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Isolation and identification of arboviruses from the Sultanate of Oman

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SUMMARY

Sentinel herds and a vector surveillance system were used to identify the presence of arboviruses in Oman. Two strains of bluetongue virus (BTV) serotype 4 and two strains of Akabane virus, were isolated and identified. Both BTV isolates and one Akabane virus isolate came from goats while the second Akabane isolate came from *Culicoides imicola*. This is the first isolation of an Akabane virus from *Culicoides* in Arabia. Vector competence studies with the Oman viruses in laboratory reared *C. variipennis* showed that after oral infection both viruses replicated in *Culicoides* and were maintained at high titre for at least 10 days post infection.

INTRODUCTION

In previous studies [1–4] serological evidence for the presence of Akabane virus (Simbu serogroup of family Bunyaviridae) and bluetongue virus (BTV) (genus Orbivirus of family Reoviridae) has been reported throughout the Arabian Peninsula but so far there have been no determined attempts to isolate these or any other arboviruses in that part of the world. The only exception is the isolation in 1984 of an untyped epizootic haemorrhagic disease virus designated EHD 318 from cattle in Bahrain (Dr W. P. Taylor, personal communication). As a sequel to the earlier epidemiological studies of Al-Busaidy and colleagues [1] a more detailed investigation was undertaken in Oman during 1987–8. This was designed to monitor arbovirus activity over a 12-month period using the sentinel herd technique described by Herniman and colleagues [5]. This technique makes use of the regular sampling of blood and serum from serologically naive animals at intervals of 2–4 weeks. Evidence of virus infection is detected initially by seroconversion. Then, because neutralizing antibodies take approximately 14 days to develop, virus isolation may be attempted from stored whole blood samples taken 2–4 weeks prior to seroconversion. Accordingly it was planned to collect blood samples from sentinel animals on a monthly basis. In addition attempts were made to isolate viruses from potential *Culicoides* vectors. When assessing vector competence one of the necessary preliminaries is to isolate the virus or viruses in question from unengorged female insects because engorged females may

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contain virus in a recent blood meal [6]. Simultaneous recovery of the same virus or viruses from known vertebrate hosts of the insects in the same geographical area would provide significant additional information.

This paper describes for the first time the isolation and identification of arboviruses from vertebrate and insect hosts in the Sultanate of Oman.

MATERIALS AND METHODS

Sentinel herds

Thirty-four farms were chosen for study. These were situated either along the Batinah Coast to Rostaq (near Nakhal) in Northern Oman or at Salalah in Southern Oman (Fig. 1). A minimum of 10 animals per farm were identified by ear-tagging. Most of the animals were goats, except at Rumais Government farm and Salalah Dairy farm, where newly born calves were also used. Monthly bleeding was undertaken from March 1987 to March 1988 but whole blood for virus isolation work was collected only during January–March 1988. After collection, heparinized blood for virus isolation procedures and clotted blood for serological testing (Al-Busaidy and Mellor, unpublished observations) were despatched on wet ice to the Central Veterinary Investigation Laboratory (CVIL) at Rumais. There the heparinized blood was centrifuged at 3000 g for 10 min and washed three times with normal saline before an equal volume of OPG (0·5% w/v potassium oxalate, 0·5% w/v phenol, 50% v/v glycerine and 50% distilled water) was added. The mixture was stored at +4 °C until transportation on wet ice to Institute for Animal Health, Pirbright, England.

Insect collection

Insects were collected at Rumais Government farm between January and May 1988 using a Pirbright modification of the Monks Wood light trap [7]. Two methods of collection were undertaken. The first method was similar to that described by Herniman and colleagues [5] where insects were collected in a Kilner jar containing a solution of Parke Davis additive media [(PDAM) 8% calcium lactobionate, 2% bovine plasma albumin, 90% Eagle's basal medium] plus 0·1% detergent as a wetting agent and the following antibiotics (0·075 mg/ml neomycin, 0·05 mg/ml streptomycin, 20 i.u./ml penicillin, 50 i.u./ml polymyxin and 25 i.u./ml mycostatin). The second method involved the collection of insects in a cage covered with 30 cm^3 gauze and containing damp paper towels to prevent desiccation [8]. *Culicoides* species were identified under a binocular stereo-microscope within 12 h of capture. Male *Culicoides* were discarded because they do not take blood and because there is no evidence to suggest the transovarian transmission of viruses by *Culicoides*. The females were separated into blood-engorged and -unengorged groups.

Unengorged females were further subdivided according to species, into three groups, *C. imicola*, *C. schulzei* group and *C. other species*. Blood-engorged female *Culicoides* were not included in the virus isolation procedures since a positive isolation may refer only to virus in the existing blood meal. All *Culicoides* were kept in 0·5 dram micro-bottles with PDAM containing antibiotic solution and stored under −70 °C until sent to IAH, Pirbright, UK in liquid nitrogen.

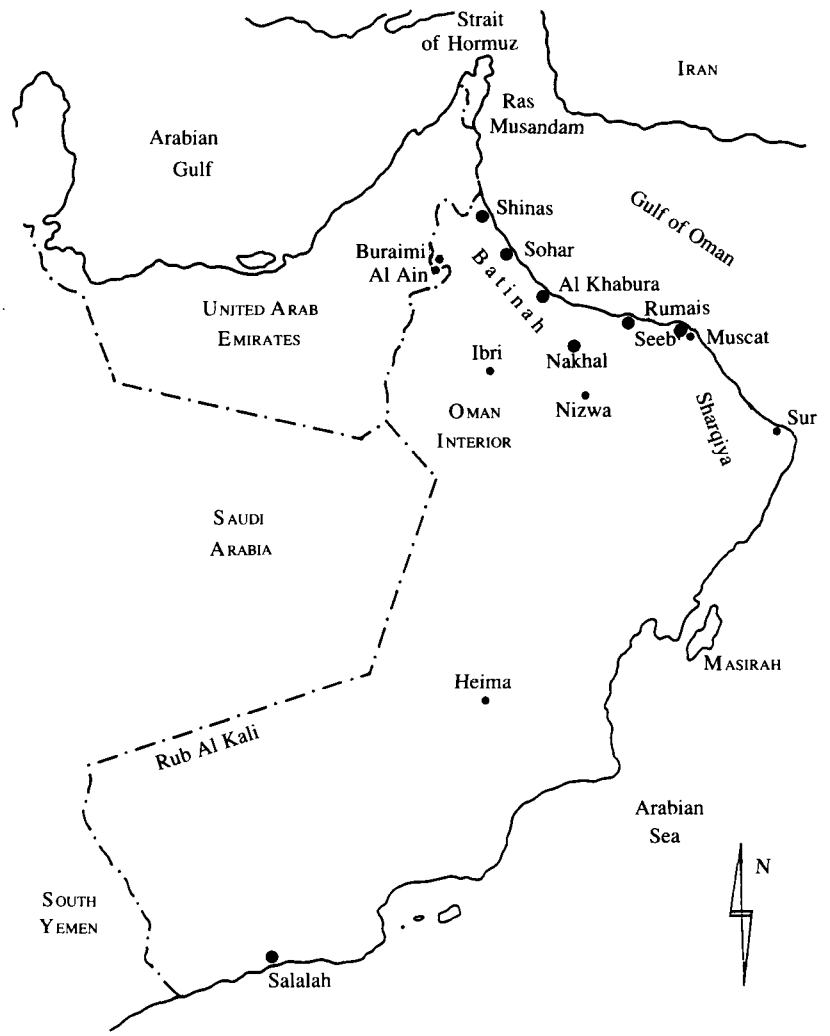

Fig. 1. The Sultanate of Oman.

Virus isolation

From blood. Blood samples were selected only from animals that had been seronegative by the agar gel immunodiffusion test (AGID) for BTV antibodies and the virus micro-neutralization test for Akabane virus antibodies at the time of the previous sampling 4 weeks earlier and had subsequently seroconverted by either or both tests. The blood samples used were those taken at the time of the last 'bleed', prior to seroconversion [1, 9, 10]. Tenfold dilutions of blood were made in phosphate buffered saline (PBS) supplemented with 0·2% bovine serum albumin (BSA) plus antibiotic stock. Virus isolation was attempted from each dilution by intravenous (I/V) inoculation into 11-day-old embryonated chick eggs, using 5 eggs per dilution [11], inoculation of BHK-21 cells [6] and

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intracerebral (I/C) inoculation of suckling mice (2–3 day old) using a 5 mice per dilution [12–14].

From insects. Sorted, unengorged *Culicoides* species were divided into groups of about 50 insects which were ground in Griffiths tubes containing 3·6 ml of 0·2% BSA in PBS plus antibiotic solution. The suspensions were clarified by centrifugation at 3000 *g* for 5 min. Virus isolation was attempted from each group using methods similar to those already described above. Attempts were also made to isolate virus in an insect cell line (*Aedes albopictus*). Eggs and mice which died between 2 and 7 days after infection and cell cultures showing cytopathic effects (cpe) within the same time interval were harvested and stored at −70 °C until required. Blood and *Culicoides* samples were passaged in eggs and cell cultures at least twice before being declared negative. Virus isolates were adapted to BHK-21 cell cultures and were then plaque purified three times. Virus titres were amplified in BHK-21 cells before being mixed with equal volumes of PDAM and stored in 1 ml aliquots at −70 °C until used for further tests.

Virus identification

Electron microscopy. Droplets of supernatants from virus infected BHK-21 cell monolayers which had been individually infected with each virus isolate and which showed 80–100% cpe were absorbed onto formvar coated copper grids for 1 min. These were then stained with either 2% methylamine tungstate or 2% phosphotungstic acid. Examination for virus particles was carried out at magnifications of up to 202K.

Agar gel immunodiffusion test. Soluble antigens for each of the virus isolates were prepared using ammonium sulphate precipitation of the supernatant following growth of the viruses in BHK-21 cell monolayers [15]. Each soluble antigen was tested against a number of known positive standard reference antisera using the method of Mohammed and Taylor [16]. The test virus soluble antigen was placed in the central well and the reference antisera were placed in the peripheral wells. The slides were examined daily for 3 days and the final reaction recorded.

Virus micro-neutralization test. The test was performed according to the method of Herniman and colleagues [5], using neutralization by heterologous antisera. All the reference antisera which had reacted with each virus antigen in the AGID group specific tests were examined for their ability to neutralize that virus in virus neutralization tests using constant serum and varying virus dilutions. Other reference antisera from the same serogroup as those antisera which gave positive reactions in the group test were included where possible. The isolates were checked against all 24 known serotypes of BTV [17], EHDV serotypes 1–4, EHDV-318 (untyped), Ibaraki, Eubenangee, Tilligerry and Akabane viruses. Where a relationship between an Oman virus isolate and a reference antiserum became apparent further testing was undertaken.

Reciprocal cross-neutralization tests. Where a relationship between an Oman isolate and one or more reference viruses became apparent in virus micro-neutralization tests a reciprocal cross-neutralization test was performed to further establish their serological identity. Antisera raised in sheep against each of the four Omani isolates and also the reference antisera which had previously neutralized each isolate were used.

Infection of colonized C. variipennis. Virus isolates (Oman 5557 and 11) which

Table 1. *Non-engorged female* Culicoides *collected for virus isolation in Oman from January–April 1988*

Species	Numbers	Percentage
C. *imicola*	5135	84·3%
C. *schultzei* (group)	403	6·6%
C. *other species**	557	9·1%
Total	6095	

* C. *other species*. The following are included in this group; *C. arabiensis*; *C. azerbajdzhanicus*; *C. badooshensis*; *C. bueltikeri*; *C. ibriensis*; *C. leucostictus*; *C. mesghali*; *C. odai*; *C. odiatus*; *C. pycnostictus*; *C. ravus*; *C. wardi*

had been plaque-purified at least three times and adapted to grow in BHK-21 cells were fed separately to colonized *C. variipennis* using the membrane feeding technique described by Mellor and colleagues [18]. After feeding to repletion on the virus suspensions, the engorged insects were incubated at 27 °C until required. Groups of flies were then assayed for virus concentration in BHK-21 cells. The method of Spearman-Karber [19] was used to calculate the virus titre ($\log_{10}$ $TCID_{50}$ per fly) in pools of infected insects.

RESULTS

A total of 261 blood samples from different animals and 6095 unengorged female *Culicoides* (Table 1) collected over a period of 4 months were analyzed for the presence of virus. Four viruses were isolated, all by inoculation of the embryonated chick eggs (Table 2) Three, Oman 5557, 2 and 47, were isolated from blood samples and one (Oman 11) was isolated from a group of 50 *C. imicola* collected during March 1988. All four viruses caused haemorrhage and death in chick embryos 2–5 days after infection. Subsequently the viruses also caused cpe in BHK-21 cells after 24–36 h.

Identification of viruses

Electron microscopy. Figure 2 shows electron micrographs of the four Oman isolates. Isolates 5557 and 2 consist of spherical virus particles between 60 and 70 nm in diameter which had the diffuse appearance typical of Orbiviruses [20]. The particles of isolates 47 and 11 were very fragile and pre-treatment with 3% glutaraldehyde was necessary to maintain particle integrity [21]. Subsequent to this treatment, spherical virus particles between 75 and 90 nm in diameter were seen. These exhibited an envelope with visible peplomers and were typical of Bunyaviruses [21].

Agar gel immunodiffusion tests. The results of the AGID tests are shown in Table 3. Each of the four soluble antigens prepared was tested for the presence of group-specific precipitation lines against known positive reference antisera to 14 orbiviruses, one Bunyavirus (Akabane), and one Rhabdovirus (Bovine Ephemeral Fever). The soluble antigen of isolate 5557 precipitated BTV serotype 1 and Tilligerry antisera while the antigen prepared from isolate 2 formed precipitation lines with antiserum to BTV serotype 1 only. The soluble antigens prepared from isolates 47 and 11 precipitated Akabane antisera only.

Micro-neutralization tests. The results of micro-neutralization tests using heterologous reference antisera are shown in Table 4. Isolates 2 and 5557 were

Table 2. *Oman viruses: sample source and date of collection*

Virus code no.	Source	Date of collection
5557	Goat blood – Rumais	February 1988
47	Goat blood – Rostaq	March 1988
2	Goat blood – Nr. Al Khabura	February 1988
11	*C. imicola* – Rumais	March 1988

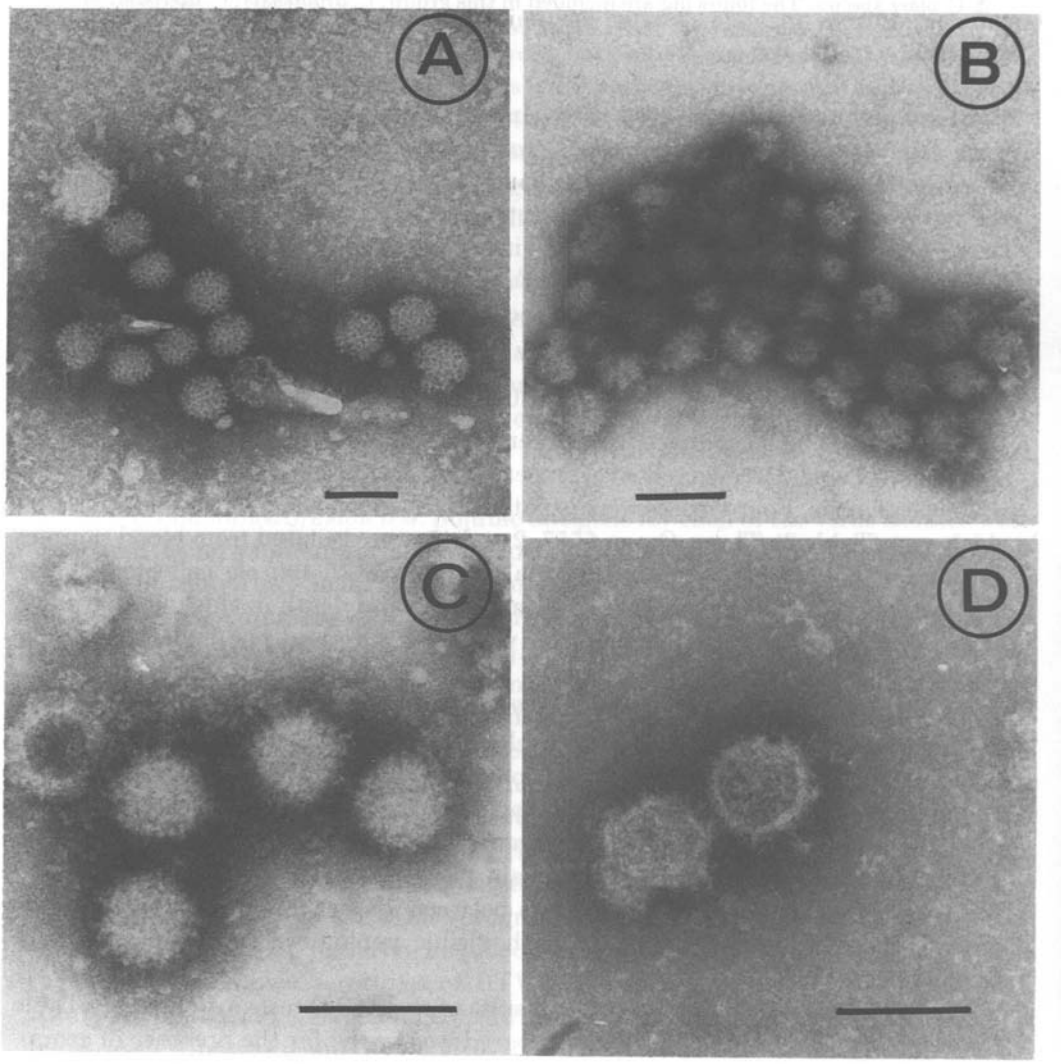

Fig. 2. Electron micrographs of: (A) Isolate 2, (B) Isolate 11 (C) Isolate 5557, (D) Isolate 47. Barline 100 nm.

neutralized only by the antiserum to BTV serotype 4 which reduced their infectivity 300 fold (2·5 $\log_{10}$ $TCID_{50}$) and 20 fold (1·25 $\log_{10}$ $TCID_{50}$) respectively. Isolates 47 and 11 were significantly neutralized by Akabane antiserum which reduced their infectivity by 30000 fold (4·5 $\log_{10}$ $TCID_{50}$) and by 3000 fold (3·5 $\log_{10}$ $TCID_{50}$) respectively.

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Table 3. *Agar gel immunodiffusion results of the soluble antigens of four Omani isolates against reference antisera to 14 orbiviruses 1 bunyavirus and 1 rhabdovirus*

	Soluble antigen			
Antserum	5557	2	47	11
AHSV1*	–	–	–	–
BTV1	+	+	–	–
Corriparta	–	–	–	–
EHDV1 (New Jersey)	–	–	–	–
EHDV2 (Alberta)	–	–	–	–
EHDV2 (Ibaraki)	–	–	–	–
EHDV3 (Ib Ar 22619)	–	–	–	–
EHDV4 (Ib Ar 33853)	–	–	–	–
EHDV-318 (untyped)	–	–	–	–
Eubenangee	–	–	–	–
Palyam	–	–	–	–
Su 48 (Palyam gp.)	–	–	–	–
Pata	–	–	–	–
Tilligerry	+	–	–	–
Akabane (bunyavirus)	–	–	+	+
BEFV (Rhabdovirus)†	–	–	–	–

* AHSV, African horse sickness virus.
† BEFV, bovine ephemeral fever virus.

Table 4. *Neutralization indices of Omani viruses against heterologous antisera**

	Viruses			
Antisera	Isolate 2	Isolate 5557	Isolate 47	Isolate 11
BTV 1-24	BTV 4 (2·5)	BTV 4 (1·25)	N.T.†	N.T.
EHDV1‡ (New Jersey)	—	—	N.T.	N.T.
EHDV2 (Alberta)	—	—	N.T.	N.T.
EHDV Ib Ar 22619	—	—	N.T.	N.T.
EHDV Ib Ar 33853	—	—	N.T.	N.T.
Ibaraki	—	—	N.T.	N.T.
EHDV (318)	—	—	N.T.	N.T.
Eubenangee	—	—	N.T.	N.T.
Tilligerry	—	—	N.T.	N.T.
Akabane	N.T.	N.T.	(4·5)	(3·5)

* Neutralization index (NI), $\log_{10}$ virus titre in normal serum minus $\log_{10}$ virus titre in the immune sera. —, NI < 0·5.
† N.T., not tested.
‡ EHDV, Epizootic haemorrhagic disease virus.

Reciprocal cross-neutralization. The results of reciprocal cross-neutralization are shown in Table 5. Isolates 2 and 5557 were shown to be serologically identical to each other and to reference BTV serotype 4 whereas isolates 47 and 11 were serologically identical to each other and to reference Akabane virus.

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Table 5. *Reciprocal cross-neutralization indices of Omani viruses and antisera against both homologous viruses and antisera, and heterologous viruses and antisera*

	Viruses					
Antisera	Isolate 2	Isolate 5557	BTV4	Isolate 47	Isolate 11	Akabane JAGar 39
2	> 3·5*	> 2·25	> 3·25	N.T.†	N.T.	N.T.
5557	> 2·0	> 1·75	> 2·5	N.T.	N.T.	N.T.
BTV4	> 2·75	> 2·25	> 3·5	N.T.	N.T.	N.T.
47	N.T.	N.T.	N.T.	> 2·25	> 2·75	> 2·0
11	N.T.	N.T.	N.T.	> 3·0	> 3·0	> 3·0
Akabane JAGar 39	N.T.	N.T.	N.T.	> 3·0	> 2·0	> 2·0

* $\log_{10}$ $TCID_{50}$.
† N.T., not tested.

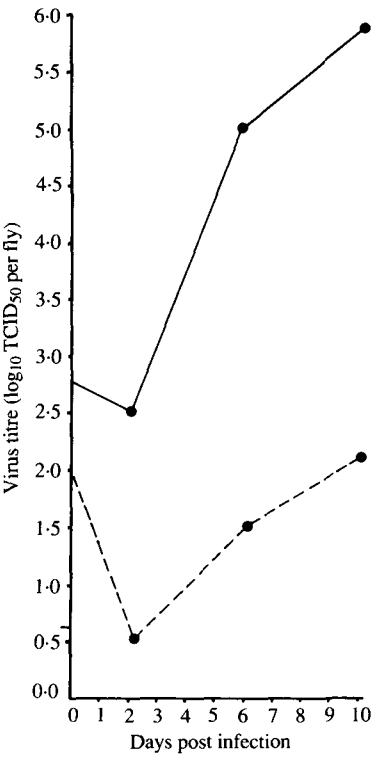

Fig. 3. Multiplication of Oman 5557 and 11 viruses in *Culicoides variipennis* after oral ingestion. ●—●, Oman 5557 virus (BTV 4); ●– –●, Oman 11 virus, (Akabane virus).

Replication of Oman 5557 *and* 11 *viruses in* C. variipennis *after oral ingestion*

Oman 5557 and 11 viruses both replicated in colonized *C. variipennis* after oral infection using artificial feeding techniques. The results are shown in Fig. 3. In flies infected with Oman isolate 5557 (BTV4) the virus titre per insect fell marginally

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during the first 48 h after the blood-virus meal and then increased rapidly to reach a mean titre of 5·9 $\log_{10}$ $TCID_{50}$ per fly after 10 days incubation. In flies fed Oman isolate 11 (Akabane virus) the virus titre fell sharply from 1·9 to 0·5 $\log_{10}$ $TCID_{50}$/fly during the first 48 h post infection and then steadily increased to reach a mean titre of 2·1 $\log_{10}$ $TCID_{50}$ per fly after 10 days incubation.

DISCUSSION

We report for the first time the isolation and identification of three arboviruses from vertebrate hosts and one arbovirus from a species of *Culicoides* in the Sultanate of Oman. This work was aided both by the serological monitoring of sentinel animals and the correlation of virus isolation with the time of seroconversion in these animals, and also by long term population studies of potential vector species of *Culicoides* (Al-Busaidy and Mellor, unpublished observations).

Isolates 5557 and 2 were identified as BTV serotype 4 and originated from goats. Isolates 47 (also from goats) and 11 (from *Culicoides*) were identified as Akabane virus. This is the first record of a strain of Akabane virus from *C. imicola* on the Arabian Peninsula, although Blackburn and colleagues [22] have previously isolated Akabane virus from this species of midge in Zimbabwe. These workers also isolated BTV-11, African horse sickness virus (AHSV-4) and a Bovine Ephemeral Fever virus from *C. imicola* in Zimbabwe and concluded that this species of midge is probably a major vector of each of these viruses in Southern Africa [22]. Our isolations of Akabane virus from *C. imicola* and from goats together with isolations of BTV4 in the Sultanate of Oman suggest that this species of midge is also likely to be a major vector of these arboviruses in the Arabian Peninsula. This likelihood is enhanced by the fact that *C. imicola* was the commonest species collected, constituting 84·3% of the total *Culicoides* catch in N. Oman with peak populations occurring in Spring, between February and May (Al-Busaidy & Mellor, unpublished observations) at the time of maximum virus transmission. However it is unlikely that *C. imicola* is the only important arbovirus vector in Oman. *C. schultzei* group midges are also common in the country and in some areas are more prevalent than *C. imicola* itself (Al-Busaidy and Mellor, unpublished observations). Virus was not isolated from *C. schultzei* in the present study, but only relatively small numbers of this species were examined. In the Sudan *C. schultzei* group midges have been implicated in the transmission of the BT-related epizootic haemorrhagic disease viruses (EHDV) [23], at least two serotypes of which occur in Oman (Al-Busaidy and Mellor, unpublished observations). Also, Akabane virus has recently been isolated from *C. schultzei* group midges in Japan [13]. *C. schultzei* midges have not been implicated as vectors of BTV and it is unlikely that they are significantly involved in the transmission of this group of viruses [23].

Unlike EHDV and BTV, which seem to be fairly specific in their vector requirements, Akabane virus has been isolated from a wide range of potential vectors, including unengorged mosquitoes [24, 25]. However, although both in the present work and previously [26], Akabane virus has been shown to replicate in species of *Culicoides*, attempts to demonstrate virus replication in mosquitoes have so far proved unsuccessful [27, 28]. Nevertheless in view of the number of

isolations from mosquitoes, this group of insects cannot yet be discounted as potential Akabane virus vectors.

Akabane virus replicates in some species of *Culicoides* but it is apparently less well adapted to this group of insects than is BTV. As reported both here and previously [26] Akabane virus invariably replicates to a lower titre and in a smaller proportion of individuals. It is therefore likely that this virus is transmitted less efficiently by *Culicoides* than is BTV. This may account for the observation that in areas where both viruses are epizootic BTV tends to occur more widely and over a longer period of time ([29], W. P. Taylor, personal communication).

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REFERENCES

1. Al-Busaidy SM, Hamblin C, Taylor WP. Prevalence of neutralizing antibodies to Akabane virus in the Arabian Peninsula. Vet Microbiol 1988; **17**: 141–9.
2. Hedger RS, Barnett ITR, Gray DF. Some virus diseases of domestic animals in the Sultanate of Oman. Trop Anim Health Prod 1980; **12**: 107–14.
3. Hafez SM, Taylor WP. Serotypes of bluetongue virus present in Saudi Arabia. In: Barber TL, Jochim MM, eds. Bluetongue and related orbiviruses. New York: Alan R. Liss, 1985; 531–7.
4. Taylor WP, Sellers RF, Gumm ID, Herniman KAJ, Owen L. Bluetongue epidemiology in the Middle East. In: Barber TL, Jochim MM, eds. Bluetongue and related orbiviruses. New York: Alan R. Liss, 1985; 527–30.
5. Herniman KAJ, Boorman JPT, Taylor WP. Bluetongue virus in a Nigerian dairy cattle herd. (a) Serological studies and correlation of virus activity to vector population. J Hyg 1983; **90**: 177–93.
6. Mellor PS, Osborne R, Jennings DM. Isolation of Bluetongue and related viruses from *Culicoides* spp. in the Sudan. J Hyg 1984; **93**: 621–8.
7. Service MW. A battery-operated light trap for sampling mosquito populations. Bull WHO 1970; **43**: 635–41.
8. Jennings DM, Mellor PS. The vector potential of British *Culicoides* species for bluetongue virus. Vet Microbiol 1988; **17**: 1–10.
9. Sellers RF, Taylor WP. Epidemiology of bluetongue and the import and export of livestock, semen and embryos. Bull Off Int Epizoot 1980; **92**: 587–92.
10. Parsonson IM, Della-Porta AJ, O'Halloran ML, Snowdon WA, Fahey KJ, Standfast HA. Akabane virus infection in the pregnant ewe. I. Growth of virus in the foetus and the development of the foetal immune response. Vet Microbiol 1981; **6**: 197–207.
11. Goldsmit L, Barzilai E. An improved method for the isolation and identification of Bluetongue virus by intravenous inoculation of embryonating chicken eggs. J Comp Path 1968; **78**: 477–87.
12. Boorman JPT, Draper CC. Isolation of Arboviruses in the Lagos area of Nigeria and a survey of antibodies to them in man and animals Trans Roy Soc trop Med Hyg 1968; **62**: 269–77.
13. Kurogi H, Akiba K, Inaba Y, Matumoto M. Isolation of Akabane virus from the biting midge *Culicoides oxystoma* in Japan Vet Microbiol 1987; **15**: 243–8.
14. Gard GP, Weir RP, Walsh, SJ. Arboviruses recovered from sentinel cattle using several virus isolation methods. Vet Microbiol 1988; **18**: 119–25.
15. Lefevre C, Taylor WP. Epidemiology of blutongue in Senegal. Revue d'Elev Med Vet Pays Trop 1983; **36**: 241–5.

16. Mohammed MEH, Taylor WP. Infection with bluetongue and related orbiviruses in the Sudan detected by the study of sentinel calf herds. Epidemiol Infect 1987; **99**: 533–45.
17. Huismans H, Cloete M. A comparison of different cloned bluetongue virus genome segments as probes for the detection of virus-specified RNA. Virology 1987; **158**: 373–80.
18. Mellor PS, Boorman JPT, Loke R. The multiplication of Main Drain virus in two species of *Culicoides* (Diptera, Ceratopogonidae). Arch ges Virusforsch 1974; **46**: 105–10.
19. Finney DJ. Statistical method in biological assay, 2nd ed. 1964, London: Griffin.
20. Mertens PPC. The genome segments of bluetongue virus: the proteins they encode and the relationship between different isolates by cross-hybridisation. Revue Sci Tech Off Epizoot 1986; **5**: 333–49.
21. Ito Y, Kurogi H, Takahashi, H, Goto Y, Inaba Y, Omori T. Electron microscopy of Akabane virus. Acta Virol 1979; **23**: 198–202.
22. Blackburn NK, Searle L, Phelps RJ. Viruses isolated from *Culicoides* (Diptera: Ceratopogonidae) caught at the Veterinary Research Farm, Mazowe, Zimbabwe. J Ent Soc Sth Afr 1985; **48**: 331–6.
23. Mohammed MEH, Mellor PS. Further studies on bluetongue and bluetongue-related Orbiviruses in the Sudan. Epidemiol Infect 1989; **105**: 619–32.
24. Oya A, Ogata T, Kobayashi I, Matsuyama T. Akabane. A new arbovirus isolated in Japan. Jpn J Med Sci Biol 1961; **14**: 101–8.
25. Metselaar D, Robin Y. Akabane virus isolated in Kenya. Vet Rec 1976; **99**: 86.
26. Jennings DM, Mellor PS. *Culicoides*, biological vectors of Akabane virus. Vet Microbiol 1989; **21**: 125–131.
27. Kay BH, Carley JG, Filippich C. The multiplication of Queensland and New Guinea arboviruses in *Culex annulirostris* (Skuse) and *Aedes vigilax* (Skuse) (Diptera: Culicidae). J Med Ent 1975; **12**: 279–83.
28. St George TD, Standfast HA, Cybinski DH. Isolation of Akabane virus from sentinel cattle and *Culicoides brevitarsis*. Aust Vet J 1978; **54**: 558–61.
29. Yonguc AD, Taylor WP, Csontos L, Worrall E. Bluetongue in Western Turkey. Vet Rec 1982; **111**: 144–6.