Efficacy of a single injection of marbofloxacin in the treatment of bovine respiratory disease

E. GRANDEMANGE1*, S. FOURNEL1, H. GIBOIN2, F. WOEHRLÉ1

1Vétoquinol Research Centre, BP189, 70204 Lure cedex, FRANCE.
2Vétoquinol S.A., 31 rue des Jeûneurs, 75002 Paris, FRANCE.

*Corresponding author: erik.grandemange@vetoquinol.com

SUMMARY

Pharmacokinetic/Pharmacodynamic (PK/PD) integration with in vitro and ex vivo testing indicates that a single injection of marbofloxacin is an effective treatment for bovine respiratory disease (BRD). The efficacy and field safety of marbofloxacin administered as a single-dose treatment to mixed breed cattle with BRD were evaluated in a masked, randomised, European, multicentre study conducted on commercial farms during 26 separate outbreaks. A total of 231 cattle were enrolled with acute, untreated respiratory disease and treated on day 0 with either marbofloxacin (10 mg/kg, intramuscular administration; n=118) or florfenicol (40 mg/kg, subcutaneous administration; n=113). Cattle were assessed on days 1, 2, 3, 4, 7 and 21 following treatment. Success rates (animal requiring no further therapy) were compared using a non-inferiority approach. Both treatments were well tolerated, a high success rate was observed on day 7, and marbofloxacin was non-inferior (P<0.00001) to florfenicol (day 7 success rate, marbofloxacin vs. florfenicol: 85% vs. 89%). Secondary parameters (day 7 clinical response, time to cure, day 21 success and overall clinical score) confirmed there was no difference between treatments (P=0.15). In conclusion, a single injection of marbofloxacin is safe and efficacious for the treatment of BRD, confirming the PK/PD prediction.

Keywords: Marbofloxacin, florfenicol, BRD, dose confirmation, field trial.

RÉSUMÉ

Efficacité d’une injection unique de marbofloxacine dans le traitement des infections respiratoires des bovins

Les calculs d’intégration pharmacodynamique/pharmacocinétique (PK/PD) réalisés à partir de tests in vitro et ex vivo suggèrent qu’une injection unique de marbofloxacine est efficace dans le traitement des infections respiratoires des bovins. L’efficacité et la tolérance d’une injection unique de marbofloxacine administrée à des bovins de différentes races présentant des signes d’infection respiratoire ont ainsi été évaluées dans une étude clinique terrain randomisée réalisée en aveugle dans différentes fermes commerciales d’Europe à l’occasion de 26 épisodes pathologiques. Un total de 231 bovins présentant des symptômes aigus d’infection respiratoire non traitée précédemment ont été inclus et traités au jour 0 avec soit de la marbofloxacine (10 mg/kg, injection intramusculaire; n=118) soit avec du florfenicol (40 mg/kg, injection sous-cutanée ; n=113). Des examens cliniques ont été réalisés 1, 2, 3, 4, 7 et 21 jours après traitement. Les taux de succès (animaux n’ayant pas nécessité d’autres traitements pour leur pathologie respiratoire) ont été comparés selon une approche statistique de non-infériorité. Les deux traitements ont été bien tolérés et des taux de succès élevés ont été observés à J7 permettant de conclure que la marbofloxacine n’a pas été moins efficace que le florfenicol (P<0.00001) (taux de succès à J7, marbofloxacine vs. florfenicol: 85 % vs. 89 %). Les paramètres secondaires (taux de réponse à J7, délai de guérison, taux de succès à J21 et score clinique global) ont confirmé qu’il n’y avait pas de différence entre les traitements (P ≥ 0.15). En conclusion, une injection unique de marbofloxacine est un traitement efficace et bien toléré des infections respiratoires des bovins, confirmant les prédictions PK/PD.

Mots clés : Marbofloxacine, florfenicol, infections respiratoires bovines, dose confirmation, essai terrain.

Introduction

Bovine respiratory disease (BRD) continues to be an important disease of cattle [3, 4, 5]. Many potential pathogens are involved, including viral, mycoplasmal, and bacterial [8, 14, 17, 19]. The ability of these pathogens to cause disease may be potentiated by contributing factors such as stress, poor housing, inadequate ventilation, high stocking density, poor nutrition and transportation [23]. Primary or secondary bacterial infections are frequently implicated in clinical outbreaks of BRD causing significant pathological changes in the lung [21]. Several species, in particular Mannheimia haemolytica, Pasturella multocida, Histophilus somni and Mycoplasma bovis are commonly isolated from clinical cases of BRD [8, 19].

The introduction of newer antimicrobials, such as the fluoroquinolones has greatly enhanced the treatment of BRD, whilst standardised laboratory techniques have improved the ability to detect clinical resistance in BRD bacterial pathogens [31]. Fluoroquinolones are used in both human and veterinary medicine. Marbofloxacin (a third generation fluoroquinolone) has been specifically developed for veterinary use. This fluoroquinolone was first approved in Europe at a dose of 2 mg/kg daily for 3 to 5 days for the treatment of BRD, caused by Pasteurellaceae and Mycoplasma strains in 1997. However, the use of antimicrobials such as fluoroquinolones in veterinary medicine remains under particular scrutiny due to any potential impact of animal-related bacterial resistance development on human medicine [22].

To minimise the risk of resistance development in cattle, a new dosage regimen for marbofloxacin has been developed. Using Pharmacokinetic/Pharmacodynamic (PK/PD) integration with in vitro modelling is common in human medicine for dosage optimization, especially for fluoroquinolones [2, 24, 27]. This approach indicated that a single injection of 10 mg/kg marbofloxacin should be effective for the treatment of BRD [28, 29].
The emergence of bacterial resistance under antimicrobial selection pressure has led to identification of PK/PD indices that better correlate with the antibacterial effect and with the prevention of antimicrobial resistance [9]. For fluoroquinolones, it has been proposed that providing higher plasma concentrations which exceed the threshold for spontaneous-drug-resistant-mutant susceptibility should prevent the selective amplification of any present mutant subpopulation [32].

Here we present the results of an international, multi-centred, randomised clinical trial conducted to evaluate the field efficacy and safety of a single injection of marbofloxacin (Forcy® Vetoquinol) administered intramuscularly at a dose of 10 mg/kg in comparison to florfenicol (Nuflor®, MSD Animal Health) for the treatment of BRD. This study was conducted as part of a product development program in accordance with Good Clinical Practice and EMEA statistics guidelines [11, 30].

Material and Methods

STUDY DESIGN

This study was conducted in compliance with VICH (International Co-operation on Harmonisation of Technical Requirements for Registration of Veterinary Medical Products) guidelines for Good Clinical Practice [30] at farms in France, Germany and Italy. Local reference laboratories were used to evaluate bacteriological and serological samples. Approval was obtained from the appropriate regulatory authorities and conformed to local animal welfare standards. Florfenicol (Nuflor®, MSD Animal Health) was chosen as the positive control because it is a licensed antimicrobial indicated for the treatment of bovine bacterial respiratory disease in EU countries, including France, Germany and Italy.

Ruminating cattle with bacterial respiratory disease requiring systemic antimicrobial therapy were randomised in a 1:1 ratio to a single treatment with either marbofloxacin or florfenicol in a masked study. Day 0 was defined as the day of treatment. Each animal was subjected to a veterinary examination and bacteriological sampling prior to antimicrobial treatment administered by a different veterinary surgeon on day 0. Animals were clinically assessed by a veterinary surgeon, who was unaware of treatment allocation, on days 1, 2, 3, 4, 7 and 21. A second bacteriological sample was collected in the event of relapse or treatment failure.

ANIMALS

Male and female cattle of various breeds which met the clinical enrolment criteria were enrolled in different farms in France, Germany and Italy (Table I). A maximum of 12 suitable animals were included from each outbreak. An outbreak was defined as a group of cattle with respiratory disease within the same airspace during the same time period. Two farms (one in France and one in Italy) experienced multiple outbreaks. The husbandry and housing of the cattle followed the standard practices on each farm and was representative of typical field conditions in each of the countries. All animals were identified by ear tags and were fed and watered appropriately for their age.

No animals had presented with bacterial respiratory disease or received antimicrobial compounds during the 30 days before inclusion in the study, and no included animals were vaccinated against Pasteurella spp. Non-ruminating cattle, lactating cows, and cattle with concomitant disease or requiring additional therapy were not included in the study.

CLINICAL ASSESSMENT

Inclusion in the study required that cattle were pyrexic (rectal temperature of \(\geq 40.0^\circ\text{C}\)), demonstrated clinical signs of respiratory disease (abnormal respiration or cough or nasal discharge score \(\geq 1\)) and were either depressed (score \(\geq 1\)) or inappetant (score \(\geq 1\), Table II). Rectal temperatures, clinical

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of Farms</th>
<th>No. of Outbreaks</th>
<th>No. of cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>13</td>
<td>15</td>
<td>111</td>
</tr>
<tr>
<td>Germany</td>
<td>7</td>
<td>7</td>
<td>72</td>
</tr>
<tr>
<td>Italy</td>
<td>2</td>
<td>4</td>
<td>48</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>26</td>
<td>231</td>
</tr>
</tbody>
</table>

\[\text{Table I: Demographics of the study.}\]

<table>
<thead>
<tr>
<th>Score</th>
<th>Rectal Temp</th>
<th>Demeanour</th>
<th>Appetite</th>
<th>Respiration</th>
<th>Nasal Discharge</th>
<th>Cough</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt; 39.0°C</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>39.0-39.5°C</td>
<td>Depression</td>
<td>Slightly reduced (&lt;50%)</td>
<td>Polypnoea</td>
<td>Sero-mucus</td>
<td>Strong and noisy</td>
</tr>
<tr>
<td>2</td>
<td>39.6-40.2°C</td>
<td>Marked depression</td>
<td>Severely reduced ((\geq 50%))</td>
<td>Dyspnoea</td>
<td>Muco-purulent</td>
<td>Weak and painful</td>
</tr>
<tr>
<td>3</td>
<td>(\geq 40.3^\circ\text{C})</td>
<td>Moribund</td>
<td>Anorexic</td>
<td>Polypnoea + Dyspnoea</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[\text{Table II: Scoring parameters.}\]
signs and presence of injection site swelling were recorded on days 1, 2, 3, 4, 7 and 21 after treatment administration. If an animal was considered to require further therapy at any time, it was withdrawn from the study, classified as a failure and received the appropriate medication.

LABORATORY EXAMINATIONS

Bacteriological samples were collected from all animals on day 0 before treatment administration. A minimum of two animals per outbreak were sampled by trans-tracheal aspiration (TTA), with the remainder sampled via nasopharyngeal swab (NPS). All animals were re-sampled in the event of treatment failure or relapse. Bacteriological samples were cultured in local reference laboratories to identify bacterial respiratory pathogens (P. multocida, M. haemolytica, M. bovis and H. somnus). Pathogens identified from any samples were subject to sensitivity analysis by antibiogram at the local laboratory [7]. For the same pathogens, minimum inhibitory concentrations (MIC) for marbofloxacin were subsequently established in a central laboratory.

A minimum of two animals from each outbreak were also blood sampled (5 mL) on days 0 and 21 for serological analysis to detect any viral aetiology at local laboratories (BVDV, Pi3V, BRSV, IBRV and eventually AD3).

INVESTIGATIONAL TREATMENT ADMINISTRATION

On day 0, cattle were randomised to receive a single injection in the neck of either marbofloxacin (10 mg/kg, intramuscular (IM) administration; 118 animals) or florfenicol (40 mg/kg, subcutaneous (SC) administration; 113 animals), administered by a veterinary surgeon who was not involved in any efficacy assessments. Pain on injection was also scored (0 = no pain; 1 = slight pain; 2 = Moderate pain; 3 = Severe pain).

ASSESSMENT CRITERIA

Animals were classified according to outcome (Table III). The primary efficacy criterion was the day 7 success rate.

### Definition | Outcome
--- | ---
**Day 7**
Success | Remained on study, and did not required further therapy.
Clinical cure | Rectal temperature < 39.5°C, cough or nasal discharge score of ≤ 1, all other parameters normal.
Clinical improvement | Rectal temperature < 39.5°C, improvement in some parameters (the number of improved parameters must be equal to or above the number of parameters which have deteriorated).
Clinical failure | Rectal temperature ≥ 39.5°C or no improvement/unsatisfactory improvement or required additional therapy or died as a consequence of respiratory disease.

**Day 21**
Success | Remained on study, and did not required further therapy.
Therapeutic failure | Required additional therapy because of respiratory disease or died as a consequence of respiratory disease.
Relapse | Animal required additional therapy for respiratory disease between days 8 and 21.

### TABLE III: Definitions of outcomes.

For each assessment criterion, two analyses were conducted. One analysis included all treated animals (Intent To Treat analysis: ITT). A second analysis (Per Protocol analysis: PP) excluded all animals for which procedures (including treatment administration and efficacy measurements) were not conducted to a sufficient standard to enable a fair comparison. Results are presented for the PP analysis only unless otherwise stated.

Power calculations indicated that a minimum of 83 cases per treatment group were needed to demonstrate non-inferiority for the primary criteria with at least 80% power and a success rate of about 75% in both groups. To test whether a single administration of marbofloxacin at 10 mg/kg IM was non-inferior to a single administration of florfenicol at 40 mg/kg SC, a 20% non-inferiority margin was defined. For the non-inferiority test, the 95% two sided confidence interval of the observed odds ratio was calculated as well as the odds ratio at the non inferiority limit of -20%. If the lower confidence bound of the confidence interval was above the predetermined lower limit of non-inferiority, then marbofloxacin was considered non-inferior to florfenicol. Furthermore a P-value was calculated to confirm non inferiority and to quantify the strength of the rejection of inferiority [10]. If the calculated P-value was lower than 2.5% i.e. 0.025 then the hypothesis of inferiority of the new compound compared to the reference one was rejected and the non-inferiority established. For all
other secondary parameters, the comparisons between treatments were performed by a variety of statistical methods dependent upon whether the data was qualitative or quantitative and repeated measure or not (qualitative variables: Chi square or Fisher’s exact test, quantitative variables: Student’s or Wilcoxon’s test, qualitative variables with repeated measurements: GEE model using GENMOD procedure, quantitative or semi-quantitative measurements: Mixed model). Statistical analyses were performed using SAS software (SAS/STAT 9.1, SAS Institute).

Results

STUDY POPULATION

A total of 231 cattle were enrolled into the study with acute, untreated respiratory disease (clinical symptoms present < 3 days). The cattle represented a wide variety of breeds and cross-breeds, including Charolais (37%), Simmental (26%), Prim’Holstein (15%), Hostein-Fresian, Blond Aquitaine, Limousin, Montbeliard and Salers. Both male (58%) and female (42%) cattle aged 14 to 515 days (mean age = 7.5 months) and weighing 50 to 496 Kg (mean weight = 215 Kg) were included. Marbofloxacin was administered to 118 cattle and florfenicol was administered to 113 cattle.

Of the 231 treated cattle, 207 animals were suitable for the per protocol analysis. The remaining 24 cattle were not included in the per protocol analysis due to at least one of the following reasons: not meeting one of the inclusion/exclusion criteria (n = 4), withdrawal due to unrelated concomitant disease (n = 3), treatment deviations (n = 12), incorrect randomisation (n = 1) and forbidden concomitant medication (n = 5). The per protocol dataset included 107 animals treated with marbofloxacin and 100 animals treated with florfenicol. All following results refer to the per protocol analyses, unless otherwise specified.

No statistical difference was found at enrolment between treatment groups for both ITT and PP populations.

CONFIRMATION OF RESPIRATORY DISEASE

From the 231 enrolled cattle, a total of 118 bacteria associated with bovine respiratory disease pathology were isolated before treatment, confirming the presence of bacterial respiratory disease (Table IV). Respiratory bacterial pathogens were isolated in 22 of the 26 outbreaks. The susceptibility of these isolates to marbofloxacin and florfenicol was determined (two M. bovis isolated were not tested due to lack of growth). All tested isolates were sensitive to both antimicrobials (marbofloxacin MIC values are presented in Table V).

Analysis of paired serology samples revealed sero-conversion for cattle respiratory viruses in the majority of the respiratory disease outbreaks (23 of 26 outbreaks). The most common viral component was bovine respiratory syncytial virus (BRSV) which was identified in 17 of the 26 outbreaks, including the four outbreaks where no pathogenic bacteria were isolated.

EFFICACY ASSESSMENTS

Day 7 Success Rate (Primary Efficacy Criteria)

There was a high day 7 success rate in both treatment groups (Table VI). A similar number of animals in each group required additional therapy. A -3.95% difference was observed between marbofloxacin and florfenicol (non inferiority test \( P < 0.00001 \); observed odds ratio = 0.70). Therefore marbofloxacin successfully achieved non-inferiority to florfenicol. Similar results were obtained with the ITT population.

<table>
<thead>
<tr>
<th>Bacteria isolated on day 0</th>
<th>Marbofloxacin</th>
<th>Florfenicol</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histophilus somni</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mannheimia haemolytica</td>
<td>14</td>
<td>20</td>
<td>34</td>
</tr>
<tr>
<td>Mycoplasma bovis</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>32</td>
<td>27</td>
<td>59</td>
</tr>
<tr>
<td>Other Mannheimia</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Other Pasteurellaceae</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

Table IV: Distribution of the bacteria isolated at inclusion according to the treatment groups (ITT population).

<table>
<thead>
<tr>
<th>Bacteria isolated on day 0</th>
<th>No of Isolates tested</th>
<th>MIC range (µg/mL)</th>
<th>MIC(_{50}) (µg/mL)</th>
<th>MIC(_{90}) (µg/mL)</th>
<th>Standard Antibiogram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histophilus somni</td>
<td>1</td>
<td>0.03</td>
<td>NA</td>
<td>NA</td>
<td>100%</td>
</tr>
<tr>
<td>Mannheimia haemolytica</td>
<td>34</td>
<td>0.015-0.5</td>
<td>0.025</td>
<td>0.18</td>
<td>100%</td>
</tr>
<tr>
<td>Mycoplasma bovis</td>
<td>7</td>
<td>1-2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>59</td>
<td>0.008-0.12</td>
<td>0.012</td>
<td>0.029</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table V: Marbofloxacin MIC range, MIC\(_{50}\) and MIC\(_{90}\) and standard antibiogram results for marbofloxacin and florfenicol susceptibility.
SECONDARY MEASURES OF EFFICACY

Day 7 Assessment

There was a high clinical response rate in both treatment groups, with a slightly higher clinical cure rate in the florfenicol treated group (Table VII). However, the clinical response rate observed in the marbofloxacin treated group was not significantly different to that obtained for the florfenicol treated group (P = 0.15).

For all animals which were defined as clinical cures on day 7, the time required to reach this status was evaluated (Fig. 1.). Whilst there was no significant difference when comparing the treatment groups (P = 0.71), more animals were defined as a ‘clinical cure’ on days 1 and 2 in the marbofloxacin treated group.

After treatment, there was an improvement in each of the clinical scoring parameters (rectal temperature, demeanour, appetite, respiration, nasal discharge and cough) which was similar for both treatment groups (treatment comparison, P ≥ 0.11 at the minimum) (Fig. 2.).

Day 21 Assessment

There was a high day 21 success rate in both treatment groups, and a similar relapse rate (Table VIII). At relapse, only two isolates of P. multocida were identified, which were sensitive to both antimicrobials. There was no significant difference between treatment groups (P ≥ 0.61). The overall clinical score (composite score of all clinical scoring parameters) significantly improved over time (P < 0.0001; Fig. 2.). Three of the cases that failed to improve after treatment (n=3, florfenicol group) subsequently died or were euthanized due to further complication of the respiratory diseases. There was no significant difference between treatment groups (P = 0.46).

SAFETY ASSESSMENT

Both treatments were well tolerated and there were no serious adverse events related to treatment. Marbofloxacin was significantly better tolerated at injection than florfenicol (no pain on injection: 75.4% vs. 54.9%, marbofloxacin vs. florfenicol; P = 0.004). Further, there was less swelling at the injection site in the marbofloxacin treated group than in the

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### TABLE VI: Day 7 Success Rate and Non-inferiority analysis for Per Protocol and Intent to Treat populations.

<table>
<thead>
<tr>
<th></th>
<th>Per Protocol (PP)</th>
<th></th>
<th>Intent to Treat (ITT)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of cattle</td>
<td>Success¹ (number)</td>
<td>Number of cattle</td>
<td>Success¹ (number)</td>
</tr>
<tr>
<td></td>
<td>107</td>
<td>85.0% (91)</td>
<td>118</td>
<td>83.1% (98)</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florfenicol</td>
<td>100</td>
<td>89.0% (89)</td>
<td>112</td>
<td>88.4% (99)</td>
</tr>
<tr>
<td>Observed Difference in Success Rate</td>
<td>-3.95%</td>
<td>-5.34%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Observed Odds ratio]</td>
<td>[0.70]</td>
<td>[0.64]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower bound of the confidence interval of the odds ratio</td>
<td>0.31</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odds ratio limit</td>
<td>0.09</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conclusion</td>
<td>Rejection of inferiority</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>(P value)</td>
<td>P &lt; 0.00001</td>
<td>P = 0.00001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Success defined as an animal which remained on study, and did not require further therapy by day 7.
²One animal treated with florfenicol was not included due to concomitant disease which confounded the day 7 clinical examination.

### TABLE VII: Day 7 clinical response rate for per protocol population.

<table>
<thead>
<tr>
<th></th>
<th>Clinical Cure¹ (number)</th>
<th>Clinical Improvement² (number)</th>
<th>Clinical Response³ (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Marbofloxacin</td>
<td>florfenicol</td>
<td>florfenicol</td>
</tr>
<tr>
<td></td>
<td>107</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Clinical Cure¹</td>
<td>75.7% (81)</td>
<td>86.0% (86)</td>
<td></td>
</tr>
<tr>
<td>Clinical Improvement²</td>
<td>5.6% (6)</td>
<td>2.0% (2)</td>
<td></td>
</tr>
<tr>
<td>Clinical Response³</td>
<td>81.3% (87)</td>
<td>88.0% (88)</td>
<td></td>
</tr>
</tbody>
</table>

¹Clinical cure defined as animals which had a rectal temperature <39.5°C, cough or nasal discharge score of ≤1, all other were parameters normal.
²Clinical improvement defined as animals which had a rectal temperature <39.5°C, improvement in some parameters (the number of improved parameters must be equal to or above the number of parameters which have deteriorated).
³Clinical Response defined as Clinical Cure + Clinical Improvement.
florfenicol treated group (day 7 swelling: 0% vs. 9.8%, marbofloxacin vs. florfenicol). Sixteen cases were reported to experience an adverse event, including diarrhoea (n=5, marbofloxacin group; n=3, florfenicol group), complication of the respiratory disease leading to death (n=3 florfenicol group), or concomitant disease (n=1, marbofloxacin group; n=4, florfenicol group).

**Discussion**

This was a controlled, masked, randomised study conducted according to VICH GCPv comparing the efficacy and safety of a new dosing regimen of marbofloxacin to an existing reference product (florfenicol, Nuflor®). This study demonstrates that a single injection of 10 mg/kg marbofloxacin is non-inferior to 40 mg/kg florfenicol in the treatment of clinical cases of bovine respiratory disease.

To maximise environmental diversity (including beef, dairy and fattening farms) the number of animals was limited to 12 per outbreak. Animals enrolled in the trial were representative of the European cattle population, including both sexes and a wide weight and age range. Clinical signs at inclusion were typical of acute bovine respiratory disease (pyrexia, respiratory signs, depression and inappetance) and had been present for less than three days. The selected cases were severely affected, as demonstrated by the high clinical scores on day 0 (Fig. 2.) and the related death of three cattle in the florfenicol group. The major respiratory pathogens were implicated across all disease outbreaks. Bacteriology confirmed that the majority of outbreaks were associated with *M. haemolytica*, *P. multocida*, *H. somni* and *M. bovis*. whilst serology also identified a viral component for many of the investigated outbreaks. Therefore, it can be concluded that the bovine respiratory disease was truly representative of that seen in France, Germany and Italy. There were few adverse events reported during the study. Less pain and swelling on injection was observed for the marbofloxacin group than the florfenicol group.

The study was well designed to evaluate the chosen BRD efficacy parameters [20]. EMEA (European Agency for the
Evaluation of Medicinal Products) statistical guidelines [11] recommend that when assessing a new treatment for a potentially serious condition, a non-inferiority approach comparing to a reference product is preferable to a conventional hypothesis test for superiority using a placebo. For this approach to be acceptable an appropriate reference product must be selected and a clinically relevant non-inferiority margin for a suitable parameter used. Florfenicol was a good reference product, not only as it is registered in Europe to treat bacterial respiratory disease in cattle, but also as a proven and robust BRD therapy [15]. In this study the efficacy of florfenicol was confirmed as it showed excellent day 7 and 21 success rates.

The primary efficacy criterion was day 7 success rate, defined as the number of animals which did not require alternative treatment for their respiratory disease from day 1 to day 7. This criterion is suitable as it reflects the field situation in which a vet can retreat an animal if in his opinion it is not sufficiently cured. Statistical analysis demonstrated that marbofloxacin was non-inferior to florfenicol. On day 7 the marbofloxacin success rate was 85% which compares favourably to a previous field study which demonstrated a day 4 cure rate of 84% following daily treatments with 2 mg/kg marbofloxacin [25]. The secondary efficacy criteria also confirmed that the new dosing regimen is efficacious. No significant differences were detected between marbofloxacin and florfenicol in any of the parameters. In particular, day 7 cure rates, relapse rates, day 21 success rates, and the overall clinical score were not clinically or statistically significantly different. In previous studies, marbofloxacin administered intravenously was shown to have a significantly more rapid onset of efficacy than tilmicosin administered subcutaneously [25]. In this study, there was no significant difference between marbofloxacin and florfenicol but there was a tendency towards a faster recovery in the animals treated with marbofloxacin. These observations of very fast onset of action of marbofloxacin on the clinical signs of BRD compared to macrolide or phenicol antimicrobials may be attributable to the rapid distribution and bactericidal action of this molecule [12, 27, 28, 29].

Administration of a high dose of a short acting bactericidal antibiotic is intended to reduce the bacterial load sufficiently to achieve clinical cure whilst minimising the emergence of resistance by reducing the overall exposure to antibiotics [2, 16]. In particular, recent PK/PD experiments of in vitro and in vivo infections have demonstrated that the size of the inoculum at the start of the antibiotic treatment and the dose level have a stronger influence on bacteriological cure rate and likelihood of resistance emergence than the number of administrations [13, 16]. Translated to bovine medicine, this would suggest that in addition to optimising treatment dosing, improving the detection of BRD in cattle to allow a focused treatment of sick animals as early as possible, would also help to minimise the development of resistance [26].

In this study, the bacteria isolated at enrolment were all susceptible to marbofloxacin and florfenicol. M. haemolytica was the least susceptible pathogen (marbofloxacin MIC\textsubscript{90} = 0.18 µg/mL) and P. multocida strains isolated on day 0 were highly susceptible (MIC\textsubscript{90} = 0.029 µg/mL). This agrees with previous epidemiosurvey results which grouped bacterial strains into one of three susceptibility groups; highly susceptible (MIC value ≤ 0.06 µg/mL), susceptible (MIC value 0.12-1 µg/mL) and resistant (>2.0 µg/mL) [18]. This survey revealed that between 1994 and 2001, 71.1% of the M. haemolytica strains were highly susceptible and 90.6% of the P. multocida strains. The MIC\textsubscript{90} here were similar to those reported by Meunier [18] indicating that there has been no shift in susceptibility. The MIC range here (0.015-0.5 µg/mL) is consistent with a combination of both susceptible and highly susceptible pre-treatment M. haemolytica isolates.

According to an in vitro PK/PD system, a single treatment of marbofloxacin at 10 mg/kg was predicted to be bactericidal against field isolates of M. haemolytica or P. multocida, and likely to eradicate them [29]. In this field study, a single dose of 10 mg/kg resulted in excellent cure rates, confirming the PK/PD predictions. Although the expected duration of exposure to the antibiotic was shorter in the group treated with marbofloxacin compared to subcutaneous florfenicol, the single injection short acting antibiotic (SISAAB) was able to control the infection in most cases just as well as the long acting product. Indeed, once the vicious cycle of bacterial lung infection is broken by the high load of bactericidal antibiotic, the natural defences and commensal flora can recover and maintain lung homeostasis [1, 6].

In conclusion, this study has demonstrated that a single intramuscular administration of marbofloxacin at 10 mg/kg is non-inferior to subcutaneous florfenicol at 40 mg/kg for the treatment of bovine respiratory disease. Further, the marbofloxacin solution was extremely well tolerated at the injection site.

Acknowledgement

The authors would like to thank the investigators, clinic staff, farmers, laboratories and monitors who took part in this international field study.

Conflict of interest

Erik GRANDEMANGE, Sandrine Fournel, Henry Giboin and Frédérique WOEHRLE-FONTAINE are employees of Vétoquinol S.A.

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