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VECTOR-COMPETENCE STUDIES FOR BLUETONGUE AND EPIZOOTIC HEMORRHAGIC DISEASE VIRUSES WITH *CULICOIDES VENUSTUS* (CERATOPOGONIDAE)

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ABSTRACT. Field-collected females of *Culicoides venustus* from New York state were tested for oral susceptibility to bluetongue (BT) and epizootic hemorrhagic disease (EHD) viruses. The infection rates obtained for females exposed to a virus meal were low (BTV 0.7% for 1/141, EHDV 2.6% for 1/38), suggesting that the species would not be an efficient vector of these viruses in New York. Females of *C. venustus* were easy to use in vector competence studies with the same methods used for *C. variipennis;* they were relatively long-lived and readily fed through a membrane and on embryonating chicken eggs. Three other species of *Culicoides* did not take a blood meal under the same conditions except for a few females of *C. stellifer* that were long-lived and assayed negative for infection with BTV.

Several species of *Culicoides* are pests of mammalian livestock in New York State (Schmidtmann et al. 1980). Four of these species, *C. venustus* Hoffman, *C. stellifer* (Coquillett), *C. biguttatus* (Coquillett) and *C. obsoletus* (Meigen), were used in experiments to determine if they could be vectors of bluetongue virus (BTV) or of epizootic hemorrhagic disease virus (EHDV). Of these, only *C. venustus,* a relatively large and robust species, was used successfully in that sufficient numbers of females took blood meals. This species is widespread in the eastern United States with its range extending westward to Wisconsin and southward to Florida (Wirth 1965).

This paper reports preliminary laboratory studies to determine whether *Culicoides* species other than *C. variipennis* (Coquillett), the primary vector of BTV throughout most of the United States (Jones et al. 1981), are potential vectors of BTV. Because the methodology for the use of a species is important, we used several test procedures to determine whether field-collected females of *C. venustus* would be easy to use in arbovirus research.

MATERIALS AND METHODS

Adult *Culicoides* were collected in 1978–79 from a pasture in Tompkins County, New York with light traps baited with CO_2 (dry ice). A serologic survey for antibody to BTV in slaughter cattle had indicated that BT was rare or nonexistent in New York State (Metcalf et al. 1981), and BTV or EHDV have never been reported from the collection area.

Female flies were separated to species, shipped alive lightly chilled, and used in experiments under the same conditions used with *C. variipennis* (Jones and Foster 1978a). They were offered an infective blood meal (1 part cell-culture-adapted virus suspension and 9 parts defibrinated sheep blood) through membranes prepared from the skins of 1-day-old chicks. The blood meal contained about $10^{7.5}$ median cell-culture infectious doses/ml. En-

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gorged females were incubated at 21 ± 1° C for 11–25 days before they were assayed for virus. Most experiments used BTV serotype 11 (strain BT-2790), although a few were conducted with serotype 11 Texas Station Strain (BT-TSS), serotype 10 (BT-8), and with EHDV serotypes 1 (NJ) and 2 (KY). The virus source was one passage in sheep and 5–6 passages in baby hamster kidney (BHK-21) cells, and virus assay was also in BHK-21 cells. Virus was isolated from infected females by inoculation of BHK-21 cells grown in 96-well microplates. The virus was confirmed by an indirect fluorescent antibody test (IFAT) against BTV- or EHDV-specific antibody (Jochim et al. 1975). Females were assayed as individuals and infection rates (IRs) were calculated as the percentage of females that became infected.

A large number of *C. variipennis* females were given BTV or EHDV infective blood meals during the same periods covered by this report; trials were with the same or similar virus stocks so that the results comprised internal controls among populations of different susceptibility from different states. Flies were assayed as individuals that were coded to avoid bias. Cell-control wells on each microplate served as contamination controls. A series of IFAT for both BTV and EHDV and for each assay date ensured that typical cytopathic effect (CPE) was caused by the viruses used.

Some groups of *C. venustus* females were used in transmission experiments with 11-day embryonating chicken eggs as the recipient host (Foster and Jones 1973). Females were offered a second blood meal via an egg feeder (unpublished). Females that fed on embryonating eggs were separated into two groups (Jones and Foster 1966): blood (included blood and blood with some allantoic fluid) and allantoic fluid (included allantoic fluid and allantoic fluid with a trace of blood).

RESULTS

In the 2 trials attempted for each species, *C. obsoletus* and *C. biguttatus* females did not take a blood meal under the conditions used for research with *C. variipennis*. In 2 of 3 trials, a few females of *C. stellifer* took a blood meal. In all 16 trials some females in each group of *C. venustus* took a blood meal. The days of extrinsic incubation, the number of flies set up, the number assayed for virus, and the percent mortality during incubation are given in Table 1 for each group of flies that took a blood meal and were assayed for virus infection. Mortality during shipping and blood feeding was often excessive, and the need to handle flies rapidly upon receipt precluded an accurate determination of the percentage that blood fed.

One of the 179 *C. venustus* females assayed (Table 1) became infected and the isolate was confirmed as EHDV by IFAT. The infection rate (IR) for EHDV was low at 2.6% (1/38). A second female probably became infected with BTV, as indicated by typical CPE for BTV along with adequate controls, but this isolate was not tested for confirmation by IFAT. The IR for BTV would also be low at 0.7% (1/141). The 5 females of *C. stellifer* that were tested, which assayed negative, were an insufficient number to

Table 1. Data for vector competence studies with bluetongue (BT) and epizootic hemorrhagic disease (EHD) viruses for *Culicoides venustus* and *C. stellifer* from New York State, 1978–9.

Virus and serotype		Extrinsic incubation (days)	No. of trials (1978–9)	Infection rate (IR)		Mortality during incubation	
BTV	EHDV			IR (%)	No. positive /no. assayed	No. set up	(%)
C. venustus							
11–2790		11	1		0/6	15	60
10		14	1		0/15	25	40
11–TSS		15	1		0/18	31	42
11–2790		15–16	2		1/9	17	47
11–2790		16	2[a]		0/6	48	88
11–2790		19–21	5[a]		0/70	194	64
11–2790		21	1		0/13	30	57
11–2790		25	1[a]		0/4	11	64
	2	15	1		0/26	72	64
	1	16	1[a]		1/12	51	76
Totals	BTV		14	0.7	1/141		
	EHDV		2	2.6	1/38		
Averages		17					64
C. stellifer							
11–2790		14	2		0/5	7	28

[a] Flies used in transmission trials with embryonating chicken eggs as recipient hosts.

show whether or not this species was susceptible to infection with BTV. The IR data for susceptible populations of *C. variipennis* that were given an infective blood meal during the same periods were routinely high; for example, 23% (59/256) for California field populations.

The single female infected with EHDV was part of a group of flies that was used in a transmission trial with a single recipient embryonating egg (Table 1). Even though this fly at 16 days of extrinsic incubation took a blood meal on the recipient host egg, EHDV did not appear to be transmitted as may have been indicated by death of the egg before the end of the 12 days of post-test incubation. However, this negative result is inconclusive as EHDV is often not pathogenic to chicken embryos and the embryo was not harvested and passaged.

The species *C. venustus* was easy to use in vector competence studies. Females readily took a blood meal through a membrane. Those females used in transmission trials with eggs fed well, with 68% (79/117) taking a second meal; 58% of the females feeding on 18 recipient host eggs took a meal characterized as a blood meal. Females were long-lived with 64% mortality at 11–25 days of incubation. A gradual loss of flies from mortality was indicated by the fact that mortality was identical for flies held 11–16 days (64%: 167/259) versus those held 19–25 days (63%:148/235). Furthermore, females lived longer than our data show as the age of females on the day of collection in light traps was not known.

DISCUSSION

The small, more delicate species *C. obsoletus* and *C. biguttatus* did not take a bloodmeal under the conditions used for research with *C. variipennis*. The results for *C. stellifer* indicated that it would be a good species for further study with minimal changes in techniques as a few females took a blood meal and a high percentage of them survived 14 days of incubation.

Culicoides venustus would be an excellent candidate for research based on the adult female characteristics that we have tested. However, the species has not been colonized. Although some females oviposited viable eggs, as expected because they were field collected, the larvae did not develop under the conditions used for *C. variipennis*. Another disadvantage is that the mating requirements of the species are unknown.

The low infection rates obtained for *C. venustus* females given infective blood meals with BTV and EHDV, two closely related arboviruses, indicate that this species would not be an efficient vector of BTV or EHDV in New York State. However, populations of a vector species can be expected to be heterogeneous in their response to oral infection with a virus, as has been shown for *C. variipennis* with BTV (Jones and Foster 1978b). The data presented here are presumably for a single population as all collections were from the same pasture. Thus, other populations might be differently susceptible to oral infection.

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