Comment on: Effect of a 5 day enrofloxacin treatment on Salmonella enterica serotype Typhimurium DT104 in the pig

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Sir,

We would like to comment on the paper by Delsol et al.1 with respect to the rather misleading conclusions which in our opinion cannot be supported by the data as presented. There are a number of inconsistencies within the paper; however, we would like to draw attention to the lack of detail regarding statistical analysis of the presented data. In paragraph two of the results it is stated that, “Treated pigs inoculated with cyclohexane-resistant S. Typhimurium DT104 (group 2) and treated pigs inoculated with gyrA mutant S. Typhimurium DT104 (group 3) consistently shed higher numbers of Salmonella than the treated pigs from their respective control groups (P<0.01 for both)”. This would certainly not be true for the gyrA mutant S. Typhimurium DT104 (group 3). For more than half the experimental period, Figure 1 shows that at some time after day 14 and likely before day 21, the treated animals were shedding fewer Salmonella than the untreated animals. The point was also made that for group 2, the Salmonella counts were 100-fold higher in treated than untreated pigs for 2 weeks post-treatment. This does not appear to be the case at day 14, although in the absence of tabulated data it is difficult to be sure. Furthermore, the statistical analysis does not address whether there are any statistical differences in counts of treated and untreated animals in both groups 2 and 3 at 35 days. This is highly relevant to the conclusions that are made by the authors, “In conclusion, our study has provided direct evidence that enrofloxacin-treated pigs could be entering abattoirs with higher numbers of quinolone-resistant bacteria compared with untreated pigs thereby increasing the risk of quinolone-resistant zoonotic bacteria entering the food chain.”

This conclusion cannot be supported by the data, as at the end of the study period the level of colonization in the treated pigs and the controls did not appear to differ. Additionally, the authors have failed to relate the experimental conditions to current understanding of development of the gastrointestinal flora in the pig and to commercial practice within the pig industry. The experiment was completed at approximately 13 weeks, whereas under commercial conditions, pigs are slaughtered at 5.5–6 months of age, i.e. at 23–25 weeks of age, which is 10–12 weeks later than the age at which the experimental trial was finished. It is therefore erroneous and highly misleading to state that this trial gives direct evidence that enrofloxacin-treated pigs could be entering abattoirs with higher numbers of quinolone-resistant bacteria compared with untreated pigs thereby increasing the risk of quinolone-resistant zoonotic bacteria entering the food chain.

The authors make no reference to the fact that the trial was performed in young weaner pigs, at an age at which the intestinal flora is still very immature. Indeed, Beleïl et al.2 make the point that there is little information about the age of contamination by ubiquitous Salmonella serotypes of growing pigs in subclinically infected herds and that longitudinal studies following the bacteriological and serological status of pigs should be carried out to determine the typical age of contamination. This is crucial because it is well accepted that the gastrointestinal flora of the young animal changes over time. Smith & Crabb3 showed this as far back as 1961, and more recently Katouli et al.4 using techniques that measured the metabolic potential of the faecal flora of pigs, showed a continuously changing flora as animals aged, although in this study analysis did not continue for more than 3 months. Understanding the development of the gastrointestinal flora and associated immunological changes in growing pigs is fundamental to making conclusions which extrapolate from a limited study such as that of Delsol et al.1 to implications for public health. In a detailed longitudinal study to describe the serological response to Salmonella enterica in growing pigs, Beleïl et al.2 showed that seroconversion occurred during the last third of the fattening phase, from 140 days to slaughter age whilst in contrast shedding was reported during the first half of the fattening period suggesting Salmonella shedding precedes seroconversion.

We make these points to illustrate the complexity of the situation and to ask that the authors re-consider their data in the overall context of an understanding of gastrointestinal development and of practices within the pig industry. This is especially important when making comments concerning public health.

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References


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**Reply**

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Silley & Froyman¹ have raised a number of issues regarding our study into the effect of enrofloxacin treatment on *S. Typhimurium* in a pig model. Many of these issues are misunderstandings due to the presentation of data in graphical rather than tabular form. It should be pointed out that the paper was provisionally accepted after peer review with the suggestion that the paper be reduced in scale to a brief report. Whilst every effort was made to retain all relevant data in the paper, it is possible that brevity and exclusion of certain information from the full paper may have contributed to the issues raised.

In their first comment, Silley & Froyman have misquoted a sentence and taken it out of context: “Treated pigs inoculated with cyclohexane-resistant *S. Typhimurium DT104* (group 2) and treated pigs inoculated with *gyrA* mutant *S. Typhimurium DT104* (group 3) consistently shed higher numbers than the untreated pigs from their respective control groups (*P* < 0.01 for both)”. Silley & Froyman argue this “cannot be true” but state this without any statistical validation. In the publication, the very next sentence explains by how much and how long the pigs treated with enrofloxacin (Baytril, Bayer) were shedding higher numbers of *S. Typhimurium*: 100-fold for 2 weeks in group 2 (for day 14, the exact values have already been given to Dr P. Silley in a personal communication confirming the accuracy of the statement) and 10-fold for 2 weeks post-treatment in group 3. In addition, the graphs show clearly the period during which the treated pigs were shedding higher levels of salmonella than the untreated pigs. Thus we contend that the data are sound and we have reported the facts accurately, and these have been analysed appropriately.

Silley & Froyman suggest that our conclusion that “our study has provided direct evidence that enrofloxacin-treated pigs could be entering abattoirs with higher numbers of quinolone-resistant zoonotic bacteria than untreated pigs” cannot be supported by our data. They suggest that we have overlooked three issues: (1) the levels of *S. Typhimurium* colonization at the end of the study period did not appear to differ between controls and treated animals; (2) the age of the animals was not relevant to the age at slaughter; (3) the endogenous gut flora of the pigs was not as mature as it would be at the commercial slaughter age.

These issues are, in our view, spurious. Our study investigated the effect of enrofloxacin treatment on organisms that possessed mechanisms of resistance to quinolones, namely *gyrA* and multiple antibiotic resistance (MAR) in a pig model. Our data are irrefutable that on and after treatment these mutants were present in significantly higher numbers in treated pigs than untreated pigs. Our main conclusion is that the use of enrofloxacin in the pig increases the number of resistant bacteria beyond the current withdrawal time and in turn increases the risk of these moving up the food chain. This point was clearly stated in the first paragraph of the discussion.

Antibiotics are powerful selective agents and we contend that the effect we observed in the model system employed in this study may apply equally at other ages. It is difficult to see how an altered flora or more mature flora would obviate the powerful selective effects of the antibiotic. Indeed, in Silley & Froyman’s discussion, not a single reference relates to the response of the native flora in pigs to antibiotic treatment and thus it is likely that the issues raised are speculative, if not unfounded. The Betleil *et al.*³ paper comments on the serological response to *Salmonella enterica* in growing pigs, whilst the Katouli *et al.*² paper specifically deals with the role of zinc oxide on stabilizing the gut flora of pig and states “there was a significant increase in both variety (*P* = 0.019) and diversity (*P* ≤ 0.001) of coliforms in control pigs compared with the ZnO treated group”. We question the relevance of these studies on *Salmonella* strains that are resistant to quinolones in the pig when subjected to selective pressure. If Silley & Froyman are questioning the presence of quinolone-resistant salmonella in pigs at slaughter, it should be pointed out that the prevalence of quinolone resistance in salmonellas from pigs in the UK is currently running at 19.8% (VLA, *Salmonella* reference laboratory, 2002).

We do not attempt to simplify the complexities of the gut flora dynamics in pigs of differing ages under differing dietary regimes but we contend that the data are unequivocal regarding the positive selective pressure mediated by the use of antibiotics. We have demonstrated that enrofloxacin positively selects for *gyrA* and MAR salmonella, and importantly that this effect outlasts the withdrawal time currently employed by the industry. Therefore, treatment with enrofloxacin increases the risk of resistant salmonellas moving up the food chain. We argue that the withdrawal time should reflect the hazard posed by resistant bacteria to meat safety, and in the case of enrofloxacin our data indicate this withdrawal time should be increased.

**References**


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