Divergent position on a CVMP opinion on an Article 13(2) of Commission Regulation (EC) No 1234/2008

Resflor solution injectable (EMEA/V/A/101)

On 24 January 2014, France presented to the European Medicines Agency a referral notification for Resflor solution injectable under Article 13 of Commission Regulation (EC) No 1234/2008. The Committee for Medicinal Products for Veterinary Use (CVMP) was requested to give its opinion as to whether *Mycoplasma bovis* can be included as a target pathogen for the treatment of bovine respiratory disease (BRD) for Resflor solution injectable and associated names. The recommended dosing regimen is a single subcutaneous injection of 40 mg florfenicol and 2.2 mg flunixin per kg body weight (2 ml/15 kg bw).

It is the opinion of the undersigned that the indication '*treatment of bovine respiratory disease associated with Mycoplasma bovis*' cannot be accepted.

There are major concerns that the product at the currently authorised single dose of 40 mg florfenicol per kg body weight will not have adequate efficacy for the treatment of bovine respiratory disease associated with *M. bovis*. In particular, the duration of the treatment effect of Resflor used as a one-shot treatment regimen is considered inadequate for treatment of *M. bovis*-associated BRD.

In addition, there is a lack of standard minimum inhibitory concentration (MIC) methodology for *M. bovis* (therefore, the 'true' MIC_{90} cannot be determined with certainty), lack of Clinical and Laboratory Standards Institute (CLSI)-approved MIC breakpoint values for *Mycoplasma spp*., and lack of well-established pharmacokinetic/pharmacodynamic (PK/PD) relationships concerning antimicrobials against *Mycoplasma spp*. Therefore, clinical studies were considered pivotal for this application.

The marketing authorisation holder has submitted two experimental challenge studies in calves conducted in 2008 and 2012, and two clinical field studies on conventional bovine respiratory infections conducted in 2004 and 2005 for which retrospective analyses were performed on the subsets of animals that cultured positive for *M. bovis* (only based on nasopharyngeal swabs) at the time of enrolment.

The results of these studies are not conclusive:

- In an experimental challenge study in 2008, Resflor was compared to a florfenicol monoproduct and to placebo over a period of 10 days. Resflor proved to be superior to placebo in terms of clinical success rate and superior to the florfenicol monoproduct in terms of reduction of pyrexia within the first 9 hours after injection, the latter indicating a considerable clinical effect of flunixin. However, antimicrobial activity in terms of reduction of *M. bovis* in the lungs could not be demonstrated in any of the treatment groups. Besides, the florfenicol monoproduct was used for the positive control, which is not authorised for BRD caused by *M. bovis* in the EU.
- In an experimental challenge study in 2012, Resflor proved to be superior to placebo and non-inferior to tulathromycin, administered together with flunixin, in terms of clinical success; however, the contribution of flunixin to the overall clinical outcome remains unknown, due to inappropriate control methods in the study design. *M. bovis* was statistically significantly reduced in the lungs of calves treated with Resflor (by two orders of magnitude on average compared to saline), suggesting antimicrobial activity to some extent. However the observation period (7 days) is considered too short and does not allow to conclude on a definite and lasting effect on *M. bovis* load in the lungs, since such an effect was not observed in the 2008 study after a longer observation period of 10-days.

- The marketing authorisation holder states that some of the typical aspects of the antimicrobial effect assessment do not apply to *Mycoplasma spp*, namely MICs (esp. lack of CLSI standards), PK/PD modelling and Mycoplasma colony forming units (cfu) counts after treatment. This limits the parameters for which non-inferiority can be assessed for the antimicrobial effect of florfenicol against *M. bovis*. In the 2008 experimental trial these remaining parameters for the clinical assessment included the primary endpoint (lung lesions), and secondary endpoints (demeanour scores, rectal temperature, respiratory scores, mortality). In the 2012 experimental trial the primary endpoint was replaced by a cumulative clinical success rate percentage and lung lesions were stated as a secondary endpoint along with additional criteria (rectal temperature, respiration and demeanour scores, changes in body weight). However, there was a further complication in both these experimental trials, in that both an antimicrobial agent and a non-steroidal anti-inflammatory drug (NSAID) flunixin were given to some groups of treated calves (i.e. florfenicol+flunixin (Resflor) and tulathromycin+flunixin groups), whereby the flunixin exerted a strong confounding statistical effect on some of the clinical parameters in these non-inferiority trials. Since a 'flunixin-only' group was not included in the study designs, then only a close inspection of the 2008 experimental trial comparing a florfenicol+flunixin-group (Resflor) to a saline and a florfenicol monoproduct group, can assist to understand which clinical parameter/s could be used to provide a more unbiased clinical assessment of the antimicrobial effect against *M. bovis*. For example, rectal temperature, and demeanours scores were significantly different between florfenicol monoproduct and Resflor groups suggesting a confounding influence from flunixin. Lung lesion scores, respiratory scores and mortality were not significantly different between florfenicol monoproduct and Resflor groups suggesting a more unbiased basis for the clinical assessment of the antimicrobial effect of florfenicol against *M. bovis*. Since cumulative success rate percentage and body weight parameters were not included in the 2008 study then it is unknown if these parameters are an unbiased basis for the clinical assessment of the antimicrobial effect of florfenicol against *M. bovis*. The key study for this type II variation is the 2012 experimental trial comparing three groups of calves, saline, Resflor, and tulathromycin+flunixin. Of the more unbiased clinical parameters identified from the 2008 experimental trial, namely lung lesion scores, respiratory scores and mortality, problems could be identified in the results of the 2012 experimental trial with lung lesions scores and mortality. Contrary to the 2008 study where mortality was a secondary endpoint, mortality was no defined assessment parameter in the 2012 study although calves that died or were euthanized due to BRD were analysed as failure. Lung lesions scores could not be used in the 2012 study because: "Due to the lack of statistical power of the non-inferiority test on this parameter, statistical comparison between treatment groups would not be meaningful and statistical conclusions could not be drawn". Respiratory scores could be utilized and no significant difference was found between treatment groups. However, the undersigned do not accept respiratory scores as the sole unbiased clinical assessment of non-inferiority because it is an imprecise parameter that is not correlated typically with clinical or bacteriological cures.
- Independent from the above, both experimental challenge studies are not representative of the field study, since acute infections with high initial mortality in saline groups were induced which is not typical under field conditions. In the field, a peak in morbidity and mortality associated with *M. bovis* is observed normally between 10 and 15 days after exposure, as well as *M. bovis* typically associated with mixed infections in BRD. In addition, the susceptibility of the challenge strain was high (MIC 1-2 µg/ml) and may not be representative for the current susceptibility distribution profile of *M. bovis* field strains.
- The re-analyses of the clinical field studies, performed ten years ago in cattle with conventional respiratory infections are not acceptable. *M. bovis* field strains with lower susceptibility have to be anticipated to date. The calculation of clinical success rates based on the animals cultured positive

for *M. bovis* at the time of enrolment is not meaningful as it considers selective results, only. Post-treatment bacteriological investigations were conducted only in case of clinical failures but not in case of clinical successes. Thus, a reliable relation between antimicrobial activity against *M. bovis* and a clinical effect could not be established. In the 2004 field study a florfenicol monoproduct was used as the control comparison, which is not authorised for BRD caused by *M. bovis* in the EU. Furthermore, all *M. bovis* cattle were identified using nasopharyngeal swabs and it is unclear if this has any relationship to lung infections or treatment success.

Results from clinical studies indicate that the single dose regime of Resflor is not suitable to achieve an efficacious treatment with regard to a lasting cure in animals suffering from BRD associated with *M. bovis*. Considering also that florfenicol is a time-dependent, bacteriostatic antibiotic, the treatment dose of Resflor and the treatment frequency may not be adequate to reach sufficiently high and long lasting levels at the target sites in calves.

This risk of underdosing is associated with a risk to animal welfare due to lack of efficacy and a risk of bacterial resistance development. Mycoplasma exposed to insufficient florfenicol levels may develop increased MICs or even become resistant. As no eradication of *M. bovis* is achieved after treatment with Resflor less susceptible/resistant Mycoplasma may persist in lungs from treated calves and could act as a reservoir of new or re-infections. It is noted that the risk of resistance development could even increase after approval of Resflor for *M. bovis*-related BRD, because this indication is expected to increase its use beyond the current extent. This assumption is supported by the marketing authorisation holder who emphasised that one of the benefits of accepting this type II variation for Resflor against *M. bovis* is that it represents a viable alternative than using critically important antibiotics for *M. bovis* associated BRD. However, this does not provide the full picture of the scenario of increased usage of florfenicol products for BRD in cattle. Resistance to florfenicol is associated with acquisition of the *floR* gene in bacteria, which has been identified in cattle bacterial isolates[1]. Bacteria will be exposed to florfenicol for treatment of *M. bovis* infections. However, current scientific observations have noted that the acquisition of the *floR* gene is always associated with multi-resistance in bacteria, due to its presence on mobile genetic elements.

Due to difficulties in isolation/cultivation techniques, lack of standardized MIC determination methods and lack of clinical breakpoints the common SPC special precaution for use in antimicrobials to perform susceptibility testing of target pathogens prior to treatment is impracticable in the field. Thus, for *M. bovis* associated BRD no specific risk mitigation measures beyond general “prudent use” measures can be proposed.

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[1] White et al., Characterization of Chloramphenicol and Florfenicol Resistance in *Escherichia coli* Associated with Bovine Diarrhea. J Clin Microbiol. 2000; 38(12): 4593–4598.