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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

Withdrawal assessment report for extension for Aivlosin (EMEA/V/C/000083/X/0055)

International non-proprietary name (INN): tylvalosin

Assessment report with all information of a commercially confidential nature deleted. Withdrawal at day 200.

Summary

An application for an extension to the Community marketing authorisation for Aivlosin was submitted by ECO Animal Health Ltd to the European Medicines Agency (the Agency) on 28 November 2012 in accordance with Article 19 of Commission Regulation (EC) No 1234/2008 and Annex I point 3 thereof for addition of a new food-producing target species.

Aivlosin contains tylvalosin (as tylvalosin tartrate), a macrolide antibiotic, and was first authorised for use in the Community on 9 September 2004. It is currently available in different pharmaceutical forms (premix for medicated feeding stuff, oral powder, granules for use in drinking water) for different target species (pigs, chickens, pheasants). This extension application concerns the addition of a new food-producing target species, chicken, to the already authorised 42.5 mg premix for medicated feeding stuff for pigs, for the following indication: Treatment of respiratory disease associated with *Mycoplasma gallisepticum* in chickens.

The application was validated on 12 December 2012 and the assessment was carried out by the Committee for Medicinal Products for Veterinary Use (CVMP) in line with the standard timetable. In response to questions, supplementary information was provided by the applicant on 9 August 2013, and oral and written explanations were provided by the applicant on 11 December 2013. At day 180 of the procedure, the CVMP considered on the basis of quality, safety and efficacy data submitted, that the product was not approvable, since major objections had been identified, which precluded a recommendation for the granting of a marketing authorisation. The major concerns were mainly in relation to the efficacy (proposed dose) of the use of the pharmaceutical form in the proposed new target species.

On 6 January 2014, ECO Animal Health withdrew the application at day 200 of the procedure. In its letter notifying the Agency of the withdrawal of application, the applicant stated the reason for the withdrawal: “CVMP considers that the data provided do not allow the Committee to conclude on a positive benefit-risk balance”.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system which fulfils the requirements of Directive 2001/82/EC, as amended. 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 or in a third country.

Manufacturing authorisations and inspection status

The active substance (tylvalosin) is manufactured outside the European Economic Area (EEA). A declaration of compliance of the manufacture of the active substance with EU good manufacturing practice (GMP) requirements for starting materials has been provided by the qualified person of the batch release site.

Manufacture of the finished product and batch release are both located in the UK. All relevant sites have valid manufacturing authorisations or valid GMP certificates as appropriate. Hence, no GMP inspections were deemed necessary within the scope of this procedure.

Scientific advice

Not applicable.

Overall conclusions on administrative particulars

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

Part 2 – Quality

The proposed product Aivlosin 42.5 mg/g premix for medicated feeding stuff for chickens is identical to Aivlosin 42.5 mg/g premix for medicated feeding stuff for pigs in all aspects of formulation, manufacture, control testing, type of packaging and pack sizes. Hence, a full part 2 dossier was not submitted and reference is made to the quality data submitted and approved previously. Only new data submitted in support of this application are discussed below.

Other Information - In-feed studies

Inclusion rate

The inclusion rate of the proposed premix in chicken feed corresponds to 3 kg Aivlosin per tonne feed, i.e. 0.3%.

Homogeneity

The proposed product Aivlosin 42.5 mg/g premix for medicated feeding stuff for chickens has been used to medicate chicken feed at two different inclusion levels (127.5 g and 170 g tylvalosin per tonne feed). Samples from across the bulk blends have been analysed and the results show adequate relative standard deviations (RSDs) of 4.4 to 6.3%.

In a second study, conducted using 208, 622 and 1,038 g tylvalosin per tonne feed, RSDs of 5.09%, 5.33% and 3.06% were obtained. In a third study, using 42.5 g tylvalosin per tonne feed, an RSD of 3.75% was obtained. These studies used more than one batch of Aivlosin 42.5 mg/g premix and more than one brand of chicken feed. It is therefore considered that the proposed product Aivlosin 42.5 mg/g premix for medicated feeding stuff for chickens can be homogeneously mixed into chicken feed.

Chicken feed is normally pelleted but, should this not be required, it has previously been shown that segregation is not a problem with Aivlosin-containing mash feeds.

Stability

The pellets generated in the homogeneity studies have been stored in 3-ply packing paper bags for 6 weeks at 25 °C/60% RH (relative humidity). This was considered appropriate as under field conditions medicated feed is usually packed in paper bags. Assays showed that after one week the decline in active substance content was up to 15% and after two weeks from 20% to 27%. There were no intermediate data. On the basis of these data, an in-feed shelf life of one week would be acceptable.

Results for three more pellet/feed stability studies were also provided. The container type was not stated. Previous data (see CVMP assessment reports for Aivlosin 42.5 mg/g premix and 8.5 mg/g premix for medicated feedingstuff for pigs) show that the mean results for the in-feed specification should fall within ± 15% of nominal values, and this criterion was also used to evaluate these studies.

Results were variable and the data suggested in-feed shelf lives for pelleted feed of 14-24 days and for meal feed of 4 weeks. The study involved two batches at each inclusion rate and all four batches contained 80%

or less of the target concentration after 2 weeks and less than 85% after 10 days. The CVMP is of the view that a one week shelf life for pelleted feed is the maximum that can be accepted.

The statements to appear in the summary of product characteristics (SPC) should be:

Shelf life after incorporation into meal feed: 4 weeks.

Shelf life after incorporation into pelleted feed: 1 week.

It was also noted that no information has been provided with regard to the shelf life after first opening. However, prior to the withdrawal of the application, the applicant had agreed to carry out an in use shelf life study.

Overall conclusions on quality

Aivlosin 42.5 mg/g premix for medicated feeding stuff has been authorised for pigs. The addition of a new target species chickens makes use of authorised packaging and pack sizes and would therefore not affect the quality of the product. Hence a full part 2 dossier was not submitted and reference was made to the quality data submitted and approved previously.
Aivlosin 42.5 mg/g premix for medicated feeding stuff has been used to medicate chicken feed at two different inclusion levels. Samples from across the bulk blends have been analysed and the results showed adequate relative standard deviations.

Stability results were variable but clearly showed that pelleted feed was quite unstable and mash feed considerably less so. The statements to appear in the SPC should be:

Shelf life after incorporation into meal feed: 4 weeks.

Shelf life after incorporation into pelleted feed: 1 week.

Part 3 – Safety

The applicant cross-referenced data that were provided in earlier submissions for two Aivlosin presentations that are already authorised; Aivlosin 42.5 mg/g premix for medicated feedingstuff for pigs, which has the same formulation as the application under consideration, and Aivlosin 625 mg/g granules for use in drinking water for chickens (and pheasants), which is indicated for the same species as the application under consideration.

Safety documentation

Since this is an application to add a new target species, chicken, to the already authorised presentation 42.5 mg premix for feedingstuff for pigs, and the pharmacology, toxicology and target animal safety have already been assessed for chickens in previous applications; no new data were provided, which is acceptable.

The toxicological profile of the active substance, tylvalosin, was assessed by the CVMP and is presented in the summary reports for acetylisovaleryltylosin (EMEA/MRL/702/99-FINAL, EMEA/MRL/794/01-FINAL, EMEA/MRL/909/04-FINAL, EMEA/CVMP/77339/2005-FINAL) and in the EPMAR for tylvalosin (EMEA/CVMP/469245/2007-Final, November 2007).

Development of resistance (food borne bacteria)

In regard to organisms of relevance for public health, Enterobacteriaceae such as *E. coli* and *Salmonella* have generally low intrinsic susceptibility to macrolides, and concerns for the development of macrolide resistant food borne pathogens is restricted to *Enterococci* spp. and *Campylobacter* spp.

In a previous extension application for the granule formulation of Aivlosin for chicken (EMA/V/C/083/X/026), the effect on the development of resistance in *Campylobacter* and enterococci to tylvalosin at the therapeutic dose of 25 mg/kg for 3 days was studied. A transient effect was observed for enterococci; however, there was no effect on *Campylobacter*. Theoretically, the new dose regimen for Aivlosin could result in an increase in the duration of the selective window. Although no specific studies have been presented to assess the impact of this on resistance development, literature studies indicate that prolonged exposure to macrolides (> 30 days, i.e. considerably longer than the intended 7 day treatment period for the premix) is needed to promote resistance development in *Campylobacter jejuni;* therefore, no further data have been requested in this respect.

Campylobacter infection is generally a self-limiting disease in man and antibiotics are not advocated in the majority of cases. Surveillance data from EFSA, MARAN and DANMAP show that levels of resistance to erythromycin in *Campylobacter* from chickens, broiler meat and humans have remained low and stable, and erythromycin still remains a drug of choice for treating this infection in humans. Levels of resistance to macrolides in *Enterococcus* spp. from chickens are high, but appear to be decreasing over recent years. Macrolides are not used to treat *Enterococcus* infections in man.

Published risk assessments estimate the general risk to public health from use of macrolides in food animals as low. Aivlosin is already authorised as an oral granule formulation to treat *M. gallisepticum* in chickens. Therefore, it is likely that the premix formulation will be used as an alternative to this formulation, i.e. the overall exposure to tylvalosin would not increase significantly. The risk for development of antimicrobial resistance of public health concern resulting from the addition of a new target species to the premix formulation was deemed acceptable.

User safety

The applicant referred to the assessment provided previously for the same product intended for use in pigs. It is considered that this is a satisfactory approach, considering the shorter duration of treatment for chickens in comparison to pigs (consecutive dosing for 7 days rather than 10 days), meaning there would be a lower potential for exposure of the person administering the product to the product. The user safety warnings approved for the authorised product were considered to be suitable for use on the product under consideration.

Environmental risk assessment

Reference was made to the environmental risk assessment (ERA) submitted in support of the approved extension for Aivlosin 625 mg/g granules for use in drinking water for chickens. For this product, a phase II ERA assessment compliant with the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guidelines was carried out with regard to use in chickens based on a dose rate of 30 mg/kg bodyweight (bw) for 5 consecutive days (i.e. an overall dose of 150 mg/kg bw). It was concluded that the risk to the environment from the use of the product in chickens was acceptable when used as recommended. No risk mitigation measures were considered necessary. No specific warnings to the environment are currently included for the authorised product with the same formulation. In line with this, no additional warnings were proposed for this new presentation either.

Overall conclusions on the safety documentation

No data were provided in part 3 of the dossier, instead reference was made to safety data provided and assessed for previous applications for the use of Aivlosin in a different formulation for chickens, or in the same formulation for a different target species (pigs).

The product was not expected to pose an unacceptable risk for the user or environment when used as recommended.

The risk for development of antimicrobial resistance of public health concern was deemed acceptable.

Residues documentation

Depletion of residues

A new residue depletion study was conducted in accordance with current guidance. Six broiler chickens per time point were administered the product (premix for medicated feeding stuff) according to the proposed SPC, with a dose rate varying widely (11.5–21.2 mg/kg bw for 7 consecutive days), but mostly attaining a dose higher than that proposed (12.75 mg tylvalosin/kg bw/day in feed for 7 consecutive days), leading to sufficient confidence in the results of the study. The birds were slaughtered at the following time points after completion of their medicated feed: 3, 12, 24, 48 and 72 hours.

At 3 hours, residues above the maximum residue limit (MRL) were found in liver only. At 12 hours, all residues were below the MRLs in each tissue analysed (kidney, liver, muscle and 'skin and fat in natural proportions'). The samples were analysed using a validated analytical method, with concurrent quality control (QC) samples demonstrating suitable accuracy and precision of the method. The applicant has confirmed that the product used in the study is the product under consideration for this application.

Maximum residue limits (MRLs)

The active substance in Aivlosin is an allowed substance as described in Table 1 of the annex to Commission Regulation (EU) No. 37/2010:

Pharmacologically active substance	Marker residue	Animal species	MRL	Target tissues	Other provisions	Therapeutic classification
Tylvalosin	Sum of tylvalosin and 3-O-acetyltylosin	Poultry	50 µg/kg 50 µg/kg	Skin and fat Liver	Not for use in animals from which eggs are produced for human consumption	Anti-infectious agents/ Antibiotics

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

Withdrawal periods

The applicant proposed a zero-day meat withdrawal period for this product. Residues of the active substance were seen to be above the MRL in liver at three hours after the final treatment was finished, but were consistently well below the MRL at all time points after that, including at 12 hours post treatment. It should be noted that birds should not be taken for slaughter during treatment, since data have not been provided for that scenario; the data that have been provided indicate that this would not be appropriate. The applicant attempted to justify the use of the 'practical zero' withdrawal period in line with relevant guidance; however, the CVMP considered that a one-day withdrawal period for meat and offal would be most appropriate for this product, due to the continuous administration of the product and the fact that residues above the MRLs were found three hours after treatment was withdrawn.

In the absence of MRLs for eggs, the product cannot be authorised for use in birds producing eggs for human consumption and should not be used in these hens within 14 days of the onset of lay.

Overall conclusions on the residues documentation

While all residues were below their respective MRLs in all tissues by 12 hours, considering that residues above the MRL in liver were found at three hours after completion of the treatment, the CVMP concluded that a one-day withdrawal period would be appropriate. In the absence of MRLs for eggs, the product cannot be authorised for use in birds producing eggs for human consumption.

Part 4 – Efficacy

Pharmacodynamics

The pharmacology of tylvalosin in chickens has been covered in detail in previous applications. The active substance in Aivlosin is tylvalosin, a macrolide antibiotic that is primarily active against Gram-positive bacteria and Mycoplasma species. Macrolides interfere with protein synthesis by reversibly binding to the 50S ribosome subunit, thus inhibiting protein synthesis.

Tylvalosin can be described as demonstrating both concentration and time dependent killing although the pharmacokinetics/pharmacodynamics (PK/PD) relationship cannot be fully explored and it is generally agreed that the dosing regimen can only be investigated in clinical studies. Studies reviewed in previous Aivlosin applications have shown that tylvalosin rapidly enters (often within 10 minutes) and is concentrated within cells such as gut epithelial cells and white blood cells.

Tylvalosin has been shown to be present in relatively high concentrations in respiratory tissue, even when the concentration in plasma is low. Macrolides have an effect on the innate immune system. Tylvalosin has been shown to have beneficial effects on monocytes and macrophages although the clinical relevance of this finding remains uncertain.

Previous applications for Aivlosin include reports of activity of tylvalosin against *M. gallisepticum*. Two minimum inhibitory concentration (MIC) studies were conducted within the last 2 years against a relatively small number of isolates of *M. gallisepticum* (37 from the UK and the US). These show that the MIC range for tylvalosin for these isolates is 0.015–0.25 µg/ml. It should be noted that the UK isolates (n=15) were collected within the last 5 years, but the US strains were collected from 8 to 18 years ago. There is also no indication whether these strains were isolated from clinical cases or during surveillance. In the US isolates, some resistance was detected to the related substances, erythromycin and lincomycin.

As diagnosis generally involves serological or molecular methods, cultures are not readily available for susceptibility testing thus contributing to the difficulties in sourcing viable *M. gallisepticum* cultures. Therefore, MIC data has only been generated for relatively few strains of *M. gallisepticum*.

Development of resistance (target pathogens)

Although the reported MICs represent a decrease in susceptibility compared to data generated in previous years, no evidence of clinical resistance in *M. gallisepticum* has been detected, even though Aivlosin has been authorised for use in chickens since 2008. Breakpoints have not been set for veterinary mycoplasmas against any of the macrolides so it is not possible to be definitive concerning clinical resistance levels. It appears that the recognised breakpoint of > 2.0 µg/ml has been rather arbitrarily chosen rather than scientifically determined. Using this breakpoint, all isolates remain susceptible to tylvalosin (MIC ≤ 0.25). The MIC distribution can currently be considered as bimodal with one distribution 0.001–0.008 µg/ml and one distribution 0.06–0.25 µg/ml.

There are generally considered to be three mechanisms of resistance to the macrolide class of compounds; this is often referred to as MLS resistance as it affects macrolides, lincosamide and streptogramins. The mechanisms involve (i) alteration of the ribosomal target site, (ii) utilisation of active efflux mechanism and (iii) production of inactivating enzymes.

In US *M. gallisepticum* isolates from 1995, it is noted that there is some *in vitro* resistance detected to erythromycin and decreasing susceptibility to lincomycin, but similar MIC values are achieved with the related active substance, tylosin.

Pharmacokinetics

Cross reference was made to pharmacokinetic data presented for a previous chicken presentation for Aivlosin.

After a single administration of an oral solution of Aivlosin to chickens (30 mg/kg), the peak mean serum concentrations of tylvalosin and its major metabolite 3-acetyltylosin (3AT) were observed 1 hour post administration. At 8 hours post administration, the mean concentration of tylvalosin was below the level of quantification, and at 12 hours post administration the mean concentration of 3AT was below the level of quantification. Rapid and wide distribution to tissues was observed. Highest concentrations were found in the liver and kidneys and respiratory tissues contained reasonably high concentrations of tylvalosin and 3AT following oral administration to chickens. *In vitro* plasma protein binding of tylvalosin in the broiler chicken was in the range 60–70%. Tylvalosin is rapidly eliminated with an elimination half-life of approximately 1 hour. Excretion is rapid with about 90% of the administered dose found in excreta after 72 hours.

Target animal tolerance

A target species tolerance study was conducted for the application for Aivlosin granules for use in drinking water for chickens. This was assessed in the previous application. This good laboratory practice (GLP) study was conducted in the UK during 2005. The results demonstrated that the administration of concentrations of Aivlosin at target doses of 30, 90 and 150 mg tylvalosin/kg bw and 30 mg/kg for 15 days (5x dose duration) had no detrimental effect on food consumption, water consumption, rate of increase in bodyweight and the final bodyweight. The final authorised dose regimen was 25 mg tylvalosin/kg bw for 3 days. The treatments had some effect on the haematology and biochemical results; however the values remained within the stated reference ranges and the changes were not reflected in the clinical examination of the chickens. There were no significant necropsy or histological changes noted after treatment with different dose rates of the product. Therefore, Aivlosin granules for use in drinking water for chickens was considered safe for 21-day-old chickens when administered at the proposed dose regimen and was shown to have a wide safety margin.

A new target animal safety study was conducted for this application using the final formulation. The GLP study was conducted in 2011 in line with VICH guideline GL43 (target animal safety). Dose rates of approximately 17 mg/kg bw, 47 mg/kg bw and 88 mg/kg bw (1.33x, 4x and 6.67x the proposed dose rate) administered for 21 days (3x the proposed dose duration), were tested in 4 groups (including a negative control group) of 10 chickens, 21-day-old.

The majority of adverse events observed were loose faeces. This occurred in a small number of birds which included those in the control group and it had no detrimental effect on the birds. It was therefore concluded that the diarrhoea was not related to administration of the product. Increasing inclusion rate had no impact on food consumption, water consumption or bodyweights. No significant differences were detected between treatment groups with regards haematology. Two clinical parameters, glucose and uric acid, were statistically significantly different in treated birds compared to the control group. However, levels remained within the overall control range and/or pre-treatment ranges and were within expected biological variability.

A slight increase in heart weights and decrease in spleen weights in the highest dose group were not associated with gross necropsy or histology findings. There were no histopathological findings attributable to test product administration.

It was concluded that the administration of Aivlosin premix at up to 88 mg tylvalosin/kg bw (6.67x the proposed treatment dose) for 21 days (3x the proposed treatment duration) is well tolerated in healthy chickens. Sufficient warnings are included in the proposed SPC regarding target species tolerance.

Dose justification

Aivlosin is currently authorised as granules for use in drinking water for chickens for treatment and prevention of respiratory disease associated with *M. gallisepticum* at a dose of 25 mg tylvalosin/kg bw/day for 3 consecutive days. The current application (for the same indication) is proposed to be administered at a lower dose over a longer time period, i.e. 12.75 mg tylvalosin/kg bw/day for 7 consecutive days. This lower dose is likely to result in reduced peak plasma levels; however, the applicant considered that tylvalosin concentrates in respiratory tissues, and that the total dose taken in by the birds with the premix is greater than that with the oral granules.

The increase of the treatment duration was chosen by the applicant, as the desired level of tylvalosin in respiratory tissues is not achieved as quickly as with water medication.

The CVMP acknowledged that the PK/PD relationship for mycoplasma/macrolides is complex. However, neither the justification to reduce the dose, nor the explanation for the longer treatment duration was considered convincing, taking into account that no rationale was given as to why similarity of the total dose given over the treatment period would be a relevant factor for dose determination. Also, in studies submitted previously for the in-water formulation, dose rates of 25 mg/kg bw and 35 mg/kg bw for 3 days were found to be more effective than lower doses, even when treatment was initiated when a smaller percentage of birds showed clinical signs at the start of the study. In addition, concerns were expressed that sub-optimal dosing, taking into account the variability in intake of a premix particularly by sick birds, might not only result in reduced efficacy but also increase the risk for the development of resistance.

Dose determination/confirmation

In support of the proposed dose regimen, two new controlled studies were provided: a dose determination study using three different dose levels over 7 days, and a dose confirmation study (127.5 mg tylvalosin/kg feed over 7 days). Both studies were performed in the USA in 2011 and 2012 under the same environmental conditions (high-altitude combined with low oxygen tension), and were well conducted (randomised, negative-controlled, blinded, good clinical practice (GCP) compliant).

Dose determination:

A total of 256 chickens were medicated with either 0, 85, 127.5 or 170 mg tylvalosin/kg feed (inclusion rate) for 7 days (64 birds/group). This corresponded to actual doses of 0, 7.36, 10.85 and 14.93 mg tylvalosin/kg bw. Medication was initiated when 18% of the chicks showed clinical signs of respiratory disease, 5 days after a virulent challenge infection of *M. gallisepticum* (MIC ≤0.06 µg/ml) was performed (21 days old).

The primary variables were combined airway lesion score at necropsy on study day 42 (9 days after treatment end) and clinical observation scores during treatment to end of study plus mortality. Both scores were statistically significantly improved when treated groups were compared to the negative control group.

For airway lesion score, there was no statistically significant difference in the improvement between the three dose groups, and the improvement compared to the control group was considered to be relatively modest.

The 'combined clinical observation score' (0 to 6) was a sum of whether a total of six signs were present or not. The pen mean number of combined clinical signs was low (less than 1) in all groups including the negative control. There was a statistically significant difference between treated and untreated groups but the clinical relevance of the small improvement of around 0.3–0.4 in the score between treated and control groups was considered modest. A statistically significant difference was seen between the clinical scores for the 7.36 mg/kg and 14.9 mg/kg dose groups.

M. gallisepticum-related mortality was 10.9% (7/64), 1.6% (1/64), 4.7% (3/64) and 0% (0/64) in the control, 7.36, 10.85 and 14.93 mg/kg groups, respectively.

Regarding secondary variables, there were statistically significant improvements in the treated groups when compared to the untreated group in some of the parameters (combined gross lesions scores on day 34, clinical signs during treatment, per cent mortality, *M. gallisepticum* serology results, daily weight gain and feed consumption). The higher dose rate groups, 127.5 and 170 mg/kg feed, also showed a statistically significant improvement in quantitative polymerase chain reaction (PCR) results. There was no statistically significant reduction in *M. gallisepticum* culture results in the treated groups compared to the control group.

The CVMP noted that the applicant's statistical criteria were met; however, the clinical relevance of the treatment benefit appeared to be modest to moderate for all doses. Although a statistically significant difference was shown only between the 7.36 and 14.93 mg/kg dose groups for clinical observations, a trend towards a positive dose response effect was seen for an improvement in necropsy airway lesion scores, clinical observations and *M. gallisepticum*-related mortality across doses of 7.36, 10.85 and 14.93 mg/kg for 7 days. In addition, there was no clear plateau effect between the two higher doses when compared to the lowest dose. Therefore, it is not clear if the dose was sufficiently investigated. It is also noted that the applicant did not compare the efficacy of the approved in-water medication (25 mg/kg x 3 days) to the proposed dose regimen for the premix, and therefore no conclusions on similarity in efficacy between the in-water and in-feed medication can be drawn.

Dose confirmation

The dose rate of 127.5 mg tylvalosin/kg feed (inclusion rate) was tested in 144 artificially challenged birds (with the same virulent strain of *M. gallisepticum* as in the previous study) against a challenged untreated group (n=144) and an unchallenged untreated group (n=24). Medication was initiated when 19% of birds showed clinical signs of *M. gallisepticum* infection (Day 26), 5 days after the challenge. Euthanasia occurred either the day after treatment end, on Day 33 (n=108) or 8 days later, on Day 42 (n=180). The observations and measurements taken during this study were the same as those taken in the previous study. The actual inclusion rates were lower than the target inclusion rates (approximately 97%). This was equivalent to a dose rate of 11.4 mg tylvalosin/kg bw.

A statistically significant benefit in the treatment group when compared to the control groups was shown for gross airway lesions (p=0.0062) and clinical observation scores (p=0.0001). *M. gallisepticum*-related mortality in treated birds (0.7%, 1/144) was lower than in the control group (4.3%, 6/144) but this was not statistically significant (p=0.066). The applicant concluded that the inclusion rate of 127.5 mg/kg feed (equivalent to a dose rate of 11.40 mg/kg bw) for 7 days is efficacious against the experimental *M. gallisepticum* infection. However, (as for the first study), the magnitude of the treatment benefits appeared to be modest in clinical terms. It was noted that in both the dose determination and dose confirmation studies, the mortality rate for an uncomplicated *M. gallisepticum* infection was higher than might be expected under usual European conditions. It was considered that the outcomes of the *M. gallisepticum* infection, especially in regards to mortality, were influenced by pathological effects brought on by the altitude at which the studies were conducted in the US (above 1,500 m) compounded by the fact that quick growing birds were used in the trials.

Field trials

The applicant considered that a conclusive outcome had been demonstrated in the clinical challenge studies conducted in the US, and did not provide any further field trials. The applicant justified the absence of field data further by stating that the MIC of the challenge strain used in the US trials was representative of the wild type population of *M. gallisepticum*. Also, as *M. gallisepticum* is usually part of a multifactorial disease, it might be difficult to find trial sites where infections are free of complications from other respiratory bacteria and viruses.

However, in the absence of field studies, it is important that there is sufficient confidence that the outcomes of the challenge studies can be seen to reflect adequately what might happen under field conditions. The dose determination and dose confirmation studies were conducted using the same single *M. gallisepticum* challenge strain, and under very similar controlled conditions at the same site. Issues such as possible delay between diagnosis and administration of the full antimicrobial dose via feeders, differences between breeds of birds, husbandry conditions, etc. that relate to field "effectiveness" are not taken into account in these types of studies. In addition, the studies were conducted in the US at a high altitude, which impacted on the study outcomes. The CVMP considered that it is difficult to extrapolate from the limited and somewhat unusual circumstances of these 2 studies to typical conditions of a European field outbreak of *M. gallisepticum*. The CVMP also noted that the applicant was able to provide two European field trials in support of the same indication (treatment of *M. gallisepticum* in chickens) in the application for the water soluble granules.

The CVMP considered that *M. gallisepticum* is a relatively common contributor to chronic respiratory disease in chickens where control programmes are not in place, and that there should not be an exemption from the need to provide either European field studies, or other sufficiently representative clinical data to support effectiveness under EU conditions, for an application concerning an important disease in a major species. Such data are therefore needed to support the effectiveness of the proposed dosage under EU conditions to treat the types of outbreaks of mycoplasma for which a premix for medicated feeding stuff formulation would be used.

It is also noted that the proposed dosing regimen for the premix differs considerably from that of the approved in-water formulation of Aivlosin, with no rationale presented. As no direct comparison of the efficacy of the two formulations and their dosing regimens has been made, extrapolation cannot be made from the field data obtained for the water soluble granules to the premix.

Overall conclusion on efficacy

The mode of action of tylvalosin and the pharmacokinetics of the product as the soluble granules, but not the premix, in chickens have been well documented in previous applications. No new kinetic data are provided for the lower dose proposed in this application but it can be assumed that the bioavailability is similar between the in-water and in-feed formulations under field conditions. As the relative pharmacokinetic characteristics of premix and water soluble formulations were not submitted as part of the present submission, and also considering that for macrolide-mycoplasma combination there is no established PK/PD rationale, the effective dose of tylvalosin should be based on clinical and other relevant criteria.

Clinical resistance in *M. gallisepticum* to tylvalosin currently appears to be low although it remains uncertain what efficacy level could be achieved in case of strains with reduced susceptibility. Although the reported MICs represent a decrease in susceptibility compared to data generated in previous years, no evidence of clinical resistance in *M. gallisepticum* has been detected, even though Aivlosin has been authorised for use in chickens since 2004.

The applicant has conducted a target animal safety study with the premix formulation and this showed that the product was well tolerated when administered to chicken at up to 88 mg tylvalosin/kg bw (i.e. 6.67x the treatment dose) for 21 days (i.e. 3x the treatment duration).

The applicant investigated the dose in a study conducted in the US using a clinical challenge model. A trend towards a positive dose-response effect was seen for improvement in necropsy airway lesion scores, clinical observation scores and *M. gallisepticum*-related mortality across doses of 7.36, 10.85 and 14.93 mg tylvalosin/kg bw over 7 days. However, the treatment benefit appeared to be modest and there was no plateau in dose-response between the two highest doses. Furthermore, the approved dose for the water formulation (25 mg tylvalosin/kg bw) was never investigated in this study. The applicant took forward a dose of 12.75 mg tylvalosin/kg bw to investigate in a dose confirmation study using the same challenge model. Taking into account the score ranges for necropsy airway lesions and clinical observations, the treatment benefit demonstrated, although statistically significant, appears to be modest.

The outcomes of both the dose determination and dose confirmation studies were complicated by the fact that the studies were conducted in the US at high altitude, resulting in a higher level of mortality than might be expected with an uncomplicated *M. gallisepticum* infection. This hinders extrapolation to European field conditions.

In conclusion, further evidence would be necessary to be provided to support the selected dose regimen, and in addition European field trial data or other clinical data representative of EU conditions would be needed to support effectiveness of the premix formulation to treat the types of outbreaks of mycoplasma for which an in-feed formulation would be used.

Part 5 – Benefit-risk assessment

Introduction

This is an extension application for Aivlosin to add a new pharmaceutical form (premix for medicated feeding stuff) for chickens, for the indication 'for the treatment of respiratory disease associated with *Mycoplasma gallisepticum'*. The proposed dose rate is 12.75 mg/kg bw for 7 days. Aivlosin granules for use in drinking water was authorised in 2008 for the same indication in chickens but with a dose rate of 25 mg/kg bw for 3 days.

Benefit assessment

Direct therapeutic benefit

Recent *M. gallisepticum* isolates indicate a possible decrease in susceptibility compared to earlier data; but no evidence of clinical resistance in *M. gallisepticum* has been detected, even though Aivlosin has been authorised for use in chickens since 2004. However, due to lack of clinical breakpoints firm conclusions with regard to strains with the lowest susceptibility could not be drawn.

Artificial infection models were used to determine and confirm the dose rate for the proposed premix formulation of 12.75 mg tylvalosin/kg bw/day for 7 consecutive days in the proposed target species, chickens. A statistically significant benefit was seen in terms of airway lesions, clinical observation scores and numerical improvement in mortality for treated groups compared to the negative control group. However, the extent of the demonstrated clinical benefit of treatment was modest to moderate, and uncertainty remained as to whether the selected dose in the dose determination study was sufficiently high as there was a trend towards a better response with a higher dose of 17 mg/kg bw/day and as the approved dose for in-water medication is 25 mg tylvalosin/kg bw/day. Although it is difficult to eliminate mycoplasma

infections with antimicrobial medication, an inadequate dosing regimen may increase the risk of relapse and the development of antimicrobial resistance.

The outcomes of both the dose determination and dose confirmation studies were complicated by the fact that the studies were conducted in the US at high altitude, resulting in a higher level of mortality than might be expected with an uncomplicated *M. gallisepticum infection*. This hinders extrapolation to European field conditions and field trial data or other clinical data representative of EU conditions are therefore essential to support the effectiveness of the proposed dosage.

Therefore, benefits remain inconclusive as the dose has not been confirmed.

Additional benefits

Aivlosin premix is proposed for administration via the feed to chickens which provides an effective means of compliance by medicating large numbers of birds without additional stress placed on birds from handling.

Risk assessment

The main risks were identified as follows:

Quality:

The shelf life after incorporation into pelleted feed is one week, and there is a risk that users will ignore this shelf life, as it is somewhat impractical. However, this risk can be mitigated by appropriate labelling.

For the target animal:

The target animal study supported that Aivlosin premix is well tolerated in healthy chickens at dose rates up to 6.67 times the proposed treatment dose for 21 days (i.e. 3x the proposed treatment duration), therefore it has a wide safety margin. As the dose has not been confirmed, a risk of major concern remains for the safe use of the product.

For the user:

User safety has been addressed in a previous application and several risk mitigation measures have been approved for the product information; these are also considered adequate for this presentation based on the proposed dose and treatment duration.

For the environment:

Environmental safety has been addressed in a previous application and no specific new risk mitigation measures were deemed necessary. The changes introduced by this application which were assessed based on the proposed dose and treatment duration will not change the risk to the environment and therefore no specific mitigation measures are required.

For the consumer:

A proposed meat withdrawal period of one day is considered appropriate. There is no MRL established for eggs; therefore the product could not be authorised for use in birds producing eggs for human consumption.

Specific potential risks:

Based on the data presented previously, there are mechanisms for resistance to tylvalosin and since macrolides are used in humans, there is a potential for the development of resistance in zoonotic foodborne bacteria (*Campylobacter, Enterococcus* spp.), macrolides are critically important antimicrobials used in human medicine. However, the additional risk for development of antimicrobial resistance of public health concern resulting from the use of the premix formulation in chickens is deemed to be low and acceptable.

Risk management or mitigation measures

Warnings and other risk management measures have been proposed for the SPC in order to address possible risks to the user, target animal, other animal species and the environment. However, on the basis of the data available it is not possible to conclude on the appropriate dose and instructions for correct use, and on risk mitigation measures possibly associated with the use of the product.

Evaluation of the benefit-risk balance

Considering that on the basis of the data available no conclusions can be drawn on the dose and therefore the treatment benefits of the product, no conclusions can currently be made on the benefit-risk balance of the application.

Conclusions

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP considered that the extension application concerning Aivlosin 42.5 mg premix to add a new food-producing species chickens for "treatment of respiratory disease associated with *Mycoplasma gallisepticum*" is not approvable at the present time since major objections remain which preclude a recommendation for marketing authorisation.