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The role of wild ruminants in the epidemiology of bovine petechial fever

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SUMMARY

After experimental inoculation of *Cytoecetes ondiri*, the agent of bovine petechial fever (BPF), multiplication occurred in impala, bushbuck, Thomson's gazelles and wildebeest, as shown by infectivity studies and clinical findings. Similar attempts to infect one eland failed.

As a sequel to this, blood and spleen samples were collected from four species of wild ruminants in an area where BPF was endemic. Isolations of *C. ondiri* were made from three of five bushbuck, but not from any other species.

INTRODUCTION

Bovine petechial fever (BPF; Ondiri disease) is caused by a rickettsia-like organism observed principally in the neutrophils, for which the name *Cytoecetes ondiri* has been proposed (Krauss, Davies, Odegaard & Cooper, 1972). BPF has been diagnosed only in Kenya at altitudes over 5000 ft., and occurs sporadically in exotic cattle, nearly always associated with grazing at forest edge or in scrub (Danskin & Burdin, 1963). Although the disease is assumed to be arthropod-borne, the vector is unknown.

It has been suggested (Haig, 1966) that the restricted distribution and sporadic occurrence of BPF might be explained by the existence of wild animal reservoir hosts. Sheep and goats are readily susceptible to experimental inoculation (Danskin & Burdin, 1963), but laboratory rodents are resistant (Cooper, 1973). There are no records of attempts to infect species of wild ruminants experimentally.

Natural infection has been diagnosed only in cattle. Attempts to isolate the organism from mongooses, genet cats, cane rats, porcupine, waterbuck and small rodents, in enzootic areas, have been unsuccessful (Report, 1958; Report, 1972; Walker, Cooper & Snodgrass, 1974).

Because only ruminants have been successfully infected with BPF, it was

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decided to determine the susceptibility of captive wild ruminants to BPF; and as a sequel to this, to attempt the isolation of *C. ondiri* from wild ruminants in an enzootic area.

MATERIALS AND METHODS

Animals

The following animals were inoculated experimentally: one impala (*Aepyceros melampus*), one eland (*Taurotragus oryx*), one bushbuck (*Tragelaphus scriptus*), two Thomson's gazelle (*Gazella thomsonii*), and two wildebeest (*Connochaetes taurinus*). Adults of either sex were used. All animals were from areas of less than 5000 ft. altitude, but the wildebeest were kept in a paddock at 7000 ft., in an area where BPF occurs. With the exception of the wildebeest, animals were housed throughout the experimental period.

C. ondiri

The N/O strain, which is pathogenic for both cattle and sheep (Snodgrass, 1974), was used to inoculate the wild ruminants. Animals were inoculated by the intravenous route with blood from a parasitaemic sheep.

Handling

The eland, impala, and wildebeest were anaesthetized before handling by administration of an appropriate dose of etorphine hydrochloride (Immobilon, Reckitt & Colman) and xylazine (Rompun, Bayer) delivered by projectile syringe (Cap-Chur Equipment, Palmer Chemical and Equipment Co., Douglasville, Georgia, U.S.A.). The bushbuck and gazelles were caught by hand.

Observations

Rectal temperatures were taken, and the animals examined for petechiae and other clinical signs of illness. Blood was withdrawn into dipotassium ethylenediamine tetra-acetic acid for haematology and infectivity studies. Blood smears were stained with Giemsa, and examined for the presence of *C. ondiri* (Krauss *et al.* 1972). At least 100 neutrophils were examined on each occasion. Total white blood cell counts were performed by electronic counter.

Infectivity titrations

The inoculum was titrated in susceptible sheep, as was blood taken from the animals before inoculation and at intervals after inoculation. Dilutions were made in phosphate buffered saline (PBS) and inoculated intravenously to susceptible sheep. One sheep was used for each dilution. The sheep were monitored for BPF infection by daily temperature-taking, and examination of blood smears for *C. ondiri* when the temperature rose over 40·5° C.

Table 1. *Reactions of captive wild ruminants to experimental BPF infection*

Animal		Inoculum (SID50)	Blood titres (SID50/ml.) after inoculation: 10 min.–24 hr.	Blood titres (SID50/ml.) after inoculation: 4–7 days*
Impala		$>10^3$	$<10^0$	$10^{2.5}$
Eland		$>10^5$	$<10^0$	$<10^0$
Bushbuck		$10^{4.5}$	$10^{0.1}$	$\geqslant 10^{3.6}$
Thomson's gazelle	1	$\geqslant 10^4$	NT	$>10^3$
	2	$\geqslant 10^4$	NT	$10^{0.5}$
Wildebeest	K213	$\geqslant 10^4$	NT	$10^{2.5}$
	4	$\geqslant 10^4$	NT	$10^{0.8}$

NT = not tested.

* Where the animals were sampled more than once, the highest titre recorded is given.

Detection of latency

One hundred ml. of blood was withdrawn into heparin at intervals after recovery, and inoculated into susceptible sheep. These were then monitored for BPF infection as above.

Field isolation

The location chosen for the study was an area near Naivasha in Rift Valley Province. This was at an altitude of 6500 ft., and contained thickly bushed escarpment interspersed with grassy areas. Cattle grazed in this area had a high incidence of BPF.

Species of wild ruminants shot and sampled were dikdik (*Rhynchotragus kirkii*), impala, bushbuck, and waterbuck (*Kobus ellipsiprymnus*).

The samples collected from the animals were spleen, and up to 100 ml. of blood in heparin. These were kept on ice and transported to the laboratory within 24 hr. An approximately 20 % suspension of spleen was prepared in 50 ml. PBS, and a sheep was inoculated with 10 ml. of this suspension by slow intravenous injection and with 40 ml. by intraperitoneal inoculation. Blood was inoculated by the intravenous route, usually to a different sheep.

To identify a strain by cross-immunity trials, sheep and cattle were infected, then challenged with N/O and N/B strains respectively (Snodgrass, 1974).

RESULTS

Experimental infection

All animals received at least 10^3 sheep infective doses (SID) (Table 1). Blood taken from the animals before inoculation was in all cases negative for BPF.

To determine the blood titres due to persistence of the inoculum, blood was taken from the impala and eland 10 min. after inoculation, and from the bushbuck 24 hr. after inoculation. The impala and eland bloods were non-infective, and the bushbuck blood had a low titre of $10^{0.1}$ SID50/ml.

To determine if multiplication of *C. ondiri* had taken place, the animals were

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bled from 4 to 7 days after inoculation, some on more than one occasion. High infectivity titres were detected in the impala, bushbuck, Thomson's gazelle 1, and wildebeest K213. Low infectivity titres were found in gazelle 2, and wildebeest 4, and no infectivity was detected in the eland.

Visible parasitaemia was observed in the impala, the bushbuck and gazelle 1.

Total leucocyte counts varied between the different species. With an arbitrary definition of leucopenia as a total leucocyte count 20% or more below the initial level, the impala, the bushbuck, gazelle 1 and both wildebeest showed a leucopenia.

Increased temperatures were recorded in the bushbuck and gazelle 1, at 41·2 and 40·5° C. respectively.

The bushbuck appeared dull during febrile reaction. Both gazelles died from 1 to 2 weeks after inoculation, with haemorrhages, oedema, and lymph node enlargement. No clinical signs were detected in any other animal.

Latency

Attempts to detect latent infections were made in only the impala and bushbuck. Latency was detected for 1 month in the impala, which then died of other causes, and for 2 months in the bushbuck.

Field isolation

Four dikdik, 3 impala, 2 waterbuck and 5 bushbuck were shot and sampled. No isolations were made from dikdik, impala or waterbuck, but isolations of an organism with the morphology of *C. ondiri* were made from the bloods of 3 of the 5 bushbuck.

The organism from one isolation was studied in more detail. In sheep and cattle it caused clinical and haematological changes consistent with those of BPF. One of 2 recovered sheep resisted challenge with N/O strain; both recovered cattle resisted challenge with N/B strain.

DISCUSSION

Infectivity titres and clinical signs indicate that multiplication of *C. ondiri* occurred in the impala, the bushbuck, gazelle 1 and wildebeest K213. There was good correlation between the different signs of multiplication, i.e. raised infectivity titre, visible parasitaemia, raised temperature, and leucopenia. The fact that visible parasitaemias were not detected in all animals is not significant, as it has been shown that visible parasitaemia occurs for a much shorter period in infected animals than does increased infectivity titres (Snodgrass, 1974). Animals sampled on one occasion only are likely to be within a period of increased titre, but not necessarily of visible parasitaemia.

The low titres detected in gazelle 2 and wildebeest 4 were probably a result of slight multiplication of the organism, but persistence of the inoculum cannot be omitted as a possibility. The eland appeared resistant to BPF, but it is not known if this is a species characteristic, or due to previous exposure of the individual to BPF.

Animals other than the gazelles were in a good state of health during the experimental period. The gazelles were newly captured, and both showed diarrhoea and weakness. The post-mortem lesions of haemorrhages, oedema and lymphoid hyperplasia were typical of BPF (Plowright, 1962). Stress may have weakened their resistance to BPF infection, and it is likely that death was due to BPF complicated by debility.

The absence of clinical disease in infected animals other than the gazelles was similar to that found in experimentally infected sheep (Dawe, Ohder, Wegener & Bruce, 1970).

The development of latent infections in the impala and bushbuck makes their potential role as reservoir hosts more significant, in that they may provide a continuing source of infection to a vector.

The results of cross-immunity tests between one bushbuck isolate and known strains of *C. ondiri* are consistent with known heterologous strain reactions in BPF, where cattle show strong heterologous strain immunity, but sheep show only weak heterologous strain immunity (Snodgrass, 1974). This isolate therefore appeared to be a strain of *C. ondiri*.

The fact that isolations were made from 3 of 5 free-ranging bushbuck indicates either a continuing reinfection cycle, or a greater degree of latency than has been found in sheep and cattle (Snodgrass, 1974). The isolations were made at the end of a prolonged dry period, so that no exceptional arthropod activity was likely to have occurred.

The susceptibility of several species of wild ruminants to BPF, in addition to the known susceptibility of domestic ruminants, probably indicates that the majority of other ruminants are similarly susceptible, and that latent infection may develop in some of them. Bushbuck in an enzootic area constitute a reservoir of BPF, and other species of wild ruminants may be regarded as potential reservoirs. Walker *et al.* (1974) found bushbuck to be one of the commonest wild ruminants in areas where BPF occurs. Other ruminants known to be present at the site near Naivasha were bush duiker (*Sylvicapra grimmia*) and buffalo (*Syncerus caffer*).

The detection of wild animal reservoirs of BPF is consistent with the similar role played by wild animals in the epidemiology of diseases caused by related organisms. *C. phagocytophila*, the agent of tick-borne fever of sheep and cattle, has been isolated from red, roe, and fallow deer, and wild goats (Foggie, 1962; McDiarmid, 1965; Foster & Greig, 1969). Similarly, jackals and wild dogs may act as reservoir hosts for *Ehrlichia canis* (Neitz & Thomas, 1938), while red and grey foxes and coyotes have been experimentally infected with this organism (Ewing, Buckner & Stringer, 1964; Amyx & Huxsoll, 1973). *Cowdria ruminantium*, the causative agent of heartwater, has been found in natural infection in springbok (Neitz, 1944), and blesbuck and black wildebeest have been experimentally infected (Neitz, 1935).

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