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Bluetongue in the Sultanate of Oman, a preliminary epidemiological study

W. P. TAYLOR[1], S. M. AL BUSAIDY[2] AND P. S. MELLOR[3]*

[1] *Ministry of Agriculture, Department of Agriculture and Cooperation, Krishi Bhavan, New Delhi 110001, India*

[2] *Department of Animal Health, Ministry of Agriculture and Fisheries, PO Box 467, Muscat, Sultanate of Oman*

[3] *Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey, GU24 0NF, Great Britain*

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SUMMARY

A group specific agar-gel immunodiffusion test was used to demonstrate that there is a frequent and widespread distribution of bluetongue virus throughout the Sultanate of Oman. The Culicoides midges *C. imicola* and *C. schultzei*, both capable of transmitting bluetongue group viruses, were recorded throughout the year. Although these studies did not establish that bluetongue is enzootic in Oman, type-specific neutralizing antibody results supported previous evidence for the existence of a Saudi Arabian bluetongue ecosystem. Variations in antibody evidence of virus activity within a restricted locality suggested a hot-spot theory concerning the perpetuation of the virus.

INTRODUCTION

Although the occurrence of bluetongue virus (BTV) in the Middle East region is well established [1–4] detailed information concerning its distribution on the Arabian peninsula is generally lacking. In Saudi Arabia, Hafez and Taylor [5] examined sera from domestic animals collected between 1977 and 1982 and attempted to deduce the virus types that had been prevalent. Hedger and colleagues [6] reported on a similar analysis undertaken in 1978 in the Sultanate of Oman.

With a view to gaining an improved understanding of the epidemiology of BTV in Oman a series of studies were started in 1983. The present paper records our initial results and provides a basis for the formulation of import policies covering the introduction of live sheep destined for slaughter.

MATERIALS AND METHODS

Serum samples

Strategies used for the collection of sheep and goat sera from the principal regions of Oman (Fig. 1) have been outlined elsewhere [7]. The same series of

*Correspondence and reprint requests should be sent to Dr P. S. Mellor.

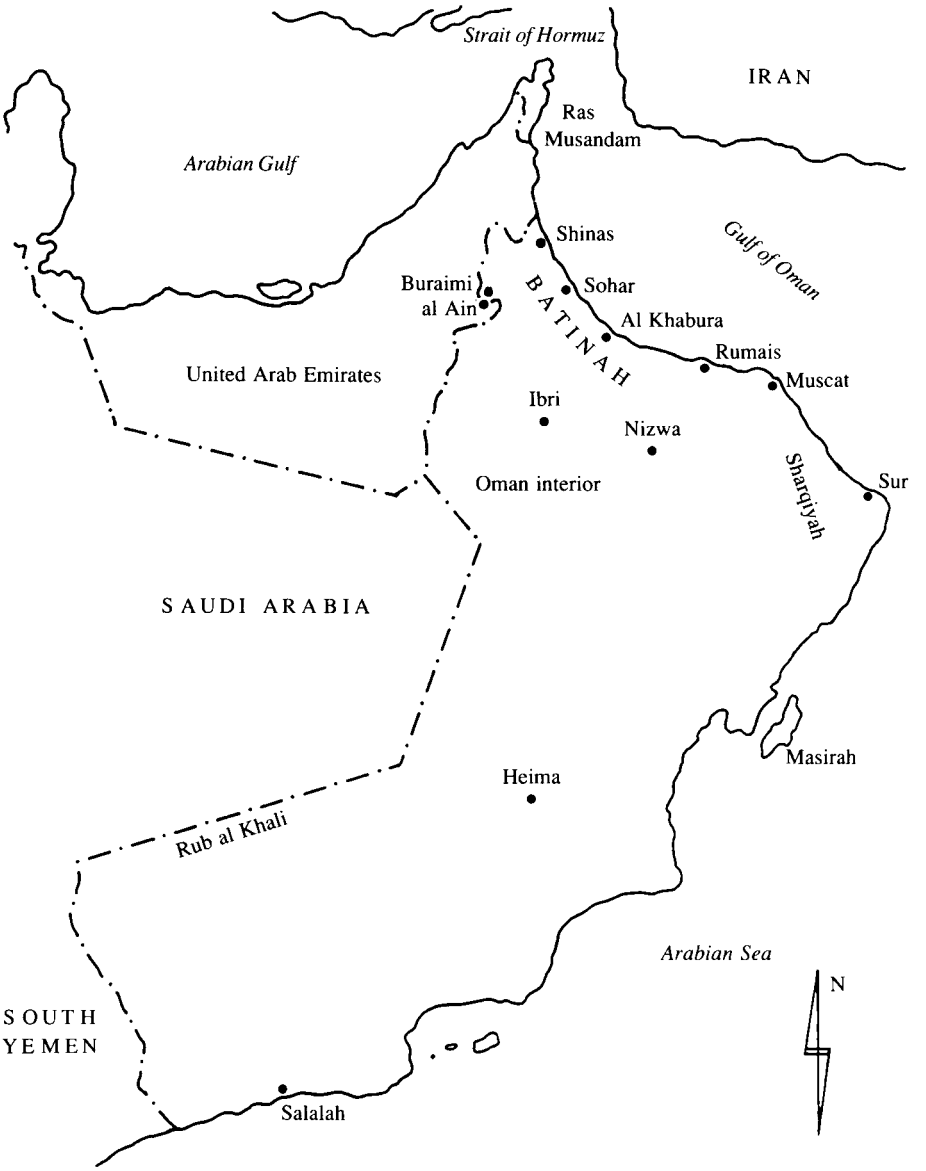

Fig. 1. The Sultanate of Oman, showing locations of serum sampling and insect collection.

samples, collected in 1983, were used in the present survey. In 1984 a small supplementary collection of goat sera was obtained from Heima, in the middle of the Omani desert.

In the northern regions of Oman (Batinah, Interior, Sharqiyah) cattle are uncommon, the entire population not exceeding 110000. Most are unimproved native animals kept for milk and maintained within household confines. Owners seldom possess more than one or two animals, which are permanently tethered and stall fed. Because of difficulties in finding and handling them these native animals

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were seldom bled. However, it was thought that established herds might play an important part in the epidemiology of bluetongue in Oman and accordingly they were sampled whenever possible. Significant collections were obtained from the Central Veterinary Investigation Laboratory (CVIL) near Rumais, the Nizwa Agricultural College and Sun Farms, Sohar.

In southern Oman the rain-fed grasslands of the Jebel al Qara, a mountain range dividing the coastal plain of Salalah from dry desert country to the north, are heavily grazed by native cattle. Although these animals were difficult to sample while on the Jebel, a destocking programme led to Jebali cattle being available for bleeding in cattle yards near Salalah. Samples were also collected from Friesian cattle belonging to Sun Farms, Salalah.

Samples were collected from the jugular vein into 20 ml draw evacuated bleeding tubes; after clarification sera were held at −20 °C. All tests were undertaken at the Institute for Animal Health, Pirbright. Sheep and goats were aged according to the pattern of incisor tooth eruption [8]. Cattle were aged from owners' records.

Serological tests

Group-specific antibodies to BTV were identified using the agar-gel immunodiffusion (AGID) test described by Lefevre and Taylor [9]. A selection of positive sera were titrated for neutralizing antibodies to BTV international serotypes 1–22 using the microplate method of Herniman and colleagues [10] and analyzed for evidence of serotype prevalence [11].

Insects

Certain species of *Culicoides* biting midges are known to be biological vectors of BTV [12]. During this study potential vector species of *Culicoides* were collected using Monks Wood light traps [13] at the following locations in Oman:

Northern Oman	Rumais (CVIL, cattle)
	Seeb (Royal Guard Stables, horses)
Central Oman	Heima (Veterinary Clinic)
Southern Oman	Salalah (Sun Farm Dairy, cattle)
	Salalah (Royal Stables, horses)

The light traps were operated when possible at the rate of two nights per week from February 1984 to February 1985. Insect collections made in this way were preserved in 10% formalin, labelled with the date and location of capture and despatched by air to the Institute for Animal Health for identification.

RESULTS

Distribution of bluetongue within Oman and species involvement

In 1983 cattle, sheep and goat sera were collected from the northern regions of Oman and from Salalah in the south. In 1984 sera were taken from goats belonging to nomadic herders in the central desert near Heima. The AGID results of these samples (Table 1) indicate that bluetongue is present in all parts of Oman, although there are no records of clinical disease.

Table 1. *Antibody evidence for the distribution of BTV in Oman*

Region	No. samples tested	Percentage AGID-positive
Batinah	336*	35·7
Interior	234*	37·2
Sharqiyah	101*	25·7
Heima	53†	90·6
Salalah	240*	40·4

*Collected in December 1983.
†Collected in November 1984.

Table 2. *Involvement of different species in the maintenance of BTV*

Species	No. animals tested	Percentage AGID-positive
Ox	264	37·5
Goat	501	49·9
Sheep	199	14·6

Table 3. *Prevalence of AGID antibodies in different age groups of Omani livestock sampled in December 1983*

Age of animal (years)	No. animals tested	Percentage AGID-positive
Yearling	138	19·6
1–1·5	320	29·4
1·5–2	151	43·7
2·5–3	57	64·9
> 3·5	248	60·5

When the positive results were analyzed by species, cattle and goats were seen to be equally involved in the maintenance of the virus but sheep were implicated to a lesser extent (Table 2).

Is BTV enzootic in Oman?

Although minor differences might occur it was considered unlikely that the basic transmission mechanisms varied between one region of the country and another. Therefore the results from all species were pooled and the incidence of infection compared with the age of the host. The results are shown in Table 3. The steady rise in the percentage of positive animals with age indicates that up to the end of 1983 BTV infection was an annual event in Oman.

Variation in the incidence of BTV within a particular region

Samples were taken at several locations in the Batinah region of northern Oman and the incidence of infection at the different sites compared (Table 4). Antibodies to BTV were to be found at each of the sampling sites but contrasting results were obtained. At Rumais and Khaburra the antibody incidence was low in the younger age groups of cattle, sheep and goats (0–18%) but higher in the older goats (41·6–62·5%). At Sohar, there had been intense BTV activity in cattle at the Sun

Table 4. *Incidence of BTV in different species from four locations on the Batinah coast*

Location	Species	Age of animal (years) 1–1·5	1·5–2	2·5–3	> 3·5	Percentage AGID-positive
Rumais	Ox	0/6	0/17	—	—	0·0
	Goat	1/24	1/14	2/4	5/8	18·0
Khaburra	Ox	1/2	0/2	1/1	—	40·0
	Goat	1/18	2/11	2/4	5/12	22·2
	Sheep	1/15	0/4	—	1/13	6·3
Sohar	Ox	17/21	19/21	11/11	7/7	90·0
	Goat	6/13	9/10	2/4	9/14	63·4
	Sheep	1/11	—	1/1	1/3	20·0
Shinas	Goat	7/8	3/4	1/1	5/5	88·9
	Sheep	0/9	0/1	—	1/4	7·1

Table 5. *Frequency of BTV neutralizing antibodies in sera from AGID-positive cattle, sheep and goats* in the Oman Interior and on the Batinah coast*

No. monospecific responses† to BTV types						
3	4	14	17	19	21	22
11	6	2	2	3	—	1
No. positive samples‡ in clusters to BTV types						
3	4	14	17	19	21	22
50	36	19	28	18	18	11

* 103 goats, 25 sheep and 9 cattle.

† Other responses were to BTV 7 (2) and BTV 20 (1).

‡ Other responses were to BTV 1 (5), BTV 2 (1), BTV 5 (4), BTV 6 (7), BTV 7 (2), BTV 8 (3), BTV 9 (2), BTV 10 (4), BTV 12 (1), BTV 16 (1), BTV 18 (3) and BTV 20 (7).

Farms involving animals of all ages. In addition a number of goats in two nearby farms had also been infected but sheep less so. At Shinas, 50 km from Sohar, goats again showed evidence of a more intensive involvement than sheep.

Serological evidence for the presence of specific types of BTV

Sera from nearly all positive sheep, goats and cattle collected on the Batinah coast and in the Interior were titrated for neutralizing antibodies to BTV types 1–22. Except for the collection of ox sera from Sun Farms, Sohar, the distribution of monospecific antibody responses and clusters are shown in Table 5. They indicate the present of BTV types 3, 4, 14, 17, 19, 21 and possibly 22 for the years prior to 1984.

As can be seen from Table 6 a pattern of infection that differed from those of other parts of the Batinah Coast or the Interior was obtained from the neutralizing antibody results from Sun Farms, Sohar (Table 6). The evidence suggested that types 4, 10, 17, 20 and 22 had been active on the farm within the 12 months preceding December 1983. No monospecific antibody responses were noted in any of the samples but BTV4 or less frequently BTV20 gave the highest antibody titre (80–640).

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Table 6. *BTV neutralizing antibody clusters in ox sera collected at Sun Farms, Sohar from animals of different ages*

Age of animals (years)	No. samples AGID-positive	No. with antibodies to types*				
		4	10	17	20	22
Yearlings	17/21	16	6	11	12	8
2	19/21	18	7	17	12	12
3–3·5	11/11	10	5	10	11	10
3·5–4	6/6	5	3	3	5	2
Combined	53/59	49	21	41	40	32

*Other responses were to BTV 2 (1), BTV 3 (6), BTV 9 (2), BTV 11 (4), BTV 12 (1), BTV 14 (8) and BTV 21 (1).

Table 7. *Precipitating antibodies in calves born in different months in 1983* in southern Oman*

Breed	Month of birth								
	Jan.	Mar.	May	June	July	Aug.	Sept.	Oct.	Nov.
Friesian	2/10	1/9	—	0/8	0/5	0/8	1/7	4/8	4/8
Jebali	3/6	1/3	2/8	0/7	—	—	—	—	—

*Sampled in December 1983.

Seasonality of bluetongue in Oman

The Salalah plains in southern Oman enjoy a seasonal monsoon between June and July during which windborne carriage of infected *Culicoides* might result in a bluetongue season. To assess this possibility cohorts of Friesian calves at Sun Farms, Salalah, born in various months during 1983 were bled to determine the time of infection. The AGID results are shown in Table 7 together with results from Jebali cattle held near Salalah.

The antibodies detected in calves born in September, October and November are assumed to be maternally derived, as these have a mean extinction time of 86 days when measured by the AGID test [14]. Possibly such antibodies would have protected calves born in June, July and August had virus been present before September but if present after September actively produced antibodies might have been found. Calves born between January and May could theoretically have been exposed to continuous infection from April to August however, the similarity in the rates of infection (16–32%) suggest the simultaneous infection of each cohort. It is clear from these results that bluetongue was present in Salalah in mid-1983 but the timing of infection cannot be deduced with greater certainty.

Culicoides

The results of the *Culicoides* collections for 1984–5 are shown in Table 8. A total of 3791 *Culicoides* comprising at least seven species were collected. Among these *C. imicola* (syn. *C. pallidipennis*), a known BTV vector from Africa and the Middle East [15, 16] and *C. schultzei* gp. midges, vectors of epizootic haemorrhagic disease

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Table 8. *Monthly totals of* Culicoides *spp from Oman*

	1984											1985	
	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Heima													
C. sp. indet.*	1	0	0	0	0	1	0	0	—	—	—	0	0
No. trap nights	4	8	8	7	9	8	8	6	0	0	0	8	4
Rumais													
C. imicola	18	29	11	53	68	75	81	93	63	118	79	102	16
C. schultzei gp.	1	6	11	29	37	197	244	344	80	24	9	21	0
C. azerbaidjanicus	0	3	7	3	0	0	13	55	12	8	0	1	1
C. mesqhalii	0	57	41	26	36	98	135	508	38	15	6	2	0
C. sp. indet.*	2	6	19	1	2	2	0	0	2	6	9	14	1
No. trap nights	3	5	5	4	4	4	8	7	8	9	8	7	1
Salalah (Sun Farms)													
C. imicola	11	9	5	0	1	1	1	9	39	6	2	1	0
C. schultzei gp.	58	49	79	40	12	5	11	4	0	3	1	2	2
C. distinctipennis	59	13	10	17	11	0	0	1	0	2	1	0	0
C. azerbaidjanicus	0	20	23	39	6	4	1	10	2	2	2	5	0
C. sp. indet.*	21	55	12	1	0	1	0	2	2	4	3	3	0
No. of trap nights	3	9	10	9	6	8	8	7	8	9	9	9	7
Salalah (Royal Stables)													
C. imicola	0	1	0	0	1	0	0	1	0	0	0	0	0
C. schultzei gp.	0	3	9	0	0	0	0	2	0	0	0	1	0
C. distinctipennis	1	5	2	0	0	0	0	0	0	0	3	0	0
C. azerbaidjanicus	0	0	0	1	0	1	0	0	0	0	3	0	0
C. sp. indet.*	2	16	3	1	1	0	0	0	0	0	0	2	1
No. of trap nights	2	9	7	9	8	7	8	7	8	8	9	9	7
Seeb (Royal Guard Stables)													
C. imicola	14	6	4	2	0	2	8	2	1	2	0	0	0
C. schultzei gp.	0	1	1	4	4	18	3	4	0	0	0	0	0
C. distinctipennis	0	0	1	1	0	0	0	0	0	0	0	0	0
C. azerbaidjanicus	0	2	0	2	3	2	0	2	1	0	0	0	0
C. mesqhalii	0	2	0	0	0	0	0	0	0	0	0	0	1
C. sp. indet.*	5	6	9	1	0	0	0	0	2	0	0	0	0
No. of trap nights	4	8	9	9	8	9	9	8	9	9	9	9	8

*Unidentified *Culicoides* species.

viruses in Africa [17] were particularly common. Both these species of midge were caught throughout the year but they were most numerous during July to September. They are present in northern and southern Oman and are more common at Rumais then elsewhere. This finding may be due in part to the heavy insecticide regimes used at both the Royal Stables and the Royal Guard Stables.

Only two specimens of *Culicoides* were recorded from Heima in central Oman, but it should be noted that the trap at Heima, unlike all other light traps, was not placed adjacent to animals.

DISCUSSION

These results and previous studies [2, 6, 14] indicate that bluetongue virus is present throughout Oman and that domestic ruminants are involved to a varying extent in its maintenance. In addition an unexpected finding was that goats in the extremely harsh desert regions of central Oman had been infected.

Evidence of rising antibody prevalences with age suggest a series of years in each of which widespread infection with bluetongue occurred. However, this evidence could result either from the continuous presence of the virus or annual reinfections of an external source. Sentinel animal studies are needed to resolve this problem which could obviously be more complex than either of these possibilities. For instance Al Busaidy and colleagues [18], found evidence to suggest that Akabane virus, also transmitted by *Culicoides* had reached Oman but was neither enzootic nor annually reintroduced. They described wind systems that could transport African insects to southern Oman during the summer months or West Asian insects to northern Oman during the winter months. The limited data from the calves held in Salalah, southern Oman, suggest a seasonal entry rather than an enzootic situation.

The results of *Culicoides* collections in 1984 and 1985 indicate that populations of adult *C. imicola* and *C. schultzei* gp. midges are present all the year in northern and southern Oman. Thus BTV could be transmitted in Oman throughout the year. If 1984 represents a typical year maximum numbers of vector midges are present during July to September. However, as the present study was not designed to investigate seasonal variations in virus activity the possibility that virus transmission might also be at its highest during these months remains conjectural.

Very few *Culicoides* were recovered from central Oman at Heima yet 37·2% of goats belonging to Bedu of this area were recorded as being seropositive to BTV in 1983 (Mellor, unpublished results) as well as the 90·6% of goats in the present study. It is probable that the light-trap collections of midges made at the veterinary clinic at Heima were not an accurate reflection of the number of *Culicoides* adjacent to the traditional watering places; where the population densities of *Culicoides* may be considerably higher.

Although *Culicoides* were recovered throughout Oman in 1984, the numbers collected were surprisingly low considering the proportions of seroconversion to BTV in cattle, sheep and goats in the preceding years. It may be that the actual numbers of *Culicoides* present in Oman are much higher than our light-trap collections suggest, a situation that could well apply since *Culicoides* frequently have a patchy distribution throughout their range. Conversely it is possible that

the *Culicoides*–BTV transmission cycle in Oman is particularly efficient or else that BTV transmission in Oman takes place at a relatively low level but throughout most of the year. Whichever suggestion is nearer the truth, the *Culicoides* data that we have at the moment suggests that the intensity of transmission is unlikely to be sufficiently high to maintain BTV in an enzootic situation in Oman. The exception may be along the Batinah coast but even here it is unlikely that BTV would be able to persist for more than a few years without periodic incursions from outside the country.

Monospecific neutralizing antibody responses and neutralizing antibody clusters in the sera of young resident animals can provide evidence of the BTV serotypes circulating in a given area [11]. In investigations on the presence of BTV in the Caribbean and Central America [19, 20] these methods suggested the possible presence of types 1, 6, 12, 14 and 17. Subsequently types 1, 6 and 12 were isolated repeatedly from different parts of the region (E. P. J. Gibbs and Jane Homan, personal communication, 1989) thus validating the cluster approach as an epidemiological tool for investigating the distribution of bluetongue serotypes.

In the present study, the antibody results in young stock revealed clusters to types 3, 4 and 17 and, when animals of all ages were assessed, additional clusters to types 14, 19, 21 and 22 became apparent. Except for type 21, clusters were backed by the presence of monospecific antibody responses.

Herniman and colleagues [2] used high-titred monospecific responses to deduce the presence of BTV types 3, 6, 17 and 20 in Oman, while the less specific communication of Hedger and colleagues [6] provided evidence for types 1–3, 14–17 and 20. A re-examination of the original data from Hedger and colleagues [6], (K. A. J. Herniman, unpublished results, 1979) indicates that clusters existed to types 15–17 and 20 (types 18 and 19 were not included in the tests), that large numbers of high monospecific responses occurred to types 16 and 20, but that evidence for the presence of types 1–3 and 14 is less convincing. Taken with the findings of Hafez and Taylor [5] a pattern of virus types common to the Arabian peninsula begins to emerge (Table 9), suggesting a discrete virus ecosystem somewhat different from that identified in Jordan, Syria and eastern Turkey [21].

The antibody investigations at Sun Farms, Sohar showed a similar intensity of infection in all age groups suggesting that a virus, or viruses, infected the entire herd in 1983 rather than in a series of infections during the preceding years. Most of the responses were to a group of four serotypes (4, 10, 17 and 20) known to share strong cross-relationships within the bluetongue group [22] and it is suggested that when a single member of this complex has recently been active in a herd, the evidence might resemble that seen at Sohar. As the responses to BTV4 were persistently higher than to any other type and it is likely that this was the principal infecting virus type.

The extent of virus activity in 1983 in cattle at Sun Farms, Sohar contrasts with other farms on the Batinah coast where activity had been either scant, non-existent or equally intense but in a different species. A similar difference in localization of virus activity was noted near Ibri in the Oman interior where, on one farm, 100% of samples came from sheep and goats that had experienced bluetongue (8/8 yearlings, 6/6 over 3·5-year-olds) while on another farm, 1 km away, only 10% of animals (all sheep) had been infected (0/5 yearlings, 1/5 over

Table 9. *Arabian bluetongue types after various authors*

Herniman et al. (1980) [2]	–	3	.	6	.	.	3	17	.	20	.	.
Hedger et al. (1980) [6] (revised)	–	.	.	.	.	15	16	17	.	20	.	.
Hafez & Taylor (1985) [5]	–	.	.	6	14	.	.	17	19	20	.	.
Oman survey: present paper (1991)	–	3	4	.	14	.	.	17	19	.	21	22

3·5-year-olds). At village level, a similar pattern was frequently seen with samples collected in northern Syria (W. P. Taylor, unpublished results).

On the African continent, and probably elsewhere bluetongue activity commences at well defined times of the year, in association with major weather changes [23, 24] which are probably responsible for translocating infected *Culicoides*. This type of virus survival may be considered to represent the macroecology of bluetongue while the patterns that unfold at farm and village level may be thought of as its microecology; it is here that something approaching an understanding may now be emerging. Hugh-Jones and colleagues [25] showed that in Louisiana State, USA, seroprevalence data could be mapped along contour lines to indicate gradients surrounding areas of intense virus activity ('hot-spots'), falling away to areas where virus activity was non-existent or at an extremely low level. Perhaps then, the whole of bluetongue microecology may be seen in terms of hot-spots. These can be defined as areas of limited size initially containing many susceptible livestock together with one or more breeding sites of a competent vector. Within these areas infected *Culicoides* ensure transmission to all or most animals and so ultimately the centre of the hot-spot must degrade through lack of susceptible hosts. If, in addition, the insects move only short distances from their breeding sites and become steadily more dispersed the further they travel it follows that, decreasing levels of activity are to be expected as the distance from the breeding site increases. However, should a female *Culicoides* infect a susceptible host in an area of low virus activity but where an uninfected population of *Culicoides* is on hand a new hot-spot may develop. It is certainly possible to view the Louisiana data as supporting the changing nature of areas of intense virus activity.

In Oman, Sun Farms Sohar, could be considered as being a hot-spot with some virus activity overflowing into neighbouring sheep and goat flocks. This suggestion might explain the puzzling variation in distribution of BTV infection that occurs in Oman and elsewhere [26].

In the short term the presence of bluetongue virus can have only limited economic significance for Omani livestock. The absence of widespread disease reporting, suggests that indigenous and adapted breeds of cattle, sheep and goats have a high degree of innate resistance to clinical disease although the situation could change if more virulent strains were to gain entry or if host resistance was to be lowered by cross-breeding. Meanwhile, it would be prudent to vaccinate imported Merinos or spray them with a long-acting insect repellent and/or insecticide.

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