Report Title: Antimicrobial Resistance (AMR) Risks Associated with Using Recycled Manure Solids (RMS) as Animal Bedding

Executive Summary

The adoption of recycled manure solids (RMS) as a bedding material for dairy cows in the UK has resulted in concerns being raised regarding the influence that such use might have on antimicrobial resistance (AMR) in micro-organisms on farm. The effect of maintaining faecal material in a "closed loop" within the cattle housing environment rather than disposing of it on land, on the development of antimicrobial resistance is unknown.

Baseline data on AMR was collected from 122 farms in Great Britain as part of a study of the risks and benefits of RMS bedding funded by Welsh Government. This study included 40 RMS farms, 41 farms bedding dairy cows on sand and 41 bedding on sawdust. Samples of used bedding and bulk tank milk collected during winter 2014/15 were analysed. Coliforms and/or *Enterococcus* spp isolated from bedding and milk were tested for resistance to a total of 22 antimicrobials using a VITEK® 2 (Biomerieux; Basingstoke UK) following the recommended protocols. This enabled direct determination of mean inhibitory concentrations (MIC) for the antibiotics selected. All farms were contacted again in 2016. Equivalent samples were obtained during winter 2016 - spring 2017, from 113 of the farms (36 RMS, 39 sand, 38 sawdust). Management information was collected using a questionnaire. Determination of MICs of coliforms and *Enterococcus* spp isolated from bedding and milk was repeated in the same manner as the 2014/15 study.

For the purposes of analysis, at each time point, MICs of each antimicrobial for each organism were ranked and differences analysed using a non-parametric approach. In addition, for each isolate, the mean MIC rank across all the antimicrobials tested was calculated, to give an overall measure of antimicrobial resistance. The change in mean rank between 2014/15 and 2016/17, on a pathogen specific basis, within farm, was then calculated, and this change was used as the outcome variable for a multivariable analysis, to determine the overall influence of bedding type on the change in MIC over time, whilst controlling for other factors. For example, the mean MIC rank for all *E. coli* isolates on an individual farm was calculated in 2014/15 and 2016/17; these mean ranks were then compared to calculate any change in mean MIC, thereby encompassing an 'overall' effect on AMR rather than considering each antimicrobial separately, which would have led to a large number of comparisons and risked the introduction of a chance finding.

Once other (management) factors were accounted for, the multivariable analysis indicated that there were differences in the coliform MICs for chloramphenicol and piperacillin that were associated with the type of bedding used. MICs for chloramphenicol were significantly lower for coliforms recovered from farms bedded on both sand and sawdust compared to RMS. However, MICs for piperacillin were significantly higher for coliforms recovered from farms bedded on sawdust compared to RMS.

For *Enterococcus* spp, multivariable analysis identified that there were differences in the MICs for cefovecin, erythromycin, enrofloxacin, marbofloxacin and benzylpenicillin. MICs for cefovecin, enrofloxacin, marbofloxacin and benzylpenicillin were significantly higher for *Enterococcus* spp recovered from farms bedded on sawdust compared to RMS. MICs for erythromycin were significantly higher for *Enterococcus* spp recovered from farms bedded on sand compared to RMS.

When considering the change in mean MIC rank, within pathogen species, within farm, between 2014/15 and 2016/17 the mean rank showed a small decrease across all sawdust farms combined (-0.08); the mean rank also fell on RMS farms (-0.03; ie -0.08 + 0.05), though this change was not significantly different from that observed on sawdust farms ($p>0.05$). The mean MIC rank of sand farms showed an increase (+0.09; ie -0.08 + 0.17) over this same period, which was significantly different from the change seen on the sawdust farms.

These study findings do not support the hypothesis that recycling manure as bedding for dairy cows, for at least two years, as currently practised in the UK, increases the overall risk of antimicrobial resistance in coliforms or enterococci, isolated from bedding material or milk, as compared with bedding cows on sand or sawdust. Whilst it is not possible, in a study of this size, to robustly refute any impact of bedding type on individual antimicrobial resistance patterns, the data suggests that if risks are present they are not consistently associated with any one type of bedding. Between 2014/15 and 2016/7, the MICs for some antimicrobials shifted higher, and some shifted lower. The shifts were sometimes consistent between bedding materials, but not for all antimicrobials, particularly for the enterococci. As demonstrated by the final model, the change in mean rank of MIC across all antimicrobials for RMS farms was intermediate between the changes for sawdust farms (which showed a small decrease) and sand farms (which showed a small increase).

DISCLAIMER

This report is the output of a study, the aim of which was to extend knowledge of the Antimicrobial Resistance (AMR) risks associated with using Recycled Manure Solids (RMS) as animal bedding. This study is inevitably constrained by the relatively short duration of RMS use in the UK, so can only provide information on the risks associated with use of RMS over an average period of 45 months. All users of RMS have to accept responsibility for their own decisions with respect to its use. The authors of this report cannot be held responsible for decisions made on the basis of the information contained herein.