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Further studies on bluetongue and bluetongue-related Orbiviruses in the Sudan

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SUMMARY

The seasonal incidence of bluetongue virus (BTV) in Central Sudan is related primarily to fluctuations in the prevalence of the vector, *Culicoides imicola*. Population densities of this midge begin to rise with the onset of precipitation and peak during October, before falling sharply at the end of the rainy season in November. These are also the months of BTV transmission. Populations of *C. schultzei*, the commonest midge in Central Sudan, are also related to the rainy season but this species does not seem to be involved with BTV transmission.

BTV serotype 2 was isolated from *C. imicola* confirming the status of this midge as a known vector but a second isolate of the same serotype was made from a mixed pool of *Culicoides* not including *C. imicola*. This suggests that BTV transmission in the Sudan may involve more than one species of *Culicoides*. Epizootic haemorrhagic disease virus (EHDV) serotype 4 and a palyam virus were isolated from *C. schultzei* which indicates that this species may be involved in the transmission of BT-related viruses. Seven further virus isolates from sentinel calves at Shambat (Khartoum) confirmed the presence of BTV serotypes 1, 4 and 16, and an untyped EHDV (designated 318) in the Sudan. All of the viruses isolated and identified during the course of this work are recorded from the Sudan for the first time.

INTRODUCTION

Bluetongue virus (BTV) is a double stranded RNA virus which causes an insect transmitted, febrile disease of ruminants. The virus has a worldwide distribution and exists in at least 24 distinct serotypes. Biting midges of the genus *Culicoides* are the only confirmed biological vectors of the disease.

Previous work [1] has shown that the incidence of bluetongue (BT) in Central Sudan is a predictable event, transmission between ruminants occurs from July to December each year and is related to the time of the rainy season. It has also been suggested [1], without confirmatory data, that disease incidence is related to the vector population.

The present work describes further studies on the epidemiology of BT and related orbiviruses in the Sudan and also records virus isolations from insect vectors and from ruminant hosts in that country.

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MATERIALS AND METHODS

Collection of insects for seasonal incidence

Potential insect vectors were collected between January and December 1981 at the University Farm, Shambat, Khartoum North. A Monks Wood light trap [2] operated from the mains supply via a transformer was used from dusk to dawn on two nights per week throughout the year. Collections were made into solutions of isotonic saline containing 0·1% Hederol detergent (Procter and Gamble) as a wetting agent and the insects were subsequently preserved in 5% formalin before dispatch to the UK for identification.

Collection of insects for virus isolation

Previous studies have shown that peak populations of *Culicoides* occur in Central Sudan during the autumn and that seroconversions to BTV are also concentrated at this time of the year [1, 3]. Therefore, insect collections for virus isolation were made during September and October 1983 both at Nyala (12° 01′ N, 24° 50′ E) in Western Sudan and at Shambat, Khartoum North (15° 40′ N, 32° 32′ E) in Central Sudan. Collections were made using Monks Wood light traps but the insects were blown directly into solutions of Parke Davies Additive Medium (PDAM) containing 0·1% detergent plus antibiotics (0·075 mg/ml neomycin, 0·05 mg/ml streptomycin, 20 IU/ml penicillin, 50 IU/ml polymyxin and 25 IU/ml mycostatin [3]. *Culicoides* species were sorted under a binocular microscope within 24 h of capture and were divided into the following groups: *C. schultzei* group (female, non-engorged), *C. imicola* (female, non-engorged), *C. 'other species'* (female, non-engorged). All *Culicoides* were preserved in PDAM and were kept at 4 °C until required for virus isolation procedures in the UK.

Virus isolation from insects

The procedures used were similar to those described by Mellor and colleagues [3]. Pools of unengorged female midges totalling 200 or fewer were ground in Griffiths tubes containing 3·5 ml of phosphate-buffered saline, supplemented with 2% bovine serum albumen and 1% antibiotic stock. The suspensions were clarified by centrifugation at 2000 ***g*** for 5 min. Tenfold dilutions were made to 10^{-2} and 0·1 ml amounts of each dilution were inoculated intravenously into 11-day-old chick embryos [4] using five eggs per dilution. Similarly each of five glass tubes (125 mm × 15 mm) containing a 24-h-old monolayer of BHK-21 cells was inoculated with 0·2 ml of the appropriate dilution of insect suspension. Following adsorption at 37 °C for 30 min, the monolayers were re-fed with Eagle's Maintenance Medium. The tubes were then rolled at 37 °C and examined daily for 8 days for evidence of cytopathic effect (CPE). During this period the medium was changed every other day.

Chick embryos dying on the first day post-inoculation were discarded but the hearts were harvested from embryos dying on subsequent days. These were ground up and passaged in further embryonating eggs and BHK-21 cells. Apparently negative samples were subcultured in eggs and tissue culture at least twice before being discarded.

Virus isolation from calves

Between October 1980 and September 1983 a sentinel calf herd was maintained at the Khartoum University Farm, Shambat [1]. Based on previous experience [1] virus isolation was attempted from individuals in this herd during September 1982 and September 1983. Blood was collected directly from the jugular vein of donor animals into 10 ml heparin lithium vacutainers (Becton Dickinson, Middlesex). This was then centrifuged at 3000 ***g*** for 10 min, the plasma discarded and the packed cells washed three times in an equal volume of isotonic saline. After the final wash the blood was sonicated for 15 s at an amplitude of 30 μm (MSE, Soniprep 150). An equal volume of OPG (0·5% w/v potassium oxalate, 0·5% w/v phenol, 50% v/v glycerine, 50% distilled water) was added and the mixture stored at 4 °C until required. Subsequently a 1/10 dilution of this mixture in PBS was used for the inoculation of eggs and BHK-21 cells as described above.

Virus plaque purification

Following adaptation to BHK-21 cells (three passages), all virus isolates were plaque-purified three times using the method of Mohammed [5]. Briefly this involved inoculating 0·2 ml amounts of tenfold dilutions of each virus onto monolayers of BHK-21 cells in 60 × 15 mm tissue culture dishes (Falcon, USA). The virus was allowed to adsorb for 40 min at 37 °C under 3% CO_2 tension and then 5 ml of overlay medium was added to each plate. The plates were inoculated at 37 °C under 3% CO_2 tension for a further 3–4 days and then 3 ml of a second overlay consisting of 0·85% saline supplemented with 0·6% (w/v) agarose indubiose A37 (IBF, France) and 0·01% neutral red added to each plate. Following incubation for a further 2 h single plaques were picked with sterile Pasteur pipettes from the plate with the least number of plaques and stored in 3·6 ml PBS containing 0·2% bovine serum albumin (BS). Two further plaque purifications following this procedure were carried out on virus grown from the harvested plaques. Virus from the third purification was stored in PDAM at −70 °C until required.

Virus identification

Preliminary virus identification was carried out as follows:

(*a*) *Electron microscopy*. Droplets of the supernatants (Eagle's Growth Medium) from Roux bottles of BHK-21 cells individually infected with each virus isolate were allowed to absorb onto formvar-carbon coated grids for 10 s and were then stained with 2% phosphotungstic acid. Examination for virus particles under a Jeol 1200 EX electron microscope was carried out at magnifications up to × 146000.

(*b*) *Agar gel immunodiffusion tests* (*AGID*). Soluble antigens from each virus isolate were prepared using the method of Lefevre and Taylor [6]. Tests were conducted on microscope slides coated with 2 ml of 1% Litex agarose in borate buffer according to the method of Mohammed & Taylor [1].

(*c*) *Microneutralization tests – neutralization indices*. Heat-inactivated sera at a dilution of 1/10 were tested against equal volumes of serial tenfold dilutions of test virus in tissue culture microtitre plates using 100 μl volumes of each according to

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the method of Mohammed [5]. The neutralization index (NI) was expressed as the log titre of test virus incubated with negative control serum minus the log titre following exposure to each of the reference antisera. Indices higher than 0·6 were regarded as being significant.

(*d*) *Microneutralization tests – reciprocal cross neutralization.* Antisera raised in guinea-pigs against each of the virus isolates and the reference antisera which had previously neutralized each isolate were all tested in reciprocal cross-neutralization tests using dilutions of each specific antiserum (1/20 to 1/640) and 100 $TCID_{50}$ of virus [5]. The SN_{50} titre was calculated using the method of Kärber [7] and was expressed as the final dilution of serum able to protect 50% of the indicator cells against 100 $TCID_{50}$ of virus.

(*e*) *Blocking ELISA for detection of BT group-specific antibodies.* The principle of this test is based upon the ability of test sera to block the pre-titrated reaction between the antigen and monoclonal antibody (Mab). The test was performed according to Anderson [8] and makes use of the BTV type 1 mouse Mab 3-17-3A. Optimal dilutions of both antigen and Mab were determined as described by Anderson [8]. Volumes of 50 μl were used throughout. An inhibition value of 50% or above was considered significant.

(*f*) *Indirect ELISA.* Inoculation and washing procedures were performed as for the blocking ELISA. The antigen was first absorbed to the microplate and this was then followed by the addition of the test sera. Specific antigen–antibody binding was detected by the addition of anti-species immunoglobulins conjugated to horseradish peroxidase. After the addition of the substrate (orthophenylene-diamine) and suitable colour development, the reaction was stopped by the addition of 1 M sulphuric acid (H_2SO_4). Optical density values were read as in the blocking ELISA.

RESULTS

Insects – seasonal incidence

A total of 103051 *Culicoides* comprising at least seven species was collected at Shambat in 1981 during the course of this study. 97·4% (100409) were *C. schultzei* gp midges, 2·0% (2024) were *C. imicola* while the remaining 0·6% (618) was made up of five other species (*C. milnei*, *C. similis*, *C. ravus*, *C. circumscriptus* and *C. distinctipennis*). Figures 1 and 2 show the seasonal prevalence of *C. imicola* and *C. schultzei* gp midges at Shambat throughout 1981. It can be seen that the population densities of both species had a strong positive correlation with rain and with the arrival of the intertropical convergence zone (ITCZ) over the area (Figs. 1 and 2). The numbers of both *C. imicola* and *C. schultzei* started to increase during March, the month of the first rainfall of the year. Populations increased to reach a peak during October but then fell sharply at the end of the rainy season during late October and early November. Low numbers of *C. schultzei* and *C. imicola* were then recorded for the duration of the dry season.

Insects – virus isolation

A total of 1806 and 2858 unengorged female *Culicoides* was collected for virus isolation from Nyala and Shambat respectively. They were sorted according to species into three main categories and then sub-divided into a total of 34 smaller groups.

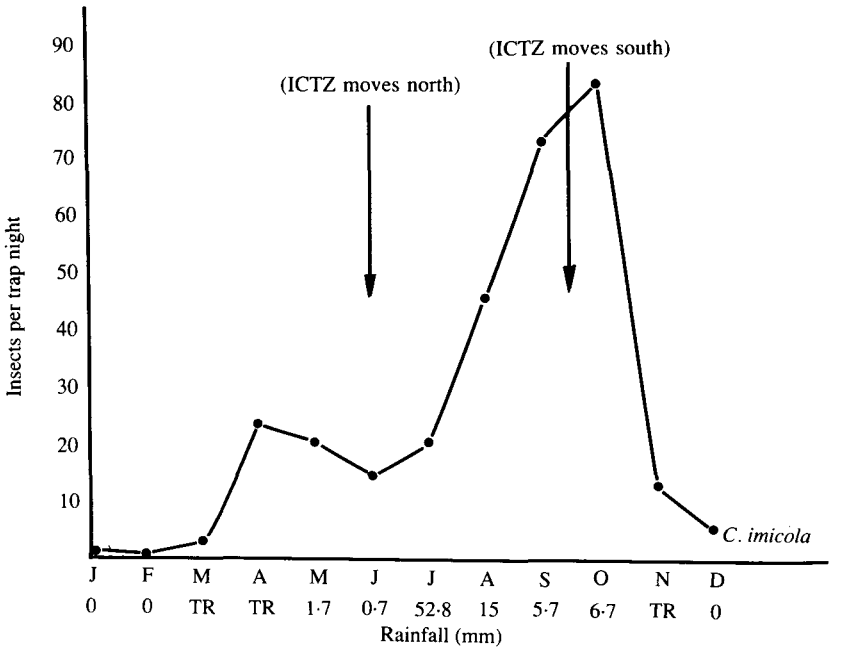

Fig. 1. Seasonal prevalence of *Culicoides imicola* at Shambat during 1981.

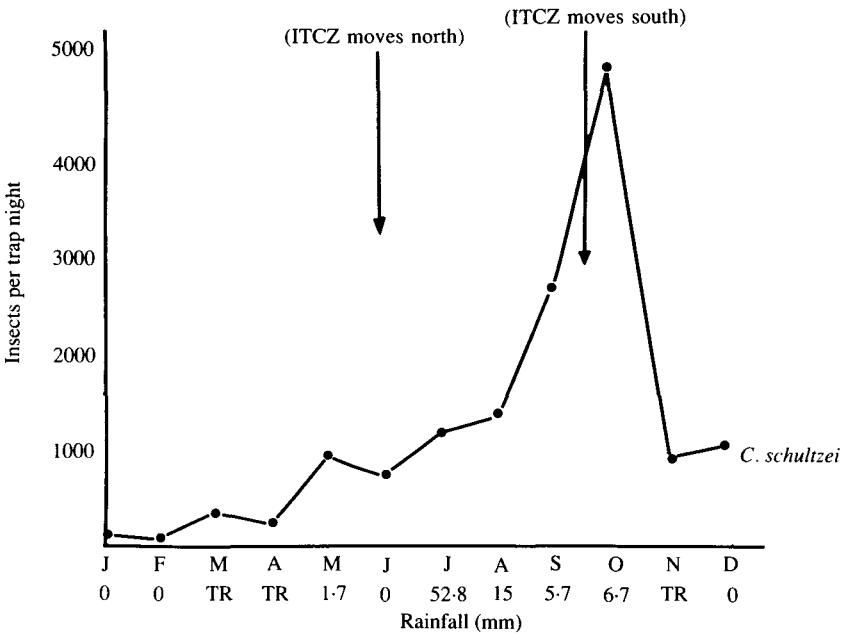

Fig. 2. Seasonal prevalence of *Culicoides schultzei* at Shambat during 1981.

Four virus isolations were made by i.v. inoculation of 11-day-old chick embryos with dilutions of ground *Culicoides*, followed by passage in BHK-21 cell cultures. Three of the isolates (Gp 4, Gp 7 and Gp 14) were obtained from pools of *Culicoides* collected in Nyala while the fourth isolate (Gp 18) was obtained from a group of 200 *C. schultzei* collected at Shambat (Table 1). Isolate Gp 4 came from 77 *C. imicola*, Gp 7 from 150 *C. schultzei* and isolate Gp 14 was obtained from a pool of

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Table 1. Culicoides *midges collected from the Sudan and the viruses isolated from them*

Location	Total numbers	Species (%)		Virus isolation
Nyala	1806	845 (46·8%)	*C. schultzei*	Isolate Gp 7 (150)*
		246 (13·7%)	*C. imicola*	Isolate Gp 4 (77)
		714 (39·5%)	*C. 'other species'*†	Isolate Gp 14 (207)
Shambat	2858	2800 (98%)	*C. schultzei*	Isolate Gp 18 (200)
		40 (1·4%)	*C. imicola*	—
		18 (0·6%)	*C. 'other species'*‡	—

* number of unengorged parous females from which the virus isolate was made.
† *C. neavei*, *C. distinctipennis*, *C. similis*, *C. circumscriptus*, *C. ravus*, *C. grahami*, *C. milnei*.
‡ *C. distinctipennis*, *C. similis*, *C. circumscriptus*, *C. ravus*.

207 *Culicoides 'other species'* (*neavei*, *distinctipennis*, *similis*, *circumscriptus*, *ravus*, *grahami*, and *milnei*).

Virus isolation from calves

A total of 29 virus isolations were made from 67 calves samples at Shambat during September 1982 (K. A. J. Herniman, personal communication). During September 1983 a further 10 virus isolates were made from 48 calves in the same herd (L. Owen, personal communication). Virus isolations were initially made either in BHK-21 cell culture or in 11-day-old chick eggs but all were subsequently adapted, as before, to grow in BHK-21 cells.

Ten virus isolates, 7 of which derived from the 1982 collection and 3 from 1983, were randomly selected for identification.

Virus identification

Each of the 4 isolates from insects and the 10 selected isolates from calves were plaque purified three times and the final clone amplified in BHK-21 cell culture [5].

(*a*) *Electron microscopy.* Electron micrographs (Fig. 3) demonstrated the presence of spherical virus particles ranging in size between 60–70 nm and which showed the diffuse appearance typical of orbiviruses for each of the 14 virus isolates [3].

(*b*) *AGID tests – insect isolates.* Soluble antigens from each of the four isolates from insects were tested against known positive antisera to 12 different orbiviruses. The results are shown in Table 2. The soluble antigen of isolate Gp 7 formed lines of complete identity with antisera to epizootic haemorrhagic disease virus (EHDV-3, EHDV-4) and with Eubenangee virus. The soluble antigen of isolate Gp 14 precipitated, BTV-1, EHDV-318 and Tilligerry antisera while the antigens prepared from isolate Gp 4 formed precipitation lines with BTV-1 and Tilligerry antisera only. Precipitation lines were also formed between the antigen of isolates Gp 18 and antisera to BTV-1 and EHDV-3.

(*c*) *AGID tests – calf isolates.* Soluble antigens from each of the 10 virus isolates from calves were tested against 9 different orbiviruses (Table 3). Five distinct patterns of reaction were seen. Monotypic reactions were detected with the antigens of isolates; 3818, NY73 and NY76 which reacted only with BTV-1 antiserum. The antigens prepared from isolates 3834 and 3890 reacted both with

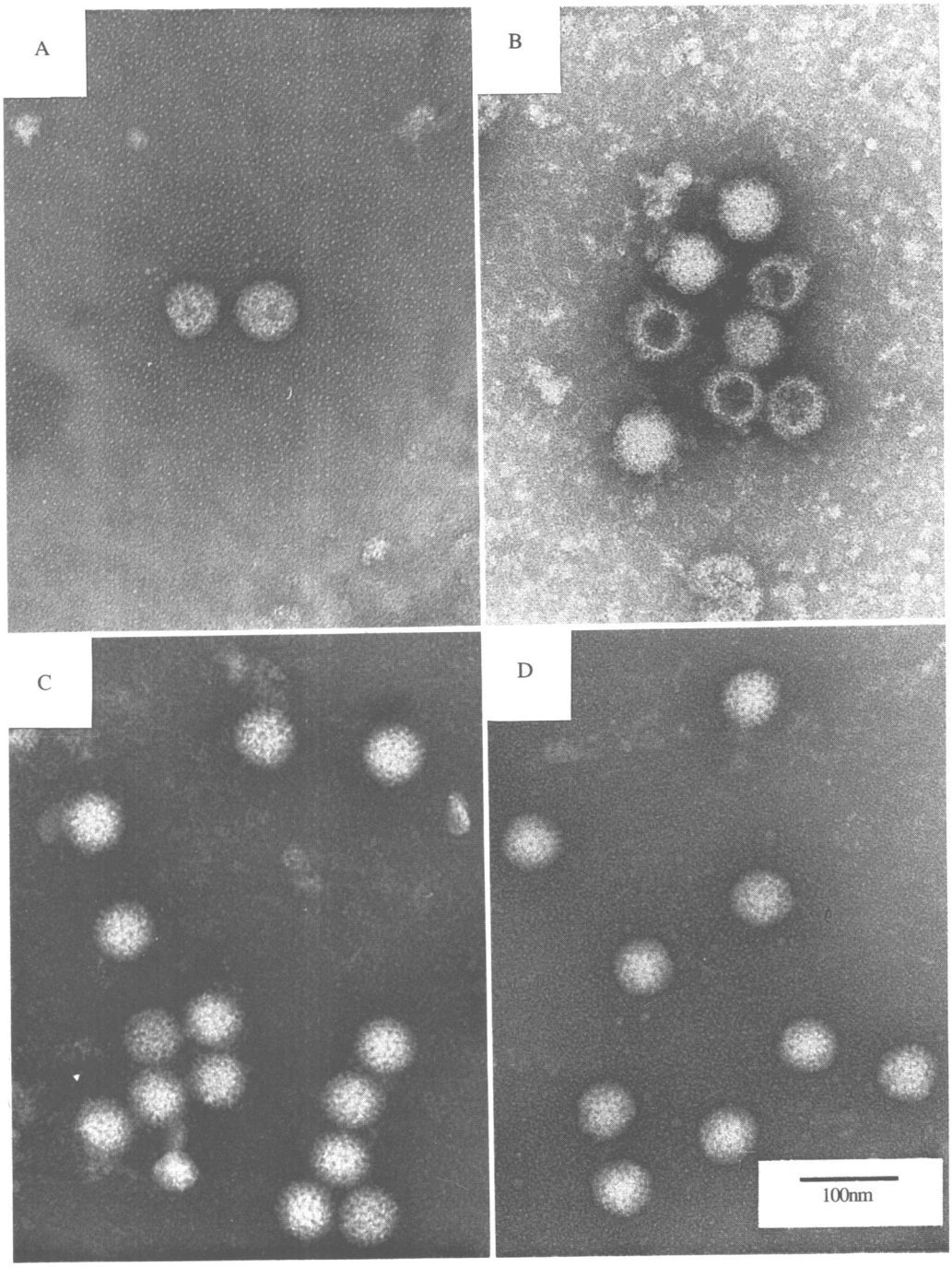

Fig. 3. Electron micrographs of Gp 7, Gp 14, Gp 18 and Gp 4 isolates from the Sudan. Particle size 60–70 nm. Barline 100 nm.

the antisera of EHDV-2 and also EHDV-318. The antigens of isolates 3838 and 3843 reacted with the antiserum of Su48 virus from the Sudan and also with the antiserum to an Australian Palyam virus, while the antigen of isolate 3856 reacted with the same two antisera and in addition with the antiserum to EHDV-3. The

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Table 2. *Agar gel immunodiffusion results of the soluble antigens of the four Sudanese isolates against 12 orbivirus antisera*

	Soluble antigen			
Antiserum	Gp 7	Gp 14	Gp 4	Gp 18
BTV-1	—*	+†	+	+
EHDV-1 (New Jersey)	—	—	—	—
EHDV-2 (Alberta)	—	—	—	—
EHDV IB Ar 22619	+	—	—	+
EHDV Ib 33853	+	—	—	—
Ibaraki	—	—	—	—
EHDV-(318)	—	+	—	—
Eubenangee	+	—	—	—
Tilligerry	—	+	+	—
Pata	—	—	—	—
AHSV-1	—	—	—	—
Corriparta	—	—	—	—

* —, No precipitation line formed.
† +, Precipitation line formed.

Table 3. *Agar gel immunodiffusion results of antigens prepared from Sudan isolates against nine orbivirus antisera*

	Antisera								
Antigen	BTV-1	EHDV-1	EHDV-2	EHDV-22619	EHDV-33853	EHDV-318	Su48	Pata	Palyam
3818	+*	—†	—	—	—	—	—	—	—
3907	+	+	+	+	—	+	—	—	—
NY20	+	+	+	+	—	+	—	—	—
NY73	+	—	—	—	—	—	—	—	—
NY76	+	—	—	—	—	—	—	—	—
3834	—	—	+	—	—	+	—	—	—
3890	—	—	+	—	—	+	—	—	—
3838	—	—	—	—	—	—	+	—	+
3843	—	—	—	—	—	—	+	—	+
3856	—	—	—	+	—	—	+	—	+

*, Precipitation line formed.
† —, No line of precipitation.

antigens prepared from isolates 3907 and NY20 were the most cross-reactive giving lines of precipitation with antisera to BTV-1 and also four EHDVs.

(*d*) *Serum virus neutralization tests – insect isolates.* (i) Neutralization indices – All the reference antisera which reacted with each virus antigen in the AGID group specific tests were also examined for their ability to neutralize specifically that virus in neutralization tests, using constant serum – varying virus dilutions. Additional reference antisera from the same orbivirus serogroup as those antisera which gave positive reactions in the group test were included where possible. The results are shown in Table 4. Isolate Gp 7 was only neutralized by the antiserum to EHDV-4 (Ib Ar 33853). Isolate Gp 14 was significantly neutralized by BTV-2 and to a lesser extent by EHDV-318 antiserum. Isolate Gp 4 was only neutralized by BTV-2 antiserum. In contrast isolate Gp 18 was not neutralized by any of the 22 BTV antisera nor by any of the 6 EHDV antisera.

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Table 4. *Neutralization indices of Sudan viruses from insects against heterologous antisera**

Antisera	Viruses			
	Gp 7	Gp 14	G4	G18
BTV (1-22)	—†	(3·75)‡	(2·5)‡	—
EHDV-1 (New Jersey)	—	—	NT§	—
EHDV-2 (Alberta)	—	—	NT	—
EHDV Ib Ar 22619	—	—	NT	—
EHDV Ib Ar 33853	3·75	—	NT	—
Ibaraki	—	—	NT	—
EHDV (318)	—	0·75	NT	—
Eubenganee	—	NT	NT	NT
Tilligerry	NT	—	—	NT

* Neutralization index (NI) = $\log_{10}$ virus titre in the normal serum minus $\log_{10}$ virus titre in the immune serum.

† NI < 0·5.

‡ Neutralized only by antiserum to BTV-2.

§ NT, not tested.

Table 5. *Reciprocal cross-neutralization indices of Sudan viruses from insects and antisera against heterologous viruses and antisera*

Antisera	Viruses					
	Gp 7	GP 14	Gp 4	Gp 18	BTV-2	EHDV Ib Ar 33853
GP 7	⩾ 4·25*	—†	—	—	—	⩾ 4·25
GP 14	—	⩾ 4·25	⩾ 4·25	—	⩾ 3·75	—
GP 4	—	⩾ 4·25	⩾ 4·0	—	⩾ 3·75	—
GP 18	—	—	—	⩾ 4·5	—	—
BTV-2	—	⩾ 4·25	⩾ 4·0	—	⩾ 3·75	—
EHDV Ib Ar 33853	⩾ 4·25	—	—	—	—	⩾ 4·0

* $\log_{10}$ virus titre in normal serum minus $\log_{10}$ virus titre in immune serum.

† —, No neutralization.

(ii) Reciprocal cross neutralization – The results of cross-neutralization tests are shown in Table 5. Isolate Gp 7 and EHDV-4 were both strongly neutralized by Gp 7 antiserum and by EHDV-4 antiserum. Isolates Gp 4, Gp 14 and BTV-2 were all strongly neutralized by each others antisera. However no serological relationship was detected between isolate Gp 18 and any of the BT and EHD viruses examined. This virus was only neutralized by homologous antiserum.

(*e*) *Serum virus neutralization tests – calf isolates.* (i) Neutralization indices – Tenfold dilutions of each test virus were reacted separately with equal volumes of those antisera that had given lines of precipitation with that virus in the AGID test. Additionally other antisera raised against viruses within the same serogroup were included and the neutralization indices recorded (Table 6). Isolate 3818 was neutralized by the antiserum to BTV-16. Isolates NY76 and NY73 were neutralized by the antisera of BTV-1 and BTV-4 respectively and their infectivities significantly reduced. Isolates 3907, NY20, 3834 and 3890 were tested against all 22 BTV antisera and all 6 EHDV antisera and were only neutralized by

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Table 6. *Neutralization indices of Sudanese calf isolates against heterologous antisera*

Isolate	Antiserum: BTV (1-22)	EHDV-318	Su48	3863
3818	BTV-16 4·34*	—†	—	—
NY76	BTV-1 3·33	—	—	—
NY73	BTV-4 2·0	—	—	—
3834	—	4·34	—	—
3890	—	3·33	—	—
3907	—	⩾ 5·0	—	—
NY20	—	⩾ 5·0	—	—
3856	—	—	3·33	—
3838	—	—	—	3·0
3843	—	—	—	3·33

* $\log_{10}$ virus titre in normal serum minus $\log_{10}$ virus titre in immune serum.
† —, Either not tested or < 0·5 (see text).

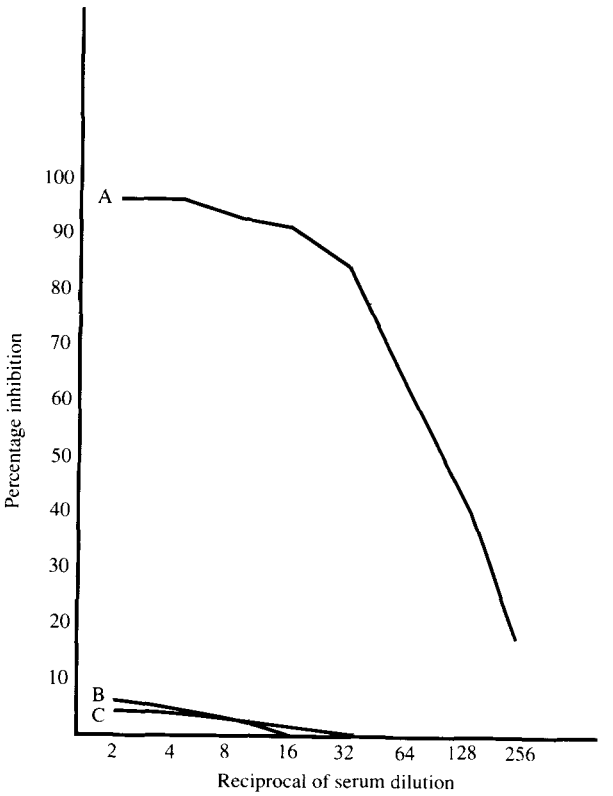

Fig. 4. Percentage inhibition values from a BTV-blocking ELISA using guinea-pig antisera against (A) BTV-1 and (B) Gp 18 viruses and (C) normal guinea-pig serum.

the antiserum of EHDV-318. Isolates 3838, 3843 and 3856 were tested against all six EHDV antisera and also against an extra antiserum raised against 3863 virus (related to Su48 [3]). Isolate 3856 was only neutralized by the antiserum Su48 while isolates 3838 and 3843 were both neutralized by the antiserum of 3863 virus.

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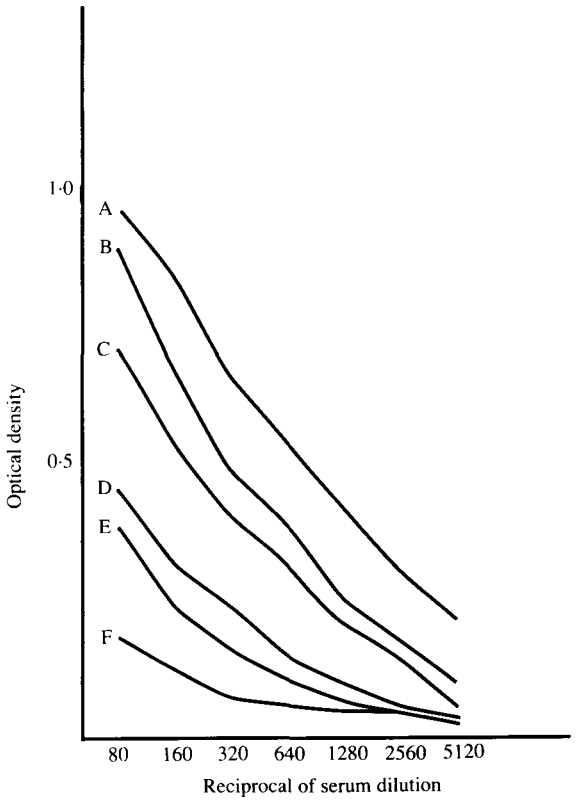

Fig. 5. Indirect ELISA showing the reactivity of serial dilutions of different orbiviruses against a constant dilution (1/320) of Gp 18 virus antiserum. A, Palyam DPP-66 antiserum; B, Palyam CS82 antiserum; C, Palyam d'Aguilar 8112 antiserum, D, BTV-1 antiserum; E, EHD CS 157 antiserum; F, normal rabbit serum.

(ii) Reciprocal cross neutralization – Antisera raised against isolates 3818, NY76 and NY73 strongly neutralized BTV-16, BTV-1 and BTV-4 respectively. Antisera raised against isolates 3834, 3890, 3907 and NY20 all neutralized EHDV-318 while the antiserum of isolate 3856 only neutralized Su48 virus. Antiserum against isolate 3838 showed no neutralizing activity against Su48 virus but did neutralize 3863 virus and also its own homologous virus. Antiserum raised against isolate 3843 strongly neutralized the homologous virus but also showed a much lesser activity against both 3863 and 3838 viruses. From the results of the neutralization tests it was concluded that isolates 3838, NY76 and NY73 are serologically identical to BTV-16, BTV-1 and BTV-4 respectively. Isolates 3834, 3890, 3907 and NY20 are serologically identical to EHDV-318. Isolate 3856 is serologically identical to Su48 virus and isolate 3838 is serologically identical to 3863 virus. Isolate 3843 is related to both 3863 and 3838 viruses but is not identical with either.

(*f*) *ELISA*. The guinea-pig antiserum of isolate Gp 18 was examined using the BTV blocking ELISA, with normal serum and BTV-1 antiserum as controls. The results are shown in Fig. 4. Gp 18 antiserum failed to react with the BTV-1 antigen while the antiserum of BTV-1 reacted specifically. The same antisera were also

examined in an indirect ELISA test using antigen prepared from isolate Gp 18. This antigen reacted specifically with its own immune serum but did not react with either the normal serum or BTV-1 antiserum. Further, the ELISA antigen prepared from Gp 18 virus was tested by the indirect ELISA against three different Palyam viruses (DPP66, CS82 and D'aguilar 8112), one EHDV and one BTV antisera. The results are shown in Fig. 5. The three Palyam antisera reacted with Gp 18 virus antigen and gave the highest OD readings while BTV-1 and EHDV antisera reacted only to low levels. These results therefore show that Gp 18 virus is not a BTV but a Palyam virus.

DISCUSSION

Two sentinel calf herds were used by Mohammed and Taylor [1] to show that the seasonal incidence of BTV in Central Sudan is a predictable event related to the rainy season. In this work we have shown additionally that the population densities of the two most common species of *Culicoides* at Shambat in Central Sudan are also related to the rainy season. Shambat enjoys a prolonged dry season extending from October–November of one year to June or July of the following year. During this period the prevailing winds are dry and are north-westerlies. The onset and duration of the rainy season is controlled by the annual north–south movements of the ITCZ which marks the boundary between the warm, dry, northerly air mass and the relatively cool, moist southerly air mass. The northward movement of the ITCZ across Shambat during June–July is therefore marked by a change in wind direction from north to south and the onset of precipitation. The ITCZ usually moves back south of Shambat during late September and a return to dry north-westerly winds is seen [1]. Population densities of both *C. schultzei* and *C. imicola* closely reflect the movements of the ITCZ and the resulting precipitation.

In 1981 the numbers of *C. schultzeri* and *C. imicola* increased steadily during March–April, the months of first rainfall, and then more rapidly during July–September, following the arrival of the ICTZ and associated heavy precipitation, to reach peaks in October. Subsequently their populations declined dramatically during November–December to 'dry season' levels. This population pattern is related partly to adult *Culicoides* being carried into the Shambat area on the humid, southerly winds that presage the arrival of the ITCZ and partly to the fact that both *C. imicola* and *C. schultzei* breed in saturated soil contaminated with cattle faeces [9, 10]. At Shambat such sites are exceptionally common during the rainy season but are very rare during the dry period at which time suitable breeding foci can only be found adjacent to a few leaky irrigation pipes and overflowing cattle troughs. The low population levels of *Culicoides* at Shambat during the dry season are therefore likely to be due primarily to an absence of suitable breeding sites.

Since *C. imicola* is the classical Old World vector of BTV [11] and has previously been shown to be involved in BTV transmission in the Sudan [3] it is likely that the seasonal incidence of BT in Central Sudan, described by Mohammed and Taylor [1], is due directly to the seasonal prevalence of this midge as influenced by

the movements of the ITCZ. Other arboviruses which may be transmitted in Central Sudan either by *C. imicola* or by *C. schultzei* will probably have, for the same reasons, a similar seasonal incidence to BTV.

During the course of the present work identification procedures which involved a battery of serological tests have confirmed the presence of; BTV-1, 2, 4 and 16, EHDV-4 and 318 (untyped) and a Palyam virus in the Sudan for the first time. Three further virus isolates could not be conclusively identified using the serological reagents at our disposal. The further characterization of these isolates and comparisons between all of the viruses isolated in the Sudan using more specific biochemical techniques will be described elsewhere.

The isolation of BTV-2 from *C. imicola* confirms the previously acknowledged status of this species as a major vector of BTV in Africa and in the Sudan [3, 11]. However, the isolation of a BTV from a mixed pool of *Culicoides* consisting of *C. neavei*, *C. distinctipennis*, *C. similis*, *C. circumscriptus*, *C. ravus*, *C. grahami* and *C. milnei* suggests that in the Sudan other species of *Culicoides* may also be involved in BTV transmission. Since *C. neavei*, *C. distinctipennis*, *C. similis*, *C. ravus* and *C. circumscriptus* do not usually blood feed upon mammals it is probable that this isolation originated from either *G. grahami* or *C. milnei*. The previous isolation of a BTV from *C. milnei* in Kenya [12] tends to support this suggestion.

C. schultzei was by far the commonest midge collected in Central Sudan comprising over 97% of the total *Culicoides* catch. However no isolations of BTV have been made from this species. As at least three isolations of BTV have now been made from other species of *Culicoides* in Central Sudan, comprising as they do less than 3% of the total [3] this strongly suggests that *C. schultzei* is not a BTV vector. Nevertheless the isolation of an EHDV and a Palyam virus from *C. schultzei* does indicate that this species is involved in the transmission of several BTV-related viruses.

Factors involved in determining the vector competence of species of *Culicoides* for arboviruses are not well understood [13]. However these factors are sufficiently specific that even when closely related viruses are involved, totally different groups of *Culicoides* may be 'selected' as the vectors. The epidemiological advantages of this in a situation where the vertebrate host is the same (i.e. cattle) is not immediately apparent.

The isolation of three different BTV strains (BTV-1, 4 or 16) and four EHDV strains (EHDV-318) from cattle at Shambat complements the earlier work of Mohammed and Taylor [1] who recorded serological evidence from 16 BTV serotypes and one or more unspecified BT-related viruses in the same area. The impact of all these orbiviruses on the various ruminant species in the area is not known although no evidence of clinical disease attributable to them was seen. From our observations it seems likely that infections caused by BTV (and BT-related viruses) in local Sudanese breeds of sheep, goats and cattle are usually inapparent. It is possible that indirect losses as a result of infection, such as loss of weight and condition, and drop in milk yield may have greater economic effects than occasional overt disease. However in recent years the Sudanese Government has been importing exotic sheep breeds from Australia. These animals are known to be highly susceptible to BT and could be severely affected if imported into Central Sudan during the BT season; between July and December. To minimize

the risks to these and other imported stock, importation should either b restricted to periods outside the BT season or the animals should be vaccinate against all the likely BTV serotypes in the Sudan, prior to introduction.

Control measures directed against vector species of insect would be extremel difficult and costly to implement in such a vast country as the Sudan and are nc a viable option; particularly as reintroduction of vectors via the wind fro surrounding territories is likely. Attention should therefore be given to measure designed to minimize contact between susceptible hosts and the vector particularly during the BT-season. Susceptible animals should be kept in shec during the flight time of the vectors, which is crepuscular, and the use of insec repellents such as diethyltoluamide (DEET), dimethylphthalate or the syntheti pyrethroids should be considered.

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