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## Research Article

### Flunixin Meglumine Transdermal Pour-on Solution as Adjunct Therapy in The Treatment of Bovine Respiratory Disease in Calves Less Than 8 Weeks of Age

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## Abstract

The safety and efficacy of a new flunixin transdermal pour-on solution for cattle, used as an adjunct therapy in the treatment of bovine respiratory disease (BRD) in juvenile calves less than 8 weeks of age, was evaluated. A total of 49 calves of less than 8 weeks of age, 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 a positive control product, carprofen (Rimadyl®; Zoetis) administered by subcutaneous injection once, on day 0. All animals received a long acting injection of 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 ( $-1.68^{\circ}\text{C}$ ) compared to the carprofen group ( $-1.02^{\circ}\text{C}$ ). The rectal temperature and clinical index (depression and respiratory signs) improved also over time. Flunixin had no negative influence on the health status. 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 occurring bovine respiratory disease in juvenile calves less than 8 weeks of age

**Keywords:** Flunixin; Bovine Respiratory Disease; Calves; 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. Respiratory disease in cattle is often presented as a disease complex that 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" [1]. 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. The inflammatory mediators may cause pulmonary lesions and affect the animal's respiratory function impairing gas exchange [2, 3]. Therefore, it is advisable that non-steroidal anti-inflammatory drugs (NSAID)

and anti-infective agents are used concurrently [4].

Flunixin is one of the most widely used NSAIDs in veterinary medicine. Flunixin is used as a meglumine salt, which 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 [4]. Its mode of action involves the inhibition of the cyclo-oxygenase (COX) that results in decreased formation of prostaglandins (PG) such as PGE2 [5], PGF2 $\alpha$  [6] and PGI2 [7], and thromboxanes (TX) such as TXB2 [8]. These inflammatory mediators are presumed to be responsible for much of the lung damage resulting from BRD [9], to produce a marked potentiation of pain [10] and are believed to be the proximal mediator of the febrile response [11, 12].

Flunixin as an injectable solution (marketed as Finadyne® or Banamine®; MSD Animal Health) has been commercially available in numerous countries worldwide for more than 30 years and its safety is well established [13]. Since its first registration, it gained several additional approvals in various livestock and other domestic species. A novel 50 mg/ml flunixin transdermal formulation has been developed (Finadyne® Transdermal; MSD Animal Health) 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 confirm the efficacy and the safety of this 50 mg/ml flunixin transdermal formulation as adjunct therapy in the treatment of naturally occurring bovine respiratory disease (BRD) in juvenile calves less than 8 weeks of age.

## Materials and methods

This field study was conducted in accordance with the Veterinary International Conference on Harmonization (VICH) guideline on good clinical practices [14].

The young calves less than 8 weeks of age reported in this article are a subset of a larger population studied in an European field study designed to confirm the efficacy of the topical application of flunixin transdermal 50 mg/mL pour-on solution.

## Animals, husbandry and pre-enrolment observation period

Calves enrolled in the study were Holstein originating from German auction markets and were monitored until a BRD outbreak occurred. Calves were pre-ruminating and were maintained according to the customary practices of the farm. They were housed grouped with adults 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 for 30 days, since their arrival at the farm. Similarly, the 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 and drug administration

At the time of the BRD outbreaks, all calves were clinically examined for depression (normal=0; mild=1; moderate=2 and severe=3) and the characterisation of the respiratory signs such as polypnoea ( $\geq 40$  breaths/minute), dyspnoea (abnormal respiration), cough and mucopurulent nasal discharge. Each respiratory sign counted for one point to establish a respiratory character score (score ranged from

0 [no respiratory sign] to 4 [4 respiratory signs described above]). The rectal temperature was also measured. All animals with a depression score  $\geq 2$ , a respiratory score  $\geq 2$  and a rectal temperature  $\geq 40.30^\circ\text{C}$  were enrolled in the study (day 0).

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 treatment 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 within 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. As the pharmaceutical form of flunixin transdermal pour-on solution is a clear red liquid, a red 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.

## Clinical assessment and treatment success

Following treatment on day 0, individual animal rectal temperature ( $^\circ\text{C}$ ) was measured at  $6\pm 1$  hours following dosing. From day 0 at  $6\pm 1$  hours to day 5, animals were examined daily, for clinical assessment including depression, respiratory characteristics, and rectal temperature measurements.

The dosing sites were evaluated twice on day 0 (prior to treatment administration and  $6\pm 1$  hours post-treatment) and once daily from day 1 to day 5. If a dosing site abnormality was present, the size of reaction (small:  $< 10$  cm, medium: 10 to 50 cm, or large:  $> 50$  cm) and the type of skin reaction (thickening of skin, alopecia, skin flakes, or other reaction) was recorded. Any other reaction observed was also recorded.

On day 5, animals with a depression score = 0 and a respiratory score  $\leq 1$  that had a rectal temperature  $< 40.00^\circ\text{C}$  were defined as treatment successes. Otherwise, animals were defined as treatment failures (depression score  $\geq 1$  or respiratory score

≥ 2, and a rectal temperature ≥ 40.00°C).

## Sampling procedure and bacteriology

On day 0, prior to treatment administration, a nasopharyngeal swab sample, was taken from each enrolled animal using a 300 mm protected swab. Animals defined as treatment failures were sampled again. Blood samples were also collected from 12 animals enrolled 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 (except *Mycoplasma* isolates) isolated were tested for cefquinome sensitivity by disk diffusion. All microbiological procedures were performed in accordance with the Clinical Laboratory Standards Institute (CLSI) recommendations. Blood samples were serologically analysed for 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.

## 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 flunixin group was compared to the carprofen group using an equivalence testing for normally distributed independent data. The secondary criterion was the overall treatment success rate on day 5. 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. 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. 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/STAT<sup>®</sup> (version 9.2).

## Results

### General observations

A total of 49 calves less than 8 weeks of age were enrolled and met all protocol criteria for analysis. Among them, 23 received flunixin and 26 received carprofen. All animals were female and Holstein (Friesian or Red). The age ranged between 31 and 56 days at enrolment. The body weight ranged from 42.5 to 87 kg. The body temperature at enrolment ranged from 40.3°C to 41.3°C with an average of 40.6°C.

### Bacteriological and serological findings

A total of 49 nasopharyngeal swab samples were collected during the study. All samples were collected on day 0, prior to treatment, and no samples collected post-treatment as no animal was classified as treatment failure. At the onset of the outbreaks, *Pasteurella multocida* (n=23 or 46.9%) and *Mannheimia haemolytica* (n=23 or 46.9%) were the most prevalent organisms present at the farm. *Mycoplasma bovis* and spp (n=8 or 16.3%) and *Histophilus somni* (n=2 or 4.1%) were also isolated. Pathogens isolated were tested for cefquinome sensitivity by disk diffusion and, with the exception of *Mycoplasma bovis* and spp which were not tested, all were found susceptible to cefquinome with a range of zone diameter from 30 mm to 45 mm.

Concomitant viral infections caused by IBR, BRSV, PI3, Adenovirus, and BVDV were detected serologically. Clinical signs associated with these concomitant viral infections were not observed by the clinicians; however, 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 to assess the efficacy of flunixin transdermal was the change in rectal temperature (°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 40.67°C in the flunixin group and 40.62°C in the carprofen group, and both groups were homogenous at this time point (p=0.5693). The mean rectal temperature at 6 hours post-treatment (Time 6h) was 38.99°C in the flunixin group and 39.60°C in the carprofen group. The change in mean rectal temperature from Time 0h to Time 6h was -1.68°C in the flunixin group and -1.02°C in the carprofen group (Figure 1). The non-inferiority was demonstrated. This difference was statistically significant (p<0.0001) and the superiority of flunixin transdermal over

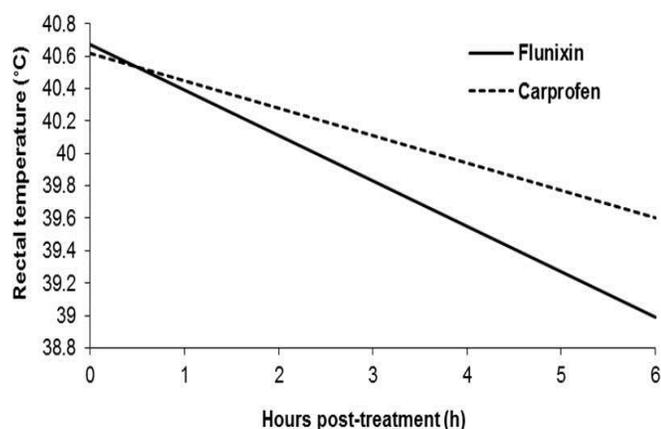
carprofen was confirmed for the pivotal variable.

**Table 1.** Summary of efficacy results

Variable	Flunixin	Carprofen
N enrolled animals	23	26
Age (days) at enrolment	42.52 ± 5.92	44.69 ± 7.13
Body weight (kg) at enrolment	62.70 ± 8.44	66.79 ± 10.54
Rectal temperature (°C) at enrolment	40.67 ± 0.29	40.62 ± 0.27
Rectal temperature (°C) at 6 h after treatment	38.99 ± 0.42	39.60 ± 0.63
Drop in temperature 6 h after treatment <sup>a</sup>	-1.68 °C	-1.02 °C
Treatment success rates on day 5 <sup>b</sup>	100% (23/23)	100% (26/26)

<sup>a</sup>The decrease in rectal temperature 6 h after treatment was statistically significantly greater in the flunixin group compared to the carprofen group ( $p < 0.0001$ ).

<sup>b</sup>The non-inferiority of the flunixin group compared to the carprofen group was statistically demonstrated.



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

### Overall treatment success on day 5

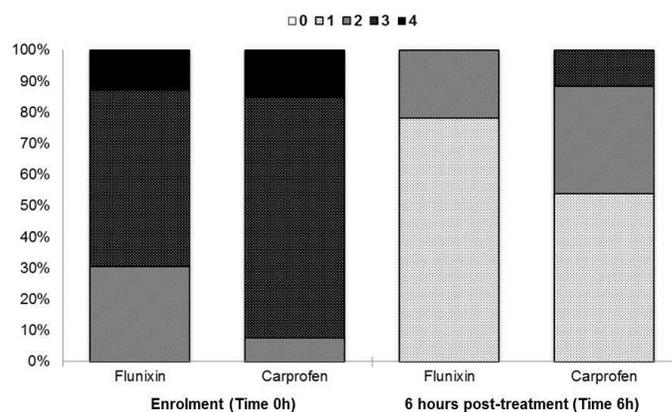
Another pivotal criterion to evaluate the clinical efficacy of flunixin transdermal in juvenile calves was the overall treatment success. The day 5 success rates were 100% (23/23) for the flunixin group and 100% (26/26) for the carprofen group. The 95% CI for the difference in success rates on day 5 was [-0.02, 0.02]. Since the lower limit was more than -0.15, flunixin transdermal was significantly non inferior to carprofen for the treatment success on day 5.

### Rectal temperature, respiratory signs and depression

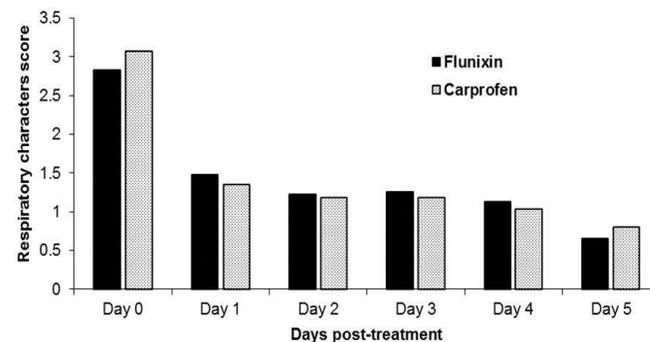
The rectal temperature was recorded from each animal from day 0 to day 5. On day 0, rectal temperature ranged from 40.30°C (which was one of the inclusion criteria) to 41.30°C. Shortly after treatment administration on day 0 (6 hours

post-treatment), a decrease in temperature was observed in both treatment groups with a lower temperature measured in the flunixin group than in the carprofen 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 respiratory characters score at 6 hours after treatment is shown in Figure 2. At enrolment, two or more respiratory signs were observed, and the mean respiratory score was 2.83 in the flunixin group and was 3.08 in the carprofen group. Six hours post-treatment, a significant decrease ( $p < 0.0001$ ) was observed, the mean respiratory score was 1.22 in the flunixin group and was 1.58 in the carprofen group. (Figure 2). In the following days, the respiratory signs greatly improved, to reach a mean respiratory score inferior to 1 on day 5 (Figure 3).



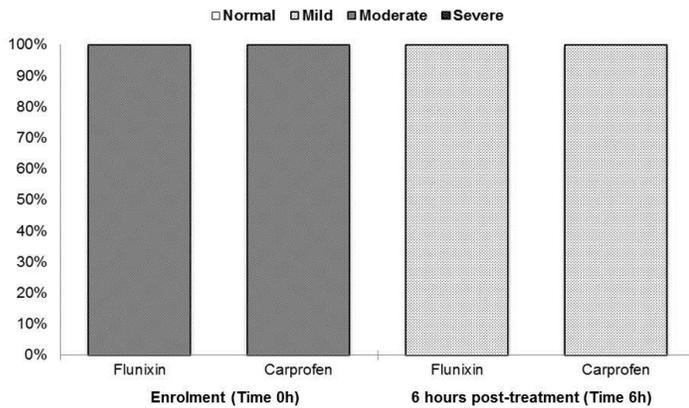
**Figure 2.** Effect of flunixin on mean respiratory score 6 hours after dosing. All animals were examined for the characterisation of the respiratory signs such as tachypnoea (or polypnoea) ( $\geq 40$  breaths/minute), dyspnoea (abnormal respiration), cough and mucopurulent nasal discharge. Each sign counted for one point to establish respiratory signs score.



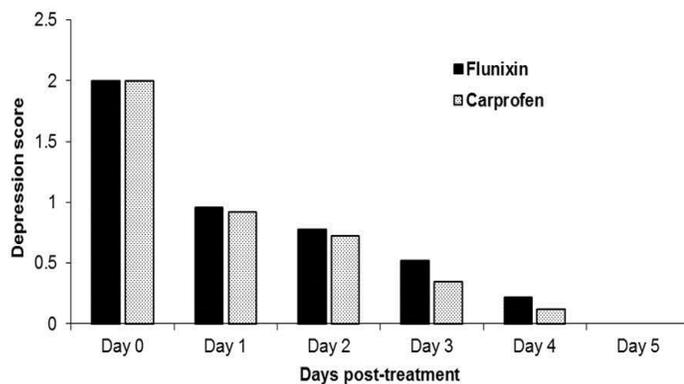
**Figure 3.** Progress of respiratory score from day 0 pre-treatment (enrolment) to day 5.

The effect of treatments on the mean depression score at 6 hours after treatment is shown in Figure 4. At enrolment, a

moderate depression was observed, and the mean depression score was 2. Six hours post treatment, a significant decrease ( $p < 0.0001$ ) in the severity of depression was observed with a mean depression score equal to 1. In the following days, a continuous decrease in the severity of depression score was observed until the disappearance of the depression signs on day 5 (Figure 5).



**Figure 4.** Effect of flunixin on mean depression score 6 hours after dosing. All animals were examined for the depression such as normal; mild; moderate and severe.



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

### Dosing site reactions and safety

For the animals treated with flunixin transdermal, only 1 erythema, measuring 10 to 50 cm, was detected on the dorsal midline on day 2. This reaction resolved spontaneously without any concomitant therapy. No other abnormal findings were observed. Flunixin and carprofen had no negative influence on the health status including appetite and faecal consistency. No animal died or was euthanased during this study.

### Discussion

The efficacy 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 [15, 16, 17, 18, 19], acute mastitis [20, 21, 22, 23, 24, 25] and musculoskeletal disorders [26]. Flunixin as an injectable solution is well-absorbed with high bioavailability, and provides onset of anti-inflammatory effects in a short time [27]. Its safety is well established in various species [13]. 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).

Because BRD also affects pre-ruminating calves and these young animals often show differences in treatment response compared to older cattle, it is relevant to report the efficacy and safety in this class of animals separately. One of the objectives of this report was to confirm the clinical efficacy of this new flunixin transdermal pour-on solution in calves less than 8 weeks of age. The statistical analysis of the data demonstrates that flunixin transdermal was able, 6 hours after treatment administration, to significantly decrease the temperature and to improve significantly the clinical index (depression score and respiratory signs). Flunixin transdermal was demonstrated to have a greater anti-pyretic effect compared to carprofen ( $p < 0.0001$ ).

It is interesting to note that the results obtained with flunixin transdermal in the current study are comparable to those recently published with an injectable florfenicol plus flunixin meglumine formulation in juvenile calves less than 6 weeks of age [32]. Indeed, a significant decrease in rectal temperature 6 hours after treatment administration ( $-1.68^{\circ}\text{C}$  for flunixin transdermal and  $-1.36^{\circ}\text{C}$  for florfenicol-flunixin injectable) and a large number of treatment successes (100% for flunixin transdermal on day 5 and 93.4% for florfenicol-flunixin injectable on day 4) were observed in both studies. However, conclusions about the comparison of the results between the two studies must be drawn with caution because the sizes of the studied population and the study conditions were different [32].

Another objective was to evaluate the safety this new flunixin transdermal pour-on solution in calves. It is known that very young calves less than 8 weeks of age are often subject to diverse disorders when administering drugs. In the current study, conducted with calves younger than 8 weeks of age and suffering from BRD, flunixin and carprofen had no negative influence on the health status including appetite and faecal consistency. It may be explained by the fact that most of the toxic effects of NSAIDs such as gastric irritation, nephrotoxicity 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 [28]. However, recently, this general assumption has been challenged by the reported increased risk of severe ad-

verse cardiovascular events induced by selective COX-2 inhibitors in humans [29] or renal failure in rats [30]. 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 [31]. 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, providing a very convenient and suitable adjunct therapy to anti-infective therapy used in cases of respiratory infections in cattle. Moreover, in this study, the temperature reduction was the pivotal criterion. This parameter is common to various disease conditions involving an inflammatory process. To conclude, flunixin transdermal 50 mg/ml pour-on solution was found to be a safe and effective therapy in juvenile calves less than 8 weeks of age to treat the signs of inflammation associated with naturally occurring bovine respiratory disease.

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#### Conflict of Interest

Julien Thiry and Philippe Brianceau are employees of Merck Animal Health. Merck Animal Health, known as MSD Animal Health outside the United States and Canada, is the global animal health business unit of Merck.

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