The Natural History of Ephemeral Fever of Cattle

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Abstract

Ephemeral fever is caused by an insect-borne rhabdovirus which is widely distributed through Africa, Asia and Australia. Based on epidemiology, and the necessity for bovine ephemeral fever (BEF) virus to be injected into a vein for disease transmission, mosquitoes of various *Anopheles* or *Culex* species are likely to be more efficient vectors of the virus than the *Culicoides* species from which BEF virus has been isolated. The definitive vectors have yet to be identified.

Ephemeral fever disease in cattle is associated with a generalised inflammation and toxæmia, possibly due to massive interferon production stimulated by BEF virus. A superimposed hypocalcaemia causes short term paralysis which can be reversed by infusion of calcium salts. The clinical signs due to inflammation can be completely prevented or resolved by the administration of anti-inflammatory drugs. The biochemical spectrum of changes in plasma zinc, iron, copper, calcium, glucose, inorganic phosphate and fibrinogen are non-specific characteristics of inflammatory diseases. There are also high plasma ammonia (NH₃) levels and non-esterified fatty acid levels during clinical disease plus elevated pH.

BEF virus probably multiplies entirely within the vascular system. The virus has been detected in plasma and neutrophils in the bloodstream and neutrophils in tissues, probably as a result of phagocytosis. The virus has not been detected in lymphocytes or erythrocytes. Natural infection of cattle appears to result in life-long immunity with no evidence of latent infection.

The first scientific account of ephemeral fever was written under the title 'Epizootic dengue fever of cattle' (Piot 1896). An epidemic of ephemeral fever swept from south to north in Egypt the previous year, affecting a third of the cattle population. Piot was struck by the similarities of the clinical signs in cattle to those of dengue in man, also a disabling disease but usually not fatal. The next scientific account of the clinical signs was by Bevan (1907), but it was Freer (1910) who, in describing the disease as 'ephemeral fever' mentioned two key points, the requirement for intravenous inoculation of blood from a clinically ill cow to transmit the disease to a susceptible cow, and that the epidemiology suggested spread by biting midges. The disease was recognised subsequently in many countries of Africa and Asia including Australia where it first appeared as a massive epidemic in 1936–37 (Mulhearn 1937). The first systematic study of the disease was that of Mackerras et al. (1940) in Australia. These authors established the parameters of the experimental disease using subinoculation of tissues and blood into susceptible cattle as the assay system. The next major advances were the isolation and characterisation of the causative agent as a rhabdovirus (van der Westhuizen et al. 1967). The first direct evidence that BEF virus multiplied in insects was supplied by Davies and Walker (1974) when they isolated BEF virus from a mixed pool of *Culicoides* species associated with cattle in Kenya.

The Disease and Apparent Anomalies

Ephemeral fever can vary in its clinical expression in individual animals. In mild cases the clinical signs may be limited to fever, loss of appetite, ocular and nasal discharge, muscle fasciculation and temporary lameness. In moderately severe cases there is depression, anorexia, loss of rumen motility, constipation, patchy subcutaneous oedema, joint swelling and a period of recumbency. In very severe cases, the animal may have paralysis of the limbs, resulting in recumbency. Salivation may be profuse. This phase
may progress to loss of reflexes, coma and death or resolve suddenly. Uncommonly, there may be air under the skin of the backline. The sudden recovery from paralysis to normal mobility and absence of disabling pathology is incompatible with major direct cellular destruction by BEF virus. Variation in clinical signs is also seen in experimental cases even when animals are injected at the same time with aliquots of the same inoculum. This paper presents the results of recent research which provides a hypothesis of the natural history of ephemeral fever.

**Fate of BEF virus after infection**

For convenience, the discussion of the biological cycle is commenced at the point when BEF virus leaves the insect vector’s mouthparts in saliva. *Culicoides* obtain their blood meal by lacerating the dermis and capillaries with their mouthparts. The insect’s saliva, which may contain virus, mixes with the blood pool, which is drained from the site via the lymphatic system. This is likely to be an inefficient route of infection as experimental evidence suggests that the virus does not appear in lymph during disease and, except for one case reported by Mackerras et al. (1940), it is not possible to infect cattle by intradermal, subcutaneous or intramuscular injection of BEF virus. Although it is possible that local multiplication of BEF virus occurs in non-vascular intradermal tissue, this is likely to be self-limiting because BEF virus is a potent inducer of interferon in bovine cells. This may explain the observation that BEF virus does not appear to multiply in cell cultures of bovine origin. In contrast to midges, mosquitoes probe the dermis to locate and penetrate small blood vessels before feeding. This parallels the experimental situation where intravenous inoculation of cattle is necessary to reliably reproduce disease. It is therefore likely that mosquitoes are more efficient vectors of BEF virus than are *Culicoides*.

After infection, the length of the initial cycle of multiplication of BEF virus is not well defined. BEF virus has been detected as early as 43 hours after inoculation using *Aedes albopictus* cell cultures as the assay system (Uren et al. 1992) and at 29 hours by subinoculation into cattle (Mackerras et al. 1940). The highest titre of virus in blood occurs approximately 24 hours before increases in neutrophil numbers or serum interferon levels and 36 hours before pyrexia and the earliest clinical signs (Uren et al. 1992).

The fraction of the blood in which BEF virus can be found in various stages of the viraemia, and the source of the virus are uncertain. Mackerras et al. (1940) found BEF virus in the leucocyte but not in the erythrocyte fraction. Young and Spradbrow (1980, 1984) found viral antigen in neutrophils. Virus has also been detected in plasma (S.S. Davis, pers. comm.).

Mackerras et al. (1940) described one case where clinical disease began at 29 hours which would mean that virus rose to a titre sufficient to induce disease in less than 24 hours. A prepatent period of 29 hours, or less, is consistent with the virus replicating through 4 or 5 cycles entirely within the vascular endothelium. The vascular endothelium is completely exposed to interferon which circulate during fever and which would inhibit BEF virus entering uninfected cells. In the period of 3-4 days between clinical recovery and antibody circulating in amounts which are adequate to neutralise free virus, the protection afforded by interferon would suppress or slow BEF viral replication. BEF virus contained on or within neutrophils in the bloodstream or serous cavities is probably there in the course of normal phagocytic function of those cells and is carried passively (Young and Spradbrow 1984).

**The clinical signs and their cause**

The clinical signs of ephemeral fever can be completely prevented without preventing viraemia by treatment with anti-inflammatory drugs (Uren et al. 1989). The clinical signs can be ameliorated by treatment in their earlier stages by anti-inflammatory drugs (Uren et al. 1989) or with calcium borogluconate or both (St George et al. 1984). As these drugs are not antiviral this clearly demonstrates that the host reaction is being modified.

The clinical signs and pathology can be considered in five groups. The first group are the toxic signs: fever, depression and cessation of milk secretion. The second group are the inflammatory signs: fever, depression, anorexia, tachycardia, permeability of blood vessels, joint swelling, subcutaneous oedema, ocular and nasal discharges and neutrophilia. The third group are those due to hypocalcaemia: namely muscular tremor, ruminal atony, bloat, bowel stasis, constipation, incoordination, temporary paralysis of the limbs, loss of swallowing reflex (excess salivation and aversion to water are consequences), loss of palpebral and other reflexes and coma. The fourth is the subcutaneous emphysema in a small percentage of cases usually in hot weather (Theodoridis and Coetzter 1979). Emphysema is probably a consequence of the mechanical breakdown of lung tissue which in turn is a product of lung oedema, obstruction of alveoli and bronchioles after 2-3 days of over-breathing. Air migrates dorsally through septal tissue. The fifth sign is the prolonged paralysis which remains after fever terminates. The cow recovers in most respects, is bright and eating normally, but cannot stand for a period of weeks, or months. Recovery
may occur. This paralysis resembles the Guillain-Barre syndrome of humans, which follows a variety of viral infections.

The first three groupings of clinical signs and pathology will be considered in more detail.

**Toxic signs**

Animals with moderate to severe ephemeral fever appears dull and unresponsive. There is a sharp fall in milk production (Davis et al. 1984). The dullness may be a direct or mediated effect of interferon in the early stages of disease. This toxaemia later may be compounded or replaced by high levels of circulating ammonia. Toxic shock syndrome of women contains the same elements of direct systemic effect, caused however by a bacterial toxin, and induction of a hypocalcaemia (Wagner et al. 1981; Wick et al. 1982).

**Inflammation**

The presence of inflammation is evident in the gross and microscopic pathology (Basson et al. 1970) and the haematology and biochemistry (St George et al. 1984, Uren and Murphy 1985, Uren et al. 1992). The same biochemical changes occur in milk fever of cattle, where a reversible paralysis is also a feature. The high levels of interferon that occur in ephemeral fever could induce the inflammation through an interleukin cascade (Uren et al. 1987). As shown in Table 1, interferon induces most of the same effects as in experimental interferon toxicity in humans (Scott et al. 1981). Both conditions are treatable with anti-inflammatory drugs.

The primary effect of inflammation is an increased permeability of small blood vessels which has been demonstrated to occur in ephemeral fever (Young and Spradbrow 1990). This produces lung oedema, a neutrophilia, plasma and cellular movement into tissues, joints, peritoneal, pleural and pericardial cavities, subcutaneous oedema and direct effects on muscle fibres. Temporary effects on fertility of bulls may be a direct effect of the generalised inflammation rather than virus effects on semen. BEF virus has not been detected in semen in natural disease (W.A. Snowden, T.D. St George, pers. comm.) Reduced semen quality is preventable by anti-inflammatory treatment. Ries can be detected on the lungs on the second day of fever when the ammonia level may be compounding oedema. The accompanying high plasma pH may slow virus replication as BEF virus deteriorates more rapidly at above or below pH 7.2 (Heuschele 1970).

The plasma biochemistry is that of an inflammatory disease (van Miert 1985) namely falls in plasma calcium, zinc, iron and inorganic phosphate, with rises in plasma copper, fibrinogen and glucose (Murphy et al. 1989; Uren et al. 1992). In experiments in mice infected with influenza virus where an inflammatory condition was created, Hurd et al. (1991) showed that zinc and iron accumulated in the liver. A rise in serum copper was attributed to increased synthesis of ceruloplasmin. Interleukin-1 was cited as the mediator.

**Hypocalcaemia**

A clinical hypocalcaemia is detectable in two ways; reduced plasma calcium (< 2.1 μmol/L) and the almost immediate specific response of the clinical signs to infused calcium borogluconate in the same sequence as described with milk fever (Blood and Henderson 1974).

The uptake of calcium from the diet is dependent on a fully functional rumen. The plasma calcium levels begin to fall for several hours before ruminal movements stop. Bloat may follow and plasma ammonia levels rise sharply as a consequence. In cows where the swallowing reflex is lost, saliva drips on the ground and does not return nitrogen to the rumen in its normal function. These cows usually cannot drink water. Ruminal stasis largely prevents calcium adsorption into the body and thus compounds the problem. The excretion of calcium into urine and faeces is not increased. This has been measured by G.M. Murphy, T.D. St George and M.F. Uren (pers. comm.) by monitoring the mineral balances in cattle with ephemeral fever in metabolism crates.

There is insufficient time during the course of the disease for mobilisation of any of the large store of calcium in the bones. Mobilisation requires a week to be effective (Kronfeld 1971). In lactating cows,
milk secretion virtually stops during ephemeral fever (Theodoridis et al. 1973; Davis et al. 1984). This is a fortunate effect as it limits calcium loss in lactating cows. Thus we have a situation where calcium input and output from the body is reduced, there is displacement of unbound calcium to the protein bound fraction driven passively by high pH; there remains an overall temporary deficit in total plasma calcium. The high pH (which may be up to 7.8) is induced initially by loss of carbon dioxide from rapid respiration in the first febrile stage and later by high ammonia levels in the blood. Some calcium is taken up by the increase in the non-esterified fatty acid in plasma (Murphy et al. 1989). It is possible that the activated neutrophils account for the loss of the remainder. When neutrophils are activated calcium is displaced from their surface to the interior portion of the activated neutrophils have left the circulation (Schalm et al. 1975). The mean time of arrival of granulocytes into the circulation is approximately seven days whether normal, or in calves given an endotoxin, and their half life in blood may be as short as 5 hours (Valli et al. 1971). The activated neutrophil population may thus function as a short term sink for calcium and would return their calcium and other minerals to the plasma as they finish their lifespan. The other temporary calcium sink could be the liver. Hurd et al. (1991) showed a rise of 33% (p<0.001) in liver calcium levels in an experiment inducing inflammation with an influenza virus in mice.

The other disease of cattle where hypocalcaemia induces paralysis is milk fever, which also has an inflammatory base. It occurs in multiparous cows, usually within 1–2 days of parturition. The same inflammatory markers are present. These include neutrophilia, eosinopenia, raised temperature (followed by a 1–2°C drop), hyperglycaemia, increased plasma copper ammonia, and fibrinogen, decreased zinc, iron and phosphate levels (T.D. St George, G.M. Murphy, B. Burran, M.F. Uren, pers. comm.). In contrast to ephemeral fever, milk secretion continues in milk fever, thus producing a high mortality in untreated cows. The triggering mechanism of the inflammation in milk fever is presently unknown. On recovery from ephemeral fever there is some overshoot and plasma calcium levels rise above normal for some hours before homeostatic mechanisms normalise them.

Immunity

The general opinion is that one episode of ephemeral fever disease confers immunity on recovery. This immunity is sterile (Uren et al. 1992). However, there are ample field reports that second bouts of disease do occur in a small percentage of cases. There are three possibilities. The first is misdiagnosis of another viral disease. The second is recrudescence. This occasionally occurs in experimental disease where clinical signs recur a few days after recovery from the fever. The effect of interferon on cells is temporary (Stewart 1981) and its effect is measured in days. If the neutralising antibody response is slightly delayed, BEF virus could enter susceptible cells again and recrudescence could follow. The third possibility is reinfection of cows where only a limited primary antibody response occurred. This is consistent with an interval of some weeks between attacks. Accumulated experience is that cattle with even low levels of homotypic antibodies to BEF, as detected by the ELISA test (Zakrzewski et al. 1992), are immune to natural or experimental disease.

A constant feature of field reports is the observation that the second episode of ephemeral fever is always more severe than the first. Possibly the presence in the bloodstream of trace levels of antibody, which while inadequate to protect, enhance the ability of BEF or a related virus to enter cells. Such an effect has been shown with flaviviruses, and has been suspected with rabies (Celis et al. 1985).

Once protective levels of neutralising antibodies are established in the bloodstream, any further BEF virus inoculated by an insect vector will be neutralised. The question remains as to whether BEF virus can re-enter the bloodstream from some other tissue within the animal months later and create a new focus for insect spread? If this occurs, then an overwintering mechanism could be provided by a delayed recrudescence of virus in cattle. There is an argument against this occurring. In two experimental cases, BEF virus was not found in serial samples of lymphocytes or lymph during viraemia and early clinical disease (M.F. Uren, T.D. St George, and S.S. Davis, pers. comm.). This apparent absence of BEF virus from lymph may be bolstered by the failure of Mackerras et al. (1940) to detect virus in mesenteric lymph nodes collected in the febrile period. Thus BEF virus, unless it infected a haemopoietic tissue, could not re-enter the bloodstream. If it were in a haemopoietic tissue it would be exposed to circulating antibodies and be unlikely to persist. Mackerras et al. (1940) using susceptible cattle as his test system, found the blood of 10 cattle to be free of infective virus on the 4th and 5th days of con-
valescence, in contrast to the first three days. In a total of 24 cases, BEF virus has not been found beyond the 3rd day of convalescence, a maximum of seven days after experimental infection by insect tissue culture inoculation (M.F. Uren, T.D. St George and S.S. Davis, pers. comm.). If these two factors are taken into consideration then sterile immunity in cattle recovered from ephemeral fever is a reasonable assumption. The presence of BEF homotypic neutralising antibodies is thus contraindicative of infection. Low levels of antibodies in cattle which will neutralise BEF virus in vitro are generated by Kimberley virus (Cybinski 1987). These are not protective in vivo (St George et al. 1984). The ELISA test distinguishes heterotypic from homotypic antibodies (Zakrzewski et al. 1992).

Vectors

BEF virus has been isolated from a pool of mixed species of African Culicoides (Davies and Walker 1974), C. brevitarsis in Australia (Cybinski and Muller 1990), a mixed pool of Culicine mosquitoes (St George et al. 1976), and twice from Anopheles bancroftii (Standfast et al. 1984; St George et al. 1976). The distribution of ephemeral fever in Africa, Asia and Australia far exceeds the combined distribution of all the species represented as sources of these isolates (StGeorge et al. 1976). There is some experimental evidence (H.A. Standfast, M.J. Muller pers. comm.) on multiplication in Culex annulirostris. BEF virus was detected between days five and eight post infection. This virus retains its virulence as demonstrated by one experiment using mosquito passed virus to infect cattle. BEF virus has been shown by these workers to multiply in C. brevitarsis when experimentally infected. Isolation of BEF virus from an insect which has digested its blood meal is strong evidence that the virus has multiplied in that insect, but does not prove it can transmit virus to a cow. The necessity for BEF virus to enter the bloodstream directly, argues for mosquitoes and against Culicoides as efficient vectors.

There is epidemiological evidence from Australia that supports Culex annulirostris as a vector in addition to An bancroftii (Muller and Standfast 1986). Knott et al. (1983), in a three-year epidemiological study of ephemeral fever on one river system in northern Australia, found that transmission of ephemeral fever and seroconversion in sentinel cattle, occurred in the wet season (summer) when mosquito populations peaked and the opposite season to that favouring C. brevitarsis in the region. The close association of ephemeral fever epidemics with recent rain or floods (Davies et al. 1975; Murray, 1970; St George et al. 1977; Uren et al. 1987) tends to indicate mosquitoes rather than Culicoides species as vectors in Australia as the response time of Culicoides brevitarsis populations is slower than mosquitoes &gt; 3 weeks. The accumulating evidence points to multiple species of mosquitoes as the efficient vectors of ephemeral fever in Australia and Culicoides species as probably inefficient vectors.

In the epidemics of ephemeral fever of 1936–37, 1955–56 and three in the 1970s, the southward movement halted for the winter and resumed movement at approximately the same latitude in the following spring and summer (Seddon 1937; St George et al. 1977; Uren et al. 1983; St George 1985). The overwintering mechanism is not known. For the reasons argued earlier in this paper it is unlikely to involve cattle, as immunity is sterile. Unless an alternative vertebrate host is identified, mosquitoes remain the prime suspect. Adult females which have had one bloodmeal may live long enough to maintain the virus through the comparatively short winters of much of Australia. BEF virus grows more slowly in Aedes albopictus tissue cultures at lower temperatures (Hoffmann et al. 1985). Transovarial transmission is the alternative explanation. The link with recent rain would support this possibility.

Hypothesis of the Natural History of Ephemeral Fever

This section attempts to draw together the experimental observations into a unified hypothesis. The cycle begins when BEF virus is transmitted, principally by mosquito species, directly into the bloodstream of cattle. After being injected into a blood vessel the virus spreads throughout the vascular system within minutes and enters and multiplies in vascular endothelial cells. Newly replicated virus is immediately dispersed as it buds from vascular cells possibly endothelium, into the bloodstream in several cycles. High levels of interferon are generated in the process which produces two effects. The interferon renders both infected and uninfected vascular endothelial cells temporarily insusceptible to infection with BEF virus and slows replication, but the interferon also induces a toxoamia and a general inflammatory response in cattle with consequent clinical signs of fever, oedema, lameness and general malaise. The activated neutrophil response due to the inflammation allows many neutrophils to pass into the tissue spaces where they phagocytose BEF virus. Neutrophils do not re-enter the circulation and virus within them would
instances lifelong. The temporary return to normal becomes permanent reflexes. As rumen regurgitation and swallowing in effective amounts. Once BEF virus is eliminated plasma calcium which effects both striated and normalise, the dependent biochemistry normalises. The return of smooth muscle control. The rapid recovery, so characteristic of ephemeral fever, follows the drop in viraemia and plasma interferon levels, the triggers for the inflammatory response and the consequential biochemical effects. Once the toxic stimulus of interferon diminishes recovery commences, BEF virus replication continues to decline due to impaired ability to enter and replicate in interferon affected target cells. The key biochemical change is a return toward normal of plasma calcium which effects both striated and smooth muscle control. The return of smooth muscle activity restores gastrointestinal function and all reflexes. As rumen regurgitation and swallowing normalise, the dependent biochemistry normalises. The temporary return to normal becomes permanent when neutralising antibodies enter the bloodstream in effective amounts. Once BEF virus is eliminated from the blood, immunity is sterile and in most instances lifelong.

References


