

Bovine Anaplasmosis Transmission Studies Conducted Under Controlled Natural Exposure in a *Dermacentor andersoni* = (*venustus*) Indigenous Area of Eastern Oregon

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SUMMARY

In *Anaplasma marginale* transmission studies conducted on the high semi-arid range of eastern Oregon during the 1974 and 1975 vector season, *A. marginale*-susceptible calves (principals) were maintained on 2 raised tick-proof platforms. Anaplasmosis-susceptible control calves of approximately the same age and latent-infected cows grazed the area surrounding the platforms. One latent-infected steer spent the entire 1975 vector season on a platform with the principals. The 28 principals did not develop anaplasmosis, whereas 15 of 30 (50%) controls became infected.

The disease was not transmitted from the latent-infected cattle to the principals exposed only to flying hematophagous insects, whereas 50% of the controls exposed to the Rocky Mountain wood tick (*Dermacentor andersoni*) = (*venustus*) developed the disease. *Dermacentor andersoni* appears to be the principal vector on this range.

Bovine anaplasmosis is enzootic in most of central and eastern Oregon. The Squaw Butte Experiment Station, a cooperative station operated by the Agricultural Research Service, US Department of Agriculture, and the Oregon State University, is located in an anaplasmosis-enzootic area. A herd of approximately 600 Hereford cattle is maintained at this station. The cattle are wintered on the 259-ha headquarters ranch in a large irrigated valley. During spring, summer, and fall, most of the herd is grazed on the high desert ranch (6,475 ha). This range is typical of the sagebrush-bunchgrass range of the western states.

The anaplasmosis card test (CT), conducted on serums of 250 cows in this herd during the fall of 1975,

revealed a 71% infection rate. The infection rates (determined by the CT) of 30 fall-born and 94 spring-born calves following their first pasture season on this range were 63 and 26%, respectively.

Only 2 species of ticks now occurring in western United States, the Rocky Mountain wood tick (*Dermacentor andersoni*) = (*venustus*) and the Pacific Coast tick (*Dermacentor occidentalis*) appear to have sufficient experimental and epizootiologic support to be considered significant anaplasmosis vectors. *Dermacentor andersoni* is indigenous to eastern and central Oregon, but *D. occidentalis* is not found in these areas. However, *D. occidentalis* is indigenous to southwestern Oregon.

It is generally believed that *D. andersoni* is the primary vector of *Anaplasma marginale* Theiler in eastern and central Oregon and in other western states where that tick is indigenous. However, because its precise role as a natural vector remains obscure, its significance as an anaplasmosis vector has been questioned.^{1,8,10,11}

In many areas of the United States, hematophagous flying insects have a major role in anaplasmosis transmission. Certain species of large blood-sucking flies belonging to the order Tabanidae are especially well-known mechanical vectors.^{3,7,12} Experimental transmission by *Psorophora* mosquitoes and stable flies (*Stomoxys calcitrans*) has also been demonstrated.³ The eye gnat (*Hippelates pusio*) Loew (Diptera; Chloropidae) is also considered a possible mechanical vector.⁹

Seventeen species of mosquitoes are recorded from Harney County, the locale of Squaw Butte Experiment Station.^{4,a,b} The genus *Aedes* contributes 12 species. Others are *Culex tarsalis*, *Culisita incidens* and *inornata*, *Anopheles freeborni*, and *Coquillettidia perturbans*.

Horn flies (*Haematobia irritans*) and stable flies (*S. calcitrans*) have also been recorded and collection records of Tabanidae indicate at least 10 species are present in the general area.^a Of the latter, 4 are deerflies, the most common being *Chrysops discalis*; and 6 are horseflies, most frequently represented by *Tabanus aegrotus*, *punctifer*, and *sonomensis*.

The role of these hematophagous insects in anaplasmosis transmission in eastern and central Oregon is not presently known. The present study was conducted to determine whether they are significant vectors or whether the chief vector is *D. andersoni*.

Received for publication May 17, 1976.

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Technical paper No. 4263, Oregon Agricultural Experiment Station, Oregon State University, Corvallis.

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^a Goulding, R. L., Oregon State University, Corvallis, Or: Unpublished data, 1976.

^b Runyan, G., Oregon State Division of Health, Portland, Or: Personal communication, 1976.

Materials and Methods

Platforms—Two identical tick-proof platforms were constructed approximately 3 km apart on this high desert range. Each had space for 10 calves or yearlings, 2 in each of 5 pens. The platforms were 9 m long, 2 m wide, and 1.5 m above ground level. Slatted floors were constructed from 5.08- by 10.16-cm boards. The ends, sides, and dividing panels were of boards spaced 20 cm apart. A manger for feed and water extended along the length of the platform across the front of each pen.

The platform was supported by 6 pipes, 10 cm in diameter, set in concrete. Two metal disks were welded on each pipe support. The lower concave disk was 40 cm in diameter, 1 m above ground level; and the upper convex disk was 50 cm in diameter, 20 cm above the lower disk. As a barrier to tick migration, oil was maintained in the lower disk and a ring of sticky adhesive material was placed around each post below the lower disk. The upper large convex disk prevented urine, feces, and other debris from accumulating in the oil (Fig 1).

To prevent direct contact between the principals and the controls and latent-infected cows, a perimeter fence was built around each platform. Approximately 2.5 m separated the platform from this fence. A water tank for control calves and latent-infected cows was placed adjacent to each fence. This was their only source of water. Protection from flying insects was not afforded platform animals, but a slatted snow fence was suspended over each platform for shade. Both platforms were used in the 1974 study and one in 1975.

1974 Experimental Animals—On April 16, 21 Holstein-Friesian male calves, approximately 6 months of age, and 2 calves 4 months of age, were purchased from a dairy located in the Willamette Valley, where anaplasmosis is rarely observed. Serums were negative from all calves tested.^c The calves were vaccinated^d and trucked to the Squaw Butte Experiment Station on April 23. Ten were unloaded on each platform, while the remaining 3 were delivered to the headquarters ranch and isolated in a drylot. They were to be used as replacements if losses occurred on the platforms. Ten *cr*-negative Hereford fall-born calves between 6 and 7 months of age were placed around 1 platform. Their dams, all but 1 of which were *cr*-positive, were placed with them. Eleven *cr*-negative fall-born calves and their 10 *cr*-positive dams were placed around the other platform (1 cow had twins). The fall-born calves were maintained as controls. Their latent-infected dams acted as a source of infection for both control and platform calves.

The principals were watered, fed pelleted feed (stored at the ranch headquarters), and observed each day for signs of illness. Every 3 weeks, blood samples were collected from each calf in a 5-ml vacutainer tube containing EDTA and 5 ml in a tube without an anticoagulant. A sterile disposable needle was used on each calf. On sample collection days, packed cell volume (%) and hemoglobin (g/100 ml) values were determined^e from each EDTA sample. Blood smears were stained by Wright's method and later examined by light microscopy for *Anaplasma* bodies. Serums were refrigerated at 4 C and later tested by the *cr*.

Three principals died suddenly during the summer, apparently from bloat (on May 21, May 29, and Aug 12).



Fig 1—Calves confined on tick-proof platform.

These were replaced by the 3 anaplasmosis-free calves which had been segregated at the headquarters ranch.

The principals were maintained on the platforms until September 23, when all cattle on this range were moved to the winter headquarters ranch. Here the principals were maintained in a drylot until the final hematologic studies were conducted on November 11.

Identical hematologic studies were conducted on control calves on July 20, September 21, and November 11. To prevent mechanical transmission of *A marginale*, all surgical procedures on principals and controls were performed with aseptic techniques. Disposable needles were used during all vaccinations or treatment procedures. Insecticides and acaricides were not applied to the animals.

1975 Experimental Animals—Four Holstein-Friesian and 4 Angus *cr*-negative calves, approximately 7 months of age, were purchased in the Willamette Valley and handled in the same manner as the 1974 platform calves. They were trucked from the valley to the Squaw Butte Experiment Station on April 29, and unloaded on 1 platform, 2 to a pen. A proved latent-infected 9-month-old steer was placed on the platform in the remaining pen. Blood sample collections, tests for hemoglobin concentration, packed cell volume, *cr*, and preparation of stained blood smears were performed in the same manner as in 1974, except that samples were collected every 2 weeks.

On April 30, 9 fall-born, *cr*-negative Hereford calves, approximately 7 months of age, were placed in the pasture surrounding the platform. Their dams, 5 of which were *cr*-positive, were placed with them. They were handled in the same manner and grazed the same area as the 1974 control calves and dams.

On July 15, grass hay was substituted for half of the pelleted feed to lessen the occurrence of bloat. The principals and the infected steer were otherwise fed, watered, and handled in the same manner as those on the platform the previous year. They were moved to the winter headquarters ranch on September 4, and the experiment was terminated October 8, 1975.

Two principals died suddenly of bloat (on June 22 and July 13). Several others required treatment during their confinement on the platform. Lack of exercise and difficulty in movement because of the slatted floors and feeding a ration of all-pelleted feed seemed to predispose the principals to bloat.

A light trap to collect night-feeding insects was installed

^c Anaplasmosis card test. Hynson, Wescott, and Dunning, Inc, Baltimore, Md.

^d Electroid, Jensen-Salsbery Laboratories, Division of Richardson-Merrell, Inc, Kansas City, Mo 64108. (A formalin-precipitated killed bacterin containing *Clostridium chauvoei*, *C septicum*, *C novyi*, *C sardelli*, and *C perfringens*, type C and D).

^e Spencer hemoglobin meter, American Optical Corporation, Buffalo, NY.

in the area and was operated during the experimental period 3 nights each week. Collected specimens were identified at Oregon State University.

Results and Discussion

The principals were protected on the platforms from *D andersoni*, but not from flying hematophagous insects, whereas the controls were exposed to both. The latent-infected cows pastured in the area surrounding the platforms maintained a source of infection for both principals and controls. In 1975, only 5 of 10 cows maintained around the 1 platform were infected. It was hoped that at least one of the noninfected cows would develop acute anaplasmosis, thus increasing the chance of *A marginale* transmission. Although transmission occurred in the controls, none occurred in the CT-negative cows. Apparently, some degree of resistance was present in these 5 noninfected cows selected from a herd with 71% adult infection.

The controls with their latent-infected dams visited the platform areas each day, since their only water source was in the tanks adjacent to the platforms. Flying hematophagous insects had the opportunity of intermittently feeding on the infected cows, the controls, the infected steer maintained on the platform in 1975, and the principals. Transmission did not occur in the principals in either the 1974 or the 1975 study. In 1974, 7 of 11 (64%) controls maintained around 1 platform and 4 of 10 (40%) around the other platform became infected. In 1975, 4 of 9 (44%) of the control calves developed the disease.

Results of the CT tests conducted on control calves are shown (Tables 1 and 2). Test results of the principals are not included since all test results were negative. All 1974 infections occurred before September 16, and all but one in 1975 occurred before September 4. Since the incubation period of anaplasmosis varies between 20 and 90 days, exposures probably occurred during May, June, and early July, the period of greatest tick activity. Although major tick activity in the area ends early in July, *D andersoni* were observed in small numbers on cattle as late as September 3.

Results in a 1970 anaplasmosis pasture-exposure study conducted in the coastal mountains of California⁵ were similar in several respects to the authors' 1974 results. In that study, 5 of 9 (55%) of the pasture-exposed cows became infected, compared to 11 of 21 (52%) of the pasture-exposed control calves in the present study. Antibodies were first detected in the California study when 1 cow became infected 9 weeks after the 1st day of exposure. The remaining 4 cows were shown to be infected 13 to 19 weeks after start of exposure. In the 1974 study, control calves that became infected also had detectable antibodies 19 weeks after the first day of exposure. The first 2 infected calves had detectable antibodies on their CT conducted 12 weeks after initial exposure. In both studies, all animals which became infected retained their infection status throughout the experiments.

In a California study conducted in 1972,⁵ 6 anaplasmosis-susceptible cows, maintained for 3 months on a raised tick-proof platform, remained free of the disease; whereas, 5 of 6 controls grazing the surrounding pas-

TABLE 1—Anaplasmosis Card Test Results of 1974 Control Calves

Calf No.	July 17	September 16	November 7
Vicinity of north platform			
465	NT	+	+
473	-	+	+
474	+	+	+
482	NT	-	-
504	-	-	-
551	-	-	-
556	-	-	-
563	-	+	+
568	+	+	+
569	-	+	+
570	-	+	+
Vicinity of south platform			
547	-	-	-
462	-	-	-
480	+	+	+
481	-	+	+
505	-	-	-
516	NT	-	-
529	+	+	+
534	-	-	-
541	+	+	+
561	-	-	-

NT = Not tested.

TABLE 2—Anaplasmosis Card Test Results of 1975 Control Calves

Calf No.	July 8	September 4	October 8
796	-	-	-
757	+	+	+
731	-	-	+
732	-	-	-
780	+	+	+
783	-	-	-
736	-	+	+
746	-	-	-
738	-	-	-

tures developed anaplasmosis. Hematophagous flying insects, as in the Oregon study, did not transmit the disease to the platform cattle. The vectors appear to be *D occidentalis* in the coastal range of California^{2,6} and *D andersoni* in eastern Oregon. *Dermacentor occidentalis* is considered a better vector, since both transovarian and transstadial transmission occurs and all stages parasitize cattle and deer. Transmission rates in the present experiments, however, were similar. It is recognized that a comparison of these transmission rates may not be valid since too few animals were involved. Also, in California, the experimental animals were well-isolated from other cattle, while latent-infected California black-tailed deer (*Odocoileus hemionus columbianus*) were the source of infection. In the Oregon study, the source of infection was latent-infected cattle.

The results of the light trap collections at Squaw Butte indicated there was little mosquito activity during the experiment; but catches did yield significant numbers of *Culicoides* (Diptera; Helidae) from which 6 species were identified. It is not known if any of the species fed upon cattle or whether *Culicoides* may act as mechanical vectors of anaplasmosis. Usually, under favorable weather conditions, *Culicoides* have been observed while feeding in the ears of cattle on this station.

Horn fly (*H irritans*) populations were moderate to heavy (200 to 500 or more per animal) during July and August on the calves adjacent to the platform and on other station cattle. The population on the platform calves was small (5 to 10 per animal) during the same period. Stable flies (*S calcitrans*) numbered 5 to 10 on

each of the platform cattle and adjacent animals. This fly was rarely observed on other station cattle. Breeding of this fly was most likely associated with feces and feed wastes beneath the platform.

Results of the present 2-year study suggest that *D andersoni* is the principal vector of *A marginale* in the high desert ranges of central and eastern Oregon. Hematophagous flying insects did not transmit the disease, although they were afforded ample opportunity. Latent-infected animals were in close proximity of the platform each day, and in 1975, one spent the entire vector season on the tick-proof platform with the *A marginale*-susceptible principals.

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