J. Hyg., Camb. (1978), **81**, 259
Printed in Great Britain

Nairobi sheep disease in Kenya. The isolation of virus from sheep and goats, ticks and possible maintenance hosts

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(*Received* 10 *January* 1978)

SUMMARY

Nairobi sheep disease was seen principally upon movement of susceptible animals into the enzootic areas. This occurred most frequently for marketing purposes near the main centres of population. Other outbreaks followed local breakdowns in tick control measures. The disease did not occur in epizootic form during the period under consideration.

Nairobi sheep disease was isolated from pools of *Rhipicephalus appendiculatus* but not from many pools of other tick species. No virus was isolated from the blood or tissues of a range of wild ruminants and rodents.

INTRODUCTION

Montgomery (1917) and Daubney & Hudson (1931) stated that most sheep and goats from *Rhipicephalus appendiculatus* infected areas, were immune to Nairobi sheep disease, but it is not clear what evidence this observation was based upon. The previous paper (Davies, 1978) has shown that the sheep and goat populations in these areas have specific antibody to NSD virus. The results presented here, however, show that the disease is not normally encountered in such areas and no unidentified losses can be attributed to the virus. Thus a situation exists where the most pathogenic virus for the sheep population in Kenya does not produce mortality in many enzootic areas. The previous paper (Davies, 1978) showed that there was a very high proportion of the sheep and goat population in the Taita-Taveta District of Kenya with antibody to NSD. The last outbreak of NSD reported in this district was in 1915 when large numbers of sheep and goats were moved into the area to feed troops stationed there in the First World War.

Earlier work (Daubney & Hudson, 1934) suggested that *Amblyomma variegatum* may have transmitted the virus in a large outbreak and they were able to show that the tick could transmit the virus in the laboratory, although inefficiently when compared with *Rhipicephalus appendiculatus*. Other reports referring to a condition with some similarity to NSD, which occurs in epizootic form in Somalia, incriminate *Rhipicephalus pulchellus* as the vector (Pellegrini, 1950; Edelsten, 1975). No characterization of the viruses causing these outbreaks was carried out however.

This paper records the results of investigations of disease outbreaks to determine the circumstances in which they occurred. Efforts have been made to examine the possibility that other tick species may transmit the virus in Kenya and to find whether other vertebrates may act as maintenance hosts for the virus.

MATERIALS AND METHODS

Virus isolation

Blood, spleen or mesenteric lymph nodes were collected from sheep or goats affected by or suspected to have died from NSD. The isolation and identification procedures for NSD which were used have been described, (Davies, Mungai & Taylor, 1977). The brains, spleen and livers of a series of rodents were treated in a similar manner for virus isolation. Blood and spleen suspensions from wild ruminant species were inoculated into unweaned mice and also into NSD-susceptible sheep for virus isolation.

The rodents were trapped in areas considered enzootic for NSD on the basis of the results of the previous paper (Davies, 1978). The wild ruminants were killed within or on the edges of such enzootic zones, where they included these in their grazing ranges at certain times of the year. The virus isolation attempts were made in an effort to determine whether these hosts might act as virus reservoirs for NSD. Rodents were suggested by Daubney & Hudson (1934) as vertebrate maintenance hosts for the virus.

Ticks were collected alive from sheep and goats, cattle and some wild ruminants in or close to areas known to be enzootic for NSD. The tick species *Amblyomma variegatum*, *Rhipicephalus pulchellus* and *Rhipicephalus evertsi*, were especially studied. These latter two ticks are generally found in drier areas than *Rhipicephalus appendiculatus*. The virus isolation attempts from ticks were to investigate the possibility that these three species may transmit the virus cyclically.

The ticks were cooled to 4 °C and sorted into species pools. All engorged specimens were discarded. The pools consisted of from 20–150 individuals. They were homogenized with sterile sand, in a cooled pestle and mortar, and triturated with an equivalent w/v of antibiotic solution containing 500 i.u. penicillin, 500 μg streptomycin sulphate, 250 μg neomycin sulphate and 200 units of mycostatin (Squibb) per ml. These were diluted with 0·75 % bovine serum albumin or 5 % fetal calf serum in phosphate buffered saline. After incubation for 1 h at room temperature, the volume was made up with the diluent to give approximately 20 % suspensions of the ticks. The suspensions were centrifuged at 400 g for 10 min and the supernatants used to inoculate litters of 2–4-day-old unweaned mice of a Swiss white variety. A sample was retained at − 70 °C for reisolation purposes.

Where any mice showed neurological or other signs of disease, the brains were harvested and passaged further in suckling mice at a 1/100 dilution of the brain harvest. After three passages the harvests were filtered through 0·22 nm millipore filters and inoculated on monolayers of BHK 21 C 13 cell cultures (MacPherson & Stoker, 1962). After adaptation to these cells the virus strains were identified by fluorescent antibody methods (FAT) and complement fixation tests (CFT), carried out with antigens prepared from unweaned mouse brain as described by Davies, Mungai & Taylor (1977).

A series of mouse ascitic fluids, which were prepared at the National Institute of Health, Bethesda, Maryland, U.S.A., were used for the identification of the virus strains.

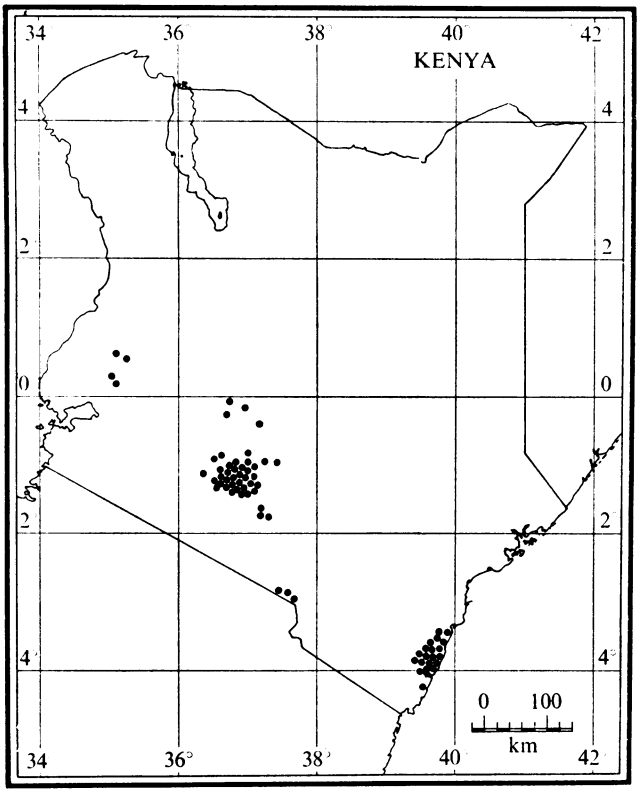

Fig. 1. The sites at which Nairobi sheep disease virus has been isolated from sheep and goats in Kenya. The clusters of outbreaks are around the principal cities of Nairobi and Mombasa.

RESULTS

Virus isolation

The sites at which NSD virus has been isolated from clinically affected sheep and goats are shown in Fig. 1. Most outbreaks have occurred around the population centres of Nairobi and Mombasa and the remainder throughout those areas where *Rhipicephalus appendiculatus* was commonly found. As many as possible were investigated to determine their origin, and a summary is given below:

(1) Most of the disease outbreaks around Nairobi and Mombasa were in trade animals which had been brought from the northern or southern pastoral areas where *Rhipicephalus appendiculatus* is not regularly found. When these animals were held in the heavily infested and infected areas near the cities, a heavy mortality frequently ensued. In one example 190 of some 240 animals died within a 4-week period.

(2) Stud rams from other countries or NSD-free parts of Kenya were highly susceptible when moved into areas enzootic for NSD. Losses were seen after such introductions.

(3) Where attempts to control the disease have been made by dipping, cases of NSD have been found after failures in its effectiveness.

Table 1

Tick species	No. of pools	NSD	Viruses isolated: Others	Viruses isolated: Unidentified
Rhipicephalus appendiculatus	47	5	Thogoto (2)	—
R. pulchellus	119	0	Kadam (4)	3
R. evertsi	69	0	—	2
R. hurtii	4	0	—	—
Amblyomma variegatum	27	0	Kadam (2)	1
A. gemma	16	0	—	—
Hyalomma truncatum	14	0	—	—
Amblyomma cohaerens	8	0	—	1

(4) Large farm units in heavily infected NSD areas, appear to become free from NSD after a long period (20–30 years) of efficient tick control in their cattle populations. Some islands of *Rhipicephalus appendiculatus* populations have been found in predominantly drier areas unsuitable for this tick, and they appear to have been free from NSD virus. The introduction of sheep and goats from NSD infected areas to such situations causes disease in the existing clean flocks or herds, which continue until an enzootic situation is achieved with a predominantly immune population.

(5) Following heavy rains and grass growth *Rhipicephalus appendiculatus* populations extend into drier zones where they are either generally absent or rare. One such situation was investigated where 390 of some 750 sheep and goats had died over a 6-week period. Sera from 30 recovered animals showed 29 with high titres indicative of recent infection and one without. Of 30 unaffected animals 29 were free from specific antibody and one had a high titre. The area was one in which *Rhipicephalus appendiculatus* was not normally found but recent rains had produced good grass cover and *Rhipicephalus appendiculatus* were found on most animals examined. This appeared to be an extension in the range of this tick but may have followed local movement of susceptible animals to take advantage of the better grazing.

Virus isolation from ticks

A summary of virus isolation attempts from tick pools is shown in Table 1. NSD virus was isolated from pools of *Rhipicephalus appendiculatus* only, and Thogoto virus was also isolated from this tick species. Many other virus strains were isolated from the other species pools, but on no occasion was NSD isolated from *Amblyomma variegatum*, *Rhipicephalus pulchellus* and *R. evertsi*. Other strains of virus were recovered from these tick species.

Virus isolation from vertebrate species

A summary of virus isolation attempts from various ruminant and rodent species is shown in Table 2. While many of the ruminant species, notably the gazelle, kongoni and wildebeest, graze outside the *Rhipicephalus appendiculatus* enzootic areas, they frequently include them in their range at certain seasons. The water-

Table 2. *Attempted isolation of NSD from wild ruminants and rodents*

Ruminants	Number	NSD virus Isolation
Waterbuck, *Kobus ellipsiprymnus*	24	—
Bushbuck, *Tragelaphus scriptus*	28	—
Reedbuck, *Redunca fulvorufula*	4	—
Impala, *Aepyceros melampus*	11	—
Wildebeest, *Connochaetes taurinus*	10	—
Cokes hartebeest, *Alcelaphus buselaphus*	10	—
Grants gazelle, *Gazella grantii*	10	—
Thompsons gazelle, *Gazella thomsonii*	10	—
Dik Dik, *Madoqua kirki kirki*	4	—
Grey duiker, *Sylvicapra grimmia*	2	—
Suni, *Nesotragus moschatus*	2	—
Steinbok, *Raphicerus campestris*	4	—
Rodents		
Otomys tropicalis	61	—
Lemniscomys striatus	12	—
Rhabdomys pumilio	1	—
Lophuromys flavopunctatus	5	—
Mastomys natalensis	13	—
Praomys jacksonii	7	—
Arvicanthus niloticus	15	—

buck, bushbuck and most other species examined were taken in areas where the vector tick is commonly found. An individual waterbuck may carry thousands of ticks of this species.

DISCUSSION

The results confirm the earlier observations (Montgomery, 1917) that NSD outbreaks occur principally upon movement of susceptible animals into enzootic areas (Davies, 1978). These are especially common near the major cities of Nairobi and Mombasa where most consumption and marketing of the sheep and goats is centred. The disease was not observed to occur in an epizootic manner during the years of this study from 1968–77. Daubney & Hudson (1934) described what may have been an epizootic of NSD in Kenya and there are reports of epizootics which may have been due to NSD in Somalia (Pellegrini, 1950; Edelsten, 1975). In these accounts *Amblyomma variegatum* and *Rhipicephalus pulchellus* were considered to be the vector ticks. The disease outbreaks described here were all in situations where *Rhipicephalus appendiculatus* was commonly found, and the evidence from this work and the preceding paper would support a conclusion that this is the only tick transmitting NSD in Kenya.

Most sheep and goats in areas enzootic for NSD in Kenya have antibody to the virus (Davies, 1978), although local pockets of susceptible animals can be found. These may be involved in limited outbreaks and reinfection of clean farms may be expected from time to time. The general trend will be to clean the higher potential areas of their tick populations by sustained effective dipping of cattle populations which are the principal hosts for the vector tick. Where the pastures are not re-infested by game animals, the tick populations will be expected to diminish.

Amblyomma variegatum and *Rhipicephalus pulchellus* have been considered to transmit NSD in epizootics. The one area in Kenya where *Amblyomma variegatum* occurs in the absence of *Rhipicephalus pulchellus*, the sheep and the goat populations are susceptible to the virus and do not have antibody. Those areas where *Rhipicephalus appendiculatus* and *R. pulchellus* overlap are many and the pulchellus populations extend into much larger areas where the sheep and goat populations are susceptible. No outbreaks of disease have been seen in these areas and if *R. pulchellus* could transmit the virus, then it is felt that epizootics would have occurred. No NSD virus was isolated from the many pools of these two tick species and it is therefore not considered likely that ticks other than *Rhipicephalus appendiculatus* account for any transmission of NSD in Kenya.

This study and the previous one (Davies, 1978) reflect a static enzootic situation for NSD in Kenya which has prevailed over the 9 years of the study period. Those factors limiting the range of the tick *Rhipicephalus appendiculatus* are primarily climatological. After heavy and prolonged rains with the associated vegetational changes, the microclimate may be altered in favour of the tick to extend its range considerably. Such populations will be built up from the existing few ticks and by reintroductions from adjacent enzootic areas. These can become infected with NSD and with the susceptible populations of the disease hosts outside the enzootic areas, conditions are ripe for epizootic spread. Yeoman (1966) has described extensions in the range of this tick species in Sukumuland in Tanzania and the epizootics of East Coast Fever which follow. In areas where previously it was difficult to find even single specimens of *Rhipicephalus appendiculatus*, animals carried up to 50 adult specimens. The areas which are marginal for the tick in Kenya are those in the nomadic pastoral areas, especially of Masailand. Lewis (1934) quoted reports from the Masai of epizootics of NSD, but was unable to confirm them. No such epizootic occurred in this study period but is clearly a possibility and the major potential requirement for the vaccine against this disease.

Rhipicephalus appendiculatus feeds principally upon cattle at all stages, as larvae, nymphs and adults. Other ruminants notably sheep and goats and wild species especially buffaloes are also preferred feeding hosts (Yeoman & Walker, 1974). There are occasional records from species other than ruminants notably dogs, equidae and rodents but the proportions are negligible compared with those feeding upon ruminants. No virus was isolated from the wild ruminants and rodents examined, and virus does not replicate in cattle (Montgomery, 1917; Davies, unpublished observations). Together with the serological data from the previous paper, a conclusion that the virus is maintained solely by a tick – sheep and goat cycle throughout the enzootic areas is considered most likely. The virus is transmitted trans-ovarially in *Rhipicephalus appendiculatus* (Montgomery, 1917; Daubney & Hudson, 1934; Lewis, 1946).

The control of NSD in Kenya can be best established by ensuring that sheep and goats travel directly for slaughter and are not held for marketing or other purposes around Nairobi and Mombasa. The major part of the losses due to the disease could be avoided in this manner. Movements of stud rams and breeding stock into

enzootic areas can be safeguarded by vaccination and this could be carried out should the disease appear again in epizootic form.

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REFERENCES

DAUBNEY, R. & HUDSON, J. R. (1931). Nairobi sheep disease. *Parasitology* **23**, 507–24.

DAUBNEY, R. & HUDSON, J. R. (1934). Parasitology. Nairobi sheep disease. Natural and experimental transmission by ticks other than *Rhipicephalus appendiculatus*. *Parasitology* **26**, 496–509.

DAVIES, F. G., MUNGAI, J. N. & TAYLOR, M. (1977). The laboratory diagnosis of Nairobi sheep disease. *Tropical Animal Health and Production* **9**, 75–80.

DAVIES, F. G. (1978). A survey of Nairobi sheep disease antibody in sheep and goats, wild ruminants and rodents within Kenya. *Journal of Hygiene* **81**, 251.

EDELSTEN, R. M. (1975). The distribution and prevalence of Nairobi sheep disease and other tick-borne infections of sheep and goats in northern Somalia. *Tropical Animal Health and Production* **7**, 29–34.

LEWIS, E. A. (1934). Investigations into the tick problem in the Masai Reserve. *Bulletin No.* 7. *Department of Agriculture, Kenya.*

LEWIS, E. A. (1946). Nairobi sheep disease. The survival of the virus in the tick *Rhipicephalus appendiculatus*. *Parasitology* **37**, 55–9.

MACPHERSON, I. & STOKER, M. G. P. (1962). Polyoma transformation of hamster cell clones, an investigation of genetic factors affecting cell competence. *Virology* **16**, 147–51.

MONTGOMERY, R. E. (1917). On a tick borne gastro-enteritis of sheep and goats occurring in East Africa. *Journal of Comparative Pathology* **30**, 28–57.

PELLEGRINI, D. (1950). La gastro enterite emorragica della pecore. *Bolletino della Societe italiana di medicina e igiene tropicale Sezione Eritrea* **10**, 164–70.

WALKER, J. B. (1974). *The Ixodid ticks of Kenya.* Published by the Commonwealth Agricultural Bureau.

YEOMEN, G. H. (1966). Seasonal studies of *Rhipicephalus appendiculatus* on bovine hosts in East Coast Fever enzootic, epizootic and free areas. *Bulletin of Epizootic Diseases of Africa* **14**, 113–40.

YEOMAN, G. H. & WALKER, J. B. (1967). *The Ixodid ticks of Tanzania.* Published by the Commonwealth Agricultural Bureau.