A combination oxytetracycline/flunixin treatment of respiratory infections in cattle

A. KEITA1*, P. POMMIER1, E. PAGOT1, A. COUPER2 and L. CROMIE2

1CTPA, ZOOPOLE développement, 2 rue Jean Rostand, Ploufragan, F-22440
2Norbrook Laboratories limited, Co Down, Northern Ireland
* Corresponding author : E-mail : alassane.keita@zoopole.asso.fr

SUMMARY

A blinded, randomised GCP study was performed to investigate the efficacy of a single injection of long-acting oxytetracycline (OXY) and flunixin (FLU) in young calves suffering from acute respiratory disease. Tilmicosine (TIL) and oxytetracycline alone were used as positive controls. Six hours post-treatment, rectal temperature in animals treated with OXY/FLU had decreased and was significantly lower than in the animals treated with both reference products (p = 0.009). This difference remained significant at 24 hours post-treatment, between OXY/FLU and OXY groups (p<0.001). Over the entire study period, the OXY/FLU treatment produced an overall clinical efficacy similar to treatment with OXY and TIL.

Keywords : Calf, respiratory disease, treatment, oxytetracycline-flunixin, tilmicosin

RESUME

Evaluation d’une association oxytetracycline/flunixine dans le traitement des infections respiratoires bovines.

Une étude BPC, randomisée et conduite en aveugle, a évalué l’efficacité d’une injection unique d’un produit longue action à base d’oxytétracycline (OXY) et de flunixine (FLU), chez des jeunes veaux atteints de maladie respiratoire. La tilmicosine et l’oxytétracycline ont été utilisées comme témoins. La température rectale 6h après le traitement a chuté dans le groupe OXY/FLU et était significativement moins élevée que celle des deux autres groupes (p = 0.009). A 24h post traitement, la température était toujours significativement moins élevée que dans le groupe OXY (p<0.001). Tout au long de l’étude, le groupe OXY/FLU a montré une efficacité clinique similaire à celle des deux autres groupes.

Mots-clés : veau, maladie respiratoire, traitement, oxytétracycline-flunixin, tilmicosine

I- Introduction

Respiratory diseases (RD) are a major disorder in cattle world-wide and represent an important cause of economic losses [7].

It is generally known that RD in calves are of multifactorial aetiology. Environmental conditions and viral diseases (bovine respiratory syncytial virus, parainfluenza virus 3, bovine herpes virus 1 etc) may facilitate the invasion of bacteria such as Pasteurella multocida, Mannheimia haemolytica or Mycoplasma spp [2]. Several injectable antibiotics can be used to treat animals with RD such as tilmicosin (TIL), florfenicol, oxytetracycline (OXY) and others [9]. The treatment of RD using OXY has been largely accepted and published [5, 14]. In addition, the use of non-steroidal anti-inflammatory drugs (NSAIDs) as adjunctive therapy in the treatment of RD is supported by several recent publications when compared to the antibacterial alone [8, 12]. The effects are related to a significant acceleration of the clinical recovery of animals. The NSAID flunixin (FLU) is commonly used as adjunctive therapy for the treatment of RD world-wide [1, 3]. However this concurrent use requires daily administration of flunixin for up to 5 days.

Norbrook laboratories limited developed their own proprietary combination comprising OXY/FLU (HEXASOL™) for treatment of RD in cattle [16]. The objective of this study was to compare the clinical efficacy of a single intramuscular injection of a combination of long-acting OXY/FLU to that of two long-acting reference products : OXY alone or TIL in young calves suffering from acute RD.

II- Materials and method

2.1. STUDY ANIMALS

One hundred and fifty pre-ruminant calves with clinical signs of lower respiratory disease and elevated rectal temperature equal or greater than 39.5°C were selected for the study. The trial was carried out at four commercial farms (designated C, G, L & Q) in Brittany, France, that had a clinical history of respiratory disease. To assess the respiratory disease status, bacteriological results were obtained from untreated affected calves by nasal swabbing and serological results were undertaken before and during the study.

At all farms calves were pre-ruminant, coming from several farms, housed in individual or collective pens and milk powder fed twice daily. The total number of calves housed at farms C, G, L & Q was respectively 550, 300, 230 and 680. Antibiotic supplement was usually used on arrival (colistin and OXY at farms G, L, colistin and trimethoprim-sulfonamide at farm C, colistin at farm Q). At time of selec-
tion all the study calves were out with any stated withdrawal periods for antibiotic supplement used.

Farms C, G, L & Q contributed 20, 17, 50 and 63 calves respectively to the study and at the time of treatment, all calves were aged between 8 and 90 days and within a weight range of 35 to 130 kg. Calves exhibiting clinical signs considered by a veterinarian to be typical of lower respiratory tract disease (Table I), with rectal temperature equal or greater than 39.5°C were included in the study. Cases with other concurrent diseases, those requiring additional treatment or those considered to be of too advanced severity to be likely to respond to therapy, including all moribund animals, were not used for the study. Homogeneity criteria (age and estimated weight) were not statistically different between groups. In the event of a treatment failure (animal meeting inclusion criteria from D3 to study end) the farmer administered another treatment.

**Table I: Clinical assessments and score range**

<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>observation</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal temperature (°C)</td>
<td>To be recorded</td>
<td>NA*</td>
</tr>
<tr>
<td>Respiratory rate (respirations/ min)</td>
<td>To be recorded</td>
<td>NA*</td>
</tr>
<tr>
<td>Hyperpnoea</td>
<td>Absent to severe</td>
<td>0 – 3</td>
</tr>
<tr>
<td>Respiratory sounds</td>
<td>Absent to severe</td>
<td>0 – 3</td>
</tr>
<tr>
<td>Coughing</td>
<td>Absent to severe</td>
<td>0 – 3</td>
</tr>
<tr>
<td>Nasal discharge</td>
<td>Absent to severe</td>
<td>0 – 3</td>
</tr>
<tr>
<td>Demeanour</td>
<td>Absent to severe</td>
<td>0 – 3</td>
</tr>
</tbody>
</table>

NA: not applicable since the values were to be recorded

Interpretation of the Scale 0 = absent  1 = slight  2 = moderate  3 = severe

2.2. BACTERIOLOGY

Nasal swabs were taken from a representative number of the study calves before any treatment. The percentage of sampled animals was 6% (1/17) at farm G, 20% at farm Q (13/63), 34% at farm L (17/50) and 100% at farm C (20/20). The samples were sent to a local microbiological laboratory on the sampling day. Pasteurella multocida and Mannheimia haemolytica were specifically cultured. Isolates were then tested by the disk diffusion method for their susceptibility to several antibiotics including OXY and TIL. Mycoplasma spp were also looked for (presence or absence without typing).

2.3. SEROLOGY

Blood samples were taken at D0 and between D21 and D28 from 3 animals at farm C, 4 animals at farm L and 13 animals at farm Q. No animal was sampled at farm G. Sera derived from the blood samples were titrated for antibodies against Mycoplasma bovis.

2.4. TREATMENT REGIMEN

Individually identified calves were selected immediately before first treatment. The calves were weighed on arrival. At time of inclusion, weight was estimated using the weight on arrival and taking into account the usual average daily weight gain for such calves. Gender was recorded and random number lists were used to allocate each calf within a block to one of the three different treatment groups. In total, fifty pre ruminant calves were treated by a single intramuscular injection of a combination of long-acting oxytetracycline and flunixin defined as group OXY/FLU (Hexasol®; Norbrook Laboratories Ltd) at a dose of 30 mg OXY per kg bodyweight and 2 mg FLU per kg bodyweight. In each of the two reference groups, fifty calves were treated either using a single intramuscular injection of a long-acting formulation of oxytetracycline defined as group OXY (Terramycin LA®; Pfizer Laboratories) at a dose of 20 mg per kg bodyweight or a single subcutaneous injection of tilmicosin defined as group TIL (Micotil®; Elanco Animal Health) at a dose of 10 mg per kg bodyweight i.e both reference products were used as per label instructions. Treatments were administered by veterinarians who were not subsequently involved in the assessments of efficacy to ensure blind conditions for the study.

2.5. ASSESSMENT OF EFFICACY

Assessments of the clinical parameters were performed on a blind basis by a veterinarian on the first day of treatment (defined as D0) and then at D0 + 6 hours post treatment, D1, D3, D7 and D10. In addition to the objective parameters (rectal temperature and respiratory rate), the subjective parameters (hyperpnoea, respiratory sounds, coughing, nasal discharge and demeanour) were recorded in a semi-quantitative scoring system. The scores for subjective parameters were based on a scoring system of increasing clinical severity and were recorded on a visual similar scale. Each score on a scale was ranged from 0 (minimum) to 3 (maximum) for the relevant clinical signs (see Table I). An overall clinical score, defined as the sum of all subjective parameter scores at a given study date, was also calculated.

2.6. STATISTICS

The statistical unit was the individual calf. Age and weight before treatment were compared using ANOVA. The course of each normally distributed parameter (rectal temperature, respiratory rate, overall clinical score) over time was compared between groups using a repeated measure ANOVA test adjusted on farms. The pre-treatment value for each parameter was taken as covariate. If a significant difference was observed on the course of a given parameter, the Tukey’s pairwise comparison test was then used at each study date to determine the signification level between groups. Differences were significant only if p ≤ 0.05.

For all non-normal distributed parameters (hyperpnoea, respiratory sounds, coughing, nasal discharge and demeanour), the Kruskall-Wallis non-parametric test was
used, at each date, to compare the study groups. By taking into account these multiple comparisons for each parameter (5 criteria and 6 study dates i.e 30 comparisons), significance level has to be taken at 0.05/30, i.e 0.0017 as suggested by Jouan-Flahault [10].

A chi-square test was used to compare re-treatment rates between groups at 0.05 as level of significance. All statistical calculations were performed using SYSTAT statistical software version 9.0.

III- Results

3.1. ANIMALS

The mean age was respectively 31.0, 31.1 and 30.5 days in OXY/FLU, TIL and OXY groups. This difference was not significant (p>0.05). The mean estimated weight was respectively 65.4, 67.2 and 65.1 kg in OXY/FLU, TIL and OXY groups. This difference was not significant (p>0.05).

3.2. CLINICAL DATA

3.2.1. Rectal temperature

The mean temperature value before treatment was respectively 40.1°C, 40.2°C, 40.0°C in OXY/FLU, TIL and OXY groups (p>0.05). The change in rectal temperature was significantly different between groups (Figure 1). ANOVA demonstrated a treatment group effect (p = 0.009) and a time effect (p < 0.0017). As the group effect and the time effect were significant, it was permissible to analyse the rectal temperature at each study date.

Over the first six hours post-treatment, body temperature decreased in all groups, respectively to a mean of 38.8°C, 39.8°C, 39.5°C in OXY/FLU, TIL and OXY groups. This difference was statistically significant (p<0.001) between the OXY/FLU group compared to both reference groups. This difference remained statistically significant at 24 hours post-treatment between the OXY/FLU group compared to the group OXY. Difference between the TIL group compared to the group OXY was also significant at 24 hours post-treatment. There was no significant difference between groups at the final three study dates (p>0.05).

3.2.2. Respiratory rate

The mean respiratory rate before treatment was respectively 64.3, 61.2, 58.4 breaths/minute in OXY/FLU, TIL and OXY groups (p>0.05). The change in respiratory rate was not significantly different between groups (p>0.05).

3.2.3. Hyperpnoea

The mean hyperpnoea score before treatment was respectively 1.0, 0.7 and 0.8 in OXY/FLU, TIL and OXY groups. This difference is significant between groups (p<0.05). There was no significant difference between groups over the five final time-points (p>0.0017).

3.2.4. Respiratory sounds

Abnormal respiratory sounds (elevated amplitude of normal sounds, crackles ..) were recorded at auscultation. The mean score pre-treatment was respectively 0.9, 0.8 and 0.7 in OXY/FLU, TIL and OXY groups (p>0.05). There was no significant difference between groups over time (p>0.0017).

3.2.5. Coughing

The mean coughing score before treatment was respectively 0.5, 0.4 and 0.3 in OXY/FLU, TIL and OXY groups (p>0.05). There was no significant difference between groups over time (p>0.0017).

3.2.6. Nasal discharge

The mean nasal discharge score before treatment was respectively 0.5, 0.4 and 0.3 in OXY/FLU, TIL and OXY groups (p>0.05). There was no significant difference between groups over time (p>0.0017).

3.2.7. Demeanour

The mean demeanour score before treatment was respectively 0.3, 0.5 and 0.3 in OXY/FLU, TIL and OXY groups (p>0.05). There was no significant difference between groups over time (p>0.0017).

3.2.8. Overall clinical score

The mean total score pre-treatment was respectively 3.2, 2.9 and 2.8 in OXY/FLU, TIL and OXY groups (p>0.05). The change in overall clinical score was not significantly different between groups (p>0.05).

3.3. RE-TREATMENTS

There was no significant difference (p>0.05) in the re-treatment rates between groups. Respectively 9, 7 and 8 calves treated with OXY/FLU, TIL and OXY required additional treatment, mostly at D3.
3.4. MICROBIOLOGICAL DATA

The distribution of the bacterial strains isolated before treatment (nasal swabs) is presented per farm in Table II. In total 29 *Pasteurella multocida* strains were isolated, among which 93% were susceptible to oxytetracycline. All of the 16 *P. multocida* strains which were tested for their susceptibility to tilmicosin were susceptible. In total 10 *Mannheimia haemolytica* strains were isolated among which 80% were resistant to oxytetracycline. All of the 5 *M. haemolytica* strains which were tested for their susceptibility to tilmicosin were susceptible. The presence of *Mycoplasma* was demonstrated by culture at farm C (17 cases) and at farm L (4 cases).

3.5. SEROLOGICAL FINDINGS

Seroconversion was observed against *Mycoplasma bovis* at 2 farms (L & Q). At farm C, animals were already positive at D0, therefore it was not possible to observe seroconversion. No sample was mistakenly analysed at farm G.

3.6. TOLERANCE DATA

No local site and no general adverse reactions were observed during the study.

**IV- Discussion**

The objective of the study was to test the efficacy of a combination of long-acting OXY/FLU in cattle suffering from respiratory infection when compared to 2 reference products, OXY alone or TIL.

The percentage of animals that were swab sampled was 6%, 20%, 34% and 100% according to farms. It was planned in the study protocol to sample about 20% of included animals. This was respected at farm Q. An insufficient number (6%) of samples was mistakenly made at farm G. At the last farm included (C), the Sponsor wished a swab sampling on all included animals.

Isolates from nasal swabs were consistent with the bacteria commonly reported in association with respiratory disease in calves [14]. According to Derosa [4], a nasal swab culture can be predictive of the bacterial pathogen within the lung when the isolates are from an acutely ill animal and can be used to determine antibiotic susceptibility. Twenty-four matched pairs of isolates of *P. haemolytica* and 3 matched pairs of isolates of *P. multocida* were isolated using a nasal swab and a transtracheal swab from individual calves, with clinical signs of bovine respiratory disease. Although the calves were sampled only once with nasal and transtracheal swab, when both samples were bacteriologically positive, the nasal swab identified the same bacterial species as the transtracheal swab 96% of the time. The nasal swab isolate was genetically identical to the transtracheal isolate in 70% of the matched pairs.

In the present study, the finding of a high number of *M. haemolytica* (8 animals /10) resistant to oxytetracycline is questionable: Of these 8 animals, 3 received a combination OXY/FLU, 4 were treated with OXY and one was treated with TIL. Only 2 cases (out of 7 treated with either OXY/FLU or OXY) were not cured and required re-treatment. MECHOR and al. [13] have demonstrated that in vitro susceptibility testing of an antimicrobial agent against common respiratory pathogens often shows little relationship to the treatment response of naturally acquired respiratory infections.

Statistical analysis took into account the multiple comparison between the study groups. According to Jouan-Flahault’s paper [10], multiple comparisons may lead to wrongfully positive conclusions. Thus, probability of obtaining at least a wrongfully significant difference is 40% by carrying out 10 tests. It increases up to 64% when 20 tests are carried out. However, this probability is based on independent supposed tests. Even if the tests used in comparative clinical studies are often positively correlated, it was not possible, in our study, to make up to 48 tests while keeping...
the effectiveness of the combination therapy since a significantly lower temperature was observed in animals treated with the combination therapy at six hours and at one day after treatment.

Unlike the comparison with one reference product, the current study also compared the OXY/FLU to OXY alone (at the registered dose of 20mg/kg). A significantly lower temperature was observed in animals treated with the combination therapy at six hours and at one day after treatment.

Long-acting oxytetracycline and tilmicosin are successfully used in the treatment of calf pneumonia worldwide [11, 14]. These drugs have a demonstrated efficacy in the treatment of bovine respiratory infection but if they are compared to each other, tilmicosin is more effective in resolving clinical signs.

MUSSER et al. [14] compared the efficacy of a single parenteral injection of TIL at 10 mg/kg with that of a single dose of a long-acting OXY at 20 mg/kg in an outbreak of undifferentiated respiratory disease in young dairy calves at 5 farms. On the basis of response to initial treatment, relapse rates and effect on growth rates, the antibiotics were determined to be equally effective. However the severity of clinical disease was significantly less for the TIL-treated calves.

LAVEN and ANDREWS [11] compared the efficacy of TIL with that of a long-acting OXY at the same doses as the previous study. They concluded that both antibiotics were effective in treating the pneumonia but a significant number of OXY-treated calves required re-treatment.

Our study confirms that TIL alone and the combined OXY/FLU treatment are more clinically effective than oxytetracycline alone. Furthermore, it also establishes that the OXY/FLU combination achieves an acceleration of the clinical recovery in comparison with TIL. A significant greater reduction in rectal temperature as quick as six hours post-treatment was observed in OXY/FLU group compared to both reference groups. Consequently the combined treatment improves animal welfare to a significant degree and supports DOHERTY et al [5] as well as PATTERSON and ORR [16] in that HexaSolTM provides an efficacious RD treatment of real practical benefit and advantage over long acting antibiotic therapy alone.

V- References