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Insects Feeding on Desert Bighorn Sheep, Domestic Rabbits, and Japanese Quail in the Santa Rosa Mountains of Southern California

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ABSTRACT: Desert bighorn sheep (*Ovis canadensis cremnobates*), a domestic rabbit (*Oryctolagus cuniculus*), and Japanese quail (*Coturnix japonica*) were used as bait animals to collect blood-feeding flies in an area of active bluetongue and epizootic hemorrhagic disease virus transmission. Precipitin tests were used to confirm the blood source where feasible. Eight species of *Culicoides*, members of the *Leptoconops kerteszi* group, *Simulium* spp., *Anopheles franciscanus*, and *Stomoxys calcitrans* were collected from the bighorn sheep. Feeding on the bighorn sheep by *Culicoides brookmani* (n = 25), *C. variipennis* (n = 6), *C. cacticola* (n = 1), and *Simulium* spp. (n = 3) was confirmed by precipitin testing. Primary species attacking the rabbit were *C. brookmani*, *C. variipennis*, and the *L. kerteszi* group. The quail were attacked primarily by members of the *C. copiosus* group and the *L. kerteszi* group.

Key words: Diptera, Insecta, *Culicoides* sp., *Leptoconops* sp., *Ovis canadensis cremnobates*, host preference, blood feeding, trapping, bluetongue, epizootic hemorrhagic disease.

Desert bighorn sheep (*Ovis canadensis nelsoni* and *O. c. cremnobates*) in southern California are known to be exposed to bluetongue and epizootic hemorrhagic disease viruses (DeForge et al., 1982; Jessup, 1985), which are presumed to be transmitted by blood-feeding midges in the genus *Culicoides* (Gibbs and Greiner, 1988). Based on surveys in the Santa Rosa Mountains using traps baited with carbon dioxide (CO_2), at least 19 species of *Culicoides* are present (Mullens and Dada, 1992). The capture of insects in a CO_2-baited trap suggests a blood-feeding habit, but says little regarding the propensity of the insects to bite a particular host species. Blood-engorged insects collected in the field are quite useful, but the identification of the source of the blood meal then is totally dependent on blood meal identification tests in the laboratory. The reliability of these tests varies with the quality and type of the antisera used, methods, experience of the investigators, and amount of antigen (size of blood meal) (Washino and Tempelis, 1983). An alternate or supplementary method is to use bait animals (Muller and Murray, 1977; Hayes et al., 1984; Gerhardt, 1986). Use of bait animals was appropriate in this setting due to lack of knowledge of *Culicoides* spp. resting sites following a blood meal, the small size of the insects (1 to 3 mm), the rugged terrain which precludes use of some collecting techniques (e.g., vehicle-mounted traps), and the low field density of desert bighorn sheep in the region. Our objective was to determine which species of blood-feeding flies fed on desert bighorn sheep. We also determined which blood-feeding flies would attack smaller vertebrates, using a domestic rabbit (*Oryctolagus cuniculus*) and Japanese quail (*Coturnix japonica*) as bait animals.

We conducted the studies at the Philip L. Boyd Deep Canyon Desert Research Center, at the base of the Santa Rosa Mountains, south of Palm Desert, California (USA) (elevation 280 m, 32°42′N, 116°22′W). This area is naturally frequented by desert bighorn sheep. A 450 m^2 enclosure with a 2.5 m high chain-link fence served to contain the animals and exclude predators. Two hand-raised peninsular bighorn sheep ewes (*O. c. cremnobates*) were acquired from Living Desert in Palm Desert, California, with the approval of the California Department of Fish and Game. A 1.3 m × 1.3 m × 1.3 m box was constructed of chain link fencing to hold a bighorn sheep during trapping periods.

A 5-m-tall support structure was erected adjacent to the box, from which a 2 m × 2 m × 2 m nylon organdy drop trap (Fig. 1) could be suspended from a horizontal cross beam. The drop trap was raised and lowered by means of a rope and pulley. With the trap raised, the animal was exposed to blood feeding insects for 10 min. During this time the investigators were 25 m away to minimize any potential bias caused by human presence. After the 10 min exposure period, the trap was lowered to enclose the box in which the bighorn sheep was held. The trap was left in place for 10 min to allow insects to complete feeding, and an investigator then entered the trap via a velcro opening. Insects were removed from the interior of the netting with a vacuum aspirator for 10 min, the trap was raised again, and the procedure was repeated. Trapping was begun 60 min before sunset, and was continued at 30 min intervals until 90 min after sunset. The bighorn sheep then was released into the larger pen area and was recaptured the following morning. Trapping was resumed from 30 min before until 90 min after sunrise. Trapping was done weekly on 14 dates between 8 March and 5 June 1990. This period includes the presumed peak of arbovirus transmission to bighorn lambs and is the period of maximum abundance of many *Culicoides* spp. in the area (Mullens and Dada, 1992). The daily trapping periods were chosen because of the crepuscular biting habits of many *Culicoides* spp. (Nelson and Bellamy, 1971).

Jackrabbits (*Lepus californicus*) were abundant in the area, and a number of wild bird species were common. To simulate these hosts, a 2 to 3 kg brown domestic rabbit and five Japanese quail were used as bait on the same trapping dates. They were held in 25 × 75 mm mesh (rabbit) or 13 mm mesh (quail) hardware cloth cages 15 to 30 m from the bighorn sheep. The cages were placed on boards in large plastic-lined boxes with 5 cm of water in the bottom to which 1 ml detergent was added to break the surface tension. The rabbit and quail were placed in the field 1.5 to 2.0 hr before sunset and remained until 1.5 to 2.0 hr after sunrise. The fluid in the pans then was strained through a 100-mesh sieve, and captured insects were retrieved.

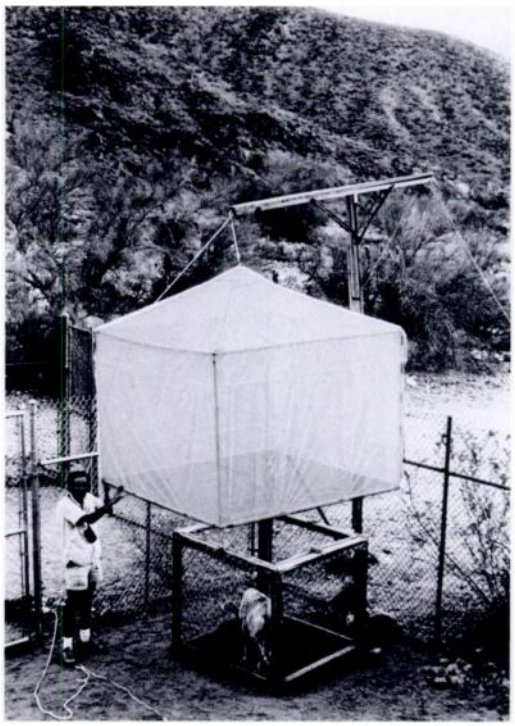

FIGURE 1. Drop trap used to collect blood-feeding insects from bighorn sheep.

Unengorged specimens were stored in 70% ethanol. Engorged specimens were frozen at −20 C. All insects were identified with the aid of Wirth et al. (1985) and reference specimens.

To test the feasibility of precipitin testing, laboratory-reared *C. variipennis* were fed on bighorn sheep blood through artificial membranes on water-jacketed feeders adjusted to 37 C (Hunt and McKinnon, 1990), and then frozen. These flies later were crushed individually in 0.1 ml of 1% NaCl in a microfuge tube. A 0.05 ml sample of the fluid was used against saline alone or commercially-available antisera (Sigma Chemical Company, St. Louis, Missouri) for rat, rabbit, dog, or sheep by agar gel precipitin and capillary precipitin

TABLE 1. Insects (Diptera) collected from peninsular bighorn sheep, domestic rabbit, and Japanese quail in Deep Canyon, Santa Rosa Mountains, Riverside County, California, March to June, 1990.

	Vertebrate species					
	Bighorn		Rabbit		Quail	
Insect species	Total recovered	Blood engorged	Total recovered	Blood engorged	Total recovered	Blood engorged
Culicoides brookmani	167	25	13	6	0	0
C. variipennis	37	6	3	0	0	0
C. copiosus group						
C. cacticola	1	1	0	0	10	2
C. ryckmani	2	0	0	0	6	0
C. sitiens	0	0	2	0	4	0
C. lahontan	2	0	0	0	0	0
C. mohave	1	0	0	0	1	0
C. (Avaritia) n. sp. nr. *pusillus*	1	0	0	0	0	0
C. n. sp. nr. lahillei	1	0	0	0	0	0
Leptoconops kerteszi group	55	1	13	6	9	2
Simulium spp.	21	3	0	0	1	0
Anopheles franciscanus	1	1	0	0	0	0
Stomoxys calcitrans	1	0	0	0	0	0

tests (Crans, 1969; Washino and Tempelis, 1983). Bighorn sheep blood reacted consistently with anti-sheep serum only.

Wild-caught engorged specimens then were similarly tested. As no animals such as domestic ruminants or deer, whose blood might react with the anti-sheep sera, were found within several kilometers of the study area, reactions with anti-sheep serum were considered confirmation of feeding on bighorn sheep. The few larger engorged insects collected (black flies, mosquitoes) were macerated in 0.3 ml saline before precipitin testing. Bird antisera were not available, and confirmation of bird blood was not done. Some engorged specimens that accidentally thawed prior to testing were not analyzed.

Thirteen species of insects were recovered from the bait animals (Table 1). Insects were not numerous due to a prolonged drought in the region (Mullens and Dada, 1992), and numbers were too low to discern possible hourly or diel differences in biting rates or species composition. The most numerous fly blood-feeding on bighorn sheep and the rabbit was *C. brookmani*. *Culicoides brookmani* was one of the main species attacking rabbits, but not quail. Several engorged specimens were removed from the ears of the bighorn sheep. Of 19 engorged *C. brookmani* tested from the drop trap or directly from the sheep, 16 reacted positively with anti-sheep serum, while three were blank. Of five engorged *C. brookmani* recovered from water under the rabbit, one reacted positively with anti-rabbit serum, while four were blank.

The second most common species engorging on bighorn sheep was *C. variipennis*. This species also was recovered from the rabbit, but not the quail. Of six engorged flies collected from the drop trap over the bighorn sheep, four reacted with anti-sheep serum, one reacted with both anti-sheep and anti-rabbit sera, and one was blank.

Remaining species of *Culicoides* were collected in lesser numbers. Of the 25 *C. copiosus* group specimens collected, 20 (80%) were from the quail, but only two specimens (*C. cacticola*) had actually engorged from that host. A single engorged female *C. cacticola* was collected from the drop trap around the bighorn sheep, and the blood reacted with anti-sheep serum. Other *Culicoides* attracted to the bighorn

sheep and found unengorged in the drop trap in very low numbers include *C. lahontan* (2), *C. mohave* (1), *C. (Avaritia)* n. sp. near *pusillus* (1) and *C.* n. sp. near *lahillei* (1).

The *Leptoconops kerteszi* group consisted primarily of *L. belkini* and *L. foulki*, based on identification of slide-mounted specimens from that site (Mullens and Dada, 1992). *Leptoconops* definitely were attracted to the sheep, but only a single blood-engorged female was collected, and blood in this fly did not react with available antisera. *Leptoconops* also were attracted to and sometimes fed on the rabbit and quail; of six engorged flies collected from the water beneath the rabbit, five were blank and one reacted with anti-rabbit serum.

Black flies (*Simulium* spp.) also fed on the bighorn, as perhaps did a single female *Anopheles franciscanus*. The black fly feeding was confirmed for two of the three engorged flies with anti-sheep serum, but the blood in the *Anopheles* did not react with available antisera. The biting stable fly, *Stomoxys calcitrans*, was collected once.

Cuclicoides brookmani was by far the most abundant species in CO_2-baited suction traps in the Santa Rosa Mountains (Mullens and Dada, 1992). Based on the lack of specimens attracted to the quail, it appears to be a mammal feeder. J. R. Anderson (in Atchley, 1970) has collected this species from the ears of deer (*Odocoileus hemionus*), indicating a similar feeding pattern on that related host. A large number of isolations of rabbit arboviruses recently have been made from pools of *Culicoides* in the subgenus *Selfia*, including *C. brookmani*, in Colorado (Kramer et al., 1990). While this is not evidence for the role of *C. brookmani* in hemorrhagic disease virus transmission, it does suggest the species possesses other attributes (e.g. longevity) that may contribute to its ability to vector pathogens (Mullens and Dada, 1992).

We confirm the mammal-feeding tendencies of *C. variipennis*, in agreement with prior studies (Tempelis and Nelson, 1971; Zimmerman and Turner, 1983). To date, *C. variipennis* is the only proven vector of bluetongue virus in North America (Gibbs and Greiner, 1988). The reaction of blood from one specimen with both anti-sheep and anti-rabbit serum may suggest double feeding within a single gonotropic cycle. While *C. brookmani* and *C. variipennis* appeared to prefer mammals, the *Leptoconops* spp. were attracted to both mammal and bird hosts. The *C. copiosus* group, which develops in rotting cactus (Ryckman, 1960), was collected most commonly from the quail, suggesting a preference for birds. The positive reaction of blood in one *C. cacticola* with anti-sheep serum, however, indicates this species also may attack bighorn sheep.

The water pan traps beneath bait animals have promise in determining host-feeding patterns of blood-feeding flies. The fully-engorged insects often cannot fly well and may apparently fall into the fluid and drown as they leave the host. The advantage of bait animal studies is that there is a high degree of probability that engorged insects collected from a given host actually fed on that animal, though there also is a possibility that the trap itself may have had an influence on insects approaching the host for a blood meal. We found that, while the water traps did collect engorged insects, the blood in these specimens was not well suited for serological confirmation of blood source. The collection of engorged insects in the pan trap also suggests that flies engorging from the bighorn sheep may have ended up on the ground near the sheep soon after engorgement. In such a case, we may have missed collecting some engorged insects which did not fly to the mesh of the drop trap in the short period involved.

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