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INFECTION OF ISRAELI *CULICOIDES* WITH AFRICAN HORSE SICKNESS, BLUENTONGUE AND AKABANE VIRUSES

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Summary. — Type 9 African horse sickness virus and type 4 bluetongue virus multiplied to a high titre in an Israeli strain of *Culicoides puncticollis* after intrathoracic inoculation. Akabane virus persisted for at least 10 days in this midge after intrathoracic inoculation but with little evidence of virus multiplication. All 3 viruses failed to multiply in *C. puncticollis* after ingestion by the oral route and all were inactivated by 4 days post infection. Five other species of Israeli *Culicoides* supported multiplication of bluetongue virus after intrathoracic inoculation.

Key words: Culicoides; African horse sickness virus; bluetongue virus; Akabane virus

Introduction

The importance of *Culicoides* midges in the transmission of disease organisms to man and animals has only recently become apparent (Kettle, 1965) and their significance as vectors of viruses has yet to be fully realised.

Three of the most important viruses isolated from *Culicoides* are: bluetongue virus (BTV), African horse sickness virus (AHSV) and Akabane virus (AKAV). The first two are orbiviruses while the other is a member of the Simbu antigenic group of bunyaviruses.

AHSV has had a wide distribution through Africa, Asia and the Middle East. It was found by du Toit (1944) to be transmitted by *Culicoides* but the species involved was not identified and no positive identification of a natural vector has been made since, though transmission of the virus has been achieved in the laboratory (Mellor *et al.*, 1975).

BTV has a very wide distribution and occurs in North and South Africa, Asia, Australia and the Middle East. The transmission of BTV by *C. imicola* (= *pallidipennis*) was first demonstrated in 1944 (du Toit) and since that time the virus has been isolated from three other species of *Culicoides* in the African and Mediterranean regions: *C. milnei* and *C. tororoensis* (Walker

and Davis, 1971) and *C. obsoletus* (Mallor and Pitzolis, 1979). The virus has also been isolated from a pool estimated to consist of 11 species of *Culicoides* in Australia (Standfast *et al.*, 1978).

AKAV occurs in Asia, Australia and the Middle East. It has been isolated from mosquitoes in Japan (Berge, 1975) and also from pools of *C. brevitarsis* in Australia (Doherty *et al.*, 1972).

All three diseases have occurred in the Middle East where the vectors, with the exception of *C. imicola* from which BTV has been isolated (Braverman and Galum, 1973; Braverman *et al.*, unpublished), are so far unrecognised. It was therefore decided to test the vector potential of several species of *Culicoides* collected by one of us (Y. Braverman) in Israel. Particular attention was given to *C. puncticollis*, a midge whose close association with cattle and large domestic animals has long caused it to be suspected of being a vector of disease to these animals (Kitaoka, 1966; unpublished; Sellers, 1975).

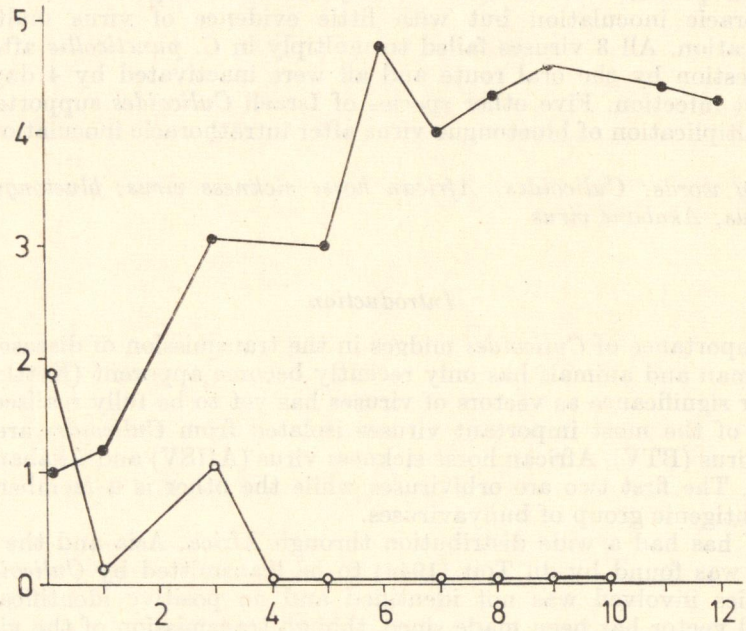

Fig. 1.

C. puncticollis infected with type 9 AHSV (titre of infecting virus 6.2—6.5 $\log_{10}$ $TCID_{50}$/0.1 ml Abscissa: days p.i.; ordinate: virus titre ($\log_{10}$ $TCID_{50}$ per fly) calculated from 5—25 insects per sample

● Intrathoracically inoculated insects

○ Membrane-fed insects

Materials and Methods

Culicoides. C. puncticollis were collected as larvae and pupae in mud samples from breeding sites along the Beer Sheva-Dimona road in Central Israel (map reference 34°13' East; 31°13' North) and were dispatched to the U.K. by air freight. On arrival at Pirbright the samples were dispensed into enamel trays. Adult midges emerged over a period varying from 1 to 49 days. The adult midges were maintained in 3-inch diameter waxed paper pill boxes in conditions similar to those described in Mellor *et al.* (1974), and 10% sucrose solution was provided as food. The other species of *Culicoides* used were collected in light traps in Israel and were transported to the U.K. as adults. These midges were also maintained in waxed paper pill boxes under conditions similar to those used for *C. puncticollis*.

Viruses. Type 9 AHSV was obtained as a freeze-dried suckling mouse brain suspension from the Veterinary Research Institute at Onderstepoort, South Africa. It was used to infect *Culicoides* after a further 19 passages in suckling mice. Type 4 BTV was isolated from sheep on the island of Cyprus during the 1969 outbreak of the disease. It was used to infect *Culicoides* after 16 passages in baby hamster kidney (BHK 21) cells. AKAV was obtained as a freeze-dried suckling mouse brain suspension from the National Institute of Animal Health, Tokyo, and was used to infect *Culicoides* after a further 2 passages in BHK 21 cells.

Virus titrations were conducted in BHK 21 cells propagated in microtitre plates, as described by Mellor and Boorman (1980). The method of Spearman-Kärber (Finney, 1964) was used to calculate the $TCID_{50}$ of each test.

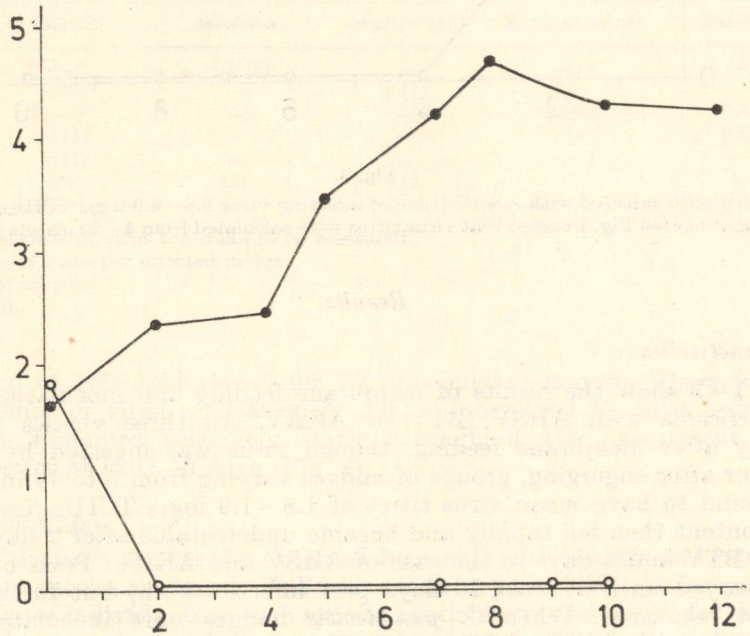

Fig. 2.

C. puncticollis infected with type 4 BTV (titre of infecting virus 5.8 $\log_{10}$ $TCID_{50}$/0.1 ml For explanation see Fig. 1, except that virus titres were calculated from 7—20 insects per sample.

Infection of Culicoides. All *Culicoides* were infected either by intrathoracic inoculation of the virus or by allowing them to engorge through a chick skin membrane on suspensions of heparinized mouse blood containing virus. Both methods have been described in Mellor *et al.* (1974). AHSV was used at a titre of 6.2—6.5 $\log_{10}$ $TCID_{50}$/0.1 ml, BTV at a titre of 5.8 $\log_{10}$ $TCID_{50}$/0.1 ml and AKAV at a titre of 5.6—6.0 $\log_{10}$ $TCID_{50}$/0.1 ml. The infected *Culicoides* were then maintained in the manner already described until required for virus assay.

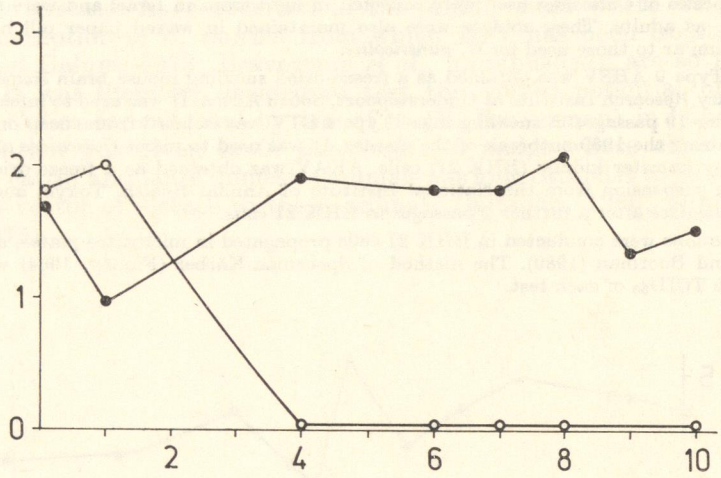

Fig. 3.

C. puncticollis infected with AKAV (titre of infecting virus 5.6—6.0 $\log_{10}$ $TCID_{50}$/0.1 ml For explanations see Fig. 1 except that virus titres were calculated from 4—20 insects per sample.

Results

C. puncticollis

Figs 1—3 show the results of membrane feeding and inoculating female *C. puncticollis* with AHSV, BTV or AKAV. All three viruses failed to multiply after membrane feeding, though virus was ingested by midges. One hour after engorging, groups of midges varying from 5 to 14 in number were found to have mean virus titres of 1.8—1.9 $\log_{10}$ $TCID_{50}$/insect. The virus content then fell rapidly and became undetectable after 2 days in the case of BTV and 4 days in the case of AHSV and AKAV. Pools of midges were assayed until at least 10 days post infection (p.i.) but further virus was not recovered. When *C. puncticollis* midges were inoculated intrathoracically with AHSV, BTV or AKAV, the virus persisted in the insects for at least 10 days. The titres of AHSV and BTV in inoculated midges increased steadily from 0 day p.i. and reached a peak after 6 days in the case of AHSV and 8 days in the case of BTV. A mean increase in virus titre per insect of over 6000-fold ($10^{3.8}$) was recorded for AHSV-inoculated

Table 1. Infection of 6 species of Israeli Culicoides either by intrathoracic inoculation or membrane feeding of type 4 BTV (5.8 $\log_{10}$ TCID /0.1 ml)

Day p.i.	Species inoculated intrathoracically				
	imicola	*circumscriptus*	*obsoletus*	*schultzei* (gp)	*newsteadi*
0	*1.1 (1)	1.3 (1)	—	0.9 (1)	—
1	0 (1)	—	0 (1)	0 (4)	—
3	—	—	0 (1)	1.2 (5)	—
4	0 (1)	—	0 (1)	0 (7)	—
5	—	3.6 (5)	2.8 (2)	2.8 (9)	3.3 (1)
6	3.4 (2)	3.2 (2)	3.4 (2)	2.7 (5)	—
7	3.1 (1)	2.9 (2)	—	T (8)	—
8	4.3 (4)	—	3.4 (2)	1.9 (1)	—
10	—	—	—	4.1 (1)	—
11	3.5 (1)	—	—	4.4 (2)	—
12	—	—	—	3.0 (1)	—
14	1.7 (4)	2.0 (3)	—	1.0 (2)	—

Day p.i.	Species membrane fed				
	imicola	*obsoletus*	*schultzei* (gp)	*distinctipennis*	*newsteadi*
0	0 (1)	1.3 (1)	—	—	—
2	—	—	T (1)	—	—
5	—	—	—	0 (3)	—
6	0 (1)	—	—	—	0 (1)
7	0 (1)	—	—	—	—
8	—	(2)	0 (2)	—	—

T Indicates a trace of virus too small to be measured.
* Mean titre of virus per infected midge.
() Number of midges.
— Not tested.

midges and of 1000-fold ($10^{3.0}$) for BTV-inoculated midges. There was no clear evidence of virus replication in AKAV-inoculated *C. puncticollis* and virus titre remained relatively static throughout the experiment, with persistence for at least 10 days (Fig. 3).

Other Culicoides

Table 1 displays the results obtained by membrane feeding and inoculating 6 other species of Israeli *Culicoides* with type 4 BTV. Unfortunately, the member of individuals of each species available was too small for detailed and reliable analysis, particularly with regard to the membrane fed midges, from which virus was only recovered once at day 2 p.i. However, all the species inoculated with BTV supported its replication and virus titre rose from $0.9-1.3$ $\log_{10}$ $TCID_{50}$/insect one hour p.i. to $3.3-4.4$ $\log_{10}$ TCID/insect after $5-10$ days of incubation at 26 °C. Two of the inoculated species, *C. imicola* and *C. obsoletus*, have already been implicated in BTV transmis-

sion but no isolates have been made from the other species, although *C. schultzei* (gp) and *C. newsteadi* are often closely associated with sheep and cattle in the wild (Sellers, 1975) and on the basis of these resuls might therefore justify further study.

Discussion

C. puncticollis has long been thought of as a possible vector of BTV and AHSV because of its close association with large domestic animals (Sellers, 1975) and because of its taxonomic relationship with *C. variipennis*, the North American vector of BTV. However, we have found no evidence in our work with an Israeli strain of *C. puncticollis* to support this assertion. We have infected a combined total of 319 *C. puncticollis* by the oral route with AHSV, BTV or AKAV but we have been unable to recover virus beyond 4 days p.i. It is possible that if a sufficiently large number of midges had been used a very low infection rate might have been recorded (Mellor and Jennings, 1980). Nevertheless, it is unlikely that this would have any significance in terms of vector potential in the wild in this instance, since the infecting titres of virus used in these experiments were considerably higher than those likely to be met in viraemic animals. *C. puncticollis* from source other than the one used by us may behave differently with respect to these three viruses. Jones and Foster (1974) were able to develop lines of total susceptibility and total insusceptibility to oral infection with BTV after one generation of selective breeding from their laboratory colony of *C. variipennis*. The circumscribed breeding habitats of many species of *Culicoides* suggest that this type of genetic selection might also occur in the wild. However, our present results indicate that the strain of *C. puncticollis* used by us is unlikely to be a vector in nature although, in areas where *Onchocerca gutturosa* occurs, double infections could transform this situation (Mellor and Boorman, 1980).

The multiplication of AHSV and BTV in *C. puncticollis* after intrathoracic inoculation is a situation that we have seen previously with *C. nubeculosus* (Mellor *et al.*, 1975). It is probably a facet of behaviour common to most *Orbivirus-Culicoides* pairs. We have shown that the barrier to infection is usually at the gut wall level (Mellor and Boorman, 1980). AKAV (a Bunyavirus), however, behaved differently. After intrathoracic inoculation the virus persisted in *C. puncti*collis for at least 10 days but there was no significant multiplication. It may be that this virus replicates in a very restricted range of tissues, only multiplies to a low titre or merely persists in the haemocoele without replication at all. In this behaviour it is similar to sandfly fever (Naples and Sicilian strains) when inoculated into *Lutzomyia longipalpis* (Jennings and Boorman, 1980.

C. obsoletus, *C. schultzei* (gp), *C. imicola*, *C. circumscriptus* and *C. newsteadi* (Table 1) all behaved in a similar way to *C. puncticollis* after intrathoracic inoculation of type 4 BTV. Virus titre rose over a period of 10 days from an infecting concentration of 0.9—1.3 $\log_{10}$ $TCID_{50}$/insect to a level varying from 3.3—4.4 $\log_{10}$ $TCID_{50}$/insect, a 100—3000-fold increase.

Unfortunately, owing to the difficulties that are commonly experienced when attempting to membrane-feed wild midges, too few individuals of these 5 species were infected by the oral route to enable any judgement on their vector potential to be made. Even the 3 orally infected specimens of the known vector of BTV, *C. imicola*, were found to be negative for virus upon titration.

None of the species of *Culicoides* infected with BTV, AHSV and AKAV during the course of this work were shown to be vectors of these viruses. *C. puncticollis* was used in sufficient numbers for us to be reasonably sure that our results are representative of the wild population from which they were collected but the other species obviously need more study. Before vector status of an insect can be reliably assessed, it is important to use as large a number of individuals as possible to avoid missing the low infection rates that are common in many virus-vector pairs (Mellor and Jennings, 1980).

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