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

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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
4 Moribund

Animals presenting with BRD before the start of the trial were treated with non-test drugs equivalent to 5 per cent or more of the cattle within the same airspace at trial conduct.

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

| Trial site | Number enrolled and treated | G* | Control† | Breeds | Age (months) | Weight (kg) | Number included in efficacy analysis | Control |
|------------|----------------------------|-----|----------|--------|-------------|------------|-------------------------------------|---------|
| France     | 43                         | 44  |          | CH, CC, L, BA, CB | 7-23 | 152-582 | 42                                   | 43      |
| Germany 1  | 60                         | 60  |          | FV     | <1-2       | 73-139    | 60                                   | 60      |
| Germany 2  | 63                         | 63  |          | FV     | <1-3       | 54-88     | 61                                   | 63      |
| Italy 1    | 121                        | 121 |          | CH, CC | 7-18       | 198-390   | 121                                  | 121     |
| Italy 2    | 113                        | 113 |          | BC, S, L, CH, CC | 4-18 | 200-430 | 113                                   | 114     |
| Total      | 322                        | 322 |          |        |            | 54-82     | 397                                  | 401     |

* Treated with a single dose of 150 mg/ml gamithromycin injectable solution at 2 ml/kg bodyweight
† Treated with a single injection of sterile 0.9 per cent saline solution at 2 ml/kg bodyweight

Recorded health observations were assessed by a veterinarian for treatment with 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).
efficacy of gamithromycin. It was considered appropriate to combine the data for ruminating and preruminating cattle from all sites because of similar gamithromycin pharmacokinetic profiles (Anon 2008) and BRD pathogenesis in both age groups.

On day 14, a treatment success was declared for cattle that were not diagnosed with BRD. Cattle that were removed from the trial for reasons other than BRD were not considered in the analysis of treatment success. The pivotal determinant of preventive efficacy was the proportion of BRD prevention treatment successes on day 14 in the gamithromycin-treated group compared with the saline-treated control group. For each individual site, the proportion of treatment successes on day 14 was compared between the gamithromycin-treated group and controls by Fisher’s exact test. The proportion of treatment successes on day 14 combined across all sites was analysed by a generalised linear model with a logit link function and a binomial distribution, where model factors included treatment as a fixed effect, and site and treatment by site interaction as random effects. A two-sided significance level of 0.05 was used for all analyses, which were performed using the procedures of SAS Version 8.2 (SAS Institute).

Results

During the trial, no animals died as a result of BRD, and no adverse events considered to be related to the gamithromycin treatment occurred. Two gamithromycin-treated animals and one saline-treated control animal were removed from the trial on welfare grounds for reasons not related to BRD (abdominal colic and non-specific enteritis), and thus were excluded from the analysis of preventive treatment success. One animal at the site in France was unintentionally underdosed and was also excluded from the data analysis. The proportion of preventive dose of 6 mg/kg bodyweight to cattle at high risk of BRD pathogenesis in both age groups.

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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 et al. 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 of 90 per cent (MIC90) 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 antibacterial effects 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 cohorts 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 defence 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’ defence status can be predicted by knowledge of the physiological and immunological characteristics of the animals, the infecting pathogens, their size, the stressors to which they are subjected, their previous exposure to disease and their vaccination status. This study was funded by Merial Limited, Duluth, GA 30096, USA.

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