
Evaluation of the effect of ketoprofen on experimentally induced ephemeral fever in dairy heifers

DC FENWICK and RCW DANIEL

Department of Farm Animal Medicine and Production, The University of Queensland, Queensland 4072

Objective To evaluate ketoprofen for the therapy of ephemeral fever.

Design A blind controlled clinical trial.

Animals Sixteen cattle (one immature Holstein bull, eight Holstein and seven Jersey heifers).

Procedure Ephemeral fever was induced by the intravenous injection of blood leucocyte layer from a clinical case. Ketoprofen solution or a coded placebo was injected intramuscularly at the rate of 3 mg/kg daily for three days.

Results Ketoprofen reversed locomotor dysfunctions significantly compared with controls, but did not have any effect on rectal temperatures, leucocyte counts, plasma fibrinogen concentrations, ionised Ca-concentrations or the presence of dyspnoea.

Conclusion Ketoprofen is a safe and effective drug for the treatment of locomotor symptoms of milk fever, but has no effect on the duration of clinical respiratory abnormalities.

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Key words: Ephemeral fever, ketoprofen, cattle.

Ephemeral fever in cattle was reviewed by Uren (1989), who claimed that it is arguably the most important viral disease of cattle in Australia. It is endemic to extensive areas of Australia from Queensland to northern Victoria. Although 99% of affected animals recover spontaneously, it has been estimated to cause losses of millions of dollars annually in beef and dairy cattle. These losses are due to loss of weight, temporary sterility of bulls, abortions, decline in lactation and deaths. Mortality is estimated at 1%.

Phenylbutazone, acetylsalicylic acid, flunixin meglumine, tripelethamine, butophosphan and tylosin have been used empirically as treatments, but evaluation of their effectiveness has been limited and of doubtful value (Hungerford 1990). Intravenous calcium solutions have also been recommended (Uren 1989). There is a need for evaluation of new drugs for treatment of this disease.

This paper records the effects of ketoprofen on heifers infected experimentally with ephemeral fever.

Materials and methods

Experimental animals

One 9-months-old Holstein bull, eight Holstein and nine Jersey heifers aged 9 to 11 months were stalled at three different periods in three insect-proofed stalls, the heifers being housed at a density of 3 per pen (3.2 m x 2.8 m) and fed mixed grass and lucerne hay *ad libitum*. All animals were weighed on entering the unit, and the Jersey heifers were also weighed at the end of the experiment.

Source of infection

In the first stage of the experiment, the 9-months-old Holstein bull was injected into a jugular vein with 3 x 2 mL vials of blood buffy coat collected from a steer at the CSIRO, Longpocket, Queensland, which had been clinically affected with ephemeral fever (strain 1864). This buffy coat material had been stored in liquid nitrogen since 1986. At the first sign of a temperature rise, 500 mL of heparinised blood was collected from the bull, centrifuged and the buffy coat transferred to plain 5 mL polystyrene tubes, which were capped and stored in liquid nitrogen.

Clinical assessments

On days 1 and 2 of the experiment, all animals had their rectal temperatures recorded and the following were noted: the presence or absence of a tucked-up abdomen, faecal characteristics, appetite, any muscle trembling, abnormality of respiration, whether there was excessive salivation and any stiffness or lameness. Inanition was regarded as present if animals were not seen to be eating immediately after fresh feed was added each day or chewing the cud during the periods that the experimenters spent with them in the stalls.

The degree of locomotor dysfunction was classified as "1" if the animals showed any sign of stiffness or lameness but could walk, "2" if they had to be forced to walk, "3" if they were recumbent and would only get up with coercion, and "4" if they remained recumbent throughout the examination and blood sampling procedure and could not be persuaded to stand.

Blood sampling

On each day a 10 mL blood sample was withdrawn from a jugular vein, with 5 mL being transferred to a plain tube and 5 mL to a tube containing dipotassium EDTA*.

Total leucocyte counts (WCC) were estimated using a Coulter Counter† according to the manufacturer's directions. Plasma fibrinogen concentration (FIBR) was determined by measuring total plasma protein with a refractometer before and after precipitating fibrinogen at 57°C, using the method of Kaneko and Smith (1967). Serum ionised Ca concentrations (ICa) were estimated using a Ca-sensitive electrode method‡.

Infection of heifers

On day 3, each heifer was injected into a jugular vein with 1 ml of thawed buffy coat obtained from the above bull.

Treatments

The two treatments were supplied in identical multidose vials and labelled as injection A and injection B. The authors

* Disposable Products, Technology Park, SA

† Coulter Counter, Model ZM, Coulter Electronics, England

‡ ICA2 Analyser, Radiometer, Copenhagen

were unaware of drug identity till completion of the trial. The young Holstein bull was treated with injection A containing 100 mg/mL of ketoprofen[§] at the first sign of locomotor dysfunction, and then alternate heifers, seen to be affected by locomotor dysfunction, were given 3 mL per 100 kg body weight of either injection B (containing base solution only) or injection A intramuscularly once daily for 3 consecutive days.

Observations were made and blood samples collected once daily till day 5. From then on observations were conducted twice daily till the heifers were obviously recovering from the ephemeral fever.

Statistical methods

The software used was the Statistical Analysis System[¶]. An analysis of variance incorporating a repeated measures design was used to determine the effects of the two treatments on rectal temperatures (RT), WCC, FIBR and ICa. Where twice daily observations were available, the results for morning and afternoon samples were averaged and used for analysis as a single daily mean reading. One Jersey heifer that did not show locomotor dysfunctions till the 11th day after infection recovered sufficiently after injection A to be included in the locomotor dysfunction analyses, but did not have sufficient post-treatment data to be included in analyses for the other observations. In these analyses, the first day of treatment was counted as day 0, regardless of which day it occurred after injection of the virus-infected leucocyte preparation.

For statistical comparison of changes in locomotor dysfunction following treatment, the number of animals affected at each examination, and the number of animals at risk but not affected, were recorded for the six observations or examinations after treatment. Chi-square analysis for likelihood ratio was performed on the data.

Student's *t*-test was used where appropriate to test the significance of the differences between paired means.

Results

Success of experimental infections

The young Holstein bull, all eight Holstein heifers, and seven of the nine Jersey heifers developed clinical signs of ephemeral fever. Two of the Jersey heifers did not show any clinical abnormalities during the experiment.

Locomotor dysfunctions after injection of ephemeral fever

The mean degree of locomotor dysfunction and the course in the two treatment groups is demonstrated in Figure 1. In addition to this classification, three animals developed lameness in a single limb. This varied from holding the limb off the ground similar to the action of an ox with an acute hoof abscess, to limited weight bearing when walking. One Holstein heifer was lame in the right hind limb and two Jerseys were lame in a left forelimb. The lameness disappeared at the same time as the generalised locomotor dysfunctions.

The times of onset of locomotor dysfunctions after injection of infected blood were 96 h (young bull), 120 h (4 Holsteins, 5 Jerseys), 132 h (1 Holstein), 144 h (1 Jersey), 156 h (3 Holsteins) and 216 h (1 Jersey).

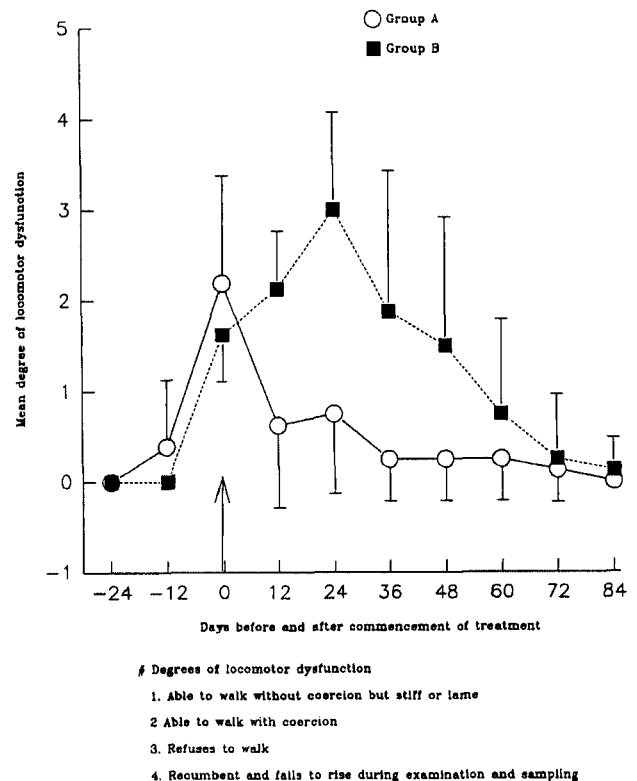


Figure 1. A comparison of the mean (\pm SD) degree of locomotor dysfunction in one group of 8-9-months-old Jerseys and Holsteins infected with ephemeral fever and treated with ketoprofen (Group A) and another similar group of 8 animals injected with base solution only (Group B) once daily for 3 days commencing on day 0.

Occurrence of "tucked abdomen"

Abdominal changes were observed in three Holstein heifers and four Jerseys. In three "tucked abdomen" was evident 12 h before the onset of locomotor dysfunction in three at the same time, and in one it was evident 12 h after the onset of locomotor dysfunction. The posture lasted for periods varying from 12 h to 2.5 days. It passed off variably during the period from 36 h beforehand to the same time as the disappearance of locomotor dysfunctions. Only one of the heifers was in the treated group, and in this case the sign appeared at the same time as the locomotor dysfunction and was evident for 12 h after the first treatment injection.

Inanition

The mean (\pm SD) periods of inanition for treated and untreated groups were 10.5 ± 7.6 and 19.5 ± 8.9 h respectively. The difference between the means approached significance ($P < 0.1 > 0.05$). All animals except two of the affected Jerseys stopped eating for periods of 12 to 36 h. The inanition occurred variably from 24 h before to 12 h after the onset of locomotor dysfunction and from 12 h before to 12 h after the highest rectal temperatures. This variability in relation to the onset of locomotor dysfunction (and thus also the time of injection) made it difficult to assess the effects of the treatments on inanition.

[§] Ketofen 100 Injectible, Rhone Merieux/Webster, West Footscray, Vic
[¶] SAS version 6.04, SAS Institute Inc., Cary, NC, 1988

Muscle trembling

Muscle trembling was observed once only in three animals. In one it was recorded at the same time as the onset of locomotor dysfunction and peak temperature (40.1°C); in another it was recorded 48 h before the onset of locomotor dysfunction and in the third (an untreated heifer) it was recorded 48 h after the onset of locomotor dysfunction.

Faecal characteristics

Because of the group penning of the heifers, information on the nature of the faeces from each animal was limited to observations of spontaneous defaecation during recording sessions and to observations of the floor litter for any abnormal faeces. No animal was affected by constipation or diarrhoea.

Changes in respiratory characteristics

All except two of the affected Jerseys showed some abnormality of respiration. Thirteen of the remaining 14 showed an increased rate of respiration commencing variably from 12 h before the onset of locomotor dysfunction and peak rectal temperature to 12 h afterwards. Three heifers also exhibited spasmodic coughing, commencing in one 2 days before the onset of locomotor dysfunction, and in the others, 12 h and 24 h respectively after the onset of locomotor dysfunction. Four animals displayed expiratory moaning or grunting at some stage, particularly when they were recumbent.

These changes in respiratory characteristics lasted a mean (\pm SD) 1.1 \pm 0.9 days in the Jerseys and a mean (\pm SD) 3.25 \pm 1.6 days in the Holsteins, and this difference was significant ($P < 0.05$).

After initiation of treatment, the changes continued for a mean 1.6 \pm 1.4 and 1.9 \pm 1.0 days per animal in the treated and untreated groups respectively.

Salivation

No animals showed excess salivation from the mouth at any stage of the experiment.

Weight of heifers

In 2 weeks, the weights of the Jersey heifers increased from 164.7 \pm 22.7 to 167.7 \pm 25.4 kg. Individual weight changes varied from -11 kg to +8 kg, with an average gain of 6.5 kg in the two heifers that did not contract ephemeral fever, and a mean gain of 3.3 kg and 4 kg in treated and untreated groups respectively.

Limb dysfunctions

The changes in limb dysfunctions are demonstrated in Figure 1. It can be seen that there was a dramatic and prompt reduction in the mean degree of locomotor dysfunction in the group that received ketoprofen injections. During the six examinations (morning and evening) after initiation of treatment, this group of seven heifers and the young bull (48 observations) had a total of nine incidents of heifers with some degree of locomotor dysfunction and 39 unaffected, while the figures for the control group were 30 and 18 respectively. Chi-square analysis for likelihood ratio was 19.8 indicating a highly significant difference in the numbers of animals still affected after the first of the three daily injections ($P < 0.0001$). One of the Jersey heifers which had progressed to a degree 4,

had no locomotor dysfunction within 12 h of receiving ketoprofen.

Rectal temperatures

Figure 2 demonstrates the changes in mean RTs. Repeated measures analysis of variance demonstrated significant changes in the overall mean RTs with time ($P < 0.001$). The means at days 4, 3, and 2 before treatment and days 2 and 3 after commencement of treatment were significantly lower than the means at day 1 before, on the day of treatment, and the day after commencement of treatment. At no stage did the ketoprofen and control groups differ significantly from one another.

Total leucocyte count

These changes are displayed graphically in Figure 3. Repeated measures analysis of variance showed no significant difference between days or between ketoprofen and control groups before or after treatment.

Plasma fibrinogen concentrations

Figure 4 demonstrates the changes in mean FIBR in the ketoprofen and control groups. Repeated measures analysis of variance showed that there were significant differences before and after treatment between the combined mean concentrations of both groups ($P < 0.005$) with the means on days 4, 3, 2 and 1 before commencement of treatment being significantly lower than the means on days 1, 2, and 3 after the commencement of treatment. There were no significant differences between the two treatment groups in the mean changes in concentration each day.

Ionised calcium concentrations

The changes in mean ICa each day are shown in Figure 5. Repeated measures analysis of variance revealed no significant differences in changes between the two groups each day, but the combined mean concentration 3 days before the commencement of treatment was significantly less than that on the day of commencement of treatment.

Discussion

This experiment confirms that the buffy coat layer from a clinical case of bovine ephemeral fever, stored for 6 years in liquid nitrogen, when thawed, can reproduce the disease, when injected intravenously. The clinical signs of increased RTs, limb stiffness and lameness and recumbency and respiratory abnormalities are all typical of the disease. However the evidence here could not confirm that the disease caused diarrhoea or excessive salivation as reported elsewhere (Uren 1989; Hungerford 1990). Three animals displayed muscle trembling to some degree, but the time relationship in two cases relative to the other major signs casts doubts on whether the trembling was related to the bovine ephemeral fever.

The clinical sign of tucked up abdomen, in 7 of the animals, appeared to be unrelated to the time of development of other signs, including limb stiffness, and its significance is obscure.

Inanition was not a major problem in either group in this study, with a maximum period of 36 h in any one animal. Because the difference in the mean time that animals were affected in the two groups approached significance, it seems that ketoprofen may have had some direct or indirect effect on the restoration of appetite.

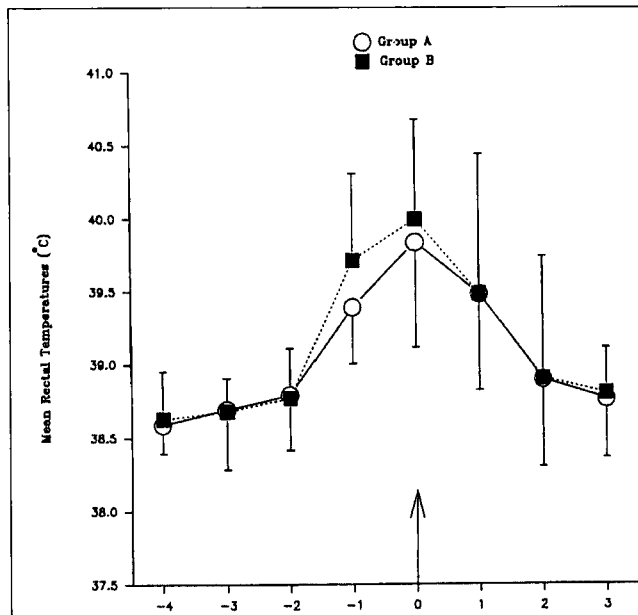


Figure 2.

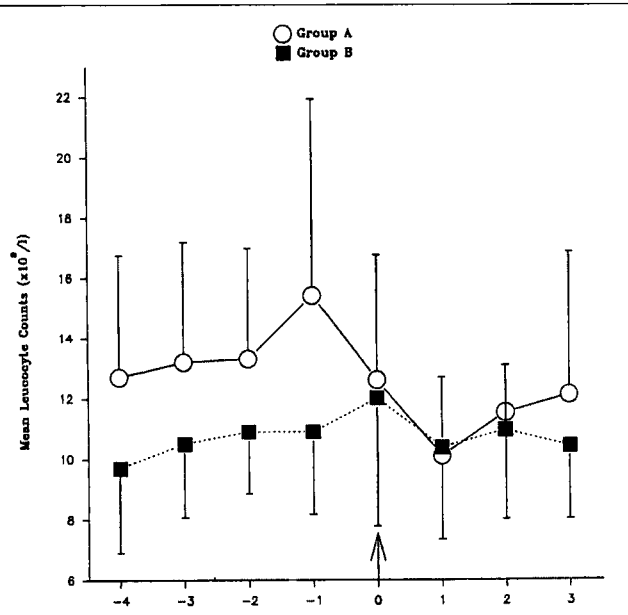


Figure 3.

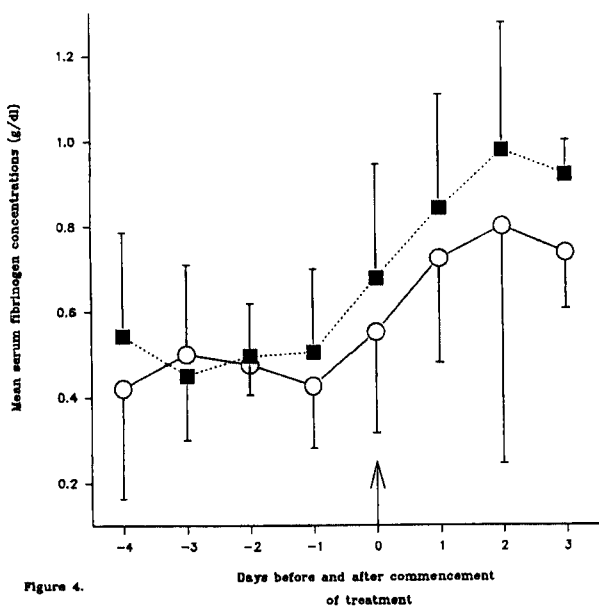


Figure 4.

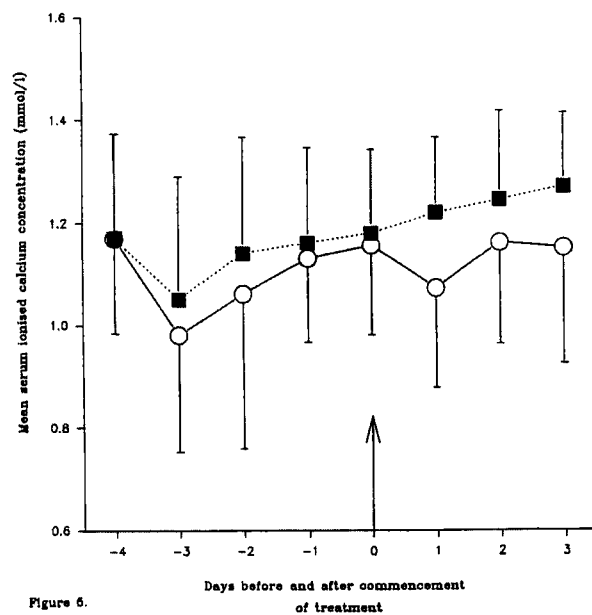


Figure 5.

Figures 2-5. Changes in mean (\pm SD) rectal temperatures, leucocyte counts, plasma fibrinogen and ionised calcium in one group of 7 (one Jersey heifer was not included in these four studies) and Holstein animals infected with ephemeral fever and treated with ketoprofen solution (Group A) and another group of similar animals infected with ephemeral fever and injected with base solution only (Group B) once daily for 3 days commencing on day 0.

The fact that two of the Jerseys were not affected at all by the disease and that those that were affected suffered respiratory effects for a lesser time, suggests that they were more resistant to ephemeral fever than the Holstein group.

Because there were no significant differences in the mean leucocyte changes in the two treatment groups and no significant differences in the weight gain of the Jersey heifers, it appears that ketoprofen had no adverse physiological effect on the animals. The expected daily weight gain for Jerseys of this age is 0.4 to 0.45 kg per day (Moss and Buchanan 1983). The two unaffected Jerseys had gains of 0.36 and 0.57 kg/day respectively, while the 3 Jerseys in the ketoprofen-treated

group gained a mean 0.23 kg/day and those in the control group gained a mean 0.29 kg/day. Considering that the heifers had been placed in a strange environment (from paddock to stalls) and had all been through a bout of ephemeral fever, this weight gain is acceptable.

This study suggested that the active ingredient had no antipyretic effect; there was no significant difference in the mean RTs of the two groups at any stage of the experiment. Furthermore there was no evidence of a biphasic temperature rise as reported by Uren (1989).

The evidence also suggests that mean FIBR concentrations increase with development of the disease but that ketoprofen

had no significant effect in reducing plasma fibrinogen concentrations.

Although there was a significant decrease in ICA three days before the onset of the clinical signs of locomotor dysfunction, this study was not able to confirm a decrease during the febrile phase as reported by Uren (1989). Even the relatively low mean concentration three days before treatment was within the normal range. Of course a different picture may have emerged if adult cows had been used in the experiment.

The most important finding was that ketoprofen significantly and dramatically reversed the locomotor dysfunction effects of experimentally induced ephemeral fever, as was demonstrated clearly from the high chi-square value for the difference in the number of affected heifers after treatment, and which was demonstrated in Figure 1. The high chi-square value was for incidence of signs only and did not even take into account the higher coded severity of the locomotor dysfunctions in the control group. Although most of the heifers were treated at the first sign of disability, one of the ketoprofen group showed a rapid recovery from recumbency within 12 h of injection.

Even though ketoprofen seemed to have a marked effect on locomotor dysfunctions, there was no significant difference in the mean time that animals in the two treatment groups took to recover from respiratory abnormalities after the commencement of injections. This suggests that the locomotor and respiratory effects may have a different underlying cause.

In conclusion it can be stated that ketoprofen had no dis-

cernible untoward effects on the animals in this experiment, and that although there were no differences in the effects of the two injections on the WCCs, RTs or respiratory effects associated with ephemeral fever, the drug markedly reversed the locomotor dysfunction effects, which were probably due to joint or muscle pain.

Acknowledgments

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Eliminating dexamethasone

The pharmacokinetic properties of dexamethasone in horse are poorly known, although this potent corticosteroid is frequently used to treat inflammatory conditions in racing horses. Dexamethasone is considered a foreign substance by most racing jurisdictions and its positive identification leads to disqualification.

FE Cunningham and coworkers (*J Vet Pharmacol Ther* 1996;19:68-71) gave dexamethasone to Thoroughbred racers intravenously and orally and studied the pharmacokinetic properties with a sensitive radioimmunoassay capable of detecting 100 pg/mg concentration. The oral bioavailability was about 0.60. The half-life of a 10 mg intravenous dose was on average 2.6 hours (range 1.4 to 4.6 h) and the 10 mg oral dose 4.4 hours (2.8-5.7 h). Most horses had eliminated the steroid below the detection level by 24 h post-administration. However, considering the varying bioavailability and elimination, the authors recommend waiting the minimum of 48 hours before racing horses that have been treated with dexamethasone.
