Fig. 2. The distribution of antibody to BEF virus in New South Wales in 1985-86.

Fig. 3. The distribution of antibody to BEF virus in New South Wales in 1986-87.

Fig. 4. The distribution of antibody to BEF virus in New South Wales in 1987-88.

Fig. 5. The distribution of antibody to BEF virus in New South Wales in 1990-91.

Fig. 6. The distribution of antibody to Akabane virus in New South Wales in 1989-90.

Fig. 7. The distribution of antibody to *C. brevitarsis*-borne arboviruses (Akabane, Palyam and EHD serogroups) in New South Wales in 1990-91.
Discussion

Bovine ephemeral fever virus has for many years shown the potential for rapid spread over very long distances. The epidemics in the period from 1930 to 1974 provide outstanding examples. In more recent times, interesting patterns of spread have also been observed. In particular, the appearance of disease in isolated foci, long distances from other cases, is of interest. Dispersal of arboviruses and their vectors by wind has been proposed (Murray 1987). An alternative hypothesis, especially for BEF virus, is the movement of viraemic vertebrate hosts into an area where effective vector populations already exist. This is the most likely explanation for the spread into northern Victoria in 1988 (Shiel et al. 1989) and into north-western New South Wales in 1991. In both cases, there are reports of the occurrence of disease soon after the arrival of cattle from the Hunter Valley and central coastal New South Wales. There were also abundant mosquito populations in both Victoria and north-western New South Wales at the time of arrival of these cattle. Provided an effective vector was present and a viraemic animal was introduced, subsequent transmission in a highly susceptible population is likely.

The vectors of BEF virus have remained elusive, mainly because of the difficulties associated with the isolation of this virus from field specimens. Nevertheless, several species of mosquitoes and Culicoides brevitarsis have been incriminated following isolations from field-caught insects (Standfast et al. 1976; Muller and Standfast 1986). Experimental infections of insects have also shown that Anopheles bancroftii, Culex annulirostris and C. brevitarsis are possible vectors (Muller and Standfast 1986) but the distribution of Anopheles bancroftii would suggest that it would only be a possible vector in northern Australia. Although BEF virus is usually transmitted during the late summer/early autumn in southeastern Australia, at a time when transmission of many arboviruses occurs, there are, nevertheless, features of the epidemiology which strongly suggest that, at least in many areas of New South Wales, this virus is spread by an insect vector other than C. brevitarsis. The low infection rates found in experimental studies with C. brevitarsis (Muller and Standfast 1986) also exclude it as a major vector in many areas.

The data presented show that there are disparities between the patterns of BEF virus transmission and the patterns of spread of a range of Culicoides-borne viruses. On occasions BEF virus infection has been confirmed at least 500 km beyond the known limits of Culicoides-borne viruses in the same season.

Within the endemic area, BEF virus infection has also been confirmed at times of the year, for example, at the end of winter, when there is virtually no C. brevitarsis activity. These patterns of infection are indicative of mosquito transmission. There is corroborating field evidence of the development of large mosquito populations at times when ephemeral fever epidemics have commenced. Other aspects of the epidemiology of BEF virus transmission which suggest a probable mosquito are the sudden onset of transmission soon after heavy rains and, in dry seasons, the close association of infection with streams and other groundwater.

In conclusion, although there is not yet definitive evidence for a mosquito vector of BEF virus, the epidemiological evidence is strong. A likely candidate, with some experimental support (Muller and Standfast 1986), is Culex annulirostris.

References


An Epidemiological Study of Bovine Ephemeral Fever in Anhui Province

W.H. Zhou*

Abstract

Ephemeral fever is known to have occurred on at least ten occasions in Anhui Province, China, during the period 1947 to 1991. The disease usually occurs in the summer and autumn months, June to October. Until 1983, when BEF virus was isolated from cattle, diagnosis in cattle and buffalo was based on clinical signs. The animals which showed the most severe clinical response were dairy cows. In the 1983 epidemic the prevalence in dairy cattle was 50%, beef cattle 35% and buffalo 0.7%.

Bovine ephemeral fever has been observed in Anhui Province on many occasions since 1947 but until 1983 the diagnosis was based on observations of clinical signs. In that year a virus was isolated in Hefei City from clinically affected cattle, and was identified as BEF virus by the Harbin Veterinary Research Institute. In 1987, a virus was isolated from cattle in Mengcheng County and was found to be a rhabdovirus by electronmicroscopic examination. In 1991, virus was again isolated from cattle in Hefei City. This isolate was identified in a neutralisation test using antiserum to BEF virus provided by the Harbin Veterinary Research Institute. This paper describes the epidemiology of ephemeral fever in Anhui Province and includes information on clinical signs, disease dynamics and investigation of probable vectors.

Geographical Features of Anhui Province

Anhui Province of China is situated between 29°41'N - 34°38'N and 11°45'4E - 11°93'7E. It is an intermediate area between the Oriental Region and the Palearctic Region according to zoogeographical classification. The Changjiang (Yangtze) River and the Huaihe River run from west to east, and divide the province into three natural zones; the Huaibei Zone (the zone to the north of the Huaihe River), the Jianghuai Zone (the zone between the Huaihe River and the Changjiang River), and the Jiangnan Zone (the zone to the south of the Changjiang River). The Huaibei Zone is a vast plain, while the Jianghuai Zone is hilly, and the Jiangnan Zone is mountainous, except for a narrow plain along the Changjiang River.

The average annual temperature in Anhui Province is 15°C, varying from minus 1°C in January to 28°C in July. In the Huaibei Zone, the average annual rainfall is 750mm, with 50–60% occurring in summer. In Jianghuai and Jiangnan Zones, the average annual rainfall is 1250 mm, with about 40–50% falling from May to July.

The Huaibei Zone is used for planting wheat and raising beef cattle while the Jianghuai Zone is used for planting rice and raising beef cattle and buffalo. To the south of the Jianghuai Zone only buffalo are raised. The Jiangnan Zone is used for planting rice and raising both beef cattle and buffalo, but there are only buffalo along the Changjiang River.

Disease Dynamics

Ephemeral fever has occurred in Anhui Province at least 10 times in the last 45 years. The years when the disease is known to have occurred are: 1947, 1954, 1958, 1966, 1970, 1976, 1983, 1987, 1988, and 1991. The average interval between outbreaks is 4.9 years. In some years (1958, 1966, 1976, 1983, 1987 and 1991) the epidemics occurred in other provinces as well as Anhui Province. Ephemeral fever usually occurs from July to October (Table 1).
The general direction of spread of ephemeral fever in Anhui Province was from west to east. It was estimated that the speed of spread was 12 km daily in 1976, and 10 km daily in 1983. The rate of spread was found to be the same, even along the Changjiang River where the buffalo population was large. The disease occurred throughout Anhui Province, despite the uneven distribution of buffalo and cattle and the varying geography.

The exact source of infection for any of the outbreaks has not been determined. When the disease did enter a farm the number of cases increased rapidly to reach a peak. The spread of disease appeared to be faster in the 1976 outbreak than in the 1983 outbreak. Once the peak number of cases occurred, there was usually a gradual decline in the number of new cases but this period was usually longer than that leading up to the peak.

The morbidity varied depending on the kind of animal affected (Table 2). Buffalo were much less likely to develop clinical disease, but whether this is due to a different rate of infection compared to cattle, is not known.

Another variable is age, with older animals being less likely to develop clinical disease (Table 3).

In an outbreak of ephemeral fever in Suixi County in 1983, the disease rates for different ages were: 29% for cattle less than one year old, 52% for cattle 1-2 years old, 62% for 3-5 year old animals, 32% for 6-7 years old, and 4.5% for animals greater than 8 years old.

### Table 1. The seasonal occurrence of ephemeral fever in Anhui Province.

<table>
<thead>
<tr>
<th>Year of occurrence</th>
<th>Date of initial case</th>
<th>Date of last case</th>
<th>Duration of occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1976</td>
<td>4 July</td>
<td>28 Sep.</td>
<td>87 days</td>
</tr>
<tr>
<td>1983</td>
<td>20 July</td>
<td>15 Oct.</td>
<td>96 days</td>
</tr>
<tr>
<td>1987</td>
<td>5 July</td>
<td>16 Nov.</td>
<td>114 days</td>
</tr>
<tr>
<td>1988</td>
<td>25 June</td>
<td>21 Oct.</td>
<td>118 days</td>
</tr>
<tr>
<td>1991</td>
<td>20 June</td>
<td>27 Oct.</td>
<td>129 days</td>
</tr>
</tbody>
</table>

### Table 2. Different classes of animals showing clinical signs in four epidemics (percent).

<table>
<thead>
<tr>
<th>Year of occurrence</th>
<th>Dairy cattle</th>
<th>Beef cattle</th>
<th>Buffalo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>49.6</td>
<td>35.3</td>
<td>0.7</td>
</tr>
<tr>
<td>1987</td>
<td>3.8</td>
<td>8.5</td>
<td>0.1</td>
</tr>
<tr>
<td>1988</td>
<td>59.5</td>
<td>20.8</td>
<td>0.8</td>
</tr>
<tr>
<td>1991</td>
<td>66.0</td>
<td>41.9</td>
<td>7.9</td>
</tr>
</tbody>
</table>

### Table 3. Different age classes of cattle showing clinical signs in seven epidemics (percent).

<table>
<thead>
<tr>
<th>Year of occurrence</th>
<th>Adults</th>
<th>Yearlings</th>
<th>Calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>1958</td>
<td>46.0</td>
<td>25.3</td>
<td>11.8</td>
</tr>
<tr>
<td>1966</td>
<td>26.1</td>
<td>12.5</td>
<td>36.4</td>
</tr>
<tr>
<td>1970</td>
<td>38.8</td>
<td>76.5</td>
<td>64.5</td>
</tr>
<tr>
<td>1976</td>
<td>35.9</td>
<td>72.1</td>
<td>36.5</td>
</tr>
<tr>
<td>1983</td>
<td>46.7</td>
<td>63.9</td>
<td>42.6</td>
</tr>
<tr>
<td>1987</td>
<td>4.1</td>
<td>3.7</td>
<td>2.7</td>
</tr>
<tr>
<td>1991</td>
<td>57.1</td>
<td>82.1</td>
<td>19.2</td>
</tr>
</tbody>
</table>

### Vector Studies

Eleven species of *Culicoides* have been collected in Anhui Province. They are: *C. antoni*, *C. arakawae*, *C. homotomus*, *C. maculatus*, *C. matsuzawai*, *C. mihensis*, *C. nipponensis*, *C. pulicaris*, *C. schultzei*, and *C. homotomus*. The dominant species were found to be *C. nipponensis* and *C. arakawae*.

*Culicoides* caught by net or light trap around stables or caught on the body surface of different animals were examined for blood-meal composition by immunoelectrophoresis. This demonstrated that *C. schultzei*, *C. homotomus* and *C. nipponensis* preferred the blood of domestic mammals (cattle, buffalo, asses, goats, sheep and swine), whereas *C. arakawae* preferred chickens.

The results of collection of *Culicoides* in the rice area of Ouzhong, Chuzhou (32°15.4′N, 118°18.3′E) are provided for the following species: *C. homotomus*, first collected on March 19, final collection on October 13 (240 days), most prevalent between May 15 to July 13 (60 days); *C. nipponensis*, first collected on March 31, final collection on October 6 (190 days), most prevalent between July 31 to August 31 (32 days); *C. schultzei*, first collected on May 2, final collection on November 20 (203 days), most prevalent between July 13 to August 15 (34 days).

Blood-fed *Culicoides nipponensis* caught from the field were kept for egg-laying, hatching, larval breeding, pupation and emergence under experimental conditions where 41-47 days was required for development from capture to emergence. The survival rate (number of adult insects emerged per number of eggs) was 4.7% with the main losses occurring during the larval stage.

To obtain more information about breeding sites and overwintering phases of *Culicoides*, 90 wet soil samples from rice and wheat fields were collected in the suburb of Heife (31°14.3′E). Larvae of *Culicoides*
were found in 50 samples. After emergence, they were identified as *C. homotomus*, *C. nipponensis* and *C. arakawae*, but no *C. schultzei* were found. Many active *Culicoides* larvae were collected in January from wet soil in rice fields, under a thin layer of ice when the air temperature was minus 5°C.

**Discussion**

Several outbreaks of ephemeral fever have occurred in Anhui Province, three of which have been confirmed by isolation of the aetiological agent. In the interval between outbreaks, ephemeral fever still occurred, but the cases were sporadic. When outbreaks did occur, they were often in summer and early autumn with rapid spread throughout the entire province. On the farms where infection is active, no infectious source could be identified, indicating that the disease is probably spread by insects. Dairy cattle and beef cattle are more susceptible to disease than are buffalo, but it is not known if this is a reflection of a difference in the infection rate. The dominant mammal feeding insect species in the region are *C. schultzei*, *C. homotomus* and *C. nipponensis* and these are the most likely vectors of BEF virus.
Bovine Ephemeral Fever in Indonesia

P.W. Daniels1, E. Soleha2, Indrawati Sendow2 and Sukarsih2

Abstract

Ephemeral fever is considered an important disease of cattle in Indonesia. Serological surveys have shown that large ruminants throughout the country are infected. Monitoring of groups of sentinel cattle at intervals of 1000 or 2000 km across the country is starting to yield information on the seasonal pattern of infections, and also to allow opportunities for isolation of viruses.

Historical Perspective

The first report of ephemeral fever in Indonesia was by Merkens (1919) who described a new clinical syndrome in dairy cattle in Bandung, West Java which was consistent with the descriptions of three-day-sickness described in southern Africa and Egypt (Plot 1896, Bevan 1907). The next report of ephemeral fever in Indonesia was by Burggraaf (1932), who described cases in an epidemic between 1928 and 1931 on the east coast of Sumatra.

In 1978, an outbreak of ephemeral fever in East Java was investigated, (Soeharsono et al. 1982). Serum neutralisation tests showed that 22 of 25 animals had antibody to bovine ephemeral fever (BEF) virus. The disease persisted in the area for several years, and mortalities were at times quite high (Ronohardjo and Rastiko 1982). Clinical cases appeared to be still frequent in East Java in 1985 (Daniels et al. 1988). More recently another large outbreak of suspected clinical BEF has been reported, from the island of Kalimantan (Soleha et al. 1993a).

Clinical Disease

The most complete clinical descriptions in Indonesia are from early reports. Merkens (1919) reported that dairy cattle said to be of Dutch and Australian origin were affected with a disease of sudden onset, high fever and increased respiration and heart rates. Inflamed conjunctiva, rumenal stasis, constipation, and muscular-skeletal lameness were also noted. Recumbent animals were observed and there were some mortalities.

Burggraaf (1932) reported a disease of sudden onset, with fever as high as 43°C. There was inappetance, rumenal stasis and constipation, salivation, inflammation of the conjunctiva, lachrymation, increased heart rates and respiration. A shifting lameness caused by pain of joints and muscles was a characteristic of the disease. Cases becoming recumbent resembled parturient paresis. Aspiration pneumonia was a problem.

Epidemiology and Economics

Burggraaf (1932) reported a species difference in susceptibility with mortality being rare in Bos indicus cattle, and higher in dairy cattle. Of 80 dairy cows in one herd, 12 showed clinical signs, five died and

1 Indonesia International Animal Science Research and Development Foundation, PO Box 94, BOUT, Bogor, Indonesia
2 Research Institute for Veterinary Science, PO Box 52, Bogor, Indonesia
four aborted. Of 27 heifers in the same herd, eight were affected and two died. No clinical signs were observed among 23 calves.

In East Java in the 1978–1982 outbreak, mortality was reported to be high, up to 36% of cases in the first year. Mortalities were fewer in subsequent years when a program of vaccination against haemorrhagic septicaemia and treatment of suspected cases with antibiotics was operational (Ronohardjo and Rastiko 1982). In the first year of the outbreak farmers were unfamiliar with ephemeral fever and may have slaughtered animals which they assumed would die.

In a rural economy based on smallholder farmers, such as in Indonesia, financial and economic costs of ephemeral fever are not only those of deaths and milk production losses. Cattle manure is an important source of fertilizer, and cattle are a major source of capital, personal wealth, fertiliser from manure and draught power (Ronohardjo and Rastiko 1982). The disease disrupts the farmer’s efficiency and also his opportunity to earn extra cash through contract ploughing.

Burggraaf (1932) found the highest incidence of disease at the beginning of the wet season, when mosquitoes were abundant. Spread of disease occurred without direct contact, suggesting an insect vector. Ronohardjo and Rastiko (1982) noted that the spread of the disease in East Java was in the direction of the prevailing winds during the wet season, when monthly incidence of disease was highest. In the recent Kalimantan outbreak, peaks of disease were reported at the beginning and end of the wet season (Soleha et al. 1993a).

### Current Studies

#### Procedures

A program for the study of arboviral infections of livestock in Indonesia (Daniels et al. 1991) includes ephemeral fever. The program is based on monitoring sentinel cattle, pigs and chickens and collecting samples for serology and virus isolation. Insects are also collected at sentinel sites, and identified to species, with the emphasis on Culicoides spp. Insects collected close to the laboratory are processed fresh for virus isolation, while those at distant sites are collected into alcohol.

Because of the problem of cross-reactions among BEF-group viruses in serum neutralisation tests (Cybinski 1987) a range of BEF group viruses for which reagents were available, were obtained from CSIRO Long Pocket Laboratories, Australia. These included BEF virus strain BB7721 (Doherty et al. 1969), Berrimah strain DPP63 (Gard et al. 1983), Kimberley virus strain CS368 (Cybinski and Zakrzewski 1983) and Adelaide River strain DPP61 (Gard et al. 1984).

#### Serological surveys

Cattle sera obtained from a serum bank were tested for neutralising antibodies to BEF virus at a dilution of 1:4 according to the method of Soleha (1991). The results, presented in Table 1, show that infection with BEF virus occurs throughout Indonesia. The results of more intensive serological surveys undertaken in Irian Jaya and Timor (Soleha et al. 1993b) are presented in Table 2. The cattle sampled in consecutive years were from the same herds. All districts studied were coastal, except for Jayawijaya, which is in the central highlands of Irian Jaya. The results confirm that BEF or closely related viruses, are widely spread in eastern Indonesia.

#### Table 1. Prevalence of serum neutralising antibodies to bovine ephemeral fever virus in cattle in several provinces of Indonesia.

<table>
<thead>
<tr>
<th>Province</th>
<th>No. tested</th>
<th>No. antibody positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aceh (Sumatra)</td>
<td>55</td>
<td>11 (20)</td>
</tr>
<tr>
<td>Lampung (Sumatra)</td>
<td>55</td>
<td>18 (33)</td>
</tr>
<tr>
<td>West Java</td>
<td>40</td>
<td>11 (28)</td>
</tr>
<tr>
<td>Central Java</td>
<td>55</td>
<td>14 (26)</td>
</tr>
<tr>
<td>East Java</td>
<td>24</td>
<td>9 (38)</td>
</tr>
<tr>
<td>Bali</td>
<td>47</td>
<td>6 (13)</td>
</tr>
<tr>
<td>Nusa Tenggara Barat</td>
<td>55</td>
<td>15 (27)</td>
</tr>
<tr>
<td>Nusa Tenggara Timur</td>
<td>29</td>
<td>8 (28)</td>
</tr>
<tr>
<td>South Kalimantan</td>
<td>55</td>
<td>14 (26)</td>
</tr>
<tr>
<td>South Sulawesi</td>
<td>18</td>
<td>3 (17)</td>
</tr>
<tr>
<td>North Sulawesi</td>
<td>39</td>
<td>7 (18)</td>
</tr>
<tr>
<td>Irian Jaya</td>
<td>55</td>
<td>9 (16)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>527</strong></td>
<td><strong>125 (23.7)</strong></td>
</tr>
</tbody>
</table>

Considering first the districts surveyed in Irian Jaya, Jayapura is a high rainfall area, 2750 mm per year, with rainfall usually not falling below 150 mm in any month. In this district the sero-prevalence in over 120 cattle in the two consecutive years was the same (24%). In the drier district of Merauke, on the south coast adjacent to northern Australia, the rainfall is 1750 mm per year and occurs predominantly in a four-month wet season, with an eight-month dry season. Here the sero-prevalence was similar each year (7–9%), but much lower than in wetter Jayapura. In contrast, the district of Kupang has a much lower rainfall (1250 mm per year), but the sero-prevalence (14%, 42%) in the two years of the study
Table 2. Prevalence of serum neutralising antibodies to bovine ephemeral fever virus in cattle in eastern Indonesia — including a comparison of data collected in two successive years.

<table>
<thead>
<tr>
<th>Province/District</th>
<th>1989 % antibody positive (No. tested)</th>
<th>1990 % antibody positive (No. tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irian Jaya</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jayapura</td>
<td>24% (122)</td>
<td>24% (156)</td>
</tr>
<tr>
<td>Jayawijaya</td>
<td>0% (9)</td>
<td>—</td>
</tr>
<tr>
<td>Merauke</td>
<td>9% (86)</td>
<td>7% (128)</td>
</tr>
<tr>
<td>Fak Fak</td>
<td>2% (28)</td>
<td>—</td>
</tr>
<tr>
<td>Sorong</td>
<td>—</td>
<td>25% (28)</td>
</tr>
<tr>
<td>Biak Numfur</td>
<td>—</td>
<td>44% (25)</td>
</tr>
<tr>
<td>Nusa Tenggara Timor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kupang</td>
<td>17% (105)</td>
<td>42% (113)</td>
</tr>
</tbody>
</table>

1 Sentinel cattle site
— Data not available

was higher than for Merauke, and showed considerable variation between years, perhaps indicating an effect of season. Merauke is more isolated than Kupang. It is separated from the Jayapura district by a wide mountainous area, Jayawijaya, where antibodies to BEF virus have not been detected (Table 2). The cattle population of Timor, where Kupang is placed, is approximately half a million, while the cattle population of Merauke is approximately 10 000. The cattle population of the south coast of Irian Jaya is being increased with imports from other provinces, especially Nusa Tenggara Timor (Kupang) and Nusa Tenggara Barat.

Seroconversions in sentinel cattle

To provide more information on the role of other viruses in the BEF virus group, detailed studies were carried out in Bali, Kupang and Jayapura, using Bali cattle (Bos javanicus). The sera were tested at a dilution of 1:4 in a serum neutralisation test (Soleha 1991).

The data available for Bali are for 1989-1990. In one animal, seroconversions were observed to Kimberley virus in March and to both Berimah virus and BEF virus in April. In December another animal seroconverted to BEF virus alone. In February individual calves seroconverted to either Kimberley virus or Adelaide River virus, and in March calves seroconverted to Kimberley virus and Adelaide River virus. It seems probable that several different BEF group viruses were circulating in the study area during the mid to late wet season. Not all animals seroconverted which may indicate inefficient vector transmission.

Seroconversions were recorded in two groups of calves at Kupang and Jayapura in 1990-1991. Patterns were similar to those in Bali. In Kupang one calf seroconverted to BEF virus, Berimah virus and Kimberley virus in the same month, while another calf seroconverted to BEF virus and Berimah virus at the same time. Several animals seroconverted to Berimah virus at the end of the wet season, and one seroconverted to Adelaide River virus in July in the early dry season.

In Jayapura, with its much wetter climate, seroconversions were again in the period March to July, the end of the wet season. Most animals in the group seroconverted to Berimah virus in June and July, with only four seroconverting to BEF virus. Also, during the observation period, most calves in the group seroconverted to Adelaide River virus, but over a longer period, January to September. In spite of the problem of virus cross-reactions complicating serological interpretations, at least two different patterns of seroconversions to different viruses were observed, which again suggests that more than one BEF group virus was circulating in the district, and also that the viruses may be preferentially spread by two different vectors.

In Bali and Kupang, separated from each other by over 1000 km, seroconversions to BEF virus and Berimah virus occurred first in December, and then in March, April and May. In Jayapura the seroconversions to these antigens were again in December, then in April and May, reaching a peak in June and July.

Discussion

Serological surveys have demonstrated that infection with BEF virus is widespread in Indonesia. No clinical disease has been reported in the sentinel animals monitored to date. Although earlier reports were of disease in Bos taurus, (Merkens 1919), with Bos taurus being more severely affected than Bos indicus (Burggraaf 1932), subsequent reports (Soeharsono et al. 1982, Ronohardjo et al. 1982) described clinical disease in local Bos indicus. Recent outbreaks of ephemeral fever-like disease in South Kalimantan involved many Bali cattle (Bos javanicus).

It is not clear whether BEF virus causes disease in water buffaloes (Bubalus bubalis) (Young 1979). None of the published reports from Indonesia mention this species, although a high prevalence of serological reactors has been found (see Soleha et al. these proceedings).


A Study of Bovine Ephemeral Fever Group Rhabdoviral Infections in West Java, Indonesia

E. Soleha¹, P.W. Daniels², Sukarsih¹ and I. Sendow¹

Abstract

The bovine ephemeral fever group of viruses includes, among others, bovine ephemeral fever, Berrimah, Kimberley and Adelaide River viruses. Serological studies of these four viruses were conducted in West Java to obtain preliminary information on the prevalence of infection in cattle and buffalo. Groups of sentinel cattle were monitored weekly to establish the seasonal incidence of infections. Viruses were isolated from sentinel cattle and from pools of Culicoides and mosquitoes. Serological evidence of infection with all four viruses has been found in cattle or buffalo. Some sheep and goat sera also contain antibodies to bovine ephemeral fever group viruses. Isolations of viruses included several that may be placed in the ephemeral fever group, subject to confirmation.

Ephemeral fever is caused by a rhabdovirus, bovine ephemeral fever virus, and has been reported from countries in Africa, Asia and Australia (St George, 1981). Clinical disease in cattle has been reported in Indonesia since early in the century, in 1918 and from 1928 to 1931 (Merkens 1919, Burggraaf 1932).

Although cattle are believed to be the primary host, antibodies to BEF virus have also been detected in some other ruminants; red deer (Cervus elaphus) and water or swamp buffalo (Bubalus bubalis) in Australia; waterbuck (Kobus ellipsiprymnus), wildebeest (Connochaetes taurinus), hartebeest (Alcelaphus buselaphus) and African buffalo (Syncerus caffer) in Africa and swamp buffalo in Malaysia (St George, 1988).

This paper reports further studies of BEF virus infections in West Java, the province where ephemeral fever was first reported in Indonesia (Merkens 1919). A serological survey of livestock in West Java for antibodies to several BEF group rhabdoviruses, BEF, Berrimah, Kimberley and Adelaide River viruses, was conducted and sentinel cattle were also monitored. Isolations of BEF group viruses were successfully attempted from sentinel cattle, and some collections of insects at the sentinel sites were made.

Materials and Methods

Sera collected in West Java from 1986 to 1992, from various species of livestock including buffalo, sheep, goats, horses, chickens and ducks, were accessed from a serum bank. A small stratified serological survey of cattle was also conducted in several districts in West Java specifically for the present study.

Sentinel cattle have been monitored in West Java since 1987 at Depok, 95 m above sea level and with an annual rainfall of 3000 mm. Holstein-Friesian cattle were bled either weekly or monthly, commencing when cattle were approximately three months of age.

Insects for attempted virus isolation were collected weekly at the sentinel site starting in 1991. Light traps were run for three to four hours at dusk, collecting into a buffer solution with detergent which was then kept cool overnight. Insects were identified and separated into species pools for virus isolation.

The first technique of virus isolation attempted was that described by St George et al. (1978), as modified by Sendow et al. (1989). Uncoagulated heparinised blood was centrifuged at 1500 rpm for 10 minutes to separate the white blood cells (WBC) from other fractions of the blood. Cell culture monolayers of $4 \times 10^5$ BHK21 cells in 2 ml of

¹ Research Institute for Veterinary Science, PO Box 52, Bogor, Indonesia
² Indonesia International Animal Research and Development Foundation, PO Box 94, BOUT, Bogor, Indonesia
minimum essential medium (MEM) (Flow Laboratories) containing 5% foetal bovine serum (FBS) (Flow Laboratories) and 200 units of kanamycin in roller tubes were inoculated with 0.1 ml of WBC from the buffy coat. The medium was changed with MEM containing 2% FBS and 200 units of Kanamycin 24 hours after inoculation. Cultures were incubated at 37°C and observed daily. After 5 days, cultures were passaged to new BHK21 roller tubes, using 0.1 ml of cell suspension as the inoculum. After three passages, cultures showing cytopathic effect were supplemented with 10% FBS, and stored at -70°C and in liquid nitrogen. Cultures not showing cytopathic effects were discarded.

Another technique for virus isolation using chicken embryos (Gard et al. 1988) has been used since 1990. Approximately 0.025 ml of whole blood diluted in phosphate buffered saline (PBS) was inoculated intravenously into embryonated chicken eggs. The eggs were incubated at 33.5°C and observed daily for 5 days. Dead embryos were harvested and the tissues homogenised and suspended in 2 ml of MEM with 5% FBS. Embryo suspensions were filtered through 450 μm (Millipore) and 0.1 ml of the filtered suspensions inoculated into monolayers of Aedes albopictus cells (C6/36) (St George 1985), and incubated at room temperature for seven days. Cell suspensions of infected C6/36 cells were passaged blind into monolayers of BHK21 cells in roller tubes.

Insect pools were processed in glass homogenisers in 2 ml of PBS at pH 7.2. Virus isolation in chicken embryos and C6/36 cells was used for virus isolation from insect suspensions.

Isolates were identified to group level by the indirect immunofluorescence (IF) test using the method of Cybinski and Zakrzewski (1983). Infected cultures of BHK21 cells on spot slides showing cytopathic effect were fixed with 50% acetone for 20 minutes. Slides were air dried. Viral antigens were detected by adding a drop of anti-BEF virus (strain BB7721) mouse ascitic fluid (maf) and incubating for 30 minutes. Slides were washed three times with PBS and air dried. A drop of rabbit anti-mouse FITC at a dilution of 1:16 was added and incubated at 37°C. After 30 minutes slides were washed three times with PBS, and once with distilled water. Stained cultures were read with a fluorescence microscope. Isolates reacting with antibodies to BEF virus in the immunofluorescence test were further tested in micro-neutralisation tests with polyclonal antibodies to BEF virus (Doherty et al. 1969), Berrimah virus strain DPP63 (Gard et al. 1983), Kimberley virus strain CS368 (Cybinski and Zakrzewski 1983), and Adelaide River virus strain DPP61 (Gard et al. 1984). Type viruses and antisera were supplied by CSIRO Long Pocket Laboratories, Australia.

Neutralisation tests were used for detecting antibodies to the BEF-group viruses in survey and sentinel sera collections, as listed above, and also for preliminary identification of isolates using methods previously described by Burgess (1974), Cybinski et al. (1978), and modified by Soleha (1991). Vero cells at a concentration of $2 \times 10^5$ cells per ml in MEM with 5% FBS were used. Viruses were propagated in monolayers of BHK21 cells and diluted to 100 TCID$_{50}$.

### Results

#### Serology

Antibodies to BEF-group rhabdoviruses were detected in several species of livestock (Table 1). Reactors to BEF virus were found in cattle (15%) and buffalo (17%); to Berrimah virus in cattle (10%), buffalo (29%), sheep (13%), and goats (3%); to Kimberley virus in cattle (20%), buffalo (29%), goats (2%), and horses (8%); and to Adelaide River virus in buffalo (2%) and goats (23%). Data on the

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cattle</th>
<th>Buffalo</th>
<th>Sheep</th>
<th>Goats</th>
<th>Horses</th>
<th>Ducks</th>
<th>Chickens</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEF</td>
<td>35/240</td>
<td>24/145</td>
<td>0/56</td>
<td>0/136</td>
<td>0/93</td>
<td>0/36</td>
<td>0/58</td>
</tr>
<tr>
<td>Berrimah</td>
<td>16/157</td>
<td>12/42</td>
<td>3/24</td>
<td>3/105</td>
<td>0/83</td>
<td>0/36</td>
<td>0/58</td>
</tr>
<tr>
<td>Kimberley</td>
<td>32/157</td>
<td>12/42</td>
<td>0/30</td>
<td>2/90</td>
<td>7/93</td>
<td>0/36</td>
<td>0/58</td>
</tr>
<tr>
<td>Adelaide River</td>
<td>0/89</td>
<td>1/42</td>
<td>0/50</td>
<td>3/13</td>
<td>0/83</td>
<td>0/36</td>
<td>0/58</td>
</tr>
</tbody>
</table>

Notes: Results are expressed as No. reactors/No. tested animals (figures in parenthesis are percentage of reactors)
distribution of infection at various altitudes showed a trend for higher prevalences at lower altitudes (Table 2). Because of the small number of sera processed and the small number of sites sampled, results were not analysed for statistical significance.

Sentinel cattle have been monitored since 1987 and tested monthly for antibodies to BEF-group viruses. Cattle were owned by various smallholder farmers, and difficulties in continuity of sampling were experienced. For example, in the period 1987–1988, the number of cattle monitored totalled 25, but only 10 cattle could be bled monthly for a full year. Antibodies to BEF virus have been detected in sentinel cattle each year. In 1987–1988 maternal antibodies were detected in 5 of 10 calves but were not detectable after cattle were 6 months of age. Seroconversions were seen in 8 of 10 cattle. In the period 1988–1989 one of 6 cattle had maternal antibodies, and 5 seroconverted. In 1989–1990, 5 of 11 cattle had maternal antibodies, and 6 seroconverted (Tables 3 and 4). The month in which seroconversions were detected each year varied but was most frequently in the period December to July (Table 4), from soon after the start of the wet season through to the end of the wet season.

Insects for virus isolation were collected in Depok and Cisarua from 1991. The insects identified and the groups for virus isolation are presented in Table 5. Culicoides spp. were identified to species, but mosquitoes to genus only.

In 1987–1988 and 1988–1989 the isolation system was inoculation of samples into BHK21 cell cultures for three passages. In subsequent years the isolation system was inoculation of samples into embryonated eggs, followed by passage in Aedes albopictus cells and three times passage in BHK21 cell cultures in rotating tubes. The number of isolates from sentinel cattle blood samples varied each year. Not all such isolates would be expected to be BEF-group viruses. Nine isolates were obtained in 1988, two in 1989, thirty seven in 1990, and three in 1991 (Table 6). A total of 51 isolates were made from blood samples

### Table 2. Detection of serum neutralising antibodies to BEF-group viruses in cattle at various altitudes in West Java.

<table>
<thead>
<tr>
<th>Place</th>
<th>Altitude (metres)</th>
<th>No. of Cattle</th>
<th>BEF</th>
<th>BRM</th>
<th>KIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garut</td>
<td>&lt;100</td>
<td>31</td>
<td>29%</td>
<td>16%</td>
<td>32%</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1240</td>
<td>18</td>
<td>6%</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Tasikmalaya</td>
<td>&lt;100</td>
<td>16</td>
<td>56%</td>
<td>31%</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>400–600</td>
<td>14</td>
<td>43%</td>
<td>21%</td>
<td>57%</td>
</tr>
<tr>
<td>Indramayu</td>
<td>&lt;100</td>
<td>28</td>
<td>46%</td>
<td>29%</td>
<td>21%</td>
</tr>
<tr>
<td>Majalengka</td>
<td>400</td>
<td>20</td>
<td>25%</td>
<td>10%</td>
<td>10%</td>
</tr>
</tbody>
</table>

### Table 3. Detection of neutralising antibodies to BEF virus in sentinel cattle at Depok, West Java in the period 1987–1988.

<table>
<thead>
<tr>
<th>No. Cattle</th>
<th>1987</th>
<th>1988</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jun</td>
<td>Jul</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>45</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>46</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>47</td>
<td>*</td>
<td>+</td>
</tr>
<tr>
<td>49</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

+ Titre more than 4
± Titre less than 4
- Negative
* Not available
collected at the Depok sentinel site in West Java. Three isolates were recovered from insect collections using the second method. Seven isolates from blood samples were characterised as belonging to the BEF virus group from samples collected in the months of February, March, April and June 1990 and from April in 1991. One of these isolates, from March 1990, was neutralised by antibodies to Berrimah virus in the micro neutralisation test.

Discussion

The study of BEF virus group infections in Indonesian livestock was commenced because clinical disease in cattle is known to occur. It was considered important to clarify the ephemeral fever status of Indonesia and to develop laboratory diagnostic capacity.

The BEF-group viruses studied serologically were those available as type antigens together with type antisera from an Australian laboratory. However, other related virus may also be present. For example, Malakal virus which was isolated from *Mansonia uniformis* in the Sudan, and Puchong virus, also isolated from these mosquitoes in Malaysia (Calisher et al. 1989). Other, undescribed, BEF-group viruses may also be present.

Serological results must be interpreted with care, because cross reactions among BEF-group viruses occur (Cybinski 1987). Hence antibodies to one BEF-group virus may be the result of infection with another BEF-group virus. None the less, the study of antibody prevalence in different species showed differences between viruses. All viruses tested were already known to infect cattle and buffalo in Australia on the basis of detection of antibodies (Cybinski and Zakrzewski 1983, Gard et al. 1984, Walker and Cybinski 1989). Losos (1986) reviewed the host range of BEF virus, and concluded that the virus does not naturally infect sheep, although they seroconvert when infected experimentally. No evidence of infection has been reported in goats, pigs or horses. However, after an epidemic of ephemeral fever in Taiwan, 1 of 16 sheep (6%) and 22 of 46

| Table 4. Seroconversions to BEF virus in four groups of sentinel cattle at Depok, West Java. |
|---|---|---|
| Year | No. of cattle | Seroconversion (%) | Month of seroconversion |
| 1987-1988 | 10 | 8 (80) | Jan, Feb, Mar, Jun, Jul |
| 1988-1989 | 6 | 5 (83) | Dec, Mar, May, Sep |
| 1989 | 7 | 4 (71) | May, Jun |
| 1989-1990 | 4 | 2 (50) | Jun, Jul |

| Table 5. Insects collected at Depok, West Java, in 1991 and sorted according to species. |
|---|---|---|
| Insects | Genus/subgenus/group | Species identified |
| Culicoides | Avarita subgenus | *C. actoni* |
| Culicoides | Avarita subgenus | *C. brevitaris*, *C. dumduni* |
| Culicoides | Hoffmania subgenus | *C. flavipunctatus*, *C. fluvus*, *C. jacobsoni*, *C. orientalis* and *C. wadai* |
| Culicoides | Trithecoides | *C. insignipenis*, *C. peregrines* and *C. sumatrace* |
| Culicoides | Meijereheleca subgenus | *C. albibasis*, *C. barnetti*, *C. gewertzi*, *C. palpifer* and *C. parahumeralis* |
| Culicoides | Shermoni group | *C. arakawaean* and *C. guttifer* |
| Culicoides | Clavipalpis group | *C. geminus* |
| Culicoides | Shortii group | *C. huffy* |
| Mosquitoes | Anopheles spp | *C. oxystoma* |
| Mosquitoes | Aedes spp | *C. shortii* |

1 Wirh and Hubert (1989)

2 Pools processed for isolation of viruses
goats (48%) had antibodies to BEF virus although the serological test protocol used was not specified (Chiu and Lu 1986).

Berrimah virus and Kimberley virus both differed from BEF virus in that antibodies were detected in small ruminants; sheep and goats in the case of Berrimah virus and goats in the case on Kimberley virus. In addition, antibodies to Kimberley virus were found in horses. A previous study in Australia failed to detect antibodies to Kimberley virus in sheep, goats and horses (Cybinski and Zakrzewski 1983). In this study, antibodies to Adelaide River virus were not found in cattle, and in only one buffalo. However other data (see Daniels et al. these proceedings) describes antibody to this virus in large ruminants in Indonesia. Goats had a higher seroprevalence for Adelaide River virus than the other viruses tested. The first report of Adelaide River virus (Gard et al. 1984), found antibodies in pigs but not in horses and goats. Clinical disease and serological reactors to BEF-group viruses in chickens and ducks have not been reported previously, and in this study there was no evidence of antibody in these species. Poultry would therefore not be useful as sentinel animals for BEF-group arboviral infections. Although the problem of serological cross reactions precludes definite interpretation of these results in a range of species, it appears that in a tropical endemic situation, Indonesia, the mammalian host range of the BEF-group viruses may be wider than previously indicated.

A sentinel animal program was considered important to allow opportunities for isolating viruses, and to start to describe the seasonal pattern of infections (Daniels et al. 1991). Depok in West Java was chosen as a high rainfall, low altitude site with a large cattle population, close to laboratory facilities. Seroconversions were observed from early in the wet season through to the end, from December to July. This pattern was somewhat different from that observed for bluetongue viral infections (Sendow et al. 1988), where the same group of sentinel animals in the two years 1987 to 1989 seroconverted to the bluetongue group orbiviruses predominantly in February and March every year. This may suggest that the vectors for these two groups of viruses differ, with some vectors of BEF virus being active earlier in the wet season. Preliminary analyses of antibodies to flaviviruses in these same calves in the 1987–1988 wet season also showed peaks of infection early in the wet season, from December to February (Sendow et al. 1988). In Indonesia flaviviruses have been isolated from Aedes spp., Culex spp. and Anopheles spp. mosquitoes.

Insect collections were made as a preliminary study of potential vectors, using insect identification and virus isolation from insects. BEF virus has been isolated from C. brevitarsis and Anopheles bancrofti in Australia, and from a pool of Culicoides spp. in Kenya. Kimberley virus has been isolated from C. brevitarsis and the mosquito Culex annulirostris. It has been suggested that Culex annulirostris may be a major vector of BEF virus ( Muller and Standfast 1986). Our insect collections showed that potential vectors such as Culicoides spp. of the Avaritia subgenus, especially C. brevitarsis and Anopheles spp. mosquitoes were present. Culex spp. mosquitoes were not caught in the light traps.

Seven virus isolates from West Java have been provisionally typed to the BEF virus group, but only one of these was neutralised by antisera to the four viruses studied, namely Berrimah virus. Further work in two-way neutralisation tests may confirm this as the first identification of a BEF-group virus in Indonesia. Of equal interest is that the other six isolates were not neutralised by antisera to any of the four type viruses, indicating that other BEF group viruses have been isolated.

References


Wirth, W.W. and Hubert, A.A. 1989. The Culicoides of Southeast Asia (Diptera Ceratopogonidae). The American Entomological Institute, Gainsville, USA.
The Natural History of Ephemeral Fever in Kenya

F.G. Davies*

Abstract

Ephemeral fever occurs in cattle in Kenya across a wide range of ecological zones, from semi-desert to temperate highland grasslands. None of the indigenous wild ruminant fauna manifest any clinical sign of disease, although serum neutralising antibody has been detected in some species. Clinical disease occurs either sporadically, or in epidemics lasting 2-3 years when there is a high morbidity in cattle. There is an association with rainfall but this is not absolute. There are clear interepidemic periods, when no clinical disease is evident anywhere in the country.

Clinical ephemeral fever in cattle was recognised in Kenya in the early years of this century. The disease was reproduced in 1913 by the subinoculation of blood from an infected to a susceptible bovine host at Kabete (Annual Report, Kenya Department of Agriculture, 1912-1913). The disease reappeared at intervals, usually in those cycles of wet years when Rift Valley fever was also encountered (Davies et al. 1985), and has continued intermittently ever since. Ephemeral fever was also reported in Uganda and Tanzania over the same periods (Annual Reports, Veterinary Departments, Uganda and Tanzania).

In indigenous zebu cattle (Bos indicus), the clinical signs of ephemeral fever are mild and do not persist for more than 1-2 days. In contrast, the disease in imported Bos taurus breeds is more severe and epidemics of ephemeral fever cause significant economic losses in milk production and depressed growth rates in beef cattle. While farmers are well aware of the effects of ephemeral fever on production, there are many other diseases which are considered to be of greater importance (rinderpest, Rift Valley fever, pleuropneumonia, East Coast fever, etc.), and little attention has been given to the problem of ephemeral fever in East Africa. This paper summarises the work on ephemeral fever carried out by the author from 1968 to 1989, at the Veterinary Research Laboratories, Kabete.

Virus Isolation

In 1972 and 1973, bovine ephemeral fever (BEF) virus was isolated from clinical cases in the Rift Valley (altitude 1600-1800 metres, 0° 28' S; 36° 13' E) in semi-arid country, defined as Ecological Zone IV in the classification of Pratt et al. (1966). At that time there were local outbreaks of ephemeral fever, involving more than 1000 cattle of Bos taurus, Bos indicus and their crosses. The outbreaks were apparently not associated with any local rainfall, but the farms were adjacent to a fresh water lake (Naivasha) and a salt water lake (Nakuru).

Blood was collected, in EDTA anticoagulant, from clinical cases on the first day when signs were evident. Samples were transported on ice to the laboratory where theuffy coat was separated and each sample inoculated into a least four litters of 1-4 day old mice by the intracerebral route (Davies and Walker 1974) within one hour of returning to the laboratory. Some isolations required two passages, and one isolate required four passages, before neurological signs became evident. In this initial study, BEF virus was isolated from at least half of the samples processed.

To reproduce clinical disease, twouffy coat samples from which virus had been isolated in mice, were inoculated into susceptible cattle. This resulted in clinical disease in one animal but only transient clinical signs in the other. Pre- and post-infection serum samples were collected for serology. Mouse ascitic fluids were also prepared from an Australian BEF virus isolate and from a Kenyan isolate.

* G2 The Court, St Mary's Place, Shrewsbury, Shropshire SY1 1DY, United Kingdom
K/86/73. These sera were used in neutralisation tests, initially in mice, and later in cell cultures.

In two-way, cross neutralisation tests (in infant mice and using pre- and post-inoculation cattle sera and ascitic fluids) no differences were evident between the Kenyan and Australian BEF viruses. Later, constant-virus varying-serum, microtitre neutralisation tests using cell cultures confirmed that the isolates from the two continents appeared to be antigenically identical.

**Mammalian Host Range**

There is relatively little information on the susceptibility of wild ruminants to BEF virus although the Asian water buffalo *Bubalus bubalis* has been shown to be mildly susceptible to ephemeral fever (Topacco et al. 1937). It is possible that BEF virus may have circulated as an inapparent infection amongst wild ruminant species and was undetected until susceptible cattle were introduced and clinical disease became evident. In order to establish whether BEF virus infection of wild ruminants occurs, sera were obtained from wild ruminants in the Rift Valley (Davies et al. 1975) and tested for the presence of neutralising antibody to BEF virus using a cell culture, microtitre neutralisation test with the K/86/73 isolate of BEF virus.

Fifty-four per cent of sera from African buffalo (*Syncerus caffer*) and 61% of sera from waterbuck (*Kobus ellipsiprymnus*) had neutralising antibody to BEF virus, whereas only 9% of wildebeest (*Connochaetes taurinus*), and 3% of hartebeest (*Alcelaphus buselaphus*) sera had antibody to BEF virus. No antibody was found in sera from impala, Grants or Thomson gazelle, even though these species were very common in the ecological zone examined. Antibody could not be detected in sera from eland or oryx, although very few sera from these two species were examined. Infection of African buffalo seems to occur frequently and it is not uncommon to see titres of 1/80-1/320 in sera from these animals.

**Ecological Range**

A template for the classification of East African rangelands was made by Pratt et al. (1966), to relate the potential for livestock use with vegetational and moisture characteristics. This classification is also relevant to insect ecology. A summary of the principal features is given by Davies et al. (1975).

To investigate the distribution of BEF virus in Kenya, sera were collected from cattle at 36 sites, representing the whole range of ecological zones in Kenya. The cattle sampled had been exposed to an epidemic of ephemeral fever in 1967-1968 which spread throughout the whole country. Sera with neutralising antibody to BEF virus were found in all the ecological zones, from desert and semi-desert to the tropical coastal zone and temperate high altitude grasslands. In individual herds, seropositive rates of 24-95% were found, and high rates were often found in the drier zones. The results show that BEF virus infection can occur with a high frequency in all the ecological zones present in Kenya.

**Interepidemic Periods**

There have been periods when clinical disease appeared to be totally absent from any part of the country. Such a period was from 1969 until 1972, during which time surveillance for clinical disease was maintained over the whole country. In addition, active weekly examination was made of a sentinel herd located on site at Kabete farm and of other cattle at the same farm, a total of about 5000 animals. Only five seroconversions were detected in 1971 in the sentinel cattle.

An isolated outbreak of ephemeral fever, which was confirmed serologically, occurred in Ecological Zone V (dry thorn bush country) after local very heavy rains. The first cases were seen 10-12 days after the rains, followed by many more cases after 18-21 days. The majority of insects trapped at the site were *Culicoides* spp. and only 15% of the total light trap catch were mosquitoes.

To further investigate the role of wild ruminants in the ecology of ephemeral fever, sera collected from wild ruminants were examined for antibody to BEF virus. More than 50% of sera from buffalo and waterbuck born in an interepidemic period (1968 to 1972) had antibody to BEF virus, suggesting that virus infection of wild ruminants occurred when there were no reported cases of ephemeral fever in cattle. Unfortunately sera were not collected from cattle in that area during the same period. However it is interesting to note that the 1972-1973 outbreak of ephemeral fever occurred in an area where there was a high frequency of infection of buffalo in preceding years.

**Vector Studies**

BEF virus was isolated from a large mixed pool of *Culicoides* spp. collected at the site where BEF virus was isolated from cattle in 1972-1973. The insect isolate was from a mixed pool consisting of *C. kingii* 67%, *C. nivosus* 24%, *C. bedfordii* 8%, *C. pallidipennis* 1% and *C. cornutus* 1% (Davies and Walker 1974). However, virus was not isolated from nine other *Culicoides* pools, nor from five mosquito pools collected at the same site.

No BEF virus isolates were made from the many pools of *Culicoides* spp. processed over a period of
years when clinical ephemeral fever was not reported (Davies et al. 1979). In addition, BEF virus was not isolated from a large series of mosquito pools which were collected during interepidemic periods, even though many other viruses were isolated from this material (Linthicum et al. 1985).

**General Observations**

An hypothesis was made early in our ephemeral fever studies that if the virus was transmitted only by a mosquito vector, then the distribution of the disease should coincide with that of the vector. Furthermore, the distribution of ephemeral fever should be similar to that of other recognised mosquito transmitted diseases such as Rift Valley fever. As mosquito species are only found in certain ecological zones in Kenya (Davies et al. 1975) then ephemeral fever should be restricted to these zones and should not be widespread. The disease proved to be widespread, with many positive sera in semidesert areas where bovine antibody to Rift Valley fever virus was rarely detected. *Culicoides* spp. were also found to be widely distributed and were invariably present when outbreaks of ephemeral fever occurred. The sentinel herd of cattle at Kabete provided evidence that most cattle seroconverted to Akabane virus (Davies and Jesset 1985) and to many strains of bluetongue virus (Davies 1978) by the time they were two years of age. Both of these viruses are transmitted by *Culicoides* spp. and it is apparent that infections with these viruses was occurring every year. However, this was not the case with BEF virus at this site, as no seroconversions were detected in sentinel cattle during many of the study years when seroconversions to Akabane and bluetongue viruses occurred. If BEF virus is transmitted exclusively by *Culicoides* spp. then it must have a lower incidence in the vector population, much lower than bluetongue or Akabane viruses.

Another explanation might be that the virus is transovarially transmitted by mosquitoes, in the same manner as Rift Valley fever virus (Linthicum et al. 1985), and emerges when the floodwater breeding species are hatched following heavy and prolonged rains. A situation where *Culicoides* spp. could also be involved, as the virus is amplified in vertebrate hosts, then becomes possible. St George and Standfast (1983) have also drawn attention to this possibility as a result of their work in Queensland. Their data show that ephemeral fever outbreaks correlate more closely with increases in numbers of mosquitoes than with *C. brevitarsis* numbers.

**References**


The 1990–1991 Epidemic of Ephemeral Fever in Egypt and the Potential for Spread to the Mediterranean Region

F.G. Davies*, A. Moussa* and G. Barsoum*

Abstract

Clinical ephemeral fever occurred in Egypt in 1990–1991 and spread throughout the whole of the Nile Valley and Delta. High morbidity (20–90%) occurred in imported Bos taurus dairy breeds. Fattening cattle of local breeds in intensive systems were also severely affected. The disease was less severe in unimproved village animals. Water buffalo (Bubalus bubalis) were mildly affected at a low prevalence. The disease was economically significant due to loss of milk production and mortality in dairy and beef animals. Infertility problems were also important. The potential for further extension of ephemeral fever into North Africa and into some Mediterranean countries is discussed.

EPHEMERAL fever has been known to occur in Egypt for many years (Piot 1896, 1909; Rabagliati 1924). Ephemeral fever has occurred throughout most of Africa to the south of Egypt, where it is endemic with periodic epidemics. In the Middle East, the disease has only been recognised in epidemic form in Israel, Iran, Iraq and Saudi Arabia. There are unconfirmed reports that ephemeral fever may have occurred in the Eastern Mediterranean region during the period 1989–1991. The 38°N latitude appears to be an ecoclimatic barrier as the disease has not been reported north of this latitude (Tanaka and Inaba 1986) except in China where it was reported at 44°N (Zhang et al. these proceedings). Clinical cases of ephemeral fever have not yet been reported in Europe, although antibody to bovine ephemeral fever (BEF) virus has been detected in serological surveys in southern Russia.

Epidemiological Observations

Distribution of clinical cases

Clinical ephemeral fever was reported in 1990 and 1991 from most of the Governates in Egypt. The Governates of Aswan, Quena, Sohag and Assiut in Upper Egypt were first affected in the summer of 1990. Further cases were described later that year from El Minya and Beni Suef. The disease was comparatively mild and occurred mainly in indigenous cattle and in buffalo as few exotic cattle were kept in those areas. Damietta in the eastern part of the Delta was affected in the autumn of 1990. In 1991 the Delta Governates experienced the disease, as well as all the Nile Valley Governates and the oases of Fayoum, Bahariya, Dakahlia and New Valley. In rice growing areas, morbidity rates appeared to be higher and cattle appeared to be more severely affected.

Epidemic pattern

Ephemeral fever occurred in the irrigated lands along the Valley of the Nile, throughout the Nile Delta and the more recently cultivated areas at the edge of the Delta. The desert oases far removed from the Nile Valley also reported the disease, as have well-head irrigation schemes. This suggests that aerial movement of the virus occurred. Clinical cases occurred in both summer and winter months. Although the prevalence of the disease was higher in June to August and fewer cases occurred in the winter months, the disease did not totally disappear in winter as has been described in some temperate zones of southern Africa.

Host Range

Domestic cattle, both indigenous and exotic to Egypt, were clinically affected. Imported Holstein cattle were more severely affected than native breeds (mostly Bos taurus) which showed milder clinical
signs of shorter duration, and had lower production losses. Morbidity rates in individual herds of imported cattle varied from 20–90%. Mortality rates in imported cattle were in the range 1.5–3%. The animals most likely to die were the largest, heaviest and highest producers. Thus the most valuable animals in the herd appeared to be the most susceptible to ephemeral fever.

Many indigenous animals which would have recovered were slaughtered in the early months of the epidemic, when they became recumbent. Other diseases which stock owners are familiar with, such as Rift Valley fever, and which cause prostration, often result in death. However, farmers soon became more familiar with ephemeral fever, where recovery was rapid and often complete, and fewer animals were slaughtered. Similar responses by farmers to outbreaks of ephemeral fever have also been reported from Indonesia (see Daniels et al. these proceedings).

The morbidity rate in buffalo was much lower than in cattle with only about 5% of buffalo showing any clinical signs. Very mild, transient and presumably subclinical infections occurred and there was little apparent fall in milk yield.

Age groups affected
Calves, weaners and young animals up to two years of age were generally far less severely affected than other age groups and cattle 2–3 years old showed only mild clinical signs. The most severe signs were seen in large, heavy milking cattle of Friesian or Holstein type, of more than 3–4 lactations, when they were in heaviest production. Large dairy bulls were frequently very severely affected.

Economic Effects
Farms with imported Holstein cattle and their crosses reported a 50% decrease in milk production over the outbreak period. The outbreaks lasted for 6–12 weeks in individual herds. Production then increased, but only to 75–85% of the expected yield.

Up to 80% of cattle in intensive feedlot systems developed clinical disease which was milder in the indigenous breeds than in the imported breeds. Heavier animals experienced more severe disease than lighter animals. Weekly weighing showed either no weight gains or loss of weight for periods of 3–6 weeks or longer after the onset of clinical signs.

Abortion rates of up to 3% were reported in Holstein cattle, usually at 4–7 months gestational age. This was probably an indirect effect as BEF virus does not infect the foetus. Detailed analysis in one herd with 80–90% morbidity showed 30% loss of foetuses of 45–105 days gestation, and some 10% loss of foetuses of 105–290 days. Laboratory tests ruled out the possibility of intercurrent brucellosis, vibriosis and trichomoniasis. Some bulls which had ephemeral fever were found to be infertile during the epidemic.

General Observations
There was a countrywide epidemic of ephemeral fever in Egypt in 1991 affecting approximately 250,000 imported cattle and a smaller number of indigenous cattle and buffalo. The disease produced its most severe clinical and economic effects in the intensive, highly productive milk and beef fattening sectors of the cattle industry. The intensive milk producing farms have a disproportionate importance in Egypt, for they supply fresh milk to the urban populations of Cairo and other cities. The rural population consumes mostly buffalo milk, and these animals were much less severely affected than imported cattle.

The disease may have entered Egypt from the south or from the east by the aerial movement of arthropod vectors. The vectors of BEF virus in Egypt have not yet been identified, but mosquito and Culicoides species are abundant, the former especially so in rice-growing areas.

Potential for further extension of ephemeral fever in the region
There have been a number of studies of the movement of insect pests and insects capable of acting as vectors for BEF virus in the Middle East and Eastern Mediterranean region (Williams 1924; Rainey 1951, 1973; Garret-Jones 1962; Dinoor and Levi 1967; Sellars 1980; Shimshony et al. 1989).

Air currents have been shown to be capable of carrying insects north from sub-Saharan Africa. These insects might be expected to carry BEF virus and other arboviruses into Egypt, Israel, Lebanon, Iran, Iraq, Syria, Turkey, Saudi Arabia, Arabia, Yemen and the Arabian peninsula. Ephemeral fever has already been described in most of these countries even though it is not a notifiable disease under the regulations of the OIE.

The potential also exists for the periodic windborne spread of BEF virus to countries such as Yugoslavia, Bulgaria, Albania, Italy, Cyprus, Greece and possibly even Spain, as well as to North African countries. A knowledge of the distribution of potential vectors of BEF virus in these countries would be valuable and surveillance should be maintained when there are epidemics in the Middle East.
References


Rabagliati, D.S. 1924. Three day's fever or stiff sickness in cattle. Veterinary Record, 4, 503-505.


Epidemiology, Clinical Findings and Treatment of Ephemeral Fever in Buffalo (*Bubalus bubalis*)


Abstract

This paper describes the epidemiology, clinical findings and treatment of ephemeral fever in buffalo (*Bubalus bubalis*) in Gujarat State, India where the disease is now endemic. The occurrence of the disease has changed from the previous pattern of severe sporadic epidemics, to a slow moving moderate epidemic, with about 10% morbidity and 1-2% mortality. Most cases occur in the summer and monsoon seasons, with occasional cases during the remainder of the year. The disease is of considerable economic importance due to a drastic reduction of up to 70% in milk production and the death of a few animals. Factors which appear to contribute to the disease include: a sudden change of weather; increase in the insect population; advanced pregnancy; parturition and high milk production. The disease is more common in well fed, healthy buffaloes. In most cases the disease is subacute. There is a sudden high temperature, anorexia, a sharp fall in milk production, profound dullness and depression, shivering, stiffness and lameness in one or more limbs. Clinical signs last for 4-5 days followed by spontaneous recovery. Some cases become complicated and finally die. Most cases recover spontaneously or with simple treatment. The acute cases respond well to calcium gluconate, dextrose, vitamins, analgesics and phenylbutazone injections. Good nursing care helps in early recovery.

Bovine ephemeral fever (BEF) is a disease of cattle and buffalo caused by an insect-borne rhabdovirus. The disease is characterised by inflammation of mesodermal tissues and manifested by muscular stiffness, lameness etc. (Blood and Radostits 1989). Ephemeral fever in buffalo has only rarely been described in other countries. This paper reports general field observations on ephemeral fever in buffaloes (*Bubalus bubalis*) in Gujarat, India.

Epidemiology

Ephemeral fever in Gujarat now occurs endemically with occasional outbreaks. However in previous times the disease used to occur as sporadic, severe epidemics. This change from epidemic to endemic behaviour has been observed in cattle in other countries (Blood and Radostits 1989). Ephemeral fever in buffalo has only rarely been described in other countries. This paper reports general field observations on ephemeral fever in buffaloes (*Bubalus bubalis*) in Gujarat, India.

Clinical findings

The symptoms of ephemeral fever in buffaloes are the same as in cattle. In most cases, the disease is

* Gujarat Agricultural University, Sardar, Krushinager-385, 506, Gujarat, India
subacute. There is sudden rise of body temperature (105-106°F), complete anorexia, sharp fall in milk yield, profound dullness and depression, constipation, ruminal stasis, drooling salivation, dry muzzle, nasal and ocular discharges, muscle shivering, weakness of limbs and lameness in one or more limbs. Some buffaloes also show respiratory signs. On the second day clinical signs are more severe than on the first day. There is more pronounced stiffness, weakness, lameness and animals adopt a posture similar to that of acute laminitis, with all four feet placed well under the body. The animal is reluctant to move and prefers to lie down quietly most of the time. Some animals are unable to get up, even on application of pain stimuli and adopt a posture similar to that of parturient paresis, that is, a sternal recumbency and head turned in to the flank. On rising most animals become recumbent again in a few minutes as they are unable to bear weight. The shifting of pain from one limb to another is characteristic.

On the third day there is usually little or no improvement. Occasionally, very severe cases remain down and adopt a posture of lateral recumbency. About the fourth day, the animals start eating and ruminating, their body temperature decreases, and they are able to rise alone showing moderate improvement. Most of the cases recover rapidly on the fifth day and completely recover on the sixth or seventh day.

Occasionally, cases of long duration develop subcutaneous emphysema, bed sores and ulcers, maggot infestation, show downer cow syndrome and finally die. Subcutaneous emphysema has been observed by Theodoridis and Coetzer (1979). Occasional cases become complicated with abortion due to high fever. Mild clinical cases occur at the end of outbreak with mild signs of fever, lameness and inappetence.

The characteristic clinical signs of fever, limb stiffness and pain are due to viral septicaemia which produce inflammation of mesodermal tissue especially joints, lymph nodes and muscles (Burgess 1971).

Treatment

Most cases recover spontaneously or with simple treatment. Prompt adequate treatment is necessary to get early recovery, to regain lost production and to avoid complications and losses. A successful treatment used in Gujarat is intravenous 20% dextrose and analgesics together with intramuscular phenylbutazone and multivitamins. Two such treatments on consecutive days are usually sufficient. Recumbent cases respond well to intravenous calcium gluconate. Good nursing care helps in early recovery. In acute cases oral medication is to be avoided. In an experimental study on the effect of phenylbutazone for the prevention of ephemeral fever, Uren et al. (1989) observed that the drug prevented fever and other clinical signs in 6 out of 16 cattle.

References

Pathology, Pathogenesis and Diagnosis

The papers included in this section reflect the present state of knowledge on the pathology of ephemeral fever. Although the disease is very serious in economic terms, the pathology has not been well described. The evidence presented in these papers confirms that it is inflammatory in nature. There is also strong support for this from biochemical indicators and the success of anti-inflammatory treatments (which is unusual for a viral disease). However, the pathologists have so far been unable to determine why fatalities occur and the site of viral multiplication has not been identified.

As the primary effects of bovine ephemeral fever virus are in the vascular system, investigations need to be focused in this tissue to identify the target cells. Improvements in technology, such as efficient virus isolation, electron microscopy and molecular probes, all increase the chances of success.
Pathological Changes in Cattle Experimentally Infected with Bovine Ephemeral Fever Virus

Chu Guifang*, Zhang Zigang*, Yin Xunnan* and Bai Wenbin*

Abstract

Ninety-three cattle experimentally infected with the Chinese (Beijing 1) strain of bovine ephemeral fever virus were necropsied 2-10 days post-infection. The principal pathological changes observed were serofibrinous arthritis (59% of cases), interstitial emphysema (31%), nephritic infarcts (69%) and bronchopneumonia (9%). The severity of the lesions appeared to be greater in cows than in steers.

Bovine ephemeral fever (BEF) is characterised by sudden illness and fever. Mortality is usually very low although the morbidity can be high. Because of the low mortality, field necropsies are infrequently undertaken and there is relatively little information available on the pathology of the naturally occurring disease. Some studies have been undertaken on the pathology of the experimental disease in other countries (Basson et al. 1970; Young and Spradbrow 1990). Experiments were carried out to reproduce the disease with a Chinese isolate of BEF virus and to describe the pathology of these experimental cases.

Materials and Methods

In all, 93 cattle (16 cows and 77 steers) were used. The ages varied from one to five years and all were proven to be free of ephemeral fever prior to experimental infection. The animals were infected by intravenous injection of 1-5 mL of the Chinese (Beijing strain) of BEF virus. Clinical signs usually appeared 3-5 days after viral inoculation and the animals were killed 2-10 days after the cessation of fever. Tissue samples were fixed with 100% formalin and then embedded in paraffin. Sections were cut, stained with haematoxylin and eosin and examined microscopically.

Results

Interstitial emphysema was observed in the lungs of a large number of animals (31.5%) and was observed mainly in the apical lobes and the anterior surface of the cardiac and diaphragmatic lobes. Lung parenchyma exhibited congestion, oedema and over-distension of alveoli. Haemorrhagic changes occurred in a few cases. Dark red, and dull purple liver-like lesions, 1-3 cm in diameter, were observed in the lung parenchyma of 9% of cases. Interstitial spaces were thickened in a number of animals. Histological examination revealed a large amount of mucus, fibrin and dead epithelial cells in bronchi as well as swollen ciliated epithelial cells. The submucosal tissues were infiltrated by a large number of lymphocytes and the alveolar spaces were reduced or obliterated. The walls of small arteries were thickened, the endothelial cells were swollen, and in some cases, the lumen was totally occluded.

In 59% of cases there was swelling of the shoulder, knee or hock joints and the volume of joint fluid was increased. In the early stages of the disease, the joint fluid was transparent or only slightly turbid but as the disease progressed the joint fluid became cloudy and grey. In 44% of cases there were fibrin flakes in the joint fluid. In later stages of the disease, fibrin was attached to the articular surfaces and joint capsules. In two cases there was blood in the joint fluid and in a third case, there was an ulcer on the articular surface. Fourteen of the 16 cows examined had a severe serofibrinous arthritis.

In 69% of cases, the kidneys were swollen and the cortex pale. Grey-white necrotic foci, varying in size...
and number were found in 43% of the cases. The necroses in the kidney surface were wedge shaped. Petecchial haemorrhage was seen in one case and cortical micro-abscesses in another. Histologically the arterial walls were thickened and endothelial cells were swollen, hyperplastic and sloughing. The lumen of some arteries was completely blocked. Focal necrosis, typical of nephroanaemic infarct, was seen in the kidney cortex.

Livers were slightly swollen and fragile in 35% of cases. Histological examination showed granular degenerating hepatocytes, swollen Kupffer cells and hepatic sinuses infiltrated with lymphocytes.

In 13% of cases, the endocardium had striped or spotted haemorrhages and the myocardium was soft and light coloured. Histological examination revealed granular degeneration of the myocardium, and in some cases, lymphocytic infiltration around the cardiac blood vessels.

The spleen in 55% of cases exhibited swelling of the pulp and indistinct splenic trabeculae. In 11% of cases which had a prolonged clinical course, a slight hyperplasia was seen in the splenic follicles. Microscopic examination revealed that the endothelial cells of splenic central arterioles were swollen, hyperplastic, sloughing and occluding the lumen. Basilar membrane cells were swollen, resulting in a thickened arterial wall. Most of follicles were ruptured or atrophic, while a few of the germinal centres in follicles were enlarged.

Lymph nodes, especially the cervical and popliteal, were enlarged. Focal haemorrhage was seen in eight cases and focal cortical necrosis in two cases. Microscopical examination revealed that the endothelial cells of small arteries were swollen and hyperplastic and there were large numbers of lymphocytes in the sinuses. Some germinal centres in the lymph nodes were slightly enlarged. Some reticular cells exhibited proliferation and some showed degeneration and necrosis.

The tonsils of most cases were swollen and yellowish. Microscopically, focal necrosis was observed in the epithelium of the crypts and the follicles were swollen. Reticular cells in the germinal centres were also swollen and necrotic. In three cases focal haemorrhage was observed in the laryngeal mucosa.

Discussion

The main pathological features in cattle experimentally infected with bovine ephemeral fever virus were serofibrous arthritis, bronchopneumonia, interstitial emphysema and nephronecrosis. The frequency and the severity of pathological lesions appeared to be more pronounced in cows than steers, although only 16 cows were infected, compared to 77 steers. The pathological manifestations in cattle inoculated with Chinese bovine ephemeral fever virus strain were similar to those observed by Basson et al. (1970) and by Young and Spradbrow (1990), in which the main pathological changes occurred in joints and muscles.

A comparison of our observations of the pathology of experimental and natural cases revealed that haemorrhagic and enteric tract changes were more obvious and serious in natural cases. Whether this resulted from coinfections is difficult to determine.

By histological examination, the main pathological change observed in our experimental cases was damage to small arteries throughout the body, resulting in endothelial cells becoming swollen, hyperplastic and detached, eventually blocking the lumen. The main effect of this damage appeared to be disruption of the circulatory system with poor nutrition of tissues and the appearance of focal necrosis. The pathological changes may indicate that BEF virus has an affinity for endothelial cells and virus replication may cause direct damage to the cells.

Similar pathological changes were observed in the blood vessels of lymph nodes and spleen, however there did not appear to be any serious damage to the immune cells. Animals killed eight days after cessation of fever had enlarged germinal centres and lymphocyte proliferation. This phenomenon indicated an increase in immune activity, which is possibly the main reason for the low mortality of this disease.

References


The Pathology of Bovine Ephemeral Fever with Special Reference to the Pathogenesis of the Joint and Skeletal Muscle Lesions and Pulmonary Emphysema

J.A.W. Coetzet1, P.A. Basson2 and J.G. Pienaar3

Abstract

The pathology of ephemeral fever has been reviewed with reference to the pathogenesis of the disease. The main lesions are found in joints, periarticular tissues, tendon sheaths and certain skeletal muscles of locomotion but the vascular, genital, respiratory and central nervous systems may also be affected. In typical cases of the disease, vasculitis, sometimes accompanied by thrombosis, is associated with serofibrinous synovitis and fasciitis and focal necrotic muscle lesions. The presence of numerous neutrophils, particularly in the affected joints and tendon sheaths, and high levels of interferon would seem to influence the severity of these lesions.

Bronchiolitis is thought to play a role in the development of pulmonary and subcutaneous emphysema which is sometimes present in cattle suffering from the disease. In animals that have been recumbent for a prolonged period, status spongiosus of certain white matter tracts in the spinal cord and brain have been reported. Sperm abnormalities and an increase in the somatic cell count in the milk occur in affected animals.

Since the first recognition of bovine ephemeral fever in Zimbabwe by Bevan (1907), surprisingly few studies have been carried out on the subcontinent. The only detailed report on ephemeral fever in the region is by Basson et al. (1970) describing joint and muscle lesions in experimentally produced cases. Although outbreaks of ephemeral fever occur almost annually in South Africa and in other countries throughout the world, few cases are presented for routine necropsy with the result that the pathology of the natural disease is poorly described. The aim of this paper is to review the pathology of ephemeral fever and to discuss the pathogenic mechanisms which play a role in the development of lesions.

Joint, periarticular, tendon sheath and skeletal muscle lesions

Typical clinical signs of ephemeral fever such as stiffness, lameness, paresis and recumbency are the result of inflammatory lesions in the joints, periarticular tissues, tendon sheaths and skeletal muscles. There is a correlation between the severity of the lesions and clinical signs. Basson et al. (1970) gave a detailed account of the joint, periarticular, tendon sheath and skeletal muscle lesions at 1-4 days, 6 days and 10-15 days after the febrile reaction in cattle infected experimentally with BEF virus. Most of the information reported below was obtained from that publication or from unpublished observations made by the authors, unless otherwise stated.

Lesions are invariably more severe in the limbs on which the animal is lame but there may be considerable variation in the severity of the joint lesions in the same animal. While two or three joints are usually severely affected, other joints may not be involved or may only show mild lesions. The inflammatory changes are usually most severe in the larger joints of affected limbs: the stifle, followed by the hip, shoulder and elbow joints with approximate

1 Department of Infectious Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa
2 State Veterinarian, P.O. Box 81, Grootsfontein, Namibia
3 Regional Diagnostic Veterinary Laboratory, Private Bag X2476, Potgietersrus 0600, South Africa
equal frequency, are most constantly involved, while the carpal, tarsal and fetlock joints are less commonly affected. In severely affected joints, excessive amounts of turbid, straw-coloured fluid (in which small yellowish flakes are suspended) and large fibrin coagula are present. The synovial membranes are oedematous and contain petechiae but the articular cartilages are normal. In less severely affected joints, diffuse congestion and a few petechiae in the synovial membranes accompanied by a slight increase in the synovial fluid are usually evident.

Fasciitis characterised by the presence of small haemorrhages and accumulation of serofibrinous exudate in the loose connective tissue is most prominent in the periarticular areas of affected joints. The lesions in the tendon sheaths are similar to those described for the joints. Microscopically, joint lesions 1-4 days after the onset of fever are characterised by fibrinopurulent synovitis and an accumulation in the joint cavity of a copious exudate comprising fibrin, numerous neutrophils and a few mononuclear cells. The lining cells of the synovial membrane are destroyed where the exudate adheres to them. Other lesions include oedema, petechial haemorrhages and a mild infiltration of mostly neutrophils, with a few lymphocytes and plasma cells, particularly around small blood vessels in the synovial membrane and synovial layers of the joint capsules. The walls of some of these blood vessels are infiltrated by inflammatory cells and in many of them there is a leukostasis, consisting mainly of neutrophils. On day four after the onset of fever there is swelling of endothelium and hyperplasia of both endothelial cells and pericytes of blood vessels in the synovial membrane. From day six the exudate consists of large masses of coagulated fibrin containing few neutrophils or other cells, and in growth of fibroblasts and capillaries into the fibrin masses where the latter attach to the joint capsule.

At the same time a mild perivascular lymphocytic infiltration is present and swelling and severe hyperplasia of endothelium culminate in partial or complete occlusion of many venules, arterioles and capillaries. Perivascular fibrosis is particularly pronounced in the synovial membrane 10-15 days after the onset of fever.

Pale, well-circumscribed areas, one to a few centimetres in diameter are present in skeletal muscles particularly near the attachments of certain muscles. Petechiae or ecchymoses are often associated with these lesions which are most commonly found in the quadriceps group but other muscles including the longissimus dorsi, biceps femoris, triceps, semimembranosus and semitendinosus may also be affected. One experimental animal infected by Basson et al. (1970) became paretic and remained recumbent for 10 days before it was killed. This animal showed conspicuous greyish areas of necrosis of almost all the muscles of all limbs and a severe cellulitis affecting the subcutis of the lower half of the limbs.

Localised serofibrinous fasciitis (sometimes accompanied by the presence of petechiae and ecchymoses) of the intermuscular connective tissue is most prominent in the large muscle groups of the fore and hind limbs and in the epimysium of some of these muscles. Localised serofibrinous cellulitis in the immediate vicinity of affected joints may be present.

Microscopically the muscle lesions at 1-4 days after the onset of fever are characterised by hyaline degeneration and necrosis; varying sized focal haemorrhages; a mild infiltration of particularly neutrophils, a few mononuclear cells, and in some necrotic muscle fibres also small numbers of macrophages; and slight proliferation of sarcolemma nuclei. From day six large numbers of mononuclear cells (predominantly macrophages), are present in and between necrotic muscle fibres, and there is also some evidence of sarcolemma nuclei proliferation, fibroplasia and mineralisation of the sarcoplasm of remaining necrotic muscle fibres. Similar but less severe vascular lesions (sometimes accompanied by fibrinoid changes of the walls of small arteries and thrombosis of small blood vessels) to those described in the synovial membrane occur near necrotic foci in the muscles. Perivascular fibrosis may also be present.

Pulmonary lesions

Signs of respiratory involvement including increased respiratory rate and respiratory distress, sometimes accompanied by subcutaneous emphysema, are occasionally present in animals which manifest typical signs of the disease or which have shown no or little stiffness previously (Theodoridis and Coetzer 1979; McFarlane and Haig 1955). These signs and death are sometimes precipitated by forced movement of animals. Animals showing severe subcutaneous emphysema may however recover fully in 2-3 weeks (Erasmus et al. 1974).

In fatal cases, severe pulmonary emphysema is the most striking lesion. The emphysematous lungs occupy almost the entire thoracic cavity, do not collapse on opening the thorax, and bullae ranging from 2-10 cm in diameter are found in the septa, parenchyma and subpleurally. Areas of atelectasis occur adjacent to these bullae. Emphysema is also evident in the mediastinum and around the pericardium and subperitoneally along the ventral part of the vertebral column, around the kidneys, spleen and other abdominal organs and in the perirectal area.

64
Microscopically, the terminal and respiratory bronchioles reveal accumulation of large amount of cellular debris comprising numerous neutrophils, macrophages, erythrocytes and fibrin and sometimes also focal necrosis and infiltration of neutrophils of the mucosa, and focal hyaline degeneration and necrosis of the muscularis mucosae. Leukostasis of small blood vessels in close proximity to affected bronchioles and an infiltrate of some neutrophils and a few eosinophils, are frequently encountered in the peribronchiolar loose connective tissue. Large bullae are found in the lung tissue as a result of rupture of alveoli and bronchioles (Theodoridis et al. 1973). The presence of an exudate in bronchioles has also been reported by Burgess and Spradbrow (1977).

Involvement of udder

It is well known that BEF virus may cause a severe reduction in milk production in cows (Henning 1956; Theodoridis et al. 1973; Davis et al. 1984) but its precise action on the udder is poorly understood. The effects of ephemeral fever on early, middle and advanced stages of lactation have been studied (Theodoridis et al. 1973). Milk production is reduced by an average of 59 ± 22% during the febrile period of the disease. Milk production of cows infected during early lactation returns to normal levels more readily than that of cows infected later, apparently because there is a tendency towards proliferative cellular activities during early lactation and involutive cellular activities during the later stages (Theodoridis et al. 1973).

The quality of the milk of acutely affected cows is altered. There is an increase in the total somatic cell counts (increased numbers of sloughed epithelial cells and mononuclear cells, but the numbers of neutrophils are reduced) and bacteria (such as Streptococcus epidermidis, Streptococcus dysgalactiae, Pseudomonas aeruginosa, Staphylococcus aureus) in the milk of some quarters of the udder. It has been suggested that the decreased numbers of neutrophils in the milk during the acute disease lower the resistance of the udder and predispose the animals to bacterial udder infections (Theodoridis et al. 1973).

Cows do not develop clinical mastitis, but according to international standards on somatic cell counts they can be diagnosed as having subclinical mastitis (Theodoridis et al. 1973). The quality of milk returns to normal 2–12 days after infection and there is no permanent damage to the udder; milk production during subsequent lactations is not affected.

Testicular involvement

Bulls may suffer from a temporary reduction in fertility for several weeks following acute clinical disease. The cause of infertility has not been studied in detail, but a drastic rise of up to 74% (compared to a normal 3–10%) in mid-piece abnormalities has been reported, commencing within the first two weeks following acute illness and reaching a peak within 3–8 weeks (Chenoweth and Burgess 1972). A higher than normal percentage of abnormal spermatozoa may be found for up to 24 weeks after clinical disease. It is not known whether sperm abnormalities are caused by virus multiplication in the testes or merely the result of the febrile reaction.

Central nervous system lesions

Lesions are not usually present in the central nervous system during the acute phase. However, motor disturbances of the hind limbs (manifested as ataxia of the hind quarters or prolonged recumbency) for a few weeks to several months following infection (with or without clinical signs) have been reported in a small percentage of animals. The appetite of these animals is usually undisturbed (Armfield 1915; Rosen 1931; Gray 1938; Mackerras et al. 1940; MacFarlane and Haig 1955; Henning 1956; Spradbrow and Francis 1969; Basson et al. 1970; Snowdon 1970; Hill and Schultz 1977).

Bilateral symmetrical non-inflammatory degeneration of varying severity of one or more of the funiculi (ventral, lateral or dorsal) of the spinal cord have been reported (Hill and Schultz 1977). The authors noted that these lesions only occurred in the cervical and/or lumbar regions. However, similar lesions may also occur in the medulla oblongata and cerebellar peduncles. The lesions in the white matter are characterised by numerous vacuoles, swollen eosinophilic axons, and the presence of some macrophages containing myelin breakdown products. Due to the similarities of these lesions to those caused by compression of the spinal cord, it has been postulated that they might be caused by extreme flexion of the occipito-atlantal and atlantoaxial joints resulting in stenosis of the vertebral canal or stretching of the cord over the ventral edge of the foramen magnum and over the bodies of the first cervical vertebrae (Hill and Schultz 1977). Heavy animals suffering from acute ephemeral fever may be very ataxic, and may fall heavily which could result in trauma to the spinal cord.

Haematological changes

Apart from the inflammatory lesions in various tissues, significant haematological and biochemical changes have been reported in cattle suffering from the disease. Clinical disease is accompanied by a marked neutrophilia of 9.6–2.5 × 10⁹/litre (Mackerras et al. 1940; St George et al. 1984) and
lymphopenia (Uren and Murphy, 1985). A decline of lymphocyte numbers, from a mean of \(10 \times 10^9/\text{litre}\) two days before the onset of clinical disease, to \(5-7 \times 10^9/\text{litre}\) on the day of the peak of the febrile reaction has been reported (Uren and Murphy 1985).

Plasma fibrinogen levels (normal values 6.0-8.0 g/litre) rise rapidly to maximum levels on the day after the febrile peak and then fall gradually over the next four days (St George et al. 1984). Serum calcium levels decline on the first day of clinical disease and reach lowest levels on the second day of disease, before returning to normal levels over the next four days (St George et al. 1984; Murphy et al. 1986; St George, in Press). The mean level of 2.13 mmol/litre is only slightly below the lower levels of the normal range of 2.25-2.75 mmol/litre (St George et al. 1984). The reason for the reduced calcium levels is not known, but rumen stasis and hypomotility of the digestive tract, by reducing calcium intake, may contribute to the reduction in calcium levels (St George et al. 1984).

**Other lesions**

Animals suffering from ephemeral fever may show a slight subcutaneous oedematous swelling around the eyes (Bevan 1912), stasis of the rumen and the intestinal tract, and slight enlargement of the lymph nodes of the limbs. Bloat, hypostatic or foreign body pneumonia may be evident in recumbent cases.

**Pathogenesis of the Most Important Lesions**

Bovine ephemeral fever virus antigen is found in neutrophils, mesothelial cells of serosal surfaces and epithelial cells of synovial membranes (Young and Spradbrow 1985). It has been suggested that BEF virus is not cytocidal and that neutrophils and high levels of interferon and substances mediated by it are important in the production of the inflammatory lesions (St George, in Press). A characteristic of the joint, tendon sheath and skeletal muscle lesions is the presence of large numbers of neutrophils, many of which contain viral antigen (Basson et al. 1970; Young and Spradbrow 1985). Neutrophils seem to influence the severity of clinical disease. Suppression of neutrophils results in much milder disease and it has been postulated that ephemeral fever may be an acute immune-complex disease (Young and Spradbrow 1980).

The vascular lesions are usually mild and occur in close association with lesions (Basson et al. 1970). However, vasculitis plays a central role in the development of the serofibrinous effusive lesions of the synovial membranes, tendon sheaths and fasciae and the focal necrotic muscle lesions (Mackerras et al. 1940; Basson et al. 1970). The inflammatory lesions in the tissues are accompanied by a two- to fourfold increase in plasma fibrinogen levels (Uren and Murphy 1985; St George et al. 1984).

Although the pathogenesis of the pulmonary emphysema is not fully understood, bronchiolitis conceivably results in rupture of bronchioles and alveoli and escape of air into the connective tissue septa and lymphatics of the lungs (Theodoridis and Coetzer 1979). From here the air extends subpleurally to the mediastinum and thoracic inlet to reach the subcutaneous tissues or passes posteriorly to the abdominal cavity and accumulates subperitoneally.

Apart from the inflammatory lesions in the joints, tendon sheaths and skeletal muscles, the reduction in the level of ionised and bound fractions of calcium in the blood and not central nervous system lesions, would seem to contribute significantly to the paretic or paralytic signs which are often present in acutely affected animals (St George et al. 1984; Murphy et al. 1986; St George, in Press).

**References**


Rosen, S.G. 1931. Ephemeral fever (three days' fever) of cattle in Palestine. Veterinary Journal, 87, 244-246.


Uren, M.F. and Murphy, G.M. 1985. Studies on the pathogenesis of bovine ephemeral fever. 2. Changes in serum calcium and enzyme levels. Veterinary Microbiology, 10, 505-515.

Young, P.L. and Spradbrow, P.B. 1980. The role of neutrophils in bovine ephemeral fever virus infection of cattle. Journal of Infectious Diseases, 142, 50-55.

Young, P.L. and Spradbrow, P.B. 1985. Transmission of virus from serosal fluids and demonstration of antigen in neutrophils and mesothelial cells of cattle infected with bovine ephemeral fever virus. Veterinary Microbiology, 10, 199-207.