Serological and Biochemical Factors in Bovine Ephemeral Fever

T. D. St George, A D. H. Cybinski, A G. M. Murphy and Corrine K. Dimmock

Division of Tropical Animal Science, CSIRO, Long Pocket Laboratories, Private Bag No. 3, P.O., Indooroopilly, Qld 4068.
 Queensland Department of Primary Industries, Animal Research Institute, Fairfield Rd, Yeerongpilly, Qld 4105.

Abstract

Clinical signs of ephemeral fever, which were observed in individual cattle during two successive epidemics in 1973 and 1976, were related to biochemical, cellular and serological changes in the blood. The rise in peripheral blood neutrophil counts in samples collected from 12 sentinel cattle on a daily basis before, during and after natural disease in the two epidemics to mean peaks of $9 \cdot 6-12 \cdot 5 \times 10^6$ per litre, and fall in counts of lymphocytes to a trough of $5-7 \times 10^6$ per litre was found to occur on the same day as the fever peak. A fall in serum calcium levels from a normal mean of $2 \cdot 55$ mmol/l to $2 \cdot 0$ mmol/l occurred on the day clinical signs were most pronounced. Serum magnesium levels were affected to only a minor degree. Plasma fibrinogen rose from a normal mean of $5 \cdot 0$ g/l to a peak of $18$ g/l on the second day of disease and fell towards normal in the week after recovery. Neutralizing antibodies to bovine ephemeral fever virus were detected up to 63 days prior to clinical disease, and the rise of antibody after recovery was secondary in pattern. Serological evidence of a prior infection with an antigenically related virus, Kimberley virus, was found in these animals. In more severe clinical cases of ephemeral fever serum calcium levels were as low as $1 \cdot 95$ mmol/l. Treatment of cattle showing clinical signs of the disease with phenylbutazone and calcium borogluconate was favourable.

Extra keywords: Rhabdovirus; paresis; hypocalcaemia; inflammation.

Introduction

Ephemeral fever, which is caused by bovine ephemeral fever (BEF) virus, is a disease of cattle and water buffaloes. It occurs in a wide band of the tropics and subtropics of Asia, Africa and Australia, with some extension into temperate zones (St George 1981), and is spread by insects, but the identity of the major vector species involved has not been established (St George 1981).

In Australia, there were a series of spaced epidemics, the first of which commenced in 1936 (Mulhearn 1937). Each of the epidemics spread in a general north–south direction through the cattle population in the eastern half of the Australian continent, though varying in apparent speed of spread. These epidemics occurred in 1936–37 (Seddon 1938), 1955–56 (Albiston 1966), 1967–68 (Murray 1970), 1970–71, 1972–74 and 1974–76 (St George et al. 1977). The clinical signs observed during epidemics have been summarized by Seddon (1938) and Morgan and Murray (1969). More detail was provided in the descriptions of the first Australian epidemic (Anon. 1937). However, the course of the disease in individual cattle has been described in more detail in experimental cases (Mackerras et al. 1940) than in natural cases. The detailed observations reported here were made on cattle which were naturally infected with ephemeral fever during the epidemics of 1972–74 and 1974–76.
Materials and Methods

Sentinel Herd, Peachester

Sentinel cattle were selected from cattle on a dairy farm at Peachester, 100 km north of Brisbane, as part of a major sentinel herd scheme in Australia described by St George (1980). The scheme involved the bleeding of a group of identified cattle on a planned program. The sampling procedures described were used in intensive studies, in the anticipation that ephemeral fever would occur in the herd within a time bracket.

1973 Outbreak

A group of 12 Australian Illawarra Shorthorn heifers, bred on the Peachester farm, were aged between 12–18 months when they were bled on a daily basis for 3–14 days before becoming ill with ephemeral fever and for 7 days after illness. The group was then bled again 7 and 14 days after the last case.

Blood was withdrawn from the jugular vein with a disposable sterile syringe and needle. The sample of blood was separated into two parts, 5 ml in ethylenediaminetetraacetic acid (EDTA) anticoagulant for haematology and 25 ml into a plastic disposable bottle where it was allowed to clot.

Blood smears were prepared from the EDTA sample within 30 min of the blood being collected then fixed with methanol after being air-dried. The blood samples were kept on ice and forwarded daily to the Long Pocket Laboratories.

The rectal temperature of cattle was taken once daily at between 0800 and 0900 h each day and any clinical signs noted. Sick sentinel animals were not treated in any way other than to provide shade and water if they became recumbent.

1976 Outbreak

In 1975, a similar group of 12 heifers, Australian Illawarra Shorthorns or Friesians, in the same herd was selected for intensive study. All were born after the previous outbreak of ephemeral fever and were aged between 11 and 20 months at the time of selection. The sentinel group was bled monthly from May to October 1975, approximately weekly from 19 November to 30 December 1975, then daily from 3 January to 28 February 1976. Observations were made and blood samples were collected as for the 1973 experiment.

Experimental Treatment

Nineteen cattle on farms other than the Peachester farm were given one of two forms of treatment. Ten cattle with clinical signs of ephemeral fever, excluding severe muscle tremor, uncoordinated gait or paralysis, were injected intramuscularly with 10 ml (200 mg/ml) of phenylbutazone (Myotone-VR Laboratories). The other nine cattle with additional signs consistent with those of hypocalcaemia as further described were slowly injected intravenously until specific clinical signs abated with a calcium borogluconate (CBG Plus, I.C.I., Australia) solution (containing 85 g calcium borogluconate, 19 g calcium hyperphosphate and 57 g of dextrose in 350 ml). A single injection of phenylbutazone followed treatment with CBG.

Laboratory Procedures

Virus isolation

Attempts were made to isolate BEF virus from the blood of 15 of the clinical cases which were treated. The method used was the intracerebral inoculation of suckling mice as described by St George et al. (1977).

Haematology

Total leucocyte counts were estimated with a Coulter counter and absolute neutrophil and lymphocyte counts were calculated from the total leucocyte count and the percentages of these cells in a 200 cell differential count in May–Grunwald Giemsa-stained smears. Haemoglobin was estimated as oxyhaemoglobin.

Serology

The serum from the clotted blood was freed and the plasma from the anticoagulated blood was separated by centrifugation.
Tests for neutralizing antibodies in the serum were carried out by the method of St George et al. (1980) except that the strain of BEF virus used in neutralization tests in Australia (BB7721; Doherty et al. 1969) and Kimberley virus (CSIRO 368; Cybinski and Zakrzewski 1983) were substituted for Peaton virus.

**Biochemistry**

Serum calcium and magnesium levels were determined by atomic absorption flame spectroscopy using a Varian Techtron AA-5R spectrophotometer. Plasma fibrinogen levels were determined by the method of Hardisty and Ingram (1965).

**Results**

**Clinical Diseases in Sentinel Cattle**

On both occasions, in 1973 and 1976, that sentinel cattle were being observed and bled daily, an epidemic of ephemeral fever occurred in other herds in the immediate district, the Peachester herd and in the sentinel group. There were no mortalities on the farm from ephemeral fever in either epidemic.

![Graph](image-url)

**Fig. 1.** Mean lymphocyte and neutrophil counts and plasma fibrinogen levels in relation to clinical ephemeral fever in 12 sentinel cattle during an epidemic of ephemeral fever in 1973. As the cattle suffered clinical disease on different dates, the day that overt characteristic signs were observed is shown as day 0.

**1973 outbreak**

The outbreak of ephemeral fever began in the Peachester herd on 20 November and finished on 13 December 1973. All of the 12 cattle in the sentinel group developed clinical signs as did 14 other cattle in the same age group. The cattle in the herd, which had experienced clinical ephemeral fever in the epidemics that occurred in the Peachester district in 1968, or 1971, did not develop disease nor did calves less than 6 months of age. None of the 12 cattle affected in 1973 developed ephemeral fever in the 1976 epidemic.
In half the sentinels, the first sign noticed was a slight change in usual behaviour 6–12 h prior to overt clinical signs of mild to moderately severe ephemeral fever. The change could be a tendency to leave the main group of the grazing herd or to come to milking in different order from the usual. The general pattern of fever is shown in Fig. 1.

The clinical signs in the mild cases were inappetence, depression, and lameness in one leg, or general muscular stiffness. The moderately severe cases showed marked depression, no interest in feed or water, ocular and nasal discharge, muscular fibrillation and were in an euthermic recumbency for 6–24 h. All recumbent animals could rise if stimulated to do so.

Two heifers exhibited signs of oestrus during the 1973 epidemic, one 4 days before and one 2 days after clinical signs of ephemeral fever were observed. Each was inseminated at that oestrus, became pregnant, and carried a normal calf to term. All the remaining heifers conceived at various intervals after the period of close observation and calved normally. The relationship between lymphocyte and neutrophil counts and plasma fibrinogen levels and clinical ephemeral fever are shown in Fig. 1. An increase in the percentage of immature neutrophils and mild degenerative changes that persisted for up to 17 days were associated with the fall in neutrophil values.

![Graph](image)

**Fig. 2.** Mean rectal temperature and lymphocyte and neutrophil counts in relation to mean total serum calcium and magnesium levels in 10 sentinel cattle during an epidemic of ephemeral fever in 1976. As the cattle suffered disease on different dates, the day that overt characteristic signs were observed is shown as day 0.

### 1976 outbreak

This outbreak began in the Peachester herd on 26 January 1976 and finished on 22 February 1976. In the sentinel group, the duration was from 29 January to 15 February 1976. Ten of the 12 heifers showed very mild to moderate clinical signs of ephemeral fever, similar to those observed in 1973. As in that outbreak, almost imperceptible signs were noted 12 h ahead of overt clinical signs in five of the animals. In each instance, a fever above 40°C was noted in an extra observation at that time. The relationship between
mean fever and leucocyte counts is shown in Fig. 2. The serum calcium and magnesium levels obtained during the 1976 epidemic are also shown in Fig. 2. Haemoglobin levels again ranged within normal limits of 10–14 g/dl (Schalm et al. 1975).

Sentinel cow serology

In 1973, neutralizing antibodies to BEF virus were found in serum from 11 of the 12 sentinel animals before clinical illness. The number of serum samples available for test depended on the number of days after sampling commenced that the individual animal became ill. This period ranged from 3 to 10 days. Titres varied from 1 to 8 in individual animals, though the antibody level fell below the level detectable in undiluted serum in the neutralization test on the day of illness. Antibody was detected the day recovery commenced, and the titre rose rapidly in the succeeding 2–3 days to maximums which ranged from 64 to 256. The level of antibody remained approximately stable for the remainder of the observation period.

![Diagram](image)

Fig. 3. (a) Antibody levels to bovine ephemeral fever (BEF) and Kimberley viruses in the serum of a heifer before and after a natural case of ephemeral fever in the 1973 epidemic. The naturally infected animal had been infected with Kimberley virus some unknown time earlier than the 3 days prior to disease for which samples were available. Day 0 is the first day of overt clinical disease. The expected pattern of the experimentally induced antibody to BEF (St George 1981) is added for comparison. (b) Antibody responses to BEF and Kimberley viruses of a heifer from which serum samples were collected for a prolonged period before the 1976 ephemeral fever epidemic. Day 0 is the first day of overt clinical ephemeral fever.

In 1975–1976, the pattern of antibodies to BEF virus being present before disease was repeated in 8 out of 12 of the sentinel cattle. There were more serum samples available prior to illness than in the 1973 series, daily sampling having begun 23 days before the first clinical case. In individual cattle, neutralizing antibodies to BEF virus were first detected in serum samples collected 15 to approximately 63 days before clinical signs of ephemeral fever were noted.
In both sentinel groups, the cattle had neutralizing antibodies to Kimberley virus at the time of ephemeral fever disease. The titre varied from 34 to 256 at the time of clinical illness and increased on the day of recovery to titres of 256 to 1024. An example of this pattern is shown in Fig. 3a. The time of seroconversion of the 1973 group was not established, as too few samples were taken prior to the occurrence of disease. Members of the 1976 sentinel group seroconverted to Kimberley virus 7 to 90 days prior to experiencing ephemeral fever. In each instance where antibodies to BEF virus were detected in the 1976 group, the animal had seroconverted earlier to Kimberley virus. An example of the antibody pattern seen in the 1976 group is shown in Fig. 3b.

Effects of Treatment

The two cows and eight bulls treated only with phenylbutazone recovered more rapidly than similarly affected untreated cattle. In each case the assessment was made by a veterinarian quite familiar with ephemeral fever.

The remaining nine animals which were treated with CBG plus phenylbutazone comprised four cows in mid-lactation and five bulls. These animals had clinical signs of hypocalcaemia in addition to fever, ocular and nasal discharge, ruminal stasis and general depression. The two cows most severely affected were in lateral recumbency and almost without reflex responses to stimuli. Both responded progressively to treatment with CBG infused intravenously over 15 min to a stage where they could sit in sternal recumbency and were attempting to rise from time to time. They were completely alert and responsive to all stimuli and drank about 20 litres of water. When revisited the day after treatment both had relapsed and they died 2 days later.

Two cows and four bulls were in sternal recumbency with head turning to, or resting on one flank, when first seen. They were quite unable to rise though reasonably moderately alert. All resumed their feet within 30–60 min of the completion of treatment. The remaining bull had a generalized muscular tremor, an incoordinated gait, and was very reluctant to move. After treatment he could walk quite normally without coaxing.

All the animals treated with CBG had a rapid, shallow respiration (90–140 per min) and an accelerated heart rate (100–160 per min). The breathing slowed to normal during treatment and the heart gradually slowed and strengthened in sound.

All the clinical cases had a neutrophilia of between 11 and $17 \times 10^9$ per litre. Neutralizing antibodies to BEF virus rose in serum samples taken on successive days, or on recovery. BEF virus was isolated from the blood of eight of the 19 cases.

Discussion

On two occasions the objective of making clinical observations and collecting blood samples from individual sentinel cattle before, during, and after ephemeral fever occurred was achieved. The close observation that the sentinel groups were under disclosed that there were early, almost imperceptible, clinical signs which could be reliably detected only by an observer completely familiar with the cattle and that these signs were accompanied by fever. The characteristic signs of ephemeral fever developed later, and were consistent with those which have been previously reported by various workers (Seddon 1938; Mackerras et al. 1940; van der Westhuizen 1967; Inaba 1968; Snowdon 1971).

Leukocyte values changed in a characteristic pattern (Figs 1 and 2). There was a decline in the lymphocyte count from a mean of $10 \times 10^9$ per litre 2 days before the onset of clinical signs to means of $5–7 \times 10^9$ per litre on the day of the fever peak. In contrast, the neutrophil count rose, and mean maximum values of 9.6 and $12.5 \times 10^9$ per litre were recorded in the 1973 and 1976 outbreaks, respectively. The peak and trough were reciprocal and occurred when clinical signs were present.
This pattern of neutrophilia in association with fever overshadowing the relative lymphopenia (Figs 1 and 2) is in accord with the pattern illustrated by Mackerras et al. (1940) and Inaba et al. (1963). The subsequent fall in neutrophil values associated with the increase in immature forms probably reflects the emigration of neutrophils from the blood stream to the site of inflammation.

The plasma fibrinogen levels (Fig. 1) which rose rapidly and reached a maximum level the day after the febrile peak fell gradually over the next 4 days. The fibrinogen level had not returned to preclinical levels by the end of the observation period. The preclinical levels lay within the range of 6·0–8·0 g/l and the higher levels of 19 g/l were consistent with the levels reported by McSherry et al. (1970) for inflammatory disease. This adds support to the statement by Mackerras et al. (1940) that, in ephemeral fever, the histopathologic changes in the endothelium of small blood vessels were inflammatory in nature.

The serum calcium and magnesium levels varied in a consistent pattern in members of the 1976 sentinel group. The serum calcium level (Fig. 2) declined on the day of clinical disease and reached its lowest point on the second day of disease but returned during the next 4 days to preclinical levels. The mean level of 2·13 mmol/l is only slightly below the lower level of the normal range of 2·25–2·75 mmol/l. In post-parturient hypocalcaemia much lower levels, even as low as 1·0 mmol/l, have been recorded (Moodie and Robertson 1961). Severe clinical signs similar to post-parturient hypocalcaemia were absent from the sentinel cattle at Peachester. The only signs in them which could be directly attributable to lowered serum calcium levels (Fig. 2) were muscle fasciculation. However, clinical signs characteristic of hypocalcaemia were present in the more severe cases that were treated on other farms and are consistent with those described by Blood and Henderson (1974). The values of serum calcium of 1·95–2·12 mmol/l which were recorded in the cattle with signs of hypocalcaemia were lower than those of the sentinel cattle and were below the normal range. The severity of the signs was inconsistent with the calcium levels, unless most of the decrease was in the ionized fraction. The reason for the lowered serum calcium levels is not known, but a respiratory acidosis may have been induced by the rapid respiration and this may have affected the ionized calcium level. No direct measurements could be made at the time to test this hypothesis. Ruminal stasis and hypomotility of the digestive tract (Moodie and Robertson 1962) also may have contributed to the lower serum calcium levels, by reducing calcium intake, but are not likely to be initiating causes.

The isolation of BEF virus from blood samples taken during clinical disease could not be attempted from all cattle due to lack of resources at the time. In the remaining cases, confirmation of the disease as ephemeral fever depended on a rise in neutralizing antibodies to BEF virus after clinical disease and the demonstration of a neutrophilia. However, the characteristic clinical signs and the epidemic situation left no doubt that the diagnosis was correct.

The presence of neutralizing antibodies to BEF virus in sentinel cattle prior to their becoming clinically ill indicated that this antibody was either not protective in vivo or was present at an inadequate level. This finding in 1973 led to blood samples of sentinel cattle being taken over a protracted period prior to the 1976 epidemic. The explanation was provided very much in retrospect by testing of the stored sera with Kimberley virus isolated elsewhere. This antigenically related virus (Cybinski and Zakrzewski 1983) must have infected the sentinel cattle prior to their infection with BEF virus. As well as inducing specific antibodies it is highly probable that it also caused the production of heterotypic antibodies which were detectable in a neutralization test by BEF virus. This prior infection may have also sensitized the cattle so that the pattern of antibody response to BEF virus and to Kimberley virus following ephemeral fever disease was secondary, not primary, and commenced on the second day of overt illness. Kimberley virus has been isolated only from healthy sentinel cattle, or insects, and has caused no clinical disease when experimentally inoculated into cattle (Cybinski and Zakrzewski 1983; St George,
unpublished data). This rapid rise in antibody to BEF virus was found in 18 out of 22 of the natural cases at Peachester, where sequential daily blood samples were taken following clinical illness. In experimental ephemeral fever, the antibody response is first detected 2–3 days after recovery, and the titre rises slowly to a maximum a further 6 days later (St George 1980). What part an accelerated antibody rise plays in recovery in natural cases is uncertain, as the experimental and field cases are otherwise very similar in their clinical course.

The development of antibodies to BEF virus in cattle as a result of infection with Kimberley virus casts some doubt on conclusions of Snowdon (1971) and St George (1980) who attributed all antibodies detected by BEF virus in serological surveys to infection with that virus.

The treatment procedures were not controlled and were assessed subjectively. Once a favourable response had been seen with the first treatments, it was decided on ethical grounds to treat all cases. The beneficial effect of phenylbutazone could be assessed only in general terms of apparent improvement over some hours. In contrast, the response to injected CBG was immediate and relieved specific clinical signs in the same pattern seen in post-parturient hypocalcaemia. The lactating cows were all in their first lactation and had calved 3–4 months previously. Thus the hypocalcaemia could not be attributed to post-parturient hypocalcaemia. Recent observations by Davis et al. (1984) have demonstrated that milk yields diminish precipitously before overt clinical signs of ephemeral fever are noticed. The lowest levels of serum calcium in non-lactating sentinel cattle (Fig. 2) occurred on the second day of overt disease. Thus a demand for calcium by the udder for milk secretion (Kronfeld 1971), one of the important metabolic requirements for calcium, cannot be the explanation of the fall in serum calcium levels in non-lactating cows, nor of the clinical signs of hypocalcaemia in lactating cows, as milk production had almost ceased by the time clinical signs were well developed. Additionally, signs of hypocalcaemia and a response to specific treatment were observed in bulls.

In summary, the clinical signs of ephemeral fever appear to be associated with inflammation, plus a hypocalcaemia. The neutrophilia, the rise in serum fibrinogen and the fall in serum calcium are objective supporting evidence. Those biochemical estimations support the rationality of the treatment which was carried out on clinical cases on an empirical basis.

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