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A preliminary survey of the epidemiology of bluetongue in Kenya

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SUMMARY

A survey of the species composition and distribution of the *Culicoides* midge populations at a range of sites where bluetongue is enzootic isolated a group of dominant species: *C. cornutus, C. grahamii, C. magnus, C. milnei, C. pallidipennis* and *C.* 23.† Monthly light-trap sampling of *Culicoides* showed that the population densities of the dominant species greatly increased after the rain seasons and that these species concentrated around flocks of sheep and cattle. The larval habitats of *C. cornutus* and *C. pallidipennis* were found associated with stock pens. Precipitin tests on blood-fed *Culicoides* showed that most of the dominant species regularly feed on sheep and cattle. Bluetongue virus was isolated from *C. milnei, C. pallidipennis* and *C.* 23. Serological surveys of wild and domestic bovids from the enzootic area showed a high proportion with antibody to bluetongue virus. The colonization of *C. cornutus*, a potential vector, is described briefly. A causal relationship between peak rainfall in April–May, peak numbers of *Culicoides* in May–June and peak bluetongue incidence in June–July is postulated. The vector status of the above species and *C. austeni* was evaluated.

INTRODUCTION

In Kenya sporadic outbreaks of bluetongue (B.T.) have been reported in sheep populations since 1909. Control measures are taken by farmers who annually vaccinate their flocks with the attenuated live virus vaccine which contains all those strains so far isolated in Kenya. The efficiency of the vaccine could be limited by the isolation of new virus strains which have to be attenuated before incorporation in the vaccine. Work by Foster, Jones & Luedke (1968) in the U.S.A. has shown that the attenuated virus, on passage through *C. variipennis*, increases in virulence to reproduce clinical B.T. when the midges feed on susceptible sheep. Indiscriminate use of the vaccine in areas where B.T. is not enzootic could spread the disease.

C. variipennis has been incriminated as the vector of B.T. in the U.S.A. (Price & Hardy, 1954; Foster, Jones & McCrory, 1963) and *C. pallidipennis* has been in-

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† A new species, *C. tororoensis*, description not yet published (see Khamala, 1968).

criminated as the vector in S. Africa (Du Toit, 1944, 1962). In both these countries B.T. is characterized by its seasonal incidence, which is associated with seasonal increases in the numbers of the vector populations (Bowne, Luedke, Jochim & Foster, 1964). On the assumption that *Culicoides* are likely to be the vectors in Kenya this study was directed at isolation of the potential vectors and natural virus reservoirs and the study of the ecology and colonization potential of selected *Culicoides* species.

MATERIALS AND METHODS

Sampling and sampling sites

Adult *Culicoides* were sampled with portable light-traps. These were copies of a modified version of the Communicable Diseases Centre mosquito trap that was supplied by A. L. Dyce. *Culicoides* were attracted to the light of a 6 V., 18 W. tungsten filament bulb at the top of the trap and then sucked through the body of the trap and into a collecting jar by a fan driven by a small 6 V. motor. Each trap was powered by a 6 V. car battery and could run for 18 hr. on one battery charge. Samples were caught in alcohol in a plastic jar or, if live samples were required, in a cubical wire-frame cage, 15 cm. dimension and covered with 50 mesh/in. nylon gauze and one clear plastic window. Both jar and cage were connected to the trap by gauze cones. The traps were suspended at 1 m. high from trees, fence wires, etc., or from a tripod of wood poles.

Experiments were carried out to determine the limitations of the traps and the best operating procedure to give standardized results. Simultaneous sampling with light-traps and a Johnson–Taylor suction trap indicated that the light-traps gave a sample of a mixed population of flying *Culicoides* that was not significantly different from the unbiased population samples obtained with the suction trap.

The effective range of the light traps for *Culicoides* was found to be 20–25 m. by performing simple choice-chamber experiments with *C. cornutus* and *C. milnei*. The traps were thus always set up at least 25 m. apart, so that the area over which a trap was independently effective was always greater than the area where it interacted with a trap close to it. Wind speeds greater than 2 m./sec. (4·5 miles/hr.) were found to reduce the flight activity of *Culicoides*, thus sampling-time in which wind speeds were greater than this was not included in the standard period of regular samplings. Strongly moonlit nights were avoided. A study of the nocturnal activity patterns of five of the common species at the Naivasha site showed that they all had a late evening peak. The period from 7 p.m. to 11 p.m. was convenient for the standardized sampling, when meteorological observations had to be made at 20 min. intervals.

To trap samples for the studies of distribution, virus isolation and blood feeding, the traps were set up at sites where large catches were expected – that is, sheltered low-lying wet areas, particularly near stock pens, and were left to run all night. Live catches were recovered soon after dawn, before strong sunlight could affect the catches, and then sorted and processed in the laboratory as soon as possible. To monitor the seasonal population fluctuations and dispersal of *Culicoides* at the fixed sites the traps were set up at least once a month in constant line transects

relative to sheep flocks or larval habitats and operated until a standard total of 20 trapping hours per month was obtained. The location of the regular sampling sites and the sites covered during the distribution survey is shown in Fig. 2.

Potential larval habitats were sampled and larvae and pupae were extracted by washing the samples with water in 100 mesh/in. sieves and then floating them from the clean filtrate with a saturated solution of magnesium sulphate. The immature stages were identified by rearing them through to adults in small emergence traps.

Male and female adult *Culicoides* were identified according to Khamala's treatise (1968).

Precipitin test

Antiovine and antibovine precipitating antisera were prepared in rabbits by the method described by Weitz (1956), and were stored without preservative at $-10°$ C. in 2·5 ml. batches. Light-trap catches of *Culicoides* were searched for specimens that appeared to be engorged; these were aspirated out from the cages, anaesthetized with CO_2, identified and stored in individual tubes at 4° C. Twelve hours before testing individual specimens were placed in separate solid watch-glasses, their abdomens were dissected off and crushed in 0·5 ml. isotonic saline (9 g./l.), where they were left overnight at 4° C. Portions of the blood meal extract were drawn up into 70×1 mm. thin-walled capillary tubes and an equal volume of antiserum was drawn up under the blood extract. An air bubble was drawn into the capillary tube, which was then sealed by pushing it into a strip of plasticine mounted on a ruler. The separate capillary tests could be identified by their position on the ruler. After incubation at 35° C. for 2 hr. precipitin rings were studied on the black stage of a binocular microscope with a narrow beam of light shining along the length of the capillary tube. The precipitate, if present, was easily recognized. Each extract was tested against antibovine and antiovine sera. Controls were set up against the homologous and heterologous normal serum at 1/200 dilution and against isotonic saline.

There were slight qualitative differences between the reactions of ovine and bovine normal sera to any one of the antisera; since blood-fed specimens could be selected from sites containing exclusively sheep or cattle the test could effectively be used to determine which species of *Culicoides* feed on sheep or cattle or both.

Virus isolation

Culicoides samples were taken at monthly intervals at Sukari cattle ranch (site 8, see Fig. 2 and Table 1) from the area around a milking shed, and excess colonization material of *C. cornutus* adults from Braemar (site 9) were added to the samples. The samples were transferred to incubation cages; these were of cardboard, $25 \times 18 \times 15$ cm., with a window and with ventilation holes covered by nylon gauze. Droplets of sugar solution and water were suspended from the gauze to sustain the midges and they were kept at about 21° C. 77 % R.H. for 2 days, exclusive of day of capture, to allow for the digestion of any recent blood meals. Batches were then

removed from the cage, anaesthetized with CO_2 and sorted into species by means of a fine aspirator. Each species was placed in a separate bottle and stored at 4° C. Within 2 days the pools of between 20 and 200 midges were ground up in sterile sand with pestle and mortar in a concentrated antibiotic solution containing 500 I.U. penicillin, 500 μg. streptomycin and 25 units mycostatin (Squibb) per ml. The emulsion was then incubated at 37° C. for 2 hr. before centrifugation at 2000 rev./min. for 10 min. Phosphate-buffered saline was added to the supernatant to give a final concentration of 100 I.U. penicillin per ml. This suspension was used as inoculum for eggs by the i.v. route or for tissue culture (BHK cells, 21 c13). These were maintained with Eagle's medium for BHK cells, with 5% horse serum, 2·5% tryptose phosphate broth and 25% sodium bicarbonate (4·4% soln.). Egg harvest material was inoculated into BHK cells. Any cytopathic agent was identified by the group-specific fluorescent antibody test (F.A.T.) (Pini, Coakley & Ohder, 1966) on flying coverslips using a direct method with conjugates prepared from anti-bluetongue and anti-Nairobi sheep disease sera.

Typing of B.T. isolates was carried out by a plaque inhibition technique (F. G. Davies, in preparation) using L cells (mouse fibroblasts) and antisera specific for the types of B.T.V. (bluetongue virus) so far isolated in Kenya. All sera were examined by an indirect F.A.T. (Pini, Ohder, Whiteland & Lund, 1968) using an anti-bovine conjugate prepared in chickens. Typing was by plaque inhibition test.

RESULTS

Bluetongue incidence

B.T. outbreaks are recorded at the Kenya Veterinary Research Laboratory but it is known that a number of cases are not reported, thus the figures are below actual incidence. The histogram of B.T. outbreaks from 1964 to 1968 (Fig. 1) shows that there is a peak during June and July and that outbreaks have occurred in every month except January. While it can be said that there is a B.T. season in Kenya it is not as clear cut as that in the U.S.A. or in S. Africa.

Postulated B.T. *enzootic area*

Fig. 2 shows the area in Kenya where B.T. is considered likely to be enzootic. It is here defined as the area enclosed by the combined boundaries of three related areas:

(1) The 30–40 in. mean annual rainfall limit (Atlas of Kenya).

(2) The limit of the contiguous ecological zones as defined by Pratt, Greenway & Gwynne (1966); zone II – the forest and grasslands at 5000–7000 ft. and the montane *Acacia* woodland; zone III – the evergreen and semi-evergreen bushland and *Combretum* woodland and savanna; zone IV – the dry transitional *Combretum* savanna and upland *Acacia* woodland.

(3) The limit of the main cattle and sheep farming areas.

The salients to the north are probably just quirks but all recorded enzootic sites have been marked on the map and it can be seen that they all lie within the postulated area.

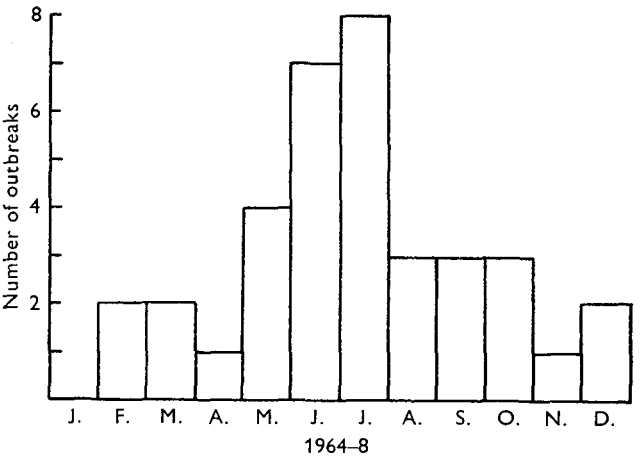

Fig. 1. Bluetongue incidence in Kenya.

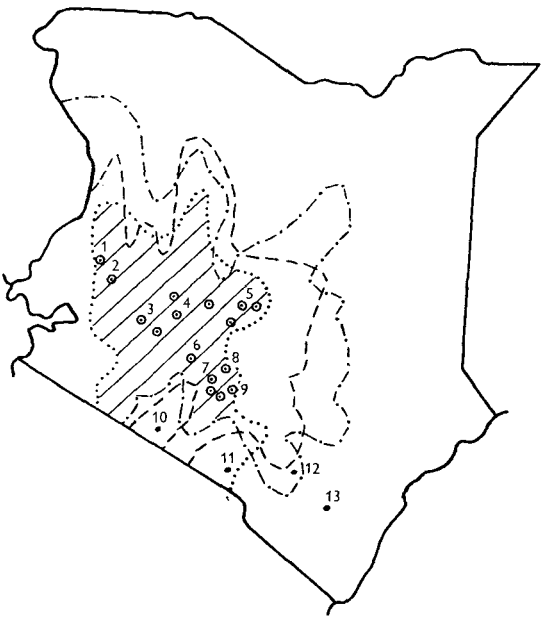

Fig. 2. Postulated enzootic area. —·—, 30–40 in. mean annual rainfall. — —, Ecological zones II, II, IV. ....., sheep- and cattle-rearing area. ⊙, Recorded enzootic site; sampling sites numbered.

Rainfall

The rainfall data of Figs. 3 and 4 were obtained from the Kenya Meteorological Department for rain-gauges that are maintained within a few miles of the sampling sites. The typical rainfall pattern in Kenya has two distinct and regular peaks: the first, the 'long rains', begin about March and last till May; the second, the less heavy 'short rains', begin in October and are usually over by December. This pattern occurs, with small variations, throughout the postulated enzootic area.

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However, the rainfall during 1968–9 was exceptional throughout Kenya. At Naivasha (Fig. 3) both the long and short rains were very heavy and long-lasting, and at Sukari (Fig. 4), although the short rains about November 1968 were normal, the long rains of 1969 virtually failed.

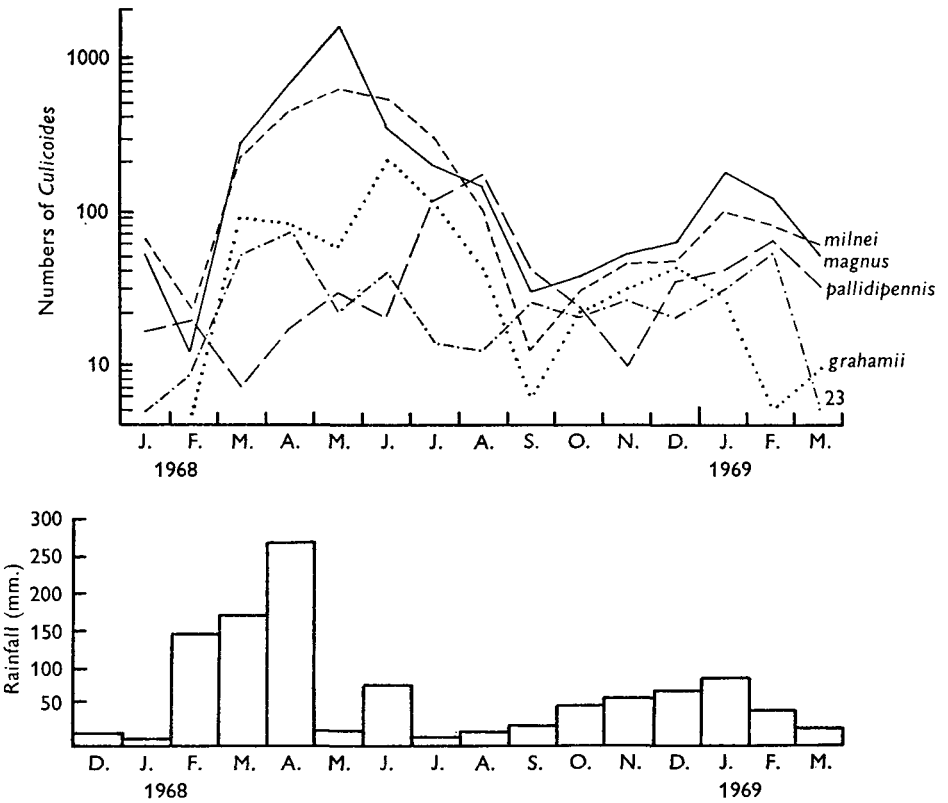

Fig. 3. Population curves of *Culicoides* – Naivasha. Rainfall pattern – Naivasha.

Distribution of Culicoides *in the enzootic area*

Table 1 shows *C. cornutus*, *C. grahamii*, *C. magnus*, *C. milnei*, *C. pallidipennis* and *C.* 23 as the dominant species within the enzootic area. The last two species, although never occurring in large numbers, were widespread and *C. cornutus*, equally widespread, was only found in especially dense localized pockets near stock pens. *C. austeni* is very similar to and closely related to *C. milnei* and it appeared to replace *C. milnei* at the far south-east margin of the enzootic area. The species composition of catches from different sites within the enzootic area were similar, but the *Culicoides* fauna from outside shows little similarity to that within the area.

There was always a preponderance of females caught in the light-traps – from 84 % to 100 %, depending on species and place. However, when immature stages were reared to adults there was always an approximate 1:1 male:female ratio; there was no reason to believe that the natural sex ratios of any of the species dealt with was other than normal.

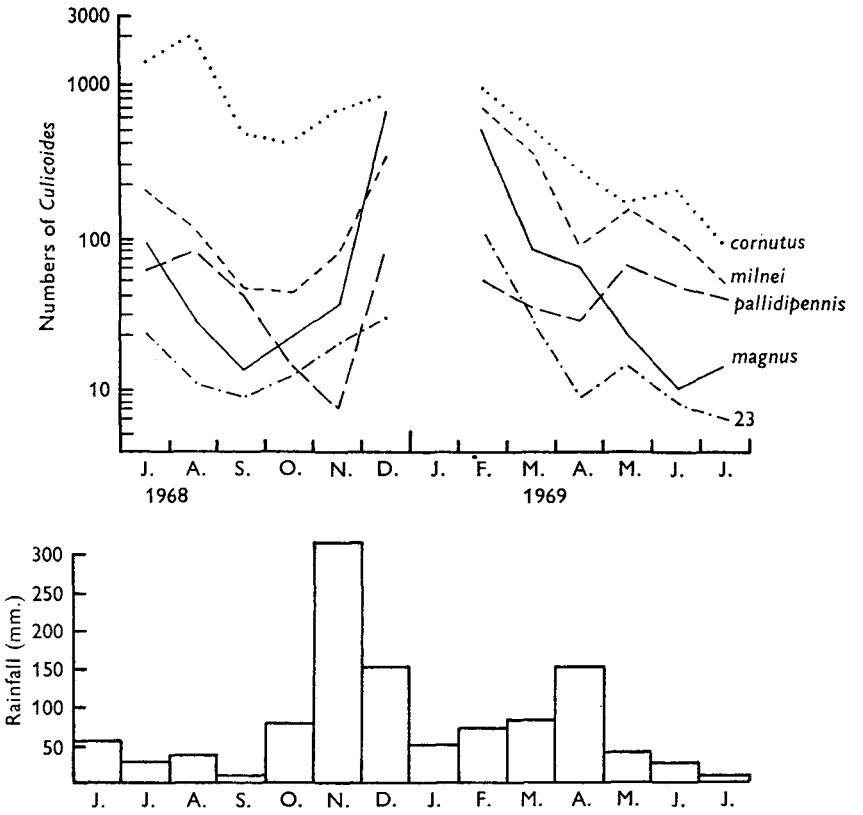

Fig. 4. Population curves of *Culicoides* – Sukari (plus *C. cornutus* from Braemar). Rainfall pattern – Sukari.

Population fluctuations

Fig. 3 shows the results of 15 months sampling at Naivasha, Ol Mogogo Veterinary Department farm (site 6), and Fig. 4 shows the results of 13 months sampling at Nairobi, Sukari Estate (site 8). In Fig. 4 is included the population curve for *C. cornutus* which was sampled simultaneously at Braemar Estate, Nairobi (site 9); no sampling could be done in January 1969. The populations of six species fluctuated considerably and a general pattern is evident in both figures if the curves of Fig. 4 are extrapolated to cover January. Reference to the rainfall histograms shows that a peak in rainfall preceded each total *Culicoides* population peak by 1–2 months and that the greater the rainfall the greater was the population peak. *C. magnus* and *C. milnei* had the most prominent and consistent pattern and together with *C. cornutus* showed the greatest periodic increase in numbers. *C. grahamii*, *C. pallidipennis* and *C.* 23 had more erratic fluctuations but still fit into the overall pattern. None of the rarer species recorded at these sites showed population fluctuations with any consistent or meaningful pattern.

The apparent positive correlation between population expansion and prior heavy rainfall is borne out by the curves for Sukari and Braemar from February 1969 onwards. Although there were rainfall peaks in April and May the amount

Table 1. *Distribution of* Culicoides *species in Kenya*

	Sampling sites*												
	Within enzootic area									Outside enzootic area			
Species	1	2	3	4	5	6	7	8	9	10	11	12	13
austeni	—	—	—	—	—	—	—	—	2	—	4	2	2
bedfordi	—	—	—	—	—	1	—	—	—	—	—	2	1
cornutus	4	2	1	—	—	—	—	—	6	—	—	—	—
fuscicaudae	—	—	—	—	—	< 1	—	—	—	—	—	—	—
grahamii	—	1	—	1	1	1	2	1	1	—	—	1	2
kibatiensis	1	—	—	—	—	1	—	—	—	—	—	—	—
magnus	1	2	3	6	4	5	3	4	1	—	—	—	—
milnei	3	5	6	3	4	3	3	4	—	—	—	—	—
naevii	1	1	—	—	—	—	—	—	—	—	—	—	—
nivosus	—	—	—	—	—	—	—	1	—	—	—	—	—
pallidipennis	2	1	1	2	2	1	3	2	2	—	—	3	2
praetermissus	—	—	—	—	—	< 1	—	—	< 1	—	—	—	—
pycnostictus	—	—	—	—	—	< 1	—	< 1	< 1	—	—	—	—
schultzei	—	—	—	—	—	—	—	—	—	10	6	5	5
23	1	1	—	1	—	1	1	1	1	—	—	—	—

The distribution of each species is shown as the relative density of the species at each site expressed as coded percentages of the total catch: < 1 = < 1 %; 1 = 1–10 %; 2 = 11–20 %; . . . up to 10 = 91–100 %.

* Sampling sites: see Fig. 2 for location. 1, Kitale; 2, Eldoret; 3, Molo; 4, Thomson's Falls; 5, Nanyuki; 6, Naivasha; 7, Nairobi, Kabete; 8, Nairobi, Sukari Estate; 9, Nairobi, Braemar Estate; 10, Magadi; 11, Amboseli; 12, Kiboko; 13, Tsavo East.

that fell was appreciably less than that falling in the short rains in November 1968 and there was almost a drought. Apart from minor fluctuations the populations of all species declined in numbers during this period.

The monthly data from Naivasha were obtained by operating the traps in a line transect with traps at 5, 25, 50, 100 and 200 m. from about 450 sheep that were always flocked at the same spot. The totals for each species for the entire sampling period for each transect trap, when plotted as numbers against distance from sheep, give a series of lines representing the distribution of each species relative to the sheep flock. In terms of total numbers caught the species fell into two distinct groups. The group of common species, *C. grahamii*, *C. magnus*, *C. milnei*, *C. pallidipennis* and *C.* 23, all showed a regression of numbers on distance from sheep and the common regression coefficient calculated as the sum of all species (logarithmically transformed to reduce variance) at each trap upon distance gave $b = -0{\cdot}059$, which was highly significant ($P = 0{\cdot}01$–$0{\cdot}001$). Thus from the aspect of populations distributed in a measured area, they concentrated around the sheep. In contrast, the group of rarer species, *C. bedfordi*, *C. fuscicaudae*, *C. kibatiensis*, and *C. pycnostictus*, treated in the same way showed a common regression that was hardly significant ($b = 0{\cdot}017$, $P = 0{\cdot}1$–$0{\cdot}05$); there was no evidence of the concentrating around the sheep. Although the larval habitats of most of the species dealt with were not discovered there was no evidence of any *Culicoides* breeding in high densities within the transect area.

Dispersal from larval habitat

At the Braemer site localized larval habitats of *C. cornutus* and *C. pallidipennis* were found in the mixture of mud and dung surrounding a cattle milking stall. The dispersal of these species was studied by setting up a line transect of six light-traps at 100 m. intervals from the stall on two consecutive calm nights. No other larval habitats were found in the transect area. *C. cornutus* was recovered up to 300 m. from the larval habitat and *C. pallidipennis* at up to 500 m. Although the larval habitats of *C. magnus*, *C. austeni* and *C.* 23 were not definitely established it is probable that they were also near the cattle stall and these species were also recovered at between 300 and 500 m. from the stall.

The dispersal coefficient, i.e. the distance at which the numbers caught fell to one-tenth of their value at the larval habitat and calculated as the reciprocal of the regression coefficient of numbers on distance (Kettle, 1951), for these species was: *C. cornutus* 89·2 m., *C. austeni* 133·3 m., *C. pallidipennis* 264·5 m., *C. magnus* 291·1 m., *C.* 23 420·2 m. These figures are an indication of the distance that each species will actively disperse relative to the others. In view of the wide distribution of B.T. antibodies in wild bovids (see below) and of B.T. enzootic sites it is likely that the vectors of B.T. will have high dispersive capabilities, although it must be remembered that *Culicoides* could be dispersed passively by wind for greater distances. Notably, *C. cornutus*, which was found to be the most localized in distribution, had the lowest dispersal coefficient.

Larval habitats

Although an extensive survey of potential larval habitats was carried out following the rainy seasons of 1968 when high larval densities were expected, the larval habitats of only two of the dominant species, *C. cornutus* and *C. pallidipennis*, were definitely established. The larvae of both species were found regularly and in high concentration in the mixture of fine mud and dung surrounding cattle pens and also in associated effluent ditches. Both *C. milnei* and *C. magnus* larvae were found in a variety of habitats characterized as fine mud with high organic detritus content at the margins of ditches and pools, but these are probably only secondary habitats. No larvae of any species were found in sites that were not at least semi-liquid mud or those that were covered by free water deeper than 1–2 cm.

Blood feeding

Table 2 shows that all of the six species found to predominate numerically and also *C. austeni* will feed on sheep and that all except *C.* 23 feed on cattle. Within the sampling areas these species feed predominantly on sheep and cattle, or possibly in a proportion of cases on closely related wild bovids. No other species were ever found engorged with blood.

Table 2. *Precipitin testing of blood from engorged* Culicoides

Culicoides species	No. tested	No. positive for		No. negative	Approximate % engorged in samples
		Sheep	Cattle		
austeni	7	3	2	2	0·77
cornutus	18	7	10	1	1·8
grahamii	12	7	2	3	2·4
magnus	14	8	5	1	0·93
milnei	43	15	20	8	1·43
pallidipennis	17	6	6	5	1·7
23	7	1	0	6	0·87

It is evident that the proportion of engorged *Culicoides* caught was very low. This is often the case with *Culicoides* (see Kettle, 1969) and could be due to differential responsiveness of engorged and non-engorged *Culicoides* to the light-traps, but tests with captive *C. cornutus* and *C. milnei* indicated that whether engorged or not they were equally susceptible to the traps when actively flying. However, it is unlikely that a midge will disperse far immediately after taking a large blood meal and this will reduce its chances of capture. A consideration of the number and length of ovarian cycles typical of *Culicoides*, each requiring only one blood meal, and the possibility that a proportion of the populations of some of the species are autogenous, leads one to expect that no more than 10 % of the female population will be blood-feeding at any one time.

Experiments with captive midges kept in standardized conditions in small holding cages and given blood meals from rabbits showed that individuals of *C. cornutus* (8), *C. milnei* (6), *C. magnus* (5), *C. pallidipennis* (3), and *C.* 23 (4) will

all take at least two blood meals separated by an ovarian cycle lasting the number of days shown in parentheses. No second ovarian cycles or third blood meals were recorded. None of the species seemed to be host-specific; they fed with equal avidity on rabbit, guinea-pig and human hosts.

Virus isolation

The results of virus isolation attempts from the main species at the sampling sites in the Nairobi area are shown in Table 3. The isolation of virus from a *C. pallidipennis* pool in August 1968 can be correlated with a population peak as seen

Table 3. *Virus isolation from* Culicoides

Culicoides species	1968					1969					
	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.
cornutus	—	—	—	—	—	—	—	—	—	—	—
grahamii	—	—	—	—	—	—	—	—	—	—	—
magnus	—	—	—	—	—	—	—	—	—	—	—
milnei	—	—	—	—	—	—	BT 1	—	—	—	—
pallidipennis	BT 4	—	BT 1	—	—	—	BT 1	—	—	—	—
23	—	—	—	NSD	—	—	BT 1	—	—	—	—

Positives are shown as BT, together with the code number of the Howell immunological type, and NSD for Nairobi sheep disease.

Table 4. *Bluetongue antibody tests on wild Bovidae*

Species	No. sera tested	Positive	%
Kongoni, *Alcelaphus buselaphus*	66	57	86
Wildebeeste, *Connochaetes taurinus*	134	81	60
Impala, *Aepyceros melampus*	36	21	58
Eland, *Taurotragus oryx*	20	8	40
Buffalo, *Syncerus caffer*	6	4	67
Topi, *Damaliscus korrigum*	38	11	29
Waterbuck, *Kobus ellipsiprymnus*	3	0	0
Reedbuck, *Redunca fulvorufula*	2	2	100
Bushbuck, *Tragelaphus scriptus*	4	0	0
Thomson's gazelle, *Gazella thomsonii thomsonii*	60	5	8
Domestic cattle	86	46	53

in Fig. 3, and similarly the isolation of virus from *C. pallidipennis*, *C. milnei* and *C.* 23 in February 1969 can be correlated with the population peak of January and February. The isolation of Nairobi sheep disease virus from a *C.* 23 pool is an enigma that prompts further study of *Culicoides* as possible vectors of this disease.

Table 4 shows that a high percentage of wild bovids in the Rift Valley (which runs through the middle of the enzootic area) contain antibodies to B.T.V. and have been infected with the virus, presumably from bites of *Culicoides* vectors. A sample of domestic bovids from the enzootic area showed 53 % with antibody to B.T.V.

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Colonization

A colony of *C. cornutus* was established in the laboratory. At least 500 wild caught specimens were placed in conical plastic containers of 20 cm. basal diameter, 8 cm. top diameter and 26 cm. high. The cages were kept at 22° C., 75 % R.H. and the adults were fed on sugar solution and given blood meals on a rabbit held in a special stock above the apical opening in the cage. Large numbers of eggs were laid on moist blotting-paper pads in the cage and these were placed direct into a larval substrate in shallow galvanized iron pans. The substrate was a simulation of the natural habitat but never proved very successful; larval mortality usually exceeded 90 %. It is likely that a microbial broth substrate as described by Jones (1969) would be better. Pupae were produced within 22 days and were extracted from the pans by water flotation. Pupae were placed direct on moist cotton pads and hatched into clean cages. Sufficient 'clean' midges could be obtained for use in transmission experiments; mean adult longevity was 10 days. An improvement of the larval substrate would probably result in a fully self-maintaining colony.

DISCUSSION

A fairly definite pattern of B.T. epidemiology emerges from the results. B.T. is confined in Kenya to one area with specific ecological characteristics. Within this area there are six species of *Culicoides* with a distinct numerical dominance and the species composition of populations from different sites is very similar. Rainfall is the major extrinsic factor affecting the populations of these dominant species. Peak rainfall in April–May facilitates high larval survival and consequent rapid expansion of adult numbers that reaches a peak in May, June and July. During the latter half of this peak there is, significantly, a peak of B.T. outbreaks.

An evaluation of the vector potential of the species studied against the criteria of distribution and time of response to rainfall and also of the nature of larval habitats, distribution about sheep, dispersive powers, blood-feeding habits and B.T.V. content, isolates two distinct groups. In order of decreasing vector potential, *C. milnei*, *C. cornutus*, *C. pallidipennis* and *C. magnus* fall into a group of high potential and *C.* 23, *C. grahamii* and *C. austeni* fall into a group of lower potential.

The number of virus isolations was insufficient to answer many of the questions posed by the study of the epidemiology of B.T. The identification of three of the seven potential vectors as being capable of harbouring B.T.V. is important. The persistence of *Culicoides*, albeit in small numbers, for the whole of the year has led the authors to suspect that in Kenya B.T.V. persists by a repeating midge to cattle or game cycle, with only a silent infection in these hosts. We have no evidence of clinical B.T. in cattle or game in Kenya, but when there is a rise in the total *Culicoides* population after the rains cattle and game probably act as amplifying hosts and after a lag phase increase the proportion of infected *Culicoides* to a stage when the relatively unattractive disease host (Du Toit 1962) is involved in the feeding pattern to a much larger extent. There is some evidence that a constant supply of susceptible amplifying hosts may not be necessary for the propagation of B.T.V.;

recurrent viraemia with the same and different virus strains have been observed in cattle (Owen, Du Toit & Howell, 1965). This might reasonably be expected to occur in the wild bovids. In South Africa and America it is probable that a distinct interepizootic period occurs when *Culicoides* are not present or do not contain the virus, and B.T. is not seen.

The game sera which were taken within the enzootic area gave a high proportion with antibody to B.T. Kongoni, Impala and Wildebeeste appear to be widely exposed to infection, presumably from the bites of *Culicoides*. They are amongst the commonest game in the enzootic area and may be the natural hosts for B.T. in Kenya together with domestic cattle. A better assessment of the role they play must await studies of the duration of viraemia that can be caused by B.T.V. in game animals.

It is felt that further work should be directed towards the colonization of potential vector species, and to transmission experiments to examine further the possibility that vaccine virus reverts to virulence after passage through *Culicoides*. A more detailed study of the ecology of the potential vector species with emphasis on blood-feeding capabilities will help to isolate the definite vectors. The role of wild game and rodents as reservoirs of B.T.V. must be examined and their significance in relation to the role of domestic cattle evaluated.

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