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Title: TRANSMISSION OF BOVINE ANAPLASMOSIS IN EASTERN OREGON BY FIELD COLLECTIONS OF DERMACENTOR ANDERSONI STILES = (VENUSTUS)

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In the spring and summer of 1975 a study was conducted under natural conditions to determine whether wild adult Dermacentor andersoni Stiles would transmit Anaplasma marginale to cattle. The ticks were collected from the range pasture of Squaw Butte Experiment Station where anaplasmosis is enzootic and D. andersoni is indigenous. Adult D. andersoni ticks were collected by flagging and by the use of CO_2 traps from pastures of Squaw Butte Station in which latent infected cows were grazing. These ticks were placed on two calves. A. marginale infection was produced when 237 adult ticks of both sexes were allowed to attach and feed on a susceptible unsplenectomized calf, but was not produced by 217 adult ticks placed on a second unsplenectomized calf.

These results show that transmission of anaplasmosis to cattle by D. andersoni ticks does occur on this range.

Transmission of Bovine Anaplasmosis in Eastern Oregon by Field Collections of *Dermacentor andersoni* Stiles=(Venustus)

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INTRODUCTION

Anaplasmosis is an infectious, transmissible disease primarily of cattle characterized by anemia, icterus and the presence of anaplasma bodies within erythrocytes.

In recent years there has been an increased interest in the age old problem of bovine anaplasmosis. This is due to the fact that the disease is endemic and causes substantial beef cattle losses in many areas of the United States including eastern, central and southern Oregon. The disease is present to some extent in nearly all states of the Union. The infection rate in cattle is high in the southern and coastal states where biting flies are the important vectors. Incidence is high too in western rangeland areas of Oregon, Idaho, Montana, Wyoming, Colorado, Utah, and California. A survey conducted by the American Cattlemen's Association considered anaplasmosis as one of the ten most important diseases of beef cattle in U.S.A. (92).

Based on morbidity reports submitted by practicing veterinarians it is estimated that between 50 and 100 thousand animals die of anaplasmosis each year (49). Though deaths resulting from anaplasmosis are staggering, they are minor as compared to weight, milk and calf losses occurring in surviving animals and the cost of treatment and control measures. It is estimated that anaplasmosis losses in the United States are about $100 million a year. In a national

anaplasmosis survey conducted between July 1, 1972 and June 30, 1973, 14% of the animals tested were found to be reactors and suspects (49). The survey was conducted by collecting market cattle serum samples in each state from slaughtering establishments at a statistically predetermined rate. Samples were collected only from backtagged cattle that could be traced to the state and county of origin.

A survey conducted in 1954 - 1955 on approximately 3500 bovine serums collected from eastern Oregon ranches revealed a within herd infection rate of 0 - 99% with an average rate of 60% (61). The survey revealed that the cattle which had remained in irrigated valleys were usually free of infection while those grazing sagebrush-covered desert range were Complement Fixation (CF) test positive. A recent study of a 168 cow herd located in this anaplasmosis enzootic area at Squaw Butte Experiment Station showed 43% CF test 4+ reactors, 28% suspects and only 29% negatives. Of 120 young calves that were moved to the sagebrush-covered desert range in April, 24.2% were CF 4+ reactors, 12.5% 3+ suspects and 1.6% 2+ suspects when removed in early September (61).

The card agglutination test (CT) conducted on 251 adult cows in the fall of 1975 revealed an infection rate of 71% (61). The infection rate in 1973 and in 1974 was found to be the same. Of 30 fall born and 94 spring born calves pastured on the same range 63% and 26% respectively were positive when removed in the fall (61).

REVIEW OF LITERATURE

History of Anaplasmosis

Smith and Kilborne in 1893 first described the etiologic agent of anaplasmosis in cattle, but considered it to represent a stage in the life cycle of Babesia bigemina (90). Theiler (1910) later named the parasite Anaplasma marginale because of the absence of cytoplasm and the typical marginale location of the parasite within the erythrocyte. He named the disease anaplasmosis (45). The disease existed in U.S.A. along with Texas fever, but for a long time was not recognized as a distinct disease entity. Following the control of Texas fever, anaplasmosis was recognized as a distinct disease.

It was first reported as a disease in cattle in the United States by Darlington (15). In 1931 Lestoquard transmitted A. marginale from bovine carriers to buffalo - presumably African buffalo (Cyncerus caffer). Neitz and Du Toit (56) demonstrated experimental infections with A. marginale in blesbuch (Damaliscus albifrons) and duider (Sylvicapra grimmi). These antelops were used to obtain pure strains of anaplasma species from mixed infections with piroplasms in cattle. Boynton and Woods (7) found deer (Odocoileus hemionus hemionus and O.h. columbianus) susceptible to experimental infection with A. marginale. Neitz (55) in 1935 reported the susceptibility of African antelope, the black wildebeest (Conochaetes gnu), to infection with A. marginale.

In another study Boynton and Woods (8) demonstrated natural occurence of A. marginale infection in California black tailed deer (O.h. columbianus). Christensen et al. (11), and Osebold et al. (57) reported a relatively high incidence of A. marginale in black tailed deer in California. However, Howe and Hepworth (36) reported a very low incidence in mule deer (O.h. hemionus) in Wyoming. Disease transmission between O.h. columbianus and cattle commonly occurs (10). Latent infections also have been found in crosses of Columbian black-tailed deer and mule deer in the Sierra-Nevada foothill area of California (9, 58) and in wild deer in Texas (43).

Other wild ruminants reported (37) to be susceptible to infection with A. marginale include the pronghorn antelope (Antilocapra americana), Rocky Mountain bighorn sheep (Ovis canadensis), and American elk (Cervus canadensis). Roberts and Lancaster (77) established the infection of A. marginale in white tailed deer (O. virginianus). However, research on native white tailed deer conducted in nine southwestern states demonstrated no natural infection or latent carriers in 262 deer examined (3). Similarly, of 49 white tailed deer sampled in Wyoming, none were infected (36).

A number of small mammals, rodents, and laboratory animals have been experimentally inoculated with virulent anaplasma to test their susceptibility. Dykstra et al. (21) reported that guinea pigs, rabbits, white mice, white rats, field mice, gray rats, dogs, cats, ferrets and chickens were refractory to A. marginale. Summers and Gonzalez (94) were unable to infect guinea pigs, mice, rats, and gerbils with A. marginale despite attempts to increase their

susceptibility by various methods.

It is not known when anaplasmosis was first recognized in Oregon. From the earliest reports it appears that A. G. Moore, a veterinarian in Ontario, Oregon received laboratory confirmation of the disease in 1920 (42). Since then anaplasmosis has been recognized as an important bovine disease in many areas of eastern and central Oregon and localized areas of southwestern Oregon (60).

Occurence

Anaplasmosis is widely distributed in the warmer parts of the world, but is spreading slowly with the transportation of latent infected cattle into more temperate areas. The rapidity of spread is largely determined by the presence of suitable arthropod vectors. The disease incidence depends upon such factors as the introduction of susceptible animals and the vector population and their capabilities of disease transmission. Expansion of vectors into previously vector free areas also occurs. The disease has been reported in Africa, European countries bordering the Mediterranean Sea, Palestine and the near East, India, Maylay Peninsula, French Indo-China, Indonesia, Australia, Mexico, South and Central America, and in the United States (19). In the United States it is enzootic in the southeastern, Gulf, lower plains, and western states, but sporadic outbreaks in northern states are not unusual.

Anaplasmosis is essentially a disease of cattle, affecting all breeds and all ages. Imported animals, however, appear to be

more susceptible than native cattle in endemic areas. Calves are generally more resistant to clinical infection and usually develop a milder form of the disease. The severity of the reaction to infection appears to be directly proportional to the age of the animal. However, in a study by Ristic (1959) it was demonstrated that one pathogenic strain of A. marginale produced equally severe clinical manifestation of infection in both young and adult cattle (74).

Character of the Disease

Anaplasmosis is an infectious disease of cattle caused by Anaplasma marginale and Anaplasma centrale, and of sheep by Anaplasma ovis. A. marginale is the most pathogenic for cattle, and is capable of causing the severest attack. Symptoms are anemia, weakness, rise in body temperature, absence of hemoglobinuria and constipation. Other signs may be icterus, inappetence, depression, dehydration, labored respiration, irrational behaviour, atony of the rumen and reduced milk flow in dairy cows (30). Pregnant cows may abort.

In range beef cattle, the disease usually is not recognized until the affected animal is extremely anemic and weak. A marked icterus often develops. Clinically affected animals often succumb from hypoxia when moved or handled for treatment (88). The mortality is quite variable. It may be greater than 50 percent or less than 5 percent. Losses are greatest in hot weather, and in older animals (31, p. 679).

Mott defined the incubation period of anaplasmosis as the interval between the time of exposure and the time the first A.

marginale bodies are detected in stained blood smears (52). Following inoculation of infected blood into susceptible hosts the incubation period varies between 17 and 45 days (30, 86). At the time clinical signs appear a few anaplasma bodies are observed in the red blood cells. Further increase occurs in a fairly constant pattern (44) the numbers being doubled every 24 hours for 7 - 11 days in an acute case (52). Hematocrit and hemoglobin value gradually decrease beginning with, or shortly after, the first appearance of microscopically demonstrable organisms. In presence of vigorous hematopoietic activity, the hematocrit reading will begin to return to normal immediately following the peak of infection (72). The malady is generally mild in calves up to 1 year of age (73, p. 523). Peracute anaplasmosis (73, p. 523) constitutes the most severe and usually fatal form of the disease. It occurs frequently in purebred animals or in high producing milk cows, which die within a few hours after the onset of infection. In addition to anemia, milk flow is suspended, extensive salivation and very rapid respiration are noted and animals so affected often exhibit irational behaviour and signs of nervousness.

Recovery from the disease is accompanied by disappearance of the parasites from the red blood cells, however, during the carrier stage of infection they may periodically reappear at irregular intervals (42). It has been consistently demonstrated that the blood of carrier animal remains infectious for long periods (4). Since carrier cattle cannot be differentiated clinically from non infected animals, it is evident that an immunologically balanced host parasite relationship exists.

Morphology as Shown by Microscopic Techniques

The classic descriptions of Anaplasma marginale were made with a light microscope and various blood stains. The organism thus described was a basophilic stained spherical body, 0.2 u. to 1.0 u. in diameter, situated within and near the margin of the erythrocyte. Many other shapes including rod, comma, ring, triangular, rough, smooth and sporoid were described by workers using light microscopy and different methods of preparation (18, 47).

Franklin and Redmond (26) have presented evidence of projections from the anaplasma body with Giemsa stained material. Definite tail-like structures projecting from anaplasma bodies toward the interior of infected erythrocytes, or in some cases extending outside the erythrocyte were evident in their pictures.

By lysing the erythrocytes with saponin and examining the wet preparations with phase contrast microscopy, Espana et al. (23) have demonstrated anaplasma with both ring and tail-like projections from the head. Occasionally, 2 anaplasma appeared to be joined by the tail structures to form a dumbbell shape.

Similar forms have been reported by Pilcher et al. (63) in anaplasma infected erythrocytes lysed by freezing and thawing and examined by phase contrast. In addition, Pilcher stated that heads were either single or divided into a number of spherical or elongated segments.

The presence in lysed erythrocytes of the forms described by Espana and Pilcher were confirmed by Madden (48), using a fluorescent

antibody specific for anaplasma. Espana, Pilcher, and Madden each reported the observation of classically shaped anaplasma in addition to the variant forms. Anaplasma showing only the classic morphology were first demonstrated with the anaplasma specific fluorescent antibody technique by Ristic in 1957 (76).

Evidence concerning the morphology of Anaplasma marginale derived from electron microscopy appears to confirm many of the characteristics previously observed. In the earliest report of anaplasma observed with the electron microscope, De Robertis et al. (16) indicated that the anaplasma body may be composed of a homogeneous mass, of a central mass surrounded by elementary bodies 0.17 to 0.22 u. in diameter, or of only the elementary bodies. The elementary bodies were also seen singly and not associated with an anaplasma body. Except for the anaplasma body with elementary particles surrounding a central mass, these descriptions agree quite closely with the smooth anaplasma composed of a single homogeneous mass, the rough form containing 8 "sporoids" and the single "sporoids" as seen by Lotze and Yiengst (47) with light microscopy.

Later workers, Espana in 1957 (22) and Ristic in 1960 (71) using the electron microscope, tended to confirm and extend these observations. Espana (22), in his preliminary report, was unable to show the single elementary body in erythrocytes. Ristic (71) was able to show single initial bodies which he tentatively identified with De. Robertis' elementary bodies. He also identified a smaller particle which he named a polyhedral body. Ristic thought

the initial bodies were the basic infective unit of anaplasmosis. From 1 to 8 of the initial bodies were found to form an anaplasma body. Ristic did show anaplasma bodies as composed of a number of initial bodies around an undifferentiated mass.

Foote et al. in 1958 (24), Scot et al. in 1961 (87) and Ristic in 1960 and 1961 (71, 75), described the anaplasma body as seen in ultra-thin sections by electron microscopy. Essential agreement was found in these reports; in cross section, the anaplasma body was composed of 1 to 8 subunits and enclosed by a membrane. Each subunit was composed of irregular masses of dense material, some containing a central dense mass, and limited by a double membrane. The subunits were identified by Ristic as the initial bodies seen on whole mounts.

From these descriptions and photographs it is possible to find general areas of agreement. The classic anaplasma body is composed of 1 to 8 subunits called sporoids by Lotze, elementary bodies by De Robertis, subunits by Foote, and initial bodies by Ristic.

Electron micrographs of anaplasma with excellent contrast were published by Simpson, Kling & Love (89). They reported that the anaplasma body contained 1 - 6 subunits, each unit was surrounded by a two layered membrane. The internal portion of the subunits appeared to be composed of a fibrillar material, and electron dense granules which were thought to be R.N.A. and D.N.A. The contour of anaplasma was either smooth or irregular indented.

In a recent comprehensive review, Ristic (73, p. 474) characterized the infectious unit of an initial body of A. marginale as a round or oval body, ranging from 300 - 400 mu. in diameter, possessing a double layered plasma membrane in addition to an outer envelope membrane, and consisting internally of an aggregation of finely granular material embedded in an electron lucid plasma. Ristic described the initial body as penetrating the erythrocyte and dividing by binary fission to produce the classic marginale body which is composed of a number of little bodies. After initial bodies were released from the erythrocytes, Ristic regarded them as the infective forms responsible for invasion of other erythrocytes.

In conclusion, there are many questions concerning the proper classification, the true morphology, and the complete life cycle of the anaplasma. Further work is necessary to answer the remaining questions.

Diagnosis

The Merck Veterinary Manual (88, p. 281) gives the following description of the diagnosis of anaplasmosis:

> In enzootic areas, anaplasmosis should be suspected in mature cattle showing anemia without hemoglobinuria. Icterus often is an important sign. The only incontrovertible evidence of the disease is demonstration of the anaplasms or marginal bodies in the erythrocytes in stained blood smears. Up to 50 - 60 percent of the red blood cells may be parasitized. In cases where blood cell destruction has been excessive and the course of the disease prolonged, there may be so few anaplasms present in the circulating red blood cells that positive diagnosis by microscopy is impossible.

Postmortem changes seen in anaplasmosis are related to the massive destruction of erythrocytes and to the effects of the resulting anemia. Typical lesion are pale mucous membranes, icterus, enlargement of the spleen up to 2 1/2 times normal size, thin watery blood, distended gallbladder, and a slightly enlarged liver with a mottled mahogany-colored surface. Epicardial and endocardial hemorrhages may be seen in acute cases, signs of constipation may be evident, lymph nodes may be enlarged and edematous, and petechial hemorrhages may occur on the visceral and parietal pleura.

In cases of sudden death the disease must be differentiated from anthrax, particularly in areas where both diseases occur. Differential diagnosis requires consideration of leptospirosis, bacillary and idiopathic hemoglobinuria, and poisoning due to rape (Brassica napus), certain crucifera and bracken fern. The absence of hemoglobinuria and hematuria in anaplasmosis should exclude these conditions.

Tests in general use are the complement fixation (CF) test and card agglutination test (CT). The CF test was developed to detect chronic or carrier cases, but a positive CF test will occur before anaplasma bodies can be found in the erythrocytes in acute cases (50). The test, though very accurate is tedious to perform, is limited to laboratory use and has other limitations (64). The CT is also accurate and is a desirable test for the routine diagnosis of anaplasmosis under field conditions.

Other methods most commonly used for laboratory confirmation of a suspected case include (1) the inoculation of blood into splenectomized or intact calves to demonstrate transmission of the disease and (2) the removal of the spleen from a suspected carrier

animal to determine whether recurrence of anaplasma bodies in the peripheral blood occurs.

Transmission of Anaplasmosis

General

Numerous arthropods have been shown experimentally to be capable of transmitting anaplasmosis. After extensive work in many parts of the world, seven genera and 20 species of ticks, 9 species of horse-flies, 1 species of stable fly and 3 species of mosquitoes have been shown to be capable of experimental transmission (30, p. 657). Dikmans stated that after years of consistent experiments the transmission of anaplasmosis in nature remain an interesting and most puzzling problem.

A carrier capable of transmitting minute quantities of blood from infected to susceptible animals is considered to be responsible for spreading the disease. The arthropods vectors (28, p. 1695) can be divided into two categories, (1) biological vectors, arthropods in whose body the infecting organism develops or multiplies before becoming infective to the recipient individual and (2) mechanical vectors, arthropods which transmit the infective organism from one host to another but which are not essential to the life cycle of the parasite.

Another unique feature of anaplasmosis is the ease with which it has been mechanically transmitted by man through use of contaminated surgical instruments (20) and hypodermic needles.

Transmission by Ticks

Failure to eradicate anaplasmosis in United States by eradication of B. annulatus indicated that the disease is perpetuated in nature by other agencies. This fact stimulated intensive research concerning the role various tick species play in transmission of anaplasmosis. Studies were conducted by permitting ticks to feed alternatively upon infected and susceptible animals. The following techniques have been used in the transmission of anaplasmosis: (1) ticks were interrupted in their feeding by removal from an infected animal and placed upon a susceptible animal before the developmental stage had changed, (2) ticks in one stage of development were allowed to engorge on an infected animal, after which they underwent further morphologic development before being placed on susceptible animals, and (3) adult female ticks were allowed to engorge on infected animals, and their progeny exposed to susceptible animals. In the latter case, transovarian passage of the etiologic agent was demonstrated in certain species.

Data accumulated from transmission studies, tabulations and literature reviews made by Neitz and de Toit (56), Sanders (84), Rees (68), Stiles (93), Dikmans (20), Piercy (62), and Howell (38), incriminate 20 species of ticks as experimental vectors. According to Dikmans (20), transovarian passage of the etiologic agent from the engorged female tick through the egg to the larval stage has been accomplished under experimental conditions by Boophilus annulatus, B. decoloratus, B. microplus, Dermacentor andersoni, D.

occidentalis, Ixodes ricinus and Rhipicephalus simus, Lotze et al. (45), identified seven tick species as possible vectors of anaplasmosis in U.S. These are Argas persicus, D. variabilis, D. andersoni, D. occidentalis, D. albipictus, Rhipicephalus sanguineus and Ixodes scapularis.

In 1940, Rees (69) fed three species of ticks, Boophilus annulatus var. australis, B. annulatus, and R. sanguineus on infected animals. An emulsion was made by grinding the engorged ticks in a physiological saline solution. Susceptible animals inoculated with this emulsion failed to become infected with anaplasmosis.

Osebold et al. (58) collected 11 male D. occidentalis and 7 male and 27 female Ixodes pacificus Cooley and Kohls from recently shot deer (O.h. columbianus). They made a suspension by grinding these in 10 ml. of 0.85% NaCl solution and inoculated the suspension into susceptible animals. After 114 days there was no evidence of anaplasmosis in the susceptible animals. However, transmission did occur after 37 days when 15 male and 13 female D. occidentalis were taken from deer and allowed to feed on a splenectomized calf.

Anthony and Roby (2) made a suspension by grinding 25 infected D. variabilis nymphs in a physiological saline solution and injected it subcutaneously into susceptible animals. This did not result in transmission of anaplasmosis; however, when nymphs of the same infective feeding group fed on a susceptible calf, they transmitted the disease.

Very little is known about the fate of anaplasma bodies after they have been ingested by the tick. Cowdry and Rees (13) made serial sections of adult D. andersoni and D. variabilis which had fed on an infected animal. They compared them with control ticks which were free of disease. No trace of the organism could be found in the tissues of the infected ticks or the control ticks.

However, in 1964, Anthony et al. (1) demonstrated anaplasmata in the tissues and excreta of D. andersoni specimens which had fed on calves infected with A. marginale. Anaplasmata were found in excreta by immunofluorescent, brightfield, and electron microscopic examinations and in the gut by immunofluorescent methods. They did not find anaplasmata in ultrathin sections of the salivary glands or reproductive organs.

Friedhoff and Ristic (27) demonstrated A. marginale in the gut contents and malpighian tubes of engorged D. andersoni nymphs using the fluorescent-antibody technique. They also indicated that there is evidence that the anaplasma organism multiplied in the malpighian tubes by the process of binary fission.

Transmission by Flying Hematophagus Insects

Sanborn et al. in 1930 reported the first successful experimental transmission of anaplasmosis by horse flies (82). During the decade following this initial report several workers (46, 51, 83, 84) reported anaplasmosis transmission by tabanids. Even in view

of these reports doubt persisted about the role of biting flies in the spread of anaplasmosis, primarily because of the absence of evidence based on carefully controlled experiments (95). However, in 1941 Howell et al. (39) reported extensive work on horse fly transmission under rigidly controlled conditions in which 15 successful transmissions were made. They showed several species of horse flies capable of transmitting the disease. Tabanus sulcifrons, T. abactor, T. venustus, T. equalis, T. erythraeus, T. americanus and T. oklahomensis were among the list of horse flies transmitting anaplasmosis. Sanders (84) incriminated T. fumipennis and Stomoxys calcitrans as experimental vectors of anaplasmosis. Morris et al. added T. atratus to the list of vectors (51). Later Wilson and Myers (96) added Tabanus fuscicostatus Hine to the list of tabanid species which have been shown to be capable of transmitting anaplasmosis under field conditions. Franklin et al. (25) while studying natural transmission of anaplasmosis in Texas found that transmission had occured in calves within 30 days from their initial exposure to high horse fly activity in May.

Roberts and Love (78) have accomplished mechanical transmission of A. marginale from eye gnats (Hippelates pusio Leow) to calves. They concluded that eye gnats are possible mechanical vectors of the disease since no evidence of a biological cycle was found.

Experimental transmission of anaplasmosis by mosquitoes was demonstrated by Howell et al. (41). Research following these

observations showed that the mosquitoes Psorophora Columbiae = (confinnis) Lynch-Arribalzaga and P. ciliata (Fabricius) in laboratory tests were capable of transmitting anaplasmosis. They concluded that Psorophora species may be the most important mosquitoes in anaplasmosis transmission because of their large size, close association with cattle and large numbers.

Accidental Transmission by Man

Accidental transmission is that which is spread by agents other than natural vectors, not including deliberate transmission. Deliberate transmission is that which is done in attempt to immunize young animals by the injection of blood from a latent carrier.

Any transfer of blood from a carrier animal to a susceptible animal is capable of producing the disease. Man is an important agent in transmitting anaplasmosis through use of contaminated surgical instruments, needles, nose tongs or other devices that penetrate the skin. Transfer of even minute quantities of infected blood from one animal to another has been responsible for numerous outbreaks (66). Unsanitary dehorning or removal of horn tips has resulted in outbreaks of anaplasmosis (33). Boynton has stated that many outbreaks of anaplasmosis transmission can be traced to unsanitary dehorning, drawing blood for brucellosis test, castrating and vaccinating in herds where carrier animals are present (5). An outbreak of anaplasmosis has been described by Crane in which the first case occurred 35 days after the cattle had been vaccinated,

were given stilbestrol implants and had their horn tips removed (14).

Common devices that are potential transmitting agents include nose tongs, pointed goads, hypodermic needles and knives used for castrating, earmarking or dewlapping.

Dermacentor andersoni Stiles as a Vector of Bovine Anaplasmosis

Hereditary Transmission

Hereditary transmission is one possibility, by which *D. andersoni* may transmit anaplasmosis in nature. Of 47 experiments conducted to demonstrate hereditary transmission by *D. andersoni* positive results were obtained in only one instance. Howell et al. concluded from their research conducted in 1941 that transovarian transmission of *A. marginale* in *D. andersoni* does occur (40). Larval progency of an engorged female was allowed to feed on an experimental cow which later developed the disease. However, numerous trials conducted by other investigators failed to prove transovarian transmission (70, 80). Of 47 experiments, a total of 36 hereditary transmission trials were conducted by Anthony and Roby (2). Eleven of these experiments were adult to adult transmission attempts and all failed.

When all their attempts to demonstrate hereditary transmission with *D. andersoni* failed, Anthony and Roby suggested a few explanations for failure to achieve hereditary transmission (2). They suggested that *D. andersoni* may have a very low vector potential. Mott (53) reported on anaplasmosis field studies in Wyoming where *D. andersoni* was the tick species of major importance on native

cattle. As a result of observations over a 2 year period, he concluded that the vector potential of D. andersoni in the Wyoming study area was very low. It may be that hereditary transmission occurs so infrequently that it would be extremely difficult to demonstrate in the laboratory. Another possibility they suggested is that D. andersoni from other anaplasmosis enzootic areas might have a greater vector potential than the specimens used in their study. Anthony and Roby realized that laboratory conditions under which ticks are raised differ greatly from the conditions occurring in the natural habitat and that differences could affect the transmission potential.

Transstadial Transmission

Another possibility is that anaplasmosis may be transmitted in nature by stage to stage transmission. It has been demonstrated by numerous investigators that stage-to-stage or transstadial transmission does occur (2, 6, 13, 67, 68).

Friedhoff and Ristic however, failed to substantiate the findings of other investigators (27). Present knowledge of the host preferences of the immature stages and the life history of the tick do not indicate that such transmission would be significant in natural spread of anaplasmosis (81).

Transmission by Interrupted Feeding

Sanborn (1937) was the first investigator to report transmission of anaplasmosis by interrupted feeding (81). In most cases numerous investigators demonstrated that positive transmission was obtained with male ticks exposed to susceptible cattle at different intervals after infection (2, 80). This suggests another way by which the disease can be transmitted in nature. Anthony and Roby showed that infection in unmated males survived in hibernating environment and one group transmitted anaplasmosis 197 days after feeding on an infected calf (2). This demonstrates that unmated males kept under hibernating environment for 63 days remain infective for 197 days. Successful transmission of anaplasmosis by males at different intervals following infection suggests this may be another method by which the disease may be transmitted. Since males may remain infected for at least six months and the infective agent can remain viable within the tick after exposure to a hibernating environment, Rozeboom et al. suggested that in nature there is every possibility males may be brushed off infected animals and reattach on susceptible animals (80). Dikmans stated that there is no information available concerning the frequency of host to host transfers (20). Anthony and Roby established that there are two periods; one before mating and one immediately after mating, when male D. andersoni do detach and move about on the host. It is at these times the male could be brushed off and reattach on a susceptible animal (2).

Anaplasmosis Transmission in Oregon by Tick Vectors

Ticks appear to be the primary vector of anaplasmosis in Oregon. Two important species found in Oregon are the Rocky Mountain Wood Tick, (D. andersoni) and Pacific Coast Tick (D. occidentalis).

The Rocky Mountain Wood Tick is believed to be the major vector in the sagebrush area of eastern and central Oregon (65). It is abundant at Squaw Butte Experiment Station. The adults are important pests of cattle, horses, several species of wild ruminants and other domestic animals. The immature stages infest rodents and other small mammals exclusively (12, 34). Muth and Goulding (54) reported that D. andersoni was the only tick consistently found on cattle in much of the sagebrush area of eastern Oregon where anaplasmosis is epizootic. They also observed that cattle maintained in adjacent irrigated pastures were quite free of ticks and relatively free of anaplasmosis. From these observations they concluded that D. andersoni was probably the principal vector. Peterson stated that Rocky Mountain Wood Tick is the only proved vector found in large numbers in much of the anaplasmosis endemic areas of the Northwest (60).

Anthony stated that in some ways D. andersoni appears to meet the requirements of an efficient vector because of its abundance throughout its habitat and long life span of the adults; some living two or more years.

Muth and Goulding (54) and Rea (65) believed that D. andersoni is the principal species of tick affecting cattle in the sagebrush areas of eastern Oregon. Since evidence indicates that D. andersoni is an important vector in the northwest states including eastern Oregon, an experiment was designed to determine whether adult D. andersoni collected on pastures grazed by latent anaplasmosis carrier cattle will transmit the disease. A trial of this type is of paramount importance in determining the potential of D. andersoni as a vector of anaplasmosis in this region.

It is pertinent to mention that a study of this type was previously conducted by Peterson (61). He collected 166 ticks from Squaw Butte Experiment Station and neighboring pastures and placed them on two unsplenectomized anaplasmosis susceptible calves. Hematocrit (PCV) and hemoglobin (Hb) values were determined. The CT was performed and blood smears were stained and examined for anaplasma bodies. Transmission did not occur. Engorged ticks were removed and placed in a desiccator jar with a desiccant. They oviposited a normal mass of eggs which hatched in 15 days.

The two calves were housed in an isolation unit at Oregon State University located in the Willamette Valley. D. andersoni is not naturally found in this valley probably because of differences in humidity, temperature, atmospheric pressure or precipitation.

Several conditions may have been responsible for lack of transmission in this trial such as (1) low infection rate (2) D. andersoni contrary to popular belief may not be an efficient vector

of anaplasmosis and (3) removal of infected ticks from their natural habitat may have had a deterimental effect on the ticks, with subsequent failure to transmit anaplasmosis (61). It has been demonstrated, for example, that environmental temperature is of paramount importance in transmission of babesiasis by Boophilus ticks (91, p. 61-84). It has also been demonstrated in Rocky Mountain spotted fever that during early spring while days and nights are cool, the rickettsia in recently emerged ticks is at its lowest ebb and tick bites grow more dangerous as the season advances (32, p. 438-439). It has also been demonstrated that the percentage of D. andersoni infected with the spotted fever agent Rickettsia rickettsi Wolbach, varies in different localities and from year to year in the same locality (59).

To reduce the numbers of experimental variables to minimum, a trial was conducted at Squaw Butte Experiment Station which is situated in an anaplasmosis endemic area where D. andersoni is indigenous.

MATERIALS AND METHODS

General

The study was conducted at Squaw Butte Experiment Station in eastern Oregon. On May 3, 1975 two unsplenectomized anaplasmosis negative calves no. 65218 (Jersey) and 718 (Holstein) approximately 8 months of age were housed in a fly proof enclosure for tick transmission trials. Both the calves had been isolated before they were transported to Squaw Butte Experiment Station from the Willamette Valley where D. andersoni is not indigenous and anaplasmosis is seldom observed.

Results of the CT conducted on both calves prior to experiment were negative. The CT was conducted at the recommended temperature between 70 degrees and 80 degrees F, using a humidifying cover on the rotator to prevent drying of the serum.

The test results were read immediately after the 4 minute rotation was completed and the card rotated twice by hand.

Two cloth patches approximately 16 cm in length and 9 cm in breadth with a central zipper were glued to the calves shaved dorsal pelvic area posterior to the tuber coxae and lateral to the sacrum. Both the calves were stanchioned to avoid loss of the patches by rubbing or licking.

Field Collection of D. andersoni and Application to Experimental Animals

From May 10, 1975 to July 19, 1975 D. andersoni ticks of both sexes were collected from pastures of the Squaw Butte Station. The

majority of tick collection were from pastures in which latent infected cows were grazing (61). The ticks were collected by dragging a flannel cloth approximately 1 meter square over the range pasture and by the use of CO_2 traps. Garcia (29) observed that CO_2 acts as a guiding stimulus for unengorged adult ticks. Based on his work a technique was developed and used to collect D. andersoni Stiles. Dry ice as a source of CO_2 was placed in boxes approximately 25 x 10 x 10 cm. with 2 holes on each side and one on each end. The holes 1.5 cm. in diameter were placed 2 cm. from the bottom of the boxes. These boxes were placed on a flannel cloth 1 meter square at 2-4 sites, 3 times a week for a period of four weeks. Ticks attracted by CO_2 migrated onto the cloth and were collected. This method of tick collection was not very satisfactory because much of the time, wind on the high desert range disseminated the CO_2 too rapidly. Most of the ticks were collected by the flagging method. Ticks adhering to the flannel cloth were removed with foreceps; counted and placed in small glass vials. Immediately following collection, they were placed on the calves in one of the cloth bags and allowed to feed.

The female ticks placed on calf 65218 and 718 engorged, fed to repletion, dropped off and were periodically removed from the bags as shown in Table 2.

Male ticks were neither removed nor counted and a substantial number of female ticks died shortly before or following complete engorgement occurred.

Table 1. Date of collection and number of D. andersoni collected and placed on calves.

Date of Tick Collection	Number of Ticks Collected	No. of Ticks Placed on Calf No. 65218	No. of ticks placed on Calf No. 718
5/10/75	19	9	10
5/11/75	52	27	25
5/12/75	81	46	35
5/13/75	49	24	25
5/14/75	4	4	0
5/15/75	0	0	0
5/16/75	3	0	3
5/17/75	0	0	0
5/18/75	0	0	0
5/19/75	0	0	0
5/20/75	0	0	0
5/21/75	0	0	0
5/22/75	23	13	10
5/23/75	0	0	0
5/24/75	0	0	0
5/25/75	5	0	5
5/26/75	4	4	0
5/27/75	19	8	11
5/28/75	0	0	0
5/29/75	13	6	7
5/30/75	0	0	0
5/31/75	0	0	0
6/1/75	0	0	0
6/2/75	0	0	0
6/3/75	25	25	0
6/4/75	22	0	22
6/5/75	0	0	0
6/6/75	0	0	0
6/7/75	0	0	0
6/8/75	0	0	0
6/9/75	22	22	0
6/10/75	8	0	8
6/11/75	58	16	42
6/12/75 to 6/24/75	0	0	0
6/25/75	2	0	2
6/26/75	0	0	0
6/27/75	0	0	0
6/28/75	0	0	0
6/29/75	0	0	0
6/30/75	13	13	0
7/1/75	12	0	12
7/2/75	9	9	0
7/3/75	5	5	0

Table 1. Continued

Date of Tick Collection	Number of Ticks Collected	No. of Ticks Placed on Calf No. 65218	No. of Ticks Placed on Calf No. 718
7/4/75	3	3	0
7/5/75 to 7/16/75	0	0	0
7/17/75	2	2	0
7/18/75	0	0	0
7/19/75	1	1	0

Total number of ticks collected from 5/10/75 through 7/19/75 = 454
Total number of ticks placed on calf No. 65218 = 237
Total number of ticks placed on calf No. 718 = 217

Table 2. A summary of engorged female D. andersoni removed and dates of removal.

Date Ticks Were Removed	Number of female ticks removed from Calf No. 65218	Number of female ticks removed from Calf No. 718
5/23/75	27	43
6/9/75	10	0
6/11/75	11	4
7/3/75	4	3
7/22/75	6	0

Total number of engorged female ticks removed = 108.

Seventy of these ticks were removed and placed in a desiccator jar without a desiccant at room temperature of approximately 70°F. This was done to determine whether females would lay the normal mass of fertile eggs.

Examination of Animals and Testing Procedures Used

After May 19, 1975 both calves were examined on an average of once in 12 days, and later on alternate days for the presence of active infection. The examination consisted of (1) determining rectal temperature (2) collecting approximately 2 ml of venous blood in a vacuum tube containing the anticoagulant EDTA (Ethylenediaminetetraacetic acid), and (3) collecting another 5 - 8 ml of blood in a tube containing no anticoagulant.

Hematocrits were conducted according to the microhematocrit method (85, P. 61), and hemoglobin values were determined by direct method (85, p. 66) using the Spencer Hb-meter. Blood samples containing 6 mg of EDTA were used for both tests. Blood smears were stained by toluidine blue staining technique (79); a new form of polychromatic stain using Gugol Blue kit rapid staining technique and Wright's method.[1] The number of anaplasma bodies and the percent infected cells were determined by light microscopy. The CT was performed on serums of all blood samples following refrigeration at 4°C for 48 hours.[2]

On August 28, 1975 the two calves were moved to isolation facilities at Oregon State University, Corvallis, where observation and testing continued until November 17, 1975.

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(1) Gugol Blue kit, Summit Hill Laboratories, Maryland.

(2) Anaplasmosis Card Test, Brewer Diagnostic kits, Hynson, Westcott and Dunning, Inc., Baltimore.

Throughout the experiment blood samples were collected in vacuum tubes using sterile disposable needles. No surgery was performed during this period and a halter rather than nose tongs was used.

Calf 65218

Calf No. 65218 developed anaplasmosis on September 12, 1975 as revealed by positive CT. However, because infection could not be proved by other routine laboratory tests a susceptible calf No. 1387 was challenged on October 1, 1975 with 10 ml of blood from calf No. 65218 inoculated subcutaneously. This calf was maintained in the isolation facility at Oregon State University for further observations. After an incubation period of 20 days, the calf was observed and examined on alternate days as described below for the presence of active infection. The temperature was recorded on alternate days. Wright's stained blood smear, PCV and Hb values were determined and CT was performed on all blood samples collected on alternate days (from October 20 to November 7, 1975). A summary of the tests conducted on calf 65218 and 1387 is presented in Table 3 and 4.

Calf 718

To determine the susceptibility of this calf to A. marginale infection it was challenged on October 27, 1975 with 5 ml of blood given subcutaneously from a known infected carrier. After the injection of the inoculum the calf was examined on alternate days for the presence of active infection. Rectal temperature was

determined, Wright - stained blood smears, PCV and Hb value and CT were performed on all blood samples.

RESULTS

As mentioned in material and methods seventy engorges females were removed and placed in a desiccator jar. Most of the engorged females placed in the jar laid a normal mass of eggs which hatched. The first larvae was observed on June 23, 32 days following removal of the engorged females.

Calf 718

In a 170 day observation period following the initiation of tick exposure, there was no evidence of A. marginale infection in the holstein calf No. 718. The CT remained negative, Hb and PCV values remained within the normal range and no anaplasma bodies were detected in the red blood cells.

To determine the susceptibility of this calf to A. marginale infection it was challenged with blood from a known infected carrier. Later as shown in Table 6, after an incubation period of 30 days, the CT became positive, a drop in PCV and Hb values was observed and anaplasma bodies were detected in red blood cells. A summary of tests conducted on calf 718 is presented in Table 3 and 4.

Calf No. 65218

Calf No. 65218 developed anaplasmosis on September 12, 1975 as revealed by a positive CT. Neither drop in PCV and Hb values were observed, nor were intraerythrocytic anaplasma bodies detected. No rise in body temperature was noticed. The C. F. test conducted

Table 3. A summary of tests conducted on calf 65218.

Date Blood Collected	Body Temperature	PCV	Hb Value	CT Results	Appearance of Anaplasma Bodies in Blood
5/20/75	100.8	30.0	12.0	-	-
6/4/75	100.6	32.0	12.2	-	-
6/10/75	100.8	34.0	12.8	-	-
6/24/75	101.2	40.0	14.4	-	-
7/3/75	101.0	38.0	14.0	-	-
7/17/75	100.8	39.0	14.0	-	-
7/25/75	101.4	36.0	12.8	-	-
8/4/75	101.2	37.0	13.0	-	-
8/19/75	101.6	36.0	12.8	-	-
8/28/75	101.6	33.0	12.6	-	-
9/3/75	100.6	31.0	10.6	-	-
9/8/75	101.4	29.0	10.4	-	-
9/12/75	100.2	30.0	11.0	+	-
9/16/75	101.0	32.0	11.6	+	-
9/19/75	101.2	30.0	10.6	+	-
9/22/75	102.5	30.0	10.5	+	-
9/26/75	102.0	30.0	10.4	+	-
9/30/75	102.5	29.0	11.5	+	-
10/2/75	100.6	34.0	11.0	+	-
10/4/75	101.0	34.0	10.8	+	-
10/6/75	102.0	32.0	10.8	+	-
10/9/75	101.6	30.0	10.2	+	-
10/10/75	101.8	30.0	10.4	+	-
10/13/75	101.8	32.0	10.8	+	-
10/15/75	101.6	30.0	11.2	+	-
10/17/75	101.0	32.0	11.4	+	-
10/20/75	101.6	32.0	11.0	+	-
10/22/75	102.0	30.0	11.0	+	-
10/24/75	101.8	36.0	12.0	+	-
10/26/75	101.8	34.0	12.0	+	-
10/28/75	102.0	32.0	11.8	+	-
10/29/75	101,8	32.0	10.8	+	-
10/31/75	101.6	32.0	10.8	+	-
11/3/75	102.0	26.0	10.6	+	-
11/5/75	101.6	28.0	9.6	+	-

by the staff of the Animal Health Laboratory, Salem, Oregon was positive. To prove whether infection has actually occurred calf 1387 was challenged. After an incubation period of 37 days a rise in body temperature occurred. The CT became positive and a drop in Hb and PCV was observed. Anaplasma bodies were observed in stained blood smears.

Table 4. A summary of tests conducted on calf 1387.

Date Blood Collected	Body Temperature	PCV	Hb Value	CT Results	Appearance of Anaplasma Bodies in Blood
10/20/75	101.8	40.0	12.5	-	-
10/22/75	102.0	40.0	12.6	-	-
10/24/75	102.0	40.0	13.2	-	-
10/26/75	101.8	38.0	13.4	-	-
10/28/75	101.4	42.0	13.4	-	-
10/29/75	102.0	40.0	13.6	-	-
10/31/75	102.0	42.0	13.6	-	-
11/3/75	101.4	36.0	13.0	-	-
11/5/75	102.0	34.0	12.0	-	-
11/7/75	103.6	34.0	10.0	+	+
11/9/75	103.0	32.0	10.0	+	+
11/10/75	102.8	30.0	9.8	+	+
11/11/75	102.4	30.0	9.6	+	+
11/12/75	102.4	30.0	9.8	+	+
11/13/75	102.4	32.0	9.6	+	+
11/14/75	102.0	31.0	9.8	+	+
11/15/75	102.0	31.0	9.6	+	-
11/17/75	102.0	28.0	9.6	+	-

Table 5. A summary of tests conducted on calf 718 from May 20, 1975 through November 5, 1975.

Date Blood Collected	Body Temperature	PCV	Hb Value	CT Results	Appearance of Anaplasma Bodies in Blood
5/20/75	101.2	50.0	11.8	-	-
6/4/75	101.0	39.0	14.6	-	-
6/10/75	101.0	36.0	13.2	-	-
6/24/75	101.2	38.0	14.0	-	-
7/3/75	100.8	39.0	13.6	-	-
7/17/75	101.0	38.0	13.4	-	-
7/25/75	101.2	37.0	13.2	-	-
8/4/75	101.4	38.0	13.6	-	-
8/19/75	101.2	34.0	12.0	-	-
8/28/75	101.2	36.0	12.6	-	-
9/3/75	101.0	30.0	11.2	-	-
9/8/75	101.2	30.0	11.0	-	-
9/12/75	101.2	30.0	10.8	-	-
9/16/75	101.3	36.0	11.2	-	-
9/19/75	100.8	30.0	10.8	-	-
9/22/75	102.0	30.0	10.6	-	-
9/26/75	101.8	30.0	10.6	-	-
9/30/75	102.5	34.0	10.8	-	-
10/2/75	101.2	34.0	11.2	-	-
10/4/75	101.4	36.0	12.0	-	-
10/6/75	102.0	32.0	11.8	-	-
10/9/75	101.8	27.0	10.8	-	-
10/10/75	101.8	29.0	10.8	-	-
10/13/75	102.0	34.0	11.0	-	-
10/15/75	101.8	34.0	11.8	-	-
10/17/75	101.4	36.0	11.4	-	-
10/20/75	101.6	32.0	11.6	-	-
10/22/75	102.0	40.0	12.2	-	-
10/24/75	101.8	36.0	12.4	-	-
10/26/75	101.6	36.0	12.2	-	-
10/29/75	102.0	36.0	10.8	-	-
10/31/75	101.8	36.0	10.6	-	-
11/3/75	101.6	36.0	11.2	-	-
11/5/75	101.6	30.0	10.8	-	-

Table 6. A summary of post challenge tests conducted on calf 718 from November 17, 1975 through December 6, 1975. Calf Innoculated October 27, 1975.

Date Blood Collected	Body Temperature	PCV	Hb Value	CT Results	Appearance of Anaplasma Bodies in Blood	% of Parasit-emia
11/17/75	101.2	30.0	10.2	-	-	
11/19/75	101.4	30.0	10.2	-	-	
11/21/75	101.2	30.0	10.0	-	-	
11/23/75	101.2	30.0	10.4	-	-	
11/25/75	105.3	28.0	9.2	+	+	5
11/27/75	103.0	25.0	9.0	+	+	10
11/28/75	102.8	24.0	8.0	+	+	8
11/30/75	102.4	20.0	8.4	+	+	5
12/2/75	101.8	20.0	8.6	+	+	4
12/4/75	101.6	20.0	9.0	+	+	1
12/6/75	101.8	20.0	8.8	+	-	

DISCUSSION

The transmission of anaplasmosis on the sagebrush bunchgrass range areas of eastern Oregon and of most Rocky Mountain and northwest states is presently not well understood. D. andersoni is indigenous to this area and is believed by most researchers to be the important vector of A. marginale. However, when Anthony and Roby failed to achieve hereditary transmission with D. andersoni they suggested that D. andersoni may have a very low vector potential. Transstadial transmission has been demonstrated but Sanborn (81) believed that transstadial transmission is of no significance in the natural spread of anaplasmosis because immature stages rarely attack cattle. This suggests that D. andersoni is likely not an efficient vector of A. marginale. Dikman (20) said that until adult to adult transmission of A. marginale is demonstrated, judgement on the role of natural transmission of anaplasmosis by D. andersoni must be reserved.

Muth and Goulding (54) and Rea (65) indicated that D. andersoni is the only tick found on cattle in much of the sagebrush area of eastern Oregon where anaplasmosis is epizootic and observed that cattle maintained in adjacent irrigated districts were quite free of ticks and relatively free of anaplasmosis. They concluded from these observations and from the seasonal occurence of anaplasmosis in the area that D. andersoni was probably the principal vector. Peterson, Raleigh and Goulding (61) demonstrated that hematophagus insects are likely not important vectors of anaplasmosis in this area. Transmission of A. marginale by D. andersoni has been demonstrated in the laboratory by numerous investigators but no reference could be found that transmission

of A. marginale by wild D. andersoni collected from vegetation in the field occurs. To determine the vector potential of D. andersoni in this area, an experiment was designed to determine whether adult wild D. andersoni collected on pasture grazed by cattle will transmit the disease.

In this trial, 454 ticks were collected on pastures grazed by a herd of cattle of which 70% of adults were latent carriers of anaplasmosis. The ticks were collected by dragging a flannel cloth approximately one meter square over the range and pasture and by the use of CO_2 traps (Table 1). Ticks collected consisted of both sexes and all appeared to be young adults. No partially engorged females were found. However, it is difficult to determine whether male ticks have fed since they do not enlarge as do partially or fully engorged female ticks. After collection, these ticks were placed immediately in cloth patches glued to two calves maintained in a fly-proof screened shelter on this range (Table 1).

One of the two calves developed anaplasmosis as demonstrated by a positive CT (Table 3), positive CF Test and by blood inoculation into a susceptible calf (Table 4). This proved that wild D. andersoni ticks do transmit anaplasmosis. In a study conducted by Howarth and Roby (35) adult D. occidentalis ticks were collected from vegetation by flagging technique in an anaplasmosis enzootic area inhabited by deer and smaller wildlife species. Anaplasmosis was produced when 435 apparently unfed adult ticks of both sexes were used to parasitize an intact cow indicating that transmission of anaplasmosis from deer reservoir to cattle by D. occidentalis does occur. However, anaplasmosis

was not produced when 64 male, 66 female and combination of 44 male and 56 female collected were allowed to attach and feed on three susceptible splenectomized calves.

Even though hematophagus flying insects are not considered important vectors in this area, this study was conducted in a fly-proof screened building. No cattle were maintained in the area surrounding the building thus again excluding the possibility of insect transmission. It has been demonstrated by Howell et al. (39) that insect transmission does not take place if repeat feeding from a carrier to susceptible animal is not accomplished within 2-5 minutes. The screened building was open on one side to allow adequate ventilation and maintain temperatures inside similar to those outside the building.

Results of this study indicate that transmission of A. marginale by adult D. andersoni ticks collected from range pasture grazed by infected cattle does occur. However, the exact method of transmission is not known. The role of hereditary transmission in D. andersoni cannot at this time be considered of major importance since to date there has been only one report of hereditary transmission while 46 other attempts have failed. Transstadial transmission has been demonstrated in the laboratory (2, 6, 13, 67, 68) but appears to be of little importance in the natural spread of anaplasmosis since the immature stages rarely attack cattle or other ruminants.

Transmission of anaplasmosis by female D. andersoni is unlikely because partially or fully engorged females were not observed in traps nor were they collected by flagging. Apparently if they are detached from the host, females oviposit and die. However, transmission

trials conducted in the laboratory have demonstrated that male D. andersoni can transmit the disease at various intervals after infection (2, 80). This perhaps is the natural method by which anaplasmosis is transmitted. It is not known why males would leave a host voluntarily, but Rozeboom et al. (80) suggested that in nature, males may be brushed off one animal and reattach to another A. marginale susceptible host, or, if an infected animal dies, the ticks may detach and seek another host. It is also possible that male ticks may leave the initial host when the animal is lying down and search for other hosts harboring unfertilized females. This might occur in areas where cattle congregate as in bedding grounds or around water tanks. Anthony and Roby (2) demonstrated that unmated males kept under hibernating environment for 63 days remain infective for 197 days. They also established that there are two periods, one before mating and one immediately after mating when male D. andersoni do detach and move about the host. Since the males are very active at these periods it seems logical to assume that they could be brushed off or voluntarily leave an infected animal and reattach on a susceptible host. Further, it has been demonstrated by Rozeboom et al. (80) that a single infected male tick is capable of transmitting anaplasmosis to a susceptible cow. The rate of transmission in nature probably depends upon the infection rate in cattle or other ruminants grazing the area and the density of the tick population. It is likely that many males may never become infected and those that become infected and detach may never transfer to another susceptible host. This may be especially true on ranges where feed is scarce and cattle graze over a large area and are widely

dispersed. This should significantly minimize the chances of animal to animal transfer of male ticks. Yet, on this typical western range, ticks apparently are responsible for a 70% A. marginale infection rate in adult cattle and a 63% transmission rate in fall born calves (61).

Further work is necessary to determine the precise role of D. andersoni in the epizootiology of anaplasmosis. The importance of hereditary and transstadial transmission in nature should be further investigated since all hereditary transmission trials were conducted under laboratory conditions which may differ markedly from conditions prevailing in the natural habitat and immature stages may parasitize cattle but because of their small size may not have been observed. There is also a possibility that a small animal indigenous to the sagebrush bunchgrass areas may act as a host of A. marginale and immature stages of ticks may become infected when parasitizing them. The importance of male D. andersoni in the natural spread of anaplasmosis with the possibility that infected males may overwinter and seek new hosts the following spring also needs further investigation.

SUMMARY

In the spring and summer of 1975 a study was conducted under natural conditions to determine whether wild adult D. andersoni Stiles would transmit A. marginale to cattle. The ticks were collected from the range pastures of the Squaw Butte Experiment Station where anaplasmosis is enzootic and D. andersoni is indigenous. Adult D. andersoni ticks were collected by flagging and by the use of CO_2 traps from pastures of Squaw Butte Station in which latent infected cows were grazing. These ticks were placed on two calves. A. marginale infection was produced when 237 adult ticks of both sexes were allowed to attach and feed on a susceptible unsplenectomized calf, but was not produced by 217 adult ticks placed on a second unsplenectomized calf.

These results demonstrated that transmission of anaplasmosis to cattle by D. andersoni ticks does occur on this range.

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