Demonstration of the metaphylactic use of gamithromycin against bacterial pathogens associated with bovine respiratory disease in a multicentre farm trial

D. Baggott, A. Casartelli, F. Fraisse, C. Manavella, R. Marteau, S. Rehbein, M. Wiedemann, S. Yoon

On five commercial cattle rearing sites across Europe, a total of 802 young cattle at high risk of developing bovine respiratory disease (BRD) associated with the bacterial pathogens *Mannheimia haemolytica* or *Pasteurella multocida* and/or *Mycoplasma bovis* were enrolled into a multicentre, controlled field trial. Half were treated with a single dose of gamithromycin at 6 mg/kg bodyweight by subcutaneous injection and half received an injection of a saline placebo as the control. All animals were observed daily for 14 days for signs of BRD as defined by set criteria. The proportion of metaphylactic preventive treatment successes, defined as animals surviving to day 14 without signs of BRD, in the gamithromycin-treated group (86 per cent) was significantly (P=0.0012) higher than in the saline-treated controls (61 per cent). Morbidity among the treated animals was reduced by 64 per cent compared with the controls.

Although BRD is multifactorial in its pathogenesis, morbidity and mortality are usually the result of the pathophysiological responses to colonisation of the respiratory tract by pathogenic bacteria, such as *M. haemolytica, P. multocida and H. somni* and *Mycoplasma* species (Mosier 1997), hence the value of antibiotic therapy. The antibacterial agent gamithromycin was developed exclusively for veterinary use as a single dose, 150 mg/ml subcutaneous injectable solution (Zactran; Merial) for the therapeutic and preventive control of BRD associated with *M. haemolytica, P. multocida and H. somni*. Gamithromycin is a novel semisynthetic macrolide of the azalide subclass. As for the macrolides in general, gamithromycin has a bacteriostatic action through inhibition of bacterial RNA-dependent protein synthesis, but based on in vitro studies it also can act in a bactericidal manner at concentrations that are reached in lung tissue (Retsema and others 1990, Jain and Danziger 2004). The macrolides generally, and the azalides even more so, achieve high concentrations for extended periods in the tissues, particularly lung tissue, compared with their concentrations in plasma (Bryskier and Bergogne-Berezin 1999). They also accumulate readily in host defence cells, including polymorphonuclear leucocytes and macrophages, and readily distribute into extracellular fluid (Mattoes and Nightingale 2002). Gamithromycin shares the dose distribution and pharmacokinetic properties of the azalides (Huang and others 2009). Coupled with its potent bactericidal activity, these properties make gamithromycin a strong candidate antibiotic for the treatment and control of BRD.

This paper describes a multicentre, randomised, controlled field trial designed to evaluate the efficacy of gamithromycin for the preventive treatment of BRD in Europe by comparing gamithromycin-treated cattle with placebo-treated cattle.

Materials and methods

The trial was conducted in accordance with Good Clinical Practice guidelines (Anon 2000) for veterinary product development with the informed consent of the owners of the cattle involved. Five commer-
Cattle meeting the criteria for BRD were removed from the study and assessed by a veterinarian for treatment with non-test drugs according to therapeutic needs. Those with depression and/or respiratory character scores ≥ 2 were assessed for euthanasia on welfare grounds.

**Depression score**
- 0 Normal: nothing unusual in the animal’s attitude
- 1 Mild depression: somewhat slow coming to the feed bunk, but did eat
- 2 Moderate depression: slight drooping of the head/ears, reluctant to move about, reluctant to come to feed
- 3 Severe depression: pronounced head/ear drooping, very reluctant to move
- 4 Moribund (recumbent)

**Respiratory character score**
- 0 Normal: no abnormal respiratory signs present. Respiratory rate and effort are appropriate for the environment
- 1 Mild respiratory distress: serous and/or slight mucous nasal or ocular discharge and/or cough
- 2 Moderate respiratory distress: mucopurulent or copious mucous nasal or ocular discharge and/or increase in respiratory rate or effort
- 3 Severe respiratory distress: marked increase in respiratory rate or effort including one or more of the following: open-mouth breathing, abdominal breathing or head extended

**Trial conduct**
At each trial site, once BRD cases had been confirmed within the airspace occupied by the trial animals, cattle that were apparently healthy (those with depression and respiratory character scores of 0; Fig 1) were paired in order of presentation within groups of animals of similar bodyweight. Animals within each pair were randomly allocated to one of two treatment groups. All enrolled cattle were treated by subcutaneous injection on the same day (day 0) at each trial site: one group with 150 mg/ml gamithromycin solution at 6 mg gamithromycin/kg bodyweight, and the other group with 0.9 per cent sterile saline solution at 2 ml/kg bodyweight, as the control.

Both treatment groups were commingled at all sites.

Animals were observed daily from the day of treatment for 14 days for signs of BRD and general health. During these health observations, a clinical assessment of each animal was made to determine its BRD status, based on depression and respiratory character scores and the rectal temperature (Fig 1). Following treatment with gamithromycin or saline, enroled cattle were removed from the study and treated as necessary with appropriate non-test drugs according to their therapeutic needs if they were diagnosed with BRD.

Cattle were diagnosed with BRD if they fulfilled the clinical criteria of BRD (depression score >0, respiratory character score >0 and rectal temperature ≥ 40.0°C) (Fig 1) for one day, or they showed clinical signs of BRD which, while not fulfilling all the clinical criteria of BRD, were considered severe enough by the attending veterinarian to justify removal on welfare grounds starting on day 1 (the day following treatment).

The trial monitoring period of 14 days was selected on the basis of an anticipated duration of antibacterial effect of gamithromycin of up to 15 days (depending on pathogen susceptibility) as estimated from pharmacokinetic, minimum inhibitory concentration (MIC) and total lung concentration data (Huang and others 2009).

**Microbiology**
At each trial site, before animals were enrolled, two nasopharyngeal swabs (Medical Wire & Equipment) from each animal displaying clinical signs of BRD were collected for culture of BRD pathogens. At each trial site, once BRD cases had been confirmed within the airspace occupied by the trial animals, cattle that were apparently healthy (those with depression and respiratory character scores of 0; Fig 1) were paired in order of presentation within groups of animals of similar bodyweight. Animals within each pair were randomly allocated to one of two treatment groups. All enrolled cattle were treated by subcutaneous injection on the same day (day 0) at each trial site: one group with 150 mg/ml gamithromycin solution at 6 mg gamithromycin/kg bodyweight, and the other group with 0.9 per cent sterile saline solution at 2 ml/kg bodyweight, as the control. Both treatment groups were commingled at all sites.

TABLE 1: Details of cattle at five sites used in a trial of the efficacy of gamithromycin in preventing clinical bovine respiratory disease

<table>
<thead>
<tr>
<th>Trial site</th>
<th>Number enrolled and treated</th>
<th>Breeds</th>
<th>Age (months)</th>
<th>Weight (kg)</th>
<th>Number included in efficacy analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>43</td>
<td>CH, CC</td>
<td>7.23</td>
<td>152-582</td>
<td>42</td>
</tr>
<tr>
<td>Germany 1</td>
<td>60</td>
<td>FV</td>
<td>&lt;1-2</td>
<td>73-139</td>
<td>60</td>
</tr>
<tr>
<td>Germany 2</td>
<td>63</td>
<td>FV</td>
<td>&lt;1-3</td>
<td>54-88</td>
<td>63</td>
</tr>
<tr>
<td>Italy 1</td>
<td>121</td>
<td>CH, CC</td>
<td>7-18</td>
<td>198-390</td>
<td>121</td>
</tr>
<tr>
<td>Italy 2</td>
<td>113</td>
<td>S, A, L, CH, CC</td>
<td>4-18</td>
<td>200-430</td>
<td>113</td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>402</td>
<td>&lt;1-24</td>
<td>54-88</td>
<td>401</td>
</tr>
</tbody>
</table>

1 Treated with a single dose of 150 mg/ml gamithromycin injectable solution at 6 mg/kg (2 ml/50 kg) bodyweight

2 Treated with a single injection of sterile 0.9 per cent saline solution at 2 ml/50 kg bodyweight

A Aubrac, BA Blonde d’Aquitaine, CB Crossbred, CC Charolais cross, CH Charolais, FV Fleckvieh (German Simmental), L Limousin, S Sålar

Data analysis: Data from each site separately and all sites combined were analysed to determine the
The ranges of MICs of gamithromycin for the pathogenic isolates are shown in Table 2. The MIC range based on one randomly selected isolate per trial site was 0.125 to 2.0 μg/ml for Pasteurella multocida, Pasteurella haemolytica, Mannheimia haemolytica, and Mycoplasma bovis. Isolates from the site in France showed a higher rate of susceptibility to gamithromycin compared to the other sites. The MICs of the isolates from the other sites were generally lower, ranging from 0.125 to 0.5 μg/ml.

The pro-inflammatory cytokine responses in sera from 14- and 28-day-treated cattle were compared to those in the control group. The cytokine levels were significantly reduced in the treated group compared to the control group. The results suggest that gamithromycin may have anti-inflammatory effects.

The prophylactic efficacy of gamithromycin was evaluated in a study involving 397 animals treated with gamithromycin at various trial sites. One animal at the site in France was excluded from the analysis due to non-BRD-related events. The overall reduction in BRD morbidity in treated animals was 72 per cent compared to 63 per cent in the control group. The reduction in BRD morbidity was statistically significant (P<0.001) at all trial sites except the site in France, where the total number of animals was relatively low compared to the other sites.

Most isolates collected from cases of BRD at the trial sites before enrolment were considered susceptible to gamithromycin based on in vitro MIC testing. The MICs of the isolates were generally lower than the MICs determined for the isolates of each BRD pathogen species shown in Table 2. The proportion of preventive treatment successes in the combined gamithromycin-treated groups (86 per cent) was significantly higher than in the combined sterile saline-treated controls (61 per cent). The overall BRD morbidity in treated cattle was statistically significant (P<0.001) at all individual trial sites except the site in France, where the total number of animals was relatively low compared to the other sites, thus reducing the statistical power for the data from this site. The overall BRD morbidity in the trial population (equivalent to the percentage of BRD-affected cattle among the controls) was 39 per cent, ranging from 72 and 63 per cent, respectively, at the two sites in Germany to 26 to 28 per cent at the sites in Italy and France.

The morbidities in the control cattle at the two sites in Germany were higher (63 to 72 per cent) than at the other three sites (26 to 28 per cent). Also at the German sites, morbidities in the treated animals (25 to 32 per cent) were higher than at other sites (5 to 19 per cent) even though the reduction in morbidity in treated animals compared with controls was high at approximately 60 per cent. At these sites, the animals studied were young calves less than three months old and had a more diverse bacterial flora than older cattle at other sites (Table 2). The bacterial pathogens isolated from the sites in Germany were shown to be susceptible to gamithromycin by in vitro MIC testing (MIC 0.125 to 2.0 μg/ml). It is assumed that the relatively high morbidity among the test animals in Germany reflect...
ed the greater susceptibility of younger animals to BRD and possibly the presence of non-susceptible respiratory pathogens, such as viruses, that were not identified.

The sites of bacterial multiplication and initial pathology for BRD pathogens are thought to be on the surface of bronchiolar and alveolar lining cells and in the adjacent pulmonary epithelial lining fluid (PELF) (Nightingale and Mattoes 2002). Although gamithromycin concentration measurements in whole lung homogenate, as reported by Huang and others (2009), do not provide a quantitative measure of the drug concentration in PELF, a further study (Giguère and others 2011) has reported rapid penetration of all lung tissues within 30 minutes of administration, with mean gamithromycin concentrations in calf PELF and associated cells peaking at 24 hours (4.6 and 17.8 μg/ml, respectively) and remaining above 0.5 μg/ml for at least seven days after treatment. It is probable, therefore, that the concentration of gamithromycin in PELF stays above the in vitro gamithromycin MIC (MIC<sub>90</sub>) for the principal target BRD pathogens (0.5 to 1.0 μg/ml) (Huang and others 2009) beyond seven days after treatment. The efficacy due to the extended duration of high levels of gamithromycin in PELF is further enhanced by the long postantibiotic effect (the antimicrobial effect in vitro after removal of the antibiotic) of up to eight hours, which is typical of modern macrolides (Diarra and others 1999).

In most regions of the world, there are strong initiatives in place to encourage the responsible use of antimicrobials in veterinary practice (European Platform for the Responsible Use of Medicines in Animals 2008, British Veterinary Association 2009). The use of antibiotics in the control of BRD can be broadly classified as either therapeutic, in which animals with clinical disease are treated, or preventive, when groups of animals are treated before the onset of disease in order to limit the potential impact of BRD. Preventive approaches can be further subdivided into prophylactic, when antibiotics are administered before the appearance of clinical disease to groups of cattle judged to be at high risk of developing BRD, and metaphylactic, in which antibiotics are administered to herds of apparently healthy animals that are in contact with clinical cases (Brumbaugh 2009). Using these definitions, the present study involved a metaphylactic approach. While the decision by veterinarians to adopt any of these approaches rests on numerous considerations, one of the most important is the welfare of the animals under their care. A therapeutic approach will generally result in the lowest level of antibiotic usage, but this requires a high level of stockmanship in order to detect BRD in its early stages, and adequate labour and facilities with which to handle, examine and treat the affected animals promptly, if their welfare is not to be compromised. When determining what approach should be used, the relative risk of infection and its consequences must be sufficient to outweigh the risks associated with using an antimicrobial drug. Risk of infection is related to the virulence of the organism(s), the amount of exposure of the animal to the organism(s) and the animal’s immune defense status (Brumbaugh 2009). The virulence of the organisms and the amount of exposure can be predicted on the basis of previous experience of the disease at the farm/facility, including specific diagnosis and isolation of pathogens, and estimates of morbidity and mortality when possible. The animals’ defense status can be predicted by knowledge of the physiological and immunological characteristics of the farm/facilities and thus, to which animals they are exposed, their previous exposure to disease and their vaccination status should all be considered. In the trial described in this paper, which employed a metaphylactic approach, the risk of BRD was based on 5 per cent or more of the cattle within the airspace having presented with clinical signs of BRD after assembly and/or commingling at the arrival site. This resulted in actual morbidity of 28 to 72 per cent among the control animals. The treatment with gamithromycin significantly reduced morbidity by 64 per cent overall (52 to 52 per cent at individual sites), confirming that the predicted consequences of not treating in terms of morbidity in untreated animals justified the use of the product by improving the welfare of the animals.

In conclusion, this European multicentre field trial has shown that gamithromycin, administered as a single treatment using a metaphylactic approach to at-risk cattle kept under a wide range of commercial conditions, significantly reduced the clinical incidence of BRD associated with M. haemolytica, P. multocida and/or M. levis over the 14-day trial observation period.

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References


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