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Committee for Medicinal Products for Veterinary Use

CVMP assessment report for ECOPORC SHIGA (EMEA/V/C/002588/0000)

Common name: Genetically modified STx2e (shiga toxin 2e) antigen

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

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Introduction

An application for the granting of a Community marketing authorisation of ECOPORC SHIGA has been submitted to the European Medicines Agency (the Agency) on 21 November 2011 by IDT Biologika GmbH in accordance with the Article 3(1) of the Regulation (EC) No. 726/2004 based on recombinant DNA technology used for the construction of the production strain.

The CVMP adopted an opinion and CVMP assessment report on 7 February 2013.

On 10 April 2013, the European Commission adopted a Commission Decision for this application.

ECOPORC SHIGA contains the recombinant Shiga toxin (Stx2e) and is intended for the active immunisation of piglets from the age of four days to reduce the mortality and clinical signs of oedema disease caused by Stx2e forming *E. coli* (STEC). The route of administration is intramuscular and the target species is pigs.

The recommended dose is 1 ml (single dose) and the vaccine is presented in packs/containers of 50 and 100 doses.

Part 1 - Administrative particulars

The pharmacovigilance system as described by the applicant fulfils the requirements of Directive 2001/82/EC, as amended and provides adequate evidence that the applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any suspected adverse reaction occurring either in the Community or in a third country.

The site of manufacturer of the active substance and batch release is covered by a current manufacturing authorisation. The site has adequate production and animal test facilities and is also covered by a current GMP certificate. Details of the batch release procedures and responsible persons have been provided and are satisfactory.

All aspects of the production of the active ingredient and finished product are conducted at a single site within the EEA. In view of the fact that the manufacturing site has been inspected during the past 2 years, no site inspection is considered necessary. A scientific advice was given in 2008 (EMEA/CVMP/487264/2008) regarding residual plasmid DNA. The question raised in the advice is however no longer relevant as the applicant has changed the production strain and production process. It is therefore acceptable that the advice given has not been followed.

Overall conclusions on administrative particulars

The dossier contains sufficient information to confirm that the requirements for administrative particulars have been met.

Part 2 - Quality

Composition

ECOPORC SHIGA is an inactivated vaccine containing genetically detoxified shiga toxin (Stx2e) adjuvanted with aluminium hydroxide. During production, the active ingredient is chemically treated with ethyleneimine to remove residual plasmid DNA and then stabilised with gluteraldehyde. Consequently the finished product may contain traces of gluteraldehyde and sodium thiosulphate (used to neutralise ethyleneimine). Recombinant Stx2e toxin is added at formulation to at least 8×10^6 $CD_{50equivalent}$ per ml. The vaccine contains at least 3.2×10^6 ELISA units (relative potency ELISA test

measured on the finished product) of the genetically detoxified shiga toxin. The vaccine is intended to be available in multidose presentations and consequently contains thiomersal as a preservative.

Container

The finished product is to be filled into both glass and polyethylene terephthalate (PET) bottles, both stoppered with bromobutyl rubber stoppers and sealed with an aluminium cap. Proposed presentations are 50 ml and 100 ml in both glass and PET bottles.

Development pharmaceutics

The development of the product is in the main well described. The construction of the master seed strain is thoroughly explained and the reasons for the genetic modifications conducted are justified. The objective was to produce a genetically modified *E. coli* strain with a plasmid carrying both sub-units of the Stx2e shiga toxin containing a specific change. As a result the toxic A subunit rendered to a non-toxic protein, whilst the B subunit responsible for cell surface receptor binding unaltered, enables the induction of an immune response which contains antibodies able to block the binding of the native toxin to cell surface receptors.

The antigen concentration in the finished product (calculated in the Stx2e ELISA), was used as the potency test. This method has been validated and the recovery rate of antigen has been estimated. A titration study was conducted, where it was concluded that a vaccine with the minimum antigen content will induce significant protection of piglets calculated 4.0×10^6 $CD_{50equivalent}$ was protective to 65% of the animals challenged 21 days after vaccination. The antigen content targeted for batch release has been defined as 8×10^6 $CD_{50equivalent}$ (calculated), which is clearly above the antigen content shown to be effective. The product specification for batch release is now set by the 95% confidence interval at blending will ensure that the right amount of Stx2e antigen is added to the vaccine at blending.

The batches comply with the criteria for the batch potency test.

Method of manufacture

Working seed cells are cultivated in a 4-stage scale up process. The duration of each passage is defined. The main fermentation is conducted in two phases; firstly growth is cultivated up to defined level, after which the culture is induced to express recombinant protein by the addition of an inducer.

Downstream processing of the main fermentation culture first involves separation of the biomass from the supernatant (containing the recombinant Stx2e antigen) by continuous flow centrifugation. The residual plasmid DNA in the sterile filtrate is chemically degraded and the active ingredient is stabilised to inactivate any proteases present in the filtrate which may affect the stability of the antigen.

Deactivated solution is tested for Stx2e content by a validated antigen capture ELISA.

The filling of the final antigen bulk occurs under sterile conditions with constant stirring to fill 50 ml bottles and 100 ml bottles and is controlled by weight and allows an acceptable overage.

Consistency of production is supported by data from 7 vaccine batches prepared from three consecutive fermentation batches, and including batches filled in each of the proposed presentations (50 ml and 100 ml glass, 50 ml and 100 ml PET). A summary of data from in-process and finished product testing has been provided. All batches comply with the proposed in-process and finished product testing specifications.

Control of starting materials

Active substance

The proposed storage conditions has been validated thorough the evaluation of the stability of the active ingredient at various stages during production.

The Stx2e antigen ELISA test will be used for measuring the amount of antigen in the finished product and will allow a better monitoring of its decrease over time. A minimum shelf life titre of ≥3.2 x10^6 $CD_{50\ equivalent}$ has been calculated based on a dose titration study conducted that showed that the administration of a single dose of ECOPORC SHIGA was protective to 65% of the animals challenged 21 days after vaccination.

Excipients

Starting materials listed in the European Pharmacopoeia (Ph. Eur.) include aluminium hydroxide, thiomersal, hydrochloric acid, sodium hydroxide, sodium chloride, silicone (anti-foam), water for injection, glycerine (vegetable) and lactose monohydrate, all of which comply with the requirements of their relevant monograph. Lactose is derived from milk which has been certified as being fit for human consumption.

Starting materials of biological origin which are not listed in the Ph. Eur. include the *E. coli* master and working seeds, and culture media components such as bacto tryptone, bacto yeast extract, LB broth and LB agar. The history and testing conducted on the seed lots is well described and the purity of the seeds has been adequately demonstrated. The risk of potential contaminants has been addressed through the thorough description of the sterilization and production processes, and the testing of the materials against relevant extraneous agents.

Starting materials of non-biological origin which are not listed in the Ph. Eur. include gluteraldehyde, ethyleneimine, sodium thiosulphate and antifoam agent SE9. Details of these starting materials is satisfactory. The in-house preparation of media and other reagents are well described.

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

The risk that the bacterial master or working seeds might be contaminated with transmissible spongiform encephalopathy (TSE) agents is negligible. The original strain was sourced from a recognised official collection of microorganisms. Materials of bovine origin used during the production of master seed and working seed are considered to pose a low risk of contamination, sourced from bovine milk suitable for human consumption.

All of the starting materials of ruminant origin used during vaccine production are considered to constitute a low risk of contamination, either because their countries of origin are considered to be free of TSE infections or/and because the tissues they are sourced from are considered to have a low risk irrespective of the country of origin. Any products from bovine milk derive from milk suitable for human consumption. The staring materials have been shown to comply with the “Note for Guidance on minimizing the risk of transmitting animal spongiform encephalopathies agents via human and veterinary medicinal products’ (EMEA/410/01-rev.3) and Commission Directive 1999/104/EEC.

Therefore, it can be concluded that the risk of TSE transmission associated with vaccinated animals is considered negligible. In addition, in contrast to ruminants and humans, pigs are not known to be highly sensitive to TSE.

Control tests during production

The description of the tests conducted at various stages during the production of the product (gram stain, sterility, purity, pH, Stx2e antigen content, sodium thiosulphate content, bioburden, fill volume) have been provided. Specifications have been proposed which are in the main adequate, providing reasonable control of the production process. Information concerning the procedures and practices in place to monitor, control and replace key test reagent and controls have been provided and the tests were adequately validated.

Control tests on the finished product

The description of the methods used for the control of the finished product (appearance, pH, aluminium content, thiomersal content, gluteraldehyde content, endotoxin content, sterility, safety, identity and potency) and the specifications were provided. The specifications proposed at release and at the end of shelf-life are in the main appropriate to control the quality of the finished product.

The results of the analysis of three consecutive production fermentation runs, formulated into 7 vaccine batches of vaccine were presented which comply with the proposed specification.

Overall, the control methods are satisfactory and they confirmed that the production and control processes generate consistent vaccine batches.

Stability

Data has been provided in support of the proposed storage periods, the conditions during the production process and for the stability of the finished production bulk. Four batches were tested at 0, 3 and 6 months, with data at 9 months available for one batch. There is evidence that antigen content will decline over time. In the main the data generated is used to set defined storage conditions and times.

Eight vaccine batches were placed on stability trial, prepared from different fermentation lots, filled in all the proposed presentations (both glass and PET bottles containing both 50 ml and 100 ml). Stability studies were designed to investigate the long term storage of vaccine in both an upright and inverted position (to investigate the effect of contact with the stopper). In addition, stability under stress conditions (30 °C for 3 days) was also investigated to determine the effect of a break in the cold chain during transport of the stability of the vaccine. There are planned stability trials to extend to 39 months batch storage.

The stability data provided for two batches of the finished product, was produced with bulk antigen stored for 9 months (proposed storage limit). There are data up to 12 months for both batches. Tests results are within specifications.

Data for six batches under inverted storage have also been provided. The stability of the product has been shown up to 18 months for four batches and 24 for two. All results are within specifications. The studies are currently on-going. The following stability data is currently available for the finished product:

Updated stability data was provided up to 21, 24 and 12 months (respectively) for three batches of 50 ml in PET containers, up to 21 months for two batches of 100 ml in PET containers and up to 24 months for one batch of 50 ml in glass containers and up to 21 months for one batch of 100 ml in glass containers. In addition, three new on-going stability studies will be established within the next production times to provide further ELISA test stability data of the vaccine since data point T0. The vaccine will be filled for additional stability studies in 50 ml PET, 50ml glass, 100ml PET and 100 ml

bottles Information regarding batch numbers and estimated dates for availability of stability results has also been provided.

At the present, a provisional shelf-life of 18 months can be granted only for PET containers, until additional data are provided. Furthermore, the in use stability of 24 hours has been demonstrated with batches stored up to 12 months.

Preservative efficacy has been tested in accordance with Ph. Eur. 5.1.3 and Monograph 0062 category C. Six batches of vaccine were tested. The vaccine does not meet the A and B criteria of Ph. Eur. 5.1.3, but is able to meet the C criteria for Ph. Eur. 0062. The applicant has justified this criteria and it is appropriate. Efficacy of the preservative data at the end of the proposed in use shelf-life is available and it is consider satisfactory.

Overall conclusions on quality

The dossier provides a comprehensive description of the development of the product, the development and validation of the production process and the development and validation of the tests used to control the production process.

A potency test was presented, measuring the antigen concentration in the finished product calculated in the Stx2e ELISA. This method has been validated and the recovery rate of antigen has been estimated. A titration study was conducted, and the antigen content targeted for batch release has been defined as 8×10^6 $CD_{50equivalent}$ (calculated). This is above the antigen content that has been shown to be effective in the study The product specification for batch release is now $\geq 5.3 \times 10^6$ CD50.

At this stage, a provisional shelf life of 18 months can be granted for PET containers. There are not sufficient stability data at present to support a shelf life claim for glass containers and therefore this container is rejected.

The stability data of two batches of finished product produced with bulk antigen stored for 9 months (proposed storage limit) have been provided. In use stability has been demonstrated up to 12 months. Further data are planned to be provided over time. Updated data for stability under stress studies are available and they are satisfactory.

Overall, the CVMP considers the analytical dossier is acceptable to show that the finished product is produced according to a consistent procedure of adequate standards and including adequate controls, to ensure that only safe and efficacious batches of vaccine are produced.

Part 3 – Safety

Safety documentation

ECOPORC SHIGA is an inactivated vaccine containing genetically detoxified shiga toxin (Stx2e antigen), adjuvanted with aluminium hydroxide and, containing thiomersal as preservative.

ECOPORC SHIGA is intended for the active immunisation of piglets from the age of four days to reduce the mortality and clinical signs of oedema disease caused by Stx2e forming *E. coli* (STEC). The vaccine is administered as a single intramuscular (IM) injection of 1.0 ml into the neck muscle behind the ear.

Laboratory tests

Three laboratory studies were carried out in compliance with the guideline for Good Laboratory Practice (GLP) to assess the safety for the administration of a single dose and a repeated administration of a

single dose, at administration of an overdose and repeated administration of an overdose and at repeated administration of an overdose with the highest endotoxin level.

Safety of the administration of a single dose

The animals in the study were administered a single vaccine dose followed by a repeated dose 14 days apart. Animals were of minimum age (4 days) and route of administration was intramuscular. Two different vaccine batches were used, one containing the target antigen content and a second with high antigen content. Both were produced according to the specifications. No systemic signs were observed apart from some few animals showing depression. No deaths were recorded post vaccination. Increase of body temperature was observed, with significant differences between treatment and control groups only post second vaccination, although the increase was transient. The maximum temperature recorded was 39.8 °C. Local reactions were observed post vaccination a few animals, which were mild swelling and disappeared spontaneously after 4 - 5 days. No significant histopathology findings were found, just those related to inflammation, which should resolve over time and without consequences for meat quality. No significant difference in weight was observed between treatment and control group.

Overall, the study demonstrates that a single dose and a repeated single dose can be safely administered to suckling piglets at the age of 4 days and older.

Safety of one administration of an overdose.

The animals in the study were administered a double vaccine dose followed by a second double dose 14 days later. The recommended vaccine schedule is only one dose and therefore the study complies with the requirements to demonstrate the safety of a double and a repeated dose. Animals were of minimum age and route of administration was intramuscularly. Two different vaccine batches were used, one containing the target antigen content and a second with high antigen content. Both were produced according to the specifications. No systemic signs were observed apart from a few animals showing depression which was also seen in the control group. Two deaths were recorded post vaccination, on days 13 (control group) and 3 (treatment group). The histopathology report has been included and concluded that the animals were crushed by their mothers, therefore not vaccine related. All temperatures were statistically equivalent for all study days between groups. The maximum temperature recorded was 40.5 °C, only in one pig at one time point, post-first vaccination.

Local reactions were observed only post-first vaccination in all groups including the control. These were mild swelling that disappeared spontaneously after 4 - 5 days. The same histopathology findings described after single dose/repeated dose administration were found, which should resolve over time and without consequences for meat quality. No significant difference in weight was observed between treatment and control group. All adverse reactions observed have been included in relevant sections of SPC. Although the serological status of the piglets at day 0 is unknown, most of them were seropositive on day 14 and all of them on day 28.

Overall, the study demonstrates that a double dose and a repeated double dose can be safely administered to suckling piglets at the age of 4 days and older.

Safety of the repeated administration of one dose

The safety of the repeated dose has been already addressed after single double dose. An additional study has been presented included the administration of a double dose followed by repeated dose using vaccine with the highest endotoxin content. This study was conducted to evaluate the safety of the maximum endotoxin content that has been proposed as the upper limit for the release of the vaccine. The animals in the study were administered a double vaccine dose followed by a second

double dose 14 days later. Two different vaccine batches were used, one containing the target antigen content, and a second with high antigen content . Both batches were spiked with the highest calculated endotoxin level. The proposed upper limit of endotoxin has been specified according to the level used in this study.

No systemic signs were observed apart from some animals showing depression. There were a total of 7 deaths recorded during the study pre and post vaccination, affecting all three groups including the control). It was concluded that the deaths were not vaccine related what was confirmed by histopathology reports for each of the dead animals.

A rise in body temperature was observed in a few animals. The maximum temperature recorded was 41.1 °C post-first vaccination and they all recovered within one day. Although the increased temperature was also observed in the control group, the causal relationship between the vaccination and the temperature increase cannot be excluded. Local reactions were observed in both treatment groups. These were mild swelling that disappeared spontaneously after 4 - 5 days.

Histopathology findings were chronic and acute inflammation, which was visible only microscopically and not in gross examination. These should resolve with the time and no consequences for meat quality should be expected.

Weight gain was not equivalent for vaccinated and control groups between study days 14 and 28. It was concluded that vaccination had no negative influence in weight development during the 14 days after each injection and the difference during the remaining period is probably not related to vaccination but to other factors. As no differences in weight were seen in previous overdose and repeated dose studies, the CVMP agrees with this view.

All adverse reactions observed have been included in relevant sections of SPC.

Although the serological status of the piglets at day 0 is unknown, most of them were seropositive on day 13 and all of them on day 28.

According to the proposed evaluation to measure the endotoxin levels, the study demonstrates that a double dose and a repeated double dose of the vaccine with the highest level of endotoxin can be safely administered to suckling piglets at the age of 4 days and older.

Examination of reproductive performance

No specific trials have been carried out and an appropriate warning has been included in section 4.7 of the SPC.

Examination of immunological functions

No specific trials have been carried out to assess the effects on the immunological functions. However, it is the aim of a vaccination to induce an immune response (specific antibodies) in the target animal. From the serological data obtained during the controlled laboratory trials to determine the safety of the vaccine ECOPORC SHIGA it can be concluded that the antigen component induces a strong serological response. There are no data which suggest that any of the components of the vaccine might adversely affect the immune response of the vaccinated animal. Furthermore, the results in the safety studies demonstrated that young suckling piglets at the age of 4 days do have the potent immune system to develop an immune response against the applied genetically modified Stx2e-antigen.

Special requirements for live vaccines

The vaccine is inactivated and therefore this is not applicable.

Study of residues

The active substance being a principle of biological origin intended to produce active immunity is not in the scope of Regulation (EC) 470/2009. The excipients, including adjuvants, included in the product 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. Considering the vaccine components a zero days withdrawal period is considered appropriate.

Interactions

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

Field studies

The three field trials conducted in Germany were in compliance with the guideline for Good Clinical Practice (GCP). They were performed to evaluate the safety and efficacy of ECOPORC SHIGA (see also part 4 of this report). Piglets of an average age of 4 days were vaccinated once intramuscularly with 1.0 ml ECOPORC SHIGA vaccine.

The following parameters were assessed at vaccination, 6 hours after vaccination and daily for the following 2 days and at day 7 and 14 after vaccination: behaviour, respiration, skin, digestion, rectal body temperature, local alterations at the injection site like swelling and / or colour change. Additionally, the piglets were weighted individually at vaccination and on study day 14.

The vaccine is considered safe for piglets from an age of 4 days on if general health of the piglets is not affected after vaccination. The mean body temperature of the vaccinated animals should not deviate from the mean body temperature of the control animals by +/- 0.5 °C at any time of measurement. Furthermore, possible local reactions at the injection site must show a tendency of declining within 2 days after vaccination and should no longer be evident 7 days after the injection. In general, local reactions should not occur in more than 10% of the animals of the vaccinated group. A clinically relevant difference is assumed, if the incidence of negative events in the Investigational Veterinary Product (IVP) group is more than 5 % higher compared to the Control Product (CP) group.

Overall, there were very few adverse findings due to the vaccination such as transient increase of body temperature and swelling at the injection site. All these finding had already been observed in the laboratory trials.

The safety of ECOPORC SHIGA is demonstrated sufficiently in the target animal (pigs) at a minimum age of 4 days under field conditions after the recommended intramuscular immunisation by the recommended administration of a single dose of 1.0 ml of the vaccine containing 8.0×10^6 $CD_{50equivalen}$t/ID.It can be concluded that ECOPORC SHIGA is well tolerated in the field when used in piglets at minimum age.

User safety

A user risk assessment was carried out, taking into account the nature of the vaccine, the type of administration, the excipients and product remainders after use.

As the ECOPORC SHIGA vaccine is a sterilised product and does not contain any live organisms, the likelihood of proliferation, persistence or excretion of vaccine organisms can be excluded. Therefore it can be concluded that the vaccine formulation does not present a zoonotic hazard to the user.

The exposure of the user during administration could occur via skin contact in case of accidental spillage or breakage of the container. The risk is considered to be low as the formulation is a ready to use one.

Accidental self-injection might occur and an appropriate warning is included in section 4.5 of the SPC. The potential risk of pathogenic effects after accidental self-injection is considered low as the modified Stx2e antigen does not play a significant role as human pathogen. Additionally the modified Stx2e antigen is genetically detoxified and no live organisms are present.

It is not expected that the adjuvants and the excipients will pose a risk to the user as they are all commonly used in veterinary and human vaccines.

To avoid any risk from remains of the vaccine after use, a respective disposal advice is given in the SPC.

Environmental risk assessment

A phase I environmental risk assessment was provided outlining that the potential exposure of the environment to the product and the level of risk associated with it is considered negligible. No phase II is considered necessary.

It is concluded that the only hazard to the environment might occur if the plasmid itself is taken up by bacteria in the environment. The likelihood of the contact with competent bacteria and uptake of intact plasmids is considered very low as the vaccine is inactivated and it is administered subcutaneously or intramuscularly. However, in case of plasmid uptake the maintenance of the plasmid in the cell is considered very unlikely as no beneficial genetic information such as antibiotic resistance genes are included in the plasmid. Furthermore, the expression of the STx2e antigen would be low due to absence of inducer and it has a reduced toxicity. To generate a hazard, the modified sequence of St2xeA would need to be reversed to the original sequence. This would only be possible by the exchange of two nucleotides which is very unlikely to occur based on the estimated mutation rate in *E. coli.* It is not expected that any hazard will occur during production as any DNA present after fermentation is treated and degraded with ethyleneimine and in addition, tests are carried out on each batch of vaccine to ensure the absence of viable organisms.

ECOPORC SHIGA is not expected to pose a risk to the environment when used as recommended. An appropriate warning is included in section 6.6 of the SPC regarding the disposal of any unused product.

Environmental risk assessment for products containing or consisting of genetically modified organisms

Although the vaccine contains a recombinant protein produced by expression from a genetically modified organism the active ingredient itself is produced by a process which eliminates the possibility of viable GMO being present (sterile filtration, chemical treatment to destroy DNA and stabilise the protein). Consequently, there is no specific requirement to address these requirements.

Overall conclusion on safety

Several controlled laboratory safety studies were submitted to show the safety of the vaccine in animals at minimum age using vaccine batches that contained the targeted fixed antigen content and an additional batch with high antigen content.

The adverse events observed in the safety studies were in general consistent through all three studies: single dose, double dose and repeat dose. The studies were carried out in animals of minimum age

(four days old). The vaccine includes a fixed concentration of antigen, but a batch with higher antigen content was also included in all laboratory safety studies. Some depressed animals were observed post-vaccination, although this effect was also observed in the control group. It could be speculated that this effect may have been caused by the stress caused to the piglets by the handling associated to vaccination; nevertheless, a warning statement has been included in the SPC. Slight increase of temperature (up to 1 °C of increase 4 - 8 hours post-vaccination in some cases) was also observed. This increase is transient and has been reflected in the SPC, although more specific information on the highest temperature observed should be included. Local reactions were seen as slight swelling of the vaccination site and have also been specified in the SPC. No significant histopathological changes were observed at necropsy.

The field trials carried out ratify the observations seen in the laboratory trials.

Although none of the batches used in the safety trials contained the maximum level of aluminium within the range specified for the vaccine, (3.5 mg), two overdose studies were conducted where double dose of vaccine was administered and therefore doses of aluminium up to 6.44 mg have been shown to be safe.

The maximum level of endotoxin specified for the vaccine has been also shown to be safe.

Overall, the vaccine has been shown to be safe when administered to animals at minimum age and no major post-vaccination reactions have been observed. Adequate advice regarding potential adverse reactions and reactions observed after overdose have been included in the SPC.

The risk for the user posed by ECOPORC SHIGA is considered low when used as recommended. To avoid any risk from remains of the vaccine after use, a respective disposal advice is given in the SPC.

ECOPORC SHIGA is not expected to pose a risk to the environment when used as recommended. An appropriate advice is included in the SPC regarding the disposal of any unused product.

There are no components that require an MRL and no specific risks for the consumer have been identified. A zero days withdrawal period is considered appropriate.

Part 4 – Efficacy

Introduction and general requirements

ECOPORC SHIGA is an inactivated, adjuvanted vaccine. It contains a recombinant *E. coli* toxin subunit, Stx2e, and is to be used for the active immunisation of young piglets against oedema disease.

According to the proposed vaccination schedule, one dose of vaccine should be given to piglets from 4 days of age by intramuscular injection. No revaccination is recommended.

The efficacy studies presented were performed according to the requirements of Annex I to Directive 2001/82/EC, the guideline on General requirements for the production and control of inactivated mammalian bacterial and viral vaccines for veterinary use, the guideline on Requirements for the production and control of porcine live and inactivated viral and bacterial vaccines, and the relevant monographs for the Ph. Eur.. It should be noted that there is no specific monograph.

Laboratory vaccination and challenge studies were provided, to establish a recommended dose, onset and duration of immunity, efficacy in the face of maternally derived antibody, and field data in support of the claims.

Laboratory trials

Study to establish a challenge model

As it was not possible to establish a reproducible challenge model with field strains of STEC, the applicant developed a recombinant toxin (rStx2e) that could be made to a defined concentration as a challenge inoculum. This is considered an acceptable approach as oedema disease is a result of the action of the Stx2e toxin. The potency of the recombinant challenge toxin was tested by inoculating graded doses intravenously into 21 day old piglets, and monitoring the clinical response. The challenge was severe, and very reproducible, in contrast to the natural challenges the applicant had attempted. The recombinant toxin was produced by an *E. coli* that is heterologous to the production strain. The coding sequence of the rStx2e used in the challenge is 100% identical with the sequence of Stx2e of oedema disease of swine which is fully documented.

A comparison was provided, between the clinical signs observed in the challenge model used for the laboratory studies and those observed during the field trials, as well as reported findings described in literature. It has been sufficiently demonstrated that the toxin challenge used for the studies is representative of field cases.

Study for the determination of the vaccine dose

In this study graded doses of laboratory produced vaccinal antigen were administered, to the youngest category of the target species, according to the vaccination scheme recommended (a single intramuscular injection in suckling piglets of 4 days old). 142 piglets, including placebo inoculated controls and environmental controls were randomly assigned to seven groups, balanced for body weight and subsequently challenged with rStx2e, at the dose rate determined in the previous study. Five groups were vaccinated, one group was an unvaccinated challenge control, and the small group was a strict control group (unvaccinated, unchallenged). Vaccinated piglets received antigen between 10.0×10^6 $CD_{50\ equivalent}$/ml and 2.0×10^6 $CD_{50\ equivalent}$/ml. Twenty-one days later the vaccinated, and the placebo inoculated piglets, were challenged and subsequently monitored for clinical signs. In addition, some piglets were not challenged, but were blood sampled for serological testing at 91 days old (87 days post vaccination). Following the challenge vaccinated piglets were protected against mortality and morbidity, in a dose dependent fashion. All doses ($\geq 2.0 \times 10^6$ $CD_{50\ equivalent}$/ml) had a statistically significant trend for protective effect, which increased with the size of dose. A dose of $\geq 4.0 \times 10^6$ $CD_{50\ equivalent}$/ml was sufficient to protect 60% of pigs from death. Seroconversion at 25 days old appeared to be dose dependant also, whereas the majority of vaccinated piglets were seropositive by 91 days old, whereas the unvaccinated piglets were not.

Study for the onset and duration of immunity

These studies were conducted in a similar way to the study of the vaccine dose determination, but utilising production vaccines, in order to determine onset and duration of immunity. In one study (that used piglets from seronegative sows), a total of 210 suckling piglets from an oedema disease free herd were randomly assigned to seven groups, balanced for body weight. Five groups were vaccinated, one group was placebo vaccinated, and the final group was the challenge control. The applicant administered graded doses of production vaccine, to the youngest category of the target species, according to the vaccination scheme recommended (one-shot vaccination of suckling piglets of 4 days old). Vaccinated piglets received antigen between 10.0×10^6 $CD_{50\ equivalent}$/ml and 2.0×10^6 $CD_{50\ equivalent}$/ml. The vaccine with 8.0×10^6 $CD_{50\ equivalent}$/ml, (the subsequently chosen concentration for production vaccine), is stated to be manufactured according to the dossier and to GMP. Piglets, including placebo inoculated controls and environmental controls were subsequently challenged with rStx2e. Challenge was carried out on sub-groups at twenty-one days later and at 105 days later.

The challenge was similar to previous studies, with a mortality of 77 - 93% in the control piglets. In comparison, vaccinated piglets were statistically significantly protected against mortality in a dose dependent fashion, with the highest vaccine dose resulting in only 15 - 21% mortality. Trend analysis was carried out and again the results were statistically significant in favour of protection for doses of ≥ 4.0×10^6 $CD_{50\ equivalent}$ /ml. The final assessment of the results taking into account PM exams and laboratory reports, together with the pair-wise statistical testing were considered adequate for this evaluation. Therefore, the CVMP supports the claims of onset of immunity at 21 days post vaccination, and duration of immunity at least 105 days post vaccination.

Study for the influence of maternal antibodies on the efficacy of the vaccine

The initial study presented to support the efficacy of vaccination in the presence of MDA was considered insufficient and therefore it was concluded that the SPC should reflect this point. Additionally, the relevance of pre-existing MDA levels to the field situation would need to be addressed should the applicant submit any further information that might result in a claim.

A new study was conducted to check if the presence of maternally derived antibodies have an impact on the efficacy of the vaccination of piglets at the age of 4 days according to the recommended regime of vaccination. One hundred and ten piglets (Large white x German Landrace) were born from seronegative and seropositive sows, from an oedema disease free herd. The piglets were distributed into groups based on the serological status of the sow and the weight of the piglets. Statistical analysis of groups demonstrated comparability for sex and weight distribution. The vaccine batch used in this study, was produced according to GMP and representative for production.

Parameters used were dead/surviving animals post challenge and “protection”. Blood samples were used to determine neutralising antibodies to Stx2e, and body weight was also assessed.

The seronegative control group suffered 80% mortality following challenge, similar to previous studies. The seropositive controls suffered 76% mortality, which was not statistically significantly different from the previous group.

There were no significant differences in mortality and protection between groups 1 and 3 (seronegative vaccinates and seropositive vaccinates) following challenge. There were also no significant differences between these groups in terms of “protection”.

Based on the efficacy study presented, the applicant has shown that MDA does not interfere with vaccination at 4 days old piglets.

Field trials

A total of three rear-fattening set ups were included in the field trials, all in Germany. Studies were carried out according to GCP following the same study design, randomised, placebo control and blinded. On each farrowing site piglets from one farrowing group were randomly assigned to vaccine or placebo group. A grand total of 518 piglets received ECOPORC SHIGA, and similar numbers received saline solution as a placebo. In accordance with the SPC piglets were vaccinated/inoculated at approximately 4 days old with a single dose. At weaning piglets were transferred to the rearing units. Each rearing site had a history of oedema disease. During the trial piglets received treatments as necessary. On the rearing sites piglets were observed daily for oedema disease during the risk period, approximately 3 weeks following weaning. Piglets that died were examined PM, and samples taken and processed. On one rearing site no clinical oedema disease was seen. Serology demonstrated seroconversion (in the absence of MDA) in the vaccinated piglets.

On the other two rearing units clinical oedema disease was seen and confirmed by PM examination and laboratory tests. On one farm none of the vaccinates showed any signs of oedema disease, nor died,

whereas 15% and 11% of placebo pigs showed signs and died. These results were statistically significant. On the other farm no vaccinates died, and only two showed signs, compared with 4 deaths (3%) and 9 (6%) ill pigs in the placebo group. Differences in morbidity, but not mortality, were statistically significant.

Overall, therefore, the field trial data are supportive of the claims to reduce the mortality and clinical signs of oedema disease caused by Stx2e forming *E. coli* (STEC) in piglets from 4 days old.

Overall conclusion on efficacy

Overall, the efficacy of the vaccine was tested by laboratory trials which included studies to determine the onset and duration of immunity, and the influence of maternal antibodies. Three field studies were also performed in order to confirm the efficacy of the vaccine and the claims to reduce the mortality and clinical signs of oedema disease caused by Stx2e forming *E. coli* (STEC) in piglets from 4 days old.

Target species

The target species are pigs. ECOPORC SHIGA is intended for use in the suckling pig, to allow immunity to develop prior to weaning in order to protect the piglet from oedema disease in the post-weaning interval.

Vaccination schedule

According to the vaccination schedule one dose of vaccine should be given from 4 days old by intramuscular injection. This vaccination schedule was successfully used in all the efficacy studies included in this dossier, and is therefore considered acceptable. No revaccination is required, as the protection afforded by one vaccination is considered to last at least 105 days (15 weeks) post vaccination. If the young pig is vaccinated this duration of immunity is sufficient to protect the animal during the period of most risk (the 2 - 4 weeks following weaning, which usually occurs at approximately 3 - 4 weeks of age).

Vaccines used in efficacy trials

The vaccines used in the efficacy trials were made in accordance with the method of preparation described in the dossier. These points have been adequately addressed. The commercial vaccine is blended to a standard amount of antigen: 8.0×10^6 $CD_{50\ equivalent}$/ml and trials have been carried out using these vaccines blended with the same quantity of antigen or less.

Minimum protective dose

Titration studies showed a marked dose-response effect, with doses as low as 2.0×10^6 $CD_{50\ equivalent}$/ml. Taking into account production constraints, safety and efficacy a higher dose of 8.0×10^6 $CD_{50\ equivalent}$/ml was agreed for the commercial vaccine. It was however shown that the minimum dose of at least 3.2×10^6 $CD_{50\ equivalent}$ provided significant protection.

Efficacy claims

The SPC claims are:

4.2 Indication for use

Active immunisation of piglets from the age of four days to reduce the mortality and clinical signs of oedema disease caused by Stx2e forming *E. coli* (STEC).

Onset of immunity: 21 days after vaccination

Duration of immunity: 105 days after vaccination

Reduction in mortality and increase in protection were consistently demonstrated in the efficacy studies, when a severe challenge with rStx2e, causing severe oedema disease was given. Statistical tests to analyse mortality and protection figures were applied. “Protection” reflects the numbers of piglets showing minimal or no clinical signs, so this can be taken as a measure of reduction in clinical signs. Onset and duration of immunity were both shown through laboratory challenges of vaccinated piglets of the minimum age. Challenges were carried out at 21 days post vaccination and at 105 days post vaccination, and statistically significant differences in mortality and clinical signs were seen. Reductions in mortality and clinical signs were also seen in the field, following a field challenge, on two farms. The claims are considered clinically relevant and important in the control of oedema disease in the post-weaning piglet, and are supported by the CVMP. It should be noted that the claims are limited to *E. coli* producing Stx2e specifically.

Immunological properties

The acceptable statement concerning immunological properties for inclusion in the SPC is that the vaccine stimulates an active immunity against Stx2e produced by *E. coli*.

Maternally derived antibodies

The applicant presents a new GCP study in an attempt to demonstrate the efficacy of the vaccine in the face of MDA. The seronegative control group suffered 80% mortality following challenge, similar to previous studies. The seropositive controls suffered 76% mortality, which was not statistically significantly different from the previous group. There were no significant differences in mortality and protection between groups 1 and 3 (seronegative vaccinates and seropositive vaccinates) following challenge. There were also no significant differences between these groups in terms of “protection”.

Overall, the data presented has shown that MDA does not interfere with vaccination in 4 days old piglets, therefore the claim is supported.

Compatibility

The acceptable statement concerning compatibility for inclusion in the SPC is that no information is available on the safety and efficacy of the vaccine when used with any other veterinary medicinal product. A decision about using this vaccine before or after any other veterinary medicinal product therefore needs to be made by the responsible veterinarian on a case by case basis.”

Part 5 – Benefit-risk assessment

Introduction

ECOPORC SHIGA is an inactivated vaccine containing genetically detoxified shiga toxin (Stx2e) adjuvanted with aluminium hydroxide, intended for active immunisation of piglets from the age of four days to reduce the mortality and clinical signs of oedema disease caused by Stx2e forming *E. coli* (STEC).

Oedema disease, also called *E. coli* enterotoxaemia, occurs worldwide and is a peracute/acute systemic disease. It generally occurs in the first two weeks after weaning and clinically affected animals usually die very quickly, within 24 - 48 hours. Those that survive will be more or less severely affected in terms of growth. Attempts to treat are often unsuccessful and uneconomic. Vaccination can be considered as an appropriate and effective tool to control the disease.

Benefit assessment

Direct therapeutic benefit

The benefit of ECOPORC SHIGA is to induce an active immunisation of piglets from the age of four days, to reduce the mortality and clinical sings caused by oedema disease

The genetically modified Stx2e antigen in the vaccine induces systemic Stx2e neutralising antibodies. The symptoms of oedema disease are caused by the toxin and therefore the neutralisation of the toxin leads to protection in vaccinated animals. Therefore, the vaccine offers specific prophylaxis against oedema disease.

The active substance is innovative.

Additional benefits

The vaccine can be administered in young pigs from 4 days of age and only need to be administered once. The duration of immunity lasts until day 105 after vaccination, which covers the rearing period in which the oedema disease usually occurs.

Prophylactic protection is likely to reduce the use of antimicrobials for the treatment of the disease.

There is sufficient evidence to support the conclusion that MDA does not interfere with vaccination.

Risk assessment

The following risks have been identified:

1. Main potential risks:

<u>For the target animal:</u>

- There is a risk of slight increase of temperature up to 1 ºC, which is common after vaccination and spontaneously resolves within one day without any treatment. Mild swelling can be observed post-vaccination, which disappear within 4 to 5 days without treatment.

<u>For the consumer:</u>

- There are no components that require an MRL and no specific risks have been identified. Additionally, the modified Stx2e antigen is genetically detoxified and no live organisms are present.

<u>For the environment:</u>

- ECOPORC SHIGA is not expected to pose a risk to the environment when used as recommended.

<u>For the user:</u>

- The risk for the user posed by ECOPORC SHIGA is considered low when used as recommended.

Risk management or mitigation measures

- Appropriate warnings have been included in relevant parts of the SPC to warn of the potential risks to the target animals, general risks to the user and environment including disposal advice, after the use of the vaccine.

Evaluation of the benefit-risk balance

The formulation and manufacture of ECOPORC SHIGA is well described and specifications has been set to ensure that product of consistent quality will be produced. The vaccine has been shown to be well tolerated by the target animals and presents a low risk for the user and the environment. Appropriate warnings have been included in the SPC and no major safety concerns have been identified.

The product has been shown to be efficacious for the indication of active immunisation of piglets from the age of four days to reduce the mortality and clinical signs of oedema disease caused by Stx2e produced by *E. coli* (STEC). The data provided support the indications proposed in the SPC.

Conclusion

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