TRANSSTADIAL AND TRANSOVARIAL TRANSMISSION OF *ANAPLASMA MARGINALE* THEILER BY *DERMACENTOR VARIABILIS* (SAY)

By

ROGER WILLIAM STICH

Bachelor of Science in Arts and Sciences

Oklahoma State University

Stillwater, Oklahoma

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Thesis Approved:

Katherine M. Kocan
Thesis Adviser

J. Alexander Hair

A. A. Irving

Norman N. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Bovine Anaplasmosis

Anaplasma marginale is the causative agent of bovine anaplasmosis. The hemoparasite invades and develops in bovine erythrocytes that are subsequently removed from circulation by phagocytic cells of the reticuloendothelial system, resulting in a mild to severe anemia.[1-3]

Anaplasma marginale is classified in the family Anaplasmataceae, order Rickettsiales, which includes those procaryotic parasites that live and multiply only within living cells.[4] *Anaplasma* organisms, as well as species in the genera *Ehrlichia*, *Cowdria*, and *Coxiella*, belong to a group of rickettsiae that are found within membrane-bound inclusions. Some of these organisms also appear to have a specific developmental cycle involving different stages.[5,6] In contrast, *Rickettsia* and *Wolbachia* are found free in the host cell cytoplasm and appear to divide only by binary fission.[5,6]

Anaplasmosis is primarily a disease of cattle and affects all breeds, but has also been reported in other ruminants including deer, antelope, buffalo, camel, elk,

wildebeest, duiker, and blesblok; some of these wild hosts may serve a significant role in the epizoology of the disease by serving as reservoirs.[3,7-14] A. marginale can be transmitted mechanically between hosts by blood-contaminated fomites and haematophagous arthropods, but only various ticks of the Family Ixodidae transmit the organism biologically.[15-17]

In the bovine host, the parasitic inclusion is usually found at the margins of red blood cells and contains 1 to 8 initial bodies within a limiting membrane.[1,6,18,19] Each initial body is an organism surrounded by a double membrane, the cellular protoplasm resembling that of Rickettsia and Chlamydia species.[18] The inclusion membrane surrounding the initial bodies is morphologically similar to the erythrocytic plasmalemma and is recognized only by anti-erythrocyte labeled immunogobulin, suggesting that it is of host origin.[18,20]

The morphology of A. marginale in bovine erythrocytes is more easily observed in hemolysed than in intact cells. In some isolates, an inclusion appendage is attached to the inclusion membrane and forms "loop", "dumbbell", or "comet" shapes.[18,19] Ferritin-conjugated antibody studies have shown that the inclusion appendage, as well as the initial bodies, contains parasite antigens. It has been suggested that these may be the particulate antigens responsible for the vertebrate immune response.[19]

A hypothetical life cycle has been proposed for A.

marginale in the vertebrate host wherein an initial body enters the erythrocyte by endocytosis, reproduces by binary fission, and is transferred to other erythrocytes.[1] Progression of the disease in cattle is correlated with increasing parasitemia, and subsequent phagocytosis of infected erythrocytes that results in anemia.[2,21] A prepatent period of approximately 21-40 days occurs, the length of which may vary with the infective dose. The site of development of A. marginale in its vertebrate host during the prepatent period has not been determined.[2,21] Once patency is reached the parasitemia rises rapidly, often doubling every 24 hours, until a peak parasitemia is reached in 5-7 days, and severest anemia occurring at 8 to 10 days.[2,21] Cattle that recover become carriers and the parasitemia may recrudesce if they are immunosuppressed.[2,5,21]

Tentative diagnosis of anaplasmosis involves clinical signs of anemia including accelerated respiration, increased cardiac rate, and pallor and icterus of the skin and mucous membranes in the absence of hemoglobinuria. History of the host (age, season, exposure to carriers) is also valuable.[2,3,22] Positive diagnosis requires demonstration of marginal bodies within the erythrocytes or positive serological tests, such as a complement-fixation or a card serological test.[2,3,22,23]

Cattle of all ages are susceptible to anaplasmosis, but those over 2 years old develop clinical symptoms often

resulting in death. Younger cattle may have moderate to inapparent symptoms and become carriers.[2,3,22] The mechanism of resistance in younger cattle is not well understood, but may involve the greater capacity of hematopoietic response characteristic of younger animals. Splenectomy renders calves susceptible and they develop severe disease with symptoms similar to those described for adult cattle.[2,24]

Anaplasma marginale is distributed world-wide and is found in the tropical and subtropical areas of every continent including Africa, Asia, Asia Minor, Australia, the Soviet Union, Indonesia, Taiwan, Philippine Islands, and the Americas.[3,25,26] Areas of the United States where anaplasmosis is enzootic include the southeastern, Gulf, lower plains, and western states, with sporadic occurrences in the northern states.[3,24] Occurrence of anaplasmosis is usually correlated with the presence of hematophagous arthropods, but A. marginale still has the greatest distribution of all known rickettsiae of veterinary medical importance, possibly due to its wide vector range.[5,25,26] Economic losses due to anaplasmosis in the United States result from weight loss, abortion, testicular degeneration and loss of libido, reduced milk production, and death.[24,27] Economic losses, including those incurred for preventive treatment and other veterinary medical services, had an estimated annual cost of $100 million to the U.S. cattle industry in 1976.[24]

Control of anaplasmosis involves isolation of carriers followed by slaughter or treatment with tetracyclines; an anaplasmosis vaccine is also available, but it must be administered repeatedly, the vaccine does not prevent infection and the immunized animal still may become a carrier.[2,3]

Transmission of *Anaplasma marginale* by Ticks

Ticks are the major biological vectors of anaplasmosis with approximately 20 species incriminated as vectors world-wide.[16,28] Transstadial and intrastadial transmission of *A. marginale* by ticks has been demonstrated repeatedly, and the transmission of *A. marginale* by ticks from chronic carrier cattle has been demonstrated experimentally and could be important in maintaining the organism in enzootic areas.[29] Transovarial transmission has been reported in some ixodid tick species, but not in *Dermacentor variabilis* and the phenomenon has not been confirmed consistently in any tick species.[16,28-34] *Cowdria ruminantium*, a rickettsia with a tick development cycle similar to *A. marginale*, has shown a low occurrence of transovarial transmission in *Amblyomma hebraeum* exposed to the parasite as larvae.[35] Similar attempts to demonstrate transovarial transmission of *A. marginale* by ticks that were exposed to the organism

as larvae, have not been reported.

Transstadial transmission of A. marginale by the tick stage following exposure has been demonstrated repeatedly, but transmission by adults infected as larvae, without reinfection as nymphs, has not been reported until recently.[16,28-34] Infection has also been caused by inoculation of susceptible cattle with gut and salivary gland homogenates from infected adult ticks.[29,31,32] Adult Dermacentor andersoni that were infected as nymphs have been reported to remain infected with the parasite up to 6 months post-molting.[36] D. variabilis is reported to remain infected up to 12 months, especially when the ticks are stored under winter conditions.[37]

Development of Anaplasma marginale in Ticks

The developmental cycle of A. marginale in ticks has been studied in D. andersoni from infection of nymphs through transmission by the subsequently-molted adults. D. andersoni nymphs become infected with A. marginale within as little as 24 hours of feeding on an infected animal.[6,36] The process by which A. marginale infects the nymphal midgut has not been determined.[39,40] Colonies of the parasite were not observed within nymphal tissue until 5 days post-repletion. Infection of nymphs is thought to occur concurrently with rapid digestion of the blood meal

after the nymphs are replete.[6,39-41]

Two distinct types of anaplasmal colonies, nymphal type 1 (Ny1) and nymphal type 2 (Ny2), along with transitional nymphal colonies (TsN) were found in the midgut epithelium as the nymphs molted into adults.[6,39-41] Ny1 colonies were the first to appear 5 days after engorgement; they contained small particles and large, round, reticulated organisms that appeared to be dividing by binary fission.[39-41] Ny2 colonies were found 20 days post-repletion, with rod-shaped reticulated organisms surrounded by a double membrane and in a dense intercellular matrix.[39-41] TsN colonies, with morphological characteristics in common with both Ny1 and Ny2 colonies, were observed on days 10 and 15 post-repletion; the organisms within the TsN colonies had variable electron densities and were within a dense matrix.[39-41]

Gut homogenate collected at days 5 to 15 post-repletion, when Ny1 and TsN colonies were present, did not cause anaplasmosis in susceptible calves, suggesting that these organisms were not infective for cattle.[38] However, gut homogenate collected at 20 days post-repletion, when Ny2 colonies were present, was infective for susceptible calves.[38]

Colonies of A. marginale have been demonstrated within the midgut epithelium of D. andersoni adults that were infected as nymphs.[6,31,32,39,42-46] These colonies were confirmed as A. marginale by ferritin- and

fluorescein-labelled antibody and peroxidase-anti-peroxidase techniques.[31,32,42] The forms found within the 5 distinct colony types included small electron-dense forms, larger reticulated forms, pleomorphic reticulated forms, and small particles.[6,39,45] The numerical order of the colony types (1-5) correlated to the mean diameter of colonies, with the smallest mean diameter for colony type 1 and the largest for colony type 5.[45]

Anaplasmal colonies in gut of unfed adult D. andersoni often appeared near the epithelial basement membrane.[6] Colonies were observed within muscle cells on the hemocoel side of the basement membrane during tick feeding; increasing numbers were found during days 3 through 5. Midgut colony densities sharply decreased on the 6th through 9th days of feeding, which corresponds to the 6-7 days of feeding required to transmit the parasite to cattle.[6,47] Organisms in muscle cells on the hemocoel side of the basement membrane are thought to subsequently infect salivary glands. A. marginale was demonstrated in the tick hemocoel by fluorescent antibody technique (FA), and within salivary glands by FA, EM, and calf-inoculation with salivary gland homogenates.[6,32,48,49]

The Research Problem

Preliminary studies have documented transstadial transmission of A. marginale by nymphal and adult D.

variabilis that were exposed to the parasite as larvae.[16,50] However, studies on the development of A. marginale in nymphal and adult D. variabilis exposed as larvae have not been reported. Furthermore, transovarial transmission of A. marginale has not been attempted with D. variabilis F1 larvae from parent ticks that were exposed as larvae.

The purpose of the present study was to test the ability of D. variabilis development stages to transmit A. marginale transstadially and transovarially, and to document development of the parasite in D. variabilis nymphs and adults exposed as larvae only. Transmission studies were done to demonstrate infection of tick stages and morphologic studies to confirm infection and to document development of the parasite in an invertebrate host.

CHAPTER II

MATERIALS AND METHODS

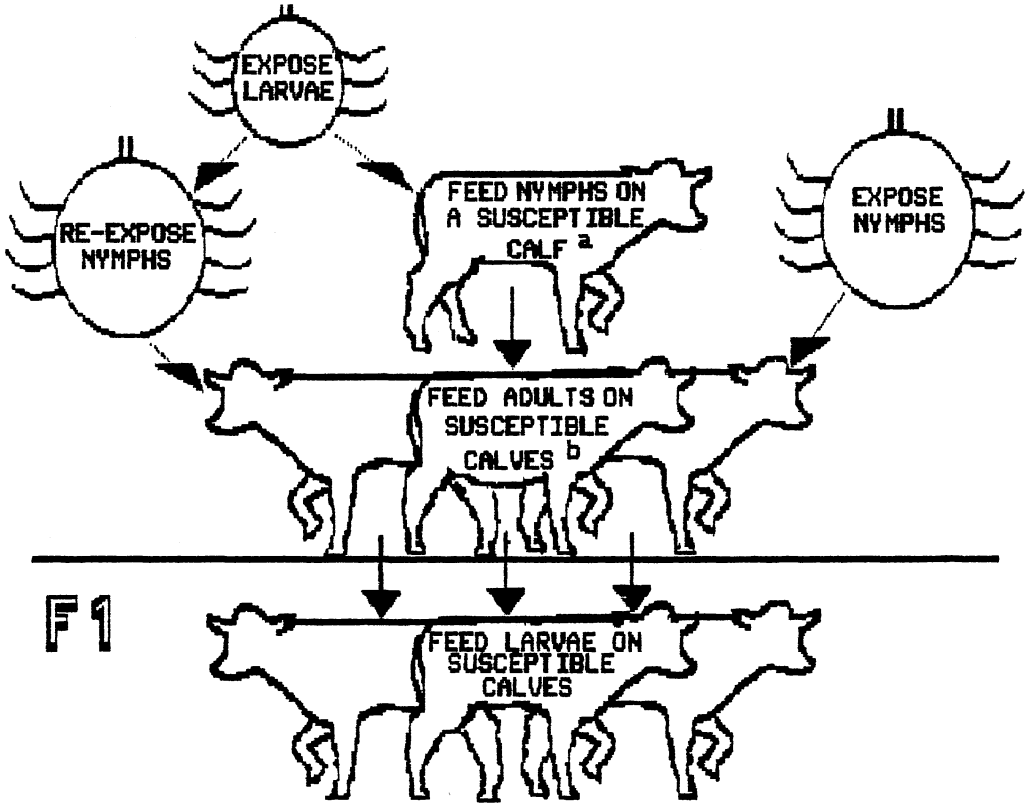
Agent

The Virginia isolate of Anaplasma marginale (VAM) was used in this study. Infected bovine blood or blood stabilate was inoculated intravenously into the donor calves that were then used to expose Dermacentor variabilis stages by allowing ticks to feed when calves developed parasitemia.

Experimental Animals

Twenty-two bull calves (2 to 4 months old) that were negative for the anaplasmosis complement-fixation (CF) test, were splenectomized and used for these studies (Figure 1). Five calves were inoculated IV with infected bovine blood or blood stabilate and served as donor calves for the infection of Dermacentor variabilis larvae and nymphs. Nine calves were used for feeding subsequently-molted D. variabilis stages that were exposed to A. marginale as larvae, and two calves were used to feed D. variabilis adults infected as nymphs. Five calves were used to feed D. variabilis F1 larvae to test for

Figure 1. Experimental Design for Transmission and Morphological Studies of *Anaplasma marginale* in *Dermacentor variabilis* Exposed as Larvae.



[a] Nymph tissue collected on days 0-6 of feeding.

[b] Adult tissue collected on days 0-9 of feeding.

transovarial transmission. The calves were monitored three times a week by examination of Wright's stained blood smears and determination of packed cell volumes (PCV). Once marginal bodies were detected in blood smears, the calves were monitored daily. One calf was used to feed uninfected control D. variabilis nymphs and larvae. One sheep was used to feed a group of control adult ticks that were exposed as nymphs only for the collection of replete females. Rabbits and sheep were used to maintain the D. variabilis ticks prior to this experiment.

Laboratory Propagation of Ticks

Dermacentor variabilis larvae, nymphs and adults were reared at the Oklahoma State University, Medical Entomology Laboratory.[51] Larvae not to be exposed to A. marginale were fed on rabbits and allowed to molt to the nymphal stage. Larvae, nymphs and adults were held in a humidity chamber (90% to 98% relative humidity) at 25C with a fourteen-hour photophase period, until used for experiments.

Tick Feeding

Dermacentor variabilis ticks were placed within orthopedic stockinettes attached to calves and allowed to feed until replete. Replete ticks were collected and

placed in a humidity chamber until they molted into the next stage. Larvae were placed in double-layered stockinettes, but nymphs and adults were placed in single-layered stockinettes. Twenty to forty-eight replete females were collected from each group of adults exposed at different stages and allowed to lay eggs; F1 larvae from these females were allowed to feed on susceptible calves as a test of transovarial transmission.

Tick Tissue Collection for Light and Electron Microscopy

Tissue was collected from various development stages of D. variabilis and fixed immediately in 2% glutaraldehyde in a 0.2 M sodium cacodylate buffer, washed with the same buffer and post-fixed with 2% osmium tetroxide in 0.2 M sodium cacodylate buffer for one hour. The fixed tissue was washed in buffer three times before dehydrating in a graded series of ethanol. Propylene oxide was the intermediate solvent for infiltration of the tissue with Dow Epoxy Resin (DER) 736. Thick and thin sections were made on a Sorval MT-5000 ultramicrotome. Thick sections were dried onto glass slides and stained with Mallory's stain for examination with a light microscope.[52] Thin sections were cut with a Diatome diamond knife, collected on 200 mesh copper grids, stained with uranyl acetate and lead citrate,[53] and examined with

a JEOL TemScan 100 CX electron microscope.

Determination of Colony Densities

Colony densities of A. marginale in gut tissues were determined as described by Kocan et al.[54] The densities were statistically compared using an analysis of variance.

Experimental Design

Infection of Ticks

Exposure of Larvae

Approximately 4,000 D. variabilis larvae were placed on each of two donor calves for Trial 1, and on one donor calf for Trial 2. These larvae were placed into cells when the donor calves had reached a 5% to 7% parasitemia.

Exposure of Nymphs

In each trial, approximately 1,000 D. variabilis nymphs that were exposed as larvae, were placed on a donor calf when its parasitemia reached 3% to 5%. One type of control was ticks that were exposed as nymphs only, and these ticks were infected the same way.

Transstadial Transmission and Development

Adult or nymphal ticks were placed in orthopedic stockinettes attached to susceptible calves. Ticks that had not attached within 24 hours were removed and discarded. In each group, gut and salivary glands were collected from 10 pairs of adult males and females on days 0-9 of feeding, and from 20 nymphs on days 0-6 of feeding. All of the susceptible calves were monitored from onset of tick feeding until termination from the project, as described previously.

Nymphs that were exposed as larvae

Approximately 1,000 D. variabilis nymphs that were exposed to A. marginale by feeding as larvae were placed into orthopedic stockinettes attached to a susceptible calf.

Adults that were exposed as larvae

Male and female ticks (150 pairs) that were exposed as larvae were fed on susceptible calves in both trials.

Adults that were exposed as nymphs

Male and female D. variabilis (150 pairs) that were fed on rabbits as larvae and then exposed to A. marginale as nymphs were fed on a susceptible calf.

Adults that were exposed as larvae and nymphs

In each trial, 150 pairs of male and female ticks that were exposed to A. marginale as larvae and then re-exposed as nymphs were fed on a susceptible calf.

Transovarial transmission studies

F1 Larvae From Adults Exposed as Larvae

Replete females (25 in Trial 1, 48 in Trial 2) were put into individual vials and placed in a humidity chamber to lay eggs. All of the larvae from 8 females in Trial 1, and approximately one half of the larvae from all 48 females in Trial 2, were allowed to feed on susceptible calves.

F1 Larvae From Adults Exposed as Larvae and Nymphs

Replete females (25 from Trial 1, 40 from Trial 2) were placed into vials within a humidity chamber and allowed to lay eggs. All of the larvae hatching from eggs deposited by 8 females in Trial 1, and approximately one half of the total larvae produced by all 40 females in Trial 2, were fed on susceptible calves.

F1 larvae From Adults Exposed as Nymphs

Twenty replete females, exposed as nymphs, that had fed on a sheep were placed in vials within a humidity chamber to ovaposit. All of the larvae were fed on a susceptible calf as a control to compare any results with the previous experiments.

CHAPTER III

RESULTS

Transstadial Transmission Studies

Nymphs that were exposed as larvae

Dermacentor variabilis nymphs that were exposed to *Anaplasma marginale* by feeding as larvae on infected calves, transmitted the organism to susceptible calves in both trials (Table I). The prepatent periods were within average range expected for the disease (Trial 1, 24 days; Trial 2, 30 days). The clinical disease that developed in the calf in Trial 1 was notably severe; parasitemia reached 76.9% and anemia was marked by a PCV reduction of 84.5%.

Adults that were exposed as larvae

Adult *D. variabilis* that were exposed to *A. marginale* as larvae, without re-exposure as nymphs, caused anaplasmosis in susceptible calves (Table I). The prepatent periods (27 days in both trials) were similar to those observed in calves fed on by nymphs infected as larvae. Clinical disease was of average intensity.

TABLE I

TRANSSTADIAL TRANSMISSION OF ANAPLASMA MARGINALE BY DERMACENTOR VARIABILIS AT VARIOUS DEVELOPMENT STAGES

Tick Stage(s) Exposed	Tick Stage Fed	Calf No.	Prepatent Period (days)a	Peak Parasitemia (%)	Percent Reduction PCV	Donor Calf Parasitemia (%)b
Trial 1						
Larvae[c]	Nymphs	B-187	24	76.9	84.52	41.6 and 47.7
	Adults	B-196	27	61.8	78.12	
Nymphs	Adults	B-204	27	34.6	73.91	51.8
Larvae[c] and Nymphs	Adults	B-197	32	51.4	70.59	41.6 and 47.7 38.6
Trial 2						
Larvae	Nymphs	B-199	30	23.8	44.83	51.8
	Adults	B-205	27	26.4	65.57	51.8
Nymphs	Adults	B-203	25	60.2	75.00	33.6
Larvae and Nymphs	Adults	B-206	30	46	57.58	51.8 33.6

a Prepatent period determined from Day 1 of tick feeding to an appearance of a 1% parasitemia in the peripheral blood.

b Peak parasitemia during feeding of ticks.

c Larvae were collected from two donor calves.

Adults that were exposed as both larvae and nymphs

Adult D. variabilis that were exposed to A. marginale as both larvae and nymphs transmitted the parasite to susceptible calves (Table I). The prepatent periods (Trial 1, 32 days; Trial 2, 30 days) and the clinical symptoms of the calves were similar to those that were exposed to adult ticks infected at a single stage.

Transovarial Transmission Studies

Parasitemias were not observed in any of the susceptible calves which were fed on by F1 larvae produced by adult D. variabilis that had been exposed to A. marginale as larvae, nymphs, or as both larvae and nymphs (Table II). These calves were all CF negative for anaplasmosis before challenge with blood from patent calves, and all but one calf developed a 1% parasitemia from the challenge in 7 to 10 days. The remaining calf was fed on by F1 larvae from adults exposed to A. marginale only as nymphs, and did not develop a 1% post-challenge parasitemia for 36 days.

TABLE II

ATTEMPTED TRANSOVARIAL TRANSMISSION OF *ANAPLASMA MARGINALE* BY *DERMACENTOR VARIABILIS* F1 LARVAE FROM ADULTS EXPOSED AT VARIOUS STAGES

Tick Stage(s) Exposed	F1 Tick Stage Fed	Calf No.	*A. marginale* Parasitemia	Post Challenge Prepatent Period (Days)
Trial I				
Larvae	Larvae	B-201	(-)	9
Larvae and Nymphs	Larvae	B-202	(-)	10
Trial II				
Larvae	Larvae	B-212*	(-)	7
Larvae and Nymphs	Larvae	B-211*	(-)	7
Control				
Nymphs	Larvae	B-213*	(-)	36

* Calves with marginal bodies similar to those of *A. marginale* in peripheral blood smears.

Colony Density Studies

Nymphs infected as larvae

Anaplasma marginale colonies were not observed in the midgut epithelium of unfed *D. variabilis* nymphs that were exposed as larvae in either trial (Table III).

Adults exposed as larvae

Anaplasma marginale colonies were not found in the midgut epithelium of unfed *D. variabilis* males in either trial. Colonies were observed in midgut epithelium of unfed females Trial 1 (Table III).

Adults exposed as larvae and nymphs

Colonies were observed in both unfed males and unfed females of each trial. No significant differences ($P > 0.13$) were observed in the mean colony density of adults that were exposed as both larvae and nymphs and the controls that were exposed only as nymphs (Table III).

TABLE III

COLONY DENSITIES OF *ANAPLASMA MARGINALE* IN MIDGUT TISSUES OF NYMPHAL AND ADULT *DERMACENTOR VARIABILIS* THAT WERE EXPOSED AS LARVAE, NYMPHS, OR BOTH LARVAE AND NYMPHS

Tick Stage Exposed	Tick Stage Examined	Sex of Ticks	Mean Colony Density[a]	Standard Deviation
Trial I				
Larvae	Nymphs	NA[b]	0	--
	Adults	M	0	--
		F	0	--
Larvae and Nymphs	Adults	M	0.69	0.71
		F	0.51	0.69
Trial II				
Larvae	Nymphs	NA[b]	0	--
	Adults	M	0	--
		F	0.11	0.14
Larvae and Nymphs	Adults	M	0.33	0.30
		F	0.44	0.43
Control				
Nymphs	Adults	M	0.56	0.52
		F	0.18	0.28

[a] No. colonies/0.1mm^2 gut tissue examined.

[b] Not Applicable.

CHAPTER IV

DISCUSSION

Dermacentor variabilis was chosen for these studies because this tick is commonly associated with cattle in Oklahoma where anaplasmosis is enzootic.[50] In previous studies in our laboratory, D. variabilis adults infected as nymphs have remained infected for as long as 12 months.[50] When infected ticks overwintered, maximal numbers of A. marginale were found in colonies approximately the seventh month post-molting.[50] The present studies were undertaken to test the importance of D. variabilis exposure to A. marginale as larvae, in transstadial and transovarial transmission.

Anaplasma marginale was transmitted transstadially between all stages of D. variabilis in the present study. Prepatent periods have been suggested as a criterion to determine infectivity of an A. marginale inoculum to the bovine host.[2] Prepatent periods of the infections in calves that developed illness in this experiment were similar regardless of which stage(s) of the ticks were infected. Calves fed on by nymphs and adults infected as larvae had prepatent periods similar to those fed on by adults infected as nymphs, and the calves fed on by

double-exposed adults had prepatent periods similar to those in the other three groups. It is interesting to note that the highest parasitemia observed in a calf in the present experiments, and one of the highest ever encountered by this laboratory, was in a calf that acquired infection from nymphs that were infected as larvae.

Previous reports on attempts to transmit A. marginale transovarially have involved exposing the parent generation as adults or nymphs, but not as larvae.[16] Cowdria ruminantium, a rickettsial organism that is transmitted by Amblyomma hebraeum and the life cycle of which is similar in some respects to that of A. marginale in Dermancentor species, has been transovarially transmitted to small numbers of tick offspring from females that had been exposed to the organism as larvae.[35] The experimental design employed in the present study was similar to that of the Cowdria experiments; unfortunately a limited number of calves was available and therefore fewer ticks were tested.

Transovarial transmission of some parasites in ticks may occur in low numbers because not every female tick will transmit the parasite to her offspring. Also, in those females in which transovarial transmission occurs, the organism may only infect a portion of the egg mass.[35] Even though parasitemias of at least 1% were not observed in the calves of this transovarial transmission

experiment, marginal bodies similar to A. marginale were observed in peripheral blood smears of both calves in trial 2 and in the control calf that was fed on by F1 larvae from adults exposed as nymphs only. A larger number of F1 larvae from more individual females were placed on the calves in Trial 2 and control calves than on the Trial 1 calves, increasing the probability of feeding any infected larvae that might have been present. The marginal bodies observed might represent a low level of transovarial transmission of A. marginale that may occur more readily in field conditions, where more compatible isolates of the parasite and strains of tick vectors probably exist. Studies may be warranted utilizing isolates of A. marginale and strains of ticks collected from an enzootic area.

Even though transstadial transmission occurred in ticks from all groups, colonies were not found in the midgut of unfed nymphs or of unfed males that were infected as larvae. Colonies were observed in females from trial 2 in which ticks were infected as larvae; however, that group had a lower colony density than the groups that were infected as nymphs. A. marginale colonies were observed in ticks from all groups of unfed adults that were exposed as both larvae and nymphs as well as those infected as nymphs alone. These observations could result from physiological differences between development stages of D. variabilis. Such differences

might allow A. marginale to develop more rapidly in the midgut cells and penetrate the salivary glands of infected larvae as they molt into nymphs. In contrast, when ticks are infected as nymphs the parasite may remain in midgut cells until the subsequently molted adults take a bloodmeal. This hypothesis is supported by the recent finding in our laboratory of A. marginale colonies within salivary glands of D. andersoni nymphs that were infected as larvae.[38]

Dermacentor variabilis is the principal vector of Rickettsia rickettsii (the causative agent of Rocky Mountain spotted fever) in the east and central portion of the United States. This tick is believed to be expanding its range and is now endemic in the northwest portion of the country.[56] D. variabilis nymphs and adults have been reported on cattle,[58-60] and the tick has been demonstrated repeatedly to become infected with A. marginale and to transmit the parasite to cattle experimentally. Thus D. variabilis has some potential to serve as a vector of the organism in North America.[16,29,34,37,42]

Studies of D. variabilis in Virginia have shown that both adults and larvae overwinter, with two larval populations arising in the season of tick activity.[57] The first population would comprise those larvae that overwintered and these ticks reach peak activity in late March; a second peak of feeding activity in October could

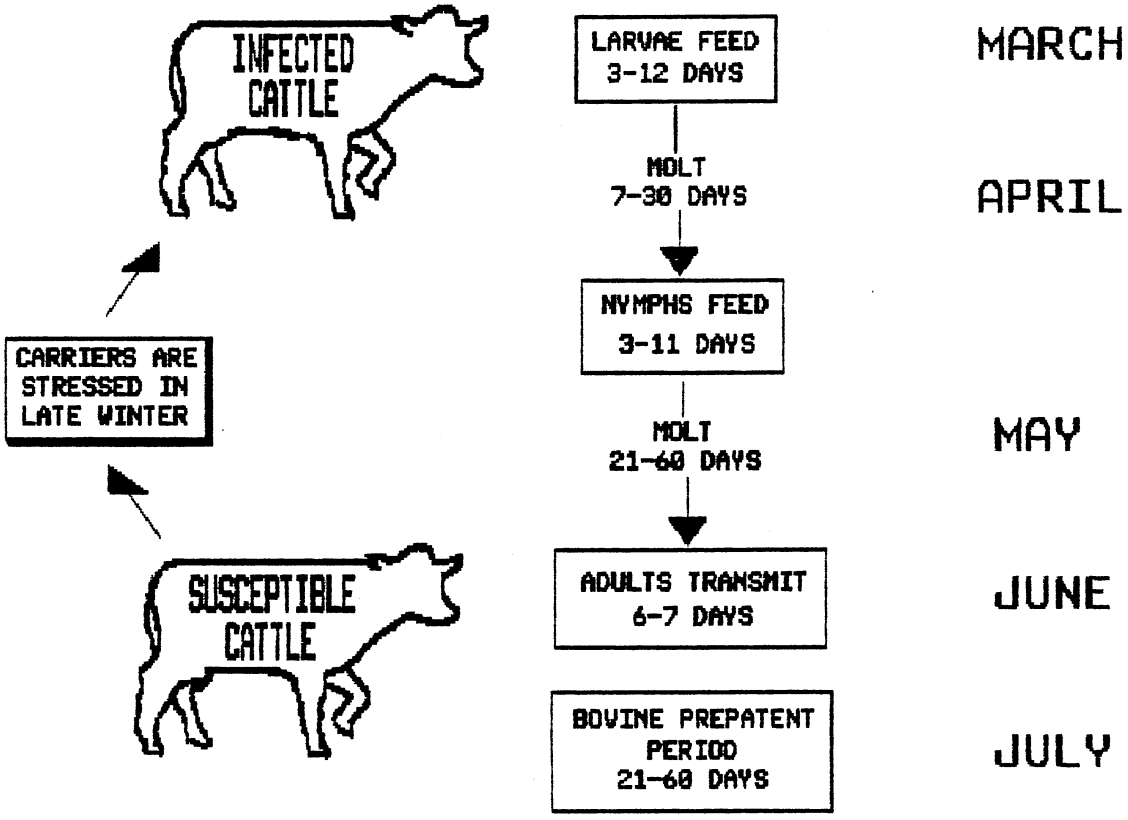
result from larvae hatching from the eggs of adults derived from the first seasonal population. A large portion of the second population of larvae probably overwinters without attaching to hosts, becoming the first generation of the following year.[57] Larvae that begin feeding in spring might acquire A. marginale from carrier cattle, resulting in infective nymphs and then adults in late spring and early summer. If D. variabilis has a similar life cycle in Oklahoma, then the peak activity of adults would correspond to the prepatent period of anaplasmosis during the season of the greatest number of reports of the disease (July, August and September).[62]

There is some question concerning whether or not D. variabilis larvae will attach to cattle under field conditions. It is generally accepted that the larval and nymphal stages of this tick prefer to feed on smaller mammals, but in our experiments larvae and nymphs have attached readily to calves. In the field (with exceptions for different species) tick behavior that leads to host attack is facilitated by odors, vibrations, visual images, touch, shadows, and radiant heat.[61] Cattle with recrudescent anaplasmosis or that are otherwise stressed might have several physiological and behavioral differences that render them more susceptible than healthy animals to attachment and feeding by ticks. Such differences might include a higher body temperature, an altered odor due to the pallor and icterus of the skin,

and a sick animal may be more likely to remain on the ground which allows for easier access by ticks as well as a higher concentration of exhaled carbon dioxide near the ground. Overwintered D. variabilis larvae may become active in late winter and early spring, when carriers of A. marginale might recrudesce due to stress.57 Further consideration that the first seasonal generation of larvae have overwintered without a blood meal, might explain possible attachment to a host that is more available than a preferred host. Once molted into nymphs these ticks probably attack their preferred hosts, but some of the subsequent adults could then attach to cattle and transmit the parasite. Thus, if D. variabilis larvae overwinter in Oklahoma, they may be exposed to A. marginale in the early spring. Peak feeding activity of the subsequent adults around mid-summer would correspond with the peak season of clinical anaplasmosis during the mid-to-late summer (Figure 2).2,21,56,62,63 Additional spread of anaplasmosis through a herd could then be facilitated by tabanids which also are present during the warm summer months. Further studies are needed to ascertain whether D. variabilis larvae overwinter in Oklahoma, if such larvae would attach to cattle under natural conditions, if these larvae would have preference for stressed animals (especially A. marginale carriers) or perhaps become infected on asymptomatic carriers of the parasite.

An epizoological study may be undertaken to examine

Figure 2. Proposed transmission cycle of *Anaplasma marginale* Within *Dermacentor variabilis* in the Field.


INFECTED CATTLE
LARVAE FEED 3-12 DAYS
MARCH
MOLT 7-30 DAYS
APRIL
NYMPHS FEED 3-11 DAYS
MOLT 21-60 DAYS
MAY
CARRIERS ARE STRESSED IN LATE WINTER
SUSCEPTIBLE CATTLE
ADULTS TRANSMIT 6-7 DAYS
JUNE
BOVINE PREPATENT PERIOD 21-60 DAYS
JULY

adult D. variabilis in the field that have been exposed to A. marginale as larvae. Such a study is partially dependent on whether or not a new DNA-probe can demonstrate that A. marginale is present in the midgut of D. variabilis adults that were exposed as nymphs, but not in the midgut of ticks exposed as larvae. Immunodot assays have been used successfully to detect African swine fever virus in the hemolymph of argasid ticks.[56] If such a method can be used to detect A. marginale in ixodid ticks, it could be used to screen infected ticks, and to test their midgut for the presence or absence of anaplasmal colonies to indicate if the ticks might have been exposed to the parasite as larvae.

CHAPTER V

SUMMARY

Anaplasma marginale, the causative agent of bovine anaplasmosis, is the cause of losses to the cattle industry world-wide. The organism is transmitted mechanically by blood-contaminated fomites and hematophagous arthropods, but ixodid ticks are the only reported biological vectors of the disease. A developmental cycle has been proposed recently for the organism in Dermacentor andersoni infected as nymphs and transmitting A. marginale as adults, but little is known about the life cycle of this organism in nymphs or adults that were infected as larvae.

Dermacentor variabilis is a common ixodid tick throughout the eastern and central United States, and is believed to be expanding its range to western portions of the country. The tick is common in Oklahoma and is associated with cattle in anaplasmosis-enzootic areas. All three development stages readily attach to experimental calves and the tick is a proven biological vector of A. marginale under experimental conditions.

The objectives of this experiment were to demonstrate the ability of D. variabilis larvae to transstadially

transmit A. marginale to subsequent stages and transovarially to subsequent generations, and to document the morphology of this parasite in ticks that were exposed to A. marginale as larvae.

Transstadial transmission was demonstrated from both larvae and nymphs. Nymphs and adults infected as larvae appeared to transmit the organism to susceptible calves as effectively as did adults that were infected as nymphs and likewise, equally as well as ticks exposed twice (i.e., as larvae and nymphs). Morphology studies did not reveal any anaplasmal colonies in the midgut epithelium of unfed nymphs or males that were exposed as larvae; colonies were observed in the midgut epithelium of unfed females in Trial 2.

Transovarial transmission did not occur from parent larvae and nymphs to F1 larvae. However, Trial 2 and control calves did have suspicious marginal bodies in erythrocytes in peripheral blood smears. The major difference between these and Trial 1 calves was that more F1 larvae, from a greater number of parental females, were placed on the Trial 2 and control calves.

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VITA

Roger William Stich

Candidate for the Degree of

Master of Science

Thesis: TRANSSTADIAL AND TRANSOVARIAL TRANSMISSION OF ANAPLASMA MARGINALE THEILER BY DERMACENTOR VARIABILIS (SAY)

Major Field: Veterinary Parasitology

Biographical:

Personal Data: Born in Tulsa, Oklahoma, September 24, 1960, the son of Roger L. and Patricia H. Stich.

Education: Graduated from Norman High School, Norman, Oklahoma, in May, 1979; received Bachelor of Science Degree in Biological Science from Oklahoma State University in December, 1984; completed requirements for the Master of Science Degree at Oklahoma State University in May, 1987.

Professional Experience: Laboratory Technician, Department of Veterinary Pathology, Oklahoma State University, 1985 to present.

Professional Associations: Oklahoma Academy of Science, Oklahoma Society for Electron Microscopy.