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EPIDEMIOLOGICAL NOTES: TWO BLUETONGUE EPIZOOTICS

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In addition to its primary goal of laboratory research on bluetongue (BT) disease of sheep, the Denver Animal Disease Laboratory serves as a diagnostic center for BT in the United States. Because of the cooperative involvement of the laboratory's veterinarians and entomologist in assisting local, state, and federal authorities in the diagnosis of the disease, some epidemiological data have been collected. This paper presents some of these data for two epizootics—one at Hudson, Colorado, and one at Billings, Montana.

HUDSON, COLORADO. This epizootic occurred in a flock of sheep in the fall of 1963 (Jones, 1965; Bowne *et al.*, 1966). Additional data are presented here because the Hudson area was selected for further study the following spring. It was selected not only because BT had occurred there the past season in both sheep and cattle, but also because the suspected primary vector, the small biting midge *Culicoides variipennis* (Coquillett), continued to breed extensively throughout the area.

This heavy breeding (Jones, 1967) continued through at least the 1966 season and indicated that *C. variipennis* was the most common biting fly in the area. This supposition was supported by data collected from an animal-bait trap (Jones, 1961) that was operated occasionally during the 1964 season. The following female biting flies were collected: 6,719 *C. variipennis*, 352 *Culicoides* spp., 549 *Leptoconops* spp., 882 Culicidae, 79 Tabanidae, and 6 Simuliidae. Of the 6,719 female *C. variipennis*, 1,348 (20 percent) had recently fed on blood, presumably from the bait sheep because the trap was the open type that was closed at intervals to collect the flies on and about the animal.

An important step in setting up the Hudson study area was to determine whether the BT infection was still present in 1964. This was accomplished by establishing a sentinel flock of sheep there from April 16 to November 17. These sheep were western white-faced ewes and their week-old lambs from a flock that had overwintered at Wellington, Colorado. The animals were considered susceptible because no serum-neutralization (SN) antibodies were detected in their serum when it was tested against the standard American BT 8 virus passed 10 times in lamb kidney. Blood for subsequent SN tests was taken from each sheep every 4 weeks. Only four sheep remained in test at the end of the season. At that time, two had developed a solid immunity against a challenge inoculation with virulent BT 8 virus, and two remained susceptible. (At 3 months, all of the 10 sheep still in test had failed to show antibodies against the BT 8 virus.) At the end of the test, the two sentinel sheep that had acquired immunity had significant SN indexes against BT 8 virus (Table 1).

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TABLE 1.—Serum neutralization (SN) indexes for two sentinel sheep that acquired immunity to bluetongue when exposed for 7 months at Hudson, Colorado, 1964.

	SN index against BT 8 at given month				
Sheep no.	July	Aug.	Sep.	Oct.	Nov.
19	0	..	..	..	3·7
23	0	0	0	2.2	3·5

In an attempt to determine whether any of the small animals indigenous to the area could be serving as reservoir hosts, we occasionally set small-animal live traps in the study area. The mammals and birds that were collected were bled at the laboratory by heart puncture, and the blood was preserved in equal parts of OPG solution (an anticoagulant-preservative solution consisting of 5 g. potassium oxalate, 5 g. phenol, 500 ml. glycerin, and 500 ml. distilled water). The blood samples were pooled according to their generic position taxonomically. These pooled samples were later determined to be negative for BT virus by inoculating each into a BT-susceptible sheep. None of the sheep developed evidence of clinical BT disease over a 21-day period, and all were subsequently susceptible to a challenge inoculation with virulent BT 8 blood virus. The number and types of animals determined to be negative for harboring BT are as follows: 37 field mice (*Peromyscus* spp.), 35 voles (*Microtus* spp.), 17 kangaroo rats (*Dipodomys* sp.), 5 rats (*Rattus* spp.), 1 rabbit (*Sylvilagus* sp.), 1 immature muskrat (*Ondatra* sp.), 2 red-winged blackbirds (*Agelaius phoeniceus*), and 6 English sparrows (*Passer domesticus*).

BILLINGS, MONTANA. The virological aspects of this epizootic in cattle have been reported by Bowne *et al.* (1968). This laboratory's initial investigation was made by the veterinarians on August 25, 1966. During their trip, they collected a sample of mud from a low swampy portion of the pasture where the epizootic had occurred —one pupa of *C. variipennis* was later recovered from this sample by the entomologist. Subsequently, three field trips for entomological research were made to the area.

Rearing sites were scarce in the epizootic area. The only two nearby water areas available for breeding were a canal bordering one side of the pasture, and the low, swampy side of the pasture, where water collected deep enough to permit mosquito breeding. Except for a few *Culicoides* pupae collected along the margin of the canal and the evidence of simuliid breeding on the grass in the canal, no biting-fly breeding was found during the entomological investigations.

The entomological equipment available was such that large numbers of flies were not collected. Collections made with two CDC miniature light traps (Sudia and Chamberlain, 1962) were small, and most of the biting flies collected during the three field trips were taken with an animal-bait trap (sheep). The data for these flies are given in Table 2.

As indicated in Table 2, many of the female flies collected were used in virus isolation tests. Flies for this purpose were returned alive to the laboratory and incubated at 21±3 C. about 3 days before use. They were identified while anesthetized with carbon dioxide, and in most cases several flies of the same species were pooled. The flies were then placed in OPG solution in TenBroeck grinders, ground, and centrifuged. The resulting supernate was inoculated intravascularly into six 10-day embryonating chicken eggs to determine whether BT virus was present. No virus was isolated in these tests, or in subsequent subpassage of pooled material into sheep.

On August 30, three sentinel sheep were taken from the susceptible flock maintained at the Denver Laboratory and established in the pasture area at Billings, Montana, where the epizootic had occurred. They were kept inside a small fenced enclosure where they could be observed daily and bled at least once a week to test for the presence of virus. Rigorous security procedures were followed in establishing this sentinel flock. On September 24, the sheep were returned to the laboratory,

TABLE 2.—Biting flies collected at Billings, Montana, after a bluetongue epizootic.

Species	Number of adults collected							
	Animal bait trap (sheep)		Light trap		Sweeping	Reared from pupae		No. used in virus isolation tests
	♀	♂	♀	♂	♀	♀	♂	♀
Culicoides variipennis (Coquillett)	12	2	2	..	..	..	1[a]	14
Culicoides sp. 1	4**	..	..	..	..	..	..	2
Culicoides sp. 2	1*	..	..	..	..	..	..	..
Culicoides denningi Foote and Pratt	..	..	3	..	..	1[b]	1[b]	2
Culicoides cockerellii (Coquillett)	1	..	..	..	..	..	..	1
Culicoides crepuscularis Malloch	..	..	..	1	..	..	..	..
Leptoconops kerteszi Kieffer	1	..	..	..	..	..	..	..
Simulium vittatum Zetterstedt	1*	..	..	..	..	..	..	..
Simulium sp. 1	1	..	..	1	..	..	..	..
Culiseta inornata (Williston)	10	..	..	..	1	..	..	9
Culex tarsalis Coquillett	1	2	2	2	..	..	..	2
Aedes dorsalis (Meigen)	8	..	..	..	1	..	..	7
Aedes nigromaculis (Ludlow)	1	..	..	..	2	..	..	..
Aedes vexans (Meigen)	16	..	..	..	5	..	..	9
Aedes trivittatus (Coquillett)	5	..	..	..	..	..	..	..
Stomoxys calcitrans (L.)	9	..	..	..	..	..	..	5

* One ♀ biting in ear of sheep.
** Two ♀ biting in ear of sheep.
[a] Reared from mud sample from pasture, collected by veterinarians, August, 1966.
[b] Pupae collected from canal margin, R. H. Jones, September 23, 1966.

blood for serum was collected, and 21 days later the immunity of each animal was challenged by inoculation with virulent BT 8 blood virus. One animal was immune to the challenge inoculation; however, the SN data for these animals were inconclusive.

An attempt was also made to determine whether there were wild animals in the area that might harbor the virus. Although small-animal live traps were used constantly during the field trips made between August 30 and September 23, no small animals were collected, and there was no evidence that small animals were present in the pasture area. The only sign of wildlife noted was the frequent sighting of antelope in the distance. It is possible, since wild ruminants are affected by BT (Karstad and Trainer, 1967; Robinson *et al.*, 1967), that antelope in the area were also infected, or could have served as a reservoir of the disease.

SUMMARY. Studies were conducted at Hudson, Colorado, the year following an epizootic and at Billings, Montana, during or immediately after an epizootic; in both cases, a portion of a flock of sentinel sheep acquired immunity. The few attempts to isolate bluetongue virus from biting flies and small mammals were negative. Data are given for the biting flies collected.

ACKNOWLEDGMENTS. The authors are indebted to the owners of the animals involved for giving permission to conduct these studies on their land: to Mr. Jack Stevens and his son at Hudson, Colorado, and to Mr. Dover Sindelar at Billings, Montana. We are grateful to Mr. L. R. Ertle who, as a summer employee, conducted the work at Hudson, Colorado, in 1964. At Billings, Montana, the Denver Laboratory's initial investigation was made by Dr. J. G. Bowne and Dr. A. J. Luedke, accompanied by Dr. W. H. Ridge, Animal Health Division Veterinarian, and Dr. Orley Arthur, the local veterinary practitioner; subsequent entomological work was conducted as follows: August 30 to 31 (R. H. Jones and S. K. Baker), September 8 to 9 (Baker), and September 23 to 24 (Jones). We are especially grateful for the help of Dr. W. H. Ridge who handled the veterinary aspects for the

maintenance of the sentinel sheep at Billings, besides tending to their daily needs during the absences of the Denver personnel. Tissue culture SN tests were performed by this section of the Animal Disease Research Laboratory at Denver, and we are grateful to Dr. M. M. Jochim for his attention to this. Finally, the authors are grateful to Dr. Alan Stone and Dr. Willis W. Wirth of the Systematic Entomology Laboratory, Entomology Research Division, USDA, and to Mr. William R. Atchley of the University of Kansas for their assistance in identifying those flies collected in Montana that were preserved for identification.

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