

## Experimental Infection of *Culicoides brevitarsis* from South-east Queensland with Three Serotypes of Bluetongue Virus

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### Abstract

Laboratory-reared *C. brevitarsis* (biting midges) were fed on sheep which had been experimentally infected with bluetongue serotype 1 (CSIRO 156), bluetongue serotype 20 (CSIRO 19) or bluetongue serotype 21 (CSIRO 154), or on cattle experimentally infected with bluetongue serotype 20 (CSIRO 19). Approximately 77 000 *C. brevitarsis* were exposed to sheep and 9000 to cattle. The average percentage feeding on sheep was 54% and on cattle 47%. In attempts to transmit virus by bite 3360 *C. brevitarsis* which had fed on viraemic sheep were held for 11-15 days before exposure to susceptible sheep. Although 11% of these insects fed, transmission of virus from sheep to sheep was not demonstrated. Estimated infection rates of *C. brevitarsis* for each serotype from sheep and serotype 20 from cattle were similar at 0.4% or lower. These low infection rates are one of the factors which make it unlikely that *C. brevitarsis* could be an efficient vector of bluetongue viruses in sheep in the field.

### Introduction

The original isolation of a bluetongue virus in Australia was made from a mixed-species pool of *Culicoides* biting midges collected at Beatrice Hill in the Northern Territory (St George *et al.* 1978*b*). The virus, CSIRO 19, was subsequently identified as a new serotype, bluetongue 20 (BLU 20), at Onderstepoort in South Africa (St George and McCaughan 1979). Since then two further serotypes of bluetongue, the CSIRO 154 strain of BLU 21 (St George *et al.* 1982) and the CSIRO 156 strain of BLU 1 (Della-Porta *et al.* 1981), have been isolated in blood samples collected from healthy cattle in the Northern Territory (St George *et al.* 1980). More recently two isolations of BLU 1 have been made from *Culicoides brevitarsis* Kieffer collected in association with cattle at Peachester, 100 km north of Brisbane (St George and Muller 1984).

Following the identification of bluetongue from the Northern Territory, Standfast *et al.* (1978), in studies to determine the vectors of bluetongue virus, attempted to infect *Culicoides* species of northern Australia and found that *C. brevitarsis* was among the species which would support the growth of BLU 20. This paper reports studies on the vector potential of *C. brevitarsis* collected in south-east Queensland for three serotypes of bluetongue virus in sheep and one serotype in cattle. In addition, attempts were made to transmit virus from sheep to sheep by the bite of *C. brevitarsis*.

## Materials and Methods

### Viruses

The bluetongue viruses used were the CSIRO 19 strain of BLU 20, the CSIRO 154 strain of BLU 21, and the CSIRO 156 strain of BLU 1.

The BLU 20 virus used to infect cattle was in tissue culture medium from the fifth passage of the original insect isolate in baby hamster kidney (BHK21) tissue cultures. For sheep the BLU 20 inoculum was blood from viraemic sheep which had been infected with virus grown in BHK21 tissue culture. The inocula of BLU 1 and BLU 21 serotypes for sheep were prepared by first growing the viruses for 10 days in *Aedes aegypti* (L.) mosquitoes by intrathoracic inoculation. For each inoculum a pool of 10 mosquitoes was homogenized in 2 ml of phosphate-buffered saline containing 10% (v/v) fetal calf serum, centrifuged and the supernatant used as inoculum.

All animals were inoculated subcutaneously and intradermally, and sheep also received at least half of the inoculum intravenously. The inoculated animals were bled daily for the duration of the experiment and the blood submitted for virus assay.

### Sheep and Cattle

The sheep were Merino or Merino crossbred ewes and wethers 6–18 months old from the New England region of New South Wales. The cattle were 1–3-year-old Friesian or Hereford heifers and one Hereford bull from Victoria. All animals were tested to ensure they had no virus antibodies which might interfere with the experimental work. Sheep and cattle were held separately in the Large Animal Insect-proof Laboratory at Long Pocket Laboratories. *C. brevitarsis* were exposed to infected sheep from day 5 to day 10 after inoculation and to infected cattle from day 7 to day 15.

### Insects and Feeding

The immature stages of *C. brevitarsis* are found in cow dung (Cannon and Reye 1966). Dung pats were collected from pasture at Samford 20 km north-west of Brisbane and adult *C. brevitarsis* were reared from them and held at 27°C and 85% R.H. as described by Standfast *et al.* (1984). *C. brevitarsis* had access to 10% (w/v) sucrose solution until 24 h before a feeding exposure on an animal.

*C. brevitarsis* were placed on the shaven ears of the animals to feed. The method described by Muller (1979) was modified to enclose the whole ear in the feeding pot, with a small 1.5 V light resting in the base of the ear. The auditory canal was plugged with paper tissue. Insects were on average 3 days post-emergence when they were exposed to the animals for 45 min in infection feeds. The duration of exposure in transmission attempts was 90 min. At the end of exposure *C. brevitarsis* were anaesthetized with CO<sub>2</sub>, removed from the ear and sorted on a refrigerated plate under a dissecting microscope. After infection feeds, blood-engorged midges were removed and held at 27°C and 85% R.H. with access to 10% (w/v) sucrose solution. Insects used only for infection assessment were held 8–10 days before being harvested. Those used in transmission attempts were held for 11–15 days, then exposed to a susceptible host and harvested immediately. The length of the holding period was determined by the number and survival rate of the insects in the experimental group; it was reduced when numbers were low or survival was poor. Of the insects fed on cattle, 350 were harvested immediately after feeding. After harvesting all insects were stored in liquid nitrogen to await processing.

### Virus Recovery

Most of the *C. brevitarsis* were processed in pools of approximately 25, enabling the infection rate to be estimated using the method of Chiang and Reeves (1962). However, for *C. brevitarsis* fed on sheep infected with BLU 20, pool size varied from 2 to 33. Of the *C. brevitarsis* that had fed on sheep infected with BLU 1, 250 were processed individually. The 350 *C. brevitarsis* harvested immediately after feeding on cattle were processed in pools of 25. Insects were processed in BHK21 tissue cultures as described by St George *et al.* (1978a).

## Results

Approximately 77 200 *C. brevitarsis* females were exposed to sheep and 9020 to cattle in infection feeds. The average percentage of *C. brevitarsis* that fed on sheep was 54% and on cattle 47%. All *C. brevitarsis* which fed engorged fully. In transmission attempts 3360 females were exposed on sheep and 370 (11%) of these fed. The results

of insect processing in tissue cultures and estimated infection rates are shown in Table 1. The infection rate of midges which fed on sheep infected with BLU 20 was estimated by assuming the single positive pool of 16 insects contained only one infected midge. Virus was not recovered from the 350 midges harvested immediately after feeding on cattle infected with BLU 20. The viraemias in the sheep inoculated with BLU 21 virus were found to be intermittent (M. F. Uren, personal communication) so that of the insects which fed on these sheep only 45% fed on days when virus was shown to be present in the blood. All seven isolations of BLU 21 came from these insects, which are the only ones shown in Table 1, and only these insects were considered in the calculation of the estimated infection rate. In all other infection feeds the host animals were viraemic on the day of feeding. For a small number of the animals on which *C. brevitarsis* fed, daily blood samples were titrated and maximum titres attained are also shown in Table 1 (K. R. E. Squire and M. F. Uren, personal communication).

**Table 1.** Infection of *C. brevitarsis* with three serotypes of bluetongue virus by feeding on experimentally infected sheep and cattle

Virus sero-type	Host	Max. virus titre in host blood (TCID <sub>50</sub> /ml)	No. of insects processed	No. of pools <sup>A</sup>	No. of isolations	Est. infection rate (%)
BLU 1	Sheep	10 <sup>5.5</sup>	1886	66 <sup>B</sup>	1 <sup>C</sup>	<0.1
BLU 20	Sheep	n.d.	364	23	1	0.3
BLU 20	Cattle	10 <sup>2.8</sup>	2071 <sup>D</sup>	72	4	0.2
BLU 21	Sheep	10 <sup>2.7</sup>	2021	78	7	0.4

<sup>A</sup> For serotype BLU 20 in sheep, pool size range was 2–33. For other serotype–host combinations pool size range was 18–30.

<sup>B</sup> Plus 250 *C. brevitarsis* processed individually.

<sup>C</sup> Processed individually.

<sup>D</sup> A further 350 *C. brevitarsis* harvested immediately after feeding were processed in 14 pools. No virus was isolated.

Virus was not recovered from any of the insects used in transmission attempts, whether they did or did not feed a second time, and none of the sheep on which they fed became infected.

## Discussion

Transmission attempts were unsuccessful because none of the *C. brevitarsis* used had become infected with virus after the first feed on viraemic sheep. Only 370 *C. brevitarsis* fed in transmission attempts and, assuming an infection rate of 0.4% or less (Table 1), no more than one or two of these could have been expected to be infected. In addition, Hardy *et al.* (1983) have reviewed evidence that with some mosquito-borne arboviruses the transmission rate is lower than the infection rate, and this may well be the case with *C. brevitarsis* and bluetongue viruses. Fewer *C. brevitarsis* fed on sheep in transmission attempts (11%) than infection feeds (54%) because after the infection feed the *C. brevitarsis* digested the blood meal to become gravid and therefore less likely to take a second blood meal.

Jones and Foster (1978), using two serotypes of bluetongue virus with an artificial feeding method, found that the infection rate of one strain of the bluetongue vector *C. variipennis* (Coquillett) fell substantially when the titre of the blood meal was

reduced 100-fold. During the experiments with *C. brevitarsis* the peak titres of virus in the blood of the hosts were up to 100-fold higher in sheep infected with BLU 1 than in sheep infected with BLU 21 or cattle infected with BLU 20 (Table 1). Despite these differences in virus concentration in the blood meal there was no obvious difference in infection rates of *C. brevitarsis* which fed on the different host-virus combinations.

There is serological evidence that the southern limit of the distribution of group-reactive antibodies to bluetongue and closely related viruses in Australia lies within the approximate southern limit of the distribution of *C. brevitarsis* (Muller *et al.* 1982; Della-Porta *et al.* 1983). In south-east Queensland and north-east New South Wales in summer and early autumn, cattle can be attacked by as many as 5000 *C. brevitarsis* per beast in the hour following sunset (Standfast and Dyce 1968; Muller, unpublished data). This high level of attack probably compensates for the low infection and transmission rate so that where it is abundant *C. brevitarsis* can act as a vector of bluetongue viruses in cattle. In south-east Queensland BLU 1 has been isolated from field-caught *C. brevitarsis* at a site where cattle seroconverted to that virus shortly after the insects were collected (St George and Muller 1984).

To date *C. brevitarsis* is the only species known to support bluetongue virus growth which is also found in areas of Australia where sheep are abundant. *C. brevitarsis* is closely associated with cattle since it breeds in cow dung (Cannon and Reye 1966), and it appears that more of them will feed on cattle where sheep and cattle are both present in the same locality (Lee *et al.* 1962; Muller and Murray 1977). These factors, combined with a low infection rate, make a transmission cycle in sheep unlikely and probably explain why clinical disease has not been found in sheep in Australia.

The only return of virus from *C. brevitarsis* fed on sheep infected with BLU 1 came from one of the 250 insects processed individually. This indicates that the processing system was capable of detecting a single infected *C. brevitarsis*.

Virus was not recovered from any of the 14 pools derived from the 350 insects stored immediately after feeding on cattle infected with BLU 20, yet the four positive pools of *C. brevitarsis* which fed on cattle came from the same feeds. The cattle were known to be viraemic on the day of feeding, on the basis of a tissue culture inoculum of 0.2 ml of blood, but virus in the blood meals taken by the insects could not be detected in the tissue culture system even in pools of 25 insects. However, after an incubation period of 8–10 days the virus had multiplied in those insects which did become infected, and virus could be detected using the same system. *C. brevitarsis* is a very small insect and the blood meal taken by a single midge is estimated to be in the order of 0.03  $\mu$ l (Muller *et al.* 1982).

*C. brevitarsis* has not been fed on cattle experimentally infected with BLU 1 or BLU 21 serotypes of bluetongue virus. However, there is now strong evidence from the field that *C. brevitarsis* can be infected with BLU 1 when feeding on cattle (St George and Muller 1984). It is likely that further testing with experimentally infected cattle will confirm this association, and also show that *C. brevitarsis* can be infected with BLU 21 by feeding on cattle as well as sheep.

### Acknowledgments

I thank I. M. Parsonson, T. D. St George, K. R. E. Squire and M. F. Uren for veterinary supervision of the animals and information on viraemias, and H. A. Standfast and M. D. Murray for constructive criticism of the manuscript. Excellent technical help was provided by P. G. Allingham, E. J. Harris, T. J. Daniel, D. F. R.

Davey and S. S. Davis. I also thank P. G. Sheaffe and L. Balfour of the CSIRO Pasture Research Station at Samford for their assistance in collection of *C. brevitarsis*.

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