18 February 2016
EMA/142811/2016
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for Poulvac E. coli for suspension for spray vaccination of chickens and turkeys (EMEA/V/C/002007/X/0008)

Common name: avian colibacillosis vaccine (live)

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

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Product profile

Invented name:	Poulvac E. coli
Active Substances:	*Escherichia coli* aroA gene deleted, type O78, strain EC34195
Target Species:	Chickens and Turkeys
Pharmaceutical Form:	Lyophilisate for suspension
Strength:	5.2 x 106 to 9.1 x 108 CFU (Colony forming units)
Therapeutic Indication:	For active immunisation of broiler chickens and future layer/breeders and turkeys in order to reduce mortality and lesions (pericarditis, perihepatitis, airsacculitis) associated with *Escherichia coli* serotype O78.
ATCvet code	QI01AE04
Pharmacotherapeutic group	Immunologicals for Aves
Applicant	Zoetis Belgium SA

Introduction

On 9 December 2014, an application for an extension to the Community marketing authorisation for Poulvac E. coli was submitted by Zoetis Belgium SA to the European Medicines Agency (the Agency) in accordance with Article 19 of Commission Regulation (EC) No 1234/2008 and Annex I point 3 thereof. The CVMP confirmed E. Werner as rapporteur and A.-M. Brady as co-rapporteur for this application.

Poulvac E. coli was first authorised in the Community on 15 June 2012 and is indicated for use by spray vaccination and in drinking water for the active immunisation of broiler chickens and future layer/breeders in order to reduce mortality and lesions (pericarditis, perihepatitis, airsacculitis) associated with *Escherichia coli* serotype O78.

This extension application is to add a new target species (turkeys).

Poulvac E. coli contains live *aroA* gene deleted *E. coli*, type O78, strain EC34195. Each dose contains 5.2 x 10^6 to 9.1 x 10^8 colony forming units (cfu) of the active substance. The pharmaceutical form is lyophilisate for suspension for spray vaccination for chickens and turkeys or for use in drinking water for chickens. Poulvac E. coli is presented in glass bottles of 10 ml for 2500 and 5000 dose-presentations and 50 ml for 10000 and 20000 dose-presentations.

On 18 February 2016, The CVMP adopted an opinion and CVMP assessment report.

On 12 April 2016, the European Commission adopted a Commission Decision granting the marketing authorisation for Poulvac E. coli.

Scientific advice

Not applicable.

MUMS/limited market status

Poulvac E. coli was established as minor use minor species (MUMS)/limited market classification by the CVMP, and the Committee at their 16 May 2013 meeting confirmed that, where appropriate, the data requirements in the appropriate CVMP guidelines on minor use minor species data requirements would be applied when assessing the application. MUMS/limited market status was granted as the application is for turkeys which are considered a minor species.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system was provided (Zoetis Pharmacovigilance System, version 1.2. dated 18 July 2013) which fulfils the requirements of Directive 2001/82/EC. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the European Union (EU) or in a third country.

Manufacturing authorisations and inspection status

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

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

Valid manufacturing licences and Good Manufacturing Practice (GMP) certificates for all sites are available.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system and the GMP certification of the manufacturing sites are considered in line with legal requirements.

Part 2 - Quality

The quality part of the product has not been changed and therefore was not submitted, and is therefore not subject for further assessment within this application. This is acceptable.

Composition

Poulvac E. coli is a live vaccine containing as active substance *aroA* gene deleted (*aroA*-) *E. 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.

Part 3 – Safety

Poulvac E. coli is a live attenuated vaccine currently indicated for use in chickens (broilers, future layers/breeders) to reduce mortality and lesions associated with *E. coli* serotype O78. No adjuvant is included. The approved titre range per dose given in the SPC is 5.2×10^6 to 9.1×10^8 cfu.

According to the proposed vaccination schedule for turkeys, one dose of vaccine should be given from 1 day of age by coarse spray administration followed by a second dose 3 weeks later.

Four (4) Good Laboratory Practice (GLP)-compliant laboratory safety trials in one day old SPF turkeys were provided, including untreated control animals. With reference to the relevant CVMP Guideline on data requirements for Immunological veterinary medicinal products intended for minor use or minor species/limited markets (EMEA/CVMP/IWP/123243/2006-rev.2) no field studies were conducted in turkeys.

Safety of the administration of one dose

To evaluate the safety of one maximum dose (9.1×10^8 cfu/dose) of Poulvac E. coli in one day old turkeys two studies were conducted.

In these two GLP-compliant laboratory studies the safety of Poulvac E. coli was tested in one day old turkeys following administration of an overdose and repeated single doses by eye drops at 3 and 5 weeks of age respectively.

The results of these studies prove the safety of the administration of the recommended maximum dose (9.1 x 10^8 cfu/dose) via oculo-nasal route to 3 weeks and 5 weeks old turkeys.

Safety of one administration of an overdose

A laboratory study was performed to evaluate the safety of a 10-fold overdose followed by two repeated single doses of Poulvac E. coli vaccine administration by eye drop to one day old turkeys. In accordance with the recommended vaccination scheme SPF turkeys at one day of age, the minimum age of administration, were vaccinated with one overdose. The tested vaccination titre of 1.7 x 10^{10} cfu per dose equates to the 10-fold maximum titre. Thirty two (32) turkeys were vaccinated oculo-nasally by eye-drop to ensure an accurate and regular delivery of the desired amount of vaccine per bird. Thirty two (32) turkeys served as control animals.

The turkeys were observed for 7 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 colibacillosis.

During the study no general reactions and at necropsy no specific findings related to colibacillosis were observed. The growth performance of the vaccinated turkeys was higher than the controls.

The observed accumulation of mortality in vaccinated turkeys (41%) during the first week after vaccination raised a need for further evaluation. In the control group a mortality rate of 16% was observed during the first week of age. No signs of colibacillosis were revealed during necropsy. Therefore, a correlation between the vaccination and the high mortality rate was deemed unlikely. Nevertheless, a further laboratory study was performed to evaluate whether there is a correlation between vaccine (over)dose and mortality as observed in the previous safety study. Also, the overdose and repeat dose safety of Poulvac E. coli, was re-assessed in one day old SPF turkeys following administration by eye drop. In each case 18 turkeys were vaccinated with doses significantly higher than planned (120-fold, 54-fold and 3-fold). A second vaccination was administered 3 weeks later with the recommended maximum dose. Another 18 turkeys served as control animals.

The turkeys were observed for 5 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 colibacillosis.

During the study no abnormal clinical signs (neither after 1st nor after 2nd vaccination) and at necropsy no specific findings related to colibacillosis were observed. The growth performance of the vaccinated turkeys was not affected.

Results from this study demonstrated absence of safety concerns after administration of an overdose (until 120-fold) of the vaccine via oculo-nasal route to one day old turkeys.

The high mortality rate in the first study was due to handling circumstances causing additional stress to birds. The improvement measures arranged in the second study reduced stress and prevented mortalities supporting that these were not related to the vaccine itself.

Safety of the repeated administration of one dose

Poulvac E. coli is intended to be given twice during the life of a turkey. Turkeys should be vaccinated with one dose of vaccine from 1 day of age followed by a second dose of vaccine 3 weeks later by coarse spray administration.

Repeated or single administration of a maximum dose was tested in two studies after vaccination with an overdose.

Results from this study demonstrated absence of safety concerns after administration of a repeated single maximum dose of the vaccine via oculo-nasal route to 3 and 5 weeks old turkeys.

Examination of reproductive performance

No studies have been performed on reproductive safety and advice is provided not to use the product in birds in lay, or within 6 weeks before the onset of the laying period. This is acceptable.

For turkeys the following vaccination scheme is proposed: one dose of vaccine from 1 day of age followed by a second dose of vaccine 3 weeks later by coarse spray administration. There is no indication for any impairment of the breeding performance of vaccinated turkeys. Therefore, the current advice not to use the vaccine during lay or within 6 weeks before the laying period, initially established for chickens, is considered to be appropriate for turkeys.

Examination of immunological functions

No studies have been performed to test the effect of the vaccine on the immune system. Effects of *E. coli* directly on immunological functions have not been reported and it is considered unlikely that the vaccine will adversely affect the immunological function of turkeys. 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

A laboratory study was conducted to evaluate dissemination, shedding and spreading of Poulvac E. coli administered by eye drop to one day old SPF turkeys.

Eighty (80) one day old SPF turkeys were vaccinated by oculo-nasal route (by eye-drop) with 1.06 x 109 cfu/0.2ml dose (recommended maximum dose). Forty (40) non-vaccinated turkeys were housed in-contact with vaccinated turkeys to serve as sentinels from four hours after vaccination on day 0. All turkeys were monitored daily for clinical signs until 3 weeks after vaccination. Four (4), 7, 14 and 21 days after vaccination, 10 vaccinated and 5 control turkeys were blood sampled and euthanised. Post-mortem examinations were performed paying particular attention to colibacillosis lesions in heart, lung, air sacs, liver and spleen. Lesions were scored. Swabs were collected from pericardium, thoracic air sacs, liver, cloaca and nasal cavity for bacterial isolation. Additionally, environmental samples from litter, feed and water were collected for bacterial isolation on days 0, 4, 7, 14 and 21.

During the study no abnormal clinical signs were recorded. Six (6) birds (4 vaccinated and 2 control birds) were found dead between day 1 and 6 after vaccination. Post-mortem examination on 5 of the 6 dead birds did not reveal any abnormal findings. *E. coli* vaccine strain was isolated from their carcasses. Due to the lack of lesions and the age of the birds, the mortalities were considered to be age related losses not linked to vaccination. No lesions typically for colibacillosis were detected in any bird at any time point (at days 4, 7, 14 and 21 after vaccination).

The *E. coli* vaccine strain could be isolated from the thoracic air sacs of 1 out of 10 vaccinated birds at 4 days after vaccination. Results from liver and pericardium samples were negative for the vaccine strain. No vaccine strain was isolated from the internal tissues of any of the contact birds at any time point.

Cloacal swabs were positive for the vaccine strain in up to 6 out of 10 of vaccinated birds (60%) and up to 2 out of 5 of contact birds (40%) for a maximum of 7 days after vaccination. Nasal swabs were positive for the vaccine strain in 3 out of 10 of vaccinated birds (30%) and up to 1 out of 5 of contact birds (20%) for a maximum of 7 days after vaccination.

The vaccine strain could be isolated from water, feed and litter 4 days after vaccination and from water also 7 days after vaccination. Section 4.5 of the SPC is amended accordingly.

In conclusion, the study demonstrates that when Poulvac E. coli is administrated by eye drop the vaccine strain is excreted by vaccinated turkeys and remains present in the environment until 7 days post vaccination as well as spreads from vaccinated birds to in contact unvaccinated birds.

Reversion to virulence of attenuated vaccines

A laboratory study was performed to evaluate reversion to virulence of the Poulvac E. coli vaccine strain by passaging in one day old turkeys.

The reversion to virulence study was carried out using a commercial batch (X+3), however, not with the master seed as requested by VICH GL 41 Target animal safety: examination of live veterinary vaccines in target animals for absence of reversion to virulence. This is due to the requirement that the initial inoculum should contain the maximum release titre expected in the recommended dose (1×10^9 cfu/ds of 0.2 ml) which was not fulfilled by the master seed (5.2×10^7 cfu/ml). The turkeys were vaccinated with 1×10^{10} cfu/dose of 0.2ml. This is the 10-fold maximum titre. Recovery of the vaccine strain was done at day 5 after inoculation of the vaccine strain. This time point was based on the results obtained in the dissemination study. In this study, *E. coli aroA*- from Poulvac E. coli vaccine was isolated from the airsacs of one vaccinated bird (1/10) at day 4 post-vaccination; and from nasal and cloacal swabs at day 4 and 7 post-vaccination. At day 5 after vaccination vaccine strain was isolated from heart, liver and air sacs of 3 out of 7 turkeys from vaccinated group. Material obtained from these 3 turkeys was pooled, identified as from the first and at once final passage (PVEC X+1), titrated (3.25 cfu/ml) and inoculated in day-old SPF turkeys (at first in 7 turkeys and then in 10 turkeys). In none of these turkeys the *E.coli* vaccine strain could be recovered.

The virulence of the commercial Poulvac E. coli batch was compared with the *E. coli* material recovered from PVEC X+1 by means of clinical signs, growth performance and post-mortem examinations. Clinical signs and post-mortem lesions could not be observed in any of the turkeys from both treatment groups. No differences in body weight were observed.

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

Biological properties of the vaccine strain

A summary of the biological properties of the vaccine strain was provided in the original dossier for the authorisation of the vaccine for chickens. No further information is included in this extension application. This is considered acceptable.

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 in the original dossier for the authorisation of the vaccine for chicken. 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 also for use in turkeys. This is considered acceptable.

Study of residues

Not required.

The active ingredient being a substance of biological origin intended to produce active immunity does not fall within the scope of Regulation (EC) No 470/2009 with regard to residues of veterinary medicinal products in foodstuffs of animal origin. In addition the other components of the vaccine are either listed in table 1 of the annex of Commission Regulation No 37/2010 or considered as not falling within the scope of Regulation (EC) No 470/2009 when used as in this product.

The withdrawal period of zero days has been justified for turkeys.

Interactions

No interaction studies have been performed neither in chickens nor in turkeys to test the effect of the vaccine on the concurrent use of any other vaccine and this is reflected in the product information.

Field studies

No field studies were carried out in turkeys. Poulvac E. coli is authorised for turkeys in the United States and in Canada and pharmacovigilance data from the United States and Canada (from June 2009 to December 2014) were provided, which give no indication for safety or efficacy concerns in the field.

User safety

A user safety assessment compliant with the CVMP guideline for user safety for immunological veterinary products (EMEA/CVMP/IWP/54533/2006) was provided.

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 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 principles and to take care in handling litter from recently vaccinated animals. The human health risk caused by inhalation of a large dose of active ingredient as well as of aerosolised endotoxin has been sufficiently addressed.

Appropriate warnings and user safety measures regarding proper protection (gloves, nose-mouth mask and eye protection) are included in the SPC. It was concluded that the user safety for this product is acceptable when used as recommended in the SPC.

Environmental risk assessment

A risk assessment has been provided in compliance with the CVMP guideline on the environmental risk assessment of immunological veterinary medicinal products (EMEA/CVMP/074/95).

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. This environmental risk assessment was provided in the dossier for the authorisation of the vaccine for chicken. 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, due to the very low environmental risk potential of the vaccine.

Through the addition of turkeys as target species no additional risks to the environment could be revealed.

In conclusion, based on the data provided the ERA can stop at phase I. Poulvac E. coli is not expected to pose a risk to the environment when used according to the SPC.

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

In addition to the requirements of Directive 2001/82/EC, 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 in the original dossier for chicken. This risk assessment does not change by adding turkeys as target species.

Overall conclusions on the safety documentation

Four (4) GLP and Ph. Eur. compliant laboratory safety trials were provided.

Poulvac E. coli is intended for use in turkeys from 1 day of age. The safety of one dose and of 10-fold overdose via oculo-nasal route has been tested in one day, 3 and 5 weeks old turkeys. The results prove the safety of the administration of one dose, an overdose and a repeated dose in turkeys from 1 day of age.

No studies have been performed on reproductive safety and appropriate information is given in the SPC.

No studies have been performed to test the effect of the vaccine on the immune system. However, Poulvac E. coli is unlikely to adversely affect the immunological function of turkeys. An appropriate precautionary measure not to vaccinate animals undergoing immunosuppressive treatment is included in the SPC.

The vaccine strain could be isolated only from the thoracic air sacs of vaccinated birds at 4 days after vaccination, but not from internal tissues of any of the contact birds at any time point. Cloacal and nasal swabs of vaccinated and control birds were positive for the vaccine strain for a maximum of 7 days after vaccination. The vaccine strain could be isolated from feed and litter 4 days after vaccination and from water until 7 days after vaccination.

In view of the ability of the vaccine strain to spread, the potential risks to other animals that might be in contact with vaccinated turkeys have been considered further and appropriate information has been included in the product information. Current warnings are deemed acceptable.

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

A detailed user safety assessment was provided in the dossier. Appropriate warnings and appropriate user safety measures regarding proper protection are included in the SPC.

The user safety for this product is acceptable when used as recommended in the SPC.

Residue studies are not required. The withdrawal period is set at zero days.

No studies have been performed to test the effect of the vaccine on concurrent use of any other vaccine and this is reflected in the product information.

No field trials were performed in turkeys. Pharmacovigilance data from the United States and Canada, where Poulvac E. coli is authorised for turkeys, were provided as supportive data. These data give no indication for safety or efficacy concerns in the field.

A phase I environmental risk assessment was conducted in the original dossier for chickens with the result that the estimation of risk can be considered very low to negligible. Through the addition of turkeys as target species no additional risks to the environment could be revealed. Therefore, the estimation of risk can be considered very low to negligible regarding turkeys as well. In conclusion Poulvac E. coli is not expected to pose a risk to the environment when used according to the SPC.

In addition to the requirements of Directive 2001/82/EC, Annex III A of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms applies for the vaccine. All points to be considered for a live genetically modified organism used as vaccine strain have been addressed in the original submitted dossier for chickens. This risk assessment does not change by adding turkeys as target species.

Overall it is concluded that appropriate documentation has been provided to demonstrate the safety of the vaccine.

Part 4 – Efficacy

Introduction and general requirements

Two (2) controlled vaccination/challenge trials were provided including untreated control animals with the aim to establish the minimum protective dose and onset of immunity (OOI). No studies were conducted to determine the duration of immunity (DOI), the impact of maternal antibodies or cross protection and no field study was performed to support the efficacy claims.

Sufficient information was given for the choice of the vaccine strain as well as the challenge strains. Their use was justified by data and publications demonstrating that *E. coli* O78 is one of the most prevalent *E. coli* types in turkeys affected by colibacillosis in Europe.

Laboratory trials

Determination of the vaccine dose

A randomized, partly blinded, negative control study was conducted in the US in order to evaluate the efficacy of Poulvac E. coli when administered to turkeys by coarse spray administration.

Two hundred and eighty eight (288) commercial turkeys were randomly assigned to one of three treatment groups. Turkeys in treatment group A (96 birds) and B (96 birds) were vaccinated twice, at 3 days and 3 weeks of age, where group A was a high titre vaccination group (~1x 107 cfu/dose) and group B was a low titre vaccination group (~1x 10^6 cfu/dose). Ninety six (96) birds (group C) served as controls (administered with sterile PBS at 3 days and 3 weeks of age). All 3 groups were challenged at 6 weeks of age (3 weeks post vaccination) with a virulent *E. coli* O78 strain and were necropsied 7 days later. At necropsy, birds were examined for the presence or absence of pericarditis, perihepatitis and airsacculitis and in addition, airsacculitis scores were evaluated.

It was clearly shown that the proportion of birds with any lesion of colibacillosis (pericarditis, perihepatitis or airsacculitis) after challenge was statistically significantly lower in groups A and B compared to group C: 0.57 (95% CI 0.46–0.67), 0.61 (95% CI 0.51–0.72) and 0.83 (95% CI 0.72–0.94) for birds vaccinated with 10^7 cfu, 10^6 cfu and controls, respectively.

The proportion of mortality due to challenge was 0.08 (95% CI -0.01–0.18), 0.18 (95% CI 0.09–0.27) and 0.29 (95% CI 0.20–0.38) for birds vaccinated with 10^7 cfu, 10^6 cfu and controls, respectively. Estimated vaccine efficacy for the prevention of mortality due to colibacillosis was 0.71 (95% CI 0.42–0.85) and 0.38 (95% CI -0.12–0.65) for birds vaccinated with 10^7 and 10^6 cfu, respectively, compared to controls. The estimates indicated that only group A had a significant difference to controls. However, it has to be taken into account that the titre of the vaccine used to vaccinate the birds of group B was approximately four times below the minimum titre of the vaccine (5.2 x 10^6 cfu/dose) and also that a significant difference in mortality was observed in study using a vaccine batch with a titre approximately 1.6 times lower than the minimum specification for Poulvac E. coli.

Justification was given for the vaccination scheme because the first vaccination was performed when the turkey pullets were 3 days old instead of 1 day which is the minimum age of vaccination. The justification is considered acceptable as maternal antibodies seem to have little if any interference with vaccine efficacy. Additional information regarding the serological status of the turkeys enrolled in the study, the challenge strain and the challenge route as well as study conditions and raw data for results after challenge were delivered and considered acceptable. Vaccinated target animals were not exposed to intercurrent field infection which could boost the immunity.

In conclusion, immunity was sufficiently demonstrated with vaccine doses less than the minimum titre. Vaccine should be given from 1 day of age by coarse spray administration followed by a second dose 3 weeks later.

Onset of immunity

A randomized, negative control, blinded laboratory challenge study was conducted to evaluate the OOI of Poulvac E. coli in turkeys when administered twice by coarse spray starting at one day of age. A total of 80, one day old SPF turkeys were randomly allocated to 2 treatment groups. Forty (40) birds (group T02) were vaccinated by coarse spray administration with Poulvac E. coli at intervals of 3 weeks where each dose was below the minimum titre of 5.2 x 10^6 CFU/dose currently approved for commercial vaccine batches and 40 turkeys (group T01) were left as controls (administered with sterile water by coarse spray with on day 0 and day 21). Three (3) weeks post vaccination all birds were intratracheally challenged with a virulent *E. coli* strain O78. Seven (7) days after challenge all surviving turkeys were euthanised, necropsied and examined for the presence or absence of typical lesions of *E. coli* septicaemia. Mortality and severe clinical signs after infection were also recorded.

The vaccination induced significant protection in terms of reduction of mortality and reduction of severity of clinical signs as well as severity of organ lesions (spleen and air sacs). The reduction of severity of colibacillosis lesions in liver and heart was not significant. There was no clear benefit in terms of the proportion of turkeys with colibacillosis lesions as all turkeys of both groups were affected due to the severity of the challenge (the challenge dose was considerably higher than targeted). This explanation is considered reasonable and it should be taken into account that the frequency of turkeys with colibacillosis lesions was clearly reduced in immunogenicity study. Therefore, OOI 3 weeks after second vaccination is considered acceptable for both the lesion (spleen and air sacs) and the mortality claim.

Duration of immunity

The DOI after vaccination of turkeys has not been studied. According to the MUMS/limited market status guideline (EMEA/CVMP/IWP/123243/2006-rev.2), for line extensions the omission of studies regarding the DOI is acceptable, provided it is made clear in the SPC that the data are not available. A sentence has been included in the SPC to reflect it and this is considered acceptable.

Influence of maternal antibodies on the efficacy of the vaccine

No studies have been conducted on the impact of maternally derived antibodies (MDA). According to the MUMS/limited market status guideline, for line extensions the omission of studies regarding the impact of MDA on the efficacy of the vaccine is acceptable, provided it is made clear in the SPC that the data are not available. An appropriate statement is already included in the current SPC section, which is considered acceptable as some serological data for turkeys was provided. The results were similar to those of chickens where it was concluded that MDA plays very little if any role in the development of, or immunity against avian pathogenic *E. coli* (APEC) associated disease.

Cross protection

No studies regarding the claim for cross protection have been conducted. Therefore, the current information regarding cross protection was restricted to chickens only.

Prevention of transplacental transmission

No studies were provided regarding prevention of vertical transmission through eggs and this is acceptable.

Field trials

No field efficacy studies have been provided. Field trials are not necessary for MUMS/limited market applications, provided sufficient laboratory studies are performed.

Overall conclusion on efficacy

Demonstration of efficacy is undertaken under well-controlled laboratory conditions by challenge of the target animal under the recommended conditions of use. The efficacy of the currently approved minimum dose of 5.2×10^6 cfu/bird is substantiated by the results of two studies. Taking all results together immunity was sufficiently demonstrated with vaccine doses less than the minimum titre.

According to the proposed vaccination schedule for turkeys one dose of vaccine should be given from 1 day of age by coarse spray administration followed by a second dose 3 weeks later. The two-shot

vaccination schedule was successfully used in all efficacy studies included in this extension dossier and is therefore considered acceptable.

OOI and DOI

The OOI 3 weeks after administration of the second dose is considered adequately demonstrated.

The DOI has not been established which is acceptable for line extensions falling within the scope of the MUMS/limited market guidance. SPC section 4.2 adequately reflects the lack of this information.

MDA

No studies have been conducted on the impact of MDA. According to the MUMS/limited market guideline, for line extensions the omission of studies regarding the impact of MDA on the efficacy of the vaccine is acceptable, provided it is made clear in the SPC that the data are not available. An acceptable statement is available in the current SPC.

No cross protection study was performed. Therefore, the claim included in the SPC is restricted to chickens only.

Part 5 – Benefit-risk assessment

Introduction

Avian colibacillosis in domestic turkeys is frequently associated with *E. coli* serotype O78. Infection commonly occurs via the respiratory tract, often after a primary bacterial or viral infection or as a result of poor husbandry practices. The disease generally affects turkeys between three and ten weeks of age and is associated with high morbidity and mortality. Carcass condemnation at slaughter is also common. The most severe manifestation of avian colibacillosis is septicaemia that is characterized by pericarditis, perihepatitis, airsacculitis and salpingitis.

The following benefit-risk assessment concerns the extension to the marketing authorisation of Poulvac E. coli to add a new food-producing target species (turkeys).

Poulvac E. coli is a live attenuated vaccine currently licenced for use in chickens (broilers, future layers/breeders) consisting of live *aroA* gene deleted *E. 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.

The dossier has been submitted in accordance with Article 12(3) of Directive 2001/82/EC.

The application has been classified as MUMS/limited market and therefore reduced data requirements have been considered in the assessment.

Benefit assessment

Direct therapeutic benefit

In well conducted and controlled laboratory studies the vaccine was shown to induce active immunisation of turkeys against *E. coli* O78 in order to reduce mortality and lesions (pericarditis, perihepatitis, airsacculitis) associated with *E. coli* serotype O78. Efficacy was demonstrated for turkeys vaccinated

twice: one dose of vaccine from 1 day of age followed by a second dose of vaccine 3 weeks later by coarse spray administration. An OOI 3 weeks after the second vaccination has been demonstrated.

Additional benefits

Poulvac E. coli is administered by coarse spray that is easy to administer and a less invasive technique for turkeys. The method enables vaccination of many birds in a short time causing minimal disturbance of animal welfare. Poulvac E. coli increases the range of available possibilities to control APEC in a minor species.

The product reduces the need for antimicrobial treatment.

Risk assessment

Main potential risks have been identified as follows:

Quality:

The formulation and manufacture of Poulvac E. coli is well described in the quality part of the original dossier submitted for the target species chickens. Specifications set will ensure that product of consistent quality will be produced.

For the target animal:

The product is generally well tolerated in the target animal.

The safety of Poulvac E. coli was adequately assessed in the minimum age group recommended for vaccination.

New data (shed, spread, dissemination and reversion to virulence) has been provided in order to assess the specific risks associated with the nature of the product when administered to turkeys:

Shed, spread and dissemination: Recovery of vaccine strain occurred in vaccinated animals from thoracic air sacs on single occasions for a short period following vaccination (up to 4 days). The vaccine strain was found in cloacal and nasal swabs of vaccinated and non-vaccinated contact birds until day 7 post vaccination. Environmental samples (feed, litter) were positive until 4 days post-vaccination, water samples until 7 days post vaccination. Section 4.5 of the SPC is amended accordingly.

Reversion to virulence: The vaccine strain of Poulvac E. coli did not show an increase in virulence after passages in turkeys.

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 low in turkeys, as in chickens.

For the user:

The risk to the user caused by inhalation of a large dose of active ingredient as well as of aerosolised endotoxin is considered low. In addition, 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 user risk assessment remains unchanged for this extension to turkeys taking into consideration that the immunisation scheme includes two vaccinations. Appropriate user safety warnings are included in the SPC to prevent exposure.

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

For the environment:

The product is not expected to pose any risk to the environment when used as recommended.

For the consumer:

The vaccine strain can be isolated from cloacal and nasal swabs of turkeys up to 7 days post vaccination which raised concern regarding the risk of carcass contamination at the time of slaughter. Appropriate information is reflected in the SPC.

Residue studies are not required. The withdrawal period is set at zero days.

Risk management or mitigation measures

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

Since the application concerns a new target animal species, the CVMP also recommended that the PSUR cycle be re-started to ensure more frequent pharmacovigilance monitoring. The data lock point (DLP) for the first 6-monthly PSUR of the re-started cycle would be 30/06/2016.

Evaluation of the benefit-risk balance

The product has been shown to have a positive benefit-risk balance overall in turkeys.

Poulvac E. coli has been demonstrated to be efficacious for the active immunisation of turkeys in order to reduce mortality and lesions (pericarditis, perihepatitis, airsacculitis) associated with *E. coli* serotype O78. An OOI has been established 3 weeks after the second vaccination.

The product is well tolerated by the target animals and presents an acceptable risk for users, consumers and the environment when used as recommended and appropriate warnings have been included in the SPC. The withdrawal period is set at zero days.

The quality of the product has been established previously.

Conclusion on benefit-risk balance

The overall benefit-risk evaluation for the product in turkeys is deemed positive with a sufficiently clear and complete SPC and product literature.

Conclusion

Based on the original and complementary data presented the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the quality, safety and efficacy for Poulvac E. coli to add a new target species, turkeys, are considered to be in accordance with the requirements of Directive 2001/82/EC. The overall benefit-risk evaluation is deemed positive with a sufficiently clear and complete SPC and product literature.

Based on the CVMP review of the data on safety and efficacy, and taking into account previous conclusions on quality, the CVMP recommends the extension of the marketing authorisation for Poulvac E. coli to add a new target species, turkeys, for Poulvac E. coli.