Epidemiol. Infect. (1991), **106**, 167–178
Printed in Great Britain

Epidemiology of bluetongue and related orbiviruses in the Sultanate of Oman

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(*Accepted 21 August 1990*)

SUMMARY

Sentinel herds at 34 farms were used to study the epidemiology of bluetongue and related orbiviruses in Oman. The results indicate that bluetongue virus (BTV) is widespread and is enzootic in Northern Oman. At least three BTV serotypes (3, 4 and 22) were present at the time of the study. Antibodies to epizootic haemorrhagic disease of deer virus (EHDV) type 2 and EHDV-318 were also detected but were less prevalent. Entomological investigations identified the presence of 16 species of *Culicoides*. The peak seasonal incidence of the BTV vector *C. imicola* and the EHDV vectors *C. schultzei* (group) midges at Rumais in Northern Oman correlated closely with the spring rains in that area. However, both species of midge were also present in lower numbers throughout the year. Four species of Omani midge, *C. arabiensis*, *C. ibriensis*, *C. neoschultzei* and *C. buettikeri* are new to science.

INTRODUCTION

Bluetongue (BT) is an insect-transmitted viral disease of ruminants with a worldwide distribution caused by bluetongue virus (BTV) the type species of the genus orbivirus (family Reoviridae). At the present time there are 24 internationally recognized serotypes of the virus [1]. Animals infected with one of these serotypes develop group specific antibodies which recognize all members of the BTV serogroup and also type specific antibodies.

Tests used to detect group specific antibodies include the agar gel immunodiffusion test (AGID) [2, 3], the modified direct complement fixation test [4] and various enzyme-linked immunosorbent assays (ELISAs) [5–7]. Methods used to detect serotype specific antibodies are the microtitre virus neutralization test [8, 9] and the plaque reduction virus neutralization test [10, 11].

The Sultanate of Oman is situated in the south-east of the Arabian Peninsula bordering both the Indian Ocean and the Arabian Gulf. Total livestock population

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of the region includes; 1·8 million cattle, 8 million sheep, 7 million goats and 0·5 million camel [12]. The general climate over the area is arid and three main types of livestock production are practised: livestock associated with irrigated agriculture; livestock associated with nomadic and semi-nomadic husbandry; large modern commercial dairy enterprises using intensive husbandry.

Although disease in the area has not been definitely attributed to BTV, congenital abnormalities consistent with the presence of viral agents such as BT and Akabane are frequently encountered in farm animals (Al-Busaidy, unpublished observations).

Some indications of the serotypes of BTV circulating in Oman have been given previously by Herniman and co-workers [13], Hedger and co-workers [14] and Al-Busaidy [15]. Hafez and Taylor [16] have also reported serological evidence of a number of BTV serotypes in neighbouring Saudi Arabia. The aim of the present study was to gain a more precise and comprehensive insight into the epidemiology of BT and related orbiviruses in Oman by the use of a sentinel herd system and a vector surveillance system operated in that country for 1 year.

MATERIALS AND METHODS

Insect collection

Insects were collected at Rumais and Nakhal (Fig. 1) using Pirbright type miniature light traps, which are a modification of the Monk's Wood light trap [17]. Traps were operated from the mains supply via a 12-volt step-down transformer from dusk until dawn. Collections were generally made at a rate of two nights each week from March 1987 to June 1988. Insects were collected in saline with a little weak detergent solution and were preserved in 5% formalin until shipment to IAH, Pirbright for identification.

All identifications of previously described species were made by comparison with specimens in the British Museum (Natural History), by comparison with descriptions or, where possible, by comparison with types.

Sentinel herds

Thirty-four farms were chosen as sites for sentinel herds, 33 along the Batinah Coast in Northern Oman and one at Salalah in Southern Oman (Fig. 1). A minimum of 10 animals, serologically negative to BTV, per farm were identified by ear-tagging. All of the animals were goats, aged between 6 an 12 months according to their dentition [18], except at Rumais Government Research and Salalah Dairy Farms where newly born calves were used.

Monthly bleeding was undertaken from March 1987 to February 1988. All animals were bled from the jugular vein into 10 ml plain vacutainer tubes (Becton–Dickinson).

Serological tests

Sera were examined for the presence of BTV group-specific antibodies using either an AGID test or a blocking ELISA. The AGID test was conducted on

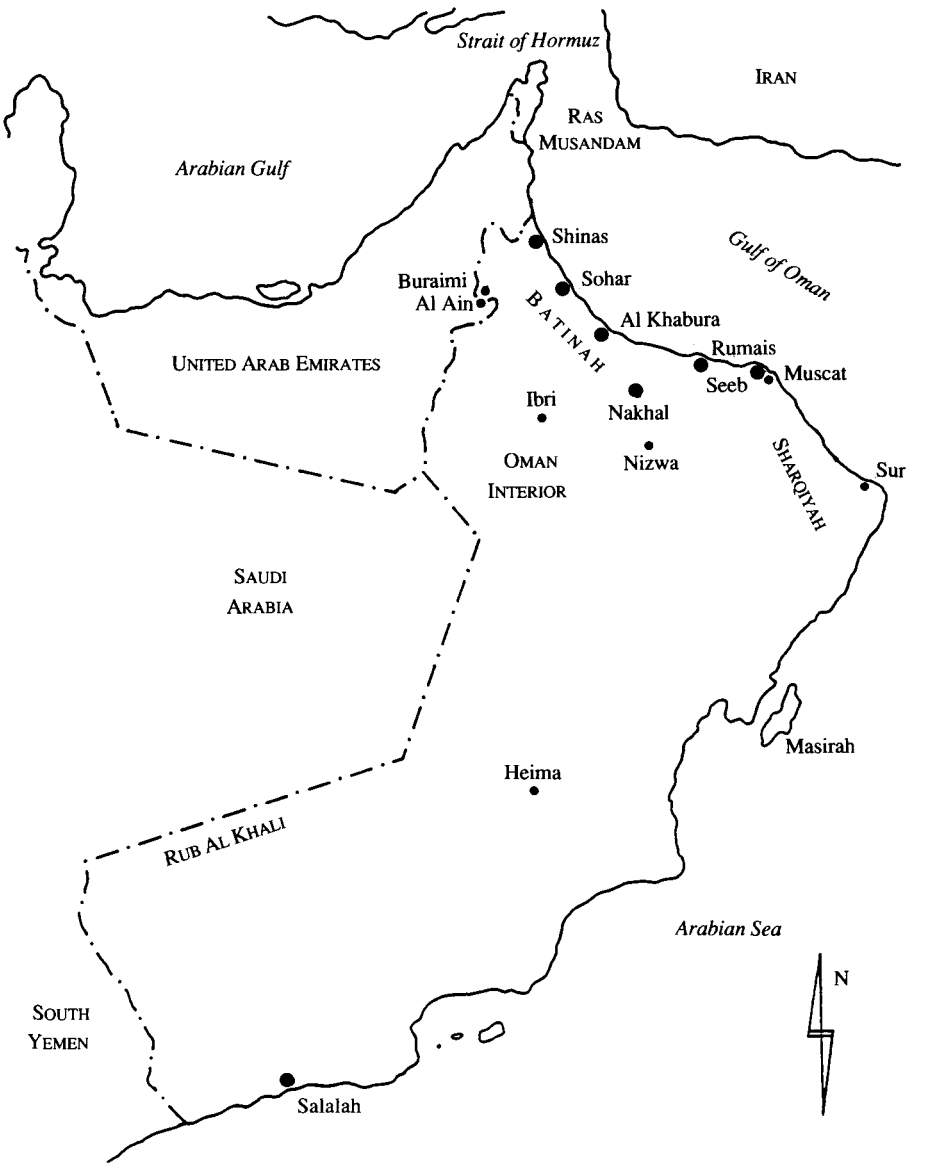

Fig. 1. The Sultanate of Oman.

microscope slides coated with 2 ml of 1 % Litex agarose in borate buffer at pH 9·2 [19]. Soluble antigen [3] was added to the central well and alternating peripheral wells received test or positive control sera. The slides were examined daily for 3 days and the final reactions recorded.

The blocking ELISA was performed according to the method of Anderson [7], using antigen prepared by the method of Manning and Chen [5] from cultures of BHK-21 cells infected with BTV serotype 1, and using a BTV serotype 1 mouse monoclonal antibody (Mab) designated 3-17-3A. Optimum dilutions of both

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antigen and Mab were determined by twofold checkerboard titrations in an indirect ELISA. Thereafter, antigen was coated onto 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 Mab. Optical density (OD) values were read on a multichannel spectrophotometer at a wavelength of 492 nm and the results expressed as the percentage inhibition, calculated from the formula: 100−[(OD in presence of test serum/OD in absence of test serum) × 100].

Selected serum samples were also examined for type-specific neutralizing antibodies in virus neutralization tests (VN) conducted in microplates. Wherever possible the first collection of serum following seroconversion was used in these tests. Samples previously inactivated at 56 °C for 30 min were screened at final dilutions of 1/20 and 1/40 against BT serotypes 1–24, using the method of Herniman and colleagues [13]. Each dilution was tested in duplicate and a positive result was recorded for those sera neutralizing 100$TCID_{50}$ of virus at a dilution of 1/20 or greater.

Criteria for the presence of BTV group-specific antibodies were a positive AGID result and/or an inhibition of 50% or more in the blocking ELISA. Criteria for the presence of BT-related Epizootic Haemorrhagic Disease Viruses (EHDV) were a positive AGID test, using soluble antigens to EHDV-1, EHDV-2 and EHDV-318, and a negative BT ELISA. Once the presence of EHDV antibodies had been confirmed the serotypes present were identified by VN conducted in a similar way to those for BTV.

RESULTS

Culicoides *collection and identification*

During the present study a total of 9128 *Culicoides* comprising 16 species were collected. *Culicoides imicola*, the classical Old World BTV vector and *C. schultzei* group midges were by far the most common, together totalling 90·8% of the catch while the remaining 14 species only comprised 9·2% (Table 1). Four of the species, *C. arabiensis*, *C. buettikeri*, *C. ibriensis* and *C. neoschultzei*, were new to science at the time of collection and have since been described by Boorman [21].

Seroconversion and vector prevalence

The monthly distribution of seroconversions to BT is shown in Fig. 2. Conversions were recorded during every month of the year except November. In those farms near Rumais (Farms 12–31) the majority of seroconversions occurred in March, April or May (Fig. 2). This pattern may be related to the seasonal incidence of the vector species *C. imicola*, whose populations peak at Rumais between February and May, just subsequent to the rains in the area (Fig. 3).

Seroconversions near Nakhal (Farms 32 and 33) were recorded in January or April and peak populations of *C. imicola* in this area correspondingly occurred between January and May (Fig. 3).

Most seroconversions to BTV in the Khadhra Al Burashid/Musanaah area (Farms 7–11) were recorded during April and August, and those at Al-Khaboura (Farms 4–6) during April, June and August to October. At Sohar (Farms 2 and 3) and Shinas (Farm 1) few conversions occurred but those that did were scattered across January, February, June and October (Fig. 2).

Table 1. Culicoides *species collected in Oman between March 1987 and June 1988*

Species	Number	Percent of total	Species	Number	Percent of total
C. imicola	4632	50·7	**C. arabiensis*	840	9·2
Schultzei group:			*C. azerbajdzhanicus*		
C. oxystoma	3656	40·1	*C. badooshensis*		
C. kingi			**C. buettikeri*		
**C. neoschultzei*			**C. ibriensis*		
			C. leucostictus		
			C. mesghali		
			C. odai		
			C. odiatus		
			C. pycnostictus		
			C. ravus		
			C. wardi		

* New species.

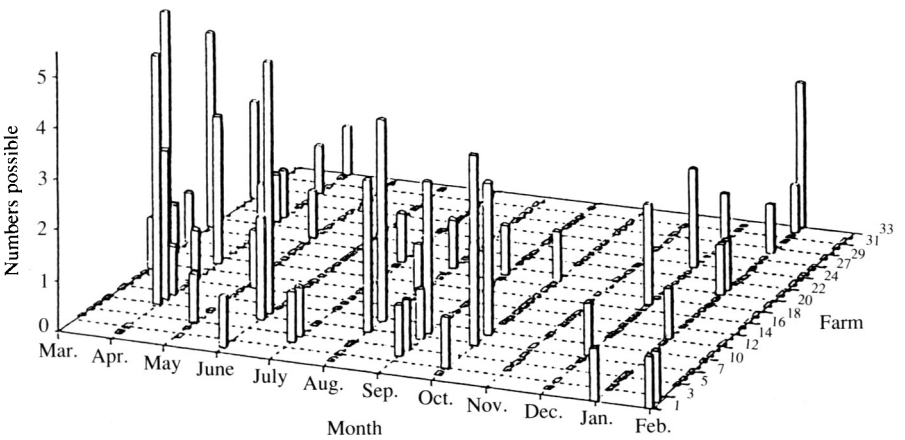

Fig. 2. Bluetongue seroconversion in sentinel herds in N. Oman from March 1987 to February 1988.

The monthly distribution of seroconversions to EHDV-2 in Northern Oman is scattered over 7 of the 12 months of the year, with most transmissions (conversion rate) being recorded between August and January (Fig. 4). This is a different pattern to that shown by BTV and may be related to the fact that these two groups of virus seem to be transmitted by different species of *Culicoides*. Strains of EHDV have been shown to be transmitted by *C. schultzei* group midges in the Sudan [20]. However, in Northern Oman peak populations of this group of insects occur at Rumais between April and November (Fig. 5) which is not the time of maximum seroconversion to EHDV-2 in the Rumais area (Fig. 4). Indeed at Rumais itself (Farm 18) no seroconversions to EHDV-2 were recorded at all, even though *C. schultzei* group midges were collected there all through the year (Fig. 5).

The incidence of EHDV-318 was extremely low and seroconversions to this virus only occurred at five farms (0·2% conversions) during January and

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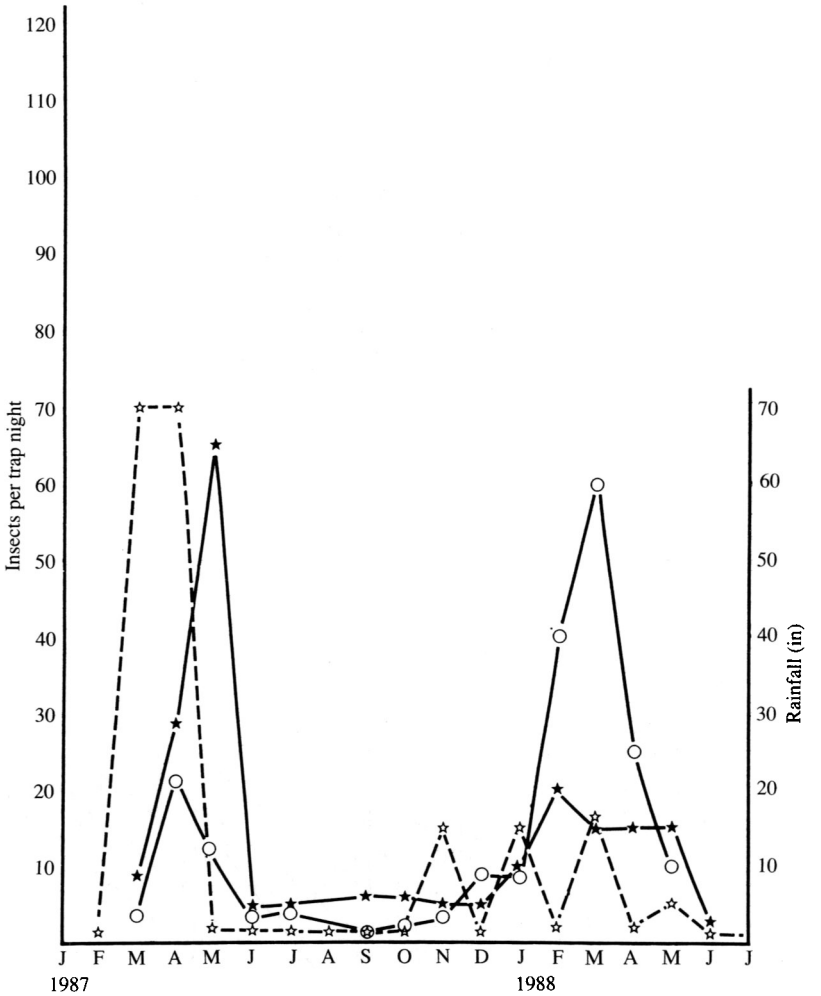

Fig. 3. Seasonal incidence of *Culicoides imicola* at Rumais and Nakhal in N. Oman. ★—★, *C. imicola* at Rumais. ○—○, *C. imicola* at Nakhal. ☆—☆, Rainfall (in) at Seeb.

February 1988. No evidence of infection with EHDV-1 was seen in any of the 34 sentinel herds in Oman during the entire period of observation.

In Salalah in Southern Oman (Fig. 1) out of 77 calves examined no antibodies could be detected to either BTV or EHDV-1 but 14·3% (11/77) and 7·8% (6/77) did have antibodies against EHDV-2 and EHDV-318 respectively.

Detection of BTV type-specific antibodies

Selected serum samples from 138 animals known to possess BTV group-specific antibodies were tested by VN against BTV serotypes 1–24. Monospecific responses were detected against BTV serotypes 3 and 4. However, most animals had developed neutralizing antibodies to between 2 and 5 BTV serotypes and antibodies were detected against BTV 3, 4, 17, 20 and 22 (Table 2).

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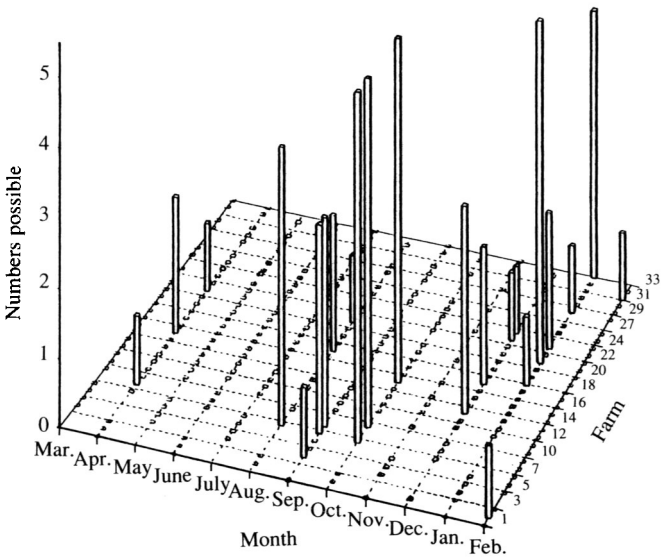

Fig. 4. EHD-2 seroconversions in sentinel herds in N. Oman from March 1987 to February 1988.

DISCUSSION

There has been much discussion on the reliability of BTV VN tests as a means of deducing the types of virus present in an area. Jeggo and colleagues [22] and Campbell [23] showed that a sequence of infections with different BTV types was likely to produce a broadly cross-reactive antibody response from which little meaning could be extracted. However, Taylor and co-workers [24] argued that in a number of instances, including some where sentinel calves unlikely to have experienced previous episodes of infection were used, credible results could be obtained. It is on the basis of Taylor's views that we have designed the sentinel herd system of serologically naive animals used here. Using this system we have detected monospecific responses to BTV serotypes 3 and 4 and we therefore believe that both these serotypes were circulating in Oman during the course of this study. However, most of the animals in the sentinel system developed antibodies to more than one serotype, and mixed responses to BTV types 3, 4, 17, 20 and 22 were recorded. Because BTV types 17 and 20 are closely related to BTV type 4 and because antibodies to these serotypes were never observed in the absence of antibodies to type 4 and were seen more rarely, it is likely that these are merely heterotypic responses and do not indicate the presence of serotypes 17 and 20. However, BTV type 22 is not closely related to any other BTV serotype. The presence of mixed responses which include antibodies specific to BTV 22 therefore do suggest that this serotype was circulating at low level in Northern Oman during this study.

Some previous serological indications of the BTV types circulating in Oman and also Saudi Arabia have been given by Herniman and colleagues [13], Hedger and colleagues [14], Al-Busaidy [15], Hafez and Taylor [16] and W. P Taylor, S. M. Al-

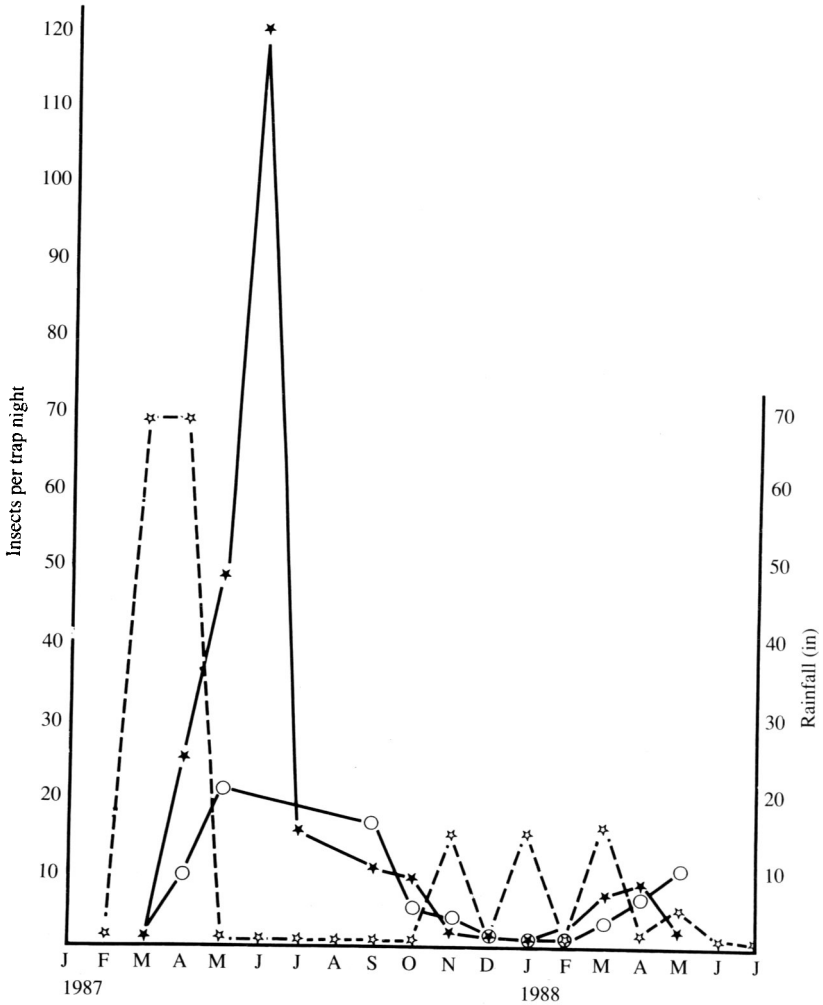

Fig. 5. Seasonal incidence of *Culicoides schultzei* group midges in N. Oman from March 1987 to February 1988. ★—★, *C.schultzei* group at Rumais. ○—○, *C. schultzei* group at Nakhal. ☆—☆, Rainfall (in) at Seeb.

Busaidy, E. Potter and P. S. Mellor (unpublished observations). Together these authors reported the presence of antibodies to 14 BTV serotypes. Monospecific responses, however, were only recorded for serotypes 3, 6, 16, 17, 19 and 20, and to these the present study has now added serotypes 4 and 22. North of the Arabian Peninsula serotypes 3, 4, 6 and 16 have also been recorded at various times in one or more of Israel, Cyprus, Syria, Jordan, and Turkey [25]. This overlap in the BTV serotypes circulating in the Arabian Peninsula and further north, suggests that there may be some relationship between the two ecosystems. However, such a relationship is likely to be fairly tenuous one since there is no evidence to suggest that BTV serotypes 17, 19, 20 and 22 from the Arabian Peninsula have ever been recorded in the countries further north. It may be that southerly movement of BTV serotypes has occurred, either through the agency of infected *Culicoides* carried on the wind as suggested by Al-Busaidy and colleagues for Akabane virus

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Table 2. *Antibodies to bluetongue virus serotypes recorded from sentinel herds in N. Oman 1987–8*

Farm no.	No. animals + ve† Total no.	%	Number of animals with neutralizing antibodies to BTV 3	BTV 4	BTV 17	BTV 20	BTV 22
1	2/25	(8·0)	1	1		1	
2	1/20	(5·0)		1*			
3	5/20	(25·0)		4	1		
4	2/11	(18·2)	2*				
5	3/10	(30·0)		1	1	1	
6	1/10	(10·0)	1*				
7	11/19	(57·9)	4	5	2	1	1
8	22/47	(46·8)	9	6	2		5
9	8/16	(50·0)	3	2			1
12	9/11	(81·8)	4	4		1	1
13	3/10	(30·0)	1	2	2		
14	1/19	(5·3)		1*			
15	8/10	(80·0)	2	5	1		
16	1/15	(6·7)		1	1		
17	5/10	(50·0)	4	1			1
18‡	1/20	(5·0)		1*			
19	4/11	(36·4)	3	1			1
20	1/16	(6·3)		1*			
21	23/30	(76·7)	16	5		3	
22	8/15	(53·3)		5		1	2
23	1/14	(7·1)		1*			
24	5/11	(45·5)		5		1	3
27	2/10	(20·0)		2	1		
28	3/20	(15·0)		2	2		
29	2/10	(20·0)		2	2		
32	1/11	(9·1)	1*				
33	5/28	(17·9)	3	4	3	1	

* Monospecific responses.
† AGID.
‡ Cattle.

[26] or by movement of viraemic animals, but northerly movements are seemingly less common. The wind systems over the area as described by Al-Busaidy and colleagues [26] would tend to encourage such southerly migrations of infected *Culicoides* and would oppose their northerly movement.

In the present study, it was noticed that sentinel herds on neighbouring farms quite often recorded wide variations in the level of BTV and EHDV activity. A similar phenomenon was observed with BTV by W. P. Taylor, S. M. Al-Busaidy, E. Potter and P. S. Mellor (unpublished observations). Possibly in Oman these viruses may operate in small ecological niches consisting of a vector breeding site and a group of susceptible animals and will remain in each niche only until most or all of the animals have become immune. During such a cycle spread to other farms can occur by movement of viraemic animals or by movement of infected vector midges. However, unless there is a local vector population present and a suitable breeding site the virus will die out. Should a suitable vector population be present then the virus may be able to establish itself at the new location until

all the susceptible hosts have been infected when it must move again. This sequence of events is well suited to the farming practice in Oman where the falaj system of flood irrigation is used on isolated farms, creating ideal local environments for vector midges to breed. Additional *Culicoides* breeding sites are also available in Oman in the form of numerous meandering wadis that occur in the mountainous terrain and some of which are filled with water all year round.

Peak numbers of vector *Culicoides* occur in Northern Oman during the spring, presumably as a result of the high rainfall usually experienced in this area at that time. In most areas of the North this will result in greatly increased numbers of breeding sites. However, it should be borne in mind that the rainfall in Oman, even in the spring, can be highly irregular and sporadic, consequently the spring rise in the numbers of *Culicoides* is also likely to be an irregular event. *Culicoides* population densities are much more likely to reflect local conditions and rainfall than the precise time of the year. Nevertheless, due to extensive irrigation is some areas (Nakhal) and the presence of water filled wadis some *Culicoides* breeding sites will always be present. As a result vector *Culicoides* can and do occur in variable numbers all through the year and this is why BTV and EHDV transmission is equally able to occur at any time. BTV and EHDV are therefore likely to be enzootic, at least in the short term, in Northern Oman.

The system of peripatetic 'virus hot spots' reported in this paper in conjunction with Oman's irregular and sporadic rainfall may help to explain the apparent lack of correlation between the population density of the EHDV vector *C. schultzei* and seroconversion to EHDV-2, in the Rumais area. *C. schultzei* midges were collected throughout the year at Rumais farm with population peaks between April and November but no evidence of seroconversion was seen at this farm. However, EHDV activity was seen on several neighbouring farms in the same area, though usually at times of the year when *C. schultzei* populations at Rumais were low. Due to the wide variations in local weather conditions that occur frequently in Northern Oman, particularly rainfall (S. M. Al-Busaidy, personal communication), it is possible that population densities of *C. schultzei* were higher at these farms during the times of EHDV seroconversion than the insect collections at Rumais would suggest. Despite this, however, it would seem that no infected midges straying from the farms where virus transmission was occurring succeeded in initiating an infection in susceptible stock at Rumais farm, or at a further 10 out of 19 other farms being monitored in the same area (Fig. 4). This is presumably so, either by mere chance, or because when it was most likely to happen (i.e. at times of high midge densities on 'infected' farms) conditions on uninfected farms were less suitable for *Culicoides* survival.

The results presented in this paper and also those of W. P. Taylor, S. M. Al-Busaidy, E. Potter and P. S. Mellor (unpublished observations) indicate that infection with BTV in Southern Oman occurs much more sporadically and at a much lower level than in the North. It is likely that Southern Oman is an epizootic area for BTV. Such evidence as is available on vector populations in Southern Oman suggests that *C. imicola* is far less prevalent at all times of the year than in the North (P. S. Mellor, unpublished observations). This is presumably the major reason why BTV has not been able to establish itself in Southern Oman since the number of susceptible vertebrate hosts in this area exceeds that in the North.

ACKNOWLEDGEMENTS

The authors wish to thank Mr J. Boorman of the British Museum (Natural History) for his expert identification of the *Culicoides* midges. They are also grateful to Miss S. Lamki of the Ministry of Agriculture and Fisheries, Oman, for typing the original manuscript and to the Staff of the Central Veterinary Investigation Laboratory, Rumais, for help in many ways. Similarly thanks are due to the Director of the IAH for permission to carry out this work at the Pirbright Laboratory, and also to the staff at that Laboratory for their willing assistance. Financial support was provided by the Government of the Sultanate of Oman and the British Council.

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