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Research Article

Effectiveness and Safety of a Novel Flunixin Meglumine Transdermal Pour-On Solution in the Treatment of Bovine Respiratory Disease

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Abstract

The safety and effectiveness of a new flunixin transdermal pour-on solution for cattle was evaluated as adjunct therapy in the treatment of bovine respiratory disease (BRD), compared to a positive control group treated with carprofen injectable solution. A total of 206 animals, showing severe signs of respiratory disease, were randomly assigned to treatment with either the test product, flunixin (Finadyne® Transdermal; MSD Animal Health) administered topically once along the dorsal midline, or the control product, carprofen (Rimadyl®; Zoetis) administered by subcutaneous injection once, on day 0. All animals received cefquinome (Cobactan® LA 7.5%; MSD Animal Health) on day 0 and day 2. The animals were observed for clinical signs of disease for 6 hours following treatment initiation and daily thereafter for 5 consecutive days. The decrease in rectal temperature 6 hours after treatment initiation was greater ($p<0.0001$) in the flunixin group (-1.30°C) compared to the control group (-0.96°C). In both treatment groups, rectal temperature and clinical index (depression and respiratory signs) improved similarly over time. The Finadyne® Transdermal 50 mg/ml pour-on solution for cattle was found to be a safe and efficacious therapy in the treatment of signs of inflammation associated with naturally occuring bovine respiratory disease.

Keywords: Flunixin ; Bovine Respiratory Disease ; Inflammation ; Field trial ; Pour-on

Introduction

Bovine respiratory disease (BRD), also known as bovine bronchopneumonia, is a disease of considerable economic significance worldwide that affects cattle of any age at all stages of production. The disease is responsible for freater production costs due to increased labor, decreased productivity, and increased losses associated with morbidity, mortality and poor growth rates [1, 2]. Respiratory disease in cattle is often presented as a complex because it results from the interactions between the microorganisms in the respiratory tract, environmental stress factors, and the animal's susceptibility, depending on its immune status and "hardiness" [3]. Transportation over long distances, delays and commingling in markets, poor appetite after arrival on the

farm, and change of ration composition, may each contribute to compromises of the immune system function [4]. Often, the disease is initiated by a primary viral infection which may impair the animal's innate immune response and trigger subsequent bacterial infection by commensal organisms [3]. However, bacteria can also act as primary pathogens.

Whether the disease-causing factor is physical, environmental, or infectious, a sequence of events occurs resulting in inflammation and ultimately activation of the innate and adaptive immune systems. Inflammation classically consists of "redness", swelling, heat, and pain, all of which can become life threatening to the host when they occur in the respiratory tract [5]. The pulmonary inflammatory reaction is usually beneficial in controlling the infectious process; however, depending on its degree of intensity, inflammatory mediators such as arachidonic acid derivatives, autacoids, cytokines, neuropeptides, and cytolytic products may be released in the respiratory tract. These inflammatory mediators may cause pulmonary lesions and affect the animal's respiratory function impairing gas exchanges [6,7]. Therefore, it is advisable that non-steroidal anti-inflammatory drugs (NSAID) and anti-infective agents are used concurrently.

Flunixin is one of the most widely used NSAIDs in veterinary medicine. Flunixin is used with meglumine that makes the drug soluble. Flunixin has anti-inflammatory, anti-pyretic and analgesic effects. This molecule is commonly used for the relief of pain and control of inflammation and pyrexia associated with diseases of different origin and nature [8]. Its mode of action involves the inhibition of the cyclo-oxygenase (COX) that results in decreased formation of prostaglandins (PG) such as PGE_2 [9], $PGF_{2\alpha}$ [10] and PGI_2 [11], and thromboxanes (TX) such as TXB_2 [12]. These inflammatory mediators are presumed to be responsible for much of the lung damage resulting from BRD [13], to produce a marked potentiation of pain [14] and are believed to be the proximal mediator of the febrile response [15, 16].

Flunixin as an injectable solution (marketed as Finadyne® or Banamine®; MSD Animal Health) has been on the market in numerous countries worldwide for more than 30 years and its safety is well established [17]. Since its first registration, it gained several additional approvals in various livestock and other domestic species and can be administered today either intravenously or, in some countries, intramuscularly. A novel 50 mg/ml flunixin transdermal formulation was developed by MSD Animal Health (Finadyne® Transdermal) and is now the first NSAID registered to be administered as a pour-on product along the dorsal midline in cattle. The objective of the present study was to determine the field effectiveness of the 50 mg/ml flunixin transdermal formulation as adjunct therapy in the treatment of naturally occurring BRD.

Materials and Methods

This field study was conducted in accordance with the Veterinary International Conference on Harmonization (VICH) guideline on good clinical practices [18].

Animals, husbandry and pre-enrolment observation period

Seven different sites (A, B, C, D, E, F and G), of different sizes, representing typical farm management practices of each region, were selected in France, Germany, and Spain. Cattle, originating from local auction markets, were monitored until a BRD outbreak occurred. Pure breeds including Aubrac, Blonde d'Aquitaine, Charolais, Gasconne, Holstein Friesian, Limousin, Mirandaise, Red Holstein, Salers and cross breeds were enrolled in the study.

Cattle were in a typical growing state, ruminating, and were maintained according to the customary practices of the farm. Cattle were housed in standard pens with natural light and natural ventilation. All animals were uniquely identified by their national identification number. Animals were fed a commercial non-medicated diet and had *ad libitum* access to water. No vaccination, antibiotics, anti-inflammatory drugs, or other medications were administered to the animals. Administration of any topical or pour-on products on the dorsal midline after arrival at the farm and prior to enrollment in the study was strictly forbidden.

Enrolment

At the time of the BRD outbreak, all animals were examined for depression (normal=0; mild=1; moderate=2 and severe=3) and the characterisation of the respiratory signs such as polypnoea (≥ 40 breaths/minute), dyspnoea (abnormal respiration), cough and mucopurulent nasal discharge (each sign counted for one point to establish respiratory character score). The rectal temperature was also measured with an electronic calibrated thermometer. All animals with a depression score ≥ 2, a respiratory score ≥ 2 and a rectal temperature ≥ 40.3°C were enrolled in the study (day 0).

Randomisation and drug administration

After clinical examination and qualification at day 0, calves were weighed to ensure accurate treatment dosage. Then, cattle were assigned to treatment groups, following a chronological order regardless of gender, using a computer-generated random code, which was given to the dispenser under separate cover to preserve masking of the clinicians. Randomisation was accomplished in advance according to a randomised complete block design based on site and order of enrolment. Enrolled animals remained in their original pen at arrival. Both treatment groups were commingled in each pen.

Once randomly allocated, each animal was treated either with the test product, 50 mg/ml flunixin (Finadyne® Transdermal; 3.3 mg/kg flunixin; 1ml/15kg; MSD Animal Health) administered topically once along the dorsal midline, or the control product, 50 mg/ml carprofen (Rimadyl®; 1.4 mg/kg carprofen; 1ml/35kg; Zoetis) administered by subcutaneous injection once, on day 0. A dye in saline solution was also administered once topically along the dorsal midline of animals treated with carprofen in order to preserve masking. Animals from both treatment groups received cefquinome

(Cobactan® LA 7.5%; 1ml/30kg cefquinome; 2.5 mg/kg; MSD Animal Health) administered by subcutaneous injection on day 0 and day 2.

To avoid observation bias, treatments were dispensed and administered by individuals not involved in the clinical assessment.

Clinical assessment and treatment success

Following treatment on day 0, individual animals had the rectal temperature (°C) measured at 6±1 hours (target: 6 hours) following dosing. From day 0 at 6±1 hours to day 5, individual animals were examined daily, for clinical assessment including depression, respiratory characteristics, and rectal temperature measurements. During this period that was considered the recovery period, animals were not considered for failure evaluation. The dosing sites was evaluated twice on day 0 (prior to treatment and 6±1 hours post-treatment) and once daily from day 1 to day 5. If dosing site abnormality was present, the size of reaction and the type of skin reaction were recorded.

On day 5, animals with a depression score ≥ 1 or a respiratory score ≥ 2 that had a rectal temperature ≥ 40.0°C were defined as treatment failures. If these criteria were not met, the animals were defined as treatment successes (depression score = 0 and a respiratory score ≤ 1 with a rectal temperature < 40.0°C).

Sampling procedure and bacteriology

On day 0, prior to treatment administration, a protected nasopharyngeal swab, 300 mm in length, was taken from each enrolled animal. Animals defined as treatment failures were sampled again. Blood samples were also collected from four animals enrolled at each site on day 0 prior to treatment; the same animals were re-sampled on day 21±2. All samples were kept at 4°C, shipped immediately to a centralised laboratory, received within 48 hours and processed within 24 hours of receipt. The microbiological examination of the samples included culture and identification of the principal BRD pathogens in Europe: *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and/or other respiratory pathogens. All pathogens (excepted *Mycoplasma* isolates) isolated from pre-treatment samples were tested for cefquinome sensitivity by disk diffusion. All pathogens (excepted *Mycoplasma* isolates) isolated from post-treatment samples were tested for determination of cefquinome minimum inhibitory concentration (MIC). All microbiological procedures were performed in accordance with the Clinical Laboratory Standards Institute (CLSI) recommendations. Blood samples were serologically analysed for paired titers of infectious bovine rhinotracheitis (IBR), bovine respiratory syncytial virus (BRSV), parainfluenza virus 3 (PI3), adenovirus and bovine viral diarrhoea virus (BVDV) to get representative information on concomitant viral infections.

Experimental unit and sample size justification

The experimental unit was the individual animal. The sample size justification was based on a test of non-inferiority of flunixin against positive control. Assuming an 86.3% success rate in both treatment groups, the minimum sample size to claim non inferiority (no higher rates of treatment failures), within a ±15% margin with 80% power and alpha of 5%, was n=83 (nQuery Advisor version 4.0). Taking into account possible exclusions, the target sample size was determined to be 93 animals per treatment group.

Statistical analysis

The pivotal criterion was the percent change between the day 0, pre-treatment (Time 0h) rectal temperatures (°C) and day 0, 6-hour post-treatment (Time 6h) rectal temperatures (°C). The secondary criterion was the overall treatment success rate on day 5. For regulatory purposes, tests of non-inferiority were performed on these criteria. For the change in rectal temperature, the flunixin group was compared to the positive control group using an equivalence testing for normally distributed independent data. For the success rates, the binomial treatment success rates on day 5 were analyzed using the equivalence testing for independent binary endpoints via the Hauck-Anderson corrected classical procedure from Tu. In addition to regulatory non-inferiority tests, the differences between groups were also analysed by a Student T-test for the change in rectal temperature and a Chi-square test for the success rates. Rectal temperature, depression score, and respiratory score were formally analyzed using mixed models analysis of covariance for a repeated measures design. No data transformations were applied. The animal's baseline response corresponded to the pre-dosing measurement. This baseline response was examined for possible inclusion in the model as it was significant at the 5% level of significance ($p<0.0001$ for rectal temperature and respiratory score, $p=0.0011$ for depression score). The appropriate covariance structures were selected using the Akaike Information Criterion. The fixed factor in the model was the treatment group and the repeated factor was the study day. The animal (experimental unit) was included as a random factor. Interactions between time and treatment group were also included. The statistical hypotheses being tested were that there were no differences between the treatment groups. All statistical analyses were performed in SAS/STATÒ (version 9.2).

Results

General observations

A total of 206 animals were enrolled in this study of which 197 met all protocol criteria for analysis. Among them, 99 were allocated to the flunixin group and 98 to the control group. The number of animals enrolled at the 7 sites ranged from 10 to 47. Of the 197 enrolled animals, 112 animals were male none castrated and 85 were female. The animals were ruminating and not pregnant. The age varied between 31 days to 22 months at enrolment. The body weight of enrolled animals ranged from 42.5 to 457 kg with an average (± SD) of 192.41 ± 119.25 kg for the 99 enrolled animals in the

flunixin group and 198.13 ± 121.45 kg for the 98 enrolled animals in the control group. The average body temperature at enrolment for both treatment groups was 40.6°C(Table 2).

Bacteriological and MIC results

A total of 229 samples (per protocol population) were collected during the study: 197 nasopharyngeal swab samples on day 0, prior to treatment, and 32 nasopharyngeal swab samples post-treatment from animals classified as treatment failure. *Mannheimia haemolytica*, *Pasteurella multocida*, and *Mycoplasma bovis* were isolated at almost all sites. In addition, *Histophilus somni* was isolated pre-treatment at sites C and E (Table 1).

At the onset of the outbreaks, *Mannheimia haemolytica* was the most prevalent (n=102 or 51.78%). *Pasteurella multocida* was the second most prevalent organism (n=81 or 41.12%), followed by *Mycoplasma bovis* and spp (n=67 or 34.01%), and *Histophilus somni* (n=3 or 1.52%). From treatment failures, the frequency of isolates was: *Pasteurella multocida* (n=4 or 12.5%) and *Mycoplasma bovis* and spp (n=29 or 90.63%). No *Mannheimia haemolytica* and *Histophilus somni* was isolated from the treatment failures.

All bacteria, except *Mycoplasma bovis* and spp, isolated during the study were susceptible to cefquinome. The 186 pathogens isolated from pre-treatment samples were tested for cefquinome sensitivity by disk diffusion and all were found susceptible to cefquinome with a range of zone diameter from 24 mm to 45 mm. Only 4 *Pasteurella multocida* were isolated post-treatment and had a cefquinome MIC between 0.06 and 0.25 µg/ml. Although no MIC breakpoint is defined by CLSI, the isolates could be considered susceptible to cefquinome as the results were in line with the current database.

Table 2.

Summary of efficacy results

Variable	Flunixin	Control
N enrolled animals	99	98
Age (days) at enrolment	157.01 ± 96.81	172.96 ± 115.27
Body weight (kg) at enrolment	192.41 ± 119.52	198.13 ± 121.45
Rectal temperature (°C) at enrolment	40.66 ± 0.33	40.69 ± 0.34
Rectal temperature (°C) at 6 h after treatment	39.36 ± 0.52	39.73 ± 0.5
Drop in temperature 6 h after treatment [a]	-1.3 °C	-0.96 °C

[a] The decrease in rectal temperature 6 h after treatment was statistically significantly greater in the flunixin group compared to the control group ($p<0.0001$).

Table 1.

Case load, treatment allocation and bovine respiratory disease (BRD) outbreak characterisation at enrolment

Site	*N* enrolled animals			*N* animals positive for BRD target pathogens			
	Flunixin	Control	Total	*Pasteurella multocida*	*Mannheimia haemolytica*	*Histophilus somni*	*Mycoplasma bovis*
A	5	5	10	6	2	0	0
B	22	21	43	10	28	0	3
C	23	24	47	14	29	1	34
D	12	12	24	12	2	0	10
E	10	10	20	11	11	2	5
F	13	14	27	18	14	0	2
G	14	12	26	10	16	0	2
Total	99	98	197	81	102	3	56

Serological findings

Concomitant viral infections caused by IBR, BRSV, PI3, Adenovirus, and BVDV were detected serologically at different study sites. Clinical signs associated with these concomitant viral infections were not observed by the clinicians. However, as a seroconversion was detected on day 21 for BoHV-1 (sites C and D), BRSV (sites A, B, C, D and G), PI 3 (sites B, C and D), Adenovirus (sites B and C) or BVD (site C); the role of these viral infections in the severity of the disease cannot be excluded.

Decrease in rectal temperature 6 hours after treatment

The pivotal criterion was the change in rectal temperatures (°C) on day 0, between pre-treatment (Time 0h) and 6 hours post-treatment (Time 6h). The mean temperatures at the pre-treatment time point (Time 0h) were 40.66°C in the flunixin group and 40.69°C in the control group, and both groups were homogenous at this time point (p=0.5291). The mean temperatures at 6 hours post-treatment (Time 6h) were 39.36°C in the flunixin group and 39.73°C in the control group. The change in mean rectal temperature from Time 0h to Time 6h was -1.30°C in the flunixin group and -0.96°C in the control group (Figure 1). The non-inferiority was demonstrated. This difference was statistically significant (p<0.0001) and the superiority of the flunixin transdermal to the control was confirmed for the pivotal variable.

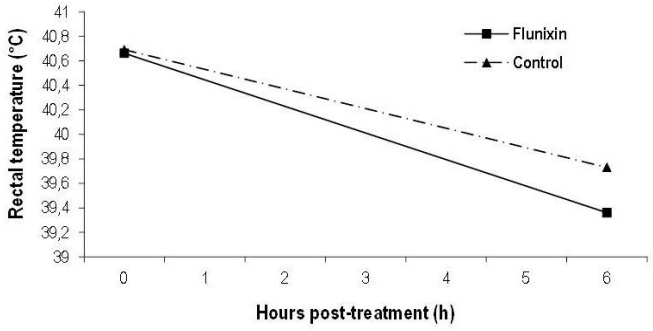

Figure 1. Effect of treatment on mean rectal temperature (°C) 6 hours after dosing.

Rectal temperature, depression and respiratory signs

The rectal temperature was recorded from each animal from day 0 to day 5. On day 0, rectal temperature ranged from 40.3°C (which was one of the inclusion criteria to 41.8°C). Shortly after treatment administration on day 0 (6 hours post-treatment), a decrease in temperature was observed in both treatment groups with a lower temperature measured in the flunixin group than in the control group (p<0.0001; Figure 1). Then, the rectal temperature leveled off in the subsequent days, and the progression of the rectal temperature over that time period was similar in both treatment groups (data not shown).

The effect of treatments on the mean depression score at 6 hours after treatment is shown in Figure 2. At enrolment, a moderate or severe depression was observed (Figure 2). The mean depression score was 2.02 in the flunixin group and 2.01 in the control group (Figure 3), and both groups were homogenous (p=1.0000). Six hours post treatment, a significant decrease (p<0.0001) in the severity of depression was observed in both treatment groups with a slightly greater improvement of animal condition observed in the flunixin group than in the control group (Figure 2). The mean depression score at this time point was 1.02 in the flunixin group and 1.1 in the control group (Figure 3). In the following days, a decrease in the severity of depression was observed in both treatment groups (Figure 3). On day 5, 80% of animals did not present any sign of depression in both groups.

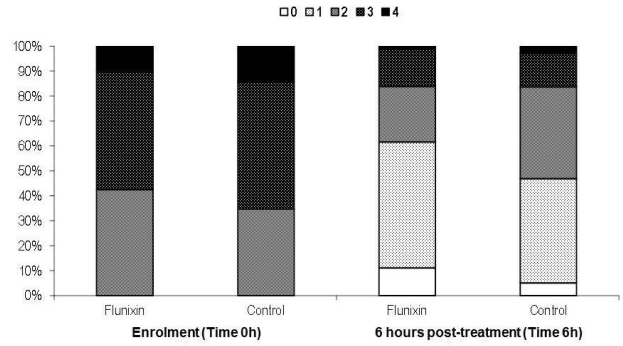

Figure 2. Effect of treatment on mean respiratory characters score 6 hours after dosing.

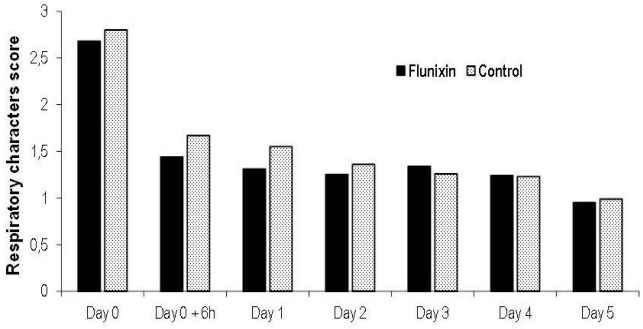

Figure 3. Progress of respiratory characters score from day 0 pre-treatment (enrolment) to day 5.

The effect of treatments on the mean respiratory characters score at 6 hours after treatment is shown in Figure 4. At enrolment, two or more respiratory signs were observed (Figure 4). The mean respiratory characteristics score was 2.68 in the flunixin group and 2.80 in the control group (Figure 5), and both groups were homogenous (p=0.4501). Six hours post-treatment, a decrease (p<0.0001) in the number of respiratory signs was observed in both treatment groups with a more pronounced improvement of animal respiratory health observed in the flunixin group than in the control group (Figure 4). The mean respiratory characteristic score at this time point was 1.44 in the flunixin group and 1.67 in the control group (Figure 5). In the following days, a decrease in the number of respiratory signs was observed in both treatment groups (Figure 5).

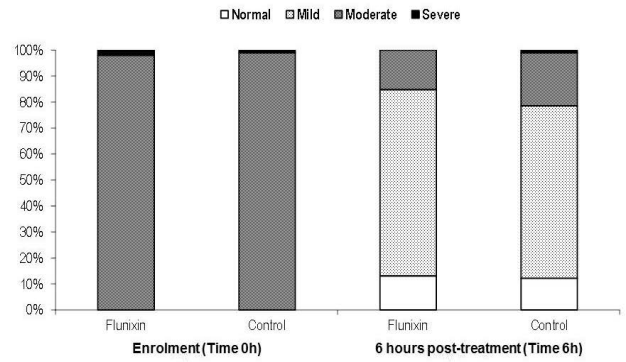

Figure 4. Effect of treatment on mean depression score 6 hours after dosing.

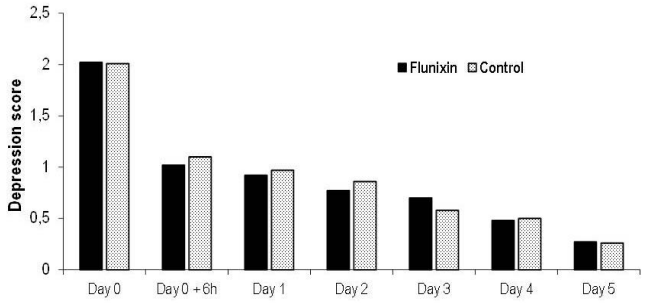

Figure 5. Progress of depression score from day 0 pre-treatment (enrolment) to day 5.

The day 5 success rates were 80.81% (80/99) for the flunixin group and 86.73% (85/98) for the control group. Although non-inferiority was not demostrated, no evidence of statistical differences were found (p=0.2595).

Dosing site reactions and safety

A very low number of treated animals presented dosing site reactions throughout the observation period. The percentage of dosing site reactions was very low: < 1% of animals in the control group who also received along the dorsal midline a red-dyed saline solution for masking, and < 4% animals in the flunixin group. These reactions were limited to skin flakes and erythema, with 1 or 2 animals showing sensitivity to palpation.

Throughout the study, 2 adverse reactions, 1 in each treatment group, were reported. Two of the animals were reported to have diarrhoea due to acidosis on day 1 and was spontaneously resolved without any concomitant therapy. The clinician stated these adverse reactions had no obvious relationship with the administered product. No animal died or was euthanased during this study.

Discussion

The effectiveness of flunixin for the treatment of inflammation and relief of pain is well established throughout the world in different cattle management systems and is considered a benchmark as adjunctive therapy in the treatment of diseases such as respiratory disease [19, 20, 21, 22, 23], acute mastitis [24, 25, 26, 27, 28, 29] and musculoskeletal disorders [30]. Flunixin as an injectable solution is well-absorbed with high bioavailability, and it provides anti-inflammatory effects in a short time [31]. Its safety is well established in various species [17]. Most of these properties were recently confirmed in cattle for the new 50 mg/ml flunixin transdermal pour-on solution (MSD Animal Health, data on file). The objective of the current study was to evaluate the safety and the clinical effectiveness of this new flunixin transdermal pour-on solution in comparison with carprofen injectable solution as positive control. The statistical analysis of the data demonstrates the superiority of the flunixin transdermal over the positive control for the decrease in rectal temperature 6 hours after treatment administration.

Based on the prevalence of the isolated pathogens in the pre-treatment samples, the outbreaks can be attributed to *Mannheimia haemolytica* and *Pasteurella multocida*. *Mycoplasma bovis* and *Histophilus somni* were also detected. These bacterial species, especially *Mannheimia haemolytica*, stimulated an inflammatory process in the lungs that resulted in a severe bronchopneumonia as demonstrated by the severe inclusion criteria met by the animals enrolled in the current study. The rapid reduction of temperature, defined as the primary effectiveness criterion, is one of the main aims in the treatment of such respiratory disease. Both treatments were effective to reduce pyrexia; however, the decrease in temperature was significantly greater with flunixin transdermal. This latter treatment acted quickly and was efficacious to prevent the deleterious effects of inflammation as it was shown by the clinical index (depression and respiratory signs) improvement. These results provide evidence that anti-inflammatory treatment is essential as it relieves pain, thereby facilitating respiratory movements, and diminishes the intensity of the febrile syndrome which further improves the feeling of comfort and allows for the return to appetite.

It is interesting to note the large number of successes in the present study where a complex aetiology was reported, knowing that the interaction between *Mannheimia haemolytica* or *Pasteurella multocida* and *Mycoplasma bovis* can often lead to more severe disease than one pathogen alone [32, 33]. These results demonstrate the benefit from prompt treatment with a NSAID and an adequate broad-spectrum antibiotic more than an antibiotic alone. The animals were immediately treated at the onset of the disease and therefore appear to have been protected from the development of severe or fatal lung lesions.

In the current study, no single animal died or was euthanased. Neither flunixin nor control had a negative influence on the health status including appetite and faecal consistency. Only two animals developed diarrhoea which was attributed to acidosis and therefore not considered to be drug related. It has been widely accepted that most of the toxic effects of NSAIDs such as gastric irritation, renotoxicity and interference with clotting mechanisms, are due to the inhibition of COX-1, while the beneficial anti-inflammatory and analgesic actions are attributable to the inhibition of COX-2

[34]. However, recently, this general assumption has been challenged by the reported increased risk of severe adverse cardiovascular events induced by selective COX-2 inhibitors in humans [35] or renal failure in rats [36]. In this study no cardiovascular or renal events have been observed by the clinician. Recently in vitro experiments demonstrated that flunixin meglumine does not have any stronger selectivity toward one of the two COX isoforms although a preferential activity against COX-1 was shown with a COX-1/COX-2 ratio < 1 [37]. This in line with the very good safety results obtained in this study as no adverse effects due to COX-1 inhibition has been observed.

In addition to the absence of adverse reaction, a formulation that is administered transdermally as a pour-on has the advantages of easier and safer administration for handlers, and reducing stress on cattle. The ease and convenience of administration of flunixin transdermal compared to other methods of administration of NSAID to cattle can certainly be an incentive for a higher number of animals to be treated with a NSAID when facing a disease outbreak, resulting in improved herd management. Moreover, side effects resulting from an injection such as but not limited to carcass damage, phlebitis and local injection site reactions would be avoided by transdermal administration.

Conclusion

Based on the study results, it can be concluded that the new 50 mg/ml flunixin transdermal solution has strong anti-pyretic effect and anti-inflammatory properties, and make it a very convenient and suitable adjunct therapy to anti-infective therapy used in cases of respiratory infections in cattle. To conclude, Finadyne® Transdermal 50 mg/ml pour-on solution for cattle was found to be a safe and effective therapy in the treatment of the signs of inflammation associated with naturally occurring bovine respiratory disease.

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Conflict of Interest

Julien Thiry, Philippe Brianceau and Vincent de Haas are employees of Merck Animal Health.

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