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Veterinary Medicines and Product Data Management

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Poulvac E. Coli (EMEA/V/C/002007)

Common name: Vaccine to reduce mortality and lesions associated with *Escherichia coli* serotype 078

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 Poulvac E. coli has been submitted to the Agency on 26 January 2011 by Pfizer Limited in accordance with Regulation (EC) No. 726/2004.

This is an application for a live vaccine containing *aroA* gene deleted *Escherichia coli*, serotype O78. The vaccine is recommended for active immunisation of broiler chickens and future layers/breeders in order to reduce mortality and lesions (like e.g. pericarditis, perihepatitis, airsacculitis) associated with *Escherichia coli* serotype O78. One dose of reconstituted vaccine ($5.2x10^6$ to $9.1x10^8$ CFU) should be administered to chickens from 1 day of age by coarse spray. The withdrawal period is zero days. The acceptable shelf life of the lyophilised vaccine is 18 months for the time being and the vaccine must be used within 2 hours after reconstitution. The product sizes marketed are 1 or 10 glass vials of 2 500, 5 000, 10 000 or 20 000 doses.

This vaccine is considered as a product for minor use / minor species (MUMS), according to the guideline EMEA/CVMP/IWP/123243/2006-rev2. Table 2 of that guideline lists *Escherichia coli* causing colibacillosis in chickens and other species, as a minor use. The vaccine contains a genetically modified organism as vaccine strain. Therefore the requirements of Directive 2001/18/EC do also apply.

There is no specific European Pharmacopoeia (Ph. Eur.) monograph for colibacillosis in chickens.

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

On 15 June 2012, the European Commission adopted a Commission Decision for this application.

Part 1 - Administrative particulars

The antigen production, in-process testing, formulation and primary packaging take place at Pfizer Animal Health, 2000 Rockford Road, Charles City, Iowa 50616, USA.

The secondary packaging, release testing and batch release are performed at Pfizer Olot, S.L.U., Ctra. Camprodón s/n "La Riba", 17813 Vall de Bianya, Girona, Spain.

Valid manufacturing licences and GMP certificates for all sites are available.

The pharmacovigilance system as described by the applicant fulfils the requirements 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.

Overall conclusions on administrative particulars

The assessment of the administrative particulars has revealed no deficiencies.

Part 2 - Quality

Composition

Poulvac E. coli is a live vaccine containing as active substance *aroA* gene deleted *Escherichia coli*, serotype O78, strain EC34195. Excipients are sodium phosphate dibasic heptahydrate, potassium phosphate monobasic, ammonium sulfate, magnesium sulfate heptahydrate and sucrose. The quantity of the antigen is 5.2×10^6 – 9.1×10^8 CFU per dose (1 dose = 0.1 - 0.5 ml).

Container

The containers are of glass type I closed with siliconised chlorobutyl rubber stoppers and sealed with aluminium caps. The validation reports for the in-house sterilisation process of the bottles and stoppers are provided.

Development pharmaceutics

The parental organism *E. coli* EC34195 serotype O78 was a wild type strain originally isolated from a clinical case of avian colibacillosis. This parent strain was selected based on its *in vivo* characteristics. The strain was further characterised by its antibiotic sensitivity pattern and presence or absence of several genetic markers. The parent strain was genetically modified in order to achieve an *aroA* gene deleted *E. coli* vaccine strain. The loss of *aroA* gene function results in attenuation of *in vivo* growth due to the requirement for aromatic metabolites. Aromatic amino acids are not synthesized by vertebrates, and are only present in tissues at very low and well-controlled levels. Efficacy and safety studies performed with the vaccine strain have been summarised and the influence of vaccination on epidemiological surveys is briefly discussed. Reasonable justification is given regarding the challenge routes chosen for studies (safety and efficacy) as well as the relevance of the chosen challenge strain and vaccine strain within the EU.

Method of manufacture

The manufacturing procedure is adequately described in detail to give sufficient confidence that the product will be safe, effective and stable.

The manufacturing process corresponds to a classical procedure. Bacteria used in manufacture are handled in a seed lot system. The vaccine strain is propagated in a scale-up system (up to X+4). After fermentation the culture may be concentrated up to 100x. At the blending step, the antigen concentrate is mixed with a stabiliser. The product is aseptically filled into defined glass bottles and afterwards the vaccine is lyophilised, closed under vacuum, labelled and packed to obtain the finished product. The consistency of the production is demonstrated on three consecutive batches.

Control of starting materials

Active substance

The master and working seeds have been produced according to the Seed Lot System. The construction of the gene deleted *E. coli* vaccine strain by genetic engineering is briefly described in Part 2 and detailed information is given in Part 3.E. of the dossier. Stability of the vaccine strain was observed during five passages. Further clarifications for the seed materials were provided, all of which are fully acceptable.

Excipients

Most of the starting materials of non-biological origin used in the production comply with Ph. Eur. monographs or are in compliance with the United States Pharmacopoeia (USP). Defined internal specifications and/or representative Certificates of Analysis (CoA) were provided for all starting materials.

EDQM certificates of suitability and/or certificates of analysis for substances of biological origin used during production were provided. Further assurance has been given that the materials of animal origin used in the manufacture of this vaccine would not be contaminated with live viruses after autoclaving.

Information regarding the qualitative and quantitative composition of all culture media and the stabiliser, their treatment processes and their storage conditions is provided in the dossier.

All starting materials are either tested for or treated to ensure that there are no contaminants or further assurance is given that there is no potential risk.

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

An appropriate TSE risk assessment for the seeds was provided showing a negligible overall TSE risk. For all other starting materials of biological origin listed in Part 2 of the dossier the TSE risk has also been adequately addressed and is considered negligible.

Control tests during production

The control tests during production include purity, viable count and endotoxin. The in-process tests are deemed to be sufficient to control all the critical steps in the manufacturing. The proposed acceptance criterion for endotoxin has been sufficiently justified.

Control tests on the finished product

Tests proposed for the final vaccine are visual inspection, vacuum test, appearance, identity *aroA*-deletion mutant, potency, safety, purity and residual humidity. Appropriate SOPs are provided. The finished product tests are deemed appropriate to control the quality of the finished product. The results of the analysis of three consecutive batches were presented and comply with the proposed specifications. Several test specific and validation questions have been answered adequately. The specifications set for the potency test (viable count: minimum release titre, end of shelf-life titre and maximum titre) are justified, based on safety/efficacy and stability considerations. In addition, the variability of the viable count measurement has been also taken into consideration for setting of the minimum and maximum batch release requirements.

Stability

The proposed shelf-life for the antigen fluids is 1 day (at 2 °C to 8 °C). For the finished product currently an 18 months shelf-life is supported by the data presented in the dossier at this stage. This is reflected in the SPC. Furthermore, an in-use shelf life of 2 hours after reconstitution is sufficiently demonstrated.

Overall conclusions on quality

The manufacturing process is described in sufficient detail to give confidence that the manufacture will yield a safe, effective and stable immunological product.
The quality of Poulvac E. coli can be considered to be adequately demonstrated.

Part 3 – Safety

Safety documentation

Poulvac E. coli is a live attenuated vaccine for use in chickens (broilers, future layers/breeders) consisting of live *aroA* gene deleted *Escherichia coli* serotype O78 (strain EC34195) and a stabiliser. No adjuvant and no preservative are included. The recommended minimum dose is 5.2×10^6 CFU/bird; the proposed maximum titre is 9.1×10^8 CFU/dose.

The vaccine is administered by coarse spray to chickens from 1 day of age. The vaccine should not be used in birds in lay and within 6 weeks before the onset of the laying period.

The applicant presented laboratory vaccination studies (safety of a single and an overdose, clearance, shed and spread of the vaccine, reversion to virulence) and a field study to support the safety.

Laboratory tests

Safety of the administration of one dose

In accordance with the recommended vaccination scheme SPF chickens at one day of age, the minimum age of administration, were vaccinated once.

Group 1 was vaccinated by oral gavage, group 2 by coarse spray which is the recommended administration route. The vaccination titre per dose of 1.7×10^9 cfu for these two groups equates to the maximum proposed titre. Group 3 was vaccinated intratracheally. The tested vaccination titre per dose of group 3 (3.4×10^8 cfu) was slightly below the maximum titre.

Animals were observed for three weeks recording death, poor health or any other clinical signs of disease. Necropsy of dead birds and examination for typical lesions of colibacillosis (airsacculitis, pericarditis, perihepatitis, cellulitis, arthritis) were performed at the end of the study.

During the study no adverse general or local reactions and at necropsy no macroscopic lesions related to *E. coli* were observed.

The results of this study proved the safety of the administration of 1.7×10^9 cfu/dose via oral gavage and coarse spray as well as of 3.4×10^8 cfu/dose via intratracheal route to one day old chickens.

Safety of one administration of an overdose

In accordance with the recommended vaccination scheme SPF chickens at one day of age, the minimum age of administration, were vaccinated once. The tested vaccination titre of 9.1×10^9 cfu per dose equates to the 10-fold maximum titre. Twenty chickens were vaccinated oculo-nasally by eyedrop to ensure an accurate and regular delivery of the desired amount of vaccine per bird. Another twenty chickens served as control chickens.

The animals were observed for three weeks recording any clinical signs or mortality. For determination of growth performance the weight was recorded. At the end of the trial the animals were necropsied with special attention to typical lesions of septicaemia: pericarditis, perihepatitis, airsacculitis and salpingitis.

During the study no general reactions and at necropsy no macroscopic lesions related to *E. coli* were observed. The growth performance of the vaccinated chickens was similar to or higher than the controls.

The results of this study proved the safety of the administration of 9.1 x 10^9 cfu/dose via oculo-nasal route to one day old chickens.

In a precursor study chicken were vaccinated with the maximum tenfold overdose. No general reactions and no macroscopic lesions were observed in the vaccinated animals at any time point during the study. The growth performance of the control chickens was higher than the vaccinated chickens. A room effect on average daily weight gain was suspected.

Safety of the repeated administration of one dose

No repeat dose studies have been performed. Poulvac E. coli is only given once during the life of a broiler or a future layer/breeder.

Examination of reproductive performance

No studies have been performed on reproductive safety and the SPC correspondingly advises not to use the product in birds in lay, or within 6 weeks before the onset of the laying period.

The safety of the vaccination for 1-day old chickens has been adequately demonstrated and it is considered unlikely that older birds will be more susceptible. However, the concerns regarding the vaccination of older birds were rather related to the lack of sufficient data to demonstrate absence of transfer of the vaccine organism to eggs. Taking these concerns into consideration the applicant has suggested the extension of the period before the onset of laying (from 4 to 6 weeks) when the vaccination must be avoided. This was considered acceptable.

Examination of immunological functions

No studies have been performed to test the effect of the vaccine on the immune system. It was noted that in publications immunosuppressive effects of *E. coli* in chicken (e.g. Hegazy AM. et al. 2010; Nakamura K. et al. 1986) were reported. However, it is considered unlikely that the vaccine will adversely affect the immunological function of chickens. Therefore it is acceptable that special tests on the immunological functions are not performed.

Additionally a warning is included in SPC section 4.3: *Do not vaccinate animals undergoing … immunosuppressive treatment*.

Special requirements for live vaccines

Spread of the vaccine strain and Dissemination in the vaccinated animal

50 birds vaccinated with the live *E. coli aroA*- vaccine at one day of age by coarse spray (2.2 x 10^8 cfu/dose) were housed together with 25 non-vaccinated control birds. The animals were observed for any clinical signs and mortality. At different days of age animals were necropsied and swabs were taken from airsacs, heart and liver to investigate for possible *E. coli aroA*- vaccine strain isolation. The vaccine strain was present in the liver of one bird at 4 days post vaccination and never in the contact group. In environmental swabs the vaccine strain could be identified 4 and 8 days post vaccination.

The dose administered by coarse spray did not contain the maximum release titre (1.0 x 10^9 cfu/dose). Using different doses may influence the colonisation and re-isolation of *E. coli* bacteria from internal organs and environmental samples as well as the spreading to contact birds. Therefore, two further studies to evaluate clearance, shedding and spreading as well as environmental persistence of Poulvac E. coli using the maximum release titre are performed.

In a first GLP study, 50 chickens vaccinated at one day of age by eyedrop (1.0 x 10^9 cfu/dose) were housed together with 25 non-vaccinated control birds. The animals were observed for any clinical signs. Animals were necropsied at different days post vaccination. Cloacal swabs, nasal swabs, swabs from different organs and environmental samples were taken for bacterial isolations until 21 days post vaccination. Neither clinical signs nor abnormal macroscopic lesions attributable to the vaccine were observed at any point in time. On a single occasion, vaccine strain was isolated from vaccinated chickens at 4 days post vaccination only and was not recovered from any of the non-vaccinated in-contact birds. Vaccine strain was detected in cloacal swabs of vaccinated animals up to 14 days post vaccination, of non-vaccinated control birds until day 11 post vaccination. In nasal swabs the vaccine strain was isolated only once in each group. Vaccine strain bacteria were recovered from the environment (from litter and feed until the end of the animal phase 21 days post vaccination, from water until day 7 post vaccination).

In a second GLP study, 20 chickens were vaccinated at one day of age by coarse spray or eyedrop. The animals were observed for any clinical signs. Animals were necropsied at day 42 post vaccination. Cloacal swabs and environmental samples were taken for bacterial isolations.

Neither clinical signs nor abnormal macroscopic lesions were observed at any point in time. Vaccine strain was detected in cloacal swabs up to 28 days post vaccination in each group. Vaccine strain organisms were recovered from the environment until day 28 post eyedrop vaccination (from water and feed) and until day 35 post spray vaccination (from litter).

The finding that the vaccine strain can be isolated from cloacal swabs up to 28 days post vaccination raised concerns regarding the risk of broiler carcass contamination at the time of slaughter and the establishment of an appropriate withdrawal period has been discussed. However, in view of all the information and supplementary data provided by the applicant it has been concluded that the vaccine strain itself is stable and safe as substantiated by the safety data and genetic stability data. As no risk to humans or the environment has been identified a special warning is rather considered suitable than a withdrawal period. Sufficient clear information regarding the detection of the strain in tissues, strain excretion as well as the persistence of the strain in the environment is provided in section 4.5 of the SPC.

Reversion to virulence of attenuated vaccines

The reversion to virulence study was carried out using the master seed. The birds were vaccinated with 3.1 x 10^7 cfu/dose (study 1), 8.3 x 10^6 cfu/dose (study 2) or 1.4 x 10^7 cfu/dose (study 3). This is well below the maximum release titre of 1.0 x 10^9 cfu/dose.

The presented reversion to virulence study is not in compliance with the requirements of VICH GL 41 for the following reasons:

- The initial inoculum should contain the maximum release titre expected in a recommended dose (1.0 x 10^9 cfu/dose).
- It is not clear whether reasonable attempts were made to repeat the test when the bacteria were not recovered from any intermediate *in vivo* passage.

The non-finding of *E. coli aroA*- strain might depend on the fact that no maximum release titre was inoculated and/or that the detection limit of the method used for recovery is insufficient. Therefore, an additional study to evaluate reversion to virulence using the maximum release titre (1.0 x 10^9 cfu/dose) was performed.

A GLP study was performed using at least the maximum release titre. Isolation of *aroA*-E.coli vaccine strain was successful after the initial vaccination. In the subsequent two passages *aroA*-E.coli vaccine

strain was not isolated. Clinical signs or lesions were not observed in any of the birds of the study, including those from the initial vaccination.

The results show that the vaccine strain of Poulvac E. coli did not show an increase in virulence.

Biological properties of the vaccine strain

The vaccine strain is unable to multiply in the environment. A summary of the biological properties of the vaccine strain was provided.

The vaccine strain is identified as *E. coli* by colony morphology and bio/chemical characterisation tests. The vaccine strain will only grow on TSA medium but not on minimal medium.

The *in vitro* survival of the vaccine strain was investigated. When tested under conditions to mimic a commercial poultry rearing environment (spiked in autoclaved chicken litter), the vaccine strain bacteria persists in such an environment for no longer than 24 hours. After spiking the vaccine strain bacteria in commercial available bedding material, water and feed, the vaccine strain persists on litter for 4 days, on feed and in non-chlorinated water for at least 42 days.

The vaccine is safe in target and non-target animals when inoculated by varying routes (eye drop, spray, oral, intra-tracheal, and intra-cerebral routes). Sufficient clarification and justification have been provided with respect to the use of pigs and mice and the duration of the studies to rule out the possibility of recombination to a more virulent form. It was clarified that pigs and mice were used as potential non-target species but not as model for human safety.

Recombination or genomic reassortment of the strains

The mechanisms for host bacterium gene transfer (conjugation, transduction and transformation), the probability of these events and the frequencies of gene transfer under laboratory and field conditions were discussed. The likelihood of recombination between the *E. coli* aroA- vaccine strain and wild-type bacteria to convert the vaccine strain to a virulent strain is considered very rare.

Study of residues

The product is a live vaccine and does not contain an adjuvant or preservative. The detailed composition of the stabiliser has been given and does not pose a risk. The withdrawal period of zero days has been justified.

Interactions

No studies have been performed to test the effect of the vaccine on the concurrent use of any other vaccine. Therefore section 4.8. of the SPC includes the common statement: *No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis.*

Field studies

A field trial was conducted as a large-scale multicentre field trial in Morocco according to Good Clinical Practice (GCP) regulations. The study was blinded. Data were collected from 224,966 birds. For statistical purposes, the experimental unit was the individual bird house.

A description of the poultry industry in Morocco was provided. Justification was given that the presented study addresses the husbandry practices and field conditions in the EU and that the generated data are also valid for the EU. This was accepted by the CVMP.

In accordance with the recommended vaccination scheme in the SPC, the commercial chickens received one vaccination at one day of age with 1.3 x 10^8 cfu/dose, which is considered as intermediate dose as recommended in the SPC. The animals were observed for two weeks for any clinical signs and mortality. Feed used, medication applied, weight development, number of antibiotic treatments were recorded.

No significant adverse events were observed throughout the study. The non-inferiority of average daily weight gain, feed conversion ratio and mortality during the first two weeks in vaccinated versus control animals was statistically demonstrated.

Overall, the study demonstrated that the vaccine Poulvac E. coli is safe when used in one-day old broiler chickens.

User safety

A user risk assessment has been provided for Poulvac E. coli including the elements: hazard identification and characterisation, exposure assessment, risk characterisation, risk management and risk communication.

An additional risk assessment for human exposure was submitted, including a review of the relationship between APEC strains and human disease based on available literature and testing data for Poulvac E. coli. The risk of Poulvac E. coli for human health is low as a potential disease causing agent or acting as reservoir for the virulence associated genes. As no information was provided on the potential risk for immune-compromised persons, an appropriate warning indicating that immunosuppressed people should not be present during administration of the vaccine has been included in SPC section 4.5. In view of the persistence of the vaccine strain in the bird and the environment additional information has been included to warn personnel involved in attending vaccinated animals to follow general hygiene principals and to take care in handling litter from recently vaccinated animals. A further risk assessment addressing the human exposure associated with the use of Poulvac E. coli has been provided. In this report the hazard in question is characterized, all potential routes of human exposure are described and the probability of contaminated carcasses is evaluated. The theoretical human health risk caused by inhalation of a large dose of active ingredient as well as of aerosolised endotoxin has been sufficiently addressed.

Appropriate operator's safety measures regarding proper protection (gloves, nose-mouth mask and eye protection) are included in the SPC.

Environmental risk assessment

Phase I assessment

The environmental risk assessment required by the EU Note for Guidance EMEA/CVMP/074/95 and the assessment required according to Directive 2001/18/EC, Annex II for veterinary medicinal products containing or consisting of GMOs cover more or less identical issues. The environmental risk of Poulvac E. coli was assessed following the recommendations of both documents mentioned above.

A phase I environmental risk assessment was conducted, including a hazard identification and assessment of the exposure to the hazard as well as the likelihood that the hazard may occur. The first phase of the assessment outlines that the potential exposure of the environment to the product and the level of risk associated with it is considered very low to negligible. The likelihood of hazard is very

low to negligible and the consequences of the occurrence of any hazard can be considered as negligible. Therefore the estimation of risk can be considered very low to negligible.

Therefore a study of Phase II has not been considered necessary or adequate, due to the very low environmental risk potential of the vaccine.

Assessment required for veterinary medicinal products containing or consisting of genetically modified organisms

In addition to the requirements of Directive 2001/82/EC as amended, Annex III A of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms applies for this vaccine.

As regards the assessment in accordance with Directive 2001/18/EC, all points to be considered for a live genetically modified organism used as vaccine strain have been addressed.

The parental organism, *E. coli* EC34195 is a wild type *E. coli* strain originally isolated from a clinical case of avian colibacillosis in the UK. The parental strain is characterised for *in vivo* characteristics in chickens and phenotypically and genotypically by using different *in vitro* methods. The parental *E. coli* strain can be identified by using morphology and bio/chemical testing methods.

The relationship between APEC and human disease and the potential for APEC to act as a reservoir of virulence genes for human pathogenic *E. coli* was considered as low.

Cloning procedures and production of the final GMO were performed by genetic modifications that are state of the art.

Verification of the final product was done by commonly used methods.

Information regarding interactions between the vaccine strain and the environment and the possible impact of the vaccine strain on the environment including non-target species has been provided.

Genetic stability has been shown by PCR. Information on monitoring techniques, control of release and waste treatment are provided.

Overall conclusion on safety

Target species

Poulvac E. coli is intended for use in chickens from 1 day of age including broilers, future layers and future breeders. The safety of one dose and of 10-fold overdose has been demonstrated in one-day old chickens.

Laboratory studies

The safety of the administration of one dose and of an overdose to one day old chickens was demonstrated.

No repeat dose studies have been performed.

No studies have been performed on reproductive safety and a warning is given in the SPC that chickens should not be vaccinated within 6 weeks before the onset of the laying period.

No studies have been performed to test the effect of the vaccine on the immune system. An appropriate warning is included in SPC section 4.3: *Do not vaccinate animals undergoing … immunosuppressive treatment.*

The number of vaccine strain positive animals decreased over time. Recovery of vaccine strain occurred in vaccinated animals on single occasions for a short period following vaccination (up to 6 days). The vaccine strain was found in cloacal swabs of vaccinated birds until day 28 post vaccination, of non-vaccinated contact birds until day 11. Environmental samples (water, feed, litter) were positive post-vaccination following both eyedrop and spray application of the vaccine, with a clear decrease in prevalence over time lasting until day 35 at the latest. Section 4.5 of the SPC was amended accordingly. The vaccine strain of Poulvac E. coli did not show an increase in virulence after passages in chicken.

A summary of the biological properties of the vaccine strain and a review of the scientific literature available on the issue of recombination and genomic re-assortment in *E. coli* is provided. The likelihood of recombination between the *E. coli* aroA- vaccine strain and wild-type bacteria to convert the vaccine strain to a virulent strain is considered very rare.

The withdrawal period of zero days has been justified. No studies have been performed to test the effect of the vaccine on concurrent use of any other vaccine. Therefore section 4.8. of the SPC includes the common statement.

Field studies

A field trial was conducted as a large-scale multicentre field trial according to Good Clinical Practice (GCP) regulations.

No significant adverse events were observed throughout the study. The non-inferiority of average daily weight gain, feed conversion ratio and mortality during the first two weeks in vaccinated versus control animals was statistically demonstrated. The study demonstrated that the vaccine Poulvac E. coli is safe when used in day-old broiler chickens. Justification is given that the presented study addresses the husbandry practices and field conditions in the EU and that the generated data are also representative for the EU.

User safety and Environmental risk assessment

A user risk assessment has been provided for Poulvac E. coli including the elements hazard identification and characterisation, exposure assessment, risk characterisation, risk management and risk communication. Appropriate operator's safety measures regarding proper protection (gloves, nose-mouth mask and eye protection) are proposed in the SPC. However, given the aerosol administration it is considered that immune-compromised persons should not administer the vaccine and therefore an appropriate warning has been included in the SPC. In view of the persistence of the vaccine strain in the bird and the environment additional information has been included to warn personnel involved in attending vaccinated animals to follow general hygiene principals and to take care in handling litter from recently vaccinated animals.

Regarding the environmental risk assessment, the first phase outlines that the potential exposure of the environment to the product and the level of risk associated with it is considered very low to negligible.

Assessment required for veterinary medicinal products containing or consisting of genetically modified organisms

In addition to the requirements of Directive 2001/82/EC as amended, Annex III A of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms applies for this vaccine. All points to be considered for a live genetically modified organism used as vaccine strain have been addressed.

Cloning procedures and production of the final GMO were performed by genetic modifications that are state of the art.

Verification of the final product was done by commonly used methods, as it was analysed via restriction analysis and PCR for efficient deletion of the *aroA* gene. The vaccine strain can be easily differentiated from the wild type strain as the mutant results in a PCR amplicon which is 100bp smaller than that of wild type *E. coli*. To detect the auxotrophic nature of the vaccine strain replica plating on supplemented versus basal media provides and additional level of specificity and reliability to identification.

Part 4 – Efficacy

Introduction and general requirements

Poulvac E. coli is a live attenuated vaccine for use in chickens (broilers, future layers/breeders) consisting of live *aroA* gene deleted *Escherichia coli* type O78 (strain EC34195) and a stabiliser. No adjuvant is included. The approved titre range per dose given in the SPC is 5.2 x 10^6 to 9.1 x 10^8 CFU.

According to the vaccination schedule one dose of vaccine should be given from 1 day of age by coarse spray. No revaccination is recommended. For administration the lyophilisate should be dissolved in chlorine-free water at room temperature: initially in a small amount in its vial (half-filled) which is then further diluted in a clean container in order to obtain an even distribution when sprayed onto the birds.

The applicant presented laboratory vaccination/challenge studies (establishment of the minimum protective dose, onset of immunity, duration of immunity, cross protection against serotype O1, O2 and O18) and a field study to support the efficacy claims.

Laboratory trials

Establishment of a challenge model

The applicant used an intratracheal challenge model. According to different publications it is well established that an intratracheal instillation results in a highly accurate reproduction of disease characteristically encountered in field situations.

The relevance of the challenge strain within the EU is sufficiently demonstrated. Furthermore, the challenge with *E. coli* O78 is considered heterologous because the challenge strain used is not identical with the production strain of the vaccine (even if the serotype of the chosen challenge strain is the same as that of the vaccine strain). Therefore, the challenge model is considered valid.

Determination of the vaccine dose

The minimum protective dose for Poulvac E. coli vaccination was determined, using the vaccination scheme recommended in the SPC (one vaccination of chickens at one day of age by coarse spray). Group 1 (30 SPF chickens) was vaccinated with 1.642 x 10^6 cfu/dose, group 2 (30 SPF chickens) was vaccinated with 8.21 x 10^5 cfu/dose, group 3 (30 birds) was not used due to laboratory error and group 4 (20 SPF chickens) was not vaccinated. The omission of group 3 does not impair the general outcome of the study and is therefore regarded as irrelevant. Groups 1 to 3 were challenged intratracheally with *E. coli* O78 at 6 weeks of age while Group 4 was the environmental control group (non-vaccinated, non-challenged). After challenge, daily observation was performed for 7 days and dead birds were necropsied and examined for the presence of. The same applied for all surviving chickens 7 days after challenge (necropsy and examination).

It was clearly shown that the occurrence of colibacillosis after challenge was statistically significantly lower in group 1 compared to group 3 (not vaccinated). In contrast, the differences between group 2 and group 3 were not significant. The mortality post-challenge was 0% in group 1 and group 2 vs. 20.7% in group 3 and thus clearly in favour of the vaccinated groups.

The study was considered valid since none of the chickens from group 4 (environmental control) showed characteristic lesions due to APEC throughout the trial. The CVMP concluded that the minimum protective dose has been established at 1.6 x 10^6 CFU/dose.

Onset of immunity

The onset of immunity was determined using one vaccination of chickens at one day of age with Poulvac E. coli by coarse spray. Groups A1, A2 and A3 (30 SPF chickens each) were vaccinated with 3.1 x 10^6 cfu/dose whereas groups B1, B2 and B3 (30 SPF chickens each) received PBS and groups C1, C2 and C3 (30 SPF chickens each) were not vaccinated. Groups C1, C2 and C3 were the environmental control groups (non-vaccinated, non-challenged). The other groups were challenged intratracheally with *E. coli* O78: Groups A1 and B1 at 1 week post vaccination, groups A2 and B2 at 2 weeks post vaccination and groups A3 and B3 at 3 weeks post vaccination. After challenge the birds were observed daily for 7 days, including necropsy of dead birds. 7 days post challenge all surviving chickens of the respective challenge groups and all chickens of one unvaccinated unchallenged control group were necropsied (i.e. A1, B1, C1) and examined for colibacillosis lesions including evaluation of airsacculitis score.

At the challenges two weeks and three weeks post vaccination the percentage of birds with colibacillosis lesions was statistically significantly lower in the vaccinated group compared to the placebo group. No difference could be revealed at challenge one week post vaccination. None of the chickens of the environmental control group C were observed with characteristic lesions. The distribution and mean group scores of airsacculitis lesions numerically favour the vaccinated group for all challenge time points and is considered supportive. In contrast the statistical evaluation of mortality data does not reveal any significant differences at any points in time chosen for challenge.

The study is considered valid because none of the chickens from the environmental control group C (non-vaccinated, non-challenged) showed characteristic lesions due to APEC throughout the trial. Although no significant difference could be revealed for mortality, the mortality claim can be considered acceptable as it is sufficiently supported by data generated from several other studies. A statistically significant treatment effect at onset in terms of protection against lesions of colibacillosis has been demonstrated. Therefore, an onset of immunity two weeks after vaccination is considered acceptable for the lesion claim. Onset of immunity has not been established for the mortality claim and this is reflected in the SPC.

Duration of immunity

The duration of immunity after one-shot vaccination of chickens at one day of age with Poulvac E. coli by coarse spray was established in this study. Groups 1a and 1b (25 SPF chickens each) were vaccinated with 1.373 x 10^6 cfu/dose whereas groups 2a and 2b (25 SPF chickens each) received PBS. All groups were challenged intratracheally with *E. coli* O78 strain 626,groups 1a and 2a at 8 weeks post vaccination and groups 1b and 2b at 12 weeks post vaccination. No environmental control group (non-vaccinated, non-challenged) was included but the applicant has given further assurance that the vaccinated target animals were not exposed to intercurrent field infection which could boost the immunity.

After challenge daily observation for mortality was conducted until 7 days post challenge, including necropsy of dead birds. At day 7 post challenge all surviving chickens were necropsied and examined for colibacillosis lesions. Occurrence of colibacillosis lesions, mortality and severity of airsacculitis was compared between the groups.

The mortality rate of vaccinates and controls following the challenge 8 weeks post-vaccination and 12 weeks post-vaccination was 3.7% vs. 63.9% and 32.4% vs. 92.1%, respectively, which is a statistically significant difference in both cases. Therefore, the duration of immunity of 12 weeks is supported for the claim “reduction of mortality”. At necropsy, incidence of typical colibacillosis lesions in vaccinates and controls following challenge 8 weeks post-vaccination and 12 weeks post-vaccination was 32% vs. 92% and 80% vs. 96%, respectively. The statistical analysis reveals a significant difference only for the challenge 8 weeks post-vaccination, whereas 12 weeks post-vaccination no significant difference occurs. Consequently, only a duration of immunity of 8 weeks is supported for the claim “reduction of lesions (pericarditis, perihepatitis, airsacculitis)”.

Overall, the results justify a duration of immunity of 12 weeks after vaccination for the reduction of mortality claim and a duration of immunity of 8 weeks after vaccination for the reduction of colibacillosis lesions claim. This is adequately reflected in section 4.2 of the SPC.

Influence of maternal antibodies on the efficacy of the vaccine

No studies have been conducted on the impact of maternally derived antibodies as all chickens have these. This was explained by the applicant that serum samples from one day old SPF chickens from four different SPF flocks were tested for the presence of antibodies to *E. coli* O78 serotype. The results of this study showed that all SPF chicks contained antibodies that reacted with *E. coli* O78 antigen in ELISA regardless of the source of parent flocks.

Based on the submitted data in combination with published literature the applicant concluded that maternal immunity plays very little if any role in the development of, or immunity against APEC associated disease. Results of use of the vaccine in the field (commercial use in the US and field trial) indicate that Poulvac E. coli is effective in the presence or absence of maternal antibodies. However, a possible negative impact on the vaccine efficacy due to high levels of maternal antibodies could not be excluded with absolute certainty. Therefore, in the SPC (section 4.4) it is stated that no information is available on the influence of extra-ordinary high levels of maternally derived antibodies on the efficacy.

Prevention of transplacental transmission

No studies were provided regarding prevention of egg transmission. This is acceptable as no SPC claim exists in this respect.

Field trials

One multicentre field trial for evaluation of safety and efficacy of Poulvac E. coli in broilers was conducted in Morocco according to GCP regulations.

A total of 224966 commercial broilers on 15 farms (36 broiler houses, minimum 1 vaccination house and 1 control house per farm) were included in this trial. In accordance with the recommended vaccination scheme in the SPC, chickens (18 broiler houses, 112490 birds) received a vaccination at one day of age by coarse spray. No challenge was conducted. Post-vaccination daily health observations were performed and mortality, feed used and medication applied were registered (daily). On days 0, 13 and 42 ≥100 randomly selected animals per house were weighted. On day 42 approximately 1000 randomly selected animals per bird house were slaughtered and scoring of

colibacillosis-like lesions (pericarditis, peritonitis, perihepatitis, airsacculitis) was performed. Data analysis was performed in compliance with the CVMP guideline on Statistical Principles for Veterinary Clinical Trials (EMEA/CVMP/816/00).

The study is considered valid as all non-vaccinated controls showed colibacillosis-like lesions, indicating that all farms were suffering from APEC. However, serotype O78 was not diagnosed in all farms. Overall, the prevalence of colibacillosis-like lesions is significantly reduced (1.7% vs. 3.5%, p=0.0054) as well as the mortality for the periods D.14 to study end (7.7% vs. 8.8%, p=0.0182) and D.0 to study end (9.3% vs. 10.3%, p=0.0203).

Besides, the results show that the average number of antibiotic treatment days was significantly reduced (0.54 vs. 1.97). Furthermore, the average daily weight gain and the percentage of animals marketed were statistically significant higher in the vaccinated group than in the control group over the whole study period (47.8g vs. 46.2g and 90% vs. 89%).

Additional studies

Compatibility

No data are available on the compatibility of this vaccine with any other, therefore the common statement according to the QRD template is included in section 4.8 of the SPC. This is deemed acceptable.

Cross protection

The cross protection of commercial broilers after vaccination with Poulvac E. coli against three strains of serotypes O1 (APEC 88), O2 (APEC 211) and O18 (APEC 351) was studied.

Commercial broilers vaccinated at one day of age by coarse spray and non-vaccinated controls were challenged at 42 days of age by intratracheal route. Each challenge strain was solely used in one vaccinated and one control group. An environmental control group (non-vaccinated, non-challenged) was included. Post-challenge the chickens were observed daily for clinical signs and mortality, including necropsy of dead birds. For determination of performance, pens and feed were weighted at 35, 42 and 47 days of age. At 47 days of age, all remaining birds were posted and lesions recorded.

The statistical analysis showed that the incidence and severity of airsacculitis are significantly reduced after vaccination compared to the non-vaccinated challenge controls for all three serotypes tested (O1, O2, O18). No statistically significant differences could be revealed for mortality and feed conversion rate.

Therefore, the CVMP concluded that the inclusion of a statement in the SPC relating to the cross protection was pertinent and the appropriate statement can now be found under section 4.2.

Overall conclusion on efficacy

Target species

Poulvac E. coli is intended for use in chickens (broilers, future layers/breeders).

Minimum protective dose

A minimum protective dose of 1.6 x 10^6 CFU/bird is adequately demonstrated.

Efficacy claims

Regarding the indications in section 4.2 the *reduction of lesions (pericarditis, perihepatitis, airsacculitis)* was consistently demonstrated throughout the efficacy studies and the claim is therefore supported by the Committee. This claim is considered clinically relevant and important for the control of colibacillosis in chickens. The claim reduction of mortality is not substantiated by data of the Onset of immunity-study. However, the mortality claim can be considered acceptable as it is sufficiently supported by data generated from several other studies.

The *onset of immunity* of 2 weeks post vaccination is supported for the lesion claim. Onset of immunity has not been established for the mortality claim.

A *duration of immunity* of 12 weeks is only supported for the SPC claim "reduction of mortality" but not for "reduction of lesions (pericarditis, perihepatitis, airsacculitis)" for which a duration of immunity of 8 weeks was demonstrated.Therefore the duration of immunity is separately included for both claims in section 4.2 of the SPC.

The *cross protection* study showed statistically significant reduction of incidence of airsacculitis and reduction of severity of air sac lesions. Therefore, the inclusion of the following statement in section 4.2 of the SPC is considered acceptable:

A cross protection study showed reduction of incidence and severity of airsacculitis caused by E. coli serotypes O1, O2 and O18. For these serotypes, no onset of immunity or duration of immunity was established.

Vaccination schedule

According to the vaccination schedule, the minimum age of vaccination is 1-day of age by coarse spray. This vaccination schedule was successfully used in all efficacy studies included in this dossier and is therefore considered acceptable.

The usefulness of immunisation of broilers, which are commonly affected by the disease between 4 and 9 weeks of age, is sufficiently substantiated: clinical signs of colibacillosis as well as mortality will be reduced at the finishing period following one vaccination. However, it is assumed that long-lived birds (layer chickens and layer/broiler breeder chickens) which are commonly affected at the peak of egg production won't be protected at this time point when vaccinated once at 1 day of age. However, the benefit of vaccination for this target group is related to the fact that more future layers/ future breeders will survive the rearing period. Therefore, it can be concluded that the vaccination increases flock health and is beneficial for all subcategories of the target animal.

Maternally derived antibodies

No studies have been conducted on the impact of maternally derived antibodies with the argumentation that all chickens have these. This explanation is based on a study where serum samples from one day old SPF chickens from four different SPF flocks were tested for the presence of antibodies to *E. coli* O78 serotype. The results of this study indicated that all SPF chicks contained antibodies that reacted with *E. coli* O78 antigen in ELISA regardless of the source of parent flocks.

Based on the submitted data in combination with published literature the applicant concludes that maternal immunity plays very little if any role in the development of, or immunity against APEC associated disease. Results of use of the vaccine in the field (commercial use in the US and field trial) indicate that Poulvac E. coli is effective in the presence or absence of maternal antibodies. However, a possible negative impact on the vaccine efficacy due to high levels of maternal antibodies could not be

excluded with absolute certainty. Therefore, in the SPC (section 4.4) is stated that no information is available on the influence of extra-ordinary high levels of maternally derived antibodies on the efficacy.

Prevention of egg transmission

No studies were provided regarding prevention of egg transmission. This is acceptable as no SPC claim exists in this respect.

Compatibility

No data are available on the compatibility of this vaccine with any other therefore the common statement is included in section 4.8 of the SPC.

Part 5 – Benefit risk assessment

Introduction

The topic of the following benefit-risk assessment is the application for marketing authorisation of Poulvac E. coli under MUMS conditions according to guideline EMEA/CVMP/IWP/123243/2006 (minor use / minor species).

Poulvac E. coli is a live attenuated vaccine for use in chickens (broilers, future layers/breeders) consisting of live *aroA* gene deleted *Escherichia coli* serotype O78 (strain EC34195) and a stabiliser. No adjuvant is included. The quantity of the active ingredient is given in the SPC with 5.2×10^6 to 9.1×10^8 per dose.

Benefit assessment

Direct therapeutic benefit

Throughout the efficacy studies a statistically significant reduction of incidence of lesions typical of colibacillosis (pericarditis, perihepatitis, airsacculitis) was consistently demonstrated for chickens vaccinated with one dose at one day of age by coarse spray. Furthermore, a significant reduction of mortality due to *E. coli* O78 infections could be detected in 3 out of 4 studies (all except the Onset of Immunity study). Both claims are supported by data generated in well controlled laboratory studies and one field study. Onset and duration of immunity of respectively 2 weeks (reduction of lesions) and 8 (reduction of lesions) or 12 (reduction of mortality) weeks after vaccination have been demonstrated. For the mortality claim onset of immunity has not been established. The benefit for future layers and breeders is related to the fact that more birds will survive the rearing period and will pass over to the subsequent reproductive phase.

Additional benefits

In addition to the direct benefits the multicentre field study revealed a statistically significantly positive effect of the vaccine on the parameters “average daily weight gain”, “number of antibiotic treatment days” and “percentage of animals marketed” (in comparison with the unvaccinated controls). The cross protection study showed reduction of incidence of airsacculitis and reduction of severity of airsac lesions caused by *E. coli* serotypes O1, O2 and O18 (for these serotypes, no onset of immunity or duration of immunity was established).

Moreover, the application by coarse spray is very user-friendly and the less invasive technique for chickens. The method enables easy vaccination of many chickens in a short time with minimal labour costs and minimal disturbance of animal welfare.

Overall, the vaccine is expected to increase animal welfare (because of reduction of disease and mortality) as well as farm efficiency and farm profitability due to decreased costs for antibiotic treatment, less labour requirements (because of lower disease incidence), increased average daily weight gain, increased percentage of animals being marketed and less carcass condemnations at slaughterhouse (because of less colibacillosis-induced lesions). The reduction of antibiotic use is also considered desirable with regard to the formation of antibiotic resistances as well as consumer demand.

Risk assessment

The following risks have been identified:

For the user:

- Appropriate operator's safety warnings are added to prevent exposure.

The theoretical human health risk caused by inhalation of a large dose of active ingredient as well as of aerosolised endotoxin has been sufficiently addressed. The risk of Poulvac E. coli for human health is low as a potential disease causing agent or acting as reservoir for the virulence associated genes. The risk of any effect of the endotoxin in the vaccine is low, too. However, taking a proportional safety approach for this product the CVMP agreed to include an appropriate warning in the SPC indicating that immune-compromised persons should not administer the vaccine, or be present during administration. To further reduce the risks to the user additional information has been included to warn personnel involved in attending vaccinated animals to follow general hygiene principals and to take care in handling litter from recently vaccinated animals.

For the environment:

- No special environmental risk could be identified. The potential exposure of the environment to the product and the level of risk associated with it are considered very low to negligible.

For the consumer:

- The finding that the vaccine strain can be isolated from cloacal swabs up to 28 days post vaccination raised concerns regarding the risk of broiler carcass contamination at the time of slaughter and the establishment of an appropriate withdrawal period has been discussed. However, in view of all the information and supplementary data provided by the applicant it has been concluded that the GMO itself is safe as substantiated by the safety data and genetic stability data. As no risk to humans or the environment has been identified a special warning is rather considered suitable than a withdrawal period. Sufficient clear information regarding the detection of the strain in tissues, strain excretion as well as the persistence of the strain in the environment is provided in section 4.5 of the SPC.
- A withdrawal period of zero days has therefore been set.

Specific risks, according to the nature of the product:

- Shed, spread and dissemination: The number of vaccine strain positive animals decreased over time. Recovery of vaccine strain occurred in vaccinated animals on single occasions for a short period following vaccination (up to 6 days). The vaccine strain was found in cloacal swabs of vaccinated birds until day 28 post vaccination, of non-vaccinated contact birds until day 11. Environmental samples (water, feed, litter) were positive post-vaccination following both eyedrop and spray application of the vaccine, with a clear decrease in prevalence over time lasting until day 35 at the latest. Section 4.5 of the SPC was amended accordingly. Reversion to virulence: The vaccine strain of Poulvac E. coli did not show an increase in virulence after passages in chicken.

- Recombination or genomic reassortment of strains: The likelihood of recombination between the *E. coli* aroA- vaccine strain and wild-type bacteria to convert the vaccine strain to a virulent strain is considered very rare.

Evaluation of the benefit risk balance

The product has been shown to have a positive benefit risk balance overall. Poulvac E. coli has been demonstrated to be efficacious for the indication for active immunisation of broiler chickens and future layers/breeders in order to reduce mortality and lesions (pericarditis, perihepatitis, airsacculitis) associated with *Escherichia coli* serotype O78.

The formulation and manufacture of Poulvac E. coli is well described and specifications set will ensure that product of consistent quality will be produced.

It is well tolerated by the target animals and presents a low risk for users and the environment and appropriate warnings has been included in the SPC.

A risk assessment addressing the human exposure associated with the use of Poulvac E. coli has been provided. In consideration of all the information and data provided by the applicant and the conclusions that the GMO itself is safe as supported by the safety and genetic stability data, a withdrawal period of zero days is considered adequately justified. The information given under 'Special precautions of use' provides the user with clear advice on how to use the product.

Conclusion on benefit risk balance

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

Conclusion

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP considers that the application for the live vaccine Poulvac E. coli is approvable.