EXPERIMENTAL TRANSMISSION OF A CANINE GRANULOCYTIC FORM OF EHRLICHIA BY TICKS

By

OSCAR SERGIO ANZIANI

Medico Veterinario

Universidad Nacional Del Litoral

Santa Fe, Republica Argentina

1975

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
December, 1988

EXPERIMENTAL TRANSMISSION OF A CANINE

GRANULOCYTIC FORM OF EHRLICHIA

BY TICKS

Thesis Approved:

R. W. Baker

Thesis Adviser

Russell Wright

William A. Drew

S. P. Irving

Norman N. Durham

Dean of the Graduate College

ACKNOWLEDGEMENTS

The writer thanks his major adviser, Dr. R.W. Barker for guidance and advice given during this project. The writer is grateful to Dr. S.A. Ewing for his help, inspiration and support. His expertise and knowledge in the area of Parasitology were invaluable. Appreciation is also extended to other committee members, Dr. R.E. Wright and W.A. Drew for their suggestions and critical evaluation of this manuscript.

A special expression of gratitude is due to the country of the writer, Argentina, for the financial support given throughout the study program.

Finally, a very special thanks goes to my wife, Haydee, and my daughter, Maria Florencia, for their support, dedication, understanding and sacrifices during my graduate studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

In domestic animals, three species of the genus Ehrlichia, Ehrlichia canis (Donatien and Lestoquard), Ehrlichia bovis (Donatien and Lestoquard) and Ehrlichia risticii (Holland, Ristic, Cole, Johnson, Baker and Goetz) are pathogens of dogs, cattle and horses and occur in mononuclear leukocytes (lymphocytes and monocytes).[1] The remaining two members of the genus, E. phagocytophila (Foggie) which is the causative agent of tick borne fever of sheep and cattle, and E. equi Lewis, Huxoll, Ristic and Johnson which causes equine ehrlichiosis, have predilection for circulating polymorphonuclear leukocytes (neutrophils and eosinophils).[1-3] Also, Ehrlichia platys, an ehrlichiae organism with unknown taxonomic relationship, has been recently described in thrombocytes of dogs.[4]

In general, the relationship among individual species of Ehrlichia and the diseases which they are reported to cause is unclear. Also, the immunological and biochemical relationships among the various species and strains are poorly understood, making a coherent approach to the study of the pathogenesis and epizootiology difficult.[2] For example, although E. canis, the type species of the genus, occurs in monocytes and lymphocytes, the isolation of neutrophilic forms of Ehrlichia has also been reported in dogs.[5-7]

This neutrophilic canine form and E. equi, which apparently not only infects horses but also dogs, sheep, goats and non-human primates, are

characterized by the presence of morulae of similar appearance in neutrophils and eosinophils.[8] Also, the agents of tick borne fever of cattle and sheep, and bovine petechial fever have marked morphological resemblances with the granulocytic form observed in dogs and horses.[3] These morphologically close resemblances, and the successful experimental infection of dogs with E. equi, led some investigators to suggest that the neutrophilic strain of E. canis and E equi may be a similar, if not the same agent.[8] However, it was described recently some pathogenic and immunogenic differences between the two granulocytic forms of Ehrlichia, thus providing a basis for postulating the weak relationship between these two agents.[9]

The route of Ehrlichia organisms transmission is also unclear and the mechanism whereby the infection persists in reservoirs have not been elucidated.[1-3] Epizootiological evidence and experimental work have incriminated ixodid ticks as vectors of most Ehrlichia organisms, [10-15] although a recent report with E. risticii (the agent of Potomac horse fever) indicated positive transmission through experimental oral inoculation.[16]

Two agranulocytic forms of Ehrlichia,(E. canis and E. risticii) are probably the organisms in which tick transmission has been most extensively studied. Transstadial transmission of E. canis by Rhipicephalus sanguineous (Latreille) has been demostrated,[12,14] but no attempts to transmit the organism by other tick species has been reported. In the case of E. risticii, transmission did not occur in a series of laboratory and field studies involving selected blood sucking insects and four different species of ticks as potential vectors of Potomac horse fever.[17,18]

Evidence for tick involvement in transmission of other Ehrlichia organisms is largely circumstancial and supporting data are still needed. The lack of transmission studies is especially marked in the granulocytic ehrlichiae. For example, tick vectors of E. equi have yet to be discovered and no attempts to transmit canine granulocytic forms has been reported.

The present study was conducted as an attempt to approach this problem by studying the ability of two Ixodid ticks, Dermacentor variabilis (Say) and Amblyomma americanum (L.), to transmit a canine granulocytic form of Ehrlichia. This information could help elucidate the epidemiology of granulocytic ehrlichial infections in domestic animals.

CHAPTER II

LITERATURE REVIEW

General background and host parasite interactions

The genus Ehrlichia is formed by a group of microorganisms of similar morphology which parasitize circulating leukocytes of humans and a variety of wild and domestic animals.[1] This genus is currently included in the family Rickettsiaceae of the order Rickettsiales.[2] The most distinguishing characteristic of members of the genus Ehrlichia is their development within a membrane-lined cytoplasmic vacuole of leukocytes.[2]

The organisms were first recognized and described in 1935 by French investigators in tick-infested dogs in Algeria.[19] Since that time, numerous reports have appeared describing naturally occurring infections in other domestic animals including sheep, goats, cattle and horses.[2]

Ehrlichia canis is the type species of the genus and the causative agent of canine ehrlichiosis, an acute febrile disease of domestic and wild canidae throughout the world. The disease is characterized by thrombocytopenia and mucosal and serosal hemorrhages.[10,20,21] Fatal cases are usually due to uncontrolled bleeding or secondary infections due to insufficient platelet or leukocyte numbers, respectively.[2] The organism occurs either singly or in compact colonies or inclusions, termed morulae, in the cytoplasm of circulating monocytes and lymphocytes.[2,10,20]

The occurrence of a milder pathogenic organism observed in

circulating neutrophils and eosinophils rather than in lymphocytes and monocytes of dogs has also been reported.[5-8] Some workers suggested that this granulocytic form of Ehrlichia and E. equi, the causative agent of equine ehrlichiosis, may be similar, if not the same.[3,8] Both organisms form inclusions, or morulae, in granulocytic leukocytes and both are responsible for a mild to moderate acute disease characterized by fever, depression, stiffness of the legs, polyarthritis, leukopenia, thrombocytopenia and mild anemia. Ehrlichia phagocytophilia, the causative agent of tick borne fever of sheep and cattle in Europe, also present morphological and pathogenic resemblances with these granulocytic forms.[2,3]

Remarkably little is known about the pathogenesis of these granulocytic Ehrlichieae and the antigenic relationships among all three agents. Recently, strong serologic cross reaction was observed between Cowdria ruminantium (Cowdry), the causative agent of a tick borne rickettsial disease of ruminants, and E. equi and E. phagocytophilia, suggesting that all these agents may be antigenically related.[22] Since E. equi has also been shown experimentally to infect dogs and ruminants,[3,8] this fact could have epidemiological importance and the possibility of serological misinterpretation should be considered.

It is unclear at this time whether the antigenic association observed among C. ruminantium, E. equi and E. phagocytophilia may also extend to the canine granulocytic form. Experimentally E. equi can infect dogs,[8] and a canine granulocytic form was transmitted successfully to horses by inoculation with whole blood.[6] However, these reports did not include systematic serological studies and therefore it would be impossible to make inferences about such a relationship. Moreover, some experimental

work,[9] and serologic studies of field cases,[7] indicate that the neutrophilic Ehrlichia agent involved in a polyarthritis syndrome observed in dogs is more closely related to E. canis than to E. equi.

Epizootiology

Most rickettsial agents are transmitted by ticks, and ixodid ticks in particular have been incriminated as vectors of several organisms of the genus Ehrlichia.[2,13] However, this historical connection between ehrlichial agents and ticks has only been demonstrated in the cases of E. phagocytophilia and E. canis. At present, tick involvement in the transmission of the remaining members of the genus, E. bovis, E. equi and E. risticii, is largely speculative.

The first and only detailed study of transmission of E. phagocytophilia by ticks dates from 1936.[11] The study showed that transstadial but not transovarial transmission occurred in the tick vector, Ixodes ricinus (Linnaeus). In the same study it was also reported that ticks transmitted the organism after the second day of attachment.

The transmission of E. canis by the brown dog tick, Rhipicephalus sanguineous, was considered as early as 1935.[19] Since then, the importance of this tick in transmitting canine ehrlichiosis has been reported in several epizootics in which dogs were infested with large number of ticks.[10,23,24] Subsequent controlled laboratory studies conclusively demonstrated transstadial transmission of E. canis by R. sanguineous,[25] and described the development of the agent in tick tissues.[14] Although the vector-parasite relationship of E. canis and R. sanguineous has been extensively studied, some questions remain unanswered. For example, the existence of transstadial but not

transovarial transmission by this tick make it difficult to explain the worldwide occurrence of canine ehrlichiosis,[26] and suggests that other vectors could be involved in transmission of the disease. The absence of transovarial transmission excludes the possibility that the vector tick is the reservoir.[20] Although studies to support this claim are lacking, chronically infected dogs and wild canidae must be considered as potential reservoirs of the disease in nature.

The vectors, reservoirs and modes of transmission of the other ehrlichial organisms reported in leukocytes of domestic animals (E. bovis, E. equi, E. risticii and the granulocytic canine form) are presently unknown. Ehrlichia bovis was first isolated from cattle naturally infested with ticks of the genus Hyalomma in Algeria. Amblyomma variegatum (Fabricius) and Boophilus decoloratus (Koch) have also been suspected as vector of this agent but confirmed information is lacking.[2] Two Dermacentor species, Dermacentor albipictus (Packard) and Dermacentor occidentalis Marx, have been associated with horses infected with E. equi.[27] However, experimental transmission with adult forms of D. occidentalis previously engorged as nymphs on acutely infected horses has been unsuccessful.[28]

Tick transmission of equine ehrlichial colitis (E. risticii), a recently recognized severe enteric disease of horses, is currently a subject of intensive investigations. Based on early field observations of affected farm areas, D. variabilis was considered the most likely candidate.[17] However, laboratory and field attemps to incriminate this tick as a vector have not met with success.[17,18] Unsuccessful laboratory attempts to transmit the organism have also been reported with D. albipictus, A. americanum and Ixodes scapularis Say.[18] The potential of

oral transmission recently reported in an experimental inoculation of E. risticii-infected monocyte tissue culture cells,[16] and its actual significance in the epizootiology of the disease requires additional confirmation. Although the mode of natural transmission of the relatively new described organism E. platys is unknown, an arthropod vector is suspected. However, no attempts to determine its potential vectors have been reported.[4]

Disease agents transmitted by **Amblyomma americanum** and **Dermacentor variabilis**

Amblyomma americanum is a three-host tick with non-specific feeding habits whose role in transmission of pathogens to human beings and animals is still unclear and the subject of some controversy. Scientific literature concerning the role of the A. americanum and the disease agents associated with this tick have sometimes been unclear and even contradictory. This apparent inconsistency "... may not necessarily indicate inaccuracy but rather pieces of a complex, even changing puzzle."[29]

Field and laboratory studies clearly indicate that A. americanum plays a very important role in the transmission of Francisella tularensis (McCoy and Chapin), the causative agent of tularemia to man and animals.[30] Transmission of Theileria cervi to white tail deer by this tick has also been demonstrated,[31] and suggests that in some areas of the United States it is the principal vector of the disease in nature.[32] On the other hand, the relationship of other rickettsial and protozoan agents with A. americanum is still unclear. For example, only indirect evidence incriminates it as a vector of murine typhus, Rickettsia typhi (Wolbach

and Todd), and apparently this tick does not play a significant role in the transmission of Rickettsia rickettsii (Wolbach), Coxiella burnetii (Derrick) or Toxoplasma sp.[29] Although Lyme disease spirochetes, Borrelia burgdorferi Johnson, Schmid, Hyde, Staigerwalt and Brenner, have been recovered from A. americanum its actual importance as a vector of the spirochete in nature is unknown. In spite of previous circumstantial evidence, the tick apparently is not a vector of Babesia odocoilei (A.A. Kocan, personal communication) and apparently it does not play an important role in the epizootiology of viral diseases.[29] The American dog tick, D. variabilis, also has a 3-host life cycle. It is one of the chief vectors of R.rickettsi, the agent of Rocky Mountain spotted fever.[33] Dermacentor variabilis has also been reported as an efficient vector of anaplasmosis.[34,35] Moreover, the causative rickettsial agent, Anaplasma marginale Theiler, can overwinter in D. variabilis, although it is unknown if overwintered ticks can transmit the disease.[36] The potential as a vector of the disease seems to be related to biological differences among A. marginale isolates which apparently extend to tick borne transmissibility.[37]

Although the importance of D. variabilis as a vector is mostly related to the rickettsial diseases above cited, the tick has also been reported, at least experimentally, as a vector of Cytauxzoon felis (Kier), a very pathogenic piroplasm of domestic and wild cats.[38]

CHAPTER III

MATERIALS AND METHODS

Experimental animals- Eight adult dogs representing both sexes (numbers 1 to 8) and two male pups of mixed breeding (numbers 0048 and 0052) were used in this study. The adult dogs were obtained from an Oklahoma animal shelter, while the pups came from an E. canis free dog colony maintained at the College of Veterinary Medicine at Oklahoma State University. Each animal had been vaccinated against rabies, distemper, parvovirosis and infectious hepatitis. Where necessary anthelmintic treatment was administered. Fifteen days before beginning the experiment, the adult dogs were dipped with an organophosphate acaricide. Freedom from infection with E. canis and E. equi was confirmed by negative indirect fluorescent antibody test (IFAT).* Before tick exposure, the test was performed twice in each of the adult dogs with an interval of fifteen days, while the pups were tested once at the onset of the study.

Agent- The granulocytic (neutrophilic) strain of Ehrlichia used was recovered originally from a dog admitted to the Veterinary Medicine Teaching Hospital at Oklahoma State University, and it was maintained by serial passages in carrier dogs. Donor dogs were experimentally infected by intravenous inoculation with 10 ml of blood in ethylene

---

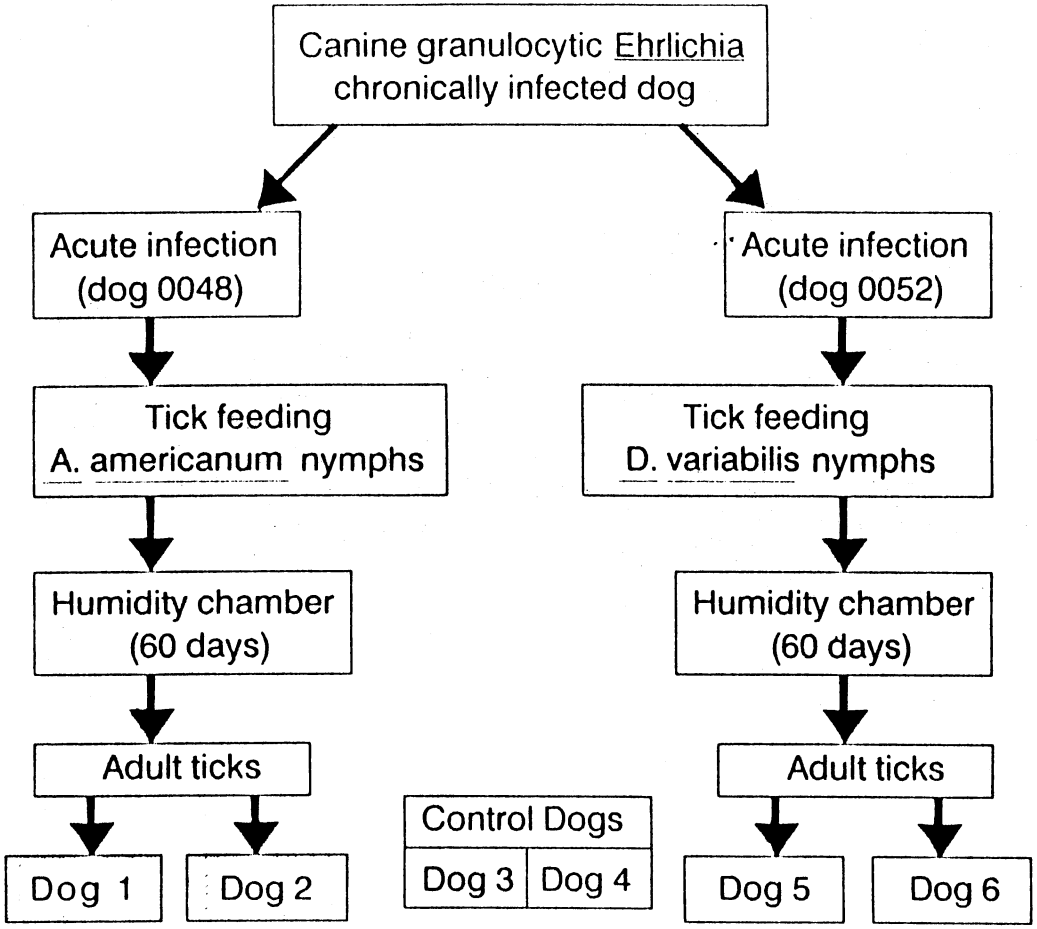
* (Test conducted at Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, Oklahoma)

diaminetetraacetic acid (EDTA) drawn from a chronically infected dog.

Ticks- The transmission studies were conducted with long-established colonies of A. americanum and D. variabilis reared and maintained at the Livestock Entomology Laboratory at Oklahoma State University. Each tick species was free of any known canine infective agent. The larval stages were fed on rabbits and allowed to develop to the nymphal stage. Ticks were held in a humidity chamber (90-95 % relative humidity) at 27 C with alternating periods of 14 hours of light and 10 hours of darkness, prior to tests.

Tick feeding methods and experimental design- A schematic diagram to test transstadial transmission of a canine granulocytic form of Ehrlichia is shown (Fig 1). The two male pups (donor dogs) were infected as described above with one dog being used for each tick species. Three and five days after the first appearance of Ehrlichia morulae in the peripheral blood, A. americanum and D. variabilis nymphs, respectively were allowed to feed until replete on the donor dogs. Thus, the phase of tick feeding was within 12 days of the first appearance of morulae. Two hundred A. americanum and 300 D. variabilis nymphs were placed directly on the neck and back of each donor dog. To avoid grooming and to facilitate tick attachment, orthopedic stockinetess sleeves were fitted around the trunk of the donor dogs and the animals were placed in enclosed, ventilated, plastic boxes for approximately 6 hours. The dogs were then placed in wire restraint cages that were suspended over water-filled plastic trays at 25°C for 8 days. Engorged nymphs were collected from the water daily and held in a humidity chamber as described above for approximately 60 days. Four weeks after molting, the resulting adults exposed as nymphs on the acutely infected dogs (donor dogs) were used for

Figure 1. Schematic diagram of the experimental design to test transstadial transmission of a canine granulocytic form of *Ehrlichia* by *Amblyomma americanum* and *Dermacentor variabilis*.


Canine granulocytic Ehrlichia chronically infected dog
Acute infection (dog 0048)
Acute infection (dog 0052)
Tick feeding A. americanum nymphs
Tick feeding D. variabilis nymphs
Humidity chamber (60 days)
Humidity chamber (60 days)
Adult ticks
Adult ticks
Control Dogs
Dog 1
Dog 2
Dog 3
Dog 4
Dog 5
Dog 6

the transmission studies. Dogs number 1 and 2 were exposed to 15 female and 11 male A. americanum adults each, while dogs number 5 and 6 were exposed to 6 female and 4 male D. variabilis adults each. Dogs number 3 and 4 were kept as unexposed controls. Ticks were allowed to feed to repletion and drop off.

Clinical evaluation and subinoculation studies- Experimentally inoculated and tick exposed dogs were examined daily by rectal temperature and examination of peripheral blood smears stained with a modified Wright** stain for a period of 40 and 80 days, respectively.

With a minimum frequency of once a week, samples of blood containing EDTA as anticoagulant were collected for clinical laboratory examination. Laboratory tests included hemoglobin and packed cell volume determination. After detection of morulae in the peripheral blood, thrombocyte and cell blood counts were performed 3 to 5 times a week.

Approximately three weeks after tick exposure serum samples were periodically tested for anti-ehrlichial antibodies by IFAT (E. equi and E. canis). In order to confirm transmission, after positive serologic evidence was observed, subinoculation of blood into susceptible dogs (number 7 and 8) and corticosteroids treatment were carried out. In the subinoculation studies each of the susceptible dogs received 16 ml of blood administered intravenously. The corticosteroid treatment consisted in the daily oral administration of 4 mg/kg of prednisone ("Prednisone", Roxane Laboratories) for 15 days.

Infection was considered to occur when typical Ehrlichia morulae were observed in the cytoplasm of circulating polymorphonuclear cells. Lack of

---

** Diff-Qick Stain Set, American Scientific Products.

transmission was confirmed by the absence of parasites and by a persisting negative serologic reaction during the 80 days of observation.

CHAPTER IV

RESULTS

Experimental inoculation of donor dogs- Eighteen days after inoculation of the canine granulocytic form of Ehrlichia, the 2 donor dogs (0048 and 0052) developed signs of infection. Morulae were first seen in peripheral blood between 17 and 18 days post inoculation and persisted for 3 to 5 days. Then, relapses occurred in both animals 4 and 5 weeks post inoculation. Morulae were observed exclusively in neutrophils. The first presence of morulae coincided with pyrexia and mild depression. During this period, a slight leukopenia was observed in dog 0048. A marked thrombocytopenia (50,000 platelets/mm^3) was observed in dog 0052 within the week that preceded the observation of organisms in blood smears. Dog 0048 experienced a mild reduction in the number of platelets. In both animals the thrombocytopenic period continued until aproximately 4 weeks post inoculacion, after which a gradual return to pre-exposure values occurred.

Ehrlichia transmission by ticks- The numbers of replete nymphs recovered from donor dogs and the resulting molted adults are presented in Table 1. All engorged nymphs had dropped off the donor dogs by 9 days post-infestation and the molting period was completed in approximately one month.

No evidence of transmission was observed in control dogs nor in dogs exposed to adult D. variabilis. In contrast, dogs exposed to adult A.

TABLE I

Number of *Amblyomma americanum* and *Dermacentor variabilis* nymphs placed on and recovered from donor dogs (0048 and 0052 and number molting to adult stage.

Tick	Nymphs placed	Nymphs recovered	Adults	
			males	females
A. americanum	> 200	57	30	22
D. variabilis	> 300	22	8	12

americanum ticks developed serologic or clinical evidences of infection. Twenty-one days after tick infestation, dog 1 showed marked thrombocytopenia accompained by fever and mild leukopenia. Dog 2 did not have a detectable clinical response except an intermittent mild fever (39.4 to 39.8°C). Although organisms were not seen in blood smears, both animals showed IFAT titers of >100 when their sera were tested for anti-ehrlichial antibodies at 50 days after tick exposure. Failure to demonstrate the organism in peripheral blood smears of these two dogs prompted corticosteroid treatment to induce recrudescence and subinoculation of their blood into two susceptible dogs.

Corticosteroid treatment and subinoculation studies- The results observed in the subinoculation studies and the corticosteroid response of dog exposed to Amblyomma americanum are presented in Fig 2. Seven and 8 days after the oral administration of prednisone, dogs 1 and 2 had morulae in their circulating neutrophils. A typical morula observed in dog 1 is shown in Fig 3. Organisms persisted in the blood smears of both animals until the end of the treatment period (15 days). Morulae were not observed in eosinophils nor in other leukocytes. The highest parasitemia was recorded 11 days after the beginning of the prednisone administration when approximately 1.5% of the neutrophils of dog 1 contained Ehrlichia morulae in the cytoplasm. The presence of morulae was not accompanied by marked clinical signs of disease in either dog; a mild depression and slight swelling of the carpus joint was observed in dog 1 but no clinical signs were observed in dog 2.

One day before starting the corticosteroid treatment in dogs 1 and 2, 16 ml of blood from each of these dogs was drawn and inoculated into dogs 7 and 8, respectively. Within 2 weeks after inoculation, dogs 7 and 8

Figure 2. Subinoculation studies and response to corticosteroid therapy in dogs exposed to Amblyomma americanum.

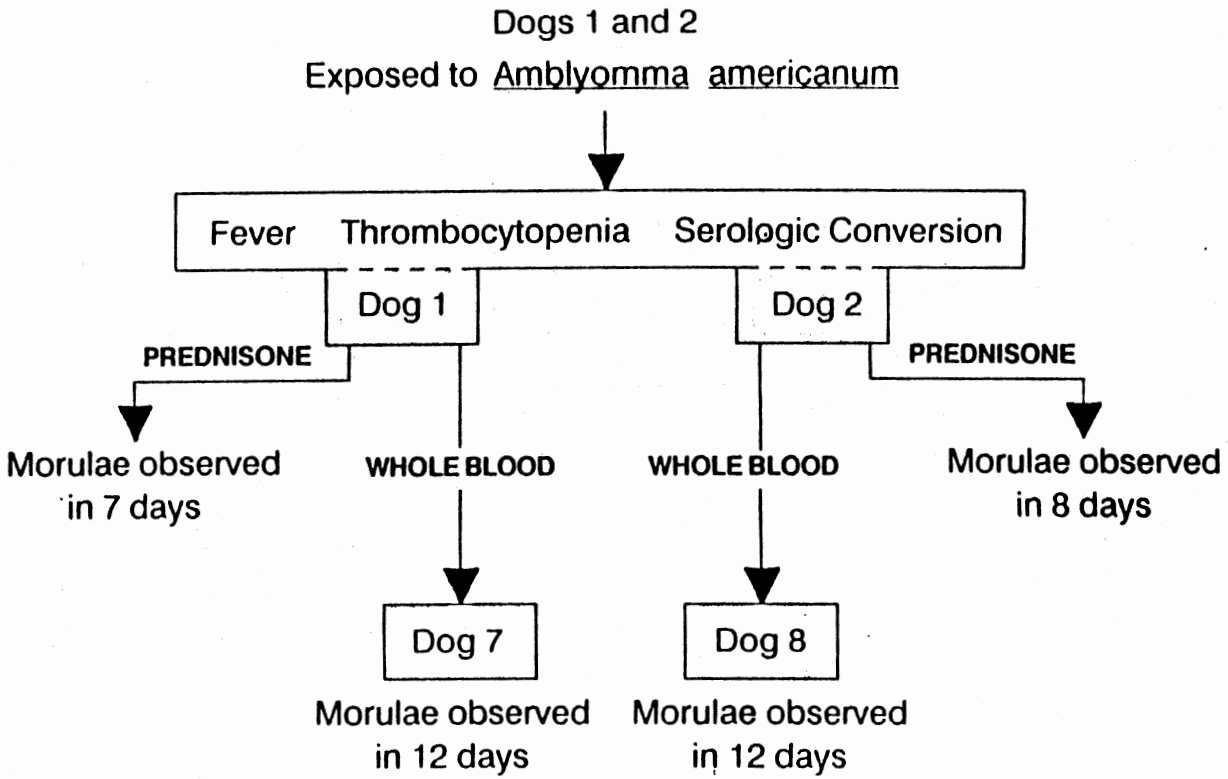
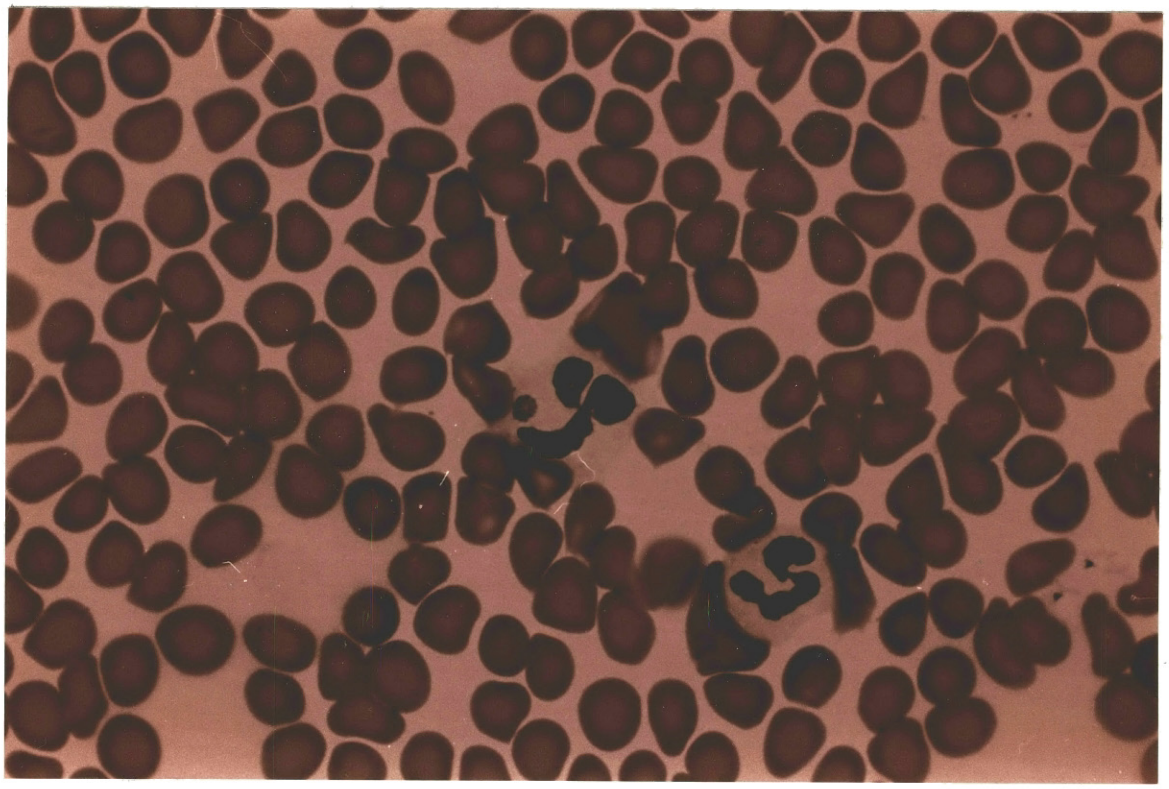

Dogs 1 and 2
Exposed to Amblyomma americanum
Fever
Thrombocytopenia
Serologic Conversion
Dog 1
Dog 2
PREDNISONE
PREDNISONE
Morulae observed in 7 days
WHOLE BLOOD
WHOLE BLOOD
Morulae observed in 8 days
Dog 7
Dog 8
Morulae observed in 12 days
Morulae observed in 12 days

Figure 3. Neutrophil containing morula of a canine granulocytic form of Ehrlichia (dog 1).



developed signs of Ehrlichia infection. In both animals morulae were observed in the neutrophils and they persisted 5 to 6 days. Morulae observation coincided with a mild febrile response (39.7 to 40.2°C) and was immediately preceded and then accompanied by severe thrombocytopenia. Platelet reduction was particularly severe in dog 7 (minimun recorded value = 6,000/mm^3) while in dog 8 the thombocytopenia was transient and not as dramatic (minimun recorded value = 70,000/mm^3). Eleven days after blood inoculation, Ehrlichia platys was detected in the platelets of dog 7. On day 12 approximately 15% of the platelets harbored inclusions of this organism and 2 days later they had disappeared. No E. platys relapses were observed during the 80 day duration of this study.

Serology- The serologic results observed in control and tick-exposed dogs are shown (Fig 4). Serum antibodies for E. canis and E. equi were not detected in either control dog nor in D. variabilis exposed dogs. In contrast, 50 days after tick exposure, a marked rise in antibody titer to E. canis (from initially < 1:10 to > 1:100) was observed in dogs exposed to A. americanum ticks. In both animals, E. canis antibodies remained at diagnostic levels until the end of the observation period. No E. equi antibodies were detected except for a low titer of >1:10 observed in one serum sample of dog 1 on day 50 after tick exposure. The titer was adequate only to be considered "suspicious".

Figure 4. Indirect fluorescent antibody response in dogs after tick feeding

E.c. = *Ehrlichia canis*

E.e. = *Ehrlichia equi*

\- = IFAT titer < 1:10

\+ = IFAT titer > 1:10

++ = IFAT TITER > 1:100

ANTIBODY RESPONSES IN DOGS AFTER TICK FEEDING

	Amblyomma americanum				No Tick Exposure				Dermacentor variabilis			
Day	Dog 1		Dog 2		Dog 3		Dog 4		Dog 5		Dog 6	
	E.c.	E.e.	E.c.	E.e.	E.c.	E.e.	E.c.	E.e.	E.c.	E.e.	E.c.	E.e.
-20	−	−	−	−	−	−	−	−	−	−	−	−
-10	−	−	−	−	−	−	−	−	−	−	−	−
0	ticks feeding								ticks feeding			
10												
20												
30	+	−	+	−	−	−	−	−	−	−	−	−
40												
50	++	−	++	−	−	−	−	−	−	−	−	−
60	++	−	++	−	−	−	−	−	−	−	−	−
70	+	−	+	−	−	−	−	−	−	−	−	−
80												

CHAPTER V

DISCUSSION

The granulocytic forms of Ehrlichia are associated with the production of a moderate to mild clinical syndrome in dogs, horses, cattle and sheep.[2,3,39] Canine granulocytic forms have been reported in field cases to cause a mild disorder characterized by anorexia, depression, fever and acute lameness.[5-7] The clinical signs and pathogenesis of the infection observed in the present study did not differ greatly from experimental inoculations previously reported with this organism.[40,41] As in earlier experimental studies, the severe inflammatory joint changes and acute lameness commonly present in field cases were not observed here, suggesting the possibility of pathogenic differences between strains or the requirement of other associated factors (e.g., severe immune suppression). Infection with a neutrophilic strain of Ehrlichia has been reported in dogs under immunosuppressive anti-cancer chemotherapy.[6]

In general, transmission of Ehrlichia organisms is poorly understood. Ticks are proven vectors for a few ehrlichial organisms, but for most of them evidence is only circumstantial.[1,2,13] The vector-parasite relationship between A. americanum and ehrlichial agents is not clear. Unsuccessful attempts to transmit Potomac horse fever (E. risticii) transtadially by this tick has been recently reported.[18]

On the other hand, the results in the present study demonstrate that A. americanum is capable of transstadial transmission of a canine

granulocytic form of Ehrlichia. Natural transmission occurred with as few as 26 adult ticks suggesting that this tick could serve as a transstadial vector of this form of Ehrlichia in dogs. These findings provide further justification for exploring its potential vector role for other ehrlichial agents. For example, it would be surprising if the tick were not involved in the transmission of E. canis, the agranulocytic form that occurs in dogs. If this were true, the existence of other potential vectors could help explain the worldwide occurrence of canine ehrlichiosis. At the present, R. sanguineous is the only proven vector for the disease, and transmission occurs transstadially rather than transovarially.

The failure to demonstrate the organisms in peripheral blood smears in two dogs with clinical and serologic evidence of infection is puzzling. Except during the short febrile phase, canine granulocytic organisms are extremely difficult to find in circulating blood,[41] and during the course of infection parasitemia levels are usually low. In the present study ticks were exposed (as nymphs) within 14 days after the first appearance of morulae, and the highest parasitemia, observed in the donor dogs occurred immediately before the tick feeding phase. A low level infection in ticks may have occurred with the consequence that the quantity of ehrlichial organisms in the blood was below the threshold of observable detection with the techniques employed. However, we have no data to confirm such speculation.

The immunosuppressive activity of prednisone in these animals was assumed to be responsible for the appearance of morulae in the peripheral blood neutrophils. Immunosuppressive actions of corticosteroids are well known and they have been described to induce acute recrudescence of such diverse agents as Toxoplasma,[42] Babesia,[43] and Anaplasma.[44] Based on the

data presented in this study, it could not be determined if the humoral or cell mediated immune systems or both, were blocked by the treatment. But undoubtedly the balance was sufficiently disturbed to produce a significant relapse of the organism. A severe clinical syndrome associated with natural infections of neutrophilic Ehrlichia forms in dogs treated with corticosteroids for prolonged periods has also been reported.[6] The recrudescence observed in the present work was in agreement with the above report and emphasized the possibility that extended usage of these drugs could result in relapses of subclinical Ehrlichia infections.

The clinical and hematological changes observed in dogs 7 and 8 agree closely with previous reports,[40,41] and with those observed in the donor dogs. However, the presence of E. platys in the blood smears of dog 7 was puzzling and remained unexplained. The dog had been inoculated with blood from dog 1 exposed to A. americanum ticks. A retrospective serologic study showed that both animals as well as the donor dog on which nymphs were fed on, were negative to E. platys before tick exposure (dogs 0048 and 1) or blood inoculation (dog 7).

One can only speculate to explain the presence of E. platys. Although the indirect fluorescent test is considered the most reliable method for diagnosis of E. platys infections,[45] there is not information about the long-term persistence of antibodies against this organism in chronically infected dogs. One remote possibility, for example, could be that dog 7 was a chronic carrier of this organism with a subclinical infection that was serologically undetectable. Thus, the stress produced by the canine granulocytic Ehrlichia infection could be responsible for a relapse of E. platys. It has been suggested that concurrent infection of this organism

with other disease agents (i.e. E.canis) could potentiate the clinical manifestations in dogs infected with both agents.[46]

Due to morphological and pathological similarities, it has been postulated that the neutrophilic canine form of Ehrlichia actually may be E. equi.[6,8,27] The serologic results of the present work failed to demonstrate a cross-antigenic relationship between E. equi and the neutrophilic canine form. In contrast, all the infected dogs had detectable titers for E. canis. These serologic results indicate that the neutrophilic Ehrlichia agent used in this study is more closely related to E. canis than to E. equi. Further studies are needed to determinate if this agent involved in the polyarthritis syndrome observed in dogs, is actually a strain of E. canis or an other ehrlichial organism.

Attempts to transmit the organism using D. variabilis were unsuccessful. A number of factors might have been responsible for the failure of transstadial transmission with this tick. With the available information it is unknown if the organism can actually survive in this tick. However, if the organism does survive in the tick, transmission could depend on the number of infected ticks feeding on a dog. The small number of D. variabilis ticks used in the present experiment could have been an important handicap in the transmission of the organism.

The possibility also exists that the neutrophilic Ehrlichia strain used was refractory to D. variabilis and vice-versa. It has been reported previously that D. variabilis can be an extremely efficient transstadial vector of some isolates of Anaplasma marginale but they can fail to transmit other isolates of the same agent.[37]

Finally, there is also a remote possibility of some interference between the immunological response of dogs to ticks and infection with the

ehrlichial organism. The contingency of previous contact between the dog used in the present study and D. variabilis ticks should not be precluded. As was suggested in the relationship Dermacentor reticulatus (Fabricius) and Babesia canis (Piana and Galli Valerio),[47] the immune response of dogs could affect the process of engorgement of ticks which in turn could also modify the passage of the organism.

CHAPTER VI

SUMMARY

Most rickettsial diseases are transmitted by ticks. However, with the exception of two members of the genus Ehrlichia, evidence for tick involvement in the transmission of ehrlichial species is largely circumstantial, and supporting data are still needed. The lack of transmission studies is especially marked in the granulocytic ehrlichiae. In the present study transstadial transmission of a canine granulocytic form of Ehrlichia was attempted with A. americanum and D. variabilis. Ticks were exposed by feeding as nymphs on acutely infected dogs; adults then fed to completion on susceptible dogs that were monitored daily for infection. No evidence of transmission was observed in control dogs nor in those exposed to D. variabilis. In contrast, dogs exposed to A. americanum developed serologic or clinical evidence of infection but organisms were not seen in blood smears. Failure to demonstrate organisms in peripheral blood smears of seropositive dogs was puzzling and prompted: a) subinoculation of whole blood into other susceptible dogs and b) steroid therapy to induce recrudescence with accompaying parasitemia. Subinoculated dogs became infected, and morulae were observed in peripheral blood neutrophils 12 days post-exposure. Moreover, morulae were found in neutrophils of the dog that had been infected by tick bite 7 days after onset of steroid therapy. Due to morphological and pathological resemblances between E. equi and the granulocytic Ehrichia

could be the same agent. The serologic results observed in the present study seem to indicate that the granulocytic form of Ehrlichia that occurs in dogs is more closely related to E. canis than to E. equi.

REFERENCES

1. Ristic M, Huxoll DL. Ehrlichieae. In: Krieg NR, Holt JH, eds. Bergey's manual of systematic bacteriology. Vol 1. Baltimore: The Williams & Wilkins Co, 1984;704-709.

2. Smith RD, Ristic M. Ehrlichiae. In: Kreier JP, ed. Parasitic Protozoa. Vol. 3. New York: Academic Press Inc, 1977;295-357.

3. Lewis GE. Equine ehrlichiosis: a comparison between E. equi and other pathogenic species of Ehrlichia. Vet Parasitol 1976;2:61-74.

4. Harvey JW, Simpson CF, Gaskin JM. Cyclic Thrombocytopenia induced by a rickettsia-like agent in dogs. J Infect Dis 1978;137:182-188.

5. Ewing SA, Roberson WR, Buckner RG, Hayat CS. A new strain of Ehrlichia canis. J Am Vet Med Assoc 1971;159:1771-1774.

6. Madewel BR, Gribble DH. Infection in two dogs with an agent resembling Ehrlichia equi. J Am Vet Med Assoc 1982;180:512-514.

7. Cowell RL, Tyler RD, Clinkenbeard KD, Meinkoth JH. Ehrlichiosis and polyarthritis in three dogs. J Am Vet Med Assoc 1988;192:1093-1095.

8. Lewis GE, Huxoll DL, Ristic M, Johnson AJ. Experimentally induced infection of dogs, cats and non-human primates with Ehrlichia equi, etiologic agent of equine ehrlichiosis. Am J Vet Res 1975;36:85-88.

9. Ewing SA, Fox JC, Johnson EM, MacAllister CG, Corstvet RE, Baldwin CJ, Mosier DA, Kocan KM, Tyler RD, Cowell RL. Canine ehrlichiosis differences between granulocytic and agranulocytic agents. (abstr) Proceedings 67th Conference of Research Workers in Animal Disease. November 14-15 of 1988.

10. Ewing SA. Canine ehrlichiosis. Adv Vet Sci Comp Med 1969;13:331-353.

11. MacLeod F. Studies in tick borne fever of sheep. Experiments on transmission and distribution of the disease. Parasitology 1936;28:320-329.

12. Lewis GE, Ristic M, Smith RD, Lincoln T, Stephenson EH. The brown dog tick Rhipicephalus sanguineous and the dog as experimental host of Ehrlichia canis. Am J Vet Res 1977;38:1953-1955.

13. Scott GR. Tick borne rickettsial disease of domestic livestock. In: Wilde JKH, ed. Tick borne diseases and their vectors. University of Edinburgh, England. Centre for Tropical Veterinary Medicine, 1978;451-473.

14. Smith RD, Sells DM, Stephenson EH, Ristic M, Huxoll DL. Development of Ehrlichia canis, causative agent of canine ehrlichiosis, in the tick Rhipicephalus sanguineous and its differentiation from a symbiotic rickettsia. Am J Vet Res 1976;37:119-126.

15. Weiss E, Moulder JW. The rickettsias and chlamydias. In: Krieg NR, Holt JG eds. Bergey's manual of systematic bacteriology. Vol 1. Baltimore: The Williams & Wilkins Co,1984;687-739.

16. Palmer JE, Benson CE. Oral transmission of Ehrlichia risticii resulting in Potomac horse fever. Vet Rec 1988;122:635.

17. Schmidtmann ET, Robl MG, Carroll JF. Attempted transmission of Ehrlichia risticii by field captured Dermacentor variabilis (Acari:Ixodidae). Am J Vet Res 1986;47:2393-2395.

18. Hahn NE, Fletcher M, Rice RM, Kocan KM, Hansen JW, Hair JA, Barker RW, Perry BD. The attempted transmission of Ehrlichia risticii, causative agent of Potomac horse fever, by the ticks Dermacentor variabilis, Rhipicephalus sanguineous, Ixodes scapularis and Amblyomma americanum. (In

preparation)

19. Donatien A, Lestoquard F. Existence en Algerie d' une rickettsia du chien. Bull Soc Pathol Exot 1935;28:418-419.

20. Huxoll DL. Canine ehrlichiosis (Tropical canine pancytopenia): a review. Vet Parasitol 1976;2:49-60.

21. Ewing SA, Buckner RG. Manifestations of babesiosis, ehrlichiosis, and combined infections in the dog. Am J Vet Res 1965;26:815-828.

22. Holland CJ, Logan LL, Mebus CA, Ristic M. The serological relationship between Cowdria ruminantium and certain menbers of the genus Ehrlichia. Ondersteport J Vet Res 1987;54:331.

23. Wilkins JH, Bowden RSF, Wilkinson GL. A new canine syndrome. Vet Rec 1967;81:57-58.

24. Huxoll DL, Hildebrant PK, Nims RM, Amyx HL, Ferguson JA. Epizootioloy of tropical canine pancytopenia. J Wildl Dis 1970;6:220-225.

25. Groves MG, Dennis GL, Amyx HL, Huxoll DL. Transmission of Ehrlichia canis to dogs by ticks (Rhipicephalus sanguineous). Am J Vet Res 1975;36:937-940.

26. Ristic M. Pertinent characteristics of leukocytic rickettsiae of humans and animals. Microbiol 1986;11:183-186.

27. Gribble DH. Equine Ehrlichiosis. J Am Vet Med Assoc 1969;157:462-469.

28. Stannard AA, Gribble DH, Smith RS. Equine ehrlichiosis, a disease with similarities to tick-borne fever and bovine petechial fever. Vet Rec 1969;84:149-150.

29. Goddard J. A review of the disease agents harbored and transmitted by the lone star tick, Amblyomma americanum. The Southwest Entomol 1987;12:158-171.

30. Hopla EC. The ecology of tularemia. Adv Vet Sci and Comp Med 1974;18:25-53.

31. Barker RW, Hoch LA, Buckner RG, Hair JA. Hematological changes in white tailed deer fawns infested with Theileria infected lone star ticks. J Parasitol 1973;59:1091-1098.

32. Durham KA, Corstvet RE, Hair JA. Application of fluorescent antibody technique to determine infectivity rates of Amblyomma americanum salivary glands and oral secretions by Theileria cervi. J Parasitol 1976;62:1000-1002.

33. Hoogstral H. Ticks in relation to human disease caused by Rickettsia species. Annu Rev Entomol 1967;12:377-381.

34. Rees CW. The experimental transmission of anaplasmosis by Dermacentor variabilis. Science 1932;75:318-320.

35. Kocan KM, Hair JA, Ewing SA, Stratton LG. Transmission of Anaplasma marginale (Theiler) by Dermacentor andersoni (Stiles) and Dermacentor variabilis (Say). Am J Vet Res 1981;42:15-18.

36. Logan TM, Kocan KM, Edwards W, Hair JA, Claypool PL, Ewing SA. Persistence of colonies of Anaplasma marginale in overwintering Dermacentor variabilis. Am J Vet Res 1987;48:661-663.

37. Smith RD, Levy MG, Kuhlenschmidt MS, Adams JH, Rzechula DL, Hardt TA, Kocan KM. Isolates of Anaplasma marginale not transmitted by ticks. Am J Vet Res 1986;47:127-129.

38. Blouin EF, Kocan AA, Glen BL, Kocan KM. Transmission of Cytauxzoon felis (Kier,1979) from bobcats, Felis rufus (Schreber), to domestic cats by Dermacentor variabilis (Say). J Wildl Dis 1984;20:241-242.

39. Woldehiwet Z. Tick borne fever: a review. Vet Res Comm 1983;6:163-175.

40. Hayat CS, Ewing SA, Buckner RG. A new strain of Ehrlichia canis in Oklahoma. Okla Vet 1972;24:19-21.

41. Hayat CS. Pathogenesis of two isolates of Ehrlichia canis in dogs. Ph.D. Thesis, Oklahoma State University 1972; Stillwater, Oklahoma. 107p.

42. Stahl W, Matsubayashi H, Akao S. Modification of subclinical toxoplasmosis in mice by cortisone, 6-Mercapto-purine and splenectomy. Am J Trop Med & Hyg 1966;15:869-874.

43. Young AS, Cox FEF. The effect of betamethasone on Babesia microti and Babesia rodhani infections on rodents. Parasitology 1971;63:447-453.

44. Kuttler KL, Adams LG. Influence of dexamethasone therapy on Analpasma carrier, splenectomized calves. In: Wilde JKH, ed. Tick borne diseases and their vectors. University of Edinburgh, England. Centre for Tropical Veterinary Medicine, 1978;557-564.

45. French TW, Harvey JW. Serologic diagnosis of infectious cyclic thrombocytopenia in dogs using an indirect fluorescent antibody test. Am J Vet Res 1983;44:2407-2411.

46. Johnson EM, Morton R. Ehrlichia platys infection in two Oklahoma dogs. Okla Vet 1987;39:68-69.

47. Martinod S, Brossard M, Moreau Y. Immunity of dogs against Babesia canis, its vector tick Dermacentor reticulatus, and Ixodes ricinus in endemic area. J Parasitol 1985;7:269-273.

VITA

Oscar Sergio Anziani

Candidate for the Degree of

Master of Science

Thesis: EXPERIMENTAL TRANSMISSION OF A CANINE GRANULOCYTIC FORM OF EHRLICHIA BY TICKS

Major Field: Entomology

Biographical:

Personal Data: Born in Zarate, Argentina, January 20, 1953, the son of Heraldo and Hermelinda Anziani.

Education: Graduated from Colegio Nacional de San Francisco High School (Cordoba) in December 1969; received Medico Veterinario Degree from Universidad Nacional del Litoral, Argentina, September, 1975; completed requirements for the Master of Science degree at Oklahoma State University, Stillwater, Oklahoma, December,1988.

Professional Experience: Fellow of World Health Organization, June through September 1978. Fellow of Food and Agriculture Organization, July to September 1986. Research worker at Instituto Nacional de Tecnologia Agropecuaria, Argentina from 1977.