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Bluetongue studies with sentinel cattle in Kenya

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SUMMARY

Bluetongue antibody of 19 different serological types was found in a group of sentinel cattle near Nairobi. A continuous challenge by these 19 strains occurred each year in this location. The sero-conversion rates were shown to vary from year to year and rainfall had no obvious effect upon the conversion rates.

The 19 different strains of virus were also shown to be active at five widely scattered sites in different parts of the country.

INTRODUCTION

Earlier work on the epidemiology of bluetongue (BT) in this laboratory, attempted to identify the *Culicoides* species most likely to be vectors for the disease in Kenya (Walker & Davies, 1971). Later studies attempted to define the maintenance cycles for BT (Davies & Walker, 1974). The distribution of antibody to BT in a wide range of ruminant species (domestic and wild) was mapped out and found to coincide with the occurrence of the probable vector species. The disease was confined to exotic wool sheep and their crosses, which have a more restricted distribution. The indigenous hair sheep and goats have antibody to BT but clinical disease is not seen in these breeds. The maintenance cycle for BT over the greater part of Kenya's bushed and wooded grassland thus persists with no signs of overt disease. The virus would seem well adapted to the environment.

Those species of *Culicoides* which are thought to transmit BT in Kenya preferentially feed on cattle and wild bovidae. This has been concluded from the analysis of blood meals (Walker & Boreham, 1976). Birds or other animal species are not fed upon to any extent by these species (Walker & Boreham, 1976).

A detailed study of BT in one locality was started at a cattle ranch near Nairobi. Virus isolation attempts were made from *Culicoides*, the biology of the *Culicoides* was studied (Walker, 1976) and cattle were monitored for sero-conversion to BT. This was designed to show which strains of BT were active in the area, to study changes in sero-conversion rates and relate these to climatic or other factors. It was hoped to be able to investigate the dynamics of infection during conditions of epidemic spread of arbo-virus infections such as had occurred in 1968, when BT, Rift Valley Fever and ephemeral fever were widespread (Davies, 1975; Davies, Shaw & Ochieng, 1975).

MATERIALS AND METHODS

Location of the study area

The ranch was situated 20 km north-east of Nairobi at an altitude of 1525 m, 1° 12′ S; 36° 58′ E. The farm is largely in ecological zone III as defined by Pratt, Greenway & Gwynne (1966) and is of moist to semi-arid *Combretum* savanna. The area is well watered with a stream and several dams. The latter have extensive swampy and muddy margins. The pastures are of good long grasses with scattered trees.

Sentinel cattle

Three groups of cattle were used, all of the Ayrshire breed.

Group 1. A group of 48 cattle which had been alive during the 1968 period of considerable vector activity, chosen to show which strains of BT had been active at this period.

Group II. This consisted of 34 females which were born in 1970. They were first bled in 1972 and again in 1973; after which most were dispersed.

Group III. This group of 34 females were born in 1972. They were bled in 1973 and each subsequent year until 1976.

All the animals were tattooed at birth, they were ear-tagged and later freeze-branded. The triple identification system allowed certain recognition of animals year after year. They were bled in February of each year and the serum stored at −20 °C.

Further sera were obtained from groups of 30 cattle, 4–6 years old in widely separated districts of Kenya. These were at the coast near Mombasa (ecological zone II), Amboselli (zone IV), Nanyuki (zone II–IV) and Kitale (zone II–III). At the latter site, a group of Friesian heifers imported from Holland were bled on arrival and after one year at this site.

Virus isolation from Culicoides

Virus isolation attempts were made at the sentinel herd site for a period of ten months during 1968–9 and these results have been published (Walker & Davies, 1971). Similar single species pools of *Culicoides pallidipennis*, *C. milnei*, *C. cornutus*, *C. grahami* and *C. magnus* were processed for virus isolation for a ten-month period April 1971 to March 1972. The methods were as described by Walker & Davies (1971) except that the suspensions were inoculated into eggs, unweaned mice and tissue culture.

Serological methods

Sera were examined initially by a group-specific indirect fluorescent antibody test (Pini, Ohder, Whiteland & Lund, 1968) and then by a type-specific plaque inhibition (PI) test (Davies & Blackburn, 1971). The zones of total plaque inhibition varied with the different homologous positive sera from 15–35 mm. With test sera from the sentinel cattle, an inhibition of 10 mm or greater was interpreted as a positive result. Inhibition to some strains was of 30 mm or greater while others were of 10–15 mm. It was suspected that the lower range of inhibition

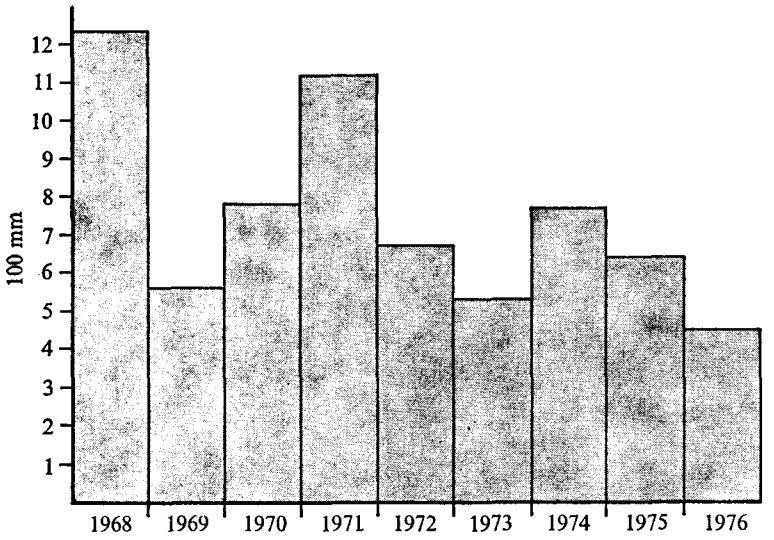

Fig. 1. The annual rainfall at the sentinel herd site for the years 1968–76.

could be due to cross relationships, and not be merely a low titre to the test virus strain. All sera were inactivated at 56 °C for 30 min before testing.

Virus strains

The attenuated strains of BT 1–16 (Howell types) were maintained in our laboratory with the exception of strains 5, 9 and 16 which were obtained from the Animal Disease Laboratory at Pirbright.

Six strains of virus have frequently been isolated from clinical disease in sheep in Kenya and they correspond to the Howell types 1, 2, 3, 4, 8 and 12. These field strains were used in the PI test. Three further strains have been isolated infrequently and are serologically distinct from the other 16. They are therefore considered to be new strains (Davies, in preparation). These are denoted Ken/BT 1, 11 and 111. One of these, Ken/Bt 11, has some relationship with Howell type 14 but is not identical. The sentinel cattle sera were thus screened against 19 strains of BT virus.

RESULTS

Rainfall

The annual rainfall for the ranch during the years of the study is shown in Fig. 1.

Culicoides studies

The results of population monitoring during the study period have been published (Walker, 1976) and also work on the longevity of the vector species (Walker, 1976). Observations of the blood meals taken from specimens at the site have been reported by Walker & Boreham, (1976). The figure 4, on page 92 of Walker (1976) is most relevant to the study period, for it shows the population fluctuations of *Culicoides* at the site over the period.

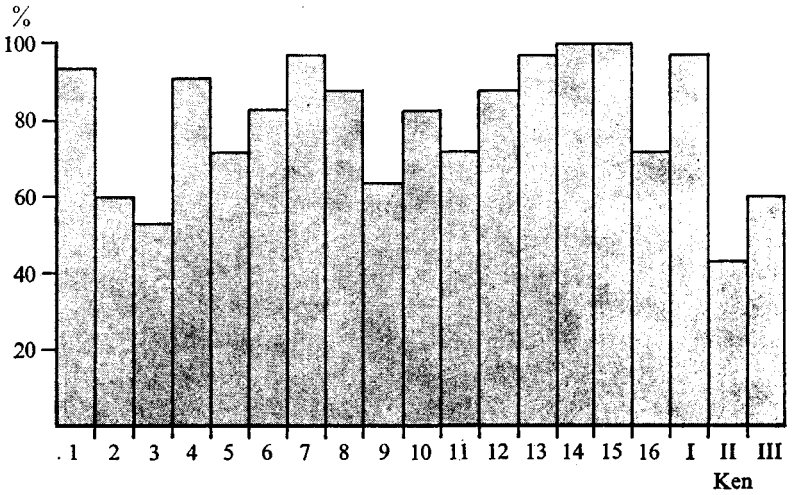

Fig. 2. The percentage of cattle alive in 1968, showing antibody to the different strains of BT found in Kenya.

Virus isolations

No isolations of BT virus strains were made during the second period, 1971–2. The isolations in the 1968–9 period have been recorded (Walker & Davies, 1971).

Antibody to BTV in sentinel cattle – Group I

The group specific antibody test showed that all 48 of these animals had antibody to BTV in their sera. The PI test results with these sera, against 19 strains of BTV are shown in Fig. 2.

Antibodies to all 19 strains were found in these sera, this being the first indication that more than 9 serotypes existed in Kenya. The results show that the further 10 serotypes, hitherto known principally in South Africa, were also common in Kenya.

Group II

The percentages of sero-conversions in this group for the years ending February 1972 and February 1973 are shown in Fig. 3. The results confirm the extent of the challenge sustained by Group I animals, with evidence of antibody to 19 strains of BT. Strains 7, 13, 14 and 15 were apparently greatly amplified in this area during 1971, for over 90% of the sentinel animals were converted.

Group III

The sero-conversions for this group for the years 1973–6 are shown in Fig. 4. Many of these years were very dry with rainfall below the mean expected, and the *Culicoides* populations were also below their calculated means (Walker, 1976). There was evidence of challenge by the 19 test strains in most years with the exception of 1973. The exposure period for this group would however be shorter

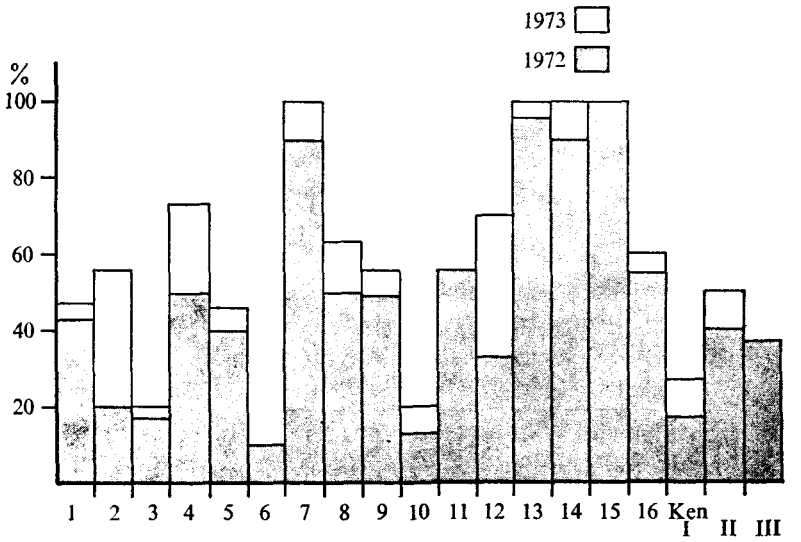

Fig. 3. The percentages of seroconversions of cattle in group II to the 19 strains of BT found in Kenya, during the years 1972 and 1973.

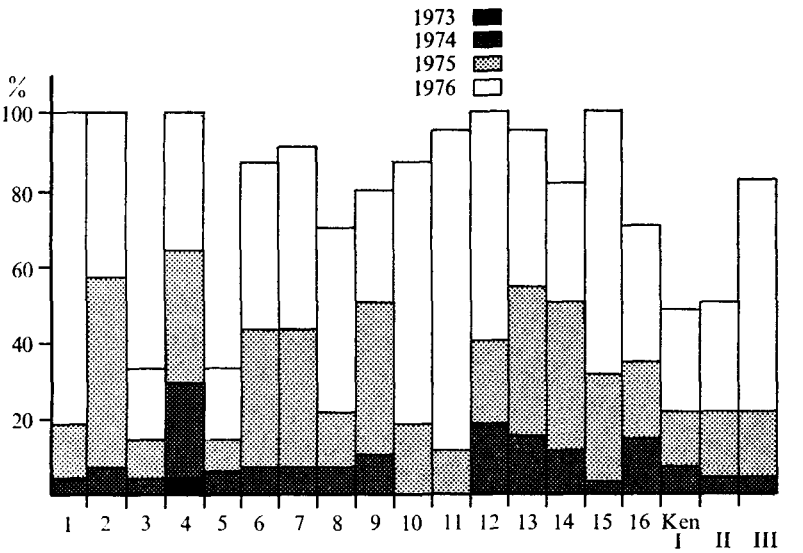

Fig. 4. The percentages of seroconversions in cattle of group III to the 19 different strains of BT found in Kenya, during the period 1973–6.

than in other years for they were all born during the year 1972 and bled in February 1973. Possible interference by passive colostral BT antibody may have altered their response to challenge by BT virus strains.

The sero-conversion rates are lower in the years 1972–3, 1973–4 and 1974–5 than in 1975–6. This latter period saw most animals sero-converted to the 19 strains in a manner similar to the Group I cattle.

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Table 1. *The differences in sero-conversion rates of sentinel cattle to 19 different bluetongue virus strains in different years*

Year ...	1972	1973	1974	1975	1976
Number sero-converted	226	56	40	119	163
Total exposed	480	582	347	307	188
Percentages	47	9·5	11·5	38	86

Table 2. *Antibody in cattle at four different sites in Kenya to 19 strains of bluetongue virus*

	Howell type																Kenya type		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	I	II	III
Kitale	5*	2	4	5	6	2	5	5	2	2	5	6	6	5	6	1	2	0	4
Amboselli	6	3	4	4	1	5	5	5	3	5	3	4	5	5	4	1	3	0	4
Mombasa	6	3	2	6	3	2	5	4	3	4	4	6	6	5	4	4	5	3	6
Nanyuki	6	5	5	6	0	6	6	6	2	3	4	4	6	4	6	1	4	0	3

* Number of pools positive of the six tested.

Sero-conversion rates per year

These are shown in Table 1 and have been derived from the animals in Groups II and III which were susceptible at the beginning of each exposure period. The results have been compared between different animals. There is no difference in the sero-conversion rates of different animals in the same year. A comparison of the percentage of sero-conversions of 20 animals in Group III gave a mean rate of $21{\cdot}8 \pm 1{\cdot}06$ S.E., for the years 1973–6.

Other sentinel groups

The results of PI tests on the cattle from different parts of Kenya are shown in Table 2. There is evidence for the presence of the 19 strains in each area. The Friesian cattle which were all negative on group specific fluorescent antibody tests to BT virus on arrival, were all positive on this test after one year. During this time no clinical signs of BT disease were observed. Their antibody conversions by the PI test are contained in Table 2.

DISCUSSION

The continuous challenge by BT of the sentinel cattle and the remainder of the herd, consisting of more than 3000 animals has not apparently been the cause of abortions, still-births or fetal abnormalities of any kind. Particular attention has been paid to the heifers in their first breeding season and the farmer has brought some fetal material to the laboratory. In 1968, between 60–70% of the pregnant animals on the farm aborted, Rift Valley fever was isolated from fetal tissue and from calves. The sero-conversions to this virus in 1968 were at a high rate (Davies, 1975). It is possible that some abortions and still-births at this time were due to

BT but the evidence indicated that Rift Valley fever was responsible. The only further problem has been Brucellosis and this was confirmed as the cause of abortion in most of the material submitted, by isolation and by serological tests on sera from aborted animals. There has been no other evidence of any effects of BT upon the fetus in the sentinel nor other cattle in this location. This is very different from the situation in the USA (Luedke, Jochim, Bowne & Jones, 1970).

In South Africa, Owen, du Toit & Howell (1965) isolated 11 different strains of BT virus from 5 cattle in a period of 18 months. Howell (1969) demonstrated sero-conversions in two sentinel cattle to 12 strains of BT in a 14-month period. Seven of these strains were recovered from these cattle during the study period; they were maintained in an area enzootic for BT in South Africa. Howell's (1969) results of virus isolation from clinical disease in sheep has also shown the plurality of strains which may be active in one area. These results support the data obtained by Howell and demonstrate the existence of a similar situation in Kenya.

The presence of the 19 antigenic types, all of which were detected at the sentinel herd site is remarkable, for only nine have been isolated from cases of disease in sheep. This is not too surprising, for the disease hosts in Kenya have a restricted distribution (Davies & Walker, 1974). BT is recognized by the sheep farmers in those areas where vaccination is widely practised, but clinical disease is not regularly reported. A concerted effort at virus isolation from sentinel cattle might reveal the existence of many other antigenic types.

These findings are relevant to the control of BT in Kenya, which is by vaccination with a polyvalent live attenuated virus vaccine containing the common serotypes 1, 2, 3, 4, 8 and 12. The value of this in a situation where up to 13 other strains are active is questionable. Flock owners are however adamant that vaccination is a valuable control measure, and the laboratory isolations do support the view that clinical disease is generally due to the six strains. The level of group specific immunity conferred by vaccination is not considered sufficient to withstand challenge by a heterologous strain. A logical control regime for BT in the enzootic areas would be to vaccinate successively with 4–5 strains at a time, until all have been covered. In those countries where BT appears in epizootic form and only one strain is involved, a killed antigen would offer a more satisfactory means of control. The widespread use of polyvalent attenuated live vaccines may allow the vector populations to become infected with additional strains of BT; for attenuation appears to be lost on passage through the vector (Foster, Jones, & Luedke, 1968).

There was no obvious correlation between the sero-conversion rates to BT and the annual rainfall during the years of this study. Walker (1976) concluded that there was no correlation between the rainfall and the populations of the potential BT vector species of Culicoides at this site over the same period. The experiences in 1968, where much of the country received more than twice the annual expected rainfall, did lead us to expect that there was a direct relationship between the disease vector populations and rainfall. This may yet be the case in years when the rainfall is extremely high, and not be evident in the years of below average rainfall, which prevailed during the study period. It would be interesting to study the

population changes of Culicoides, the sero-conversions to BT and virus amplification in a year of very heavy prolonged rainfall.

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