Epidem. Inf. (1987), 99, 533–545
Printed in Great Britain

Infection with bluetongue and related orbiviruses in the Sudan detected by the study of sentinel calf herds

By M. ELFATIH, H. MOHAMMED* and W. P. TAYLOR†
Animal Virus Research Institute, Pirbright, Woking, Surrey GU24 0NF, UK.

(Accepted 11 February 1987)

SUMMARY

Using two sentinel calf herds, the seasonal incidence of bluetongue in central Sudan was shown to be a predictable event related to the rainy season and to vector population. Seroconversions to bluetongue virus (BTV) were detected only between July and December. Neutralizing antibodies to 16 BTV serotypes (1–10, 12, 14, 17, 20–22) were present in animals from both sentinel herds. The use of a blocking enzyme-linked immunosorbent assay made possible the distinction between infections due to BTV and those due to infections with other orbiviruses. Sera from 13 calves at Shambat and from 17 at Um Benein gave agar-gel tests indicative of BTV infections not attributable to any of the 22 serotypes, suggesting the presence of new serotypes of BTV. Sera from 30 calves gave results interpreted as evidence of infection with BTV-related viruses, rather than BTV serotypes. Comparing these results with those reported from Kenya, we suggest that the pattern of the disease and the types involved are similar in both countries and that the movements of the Intertropical Convergence Zone (I.T.C.Z.) may carry infected midges northwards into the Sudan at the start of the rainy season and southward at the end of this period.

INTRODUCTION

Bluetongue (BT) is an insect-transmitted viral disease of ruminants with a world-wide distribution caused by bluetongue virus (BTV), the type species of the genus orbivirus (family Reoviridae). The genus is subdivided into 12 subgroups, each containing a variable number of serotypes (22 in the case of BTV) (Matthews, 1982). Low levels of cross-relatedness have been reported between BTV and viruses in other subgroups within the Orbivirus genus.

In the Sudan the disease was first reported in 1953 in sheep in the central Sudan, and material sent to the Veterinary Research Institute at Onderstepoort was shown to contain BTV (Anon., 1953). Unconfirmed BT was suspected in sheep at Khartoum University farm based on clinical symptoms and general epizootiology,

* Permanent address: Department of Preventive Medicine and Veterinary Public Health, Faculty of Veterinary Science, University of Khartoum, Khartoum North, P.O. Box 32, Sudan.

† Present address: F.A.O. P.O. Box 30470, Nairobi, Kenya.

Address for correspondence: Dr B. W. J. Mahy, The Animal Virus Research Institute, Pirbright, Woking, Surrey GU24 0NF.

but attempted virus isolations were unsuccessful (Pillai, 1961). Although the breed of sheep was not specified, we suspect that they were indigenous animals. Eisa *et al.* (1980) reported another outbreak of bluetongue involving indigenous sheep from western Sudan and were able to isolate the virus. The authors concluded that the stress to which these animals had been exposed enhanced the severity of the disease. More recently, Abu Elzein & Tag Eldin (1985) reported a BT outbreak in indigenous lambs 3–6 months old in Khartoum province in 1982. They isolated the virus and were able to reproduce the disease experimentally. To the best of our knowledge, the type specificity of none of these isolates is known.

Bluetongue group-specific antibodies were detected in field sera (Eisa, Karrar & Abdel Rahim, 1979; Eisa *et al.* 1983) throughout Sudan, suggesting a widespread virus distribution. The aim of the present study was to gain an insight into the epidemiology of bluetongue in the Sudan and, through the use of sentinel calf herds at Shambat and Um Benein, to investigate the seasonal incidence of virus activity during a 2-year study period.

MATERIALS AND METHODS

Sentinel herds. Between October 1980 and September 1983 sentinel calf herds were established at the Khartoum University farm at Shambat, Khartoum North (15° 40′ N, 32° 32′ E) and at Um Benein Animal Research Station (13° 15′ N, 34° E) some 250 miles south of Khartoum on the Blue Nile. Calves were bled for serum during the first month of life and at approximately monthly intervals thereafter until excluded from the experiment; where possible, dams were also bled when their calves were first sampled. All calves born during the period of the study were included in the trial, giving totals of 67 calves from Shambat and 94 from Um Benein. Most calves in Um Benein were sampled for 1 year, while in Shambat the majority of calves were sampled for almost 2 years and a few animals for longer.

Serological tests. Sera were examined for the presence of BTV group-specific antibodies, using either an agar gel immunodiffusion (AGID) test or a blocking enzyme-linked immunosorbent assay (ELISA) test.

The AGID test was conducted on microscope slides coated with 2 ml of 1% Litex agarose in borate buffer at pH 9·2. Soluble antigen (Lefevre & Taylor, 1983) was added to the central well, and alternating peripheral wells received test and positive-control sera respectively. Samples were also tested against negative-control antigen prepared from uninfected BHK cells. The slides were examined daily for 3 days and the final reactions recorded as follows.

Negative (−). No deflection of the control precipitation reactions in the area adjacent to the test well.

A one-plus hook (*h*+). A very slight deflection of the control reaction in the direction of the test well.

A two-plus hook (*h*++). A moderate to strong deflection of the control reaction in the direction of the test well.

A three-plus hook (*h*+++). A reaction in which the inwardly deflected reactions unite immediately in front of the test well.

Positive (+). A clear-cut precipitation line midway between the test serum well and the central antigen well.

https://doi.org/10.1017/S0950268800068035 Published online by Cambridge University Press

The blocking ELISA was performed according to the method of Anderson (1984) with antigen prepared by the method of Manning & Chen (1980) from cultures of BHK cells infected with BTV type 1. Using a BTV type 1 mouse monoclonal antibody designated 3-17-3A (Anderson, 1984), optimum dilutions of both antigen and monoclonal antibody were determined by twofold chessboard titrations in an indirect ELISA test. Thereafter, antigen was coated to the solid phase at the optimal dilution and test sera were examined for their ability to block the pre-titrated reaction between the antigen and the monoclonal antibody. Optical density (OD) values were read in a multichannel spectrophotometer at a wavelength of 492 nm and results expressed as the percentage inhibition calculated from the formula:

$$100-[(\text{OD in presence of test serum}/\text{OD in absence of test serum})\times 100]$$

Selected serum samples were examined for type-specific neutralizing antibodies in a serum-virus neutralization (SVN) test conducted in microplates. Samples previously inactivated at 56 °C for 30 min were screened at a final dilution of 1/20 and 1/40 against bluetongue serotypes 1–22, using the method of Herniman, Boorman & Taylor (1983); each dilution was tested in duplicate. Sera which contained antibodies to any particular serotype at a dilution greater than 1/40 were titrated using doubling dilutions from 1/20 to 1/640. The SN_{50} titre was expressed as the final dilution of serum able to protect 50% of the indicator cells against 100 $TCID_{50}$ of virus.

RESULTS

Group-specific antibodies. Sera were examined for BTV group-specific antibodies in a system that simultaneously looked at all the monthly sera from each individual calf. Antibodies present immediately after birth were interpreted as being maternal, and the acquisition of group-specific antibodies following an antibody-free state as seroconversion (Herniman, Boorman & Taylor, 1983). Criteria for group-specific antibodies were a positive AGID result, with a minimum of a two-plus hook (a one-plus hook was not considered positive) and/or an inhibition of 50% or more in the ELISA.

Sera from 63 dams from Shambat and 48 dams from Um Benein were found to contain bluetongue group-specific antibodies. In the ELISA test maternal antibodies were detected in all calves examined, with the exception of one calf in Shambat and three in Um Benein, while the AGID failed to detect maternal antibodies in 19/161 (11·8%) of the total number of calves examined. Waning maternal antibodies were detected by the AGID test for up to 3 months after the first sampling and for up to 6 months in some cases by the ELISA test.

In both 1981 and 1982 in Shambat and Um Benein, seroconversions were shown to be seasonal, occurring only between July and December with a peak incidence in September, October and November. In a number of calves, mostly from Shambat, AGID reactions following seroconversion were inconsistent and waned within 1 or 2 months of onset. In other calves, seroconversions were followed by either a series of weak positive reactions or alternating positive–negative reactions before becoming completely negative. Table 1 shows the results of both AGID and ELISA tests with sequential sera from animal NY 78. Two distinct seroconversions were detected by AGID, one in August 1981 and the other in October

Table 1. AGID and ELISA results on sera from calf NY 78

Date of sample	AGID	ELISA	Remarks
October 1980	+	84*	Maternal antibodies
December 1980	+	78	
March 1981	−	62	
June 1981	−	28	
August 1981	h++	25	Infection with BT-related virus
September 1981	h+++	29	
October 1981	+	30	
November 1981	+	27	
January 1982	+	24	
March 1982	h+	16	
April 1982	−	21	
September 1982	−	10	
October 1982	+	63	Infection with BTV
November 1982	+	78	
February 1983	+	83	
June 1983	+	75	

* Percentage inhibition of group-specific monoclonal antibody 3-17-A3.

1982, yet sera collected between April and September 1982 gave negative reactions. The ELISA detected only one seroconversion corresponding to that of October 1982 and all the positive AGID samples from the first seroconversion were negative by this test. From these results we have concluded that this animal was infected with a bluetongue-related virus in August 1981 and with bluetongue itself in October the following year. Analysing all AGID results in this way we have been able to distinguish between bluetongue and bluetongue-related infections with the results shown in Table 2 for Shambat in 1981 and 1982. Eleven of 31 calves positive in the AGID test in 1981 and 11 out of 34 reacting in 1982 were considered to have been infected with a bluetongue-related virus. At Um Benein in 1982, 8 of 28 calves (28·6%) were considered to have developed reactions to BTV-related viruses but none was detected in 1981. In both years, in both locations, BTV seroconversions were distinctly seasonal, occurring only between July and December; in all other months sentinel calves failed to show any evidence of BTV activity. Similarly, BTV-related virus activity was recorded only between August and November.

Khartoum enjoys a prolonged dry season extending from September of one year to June or July the following year. During this period the prevailing winds are dry and from the north. The onset and duration of the rainly season is governed by the annual north–south movements of the Intertropical Convergence Zone (I.T.C.Z.), marking the boundary of the dry continental air mass conveyed by the northeast trades of the northern hemisphere and the relatively cool and moist air mass associated with the south-east trades of the southern hemisphere. Figs 1 and 2 attempt to correlate wind direction, rainfall and BTV activity at Shambat and Um Benein. The northerly movement of the I.T.C.Z. across Shambat is marked by a change in wind direction from north to south and the onset of precipitation. At the same time a 4- to 6-month period of BTV activity commences. The I.T.C.Z.

Table 2. *Seasonal activity of bluetongue and bluetongue-related viruses at Shambat in 1981 and 1982*

Year	Month	AGID	ELISA	
			BTV	BTV-related
1981	July	0/29 (0·0)*	0/29 (0·0)	0/29 (0·0)
	August	6/33 (18·0)	4/33 (12·1)	2/33 (6·1)
	September	16/29 (55·0)	12/29 (41·4)	4/29 (13·8)
	October	5/14 (35·7)	3/14 (21·1)	2/14 (14·3)
	November	4/9 (44·4)	1/9 (11·0)	3/9 (33·3)
	December	0/11 (0·0)	0/11 (0·0)	0/11 (0·0)
1982	June	0/26 (0·0)	0/26 (0·0)	0/26 (0·0)
	July	1/31 (3·2)	1/31 (3·2)	0/31 (0·0)
	August	8/38 (21·0)	6/38 (15·8)	2/38 (5·3)
	September	10/32 (31·3)	4/32 (12·5)	6/32 (18·6)
	October	10/21 (47·6)	8/21 (38·1)	2/21 (9·5)
	November	4/12 (33·3)	3/12 (25·0)	1/12 (8·3)
	December	1/9 (11·3)	1/9 (11·1)	0/9 (0·0)

* Numerator, number of seroconversions. Denominator, number of BTV-susceptible calves present during that month. Figure in parentheses gives percentage of susceptible calves infected.

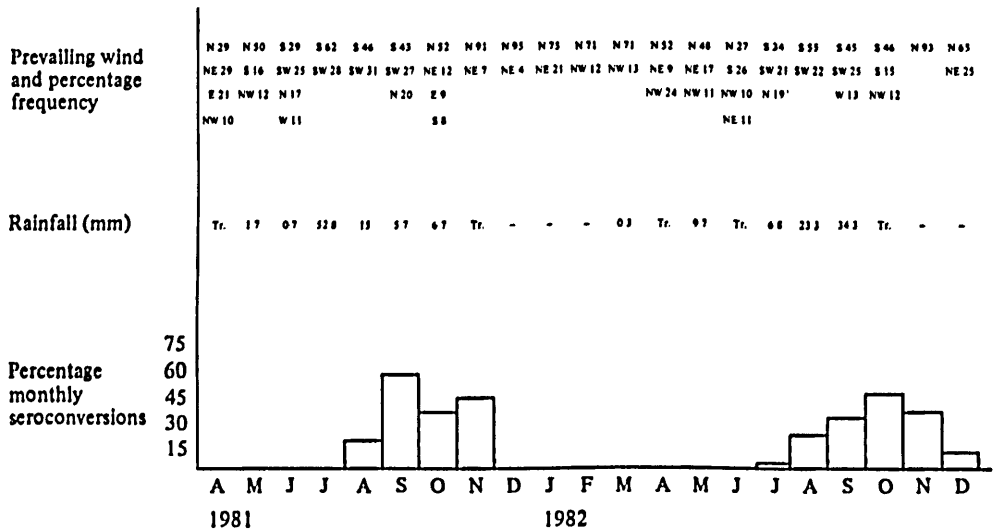

Fig. 1. Correlation of rainfall, wind direction and bluetongue activity at Shambat in 1981 and 1982.

moves back to the south of Khartoum in late September and a return of the dry northerly winds is seen. This does not signal an immediate cessation of BTV activity but instead the number of seroconversions declines in a somewhat erratic manner, dying out eventually in November or December. Similar correlations between wind direction and viral activity were seen at Um Benein.

Bluetongue type-specific antibodies. Serum samples from all infected animals were tested in the SVN test. Wherever possible, these included the last serum

https://doi.org/10.1017/S0950268800068035 Published online by Cambridge University Press

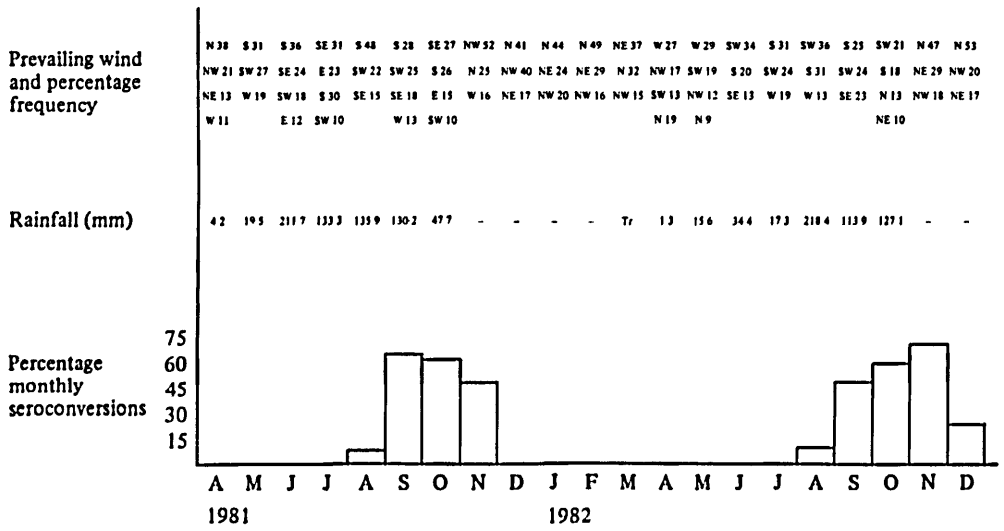

Fig. 2. Correlation of rainfall, wind direction and bluetongue activity at Um Benein in 1981 and 1982. (Wind and rainfall observations were recorded at Abu Na'ama Agrometeorological Station (12° 44′ N, 34° 07′ E).)

sample collected before seroconversion and the first sample collected after seroconversion. Usually another sample collected several months later was also included, and additional sera were examined if the animal remained in the experiment for a further year. The type specificity of the infection was considered to have been established if neutralizing antibodies to a single virus type appeared in the first post-conversion sample and endured through subsequent samplings.

A number of animals from both sentinel herds had neutralizing antibodies to a single type from the range 1–5, 7–9, 12 and 14. Other animals had antibodies to more than one virus type, and the results of four such animals are shown in Table 3. Animal NX 54 developed neutralizing antibodies to type 5 and 9 commencing at the same time and to almost the same levels, which was interpreted as evidence of a dual infection by both serotypes. Animal NY 55 had substantial levels of neutralizing antibodies against types 4 and 17 in November although no neutralizing antibody was detected in the serum of October 1981, possibly because any antibodies present were below the threshold of sensitivity in our test. The results were interpreted in the same way as for NX 54. Animal NY 80 seroconverted to type 7 in October 1982 and to types 4 and 20 in November. From the low titres observed for type 20 and from the close serological relationship reported between types 4 and 20 (Della-Porta, Herniman & Sellers, 1981) we have assumed that this animal was infected with serotype 7 in October 1982 followed by type 4 only the following month. Interestingly, the first two samples of August and September 1982 which were positive on AGID and negative on ELISA did not neutralize any of the 22 serotypes tested, including 4, 7 and 20, and the antibodies were possibly due to exposure to a bluetongue-related virus during that period. Similarly, animal NY 95 may have been infected with a bluetongue-related virus in September 1981, with type 14 in November and during the following month with types 3 and 21. This animal was also infected with type 12 in September 1982

https://doi.org/10.1017/S0950268800068035 Published online by Cambridge University Press

Table 3. *Animals with neutralizing antibodies to more than one virus serotype*

Animal no.	Date bled	AGID/ELISA	SN_{50} titre to types detected									
			3	4	5	7	9	12	14	17	20	21
NX 54	21 Sept. 81	−	—	—	—	—	—	—	—	—	—	—
	1 Oct. 81	+	—	—	120	—	160	—	—	—	—	—
	29 Oct. 81	+	—	—	80	—	160	—	—	—	—	—
	3 Dec. 81	+	—	—	60	—	80	—	—	—	—	—
NY 55	6 Sept. 81	−	—	—	—	—	—	—	—	—	—	—
	4 Oct. 81	+	—	—	—	—	—	—	—	—	—	—
	3 Nov. 81	+	—	180	—	—	—	—	—	80	—	—
	7 Dec. 81	+	—	80	—	—	—	—	—	80	—	—
NY 80	8 July 82	−	—	—	—	—	—	—	—	—	—	—
	8 Aug. 82	+*	—	—	—	—	—	—	—	—	—	—
	5 Sept. 82	+*	—	—	—	—	—	—	—	—	—	—
	20 Oct. 82	+	—	—	—	640	—	—	—	—	—	—
	14 Nov. 82	+	—	240	—	640	—	—	—	—	20	—
	19 Dec. 82	+	—	160	—	480	—	—	—	—	30	—
	19 Jan. 83	+	—	240	—	320	—	—	—	—	20	—
	13 Jun. 83	+	—	120	—	160	—	—	—	—	40	—
NY 95	8 Aug. 81	−	—	—	—	—	—	—	—	—	—	—
	6 Sept. 81	+*	—	—	—	—	—	—	—	—	—	—
	4 Oct. 81	+*	—	—	—	—	—	—	—	—	—	—
	3 Nov. 81	+	—	—	—	—	—	—	320	—	—	—
	7 Dec. 81	+	60	—	—	—	—	—	640	—	—	80
	1 Apr. 82	+	30	—	—	—	—	—	480	—	—	80
	5 Sept. 82	+	60	—	—	—	—	80	480	—	—	60
	19 Dec. 82	+	60	—	—	—	—	240	160	—	—	80
	19 July 83	+	80	—	—	—	—	120	240	—	—	160

* Positive by AGID only.

and thereafter remained positive to the four bluetongue virus types until excluded in September 1983. A broadly cross-reactive antibody response was not seen.

Results of bluetongue SVN tests and the type-specific antibodies detected in both sentinel herds are shown in Table 4. We have detected neutralizing antibodies to 15 virus types in Shambat. In 1981 neutralizing antibodies to types 1, 3, 4, 5, 6, 8, 9, 14, 17 and 21 were detected, while in 1982 neutralizing antibodies were to the same types, except for types 6 and 9 but with the addition of types 2, 7, 12, 20 and 22.

A similar pattern was observed in Um Benein. With the exception of type 6 and the addition of type 10, the neutralizing antibodies present were found to be directed to the same group of virus types previously detected in Shambat. In all, antibodies to 16 virus types were detected across the two sentinel groups, with a high incidence to types 1, 3, 4, 8 and 17. Sera from 13 calves from Shambat and 17 calves from Um Benein which were positive for bluetongue group-specific antibodies by both AGID and ELISA tests failed to neutralize any of the 22 virus types tested. This suggests the presence of one or more new bluetongue serotypes in these sentinel herds.

From the monthly seroconversions detected by neutralization in the sera from calves at Shambat (Table 5) it is evident that infections with any particular serotype were spread over the full bluetongue season.

Sera from most calves regarded as infected with bluetongue-related viruses did not neutralize any of the 22 bluetongue virus serotypes with the exception of two calves from Shambat and four from Um Benein. Three of the four calves from Um Benein had neutralizing antibodies to serotype 3 and the fourth calf was found to contain antibodies to serotype 7. On the other hand, one of the two calves from Shambat had neutralizing antibodies to serotype 3 and the other one had neutralizing antibodies to serotype 6. SN_{50} titres for these virus serotypes were in a range from 1/20 to 1/80. From this we have concluded that low levels of neutralizing activity may occasionally follow infection with a virus not specifically in the BTV subgroup.

DISCUSSION

Various tests have been employed by several authors to study the degree of relatedness between different orbivirus subgroups. Moore & Lee (1972) used the complement fixation test to demonstrate a relationship between the bluetongue and epizootic haemorrhagic disease (EHD) subgroups, and Borden, Shope & Murphy (1971) used the same test to link together the BT, EHD and Eubenangee subgroups; similar cross-relationships may be seen in the AGID test (Della-Porta, Parsonson & McPhee, 1985; Taylor & Gumm, 1985). Clearly, therefore, these cross-relationships can cause interpretation problems in field surveys in areas where members of several different subgroups are active at the same time (Della-Porta *et al.* 1983). In view of the reported isolation of both bluetongue and EHD from Sudan (Eisa *et al.* 1980; Mellor, Osborne & Jennings, 1984) and the presence of Pata, a Eubenangee subgroup member in the neighbouring Central African Republic (Karabatsos, 1985), we were not surprised when preliminary AGID tests showed that some animals were positive by this test for only a few months before

Table 4. *Number of animals positive against each BTV serotype*

Year	Location	BTV																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1981	Shambat	1	—	3	2	1	1	—	4	3	—	—	—	—	2	—	—	3	—	—	—	2	—
	Um Benein	5	1	11	9	3	—	—	5	3	2	—	2	—	1	—	—	4	—	—	—	1	3
1982	Shambat	2	1	7	3	1	—	3	4	—	—	—	2	—	1	—	—	3	—	—	2	2	1
	Um Benein	5	1	8	4	—	—	1	3	1	—	—	1	—	1	—	—	—	—	—	1	2	—
1983	Shambat	—	—	1	—	—																	
	Um Benein	—	1	—	—	1																	

Table 5. *Monthly distribution of bluetongue-seropositive animals – Shambat*

Month	Year	BTV types																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Aug.	1981	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—
Sept.	1981	—	—	1	1	—	—	—	2	2	—	—	—	—	—	—	—	2	—	—	—	—	—
Oct.	1981	1	—	1	1	—	—	—	1	—	—	—	—	—	—	—	—	1	—	—	—	—	—
Nov.	1981	—	—	—	—	—	—	—	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—
Dec.	1981	—	—	1	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—
Jan.	1982	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Aug.	1982	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	—	1
Sept.	1982	—	1	4	—	—	—	—	1	—	—	—	2	—	—	—	—	—	—	—	—	1	—
Oct.	1982	1	—	—	—	—	—	2	3	—	—	—	—	—	1	—	—	2	—	—	1	—	—
Nov.	1982	1	—	2	2	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
Dec.	1982	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Jan.	1983	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

https://doi.org/10.1017/S0950268800068035 Published online by Cambridge University Press

the antibodies faded to leave a seronegative animal. Believing that bluetongue confers an enduring AGID response (Taylor, Herniman & Owen, unpublished results), we took these reactions to be evidence for the presence of a related virus or viruses. Anderson (1984) developed an ELISA test that apparently detects only antibodies against BTV subgroup viruses, thereby suggesting a method of differentiating bluetongue serological responses from those of other subgroup viruses. Clearly, the ELISA test on its own would provide a record of bluetongue activity in a group of animals, but by using the less specific AGID test in parallel and by examining serial samples from groups of sentinel calves we were able to draw the additional distinction between the enduring AGID responses to BT and the transient AGID responses to cross-related viruses. Nonetheless, in our system, the presence of an AGID response to a cross-related virus is masked if a simultaneous BT infection occurs and a full investigation of these agents requires considerable additional testing; this work is in hand and will be reported later.

There has been much discussion on the reliance that can be placed on the results of bluetongue neutralization tests as a means of deducing the BTV types present in an area. Campbell (1985) pointed out the possibility that a sequence of infections with different virus types could produce a broadly cross-reactive antibody response from which little meaning could be extracted. On the other hand Taylor *et al.* (1985) argued that in a number of specific instances, including the use of sentinel calves, credible results could be obtained. From the results of the present work we would again argue that sera collected before and after seroconversion can clearly point to the virus type involved. Further, in a number of individual bovines we were unable to detect a broad spectrum of antibody response following simultaneous infection with two different viruses or even following a sequence of infections covering two different years. Using the present approach we were able to build up a comprehensive picture of the virus types that were active in each of the sentinel groups studied and to show that, of the large number of types present, most persisted throughout the entire period of virus activity in both areas.

Between the two sentinel groups we detected antibodies to 16 different BTV types. During the same period the only viruses isolated were BTV 5 (Mellor, Osborne & Jennings, 1984), and BTV 22 (Herniman, unpublished result of the original isolation). However, as the antibody response was monospecific to 10 of the viruses we are confident that our results reflect the discovery of a bluetongue epizootic in both herds made up from a large number of virus types. The fact that several BTV-positive sera in both herds failed to neutralize any of the 22 serotypes examined suggests the possibility of further new types of BTV circulating in Sudan.

Sellers (1984) reported preliminary results from the present study and indicated that seroconversion of calves at Khartoum and Um Benein occurred between July and October. While still strictly seasonal, our present more extended results clearly show that activity can stretch from July to December. Enhanced breeding of the vector species of culicoides (*C. imicola* and possibly *C. schultzei*) occurs in the rainy season (Mellor, personal communication), the duration of which is largely governed by the movement of the I.T.C.Z. The northerly limit of the I.T.C.Z. is between 18 and 20° N, and this point is reached and held in July and August; in September it retreats southwards. Wind directions are also governed by the

position of the zone, and for as long as it lies south of Um Benein or Khartoum dry northerly winds prevail. However, moist southerly winds occur as soon as the I.T.C.Z. passes north of these points, and there is a clear correlation between this alteration in wind direction and the onset of the bluetongue season. While the reverse situation is also found, the resumption of northerly winds does not immediately terminate the bluetongue season and a slow tailing off is seen. This presumably reflects a gradual decline in the insect population as the number of breeding sites diminishes. In the ensuing dry months no bluetongue sero-conversions were detected, and our conclusion is that the onset of virus activity is due to the windborne dispersal of infected culicoides from areas of Sudan to the south of Um Benein and Khartoum. Vector species of culicoides are on the wing throughout the year in the Khartoum area, but their numbers are so low during the dry season that they are presumably insufficient to maintain the cycle of bluetongue transmission (Mellor, personal communication).

In Kenya there is little evidence of BTV seasonality, and Walker & Davies (1971) describe the presence of disease outbreaks in all months of the year except January. The virus isolates that have been typed in Kenya are BTV1, 2, 3, 4, 8 and 12, while additional untyped viruses are known to occur (Davies & Blackburn, 1971). It is interesting to speculate that this truly endemic area acts as a partial source of infected insects for countries lying to the north. During the wet season components of the surface winds in the south and central parts of Sudan are generated over either central or eastern Africa (Fig. 3) and could carry infected culicoides from the Central African Republic or Kenya. Although data are lacking for the former country, it may be noted that in Kenya four of the six typed viruses were the commonest infecting serotypes at Shambat and Um Benein. While it is unclear to what extent and over what distances culicoides midges can migrate, it is apparent that some movement does occur or there would be no bluetongue season in non-endemic countries and, indeed, it is known that some culicoides species can move with the wind over distances greater than 200 miles (Hayashi *et al.* 1979). It is also difficult to be certain if a return migration accompanies the southward retreat of the I.T.C.Z., but plainly this would have limited value in terms of virus survival as most animal populations to the south would be immune to the viruses returning from the north.

We wish to thank Mr J. J. McGrane and Mr S. Kenyon of the Overseas Development Administration for their prodigious efforts in maintaining the sentinel herds used in these studies. The same organization provided a grant which was responsible for initiating the project. We would also like to thank Professor Dr Babiker El Hag Ali of the Central Veterinary Laboratory, Soba, Khartoum for his support and encouragement throughout the collecting period, Dr J. Anderson for kindly supplying monoclonal antibody and Mr C. Timms for preparing the figures. All meteorological data was kindly supplied by Mr B. D. Castle, Meteorological Office, Bracknell, Berkshire, RG12 2SZ. The senior author is in receipt of a study grant from the Sudanese Government.

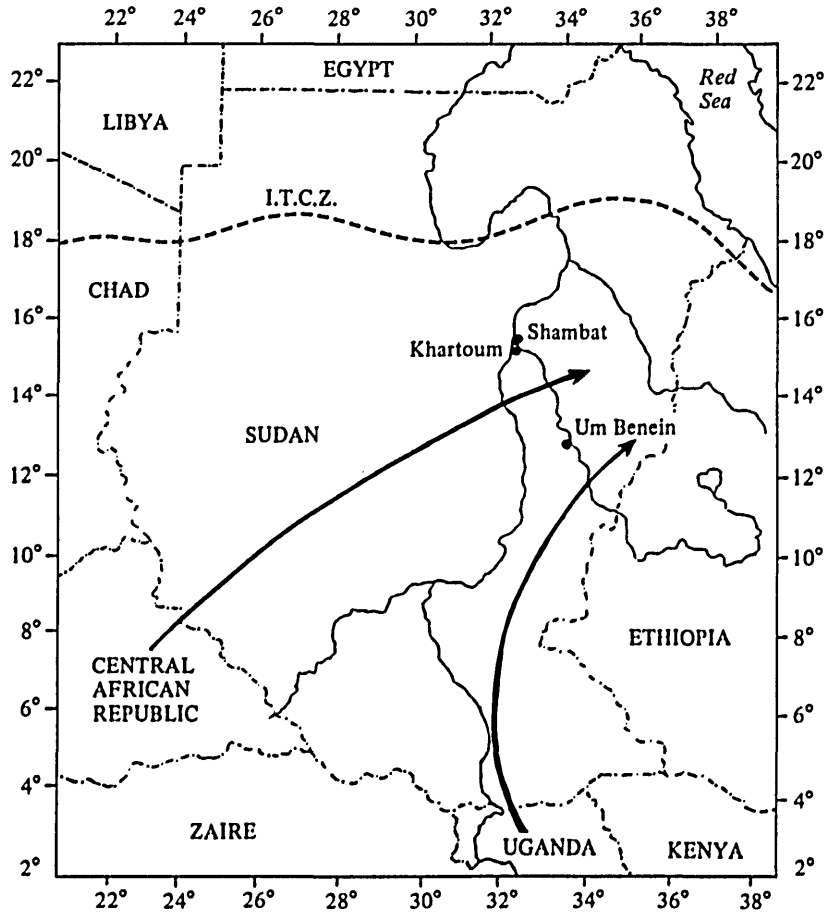

Fig. 3. Direction of surface winds and position of I.T.C.Z. over Sudan during July.

REFERENCES

Abu Elzein, E. M. E. & Tag Eldin, M. H. (1985). The first outbreaks of sheep bluetongue in Khartoum Province, Sudan. *Revue Scientifique et Technique de l'O.I.E.* **4**, 509–515.

Anderson, J. (1984). Use of monoclonal antibody in blocking ELISA to detect group specific antibodies to bluetongue virus. *Journal of Immunological Methods* **74**, 139–149.

Anon. (1953). Department of Animal Production, Sudan Government, annual report.

Borden, E. C., Shope, R. E. & Murphy, F. A. (1971). Physicochemical and morphological relationships of some arthropod-borne viruses to bluetongue virus – a new taxonomic group: physicochemical and serological studies. *Journal of General Virology* **13**, 261–271.

Campbell, C. H. (1985). Bluetongue: diagnostic/antigenic interpretation. In *Bluetongue and Related Orbiviruses* (ed. T. Barber and M. Jochim), pp. 435–443. New York: Alan R. Liss.

Davies, F. G. & Blackburn, N. K. (1971). The typing of bluetongue virus. *Research in Veterinary Science* **12**, 181–183.

Della-Porta, A. J., Herniman, K. A. J. & Sellers, R. F. (1981). A serological comparison of the Australian isolate of bluetongue virus type 20 (CSIRO 19) with bluetongue group viruses. *Veterinary Microbiology* **6**, 9–21.

Della-Porta, A. J., Sellers, R. F., Herniman, K. A. J., Littlejohns, I. R., Cybinski, D. H., St George, T. D., McPhee, D. A., Snowdon, W. A., Campbell, J., Cargill, C., Corbould, A., Chung, Y. S. & Smith, V. W. (1983). Serological studies of Australian and Papua New Guinean cattle and Australian sheep for the presence of antibodies against bluetongue group viruses. *Veterinary Microbiology* **8**, 147–162.

Della-Porta, A. J., Parsonson, I. M. & McPhee, D. A. (1985). Problems in the interpretation of diagnostic tests due to cross-reactions between orbiviruses and broad serological responses in animals. In *Bluetongue and Related Orbiviruses* (ed. T. L. Barber and M. Jochim), pp. 445–453. New York: Alan R. Liss.

Eisa, M., Karrar, A. E. & Abdel Rahim, A. H. (1979). Incidence of bluetongue virus precipitating antibodies in sera of some domestic animals in the Sudan. *Journal of Hygiene* **83**, 539–545.

Eisa, M., Osman, O. M., Karrar, A. E. & Abdel Rahim, A. A. (1980). An outbreak of bluetongue in sheep in the Sudan. *The Veterinary Record* **106**, 481–482.

Eisa, M., McGrane, J. J., Taylor, W. P. & Ballouh, A. (1983). Survey of precipitating antibodies to bluetongue virus in domestic animals of the Sudan. *Bulletin of Animal Health and Production, Africa* **31**, 95–99.

Hayashi, K., Suzuki, H., Makino, Y. & Asahina, S. (1979). Notes on the transoceanic insects captured on East China Sea in 1976, 1977 and 1978. *Tropical Medicine* **21**, 1–10.

Herniman, K. A. J., Boorman, J. P. T. & Taylor, W. P. (1983). Bluetongue virus in a Nigerian dairy cattle herd. 1. Serological studies and correlation of virus activity to vector population. *Journal of Hygiene* **90**, 177–193.

Karabatsos, N. (1985). *International Catalogue of Arboviruses*, 3rd edition, pp. 1147. San Antonio, Texas: American Society of Tropical Medicine and Hygiene.

Lefevre, P. C. & Taylor, W. P. (1983). Situation épidemiologique de la fièvre catarrhale du mouton (Bluetongue) au Sénégal. *Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux* **36**, 241–245.

Manning, J. S. & Chen, M. F. (1980). Bluetongue virus: detection of antiviral immunoglobulin G by means of enzyme-linked immunosorbent assay. *Current Microbiology* **4**, 381–385.

Matthews, R. E. F. (1982). Classification and nomenclature of viruses. *Intervirology* **17**, 1–199.

Mellor, P. S., Osborne, R. & Jennings, D. M. (1984). Isolation of bluetongue and related viruses from Culicoides spp. in the Sudan. *Journal of Hygiene* **93**, 621–628.

Moore, D. L. & Lee, V. H. (1972). Antigenic relationship between the virus of epizootic haemorrhagic disease of deer and bluetongue virus. *Archives of Virology* **37**, 282–284.

Pillai, C. P. (1961). Suspected cases of bluetongue in sheep. *Sudan Journal of Veterinary Science and Animal Husbandry* **8**, 47–50.

Sellers, R. F. (1984). Bluetongue in Africa, the Mediterranean region and Near East – disease, virus and vectors. *Preventive Veterinary Medicine* **2**, 371–378.

Taylor, W. P. & Gumm, I. D. (1985). Is the AGID test group-specific for epizootic haemorrhagic disease virus? In *Bluetongue and Related Orbiviruses* (ed. T. L. Barber and M. Jochim), pp. 513–514. New York: Alan R. Liss.

Taylor, W. P., Gumm, I. D., Gibbs, E. P. J. & Homan, J. (1985). The use of serology in bluetongue epidemiology. In *Bluetongue and Related Orbiviruses* (ed. T. L. Barber and M. Jochim), pp. 461–468. New York: Alan R. Liss.

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