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Bluetongue virus in a Nigerian dairy cattle herd

1. Serological studies and correlation of virus activity to vector population

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SUMMARY

Newborn calves were bled at monthly intervals and examined for serum antibodies to bluetongue virus (BTV). Maternal immunity persisted for 3 months and it was possible to calculate decay rates for virus neutralizing antibody. Calves were subclinically infected with BTV within a few months of becoming susceptible and neutralization tests were used to deduce the serotype responsible. A profile of virus activity was built up over a 12 month period. Frequent light trap catches were used to examine the population dynamics of suspected Culicoides vector species. Two species, *imicola* and *schultzei* were present throughout the wet and dry seasons and survival rates were sufficiently long to account for virus transmission at any time of the year.

INTRODUCTION

In a previous study (Taylor & McCausland, 1976) group-specific serological evidence of bluetongue virus (BTV) (genus Orbivirus, family Reoviridae) activity was reported in young and adult Friesian cattle of the dairy herd at the National Veterinary Research Institute (NVRI), Vom, Nigeria. These results suggested a more detailed investigation of this herd to monitor virus activity over a 12-month period.

As the acquisition of BTV group-specific agar gel precipitating antibodies indicates that an animal has undergone BTV infection, by narrowing and regularizing sampling intervals it should be possible to attribute a given seroconversion to a particular time. Therefore, it was planned to collect serum samples from a substantial number of animals on a monthly basis. The BTV group-specific antibody appears to be long lasting (Jochim & Chow, 1969) and hence only its first appearance can give a reliable indication of recent infection. For this reason all studies were confined to calves. All sera were also examined for BTV type-specific neutralizing antibodies in the hope that a picture of the virus types circulating in this group of animals would emerge.

In addition entomological investigations were undertaken into the population dynamics of potential Culicoides vectors of BTV. Boorman & Dipeolu (1979) recorded 55 species of Culicoides in Nigeria of which 44 have been taken at Vom. It was unlikely that more than a few species would be involved in transmitting BTV and attention was directed to those African species occurring at Vom on which suspicion had previously fallen. *C. imicola* Kieffer (syn. *C. pallidipennis*) was

implicated by Du Toit (1944) and Nevill (1971) while Walker & Davis (1971) added *C. milnei* to the list of vectors. Although not a proven vector *C. schultzei* is associated with domestic livestock (Walker, 1977*a*) and yielded an isolate of the bluetongue related Epizootic Haemorrhagic disease of Deer in Ibadan, southern Nigeria (Lee, 1979). Each of *imicola*, *milnei*, and *schultzei* may be placed with a few close relatives, distinguishable only with difficulty, into species groups. It was impractical to differentiate species on a project of this scale and accordingly these insects were enumerated in terms of species groups, details of which are discussed later; for convenience these species groups are referred to below simply as *imicola*, *milnei* or *schultzei*.

Finally, when information had accumulated to indicate the period of maximum virus activity, we attempted a limited number of virus isolations using calves as a source of viraemic blood; this work will be reported separately.

MATERIALS AND METHODS

Experimental animals

The NVRI dairy herd at Vom consisted largely of pure-bred Friesians together with a few pure-bred Fulanis and cross-bred animals. Calving was throughout the year in large loose-boxes. The calves remained with their mothers for the first three to 4 days of life, during which time they received colostrum. Thereafter, the mothers were returned to the milk herd and calves were bucket fed with normal milk. At about 3 weeks of age calves were moved to a rearing shed where they were individually penned and where they remained until 3 or 4 months old. They were then weaned and brought on in groups in partially covered yards. In both the rearing shed and in the yards a variety of *Culicoides* species had free access to the animals.

Calves were bled for serum during the first week of life and then at approximately monthly intervals until discarded from the experiment. Wherever possible, the mother was bled at the time the calf was first sampled.

Insect collection

Insects were attracted to a 125 watt mercury vapour lamp and impelled into a Kilner jar by means of a 9-inch Ventaxia fan. Within the jar insects were trapped in water that filled the bottom quarter and to which had been added a little household detergent to assist wetting. Collections were generally made every 3–5 days using a standard attracting period from 19.00 to 22.00 h at a domestic residence approximately 2 km from the cattle herd. Insects were fixed by the addition of formalin to give a final 5 % (v/v), after which excess fluid was discarded and the catches transferred to glass Universal bottles until sorted and counted.

Serological tests

Agar gel precipitation tests [AGPT] were performed in Petri dishes using mouse brain antigen (Taylor & McCausland, 1976) in the central well and a control positive serum in alternate peripheral wells. Although sera were usually tested individually shortly after collection, results were not assessed until all samples from a particular animal had been tested simultaneously. All sera were stored at −20 °C.

Microneutralization (VN) tests were undertaken with each BTV international serotype from 1 to 20.

Screening tests were carried out in tissue culture grade microplates with flat-bottomed wells using a master stock of serum diluted 1 in 10 in phosphate buffered saline containing 0·2% (v/v) bovine plasma albumin, fraction V (BA/PBS) and inactivated at 56 °C for 30 min. In a two well test a 0·1 ml volume of each virus serotype diluted in BA/PBS to contain $10^{3\cdot0}$ $TCID_{50}$/ml was added to an equal volume of a 1 in 10 dilution of serum. The mixtures were sealed and incubated for 1 h at 37 °C then overnight at 4 °C. The following morning BHK cells suspended in Glasgow modified Eagle's medium, supplemented with 10% tryptose phosphate broth and 2% adult ox serum, were added to each well in 0·05 ml volumes containing $2\cdot5 \times 10^4$ cells. Plates were resealed and incubated at 37 °C for a further 5 days. Microscopic readings were made at 3, 4 and 5 days. Serum dilutions were referred to the final virus-serum mixture and if one of the two wells was protected the titre was taken as 20; if both wells were protected the titre was > 30 and the sample was retested in a titration. Sera which did not contain virus neutralizing antibody at a dilution of 1 in 20 were regarded as negative.

Serum titrations were carried out using a doubling dilution series from 1 in 10 to 1 in 320 (1 in 20 to 1 in 640 final) using two wells per dilution.

All samples were coded and until all routine testing had been completed none of the operators knew the origin of any sample.

Blood meal identification tests

Latex agglutination and precipitation tests used to identify the source of blood meals for engorged female Culicoides have been described (Boreham, 1975; Boorman *et al.* 1977).

Rainfall

Rainfall data were obtained from a small meteorological station located at Vom.

RESULTS

Forty calves born between May and September 1975 were bled at approximately monthly intervals from their date of birth. Between October 1975 and February 1976 a further 38 calves were included as trial animals showed BTV seroconversion and required replacing; sampling ceased in June 1976.

Maternal antibody

No pre-colostral sera were obtained but 66 of 78 (85%) serum samples collected from calves within a few days of birth were positive by the AGPT. As BTV group-specific antibody was common in the adult animals of this herd, these antibodies were assumed to be colostral in origin. This was confirmed when it was found that they disappeared from the circulation of most calves within a few weeks of birth. Using data from 63 calves, the average period over which individuals remained AGPT-positive was 95 days, although there was considerable variation as may be judged by the standard deviation of 41 days. In 13 instances it was impossible to determine the final decline of maternal antibody as seroconversion

occurred (*vide infra*) even though the previous monthly sample still contained group-specific antibody.

Maternally conferred BTV neutralising antibody was less enduring than group-specific antibody. Taking a threshold titre of 20, the average period over which VN antibody could be detected was 56 ± 24 days. Half-lives were calculated in every instance where the initial serum titre exceeded 100. There did not appear to be any significant difference in decay rates between serotypes and an analysis of all results showed an average half-life of $22{\cdot}5 \pm 7{\cdot}9$ days.

Bluetongue virus infection

In a number of instances, AGPT examination of serial monthly samples from individual calves showed that they had undergone seroconversion. No clinical sign of bluetongue was ever seen so accurate determination of the time of infection was not possible. However, results from experimental bluetongue infections in cattle show VN and group antibody appearing between 12 and 14 days after infection (Jeggo, unpublished results). Thus detection of seroconversion early in a given month is actually indicative of an infection acquired the month before. To produce a working system it was decided to attribute seroconversions occurring before the 15th of the month to the previous month and those after the 15th to the month in question.

Usually infection did not occur until maternally derived VN and precipitating antibodies had vanished completely. Then, after a variable period, usually of 1 or 2 months but not exceeding 6 months, a sample would be found to have seroconverted by the AGPT and to contain high levels of VN antibody against a single BTV type. In such instances the animal was considered to have undergone infection with the BTV serotype inducing the high level of specific antibody. The

Table 1. *Bluetongue antibodies in serum of calf 1877 at various times after birth*

Neutralizing antibody to serotypes*	Age of calf (days)						
	25	32	42	59	97	134	229
1	110†	320	80	40	—	—	450
3	20	30	20	—	—	—	—
4	20	—	20	—	—	—	—
5	110	160	20	—	—	—	—
6	40	30	20	20	—	—	20
7	—	—	—	20	—	—	—
9	—	20	20	—	—	—	—
11	20	—	—	—	—	—	—
12	30	—	20	—	—	—	20
13	—	20	—	—	—	—	20
14	80	60	20	20	20	—	—
15	—	20	—	—	—	—	—
16	20	—	—	—	—	—	—
17	20	—	—	—	—	—	20
18	20	—	—	—	—	—	—
Precipitating antibody	+	+	+	+	+	−	+

* Serotypes against which no antibody was detected have been omitted.
† Reciprocal of 50% end point.

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Table 2. *Bluetongue antibodies in serum of calf 1813 at various times after birth*

Neutralizing antibody to serotypes*	Age of calf (days)							
	4	35	65	104	145	158	205	215
1	—	—	—	—	—	—	20†	20
2	—	—	—	—	—	—	160	220
4	—	20	—	—	—	—	—	30
7	—	—	—	—	80	450	30	30
10	—	—	—	—	—	—	—	40
18	20	—	—	—	—	30	—	—
20	—	—	—	—	—	30	30	60
Precipitating antibody	−	−	−	−	+	+	+	+

* Serotypes against which no antibody was detected have been omitted.
† Reciprocal of 50% end point.

results obtained with calf 1877 (Table 1) illustrate this sort of response. Maternally derived AGP and VN antibodies were present from day 25 to day 97 but not on day 134. By day 229 fresh precipitating antibodies had been formed and the calf had a VN_{50} titre of 450 against BTV type 1, which was considered to have been the infecting virus. The low levels of VN activity against types 6, 12, 13 and 17 were probably due to cross reactivity between these viruses and type 1 neutralizing antibody.

In several calves VN antibody appeared at high levels to a second BTV type some time after seroconversion to a first type had already occurred. In these cases the calf was considered to have had two separate infections, each of which could be timed from the month when specific VN antibody first arose. Calf 1813 (Table 2) lacked VN or precipitating antibody between days 4 and 104 but was infected with BTV type 7 around day 145 and type 2 around day 205. Again low levels of cross neutralizing antibody were recorded against types 18 and 20 following infection with type 7 and against types 1, 4 and 10 following infection with type 2. On its own the AGPT would have detected the infection only at day 145.

Finally, two calves were found with high levels of VN antibody to two different serotypes in the serum sample corresponding with the first appearance of precipitating antibody; these animals were considered to have undergone a dual infection during the preceding month.

Obviously, with time, calves would be exposed to further serotypes or re-exposed to ones they had already experienced. However, in this trial sampling ceased shortly after it was known that seroconversion had occurred and we were unable to follow the serological picture beyond the stages just described.

Serotype detected

Using the interpretations outlined above, it appeared that a number of BTV serotypes were infecting the calf herd at Vom. Seroconversions were seen that could be attributed to types 1–7 inclusive, together with types 11, 12, 13 and 20; Table 3 lists the number of times different serotypes were detected in each month.

Table 3. *Frequency of serotype activity throughout a 12-month period*

BTV serotype*	Jan.	Feb.	Mar.	Apr.	May	June		July	Aug.	Sept.	Oct.	Nov.	Dec.	Total no. of appearances
1	—	—	—	—	2	2†	1‡	—	—	3	1	—	1	10
2	—	—	1	—	—	—	1‡	—	—	3	1	1	—	7
3	—	—	—	—	1	—	—	—	—	1	1	2	—	5
4	—	—	1	—	1	—	—	—	—	—	3	2	—	7
5	—	—	—	—	1	—	—	—	—	—	1	—	—	2
6	—	—	—	—	—	—	—	—	1	1	1	—	—	3
7	—	—	—	—	—	1†	—	—	—	1	—	—	—	2
11	—	—	—	—	1	—	—	—	1	—	2	—	—	4
12	—	—	—	—	—	—	—	—	—	1	—	1	1	3
13	—	—	—	—	—	—	—	—	—	1	—	—	—	1
20	—	—	—	—	1	—	—	—	—	—	—	—	—	1
X	—	—	4	—	2	5†	—	—	—	2	—	—	—	13

* Serotypes never detected in calf sera have been omitted.

† These calves were not bled in May and might have been infected in May or June.

‡ These calves were not sampled in April or May and might have been infected in April, May or June.

The sera of 13 AGPT positive animals failed to neutralize types 1–20 and the frequency with which such reactions occurred suggested the presence of an unrecognized BTV serotype. Accordingly, these reactions were attributed to serotype X although it was recognized that X might be more than one virus type. Interestingly, the BTV isolation programme conducted at Vom has yielded a suite of at least six isolates that do not correspond to any of the 20 known serotypes (Herniman, unpublished results). Some serotypes occurred more frequently than others during the course of our observations (cf. types 1 and 13) or were found over a larger number of months (cf. types 2 and 20).

Seasonal occurrence of infections

One of the reasons for undertaking this work was to see if there was any seasonal variation in virus activity. It was felt that the somewhat harsh nature of the northern Nigerian climate, alternating between 6 months of dry season and 6 months of rains, might affect Culicoides populations and therefore the virus.

Table 4 shows the total number of bluetongue infections recorded each month together with rainfall and season and it is clear from this that there did appear to be fluctuations in activity. A large number of infections occurred in 1975 at the end of the wet season in September and October. The virus was still active in November but decreased in December and could not be detected at the height of the dry season in January and February. Infections were detected in March, prior to the real onset of rain, but not in April which is the first wet season month. Activity became prominent during May and June but appeared to diminish in July.

Relationship between antibodies in dam and calf

During the course of the trial group specific AGPT examinations were carried out on 44 pairs of sera from mothers and their colostrum fed calves aged less than 1 week. In 36 instances both mother and calf were positive, in six pairs both sera were negative while in two pairs the mother was negative but the calf was positive. In these last animals it is possible that calves received colostrum from another dam.

Many dams carried VN antibodies to BTV in their sera but three had high levels

Table 4. *Monthly incidence of bluetongue virus infections in relation to season*

Year	Month	Season	Rainfall (mm)	Fully susceptible calves each month	Bluetongue seroconversions
1975	August	Wet	374·8	12	2
	September	Wet	187·3	16	13
	October	Dry	6·8	17	10
	November	Dry	Nil	10	6
	December	Dry	Nil	9	2
1976	January	Dry	Nil	14	0
	February	Dry	Nil	24	0
	March	Dry	10·8	21	6
	April	Wet	182·8	28	0
	May	Wet	189·0	31	9
	June	Wet	186·5	21	8* 2†
	July	Wet	303·8	6	0

* May have been infected in May or June.
† May have been infected in April, May or June.

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Table 5. *Neutralizing antibody responses in dams and their colostrum fed calves*

Animal no.	Titre of VN antibody to BTV types															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Mother 0064	—	—	—	—	—	—	—	240	—	—	—	—	—	—	—	—
Calf 1821	—	—	—	—	—	—	—	160	—	—	—	20	—	—	—	—
Mother 3803	320	160	110	40	20	40	20	40	30	40	20	80	40	—	—	60
Calf 1814	160	30	160	160	—	20	—	20	—	30	—	80	—	—	30	30
Mother 0026	160	—	—	120	—	—	—	—	—	—	20	40	—	—	—	30
Calf 1863	20	> 640	40	450	20	20	—	20	20	20	450	220	20	—	20	20

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of VN antibody to a single serotype. These were animals that had presumably been infected by types 1, 4 and 8. Based on VN antibody titres of > 100 there was evidence that, as a group, the mothers had been infected by types 1, 2, 3, 4, 5, 8, 9, 10, 15 and 16, but as several contained high levels to two or more serotypes the possibility that some of these results may represent cross reactions cannot be ignored.

The type specific antibodies most frequently found in a mother's sera were also the commonest in the young calves. In several instances there was convincing evidence that a calf had acquired a passive immunity identical in range to that of its dam. At times this was associated with a monotypic antibody response (0064; Table 5) but in other instances several type specificities were individually conferred (3803; Table 5). A more common picture was partial correlation between the VN antibodies of the calf and the dam (0026; Table 5). This part of the investigation was completed before BTV types 17–20 had been acquired, consequently the results refer to types 1–16 only.

Culicoides behaviour

From 25th January until 31st December, 1975 a total of 23346 male and 50070 female Culicoides were taken: these included 4885 male and 14352 female *imicola* group; 1096 male and 3018 female *milnei* group and 1262 male and 2465 female *schultzei* group. Among other species *C. brucei*, *C. distinctipennis*, *C. exspectator*, *C. fulvithorax*, *C. leucostictus*, *C. neavei* and *C. similis* were the most common. Of these seven species the animal hosts of *brucei* are unknown; the other six are thought usually to feed on avian hosts and all were omitted from further consideration.

The seasonal distributions of *imicola*, *milnei* and *schultzei* were plotted as 20 day running arithmetic means (total catch over a 20 day period divided by the number of catches during that period). *C. milnei* did not appear in our catches until 17th April and was last taken on 12th December (Fig. 1). For this species-group the population was at a very low level until early in May when it began to rise slowly. There was a small peak in late June followed by a drop but after mid-July the population rose rapidly to a peak in late August. Thereafter the numbers declined sharply towards mid September, followed by a further small peak and decline through October, to a very low level in November and early December.

In contrast both *imicola* and *schultzei* were present throughout the year (Figs 2 and 3). Both showed two large population peaks in late August through September; in addition *schultzei* showed small peaks in April and June and a smaller and less obvious peak in early February while *imicola* also showed small peaks in February and June.

The flight pattern of these species was studied throughout a night in late October. For *C. milnei* peak activity occurred around or just before midnight with no activity after 05.00 h while the majority of *imicola* arrived at the light trap from just after dark until just after midnight with a peak at 20.00–21.00 h: scarcely any *imicola* arrived after 03.00 h. In *schultzei* there was a peak at 20.00–21.00 h but activity continued throughout the night falling off gradually towards dawn.

In September, 1977 a light trap was operated in the vicinity of the calf rearing yards and as expected, Culicoides were particularly numerous. Of a sample of 2017 individuals from a single all night catch there were 721 *imicola*, 1063 *milnei* and

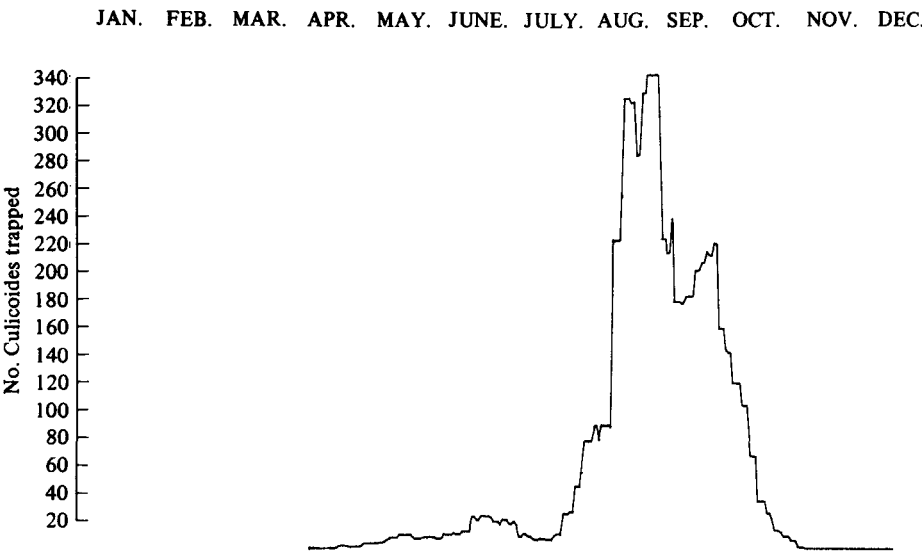

Fig. 1. Variation in population densities of *Culicoides milnei* caught at Vom, northern Nigeria over a 12-month period.

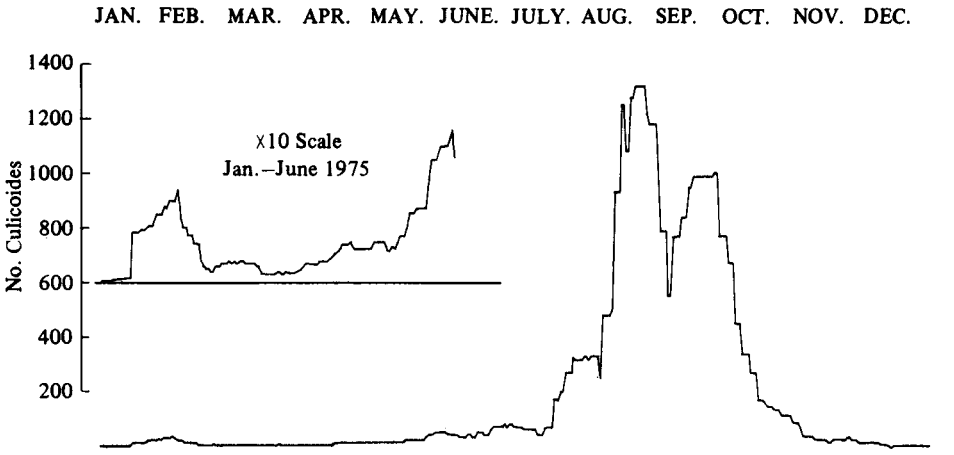

Fig. 2. Variation in population densities of *Culicoides imicola* caught at Vom, northern Nigeria over a 12-month period.

200 *schultzei*; other species accounted for only 33 specimens. Eight per cent of the *imicola*, 16% of the *milnei* and 2% of the *schultzei* had recently blood-fed.

The results of a host-meal identification tests on female *imicola*, *milnei* and *schultzei* collected over several nights during September and October, 1977 at different locations on the Vom farm are shown in Table 6 (P. Boreham, unpublished data). Both *imicola* and *milnei* showed a clear preference for cattle blood but this source provided only a minor part of the feeding requirements of *schultzei*.

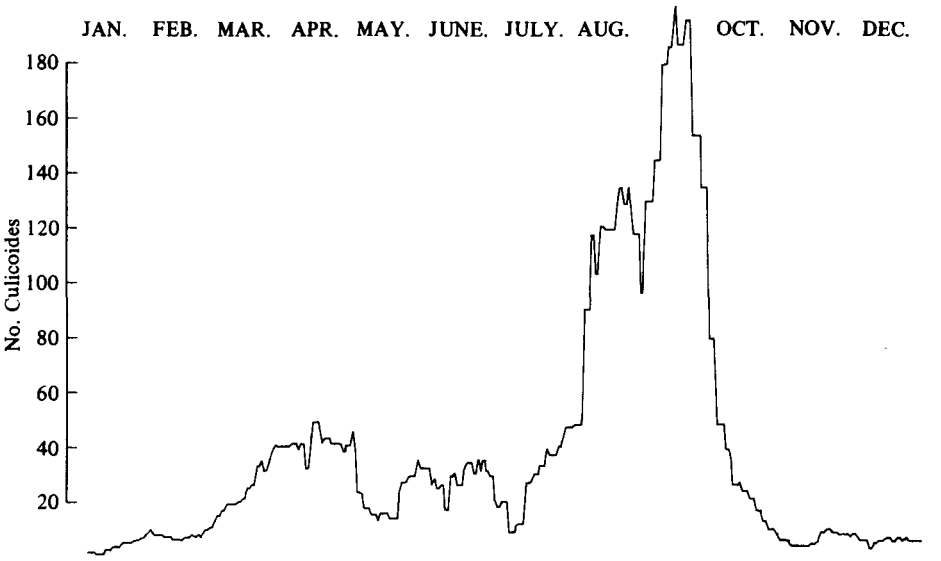

Fig. 3. Variation in population densities of *Culicoides schultzei* caught at Vom, northern Nigeria over a 12-month period.

Table 6. *Blood meal results for* Culicoides imicola, milnei *and* schultzei *caught at Vom*

Species	Sample size	Number feeding on				
		Ox	Pig	Mammal	Fowl	Other*
C. imicola	60	25	2	10	1	22
C. milnei	30	15	6	3	0	6
C. schultzei	26	5	3	4	0	14

* Blood-fed but source of meal not identified; includes tests for sheep and goats.

Females of *imicola* and *schultzei* (but not *milnei*) were graded as nulliparous or parous on the basis of the presence or absence of red abdominal pigment (Dyce, 1969) so that an analysis of survival rates could be obtained. Since our series of insect catches was discontinuous, the method of Birley & Boorman (1982) could not be used as this requires a continuous series of daily catches. However, the mean parous rate 'P' is a measure of survival over the period of a gonotrophic cycle assuming constant recruitment and constant survival rates (Macdonald, 1952). Daily survival rates (S) may then be computed from the expression $S = P^{1/n}$, where n is the length of the gonotrophic cycle.

For *imicola* the parous rate was low during January, February and March (Table 7) but rose abruptly in April. Apart from June and July high levels were then maintained until the end of the year. The figures for *schultzei* showed a broadly similar pattern except for January when only one individual was taken.

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Table 7. *Monthly parous rates for* C. imicola *and* C. schultzei *caught at Vom between January and December 1975*

	C. imicola		
Month	Nulliparous count, N	Parous count, P	Parous rate, P $P = P/(N+P)$
January	3	1	0·25
February	117	40	0·25
March	26	12	0·32
April	19	49	0·72
May	35	73	0·68
June	109	133	0·55
July	147	289	0·41
August	1798	4192	0·70
September	1505	2840	0·65
October	675	1927	0·74
November	72	192	0·73
December	44	54	0·55
Overall	4550	10172	0·69
	C. schultzei		
Month	Nulliparous count, N	Parous count, P	Parous rate, P $P = P/(N+P)$
January	0	1	1·0
February	17	12	0·63
March	37	53	0·59
April	98	132	0·57
May	82	41	0·33
June	97	55	0·36
July	66	42	0·39
August	142	257	0·64
September	248	637	0·69
October	90	280	0·76
November	20	21	0·51
December	20	21	0·51
Overall	917	1552	0·63

DISCUSSION

Epidemiological studies of BTV usually involve virus isolation either from viraemic blood or from infected midges. We would now suggest that the sentinel calf approach offers a valuable alternative method for undertaking detailed investigative studies. The AGPT can be used to deduce the frequency of virus infection at different times of the year, while the micro-neutralization test expands this basic information by identification of the serotypes responsible for individual infections. Perhaps the most surprising aspects of this study were the confidence with which a given seroconversion could be allocated to a specific virus type, and that among our group of animals it was also possible to identify more than one type specific infection in the same animal. This was unexpected as work with sheep has shown that cross-neutralizing antibodies may be stimulated following infection with two BTV types (Jeggo, unpublished results). It is perhaps worth emphasizing

that the two features necessary for sentinel work of this nature are regular bleedings, which at least allow seroconversions to be allocated to a given month, and the use of young animals which show clear cut type-specific neutralizing antibody responses. In our opinion, older cattle, which may have undergone repeated infections with a variety of serotypes and may well have produced cross-reacting antibody, cannot give a reliable indication of the types present in a given area, and certainly cannot contribute to a study on virus dynamics.

As a study method, the use of sentinel calves has advantages over the isolation of virus from insects or animals as a means of determining the presence and prevalence of different types in a given area. Culicoides trapping involves skilled staff to sort insects. To be effective this has to be done rapidly and requires the use of liquid nitrogen or freezer cabinets in which to store specimens prior to testing. Catches of insects vary with climatic conditions and certainly Vom was sparsely populated with Culicoides during the dry season, under which circumstances it is doubtful if many virus isolations would have been made. Perhaps one advantage in using the mammalian hosts as the detector system is that over a period of time they will focus the attention of large numbers of insects, probably more than could easily be caught and processed, so that even when Culicoides are scarce virus activity may still be monitored.

Virus isolation from viraemic blood can be readily accomplished but to be fully effective it requires frequent sampling, usually at intervals of not more than a few days. This causes considerable and often unacceptable disturbance of farm routines. Although it would be advantageous to collect sentinel serum more frequently, even at monthly intervals considerable detail can be accumulated with negligible interference.

Maternally conferred VN antibodies apparently only lasted about 56 days compared to precipitating antibody which lasted 95 days. While this result may have been due to different decay rates for the two types of antibody, it could also be a reflexion of the differing sensitivity of the tests involved, especially as neutralization titres below 20 were regarded as negative. While this working rule avoids false positive results due to non-specific inhibition, it also undoubtedly ignores low levels of specific activity. The neutralizing antibody half-life of 22·5 days is similar to the range of half-lives (12·5 and 14·3 days) that may be calculated for bovine maternal antibody, using the data of Luedke, Jochim & Jones (1977); similar figures are available for sheep (Richardson *et al.* in preparation).

The results of the light trapping showed that both *C. imicola* and *C. schultzei* were present and could have transmitted virus throughout the year. *C. milnei*, although abundant in the vicinity of cattle, was not present in our catches between January and March and only ever reached substantial levels between August and October.

In January and February, under severe dry season conditions, *imicola* were more numerous than *schultzei* and, although the reverse was true from March to April, after June *imicola* was the dominant species in all catches. At the population peak in August and September, *imicola* were four times more numerous than *milnei* and five or six times more numerous than *schultzei*. It was concluded that transmission of BTV could be attributed to *imicola* group midges alone although *schultzei* remained a strong alternative candidate and a role for *milnei* could not be

excluded. For either *imicola* or *schultzei* survival rates were such that, assuming a midge took a first blood meal 24 h after emergence, entered a gonotrophic cycle of 4 days but had a virus incubation period of 7 days, virus transmission could have taken place during any month of the year.

Although there are no estimates of the length of the gonotrophic cycle for *imicola* or *schultzei* in Nigeria, Walker (1977*b*) established a value of 4 days for both these species in Kenya. In fact, transmission was not demonstrated in either January or February when the parous rates for *imicola* were at their lowest and the daily survival rate over 4 days was 0·71. However, as virus isolation was not attempted, the evidence that any of these species is involved is entirely circumstantial and the possibility that other species may participate in transmission cannot be excluded.

In Vom *C. imicola* is a member of a group of closely related species which includes *C. brosseti* Vattier and Adam, *C. grahamii* Austen and *C. kibatiensis* Goetghebuer. *C. milnei* is similarly part of a group containing *C. hortensis* Khamala and Kettle, *C. krameri* Clastrier, *C. moreli* Clastrier and *C. murtalai* Boorman and Dipeolu. Finally, *C. schultzei* is represented by species A and B (sensu Boorman & Dipeolu, 1979). In the catches from which our population dynamics were determined, the proportions of *imicola: brosseti: grahamii: kibatiensis* were approximately 2:1:1:05; of *milnei*: others approximately 3:1 and of *schultzei* A:B about 1:1. These proportions remained much the same throughout the period of this study.

The relative importance of individual members of the *imicola* and other groups is unknown but it is clearly possible that differences in feeding habit or vector competence might differentiate between the species.

Virus activity was not recorded in either January or February, the two most arid months of the year, in spite of the presence of substantial numbers of susceptible calves and the continuous availability of insects. By contrast six infections were detected in March although this is essentially a dry month and seasonal change does not become obvious until April, a month in which virus was again undetectable; activity reappeared and maintained a high level in May and probably in June. These early wet season fluctuations in virus activity fail to correlate clearly with any substantial increase or decrease in the population of Culicoides. Both *imicola* and *schultzei* were present throughout this period and although some minor fluctuations were seen in the population levels of *schultzei* neither species showed any dramatic increase in numbers with the return of wet season conditions that could explain the upsurge in virus activity. In Kenya, Walker & Davis (1971) showed that a population of *imicola* (syn. *pallidipennis*) responded poorly to the onset of the main wet season and did not peak until 4 months after the end of the long rains. Interestingly, the only documented outbreak of clinical bluetongue in Nigeria in recent years was seen in sheep at the start of the rains (Bida, Njoku & Eid, 1975).

In July and August virus activity was less marked. It is difficult to know if this lull is real, or an artifact caused by the low number of susceptible calves available. Insect numbers were not markedly lower than in the preceding 2 months although survival rates for both *imicola* and *schultzei* were relatively low during July.

A second peak of virus activity occurred in September, October and November.

Each of the potential vector species examined reached an annual population peak in early to mid September followed by a rapid decline through October to reach dry season levels by early November and at this stage of the year the association between insect numbers and virus activity appears convincing. Some further decline in activity was seen in December followed, as has been seen, by a quiescent period in January and February.

Flying insects migrate to achieve dispersal and colonization (Dingle, 1972) by means of persistent, undistracted flights which are aided but not dependent on wind displacement. Apart from large species such as butterflies many small insects are also involved and detailed studies by Duviard (1977) and Magor & Rosenberg (1980) in West Africa have shown migratory activity by cotton stainer bugs (*Dysdercus* spp.) and by the blackfly *Simulium damnosum* (*Diptera: Simulidae*). The movement of these two species is greatly influenced by the wind patterns following the Inter Tropical Convergence Zone (ITCZ). This zone occurs where the dry north-east trade winds from the Sahara meet the moist south-west monsoon winds from the Atlantic. Between February and July the ITCZ moves northwards across West Africa from latitude 7° N to 22° N; a similar southern movement occurs between September and December. Broadly speaking, the monsoon winds following behind the northern passage of the ITCZ promote vegetation growth, insect multiplication and insect dispersal. Although there is no direct evidence for the migration of Culicoides in West Africa, Sellers (1980) has reviewed the considerable volume of circumstantial data that link the spread of vector transmitted viral disease with wind and suggest that migration does occur.

In spite of the obvious lack of detail it is worth considering the general outlines of the present results against the possibility that Culicoides migration influences the distribution of BTV. The peaks of infection in May and September could be coincident with the arrival of infected migratory insects carried on winds associated with movements of the ITCZ somewhat south of the surface of separation (see Duviard, 1977). The earlier peak in March could be due to arrival on other winds (Magor & Rosenberg, 1980) or due to a species migrating at a different time (Duviard, 1977). Other factors may also be involved such as the decline in insect numbers due to the decrease in breeding sites at the end of the rainy season, which could affect the reduction in infection late in the year. However, it is difficult to explain the apparent lack of infection in the middle of the rainy season around July.

The present study greatly increases the number of serotypes of BTV believed to be present in Nigeria although confirmed isolations are restricted to types 6, 7, 10 and 16 (Lee, Causey & Moore 1974). Calves at Vom showed serological evidence for the presence of serotypes 1 to 7 inclusive, 11, 12, 13 and 20 together with at least one as yet unidentified virus type. In contrast the mothers of these calves, which were each bled once shortly after calving, showed no evidence of having been infected with types 6, 7, 11, 13 or 20 but did appear to have been infected with types 8, 9, 10, 15 and 16. Possibly there is variation in the spectrum of serotypes present in different years as might be expected if the chance of infection depended on the arrival of migratory insects. In such a way then, BTV survival may depend on migration with its insect vector to exploit groups of susceptible host animals such as may accumulate in different parts of the country at different times of the

year but detailed long term studies will be necessary before virus survival can finally be linked to climatic change.

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