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FAILURE OF ANAPLASMA MARGINALE THEILER TO SURVIVE NATURAL WINTER CONDITIONS ON A DERMACENTOR ANDERSONI = (VENUSTUS)

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INTRODUCTION

Bovine anaplasmosis is enzootic in the semi-arid sagebrush-bunchgrass area of eastern and central Oregon where the Rocky Mountain wood tick (Dermacentor andersoni) = (venustus) is the major Anaplasma marginale Stiles vector (7, 9). This is a hardy northern three host tick which is resistant to severe cold and is found in the Rocky Mountain and Intermountain regions of the United States at elevations of 500 to 9000 feet (0.152 km to 2.74 km). Small mammals serve as hosts of the larvae and nymphs while adults require large mammals, preferably cattle and horses, to complete their development (3, 6). All stages are hematophagous and must ingest a blood meal to survive. The life span of the tick is 2 to 6 years.

It has been suggested that transovarian and transstadial transmission of A. marginale occurs in D. andersoni and that under favorable conditions the organism may be picked up and transferred to all succeeding stages of the tick for at least 2 generations and still be capable of infecting susceptible cattle (6, 13). Ricketts demonstrated that the etiologic agent of Rocky Mountain Spotted Fever (Rickettsia rickettsii) was transmitted transovarially and transstadially by D. andersoni (3). Howell demonstrated transovarian transmission of A. marginale by D. andersoni in a single experiment (4). However, numerous similar trials by other researchers failed (1, 11, 12). These trials were not conducted in a D. andersoni indigenous area and altered environmental conditions may have influenced the results. Whether transovarian transmission occurs in nature, and if so, to what extent, is presently not known.

Transstadial transmission has been demonstrated in the laboratory (1, 2, 10) but this type of transmission should be of little significance since under natural conditions the larvae and nymphs parasitize small mammals rather than larger ruminants (3, 6). To date, no species of small mammals has been incriminated as a reservoir host of A. marginale.

Since under natural conditions transovarian and transstadial transmission appear to be of no significance, adult D. andersoni by repeat feeding must act as the vector of A. marginale. Repeat feeding of adult females is unlikely as females quickly attach to the host,

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begin feeding and usually remain attached until replete. Following engorgment and fertilization 14 to 17 days after attachment they drop from the host and seek a sheltered place for oviposition which may begin in 3 to 5 days (6). Following oviposition the female dies. Repeat feeding by males does occur as they move about the host seeking females (6). As suggested by Roseboom et. al. (11) at this stage the males may leave the host and later parasitize another. Since D. andersoni is a biological vector, infected ticks may transmit A. marginale for a considerable period of time. It has been demonstrated in the laboratory that A. marginale will remain alive in the adult male tick under partial hibernating conditions for as long as 197 days (1). If it is capable of remaining alive and viable under natural conditions throughout the entire winter dormant period of the tick, control will be difficult since pastures will remain infected from year to year.

To determine whether under natural condition A. marginale survives the long winter dormant period of D. andersoni a two year study was conducted on the Squaw Butte Experiment Station. This station located in the anaplasmosis enzootic area of Eastern Oregon, consists of two ranches, the Squaw Butte Range of 16,000 acres (6,475 ha) situated in the semi-arid sagebrush-bunchgrass area and the winter headquarters ranch of 640 acres (259 ha) located 40 miles (64 km) distant on a flood plain meadow. Dermacentor andersoni have not been found on the headquarters ranch although numerous collection attempts by flagging and CO_2 trapping have been made. Undoubtedly the tick is continually introduced into this meadow habitat on both domestic and wild animals. Apparently the habitat will not support D. andersoni populations. The tick is indigenous to the Squaw Butte Range.

A herd of approximately 600 Hereford cattle is maintained by the station. Most of the animals are grazed from the latter part of April until October on the Squaw Butte Range. All are wintered at the headquaters ranch. Numerous Anaplasmosis Card Agglutination Tests (CT) and Standard Complement-fixation Tests (CF) conducted on this herd during the past 4 years have demonstrated that approximately 70% of adults are latent A. marginale carriers.

MATERIALS AND METHODS

1975 Trial--On April 29, 1975, 35 Holstein-Friesian steers (principals) 7 to 9 months of age were trucked to the Squaw Butte Range and unloaded directly on a double fenced sagebrush-bunchgrass pasture which had contained no cattle since October, 1974. The principals were purchased from a large dairy located in the Williamette Valley of Oregon where anaplasmosis is seldom observed. On March 18 and April 28 prior to purchase a 5 ml blood sample was collected from each in a vacutainer containing no anticoagulant. After clotting, the samples were refrigerated at 4 C. The CT following the method of Hynson, Wescott and Dunning[a] was conducted on the serums 72 hours following collection. All tests were negative.

These anaplasmosis free principals grazed the entire season until October 3 on 5 double fenced sagebrush-bunchgrass pastures totalling 1,200 acres (486 ha). These pastures had been grazed the previous summer and for years prior to that by the Squaw Butte herd. To avoid direct contact with the latent A. marginale infected herd, each pasture grazed by the principals was double fenced. An area 15 to 40 feet (4.6 m to 12.2 m) wide separated the fences. A portable corral and chute were used for examining the principals and collecting blood samples. The principals were observed daily for signs of illness and at 2 week intervals 10 ml of blood was collected from each, 5 ml in a vacutainer containing EDTA and 5 ml containing no anticoagulant. A sterile disposable needle was used on each animal and nose-tongs were disinfected between animals. All surgical procedures were performed using aseptic techniques. On collection day, packed cell volume percent (PCV) and hemoglobin (Hb) values (gm/100 ml as measured by the Spencer Hb. Meter)[b] were determined for each sample. Blood smears were stained by modified Wright's method and later examined for A. marginale bodies by light microscopy. Serums were refrigerated at 4 C and later tested by the CT method. Positive control serum was collected for each series of tests from a latent infected steer maintained at Oregon State University, Corvallis, Oregon.

On April 23, blood was collected from thirty, 6 to 8 months old Squaw Butte fall-born Hereford calves in the same manner as from the principals. Serums were tested by the CT method and all were negative. On April 28, these calves (controls) with their dams and the main herd were trucked to the Squaw Butte Range. During the grazing season they grazed pastures surrounding those containing the principals. There was no observable difference in these pastures regarding vegetation, animal life or topography. Serum samples were collected from the controls on September 4 and October 8 and the CT conducted on September 7 and October 13 respectively. As with the principals, nose-tongs were disinfected between each control and a sterile disposable needle was used on each. All necessary surgical procedures were performed with aseptic techniques. No insecticides or acaricides were used on either the principals or controls. The controls were weaned on September 4 and 25 were moved to a feedlot on the headquarters ranch and 5 to a feedlot at Corvallis, Oregon. The final tests were conducted on both controls and principals on October 13.

On June 26, July 23, August 5 and September 3 the principals were examined in a squeeze chute for the presence of D. andersoni by digital palpation and visual observation. Tick numbers were recorded and ticks classified as either flat or engorged. No ticks were removed or detached.

[a]Anaplasmosis Card Test, Hynson, Wescott, and Dunning, Inc., Baltimore Md.
[b]Spencer hemoglobin meter, American Optical Corp., Buffalo, New York 14215.

1976 Trial--Eighteen purebred Red Polled females varying in ages from 1 to 10 years were shipped from the Willamette Valley to the Squaw Butte headquarters ranch during February. Blood samples were collected from each on February 26 and the serums were tested for anaplasmosis by both the CT method and CF test on March 4. All tests were negative. On April 23 serum samples were again collected. All CTs were negative and on April 26 the cattle were trucked to the Squaw Butte Range where they were unloaded on a double fenced pasture grazed in 1975 by the main herd. Ten of the Red Polled cows were nursing spring born calves.

On April 28, blood samples were collected from 8 aged Hereford non-pregnant cows at the Eastern Oregon Agricultural Research Center located at Union, Oregon. This center is located in an area where anaplasmosis is not enzootic and a previous 1975 herd test indicated no latent A. marginale carriers. The CT conducted on the serums of the 8 Herefords were negative. The cows were trucked to the Squaw Butte Range on May 4 and were unloaded on the pasture containing the Red Polled cattle. This anaplasmosis free herd of 26 head was maintained as the experimental herd (principals) and was handled and tested in an identical manner to the 1975 principals except that blood collections and tick examinations were made at 3 week intervals and tick examination began on May 20.

Throughout the summer pasture season the principals grazed 2 double fenced pastures totaling 1640 acres (663.7 ha) all of which had been pastured the previous summer by the 1975 controls, their dams and the main herd. The spring born Red Polled calves remained with their dams, throughout the summer. They were tested by the CT method only at the trial's termination when they were weaned.

On April 29, 30 fall born Hereford CT negative calves (controls) were moved from the headquarters ranch along with their dams and the main herd to the Squaw Butte Range. Here they were maintained during the summer grazing period on D. andersoni infested pastures similar to those grazed by the principals. Serum from each control was collected on May 10, July 12, August 4 and October 19 and tested by the CT method. These controls were otherwise handled in the same manner as those in the 1975 trial.

Results

1975 trial--No signs of illness were noted in the principals during the daily observation periods. The CTs remained negative during the entire trial, no A. marginale bodies were observed in the stained blood smears and PCVs and Hb. values (Table 1) remained within normal ranges.

The control calves were not observed daily since they grazed with the main herd over a large area. However, when observed, none demonstrated clinical signs of anaplasmosis. Thirteen controls developed positive CT reactions by September 4 and 18 of 30 (60%) by October 8 (Table 5).

Dermacentor andersoni counts conducted upon the principals demonstrated that tick activity decreased after July 1, but a few ticks persisted into August and 1 into September (Table 3).

1976 trial-- The principals remained clinically healthy during the entire trial as no signs of anaplasmosis or other illness were noted during the daily observation periods. The CTs again remained negative no A. marginale bodies were observed in stained blood smears. None of the PCVs and Hb. values were reduced--generally they were slightly higher than normal possibly due to dehydration resulting from heat stress (Table 2). Serum from the ten spring calves were also CT negative when tested at the trials termination.

The control calves were not observed daily. However, none were observed ill during the grazing season and all appeared healthy when weaned and removed from this range on August 4. Seven of the 30 controls (23%) developed positive CT reactions by October 21 (Table 6). Four were CT positive on July 12, another developed a positive CT reaction by August 4 and 2 more by October 21. All calves elicitating a postive reaction continued to react in succeeding tests.

Dermacentor andersoni counts (Table 4) as expected were highest during May but ticks were still active on July 22. None were observed on the August 2 count.

To avoid possible interference with A. marginale transmission, ticks were not removed during either the 1975 or 1976 counting procedure. An accurate sex determination of the ticks was therefore not possible. All engorged ticks were females but flat ticks may have been either males or engorging females which had recently infested the host.

Discussion

In both 1975 and 1976 trials the anaplasmosis-susceptible principals remained free of anaplasmosis throughout the summer grazing period which extended from late April or early May into October. During both periods, they grazed D. andersoni infested pastures which had been grazed the previous year by a herd of cattle heavily infected with anaplasmosis. In contrast, 60% of the 1975 and 23% of the 1976 control calves developed positive-CT reactions. They were maintained during the grazing periods on similar pastures but in direct contact with infected cattle. The high transmission rates observed in the controls likely resulted from male D. andersoni ingesting one or more blood meals from infected cows prior to parasitizing the susceptible controls.

Results of these trials demonstrated that A. marginale failed under natural range conditions to survive the winter season on D. andersoni infested pastures. It did not remain viable in either nymphs or adult ticks during their long dormant periods. Transovarian and transstadial transmission, if they occurred, were of no significance since A. marginale infection did not continue into the adult tick stage.

No species of small mammals was involved as an A. marginale reservoir host since identical species must have been present in the adjacent pastures grazed by the principals and controls. In 1976 the principals grazed the identical pastures grazed by the controls in 1975. Mule deer (Odocoileus hemionus hemionus) present on this range in moderate numbers were of no significance as reservoir hosts of A. marginale. It has been demonstrated in Idaho (5) but not in Oregon (8) that mule deer may act as natural reservoir hosts of A. marginale. Mule deer commingling on this range for years with a herd of heavily infected cattle failed to play a role in A. marginale transmission. Apparently A. marginale survives the winter season in this area only in latent infected cattle.

The higher infection rate in the 1975 controls (60%) compared to the 1976 controls (23%) cannot be explained. It is known that the incidence of bovine anaplasmosis differs from year to year in a given area. In previous experiments on this range infection rates in similar controls have varied from 40% to 63%. Whether the population of D. andersoni, the only proven A. marginale vector on this range (9) was greater in 1975 than in 1976 was not definitely determined. Tick counts were performed on the principals both years, however, in 1975 the first count was conducted on June 26, past the peak of the vector season. (The reason for counting the ticks was to determine when tick activity ceased). However, a comparison of this 1975 count with the July 1, 1976 count shows a greater number of ticks in 1976 (Tables 3-4). Assuming a similar tick population in the controls it would appear that the tick population in 1976 was at least as great as in 1975.

It has been stated that D. andersoni are not active after July 1 (3). However, Dermacentor andersoni activity in both the 1975 and 1976 trials extended into the latter part of July. In 1975, results suggest that the vector season in this area must be considered to extend from early spring through August. Finally the results of these two trials demonstrated that anaplasmosis could be controlled or eliminated on selected ranches in this area. Recommendations similar to those used in the south and southeastern states for the development of anaplasmosis free herds would be applicable on ranches in central and eastern Oregon where the cattle are maintained separately and do not intermingle with other cattle of unknown status. These recommendations would be especially applicable to purebred herds where the owners wish to sell animals interstate and internationally. Cleaning up heavily infected herds at the present will be difficult and it probably is not advisable in herds that mingle with infected cattle. However, with the development of new, more effective, economical and more easily administered drugs, treatment to eliminate latent infected carriers from these herds may become practical.

SUMMARY

Anaplasma marginale did not survive the natural winter range conditions of eastern Oregon on D. andersoni infested pastures. Apparently the long dormant period of D. andersoni was lethal to A. marginale. Transovarian and transstadial transmission, if they occurred, were of no significance as infection did not continue into unfed adult ticks. No species of small mammal was involved in the transmission cycle since identical species must have been present in the adjacent pastures grazed by the principals and controls. Mule deer (Odocoileus hemionus hemionus) present on this range in moderate numbers were of no significance as A. marginale reservoir hosts. Apparently A. marginale survives the winter in this area only in infected cattle and is transmitted to susceptible cattle by D. andersoni parasitizing infected cattle and later during the same vector season parasitizing noninfected cattle. Results of these trials indicate that anaplasmosis could be eliminated in selected herds.

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Table 1. Packed Cell Volumes and Hemoglobin Values of the 1975 Principals - 35 Holstein-Friesian Steers

Date	5/15	5/29	6/12	6/26	7/10	7/23	8/4	9/20	10/4
					PCV (%)				
Mean	32	32.2	34	38	35.5	35.9	34.7	33.2	33.3
Min.	26	24	30	32	31	30	28	28	27
Max.	40	38	39	45	46	42	41	40	40
					Hb. (g/dl)				
Mean	12.9	12.4	12.4	13.5	12.9	12.5	12.3	11.3	12.3
Min.	10.8	11.0	11.0	11.6	11.2	11.0	10.2	10	10
Max.	14.4	14.4	14.5	15.0	15.0	14.6	14.2	13.8	23

*Steers were 7 to 9 months old at the beginning of the trial

Table 2. Packed Cell Volume and Hemoglobin Values of the 1976 Principals - 26 Red Polled and Hereford Females

Date	5/20	6/9	7/1	7/22	8/12	9/12	9/23	10/21
					PCV (%)			
Mean	36.0	37.1	39.0	41.2	39.8	40.7	40	38
Min.	31.0	30.0	35.0	36.0	35.0	37.0	36	30
Max.	40.0	45.0	45.0	48.0	46.0	45.0	46	44
					Hb. (g/dl)			
Mean	15.3	15.5	17.0	14.2	15.2	16.0	14.3	14.9
Min.	13.0	13.0	14.5	12.0	12.0	13.0	12	13
Max.	17.5	17.5	19	17.0	17.5	17.5	17	17

Table 3. Dermacentor andersoni Observed Parasitizing 1975 Principals

Date	6/26	7/23	8/5	9/3
Flat Ticks	23	20	3	1
Engorged Ticks	82	12	3	0
Total	105	32	6	1
Mean/steer	3.1	.94	0.14	0.028

Table 4. Dermacentor andersoni Observed Parasitizing the 1976 Principals

Date	5/20	6/9	7/1	7/22	8/12	9/2
Flat Ticks	244	201	218	103	0	0
Engorged Ticks	328	72	158	41	0	0
Total	582	273	376	144	0	0
Mean/Animal	23.3	10.5	14.5	5.5	0	0

Table 5. Anaplasmosis Card Agglutination Test Results of 30, 1975 Control Calves

Calf No.	4/16	9/4	10/8
731	-	-	-
740	-	-	-
743	-	+	+
745	-	+	+
748	-	-	-
752	-	-	+
761	-	-	-
765	-	+	+
766	-	NT	+
770	-	-	+
774	-	+	+
781	-	+	+
789	-	NT	-
797	-	+	+
798	-	+	+
807	-	+	+
812	-	-	+
814	-	+	+
820	-	-	-
822	-	-	-
823	-	-	-
826	-	-	-
829	-	-	-
844	-	-	-
847	-	-	+
850	-	+	+
851	-	+	+
853	-	+	+
863	-	-	-
865	-	+	+
Total positive reactors	0	13	18

NT=Not Tested

Table 6. Anaplasmosis Card Agglutination Test Results of 1976 Control Calves

Calf No.	5/10	7/12	8/4		10/20
G 19	-	-	-		-
G 20	-	-	-	-	
G 21	-	-	-	+	
G 24	-	+	+	+	
G 27	-	-	-	-	
G 31	-	-	-	-	
G 34	-	-	-	-	
G 46	-	-	-	-	
G 47	-	-	-	-	
G 48	-	-	-	-	
G 51	-	-	-	-	
G 54	-	+	+	+	
G 60	-	-	-	-	
G 64	-	-	-	-	
G 65	-	-	-	-	
G 68	-	-	-	-	
G 78	-	+	NT	+	
G 82	-	-	-	-	
G 93	-	-	-	-	
G 96	-	-	-	-	
G 103	-	-	-	+	
G 106	-	-	-	-	
G 108	-	-	-	-	
G 111	-	-	-	-	
G 113	-	-	-	-	
G 117	-	-	-	-	
G 122	-	-	+	+	
G 140	-	-	-	-	
G 147	-	+	+	+	
G 150	-	-	-	-	
Total positive reactors	0	4	4		7

NT = Not Tested

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