J. Hyg., Camb. (1974), **72**, 265
Printed in Great Britain

The distribution in Kenya of bluetongue virus and antibody, and the *Culicoides** vector

By F. G. DAVIES and A. R. WALKER
Veterinary Research Laboratory, P.O. Kabete, Kenya

(*Received* 7 *August* 1973)

SUMMARY

Sera from domestic cattle, sheep and goats and 11 wild bovid species taken at 41 locations throughout Kenya were screened for antibody to bluetongue virus using the indirect fluorescent antibody method. Positive sera were found at 39 locations and in all the species tested. The distribution of clinical disease was mapped and found to be more restricted than the distribution of antibody. *Culicoides* were collected in light traps at 55 locations throughout Kenya. The presumptive vector of bluetongue in Kenya, *C. pallidipennis,* was the most widespread species, found in 38 locations. Both the distribution of antibody and *Culicoides* have been tabulated by ecological zones to demonstrate that bluetongue virus in its natural state in cattle and wild bovids is far more widespread than clinical bluetongue disease seen in exotic sheep of restricted distribution; and that *C. pallidipennis* has great ecological range.

INTRODUCTION

Bluetongue in Kenya is thought to exist largely in its natural biological state. The virus is transmitted by *Culicoides* midge vectors between wild bovids, indigenous cattle and sheep without apparent clinical disease. The disease hosts are generally exotic wool sheep, which are protected in Kenya by a multi-strain egg-attenuated vaccine. The reservoir and amplifying mammalian hosts, both wild and domestic, are widely distributed throughout Kenya, but little is known of the distribution of bluetongue virus and its vectors.

Walker & Davies (1971) gave results from a small number of locations and postulated an apparent enzootic area which has now been shown to be too restricted. They listed domestic cattle and sheep and eight wild bovid species as containing antibody to bluetongue virus; and *Culicoides milnei, C. pallidipennis* and *C. cornutus* as probable vectors. Bluetongue virus was isolated from the first two species and *C. tororoensis* (previously *C.23*), and *C. pallidipennis* is known as a vector of bluetongue in South Africa (Du Toit, 1944; Nevill, 1971). Khamala (1971) made an analysis of collections of East African *Culicoides* which were obtained for a taxonomic survey (Khamala & Kettle, 1971), by distribution in ecological zones. This indicated that the probable vector species were more widespread than

* Diptera, Ceratopogonidae.

https://doi.org/10.1017/S0022172400023470 Published online by Cambridge University Press

expected, but the range of collection locations was limited by lack of battery-powered traps.

This work has been carried out as an extension of the earlier surveys, with the intention of defining the ecological zones in which the virus, its vectors and mammalian hosts can be found. The work has been hampered to some extent by the dry conditions prevailing since the heavy rainfall year of 1967–8 which has kept the populations of *Culicoides* at a lower level than in 1968 when large numbers of domestic and wild bovids were infected. This has been shown by regular monitoring since 1968 of a sentinel cattle herd (F. G. Davies, to be published).

METHODS

Serology

The indirect fluorescent antibody method described by Pini, Ohder, Whiteland & Lund (1968) has been used to screen most sera for bluetongue antibody. This is a group-specific test. There were some modifications in the preparation of anti-ovine and anti-bovine gamma globulins. The gamma globulins were precipitated with 2·25 M sodium sulphate from ovine or bovine sera. The precipitate formed was resuspended in phosphate buffered saline (PBS) and precipitation repeated twice more. The precipitate was finally resuspended in PBS and dialysed against PBS for 24 to 48 hr., at 4° C. The protein concentration was adjusted to 1% for conjugation.

Conjugation with fluorescein isothiocyanate was at a ratio of 1 mg. of dye to 50 mg. of protein. The dialysis conjugation method of Clark & Shepard (1963) has been found to be better than the earlier conjugation method at 4° C. overnight. Conjugates were further dialysed against PBS and filtered down a column of Sephadex G 25.

Gel precipitation tests with the wild bovid sera and ovine and bovine sera showed lines of identity against the anti-bovine gamma globulin. All sera were stored at −20° C. and were routinely inactivated at 56° C. for 30 min. The wild bovid sera were kindly supplied by various farmers and the Food and Agriculture Organisation Wildlife team at the Veterinary Research Laboratory, Kabete. Sera from domestic animals were collected by members of the Kenya Veterinary Department. Collections of sheep and goat sera were generally made from mixed flocks, described in Kenya as 'shoats'. There was no difference in the results obtained from these when tested separately as goat or sheep sera, and for the purposes of this paper they are presented together. The tissue culture and staining procedures were largely as described by Pini *et al.* (1968).

Entomology

Culicoides were caught in battery-powered light and suction traps similar in construction to that described by Service (1970). Typically, collections were made in sites considered to be favourable to *Culicoides* such as stock pens or waterholes, using from two to six traps with a variety of lights (6W ultra-violet or white light tubes or 18W incandescent bulbs) and augmented with carbon dioxide from dry

https://doi.org/10.1017/S0022172400023470 Published online by Cambridge University Press

ice in plastic bags on the traps. Most locations were visited for one or two nights only and regardless of season. The intention was to cover as wide an area as possible but the north-east area of Kenya is remote and difficult of access and was not covered as well as other areas.

Ecological zones

The zones into which the insect and serum sampling locations are allocated are after a classification by Pratt, Greenway & Gwynne (1966). These zones are defined by climate and described by vegetation and land use. They are summarized below.

Zone II. Climate humid to dry subhumid, moisture index not less than -10. Vegetation forests and derived grasslands and bushlands.

Zone III. Climate dry subhumid to semi-arid, moisture indices -10 to -30. Vegetation moist woodland/bushland/wooded grassland, trees typically broad-leaved *Combretum*.

Zone IV. Climate semi-arid, moisture indices -30 to -40. Vegetation dry woodland and wooded or bushed grassland, trees typically *Acacia*.

Zone V. Climate arid, moisture indices -40 to -50. Vegetation dry woodland/bushland, trees typically *Commiphora* and *Acacia*.

Zone VI. Climate very arid, moisture indices -50 to -60. Vegetation dwarf shrub grassland or dry bushed grassland, including barren land.

The zones are approximately delineated in Figs. 1 and 2. They are meant to indicate the potential of areas for use as range land by domestic and wild ungulates and as such are better suited to the purposes of this paper than vegetation maps based on physiognomic characters. The range of ecological zones in Kenya is very great. Zone II areas (except for the coastal forests) are similar in many respects to world cool temperate latitudes because of the high altitude (1700–2200 m.), although obviously insolation is higher and frosts are rare. Zone VI areas are 'semi-deserts'. Zone I is the afro-alpine zone confined to mountain slopes above 3300 m.

Bluetongue disease

Bluetongue virus has been isolated in these laboratories from field material either by sheep inoculation or directly in embryonating eggs or tissue culture (BHK cells). Laboratory records for the period 1950 to 1972 have been used to plot the distribution of clinical disease.

RESULTS

Entomology

Of the 30 species of *Culicoides* found only those 15 found in five or more of the 55 collection locations are included, in order of frequency in Table 1. Fig. 1 shows the collection locations. Numbers caught have not been shown because of the very large and erratic population fluctuations with time which make comparisons

https://doi.org/10.1017/S0022172400023470 Published online by Cambridge University Press

Table 1. *Distribution of* Culicoides *species*

Species	II (13)	III (9)	IV (18)	V (12)	VI (3)	Totals per species
	Zones. No. of locations per zone in parentheses					
C. pallidipennis	9	9	11	8	1	38
C. nivosus	4	5	17	5	1	32
C. grahamii	7	5	7	3	0	22
C. milnei	9	4	7	1	0	21
C. pycnostictus	5	2	9	4	1	21
C. schultzei	3	4	5	7	0	19
C. magnus	7	3	8	0	0	18
C. praetermissus	0	1	8	7	0	16
C. cornutus	5	2	7	1	0	15
C. trifasciellus	6	2	6	1	0	15
C. kingi	0	2	6	4	2	14
C. neavei	3	5	2	1	0	11
C. similis	0	2	3	4	1	10
C. tororoensis	2	2	3	2	0	9
C. kibatiensis	3	2	3	0	0	8

Number of locations where species found are shown across the table.

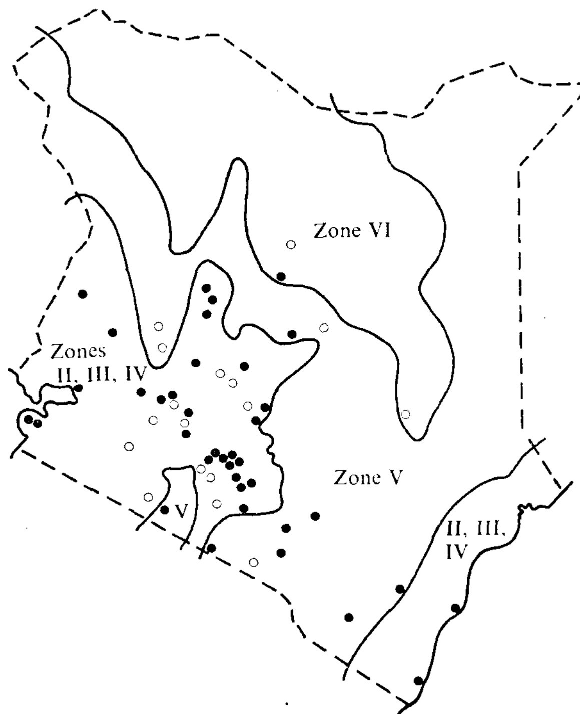

Fig. 1. Distribution of insect collection locations. ○, ●, Locations; ●, locations where *C. pallidipennis* found, ——, ecological zone boundaries; – – –, Kenya boundary.

https://doi.org/10.1017/S0022172400023470 Published online by Cambridge University Press

Table 2. *Distribution of sera with antibody to bluetongue virus*

Species	Zones. No. of locations per zone in parenthesis				
	II (9)	III (7)	IV (13)	V (10)	VI (2)
Domestic cattle	90/129	72/138	47/144	62/188	18/35
Domestic sheep and goat	25/60	14/78	142/339	53/214	5/8
Coke's hartebeeste, *Alcelaphus buselaphus*	4/5	—	46/60	29/39	—
Wildebeeste, *Connochaetes taurinus*	6/10	—	42/58	27/42	—
Eland, *Taurotragus oryx*	—	—	1/7	7/17	—
Impala, *Aepyceros melampus*	1/10	—	29/91	6/13	—
Grant's gazelle, *Gazella grantii*	1/10	—	6/20	6/15	—
Thomson's gazelle, *Gazella thomsonii*	1/10	—	20/137	1/4	—
Waterbuck, *Kobus ellipsiprymnus*	—	—	10/25	1/6	—
Reedbuck, *Redunca fulvorfula*	—	11/28	0/7	—	—
Oryx, *Oryx beisa*	—	—	—	2/2	—
Buffalo, *Syncercus caffer*	—	—	7/19	—	—
Oribi, *Ourebia ourebia*	—	2/14	—	—	—

Results are shown as number positive over the sample number. Dashes indicate that no samples were taken.

between individual species at individual locations of little value. Lack of records of a species at a location means only that it was not found in these collections.

Of the species of *Culicoides* previously considered to have potential as vectors of bluetongue (Walker & Davies, 1971) *C. pallidipennis* was found at a greater number and range of sites than *C. grahamii*, *C. magnus*, *C. cornutus* and *C. tororoensis*, which were found more sporadically. In terms of numbers caught, on average, *C. pallidipennis* was also dominant.

Few species show a marked restriction to certain zones, but *C. milnei* is conspicuous for being found only once in zones V and VI. *C. nivosus* was very widespread, which was in contrast to the earlier studies. Changes in trap design could possibly account for the higher frequency of capture. However, only five specimens were found engorged with blood and none gave positive results against bovid precipitating antisera (tests performed by P. F. L. Boreham). *C. grahamii* was never caught in large numbers and only at a moderate number of locations; this contrasts with Khamala's results where *C. grahamii* was the dominant species in numbers and distribution. The greater range of sites in the present study may account for this discrepancy.

Serology

The results are summarized in Table 2 and the sampling locations are shown in Fig. 2. Of the 41 locations there were 39 at which sera gave positive results for bluetongue antibody. The sera were collected over the period 1967–72. During

https://doi.org/10.1017/S0022172400023470 Published online by Cambridge University Press

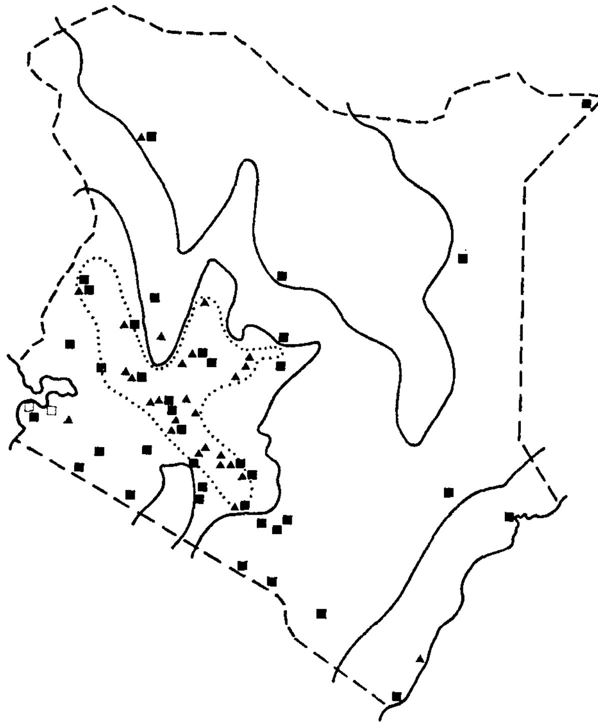

Fig. 2. Distribution of serum sample locations and clinical disease. □, ■, serum sample locations; ■, locations where sera were positive for bluetongue antibody; ▲, locations of outbreaks of clinical bluetongue disease; . . ., exotic wool sheep rearing area; ——, – – – –, as in Fig. 1.

the early part of this period there was considerable rainfall and arbovirus diseases of cattle and sheep such as Rift Valley fever and ephemeral fever were prevalent. Considerable biting activity of bluetongue-infected *Culicoides* occurred during this period, which has been confirmed by serological studies in a sentinel herd (F. G. Davies, to be published). The results taken from the earlier years gave higher percentages of positive sera than those taken in 1971–2, especially when young animals were bled. The serological results are intended to show the universality of contact with bluetongue throughout the range of ecological zones in Kenya.

Occurrence of clinical bluetongue

The distribution of locations where there have been outbreaks of clinical bluetongue disease is shown in Fig. 2. Clinical disease has occurred in all ecological zones, as might be expected from the results of the entomological and serological surveys. However, the proportions of outbreak locations per zone (zone II = 10, zone III = 6, zone IV = 9, zone V = 1, zone VI = 1) indicate that factors other than the distribution of virus and vector influence the actual appearance of clinical disease. Fig. 2 shows an approximate boundary of the current wool sheep rearing area in Kenya as derived from data in the *National Atlas of Kenya* (1970). Disease is seen almost entirely within this boundary, affecting exotic breeds of

https://doi.org/10.1017/S0022172400023470 Published online by Cambridge University Press

wool sheep, with rare anomalous cases such as clinical bluetongue in introduced goats at Lodwar, the location marked in the far north-west.

DISCUSSION

The distribution of an arbovirus vector must be contiguous with the distribution of the virus. In a survey of this type negative results mean little and there are locations at which sera contained antibody to bluetongue virus and yet no *C. pallidipennis* were found. However, the results are adequate to show that *C. pallidipennis* fits the vector criterion well and that it is a very adaptable and viable species. The viability may be due to the larval habitat being in bovid dung pats, as Nevill (1968) has shown in South Africa.

The results of the serological survey demonstrate the widespread distribution of bluetongue antibody throughout Kenya. The disease occurs almost entirely in exotic sheep which are kept in a limited area. The exotic sheep appear to be more susceptible than the indigenous sheep and goats. No bluetongue disease is recognized by the pastoral tribes, such as the Maasai, who are familiar with all the other cattle and sheep or goat diseases in their areas. There is antibody to bluetongue virus in many areas of Maasai land. Bluetongue virus has been isolated from indigenous sheep, this has been adventitious however and had not been associated with any clinical signs of disease. Further work will be aimed at confirming the resistance of indigenous sheep and goats to bluetongue.

Factors other than breed susceptibility clearly influence the occurrence of disease. We suspect that the increase in vector population with amplification of the proportion infected with virus is the principal one (Walker & Davies, 1971). Further studies after a heavy period of rainfall such as was experienced in 1967–8 should serve to answer these questions.

We have no evidence of any antigenic variation amongst the bluetongue strains in Kenya. All virus strains isolated to date have been typed as one of the seven thus far recognized in Kenya (F. G. Davies, unpublished data). Further work will be aimed at typing the positive sera from different parts of Kenya from domestic and wild bovids.

No pathogenicity experiments have as yet been carried out with the game species shown to contain antibody to bluetongue virus. Plaque neutralization tests carried out by the method of Howell (1970) show that titres of antibody in game animals are comparable with those in domestic animals. They are in the range of $10^{2\cdot5}$ to $10^{4\cdot5}$ neutralizing indices (F. G. Davies, unpublished data). There is abundant evidence (Barzilai & Tadmor, 1972; Thomas & Trainer, 1970) that wild ruminants elsewhere sustain a viraemia of sufficient titre to infect *Culicoides* (Luedke, Jones & Jochim, 1967).

This paper is published with the permission of the Director of Veterinary Services, Kenya.

https://doi.org/10.1017/S0022172400023470 Published online by Cambridge University Press

REFERENCES

Barzilai, E. & Tadmor, A. (1972). Experimental infection of the Mountain Gazelle (*Gazella gazella*) with bluetongue virus. *Refuah Veterinarith* **29**, 45–50.

Clark, H. F. & Shepard, C. C. (1963). A dialysis technique for preparing fluorescent antibody. *Virology* **20**, 642–4.

Du Toit, R. M. (1944). Transmission of bluetongue and horsesickness by *Culicoides*. *Onderstepoort Journal of Veterinary Science and Animal Industry* **19**, 7–16.

Howell, P. G. (1970). The antigenic classification and distribution of naturally occurring strains of bluetongue virus. *Journal of the South African Veterinary Medical Association* **41**, 215–23.

Khamala, C. P. M. (1971). Ecological distribution of East African *Culicoides* as shown by light traps. *Bulletin of Entomological Research*, **60**, 549.

Khamala, C. P. M. & Kettle, D. S. (1971). The *Culicoides* of East Africa. *Transactions of the Royal Entomological Society of London* **123**, 1–95.

Luedke, A. J., Jones, R. M. & Jochim, M. M. (1967). Transmission of bluetongue between sheep and cattle by *Culicoides variipennis*. *American Journal of Veterinary Research* **28**, 457–60.

National Atlas of Kenya (1970). *Survey of Kenya*, Nairobi.

Nevill, E. M. (1968). A significant new breeding site of *Culicoides pallidipennis*. *Journal of the South African Veterinary Medical Association* **39** (part 3), 61.

Nevill, E. M. (1971). Cattle and *Culicoides* midges as possible overwintering hosts of bluetongue virus. *Onderstepoort Journal of Veterinary Research* **38**, 65–72.

Pini, A., Ohder, H., Whiteland, A. P. & Lund, L. J. (1968). Studies on the fluorescent and neutralising antibodies to bluetongue virus in sheep. *Archiv für die gesamte Virusforschung* **25**, 128–36.

Pratt, D. J., Greenway, P. J. & Gwynne, M. D. (1966). A classification of East African rangeland, with an appendix on terminology. *Journal of Applied Ecology* **3**, 369–82.

Service, M. W. (1970). A battery-operated light-trap for sampling mosquito populations. *Bulletin of the World Health Organization* **43**, 635–41.

Thomas, F. C. & Trainer, D. O. (1970). Bluetongue virus in white tailed deer. *American Journal of Veterinary Research* **31**, 271–8.

Walker, A. R. & Davies, F. G. (1971). A preliminary survey of the epidemiology of bluetongue in Kenya. *Journal of Hygiene* **69**, 47–60.

https://doi.org/10.1017/S0022172400023470 Published online by Cambridge University Press