antigens, and almost all were seronegative for the *C. perfringens* beta toxin. Mean colostrum titres show a clear antibody response to all antigens. There were no statistically significant differences between the two vaccinated groups for the five *E. coli* antigens, but the beta toxin response was significantly higher in the Porcilis ColiClos (strain CN 883 antigen) group. A higher response was also found when pooled colostrum was tested in the Ph. Eur. toxin neutralisation test. Antitoxin levels of 4 IU/ml and 40 IU/ml were found for Porcilis ColiClos (strain 578) and Porcilis ColiClos (strain CN 883), respectively.

It was noted that in two animals in each of the Porcilis ColiClos groups the colostrum antibody titres to almost all antigens were lower or just slightly higher than the serum antibody levels at the time of booster vaccination, whereas in all other vaccinated animals the colostrum titres were in general 2-4 log2 higher than these serum titres. This is unexpected and suggestive of incorrect colostrum sampling as antibody levels in colostrum/milk drop after each feeding of the piglets. Therefore, the statistical analysis was repeated excluding these four gilts: removal of these four

animals from the data set did not result in a different outcome of the statistical analysis, the mean anti-beta toxin titre in the group vaccinated with Porcilis ColiClos containing *C. perfringens* type C antigen produced at the proposed site was still significantly higher.

### *Overall conclusion on efficacy*

The serological efficacy of Porcilis ColiClos with the beta-toxoid from strain CN 883 was compared with that of Porcilis ColiClos containing beta-toxoid from strain 578 produced at current production site. Study results showed that antibody responses against *E. coli* antigens were similar between the test groups. The antibody titre against beta-toxoid in colostrum were significantly higher in the Porcilis ColiClos strain CN 883 group compared to the Porcilis ColiClos strain 578 group. These results indicate that Porcilis ColiClos containing beta-toxoid from strain CN 883 is at least as efficacious as the currently registered vaccine containing strain 578 antigen and the relevant efficacy study has been included in the respective part of the dossier.

## Part 5 – Benefit-risk assessment

### *Introduction*

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

The proposed variation is to replace the *C. perfringens* type C master seed by a new strain, to replace the current authorised site for the beta-toxoid antigen manufacturing process and in process control tests by a new site, and to adapt the production process and in process control tests for the beta-toxoid antigen.

### *Benefit assessment*

### Direct benefit

With this variation, the applicant wishes to replace the currently authorised *C. perfringens* type C master seed by one based on a new strain. Moreover, it is proposed to change the currently authorised site for the beta-toxoid antigen manufacturing process and in-process control tests by a new site. As a consequence, the production process and in-process control tests for the antigen are proposed to be adapted. A well-designed GLP laboratory study was conducted to evaluate the safety and the efficacy of the beta-toxoid from new strain CN 883 of *C. perfringens* Type C.

The benefits of the product remain unaffected by this variation.

### *Risk assessment*

The main potential risks are identified as follows:

*Quality*

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

*Safety*

*Risks for the target animal*

The safety of Porcilis ColiClos to provide passive immunity to pigs (pregnant sows and gilts) was confirmed in a GLP safety study. Vaccination with Porcilis ColiClos containing beta-toxoid from strain CN 883 resulted in a maximum transient temperature increase of 2.2 °C. Local reactions, soft and hard, were observed varying from 0.5-5 cm and disappeared in most cases within the follow-up period.

These results are reflected in section 3.6 of the SPC and section 7 of the package leaflet.

Administration of Porcilis ColiClos with beta-toxoid from strain CN 883 to pigs in accordance with SPC recommendations is generally well tolerated.

*Risk for the user*

The risks for the user remain unaffected by this variation.

*Risk for the environment*

The risks for the environment remain unaffected by this variation.

*Risk for the consumer*

The risks for the consumer remain unaffected by this variation.

## *Risk management or mitigation measures*

Risk management or mitigation measures remain unaffected by this variation.

*User and environmental safety:*

The risks for the user and the environment remain unaffected by this variation.

## *Evaluation of the benefit-risk balance*

No change to the impact of the product is envisaged on the following aspects: quality, safety, user safety, environmental safety, consumer safety, target animal safety and efficacy.

The benefit-risk balance remains unchanged.

## *Conclusion*

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Veterinary Medicinal Products (CVMP) considers that the application for a variation to the terms of the marketing authorisation for Porcilis ColiClos is approvable. The benefit risk balance remains unchanged.

Changes are required in the following Annexes to the Union marketing authorisation:

I and IIIB

Please refer to the separate product information showing the final version.

As a consequence of these variations, section 2 of the SPC and of the Package Leaflet are updated accordingly.