EXPERIMENTAL TRANSMISSION OF

EHRLICHIA SPP.

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PREFACE

This study dealt primarily with experimental transmission of Ehrlichia canis, an agranulocytic ehrlichial parasite of canids, to dogs and horses through transfusion of rickettsemic canine blood and through replete feeding of experimentally exposed adult Dermacentor variabilis on dogs and horses. The primary objectives were: to determine the susceptibility of horses to E. canis by inoculation with rickettsemic canine whole blood; to determine the vector potential of D. variabilis for E. canis by transstadial transmission to dogs and horses through adult ticks exposed as nymphs; and to demonstrate E. canis in tissues of experimentally exposed adult D. variabilis by light and electron microscopy.

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CHAPTER I

INTRODUCTION

Explanation of the Problem

Two forms of Ehrlichia, an agranulocytic form (AF) and a granulocytic form (GF), infect both dogs and horses in the United States, and are associated with two forms of ehrlichiosis, a severe clinical syndrome (AF) and a mild clinical syndrome (GF), in both host species.[5,6,9,12,14,15,18,21,30,36,44,68,69,78,87,102,108] Recently, reports of human ehrlichial infections in North America, one of which involved both an agranulocytic form and a granulocytic form, have appeared in the literature.[26,71,104,106] An agranulocytic Ehrlichia, E. sennetsu, has also been found in human beings in Japan and Malaysia.[43,91,93] The relationships, taxonomic and etiologic, among these ehrlichiae are unclear.

Ehrlichia canis (AF), the etiologic agent of canine ehrlichiosis, has been shown to cross-react serologically with E. risticii, E. equi, several canine granulocytic ehrlichial isolates, and the human ehrlichial agents.[5,9,26,44,45,71,102,104,106] E. risticii (AF), the etiologic agent of Potomac horse fever, has been shown to cross-react with E. canis, E. sennetsu, and the North

American human agent(s).[44,71] E. equi (GF), the etiologic agent of equine ehrlichiosis, has been shown to cross-react with E. canis and the North American human ehrlichial agent(s), but not with E. risticii.[44,71] E. sennetsu (AF), the etiologic agent of infectious mononucleosis in Japan, has been shown to cross-react with E. canis, E. risticii, and the North American human ehrlichial agent(s).[44,45,71] The North American human ehrlichial agent(s) (AF and GF) cross-reacted with E. canis, E. risticii, E. equi, and E. sennetsu.[71] The antigenic affinities of the canine granulocytic ehrlichial (CGE) agents have not been established fully; however, cross-reactions with E. canis and E. equi have been reported.[5,9,24,68,102]

Attempts to determine the vertebrate host range of E. canis have included studies of blood transmission to monkeys, wild canids, cats, sheep, goats, and laboratory rodents and lagomorphs. Only canids have proved to be susceptible.[2,14,15,20,21,31,33,37,38,48,49,82] E. risticii has been shown to infect mice, non-human primates, cats, and dogs as well as horses.[13,50,94,101] E. equi has a wide host range, infecting horses, burros, dogs, cats, sheep, goats, and non-human primates.[30,64,65] A canine granulocytic ehrlichial isolate was transmitted successfully to horses by inoculation with whole blood.[68] E. sennetsu has been transmitted to monkeys, dogs, and mice.[43,45,50,101] Experimental transmission of E. canis to horses has not been reported, but was attempted in the

present study because: serologic evidence of its antigenic similarity to both equine ehrlichiae exists; both E. equi and E. risticii have been transmitted to dogs; and a California isolate of a canine granulocytic Ehrlichia was transmitted to horses.

Ticks are proven vectors of several ehrlichiae and suspected vectors of others.[91] Transstadial transmission of E. canis to dogs by Rhipicephalus sanguineus, a three host tick which feeds almost exclusively on dogs in the U.S., has been demonstrated.[31,34,39,66,75] No attempts to transmit E. canis by other tick species have been reported; nor has there been any effort to study tick transmission to non-canid vertebrate hosts. Dermacentor variabilis, a three host tick which feeds on dogs, horses, and man as an adult tick, has been associated with natural infections of E. risticii and is a major vector of Rocky Mountain spotted fever to man in the eastern U.S.[34,95] Dermacentor species have also been associated with E. equi infections.[30,65] Transmission of E. canis to dogs and horses by D. variabilis was attempted in the present study because under natural conditions this tick represents a connecting link between these hosts and ehrlichial organisms. It may also be involved in the transmission of ehrlichial agents to man in North America.

Purpose of the Study

The primary purposes of the study were: to determine the susceptibility of horses to E. canis by inoculation with rickettsemic canine whole blood; to determine the vector potential of D. variabilis for E. canis by transstadial transmission to dogs and horses through adult ticks exposed as nymphs; and to demonstrate E. canis in tissues from adult D. variabilis exposed as nymphs by electron microscopy. Secondary findings have been included related to: 1) the incidental discovery of an ehrlichial organism, E. platys, in the platelets of naturally infected dogs, and 2) preliminary findings related to attempted blood transmission of an Oklahoma isolate of a canine granulocytic Ehrlichia species to a horse and to mice.

CHAPTER II

LITERATURE REVIEW

Ehrlichia canis is a small pleomorphic rickettsial organism found in the cytoplasm of monocytes and lymphocytes of canids worldwide.[7,14,17-19,21,41,47,48,52,76,99] Attempts at experimental transmission to laboratory animals have been unsuccessful except for an unsubstantiated report of transmission to monkeys.[2,14,20,21,33,37,38,49,82] The disease it produces, canine ehrlichiosis, consists of three phases: an acute phase, a subclinical phase, and a chronic carrier phase. The disease has been characterized by fever, progressive pancytopenia, and hypergammaglobulinemia.[6-8,10,14,18,19,46-49,73,107]

Multiple strains of E. canis were thought to exist because organisms morphologically similar to those found in agranulocytes were observed in granulocytes of naturally infected dogs.[22,36] Experimentally, the clinical syndrome produced by the canine granulocytic isolate was milder than that produced by E. canis.[22,35,36,74] Convalescing dogs were not protected from E. canis when challenged 81 days after exposure to the canine granulocytic isolate.[74] Canine granulocytic ehrlichial infections have been reported from dogs in Arkansas, Oklahoma, Tennessee,

Missouri, and California.[5,9,12,22,24,36,68,102] Several of these isolates have been associated with development of polyarthritis in naturally infected dogs.[5,12,102] The California isolate was transmitted from a dog to horses and produced disease typical of equine ehrlichiosis.[68]

Ehrlichia equi occurs in granulocytes of horses and causes equine ehrlichiosis, a seasonal disease characterized by fever, leucopenia, thrombocytopenia, limb edema, and ataxia.[30,65,68,97] E. equi has been experimentally transmitted to dogs, cats, sheep, goats, burros, and non-human primates.[30,64] It has been diagnosed in horses from California, Colorado, Illinois, Florida, Washington, and New Jersey.[69] The disease produced in dogs was similar to that described for canine granulocytic ehrlichial infections.[64] E. equi-infected dogs challenged with E. canis were not protected from infection. It has been suggested that the canine granulocytic isolates may actually be E. equi.[65,68] Serological tests, specifically the indirect fluorescent antibody (IFA) tests, for detection of antibodies to Ehrlichia species were not readily available when experimental studies with E. equi and an Oklahoma CGE were being conducted.[22,30,64,77,89,90,92]

In recent years, serologic evidence regarding canine granulocytic ehrlichial infections has been inconsistent. Two naturally infected California dogs were serologically negative for E. canis.[68] Subinoculated horses became infected with the canine granulocytic isolate and developed

clinical symptoms typical of equine ehrlichiosis. Several Missouri dogs naturally infected with a granulocytic Ehrlichia were serologically positive for E. canis and negative for E. equi by the IFA tests.[102] Oklahoma and Tennessee dogs infected with a granulocytic Ehrlichia also seroconverted to E. canis.[4,24] The IFA tests were considered to be sensitive and specific.[89,90] However, investigations of the etiology of Potomac horse fever demonstrated that serologic cross-reactions occur between Ehrlichia species.[44,92] Cross-reactions between E. equi and E. canis with Cowdria ruminantium, another member of the tribe Ehrlichieae which infects cattle and causes heartwater disease, have also been demonstrated.[67] Attempts to transmit all of the different geographic isolates of canine granulocytic ehrlichiae occurring in the U.S.A. to horses have not been made.

Ehrlichia risticii, a rickettsial organism demonstrated by electron microscopy and cell culture techniques to parasitize blood monocytes as well as macrophages and epithelial cells of the large colon of horses with Potomac horse fever, has been propagated successfully in murine macrophage, human histiocyte, and canine monocyte cell cultures.[15,44,87,88] Potomac horse fever occurs seasonally and is characterized by anorexia, depression, fever, leucopenia, profuse diarrhea, dehydration, and occasionally laminitis; and it has a 30% mortality rate.[16,108,110] The disease has been produced in

susceptible horses by intravenous inoculation with infective whole blood and by intravenous and subcutaneous inoculation with infective cell culture materials.[15,44,80,87,87] Mice, monkeys, dogs, and cats have been infected with E. risticii.[13,50,94,101] Results of a serologic survey indicated that the infection is not limited to the eastern United States as originally thought.[92] Subsequent studies of experimental infections in horses indicated that subclinical infections occur and detectable antibody levels persist for an extended period.[16,81,110]

The first case of a human ehrlichial infection in the United States was reported in 1986.[71] The disease was similar to Rocky Mountain spotted fever (RMSF); however, there was no rash nor seroconversion in acute and convalescent serum samples to Rickettsia rickettsii. Morulae were detected in peripheral lymphocytes, neutrophils, and monocytes by light and electron microscopy. Serum samples demonstrated a declining antibody titer to E. canis. The investigators concluded that this represented a human infection with E. canis. Other investigators questioned this conclusion because: evidence indicating that E. canis can be transmitted to primates is inconclusive; the only known vector of E. canis is R. sanguineus, a three host tick which in the U.S.A. feeds almost exclusively on dogs; E. canis is reported to parasitize agranulocytes; no attempt was made to transmit the human agent(s) to dogs; and the species of

tick that fed on the infected man was not determined.[23] Subsequent reports indicated that the prevalence of human ehrlichial infections may be as common as RMSF in the southcentral and southeastern United States based on serologic evidence of rising or declining antibody levels to E. canis in people with symptoms similar to RMSF.[26,104,106] Most cases have included a history of tick bite 1 to 3 weeks prior to the onset of clinical signs.

The seasonal occurrence of Potomac horse fever, equine ehrlichiosis, and human ehrlichial infections suggest that hematophagous arthropods are vectors of E. risticii, E. equi, and the human ehrlichial agent(s).[26,30,106,108] D. variabilis was suspected to be a vector of E. risticii because adults were frequently found on horses at farms where Potomac horse fever was endemic.[96,108] Dermacentor species were also associated with horses naturally infected with E. equi.[30,65] However, attempts to transmit E. risticii with field-captured D. variabilis from endemic farms and with experimentally exposed laboratory-reared D. variabilis and Ctenocephalides felis failed.[95,96] Attempts to transmit E. equi with experimentally exposed D. occidentalis also failed.[30,65]

The seasonal pattern of human ehrlichial infections has been similar to that of RMSF.[106] The known important vectors of Rocky Mountain spotted fever in the United States are D. andersoni, D. variabilis, and Amblyomma americanum.[34] Dermacentor variabilis and A. americanum

should be suspected as vectors of the human ehrlichial agent(s) because the majority of cases occurred in the southeastern and southcentral United States where these tick species readily feed on man in one or more stages of their life cycle and are considered to be important vectors of RMSF.

Transstadial transmission of E. canis to dogs by R. sanguineus, the tick associated with natural outbreaks of canine ehrlichiosis, has been demonstrated.[14,31,66] Development of E. canis in hemocytes, midgut epithelium, and salivary glands of adult R. sanguineus was demonstrated by immunofluorescence and electron microscopy.[100] Transmission of E. canis by adult R. sanguineus occurred only when the ticks engorged as larva or nymphs on a rickettsemic dog.[31,66] Attempts to transmit E. canis with adult ticks which fed to repletion as nymphs on chronic E. canis carriers were unsuccessful.[66]

Infectious cyclic thrombocytopenia of dogs is caused by a small rickettsial organism, tentatively referred to as E. platys, which resides in platelets.[32] This disease has been characterized by cyclic episodes of parasitemia and thrombocytopenia at 8- to 15-day intervals. Initial parasitemias in experimentally infected dogs were the greatest and were followed in 2 to 3 days by a precipitous decline in the number of circulating platelets. Subsequent parasitemias were of a much lower magnitude, but thrombocytopenias which lasted for 2 to 3 days were as

severe as that of the initial cycle. Eventually, the disease became recognized by a slowly resolving thrombocytopenia with only sporadic presence of the organism. An IFA test was developed for the detection of antibodies to E. platys.[27] The infection has been recognized in dogs from Florida, Louisiana, and Oklahoma.[28,32,51] However, serologic evidence indicated that the distribution of E. platys is broader since serum samples from dogs in 10 states of the U.S. were positive for antibodies to the organism.[27] Pathologic findings in experimentally infected dogs consisted of lymphoreticular hyperplasia of lymph nodes and splenic follicles and lymphocytic infiltrations of the liver, spleen, kidney, and bone marrow.[4] These findings were similar to those seen with E. canis infections but were of a milder nature.[42,83,84] The host range for E. platys has not been established. It is also not known whether a carrier state exists or if protective immunity develops. A hematophagous arthropod is suspected to be the vector of this agent to dogs because it is a rickettsial organism and reports of natural infections have included a history of tick infestation prior to detection of the disease.[28,32,51]

CHAPTER III

MATERIALS AND METHODS

General Experimental Methods

These studies consisted of primary experiments and secondary ones as stated in Chapter I under the Purpose of the Study. The primary experiments were conducted in two trials. The dogs used in Trial 1 were not treated with an insecticide prior to initiation of the experiments and were not sedated before placement in tick attachment boxes. Intravenous blood transmission of E. canis to ponies was attempted only during Trial 1. Intraperitoneal blood transmission of E. canis to a horse was attempted only during Trial 2. The dogs used in Trial 2 were dipped with an organophosphate insecticide two weeks prior to initiation of the experiments to remove ectoparasites. Dogs in Trial 2 also were sedated with a mixture of ketamine hydrochloride and xylazine given intramuscularly fifteen minutes before being placed in the tick attachment boxes.

In Trial 1, tick transmission to dogs was attempted by allowing adult D. variabilis exposed as nymphs to feed to repletion on susceptible dogs. In Trial 2, the same approach was used and tick transmission was also attempted

through intravenous inoculation of dogs with a mixture of salivary gland and midgut from unfed, adult D. variabilis and incubated, unfed, adult D. variabilis which had been exposed as nymphs. All other aspects of the experimental methods and design were the same for both trials.

Animals

Eighteen adult mix-breed dogs, nine yearling ponies, one adult quarter horse, and three male white mice were acquired from and maintained by the Laboratory Animal Resource Unit (LARU) of Oklahoma State University. They were vaccinated for infectious diseases and allowed a one-week acclimation period prior to the start of the experiments.

Dogs were confined in individual, fenced runs with concrete floors in a temperature controlled building. Ponies were housed in a temperature controlled building with concrete-floored stalls and bedded with straw. The mice were housed in individual isolation cages in the LARU rodent rooms. The animals were fed and watered daily. The cages, runs, and stalls were cleaned daily.

Agents

Trial 1 primary experiments were conducted with an agranulocytic isolate of Ehrlichia canis from Louisiana. Trial 2 primary experiments were conducted with an agranulocytic isolate of E. canis acquired from tick

transmission experiments in Trial 1. Secondary experiments were conducted with an Oklahoma isolate of E. platys recovered from research dogs acquired for use in the primary experiments and an Oklahoma isolate of a canine granulocytic Ehrlichia recovered from a dog with polyarthritis.

Infection of *Dermacentor variabilis* Nymphal Ticks

Dermacentor variabilis nymphs, reared and maintained at the Oklahoma State University Department of Entomology Tick Laboratory, were allowed to engorge until replete (6 days) on rickettsemic dogs infected with E. canis.[79] The dogs and nymphal ticks were placed in an enclosed, ventilated, wooden box for 6 to 12 hours to facilitate tick attachment. The tick infested dogs were maintained in separate metabolism cages to facilitate recovery of replete nymphs. The engorged nymphs were kept in humidity chambers and allowed to molt (60 days). Exposed adult ticks and associated controls were used in tick transmission studies and histologic studies. The procedure described above was also used for the attachment and feeding of adult ticks on dogs. Feeding of adult ticks on ponies was facilitated by orthopedic stockinettes glued to the shaven skin.

Collection of Tick Tissues for Light and Electron Microscopy

Salivary gland, midgut, and gonad dissected from unfed and engorging adult D. variabilis (infected and controls) were placed in 0.2M cacodylate-buffered glutaraldehyde, post-fixed in cacodylate-buffered 2% osmium tetroxide, and processed for electron microscopy according to the procedure of Kocan et al.[54] Thick sections (1um) were stained with Mallory's stain and examined by light microscopy.[86] Thin sections were cut with a diamond knife, placed on 300 mesh copper grids, stained with uranyl acetate and lead citrate,[105] and examined and photographed in a JEOL-100 CXII electron microscope.

Indirect Fluorescent Antibody Testing of Animal Sera for *Ehrlichia canis*, *Ehrlichia risticii*, *Ehrlichia equi*, *Ehrlichia platys*, and *Rickettsia rickettsii*

Indirect fluorescent antibody (IFA) tests were conducted for the detection of antibodies to E. canis, E. risticii, E. equi, and R. rickettsii in sera of experimental animals prior to the onset of the experiments and again at the end of a 30-day observation period following intravenous inoculation with whole blood from E. canis-infected dogs or E. canis-exposed ticks, or after exposed ticks fed to repletion. The IFA tests were

conducted by the Oklahoma Animal Disease Diagonostic Laboratory according to the procedure of Ristic et al.[89,90,92]

Serum samples of dogs naturally or experimentally infected with E. platys were collected at the time of initial discovery of E. platys in the blood or prior to exposure and again 10 to 14 days after discovery or exposure. The serum samples were tested for antibodies to E. platys with an IFA test conducted according to the procedure of French et al.[27]

Primary Experiments

Transmission of *Ehrlichia canis* to Dogs by Intravenous Inoculation with Rickettsemic Canine Whole Blood

Dogs 2, 3, and 0924 were inoculated intravenously with 10 milliliters (mls) of whole blood from an E. canis carrier. Dogs 1 and 0925 served as controls. These dogs were monitored daily for infection by 3 methods: microscopic examination of Romanovsky-stained blood films and buffy coat smears, and rectal temperature. They were monitored weekly by determination of packed cell volumes (PCV), white blood cell counts (WBC), and platelet counts. When characteristic morulae were detected, 100 mls of whole blood was collected in an anticoagulant from dogs 2 and 3 for subinoculation of ponies 1, 2, 3, and 4. Dogs 2 and 0924 were also used to expose nymphal D. variabilis to E.

canis by allowing them to feed to repletion on these dogs when E. canis morulae were detected in the dogs' blood. Associated control ticks were fed on the control dogs.

Attempted Transmission of *Ehrlichia canis* to Horses by Intravenous or Intraperitoneal Inoculation with Rickettsemic Canine Whole Blood

Ponies 1, 2, 3, and 4 were inoculated intravenously with 50 mls of the rickettsemic canine blood acquired from dogs 2 and 3. Pony 9 was inoculated intraperitoneally with 50 mls of blood from an E. canis carrier (dog 4). The ponies were monitored daily for infection by: examination of Romanovsky-stained blood films and buffy coat smears, and determination of PCV, WBC, platelet counts, and rectal temperature for 21 to 30 days.

Attempted Transmission of *Ehrlichia canis* to Dogs by Adult *Dermacentor variabilis* Exposed as Nymphs

Twenty-five pairs of adult D. variabilis exposed as nymphs on dogs 2 and 0924 were fed to repletion on each of four dogs (4, 0962, 0926, and 0984). Associated control ticks were fed on each of two dogs (7 and 0983). All dogs were monitored for ehrlichial infection as previously described for 30 days. Dog 0984 was challenged with 10 mls of whole blood from an E. canis carrier (dog 4) 21 days

after the tick transmission experiment was completed. After challenge, dog 0984 was monitored for infection by daily examination of blood and buffy coat smears, and was subjected to necropsy examination on day 12 post-challenge.

Attempted Transmission of *Ehrlichia canis* to Dogs by Intravenous Inoculation with Tissues from Unfed, Adult *Dermacentor variabilis* Exposed as Nymphs

Dog 0925 was inoculated intravenously with a suspension of salivary gland and midgut collected from 8 pairs of unfed, adult D. variabilis exposed as nymphs. Dog 0933 was inoculated with associated control tick tissues. Dog 0999 was inoculated intravenously with a suspension of salivary gland and midgut collected from 8 pairs of unfed, adult D. variabilis exposed as nymphs which had been incubated at 37°C for 2.5 days according to the procedure of Kocan et al.[56] Dog 0961 was inoculated with associated control tick tissues. All the dogs were monitored for infection as previously described for 30 days. Dog 0999 was challenged with 8 mls of whole blood from an E. canis carrier (dog 4) after the tick transmission experiment was completed. Dog 0999 was then monitored daily for infection by examination of Romanovsky-stained blood and buffy coat smears. When morulae were first observed in monocytic cells, a mixture of ketamine hydrochloride and xylazine was administered intramuscularly

to mimic the administration of these anesthetic agents to dogs of Trial 2 before placement in tick attachment boxes. Dog 0999 was then monitored for an additional three days. Before euthanasia and necropsy examination, 10 mls of whole blood was collected from dog 0999 and inoculated intravenously into dog 1247 to determine if the anesthetic agents had an effect on the transmissibility of E. canis. Dog 1247 was then monitored for infection with E. canis by daily examination of blood and buffy coat smears.

Attempted Transmission of *Ehrlichia canis* to Horses by Adult *Dermacentor variabilis* Exposed as Nymphs

Twenty-five pairs of adult D. variabilis exposed as nymphs on dogs 2 and 0924 were fed to repletion on each of three ponies (6, 7 and 8). Associated control ticks were fed on pony 5. The ponies were monitored for infection as previously described.

Light and Electron Microscopic Examination of Selected Tissues of Adult *Dermacentor variabilis* Exposed as Nymphs

During Trial 1, tissues were collected from 5 unfed, adult D. variabilis exposed as nymphs on dog 2 and from 5 associated controls fed on dog 1 as nymphs on each of days 30, 35, and 40 post-repletion for histological studies.

Histological studies of engorging adult ticks were conducted by feeding 50 pairs of adult D. variabilis exposed as nymphs on dog 8. Associated controls were fed on dog 7. One pair of adult ticks per dog were collected daily during engorgement (9 days). Dissected tick tissues were fixed, processed, and examined by light and electron microscopy for inclusions of E. canis. These dogs were monitored for ehrlichial infection as previously described.

During Trial 2, tissues were collected for histological studies from 5 pairs of unfed, adult D. variabilis exposed as nymphs on dog 0924 and 5 pairs of associated controls fed as nymphs on dog 0925 on day 35 post-repletion. Histological studies of engorging adult D. variabilis exposed as nymphs were conducted by feeding 50 pairs of adult D. variabilis on one sheep. Associated controls were fed on another sheep. Five pairs of ticks per sheep were collected daily during engorgement. Dissected tissues were fixed and processed for microscopic examination as described previously.

Experiments Conducted with a Rickettsial Organism, Ehrlichia platys, Recovered from Dogs Acquired for Research Purposes

These experiments were conducted with an isolate of E. platys recovered from two sibling dogs (5 and 6) acquired for use in the primary experiments.[51] These two dogs were

rejected for use in the primary experiments because of patent infections with E. platys. They were monitored as previously described for periods of 17 and 43 days.

Transmission of *Ehrlichia platys* to a Dog by Intravenous Inoculation with Rickettsemic Canine Whole Blood

Dog 7 was inoculated intravenously with ten mls of whole blood collected from dog 6 forty-three days after E. platys was first detected. Dog 7 was monitored daily for infection for 23 days by determination of PCV, WBC, rectal temperature, platelet count, and examination of Romanovsky-stained blood and buffy coat smears. Indirect flourescent antibody tests were conducted for detection of antibodies to E. canis and E. platys prior to inoculation and on post-infection day 22.

Attempted Transmission of *Ehrlichia platys* to a Dog by Intravenous Inoculation with Whole Blood from a Dog One Year after Experimental Infection

Dog 0983 was inoculated intravenously with ten mls of whole blood collected from dog 7 one year after experimental infection with E. platys. Dog 0983 was monitored for infection for 30 days by daily examination of Romanovsky-stained blood and buffy coat smears. The dog

was then challenged with ten mls of rickettsemic blood from another dog (1221) with a patent infection of E. platys. Dog 0983 was then monitored an additional 11 days.

Challenge Exposure of a Dog Recovering from an *Ehrlichia platys* Infection with *Ehrlichia canis*

Dog 0999, which was discovered to be naturally infected with E. platys at the time of procurement, was inoculated intravenously with 8 mls of whole blood from an E. canis carrier (dog 4) 60 days after the E. platys infection was detected. The dog was monitored for infection with E. platys and E. canis for 17 days by daily examination of Romanovsky-stained blood and buffy coat smears.

Attempted Simultaneous Transmission of *Ehrlichia platys* and *Ehrlichia canis* to a Dog by Intravenous Inoculation with Whole Blood from a Dog Previously Infected with Both Agents

Dog 1247 was inoculated intravenously with 10 mls of whole blood collected from dog 0999 seventy-seven days after it was found to be infected with E. platys and 17 days after it was challenged with E. canis. This dog was monitored daily for infection with both agents by

examination of Romanovsky-stained blood and buffy coat smears for 11 days.

Experiments Conducted with a Granulocytic *Ehrlichia* Species of Canine Origin

Attempted Transmission of a Granulocytic *Ehrlichia* Species of Canine Origin to White Mice by Intraperitoneal Inoculation with Rickettsemic Canine Whole Blood

Two male white mice were inoculated intraperitoneally with 1 ml of rickettsemic whole blood from a dog infected with a granulocytic *Ehrlichia*. A third male mouse served as a control. The 3 mice were monitored for infection by examination of Romanovsky-stained blood smears prepared every other day for 30 days and by observation of their general condition and behavior.

Attempted Transmission of a Granulocytic *Ehrlichia* Species of Canine Origin to a Horse by Intraperitoneal Inoculation with Rickettsemic Canine Whole Blood

An adult quarter horse (horse 10) was inoculated intraperitoneally with 50 mls of rickettsemic canine whole blood collected from a dog experimentally infected with a granulocytic *Ehrlichia* of canine origin. The horse was

monitored for infection by daily examination of body temperature, Romanovsky-stained blood and buffy coat smears, and PCV for 21 days. The horse was also monitored by weekly determinations of WBC and platelet counts.

CHAPTER IV

RESULTS

Transmission of *Ehrlichia canis* to Dogs by Intravenous Inoculation of Rickettsemic Canine Whole Blood

Attempts to infect dogs 2, 3, 0924, 0984, 0999, and 1247 with whole blood from an *E. canis* carrier were successful. Incubation periods were 10, 11, 15, 12, 14, and 8 days, respectively. The infections of dogs 2, 3, and 0924 were characterized by elevations of rectal body temperature between 40.0°C (104.0°F) and 41.1°C (106.0°F) one to two days prior to detection of *E. canis* morulae in monocytic cells of the peripheral blood or on the day of detection. Platelet counts dropped from pre-exposure values ranging between 192.0 x 10^3/µl to 597.0 x 10^3/µl to values ranging between 29.0 x 10^3/µl to 85.0 x 10^3/µl four days prior to detection of morulae or on the day of detection. Total white blood cell count values decreased from pre-exposure levels ranging between 12,540/µl and 18,370/µl to levels ranging between 5,170/µl and 9,570/µl on the day morulae were detected. Packed cell volume values progressively decreased from pre-exposure levels but

remained in the accepted normal range. Daily body temperature and weekly PCV, WBC, and platelet count values of dogs 2, 3, and 0924 are listed in Tables I, II, and III, respectively, pages 99, 100, and 101 of the Appendix. Daily body temperature and weekly PCV, WBC, and platelet count values of the control dogs (1 and 0925) are listed in Tables IV and V, respectively, pages 102 and 103 of the Appendix. Erythrophagocytosis and vacuolization of the cytoplasm of monocytic cells were evident in buffy coat smears 2 to 3 days prior to detection of morulae. The principal clinical signs evident in these dogs were mucopurulent ocular discharge, lymphadenopathy, and malaise.

Gross necropsy findings in dogs 0984 and 0999 consisted of generalized lymphadenopathy and enlargement of the spleen. Histopathologic findings consisted of lymphoid hyperplasia and lymphoplasmocytic infiltration of tissues of the brain, lung, liver, kidney, spleen, and heart.

Attempted Transmission of *Ehrlichia canis* to Horses by Intravenous or Intraperitoneal Inoculation of Rickettsemic Canine Whole Blood

Attempts to infect ponies 1, 2, 3, 4, and 9 with blood from dogs with acute or chronic infections of *E. canis* were unsuccessful. Daily PCV, WBC, platelet count, and body

temperature values of ponies 1, 2, 3, 4,and 9 remained within the normal ranges. Daily PCV, WBC, platelet count and rectal temperature values of ponies 1, 2, 3, and 4 are listed in Tables VI, VII, VIII, and IX, respectively, pages 104, 105, 106, and 107 of the Appendix. Daily body temperature, PCV, WBC and platelet count values of horse 9 are listed in Table X, page 108 of the Appendix. Morulae were not detected in peripheral blood cells on blood or buffy coat smears prepared daily during the 30-day observation period.

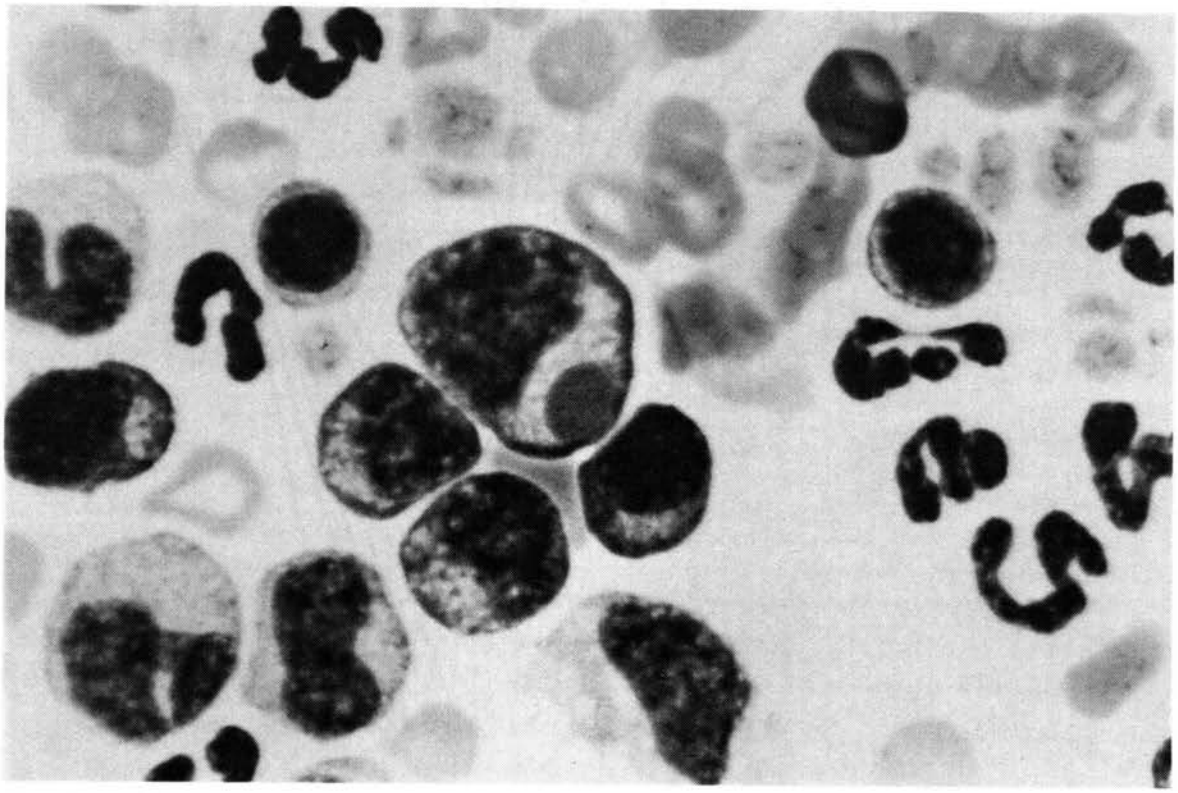
Attempted Transmission of *Ehrlichia canis* to Dogs by Adult *Dermacentor variabilis* Exposed as Nymphs

Seven pairs of *D. variabilis* among each of 25 pairs placed on dogs 7 and 8 of Trial 1 attached and fed. One pair of ticks was removed daily from each dog for histologic studies. Dog 7 served as the tick transmission control and as the control for histologic studies because control ticks were in short supply. Dog 8 was infested with exposed adult ticks that were used for histologic studies; but was also monitored for infection because of a shortage of exposed adult ticks. In the 30-day observation period there was no evidence of transstadial transmission in either dog 7 or dog 8. Morulae of *E. canis* were not detected in monocytic cells on blood or buffy coat smears of the peripheral blood. Body temperature, PCV, WBC and

platelet count values remained within the normal ranges or at pre-exposure values. Daily body temperature and weekly PCV, WBC, and platelet count values of dogs 7 and 8 are listed in Tables XI and XII, respectively, pages 109 and 110 of the Appendix.

Twenty-five adult D. variabilis exposed as nymphs, 14 females and 11 males, fed to repletion on dog 4 of Trial 1 within 14 days. A total of 4 feeding male R. sanguineus were recovered from dog 4 on days 5, 6, and 8 of tick feeding; and one replete female R. sanguineus was recovered on the third day that experimental ticks fed. Morulae (Figure 1, page 30) of E. canis were detected in monocytic cells of the peripheral blood 17 days after attachment of the exposed adult D. variabilis. Morulae persisted for the 8 remaining days that the dog was monitored. The body temperature rose to 39.8°C (103.6°F) on day 17 after tick attachment and peaked at 40.7°C (105.2°F) on day 20. The WBC and platelet counts dropped from initial values of 20,350/µl and 344.0 x 10^3/µl, respectively, to low levels of 9,130/µl and 31.0 x 10^3/µl by day 21 after attachment of experimental ticks. Erythrophagocytosis and vacuolization of the cytoplasm of monocytic cells was evident on day 15 after attachment of experimental ticks. Values for PCV remained at pre-exposure levels. Daily body temperature, PCV, WBC, and platelet count values of dog 4 are listed in Table XIII, page 111 in the Appendix.

Figure 1. Photomicrograph of Ehrlichia canis Morula in a Monocytic Cell of Dog 4, Buffy Coat Smear. X 4,000.



Thirty replete adult D. variabilis exposed as nymphs, 21 females and 9 males, were recovered from dog 0962, Trial 2, in 11 days of tick feeding. Thirty-three replete adult D. variabilis exposed as nymphs, 20 females and 13 males, were recovered from dog 0926, Trial 2, in 15 days of tick feeding. Twenty-four replete adult D. variabilis exposed as nymphs, 18 females and 6 males, were recovered from dog 0984, Trial 2 in 10 days of tick feeding. During the 30-day observation period there was no evidence of transmission of E. canis to any of these dogs. Daily rectal temperature and weekly PCV, WBC, and platelet count values remained near the pre-exposure levels. Daily body temperature and weekly PCV, WBC, and platelet count values of dogs 0962, 0926, and 0984 are listed in Tables XIV, XV, and XVI, respectively, pages 112, 113, and 114 of the Appendix. Morulae of E. canis were detected in monocytic cells of the peripheral blood on buffy coat smears of dog 0984 twelve days after challenge with blood from an E. canis carrier.

Eighteen replete non-exposed adult D. variabilis, 12 females and 6 males, were recovered from dog 0983 in 10 days of tick feeding. Throughout the 30-day observation period, no remarkable changes were observed in this dog. Daily rectal temperature and weekly PCV, WBC, and platelet count values of dog 0983 are listed in Table XVII, page 115 of the Appendix.

Attempted Transmission of *Ehrlichia canis* to Dogs by Intravenous Inoculation with Tissues from Unfed, Adult *Dermacentor variabilis* Exposed as Nymphs

Attempts to transmit *E. canis* to dogs of Trial 2 by intravenous inoculation of tick tissues from unfed, adult *D. variabilis* or incubated, unfed, adult *D. variabilis* exposed as nymphs were unsuccessful. No remarkable changes were observed in the principals (dogs 0925 and 0999) or control dogs (0933 and 0961). Morulae of *E. canis* were not detected on blood or buffy coat smears of peripheral blood from these dogs during the 30-day observation period. Daily body temperature and weekly PCV, WBC, and platelet count values of dogs 0925, 0999, 0933, and 0961 remained at pre-exposure values. Data for these dogs are listed in Tables XVIII, XIX, XX, and XXI, respectively, on pages 116, 117, 118, and 119 of the Appendix.

Dog 0999 was challenged with whole blood from an *E. canis* carrier (dog 4) 30 days after completion of the experiment involving injection of tick tissues. Morulae of *E. canis* were detected in monocytic cells of the peripheral blood on day 14 post-challenge. At this time, a combination of ketamine hydrochloride and xylazine was administered intramuscularly to dog 0999 to see if these agents could have an effect on the parasitemia and transmissibility of *E. canis*. Morulae were detected for three consecutive days after the administration of the

anesthetic agents in monocytic cells of the peripheral blood. On day 17 post-challenge, 3 days after ketamine and xylazine were administered, 10 mls of blood were collected from dog 0999 and inoculated intravenously into dog 1247. Dog 0999 was then subjected to necropsy examination and numerous morulae were found in lung impression smears. Morulae were also detected in monocytic cells of the peripheral blood of dog 1247 eight days after it was subinoculated with blood from dog 0999.

Attempted Transmission of *Ehrlichia canis* to Horses by Adult *Dermacentor variabilis* Exposed as Nymphs

Attempts to transmit *E. canis* to ponies 6, 7, and 8 by allowing adult *D. variabilis* exposed as nymphs to feed to repletion on them were unsuccessful. No remarkable changes were observed in the control pony (5) or ponies 6, 7, and 8. Morulae of *E. canis* were not detected on blood or buffy coat smears of peripheral blood, and daily body temperature, PCV, WBC, and platelet count values remained in the normal ranges during the 30-day observation period. These daily values for ponies 5, 6, 7, and 8 are listed in Tables XXII, XXIII, XXIV, and XXV, respectively, pages 120, 121, 122, and 123 of the Appendix.

Four engorged, non-exposed, female *D. variabilis* and 6 males were recovered from the capsules placed on pony 5. Fourteen engorged females and 11 males exposed as nymphs

were recovered from pony 6. Twenty-five engorged females and 25 males exposed as nymphs were recovered from both horse 7 and horse 8.

Indirect Fluorescent Antibody Titers

Antibodies to E. canis, E. equi, and E. risticii were not detected by the IFA tests in pre-exposure serum samples of dogs 1, 2, 3, 4, 5, 6, 7, and 8 of Trial 1. Antibodies to R. rickettsii were detected in pre-exposure serum samples of dogs 4, 7, and 8. Rising antibody titers to E. canis were detected in dogs 2, 3, and 4 thirty days after exposure to infective blood or exposed adult D. variabilis. Two-fold decreases in antibody titers to R. rickettsii were observed in dogs 4 and 7, respectively, thirty days after attachment of exposed and control adult D. variabilis. The antibody titer to R. rickettsii in the serum sample of dog 8 collected 30 days after attachment of adult D. variabilis exposed as nymphs remained unchanged.

Antibodies to E. canis and E. risticii were not detected by the IFA tests in pre-exposure serum samples of dogs 0924, 0925, 0933, 0926, 0962, 0983, 0984, 0961, 0999,and 1247 of Trial 2. Antibodies to E. equi were detected in the pre-exposure serum sample of dog 0999. Antibodies to R. rickettsii were detected in the pre-exposure serum samples of dogs 0933, 0961, and 0999. A rising antibody titer to E. canis was observed in the serum sample of dog 0924 thirty days after exposure to infective

blood. A two-fold increase in antibody titer to E. equi was observed in the serum sample of dog 0999 thirty days after inoculation with incubated, unfed, exposed, adult D. variabilis tissues. Post-exposure serum antibody titers to R. rickettsii for dogs 0933, 0961, and 0999 remained unchanged. Pre-exposure and post-exposure IFA test titers for dogs of Trial 1 and Trial 2 are listed in Table XXVI, page 124 of the Appendix.

Antibodies to E. canis, E. equi, E. risticii, and R. rickettsii were not detected in either the pre-exposure or post-exposure serum samples of horses 1, 2, 3, 4, 5, and 6 of Trial 1. Antibodies to E. canis, E. equi, and E. risticii were not detected in either the pre-exposure or the post-exposure serum samples of horses 7, 8, and 9 of Trial 2. Antibody titers to R. rickettsii were observed in pre-exposure serum samples of horses 7, 8, and 9. The post-exposure antibody titers to R. rickettsii for horses 7, 8, and 9 remained unchanged. Pre-exposure and post-exposure IFA test titers for horses of Trial 1 and Trial 2 are listed in Table XXVII, page 125 of the Appendix.

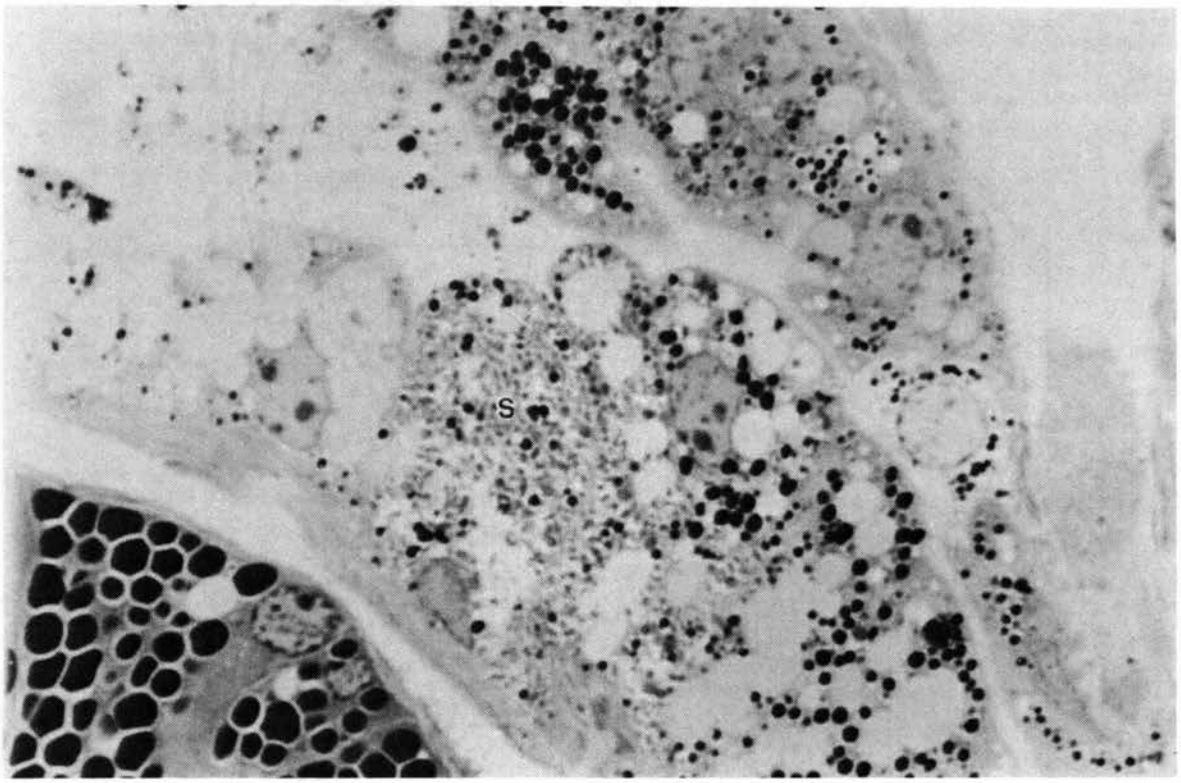
Antibodies to E. platys were detected in the serum sample collected from dog 5 during the preliminary examination. Rising antibody titers were observed in dogs 5 and 6 seventeen days after initial examination. Serum antibodies to E. canis were not detected in either of these dogs. Serum antibodies to E. canis and E. platys were not detected in the pre-exposure serum samples of dogs 7, 0983,

and 1247. A rising titer to E. platys was observed in the serum sample of dog 7 twenty-three days after intravenous inoculation with blood from dog 6. Antibodies to E. platys were not detected in serum of dog 0983 collected 30 days after exposure to blood from dog 7 which had been experimentally infected with E. platys 1 year previously. After challenge of dog 0983 with blood from a dog (1221) acquired for research purposes with a patent infection of E. platys, the antibody titer for dog 0983 then rose. Antibodies to E. platys were detected in both the pre-exposure and post-exposure serum samples collected from dog 0999. The post-exposure antibody titer to E. platys for dog 1247 was not determined.

Light Microscopic Findings in Selected Tissues of Adult Dermacentor variabilis Exposed as Nymphs

Colonies resembling those seen in tick tissues infected with Anaplasma marginale or Cowdria ruminantium were not detected in thick sections of midgut or salivary gland of adult D. variabilis exposed to E. canis as nymphs. Colonies were not observed in sections of tick tissues collected on days 30, 35, and 40 after replete feeding as nymphal ticks and during days 1 through 7 of engorgement as adults.[57,59-62] Clusters of rickettsia-like organisms were observed in sections of midgut of both control and exposed adult ticks (Figure 2, page 38).

Figure 2. Photomicrograph of Clusters of Symbiotic Rickettsial Organisms (S) in the Midgut of Adult Dermacentor variabilis. X 4,950.


S

Flagellated organisms were observed in one block of control female tick tissues and in several blocks of tissues from female and male ticks which were exposed as nymphs. The flagellates were found in the midgut (Figure 3, page 41) and in the seminal vesicle (Figure 4, page 43) of ticks.

Electron Microscopic Findings in Selected Tissues of Adult *Dermacentor variabilis* Exposed as Nymphs

Selected blocks of male and female tick tissues collected 35 days after repletion as nymphs and on days 1, 2, and 7 of feeding as adults, which were observed by light microscopy to contain flagellates, were thin sectioned and stained for examination with the electron microscope. The flagellates (Figure 5, page 45) were observed to have a membrane-bound leaflike body with a vesicular nucleus (N). Subpellicular microtubules (MT) were present beneath the outer membrane. A single free flagellum (F) arising from a kinetosome (BB) was observed anterior to a kinetoplast (K). A mitochondrion (M) extended along the whole length of the body. No undulating membrane was observed. These flagellates appeared to invade the basement membrane of the midgut and to enter the hemocoel. Some were observed free in the spaces between the salivary gland alveoli. Others had invaded the seminal vesicle of male ticks where they were dispersed between spermatophores (Figure 6, page 47).

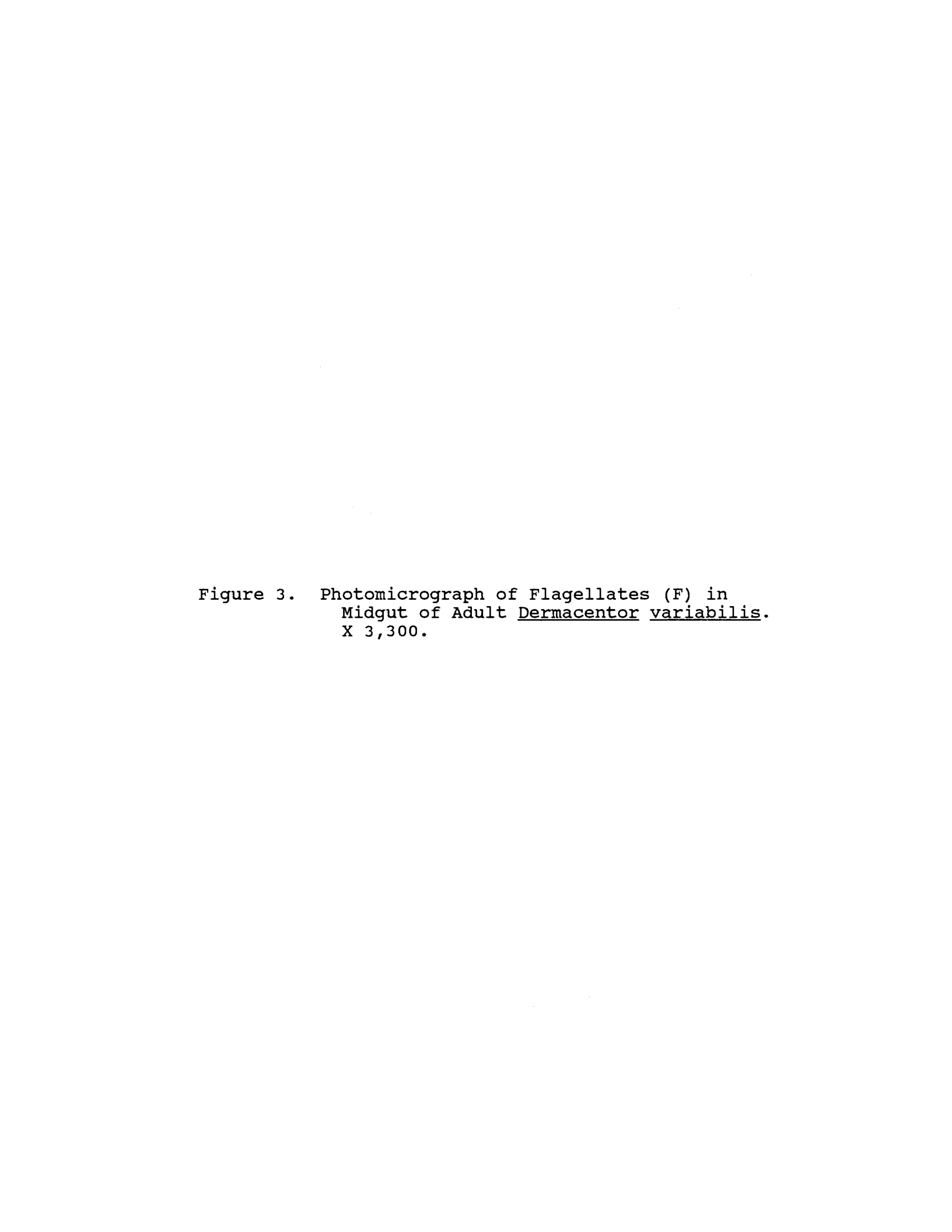
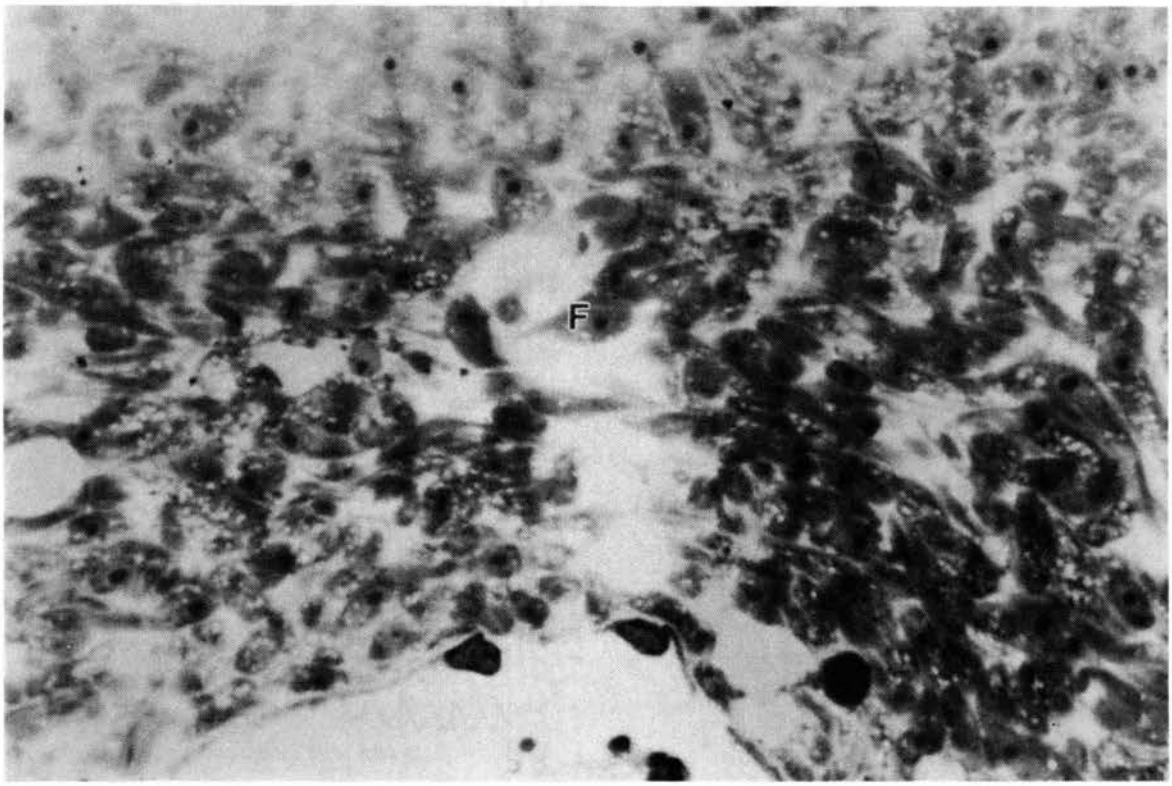


Figure 3. Photomicrograph of Flagellates (F) in Midgut of Adult Dermacentor variabilis. X 3,300.


F

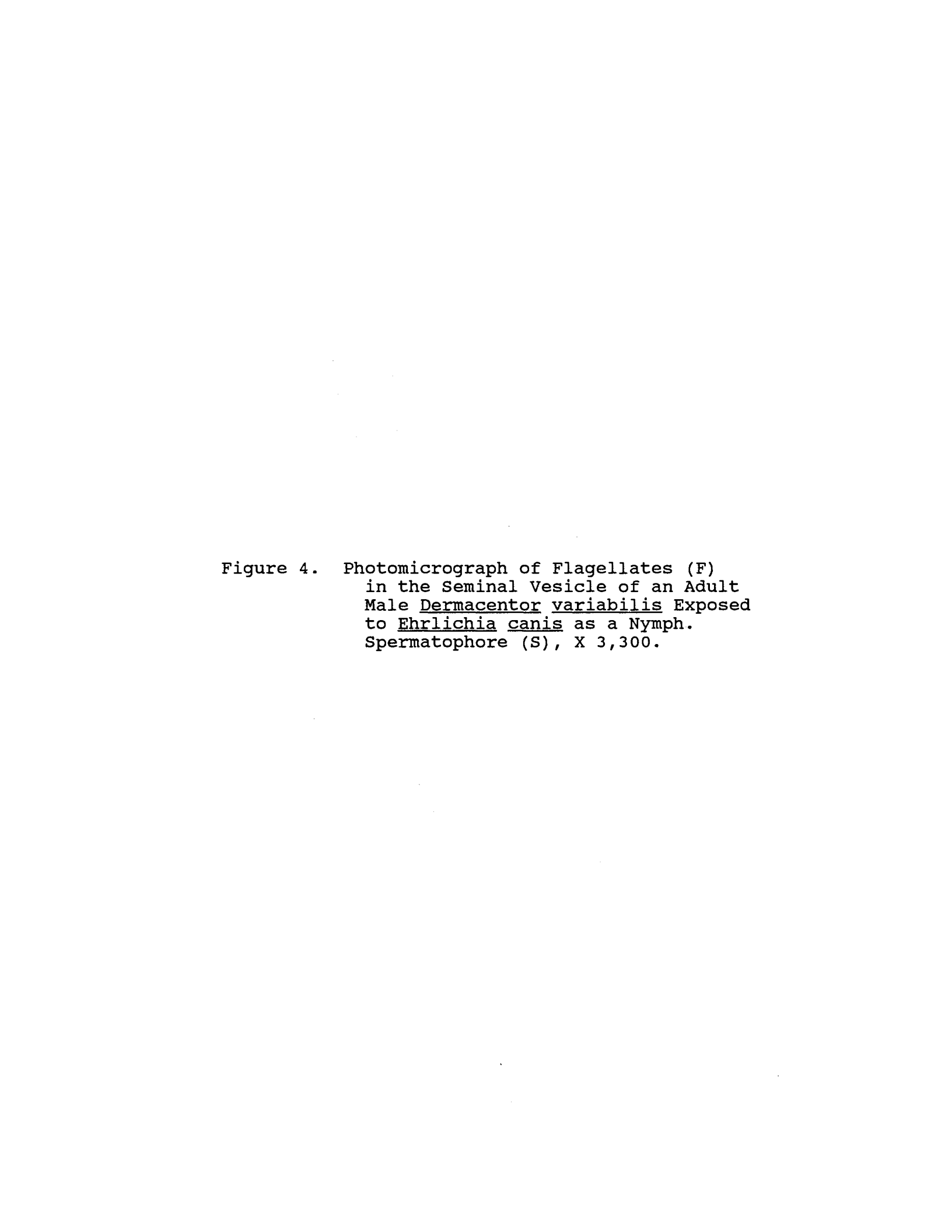


Figure 4. Photomicrograph of Flagellates (F) in the Seminal Vesicle of an Adult Male *Dermacentor variabilis* Exposed to *Ehrlichia canis* as a Nymph. Spermatophore (S), X 3,300.

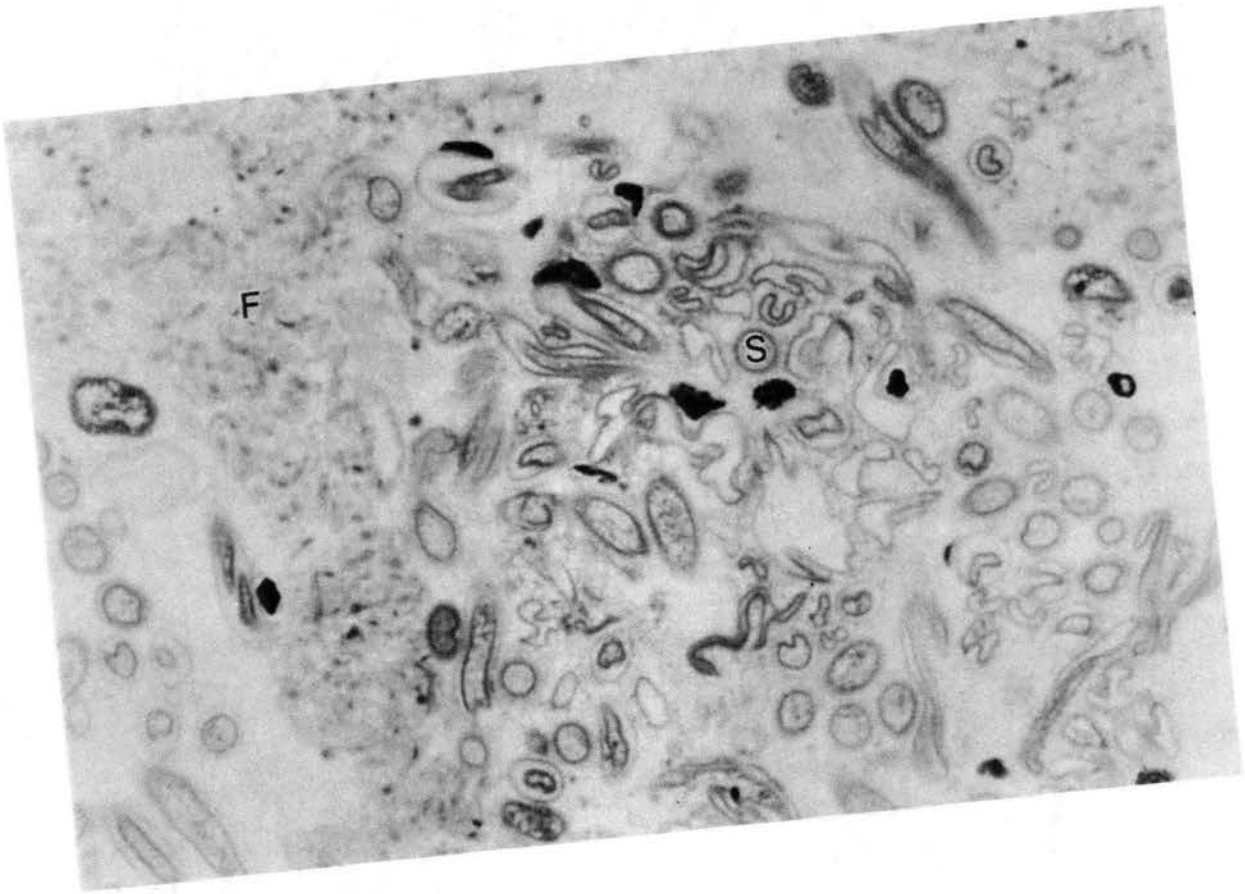
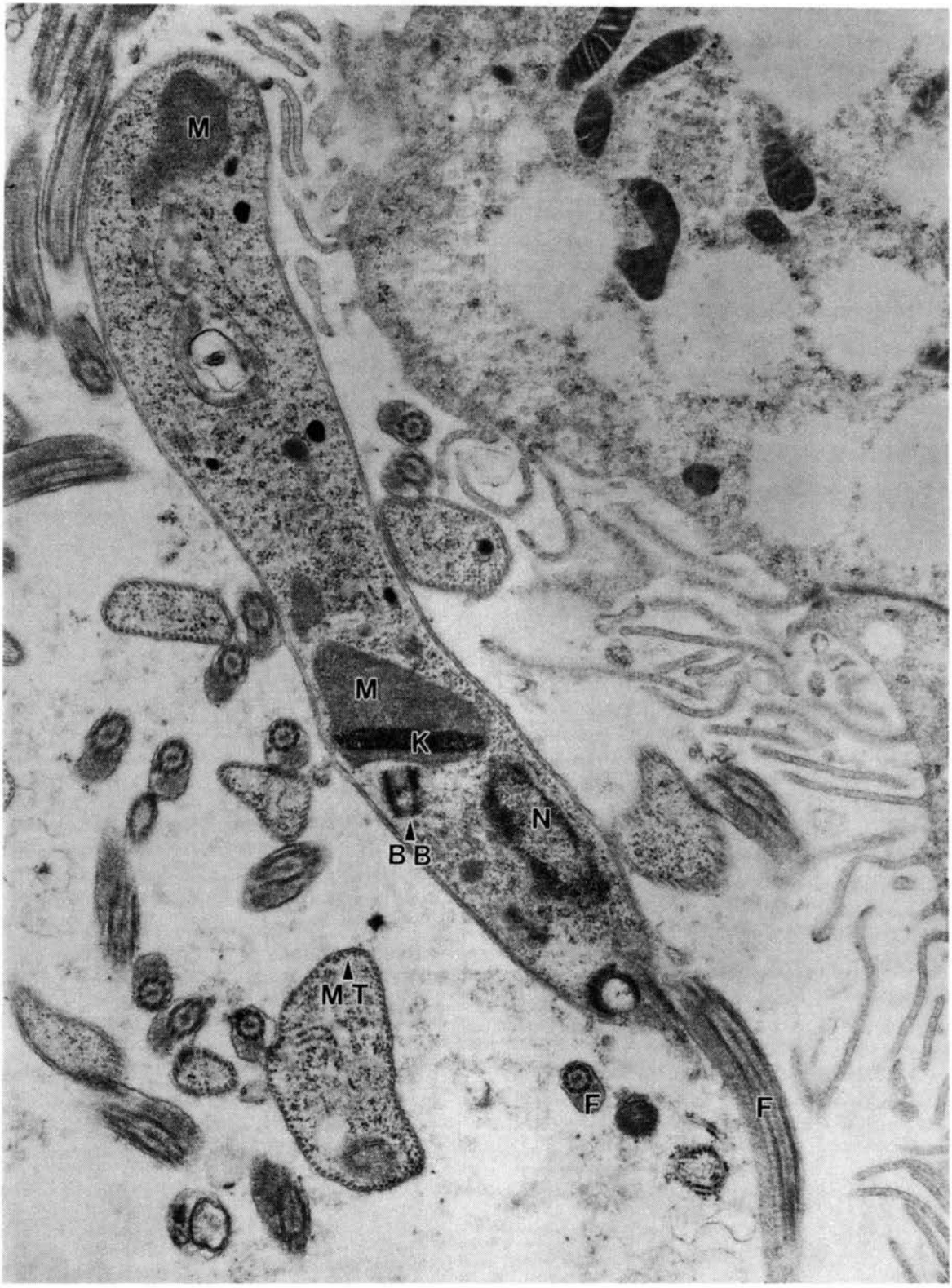

F
S

Figure 5. Electron Micrograph of Trypanosomes in the Midgut of an Adult Dermacentor variabilis Exposed to Ehrlichia canis as a Nymph. Nucleus (N), Subpellicular Microtubules (MT), Flagellum (F), Kinetosome (BB), Kinetoplast (K), Mitochondrion (M). X 10,000.


M
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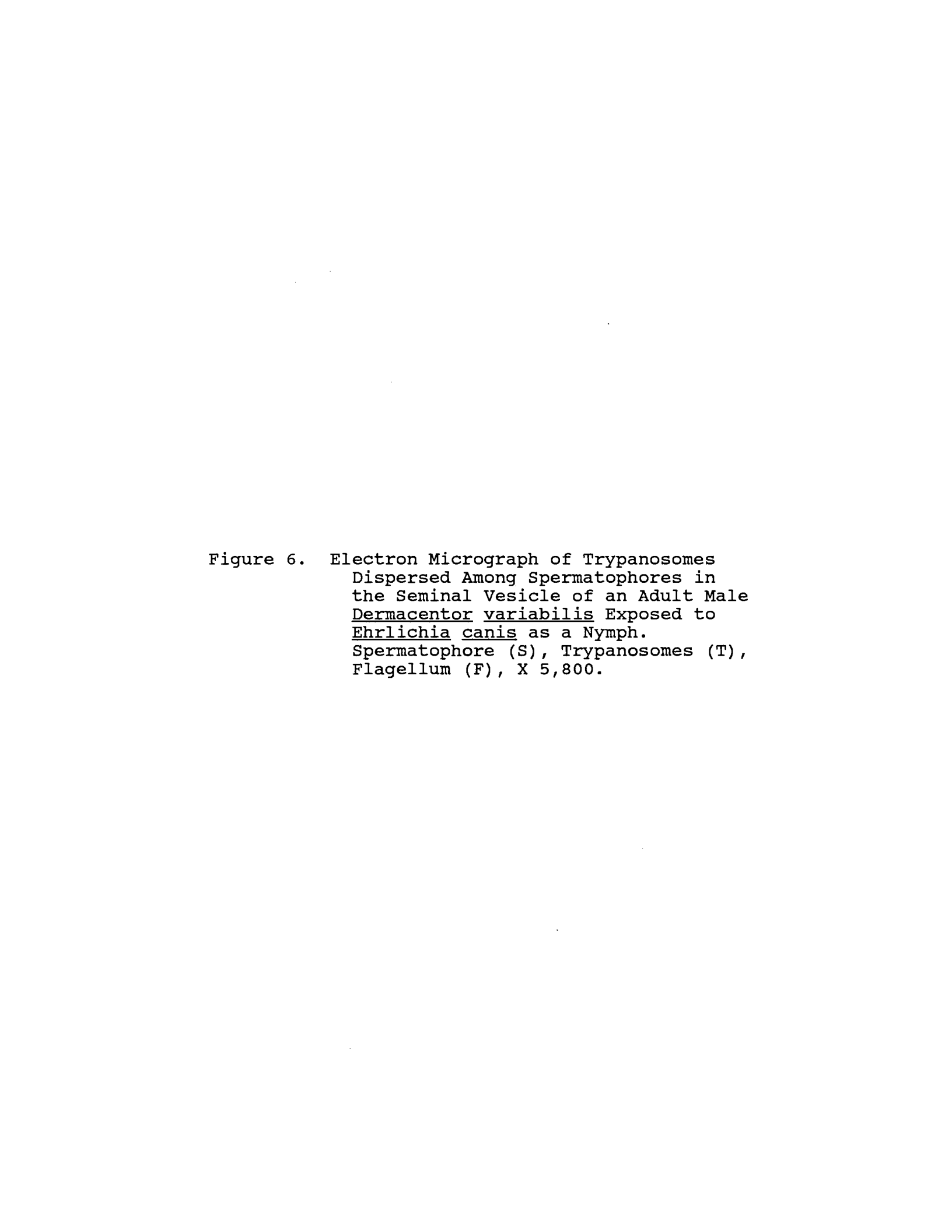
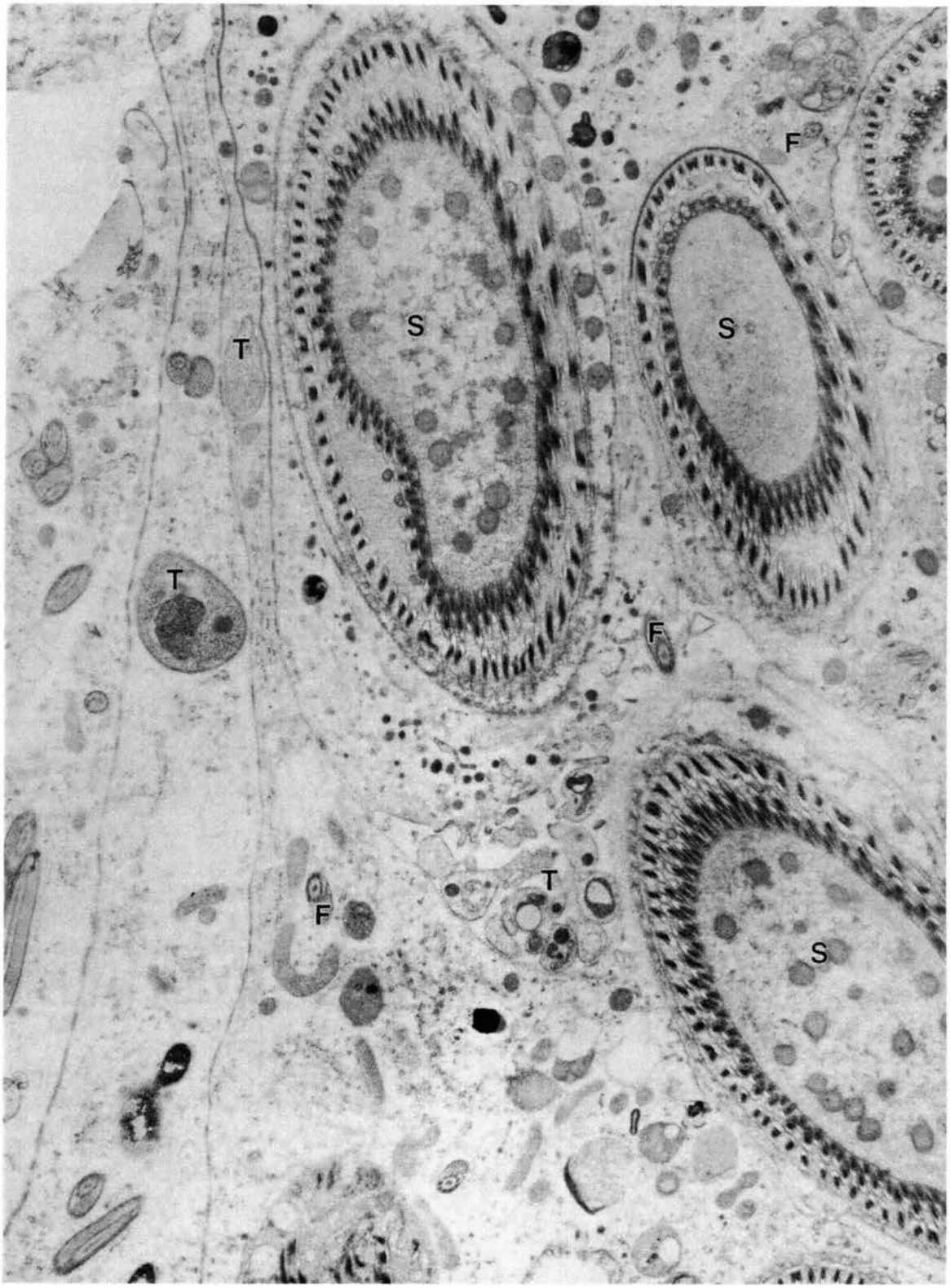


Figure 6. Electron Micrograph of Trypanosomes Dispersed Among Spermatophores in the Seminal Vesicle of an Adult Male Dermacentor variabilis Exposed to Ehrlichia canis as a Nymph. Spermatophore (S), Trypanosomes (T), Flagellum (F), X 5,800.


F
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S

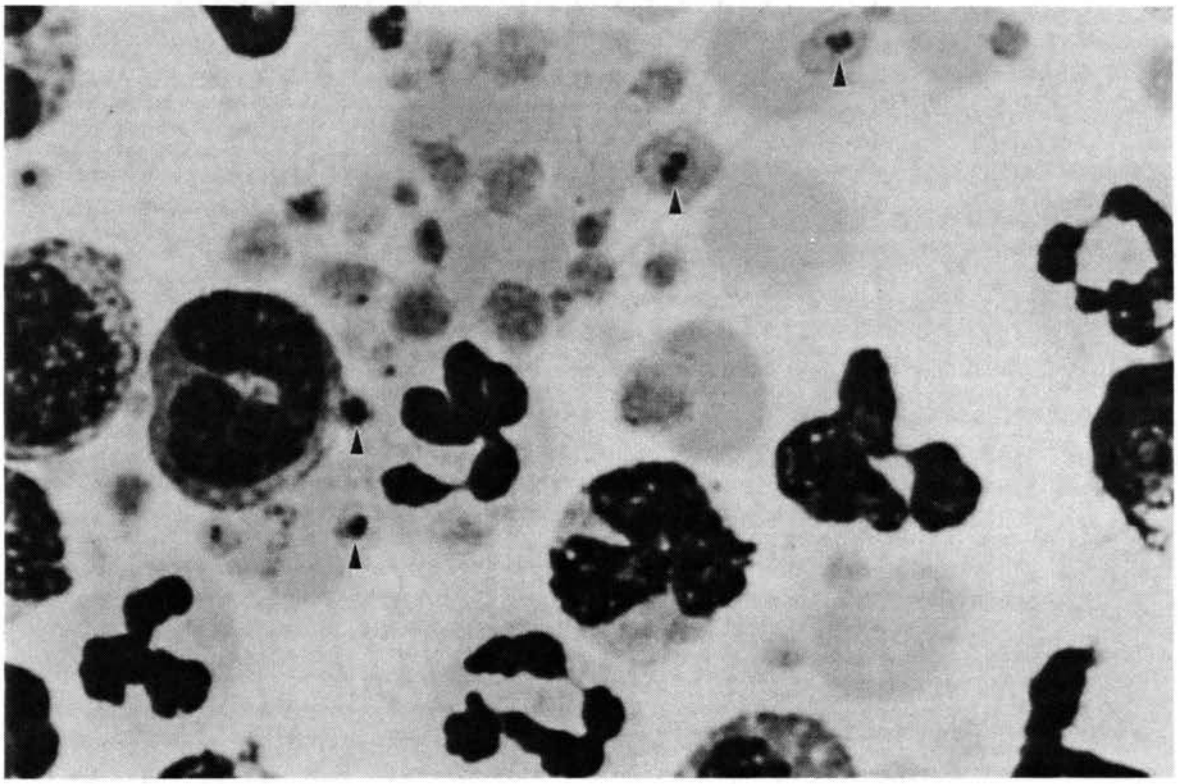
Membrane-bound colonies of rickettsiae similar to those described for E. canis, C. ruminantium, and A. marginale were not observed in these thin sections of midgut and salivary gland.[54,57,59-62,100] Clusters of individual rickettsia-like organisms were observed in midgut cells of male ticks exposed as nymphs on day 7 of tick feeding.

Identification of Ehrlichia platys Infections in Dogs Acquired for Research Purposes

Two adult sibling dogs (5 and 6) which were acquired from an animal shelter in north central Oklahoma for use in this research project were held in isolation for 5 days before being released for use. Preliminary determinations of each dog's PCV, WBC, platelet count, and rectal temperature were conducted; and Romanovsky-stained blood and buffy coat smears were examined.

Both dogs appeared alert and healthy but had elevated rectal temperature values of 39.7°C (103.4°F) and 40.0°C (104.0°F). Values for PCV and WBC were within the normal accepted ranges but platelet counts were low. Dog 5 had a platelet count of 46.0 X $10^3/\mu l$ and dog 6, 49.0 X $10^3/\mu l$. Small, dense, basophilic inclusions (Figure 7, page 50) were seen in platelets on the blood and buffy coat smears of both dogs. Fluorescent antibody tests performed on unfixed buffy coat smears were negative for canine distemper virus.

Figure 7. Photomicrograph of Ehrlichia platys Morulae (arrows) in Platelets of Dog 6, Buffy Coat Smear. X 3,300.



The dogs were monitored periodically for 17 (dog 5) and 43 (dog 6) days.

Cyclic episodes of thrombocytopenia occurred at one- to two-week intervals. Few inclusions were present when platelet counts reached minimal values. The minimal value observed in dog 5 was 18.5 X $10^3/\mu l$ and for dog 6, 9.0 X $10^3/\mu l$. Several days after platelet counts returned to normal levels, the number of platelets containing inclusions increased. Values for PCV, WBC, and rectal temperature remained within the accepted normal ranges. Serum samples collected during the initial examination and again after the periods of observation were tested by the IFA tests for antibodies to E. canis and E. platys. Rising antibody titers to E. platys were observed in both dogs. The titer for dog 5 rose from 1:100 initially to >1:1000, and that of dog 6 from <1:10 initially to >1:1000. Buffy coat pellets from dog 6 were fixed and processed for electron microscopy 3 days after detection of the inclusions in platelets. Double-membraned, reticulate rickettsial organisms (Figure 8, page 53) were found in single-membrane-lined vacuoles of platelets.

Transmission of Ehrlichia platys to a Dog by Intravenous Inoculation with Rickettsemic Canine Whole Blood

Dense basophilic inclusions were detected in platelets on buffy coat smears of dog 7 four days after intravenous

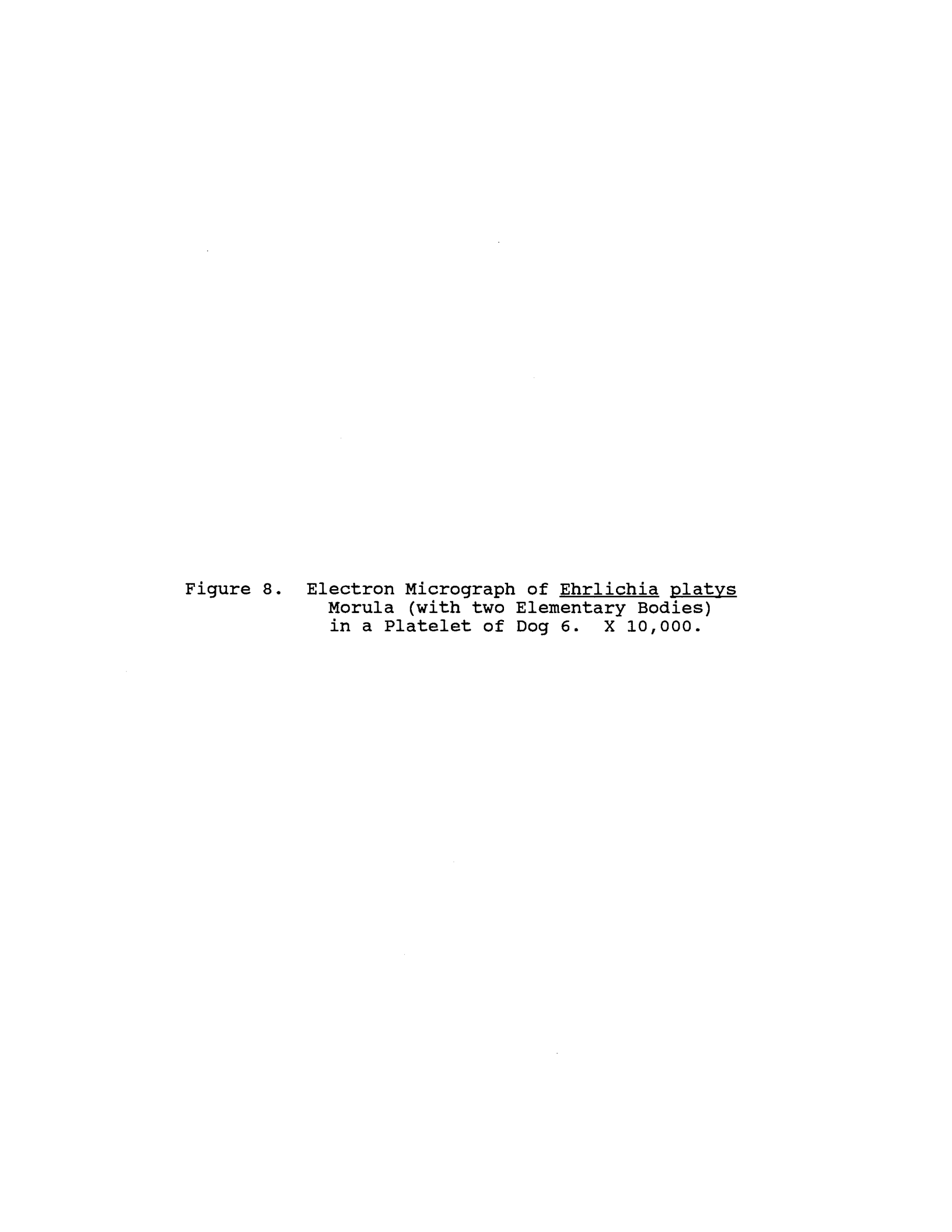
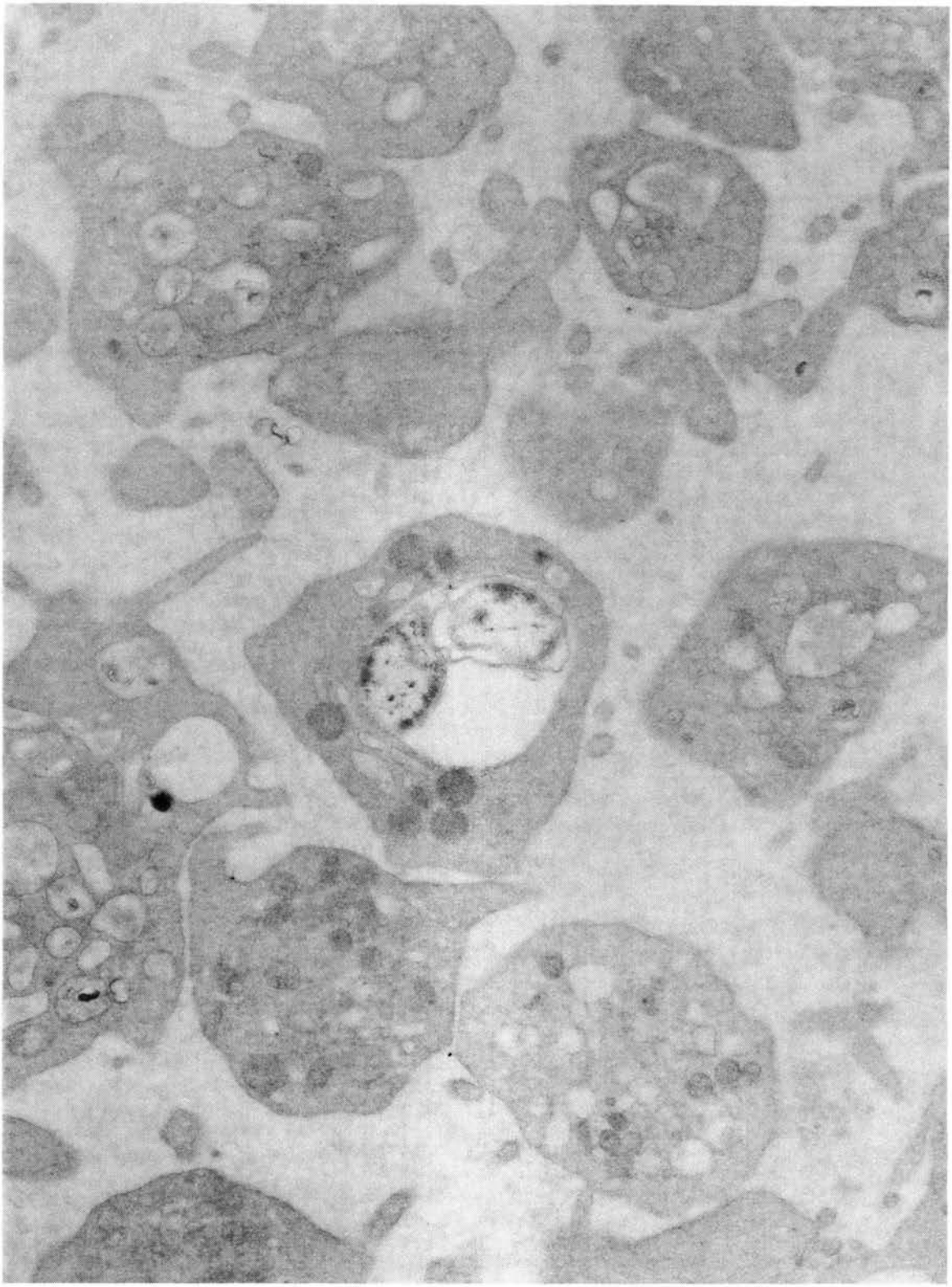


Figure 8. Electron Micrograph of Ehrlichia platys Morula (with two Elementary Bodies) in a Platelet of Dog 6. X 10,000.



inoculation with whole blood from dog 6. Maximal parasitemias were observed at 7 to 8 day intervals. The PCV, WBC, and rectal temperature values remained within normal limits during the 23-day observation period. Maximal parasitemias were followed in 2 to 3 days by marked thrombocytopenia. Minimal platelet counts were observed on days 11, 18, and 19 post-exposure. Daily platelet count values and parasitemias are depicted in Figure 9 on page 55. Platelet counts did not return to pre-exposure levels during the period of observation. Two cutaneous ecchymotic hemorrhages, 1 centimeter in diameter, were detected on the abdomen and perianal region on post-exposure day 18 when the platelet count was 8.0 X $10^3/\mu l$.

Antibodies to E. canis and E. platys were not detected in a pre-exposure serum sample. A rising antibody titer to E. platys from <1:10 to >1:1000 was observed by post-exposure day 22. The dog was active and, aside from the ecchymotic hemorrages observed when the platelet count was lowest, appeared healthy during the observation period.

Attempted Transmission of Ehrlichia platys to a Dog by Intravenous Inoculation with Whole Blood from a Dog One Year after Experimental Infection

Morulae of E. platys were not detected in platelets on buffy coat smears of dog 0983 during 30 consecutive days of

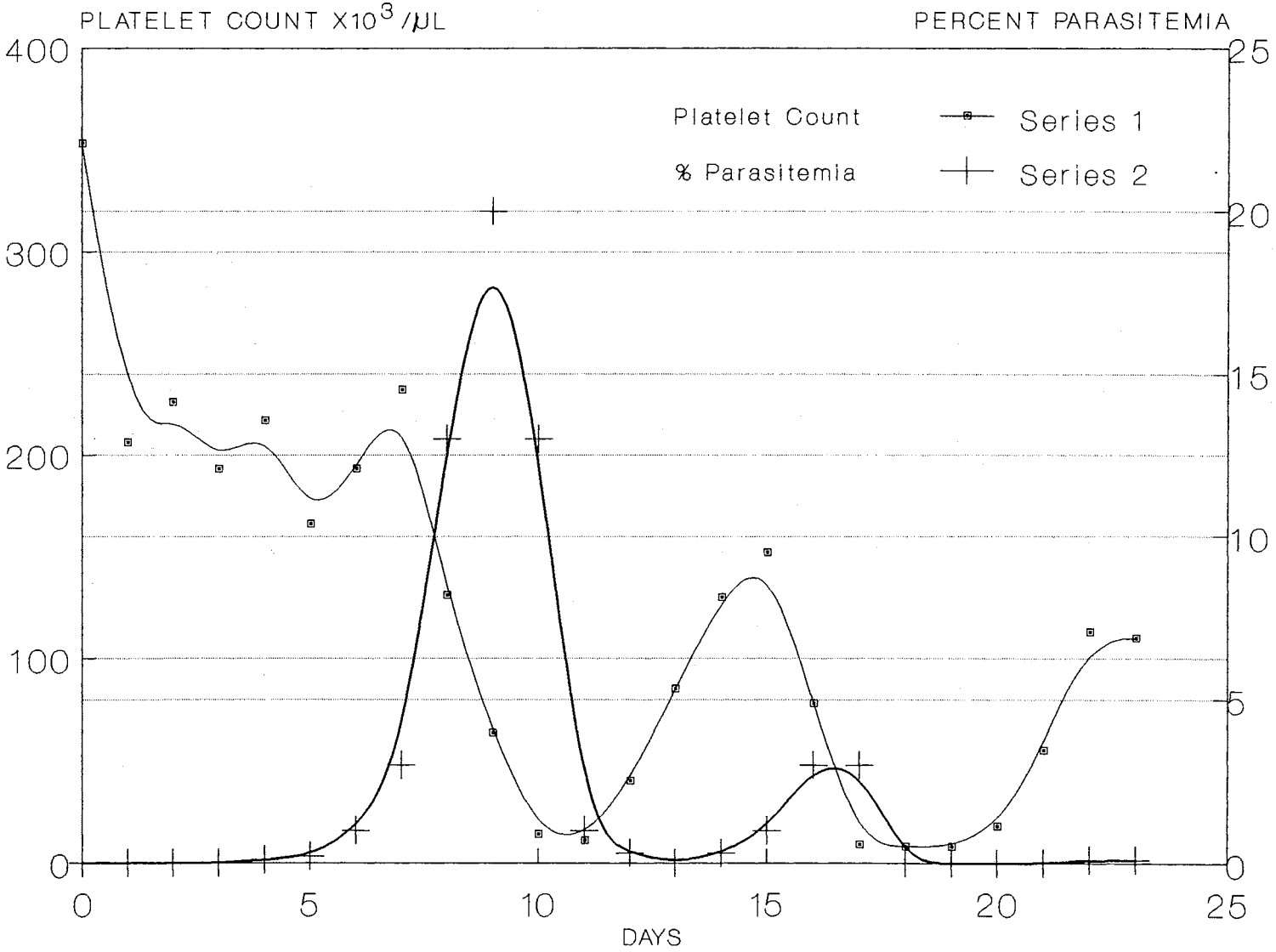


Figure 9. Daily Platelet Count And % Parasitemia Of Dog 7

monitoring after intravenous inoculation of whole blood collected from dog 7. Dog 7 had been experimentally infected with E. platys one year previous to this time. Serum antibodies to E. platys were not detected in either the pre-exposure or post-exposure serum samples of dog 0983. When dog 0983 was challenge exposed with rickettsemic blood from dog 1221, morulae were detected in platelets on buffy coat smears by day 4 post-challenge. Maximal parasitemia was observed on day 6. Thrombocytopenia was evident on day 8 at which time platelets were difficult to demonstrate in buffy coat smears. The antibody titer of dog 0983 to E. platys rose from <1:10 to >1:100 after challenge with blood from dog 1221.

Challenge Exposure of a Dog Recovering from an Ehrlichia platys Infection with Ehrlichia canis

Morulae of E. canis were detected on buffy coat smears of dog 0999 fourteen days after challenge with whole blood from dog 4, an E. canis carrier. Dog 0999 was determined to be infected with E. platys 60 days prior to challenge with E. canis. Morulae of E. platys were not observed in platelets during the 17 day post-challenge observation period.

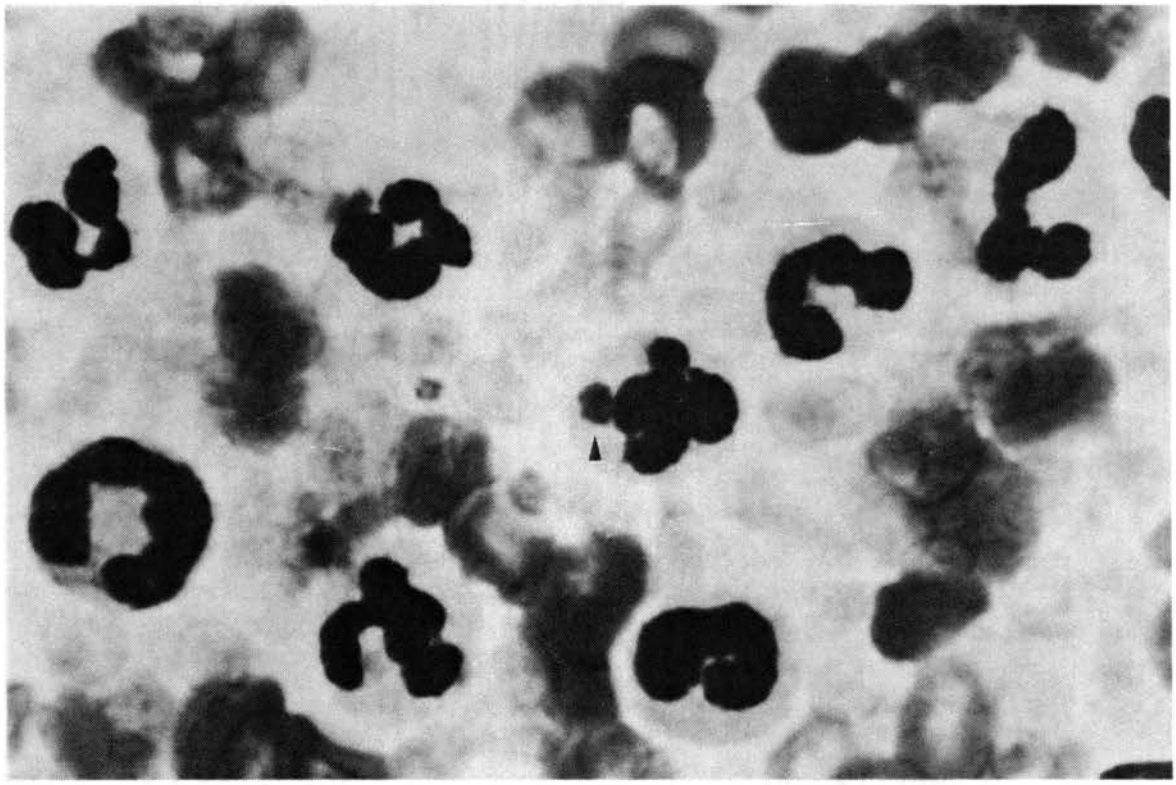
Attempted Simultaneous Transmission of *Ehrlichia platys* and *Ehrlichia canis* to a Dog by Intravenous Inoculation with Whole Blood from a Dog Previously Infected with Both Agents

Morulae of *E. canis* were detected on buffy coat smears of dog 1247 eight days after intravenous inoculation with whole blood from dog 0999 that had been naturally infected with *E. platys* and was carrying *E. canis* as a result of experimental, challenge infection. Morulae of *E. platys* were not observed during the 11 day observation period. Morulae of *E. canis* were detected in monocytic cells of the peripheral blood of dog 1247 from day 8 through day 11 post-exposure.

Attempted Transmission of a Granulocytic *Ehrlichia* Species of Canine Origin to White Mice by Intraperitoneal Inoculation with Rickettsemic Canine Whole Blood

Morulae (Figure 10, page 59) of a canine granulocytic *Ehrlichia* were not observed in peripheral blood leucocytes of two male mice inoculated intraperitoneally with whole blood from a dog exhibiting numerous morulae in neutrophils. No evidence of infection was detected during the 30 days of observation. The control mouse also

Figure 10. Photomicrograph of a Morula (arrow) of an Oklahoma Isolate of a Canine Granulocytic Ehrlichia in a Neutrophil. X 4,950.



remained unchanged, all three mice remaining active and apparently healthy during the observation period.

Attempted Transmission of a Granulocytic *Ehrlichia* Species of Canine Origin to a Horse by Intraperitoneal Inoculation with Rickettsemic Canine Whole Blood

Morulae of a canine granulocytic *Ehrlichia* were not observed in peripheral blood leucocytes of horse 10 for a period of 21 days after intraperitoneal inoculation with rickettsemic canine blood. The PCV, WBC, platelet count and body temperature values remained in the normal ranges for the 21-day observation period. Daily body temperature and PCV, and weekly WBC and platelet count values are listed in Table XXVIII, page 126 of the Appendix.

CHAPTER V

DISCUSSION

Blood Transmission of *Ehrlichia canis* to Dogs

All six attempts to transmit *E. canis* to dogs by intravenous inoculation of whole blood from an *E. canis* carrier were successful. Successful transmission was considered proved by demonstration of morulae in monocytic cells of the peripheral blood, by demonstration of seroconversion to *E. canis* with the IFA test, and by demonstration of consistent clinical manifestations of the disease such as fever, leucopenia, and thrombocytopenia. The clinical signs, incubation periods, and hematologic abnormalities in all dogs were consistent with those reported previously for experimentally induced infections.[7,19,47,49,107] Observations were limited to the acute stage of the disease. Erythrophagocytosis and vacuolization of the cytoplasm of monocytic cells, observed on buffy coat smears of the peripheral blood, were the most consistent indicator of infection before detection of morulae.

Attempted Blood Transmission of *Ehrlichia canis* to Horses

All five attempts to transmit *E. canis* to horses by intravenous or intraperitoneal inoculation of rickettsemic canine blood were interpreted to be unsuccessful because morulae were not found in leucocytes of peripheral blood, clinical manifestations of disease were not detected, and seroconversion to *E. canis*, *E. risticii*, or *E. equi* did not occur. Previous efforts to transmit *E. canis* experimentally to other non-canid host species through blood transfusion also have been unsuccessful, except for an unsubstantiated report of transmission to monkeys.[2,20,21,31,33,37,48,49] Only wild canids such as foxes, jackals, coyotes, and wolves have been successfully infected. Mice, guinea pigs, rats, rabbits, hamsters, sheep, cattle, and cats have all been found refractory to infection. The original report of transmission to monkeys was accomplished by inoculation of tissue emulsions of *R. sanguineus* collected from infected dogs, emulsions of organs from infected dogs, or by inoculation of infective dog blood.[14] Later attempts by other workers to transmit *E. canis* to monkeys via infective canine blood failed.[31,38] The findings of the present experiment and those reported previously support the commonly held opinion that *E. canis* is very host specific with host range being limited to canids and possibly certain primates.

The methods used for exposure of horses to *E. canis*

have been used to study the host ranges of E. canis and E. equi.[2,20,21,30,33,37,38,48,49,64,65,68] The diagnostic procedures used to detect infection in the horses have been employed in studies involving transmission of E. canis, E. equi, E. risticii, and E. sennetsu to natural and experimental hosts.[7,13-15,19,30,44,45,47,49,64,87,90,94,101] It seems unlikely, therefore, that failure to infect horses with E. canis or failure to detect infection of the horses after intravenous or intraperitoneal inoculation of rickettsemic blood resulted from the methodology employed in these experiments. Another method which has been used to diagnose and confirm infections with E. risticii in experimentally infected horses and other host species has been to establish monocytic cultures of the animals' blood with isolation of E. risticii.[13,44,94,101] Culture methods might have been employed in the present experiments to insure that an infection with E. canis did not go undetected. However, the methods employed here have consistently identified successful transmission of other ehrlichial agents to aberrant vertebrate host species.

Attempted Transmission of Ehrlichia canis to Dogs by Adult Dermacentor variabilis Exposed as Nymphs

The results of tick transmission Trials 1 and 2 were inconclusive. The apparently positive result of transstadial transmission of E. canis by D. variabilis to

dog 4 of Trial 1 was complicated by the presence of extraneous adult R. sanguineus of unknown origin. These unwanted ticks were found feeding on this dog at the same time as the experimentally exposed adult D. variabilis. Intrepretation must also be tentative owing to the failure to demonstrate colonies of E. canis in tissues of companion ticks with light or electron microscopy. The probability of transmission occurring from random source R. sanguineus is rather low; but because this species is the known vector of E. canis and the probable origin of the extraneous R. sanguineus was the animal shelter, it cannot be stated with absolute confidence that the experimentally exposed D. variabilis were responsible for transmission of E. canis to dog 4.

The flagellates found in the adult ticks exposed to E. canis as nymphs on dog 2, Trial 1 had morphologic features of trypanosomes. Their presence in laboratory-reared ticks was puzzling; however, they probably did not interfere with transmission of E. canis given that transmission to dog 4 may have occurred from companion ticks. Proliferation of trypanosomes in the tick tissues may have interfered with demonstration of colonies of E. canis either by destruction of the normal architecture of tissues or by otherwise obscuring the presence of colonies.

Dog 8 of Trial 1 was also monitored for infection after the attachment and feeding of exposed adult D. variabilis. Tick attachment on this dog was poor with only

7 of 25 pairs feeding; furthermore, one pair was removed daily for histological studies. Failure to transmit Ehrlichia canis to dog 8 may have resulted from the poor attachment rate of ticks or from interruption of feeding before the rapid phase of engorgement was reached. Kocan et al. have demonstrated that D. andersoni does not transmit Anaplasma marginale before day 7 of engorgement.[58] It is not known at what point during the feeding process E. canis is transmitted by R. sanguineus.

Three attempts to transmit E. canis to dogs through replete feeding of adult D. variabilis exposed as nymphs in Trial 2 were unsuccessful. There was no evidence of transmission in any of these dogs during the 30-day observation period. The experimental methods employed were the same as in Trial 1 except that the dogs were treated with an organophosphate insecticide 2 weeks prior to placement in the tick attachment boxes, and a mixture of ketamine hydrochloride and xylazine was administered intramuscularly immediately prior to placement in the boxes. Use of the anesthetic combination of agents, ketamine hydrochloride and xylazine, to sedate the dogs of Trial 2 was an additional factor which could have influenced the results. This explanation seems unlikely, however, because there was no effect on the parasitemia after intramuscular administration of these agents to a rickettsemic dog (0999) nor on the ability to transmit E. canis to a susceptible dog (1247). Morulae were observed

for 3 days in the peripheral blood of dog 0999 after the anesthetic agents were given, and numerous morulae were detected in lung impression smear made during necropsy examination. Furthermore, dog 1247 was subinoculated with blood from dog 0999 (collected 3 days after administration of the anesthetic agents) and developed a patent infection with E. canis within 8 days of exposure. A combination of ketamine, xylazine, and atropine administered intravenously to dogs can cause hypoventilation and increases in arterial blood pressure, left atrial pressure, and peripheral resistance; usually for a period of 30 minutes.[98] Without atropine, bradycardia is frequently observed. When the combination is administered intramuscularly, the incidence of cardiopulmonary depression is reduced. Because of the short duration of the cardiopulmonary effects and the reduced incidence of these effects when combinations of ketamine and xylazine are administered intramuscularly, failure to infect ticks in Trial 2 probably was not influenced by changes in peripheral circulation resulting from administration of these agents.

One of the dogs (0984) of Trial 2 which failed to become infected with E. canis after the exposed ticks had fed to repletion was challenged with infective blood to establish that the dog was actually susceptible to infection. Typical morulae of E. canis were detected 12 days after challenge. The number of adult ticks that fed on each of the 3 dogs of Trial 2 was comparable to the

number that fed on dog 4 of Trial 1.

Two attempts to infect dogs (0925 and 0999) of Trial 2 through inoculation of tissues of unfed, adult D. variabilis which were exposed as nymphs also were unsuccessful. A method involving incubation of exposed ticks at 37°C before tick gut homogenates were inoculated intravenously has been used by Kocan et al. to infect cattle with A. marginale.[56] The shortest prepatent periods were observed in cattle which received inocula from ticks incubated for 2.5 days. Failure to transmit E. canis with the tissues of exposed ticks that had been incubated at 37°C for 2.5 days suggests that the ticks of Trial 2 did not become infected with E. canis. One of these dogs (0999) proved to be susceptible to infection upon challenge exposure to E. canis through blood transfusion.

Failure to transmit E. canis to 5 dogs in Trial 2 casts doubt upon the possibility that transmission by D. variabilis did occur in Trial 1. However, other factors could have contributed to failure in Trial 2. The prepatent period for the dog of Trial 2 (0924) on which nymphs fed was prolonged (16 days) whereas that of the dog of Trial 1 (2) was 10 days. Nymphs were placed on dog 2 of Trial 1 eleven days after exposure to E. canis. Nymphs were placed on dog 0924 of Trial 2 on day 17 post-exposure to E. canis. Groves et al. stated that transstadial transmission with R. sanguineus occurred when nymphs began feeding on days 12 and 14 but not on day 9 after dogs were

inoculation with E. canis.[31] Smith et al. were able to infect R. sanguineus by allowing them to feed on febrile dogs when morulae were detected in buffy coat smears.[100] Lewis et al. observed successful transmission with R. sanguineus when uninfected nymphs were fed on a dog 15 days after the dog was first exposed to E. canis.[66] Transmission did not occur when nymphs fed on dogs 25, 41, and 75 days after the dogs were first exposed to E. canis. Based upon the findings of these studies, it appears that the timing for tick attachment after exposure of dogs to E. canis is critical to infection of the ticks. Although nymphal feeding was initiated in the present study immediately following initial detection of characteristic morulae in monocytic cells of the peripheral blood, the extended prepatent period for dog 0924 may have inadvertently affected infection of nymphal ticks of Trial 2.

Although results of the present experiments were inconclusive, transstadial transmission of E. canis by adult D. variabilis exposed as nymphs on rickettsemic dogs probably was successful in Trial 1. Subsequent attempts to repeat these experiments by S. A. Ewing have achieved successful transmission.[25]

Attempted Transmission of *Ehrlichia canis* to Horses by Adult *Dermacentor variabilis* Exposed as Nymphs

Three attempts to transmit *E. canis* transstadially to ponies with experimentally exposed adult *D. variabilis* were unsuccessful. There was no evidence of infection in any of these ponies during the 30-day observation period. In view of the inconclusive results with transmission to dogs, it cannot be stated whether transmission failed because the horse is not susceptible to infection with *E. canis* or because the ticks were not infected with the organism. Considering that attempts to transmit *Ehrlichia canis* to horses with rickettsemic blood were also unsuccessful, it is reasonable to conclude that if the ticks from Trial 1 were infected, transstadial transmission to horses failed because horses are not a suitable host for *E. canis*. This conclusion seems justified and is supported by findings of other investigators who employed similar methods to study the host ranges of *E. canis*, *E. equi* and *E. risticii*.[13,30,50,64,94,101]

Serologic Findings

Antibodies to *E. canis* were not detected in any pre-exposure serum samples of the animals used for these experiments. Seroconversion to *E. canis* occurred in dogs 2, 3, 4, and 0924 all which were infected either through blood transmission (dogs 2, 3, and 0924) or apparent tick

transmission (dog 4). Seroconversion did not occur in any of the dogs and horses that remained clinically normal after exposure to infected blood, nor in those fed upon by or inoculated with tissues of exposed ticks.

There was no evidence of cross-reactions with E. risticii, E. equi, or R. rickettsii in the present studies. Holland et al. and Logan et al. demonstrated that E. canis cross-reacts with E. risticii, E. equi, E. sennetsu, and C. ruminantium.[44,67] Cross-reactions between members of the genus Rickettsia and members of the genus Ehrlichia have never been demonstrated.[44,67,89,90,92] Differences in lengths of the infections may account for the descrepancies between previous findings and results of the present experiments. Pretzman et al. demonstrated through the use of an enzyme-linked immunosorbent assay that specific antibodies to E. risticii in the immunoglobulin M fraction of sera from experimentally and naturally infected horses were detectable earlier but were short lived, falling to undetectable levels by day 60 post-inoculation. Specific antibodies in the immunoglobulin G fraction of sera rose more slowly and persisted for more than a year.[81] Weisiger et al. demonstrated a similar immunoglobulin response with canine ehrlichiosis.[109] It has not been determined which fraction of immunoglobulins contains the cross-reactive antibodies for E. canis or any of the other species of Ehrlichia.

Antibody titers to R. rickettsii in pre-exposure serum

samples of dogs 4, 7, 8, 0933, 0961, and 0999 indicated previous exposure to R. rickettsii. There was no change in the post-exposure titers, giving little indication of the status of the infections. Antibodies to R. rickettsii can persist in the blood of dogs for up to 10 months.[29] Antibody titers in the pre-exposure and post-exposure serum samples of ponies 7, 8, and 9 also indicated that these horses had been previously infected with this agent. Again, the response was static and gave no indications of the course of the infections. There was no cross protection to dogs and presumably none to horses against E. canis from the antibodies to R. rickettsii, since 2 of these dogs (4 and 0999) became infected with E. canis after tick feeding or blood transfusion.

An antibody titer to E. equi in the pre-exposure serum sample of dog 0999 also indicated previous exposure to this agent or a cross-reaction with a similar agent. There was a two-fold increase in this titer after inoculation with tissues of unfed, incubated, exposed adult D. variabilis. The rising antibody titer would indicate an ongoing infection with E. equi. However, morulae of E. equi were never observed in peripheral blood. Moreover, the organism was not detected in the blood of dog 1247 which was inoculated with blood from dog 0999 seventy-seven days after procurement. Dog 0999 was infected with E. platys at the time of procurement and later challenged with E. canis before dog 1247 was subinoculated. The antibody titer for

dog 0999 could have represented a cross-reaction with E. platys; or expression of the E. equi infection could have been obscured by the E. platys infection. French and Harvey demonstrated that E. platys does not cross-react with E. canis but did not state whether it cross-reacts with other ehrlichial agents.[27] Recently, Ewing et al. showed that dogs infected with E. canis were protected from a canine granulocytic ehrlichial infection but this immunity was abrogated with steroid therapy.[24]

Light and Electron Microscopic Findings in Selected Tissues of Adult Dermacentor variabilis Exposed as Nymphs

Colonies similar to those described in midgut and salivary glands of D. andersoni and D. variabilis infected with A. marginale, and Amblyomma variegatum and A. hebraeum infected with C. ruminantium were not detected in thick sections of tissues of adult D. variabilis exposed to E. canis as nymphs.[57,59-62] As many as 20 sections were examined from each block of tick tissue collected on days 30, 35, and 40 post-repletion as nymphs and on days 1 through 7 of engorgement as adults. Because clusters of individual rickettsia-like organisms were detected in thick sections of unexposed controls as well as in exposed tick midgut tissues, it was concluded that these organisms were symbiotes. The symbiotes were demonstrated by electron

microscopy in midgut tissue of exposed male ticks on day 7 of engorgement. It is regrettable that immunofluorescent methods were not employed to detect E. canis in the tissues of exposed adult D. variabilis of Trial 1. Such a method was used to demonstrate E. canis in R. sanguineus and would have been a good method to screen for E. canis colonies in the present study.[100] Immunofluorescent techniques also would have helped to distinguish symbiotes from the parasitic agent. Tissues collected from ticks of Trial 2 were not examined for E. canis because there was no evidence that transmission of E. canis occurred during that trial.

The flagellated organisms observed in one block of tissue from a control female tick and several blocks of tissue from exposed females and males had morphologic structures typical of trypanosomes.[63] The origin of these trypanosomes was not determined. Ticks used in these experiments were laboratory-reared. Ticks from the colony have been used in experiments for transmission of other arthropod borne disease agents at Oklahoma State University for several years without previous detection of trypanosomes.[53-55,62] The dogs (1 and 2) on which nymphs fed were thirteen-week-old sibling puppies in excellent health; they were born in February, 1986, and were acquired from a private individual. There was no evidence of any ectoparasitic infestation of these dogs when they were acquired. Dog 2 was infected with E. canis through

blood transfusion from a carrier dog (0243) in June,1986. Dog 0243 may have been the source of the trypanosomes, but retrospective efforts to determine this were not productive.

Dog 0243 was acquired in good health in October, 1985. She was splenectomized and inoculated intravenously with a stabilate of E. canis acquired from Louisiana State University. This dog was maintained at Oklahoma State University as a carrier of E. canis for future studies. In April, 1986, dog 0243 developed a raised, moist, ulcerated lesion approximately 2.0 centimeters in diameter on the occipital region. By June, 1986, she developed a generalized loss of body hair and body condition typical of that seen with generalized leishmanial infections of dogs.[103] This dog was humanely killed and a necropsy examination was performed in September, 1986, because her condition was progressively deteriorating. The gross and histological findings were compatible with a chronic E. canis infection and with that of visceral leishmaniasis.[42,103] However, there was no evidence of amastigote forms of Trypanosoma cruzi or Leishmania species in heart or lymphatic tissues examined. Moreover, no trypanosomal forms nor leishmanial bodies were observed during the 30 days of daily monitoring of blood and buffy coat smears nor on lung impression smears prepared at necropsy. Finally, no trypanosomal forms nor leishmanial bodies were detected in blood or buffy coat smears of 4

dogs exposed to blood from dog 0243. It must be remembered, however, that leishmanial infections of dogs often are subclinical and go undetected.[72,103] Demonstration of the organism in peripheral blood and other tissues can be extremely difficult and often requires cultures of blood and tissues.[72,103] Clinical manifestations of leishmanial infections of dogs often do not become apparent until hosts are immunosuppressed by concomitant disease or immunosuppressive therapy. The source and identity of these trypanosomes might have been determined retrospectively given access to appropriate serologic methods, isoenzyme techniques, or DNA probes for Trypanosoma species and Leishmania species.[72] The serologic tests for Trypanosoma and Leishmania, once routinely conducted at Oklahoma State University, were no longer available when efforts were made to test the sera of dogs used in the present experiments. It is possible that the one block of control tick tissue that contained trypanosomes resulted from contamination during processing for electron microscopy.

In view of the fact that transmission of E. canis to dog 4 by D. variabilis probably occurred in Trial 1, failure to demonstrate E. canis in tick tissues is disappointing. It is possible that the E. canis infection may have been obscured by presence of trypanosomes in the tick tissues.

Blood Transmission of *Ehrlichia platys* to Dogs

Harvey et al. first described *E. platys* infections in naturally and experimentally infected dogs.[32] These workers experimentally infected dogs by inoculation with 2 mls of frozen blood collected from a subinoculated dog during its initial parasitemic episode. Glaze and Gaunt also infected dogs with frozen rickettsemic blood collected from a naturally infected dog.[28] Baker et al. used frozen platelet-rich plasma containing $3.0 \times 10^5/\mu l$ platelets with 5% of platelets containing inclusions of *E. platys*.[4] In the present experiments, blood transmission of *E. platys* via rickettsemic whole blood was also successful. Dog 7 was infected with rickettsemic blood from dog 6 forty-three days after the organism was first detected in the donor. Dog 0983 was challenged with rickettsemic blood from dog 1221 and became infected. The longevity of *E. platys* infections is not known. Harvey et al. followed infections in experimentally infected dogs for 75 days.[32] The initial parasitemic episode was the highest. Subsequent parasitemias were lower and eventually parasites were difficult to demonstrate. On day 70 post-infection the parasitemia level was 1%. There are no reports of attempted transmission with blood from recovering animals.

In the present experiments blood from dog 7, infected 1 year previously, was inoculated into a susceptible dog (0983). There was no evidence of infection in this dog

(0983) during a 30-day observation period. Morulae were not detected in platelets on buffy coat smears, platelet count values remained at pre-exposure levels, and no serologic evidence of infection with E. platys was found with the IFA test. When dog 0983 was challenged with rickettsemic blood from an E. platys-infected dog (1221), morulae were observed in platelets by day 4 post-challenge and antibodies to E. platys were detected with the IFA test by day 11 post-challenge. Thrombocytopenia was detected subjectively by day 7 post-challenge when platelets were difficult to find on buffy coat smears. Attempted simultaneous transmission of E. platys and E. canis from dog 0999 to dog 1247 seventy-seven days after detection of the E. platys infection in dog 0999 and 17 days after challenge exposure with E. canis resulted in a patent infection with E. canis but not with E. platys. These findings suggest that an infectious carrier state for E. platys does not exist. This situation contrasts sharply with E. canis infections which persist for prolonged periods and immunity is dependent upon continuous presence of the organism.[7,19] A similar situation to that seen with E. platys is seen with E. equi infections. Attempts to produce equine ehrlichiosis by inoculation of blood or organ homogenates from recovered ponies have been unsuccessful.[77] Recovered ponies were resistant to reinfection after inoculation with rickettsemic blood even after treatment with tetracyclines. E. canis-infected dogs

that were treated with tetracyclines and challenged with E. canis were susceptible to infection.[7] Protective immunity to E. risticii develops in horses.[92,108] However, it has not been determined if a carrier state exists. No attempt was made during these experiments to determine if dogs which had recovered from E. platys or which had been treated with tetracyclines were susceptible to reinfection. Therefore, it is not known from the present studies or from the work of others whether dogs develop protective immunity to E. platys.

Challenge Exposure of a Dog Recovering from an Ehrlichia platys Infection with Ehrlichia canis

Previous infection with E. platys did not produce protective immunity against E. canis. Dog 0999 which was naturally infected with E. platys at the time of procurement developed E. canis infection upon challenge exposure. Blood collected from dog 0999 and inoculated into dog 1247 proved to be infective; E. canis infection developed but E. platys did not. French and Harvey conducted a serologic survey for E. platys and found the highest occurrence of antibodies to E. platys in dogs that were seropositive for E. canis as well.[27] However, dogs experimentally infected with E. platys seroconverted to E. platys only. When they inoculated whole blood from a

thrombocytopenic dog that was serologically positive for both agents into a susceptible animal, an E. canis infection developed but not E. platys. When they inoculated whole blood from a thrombocytopenic dog that was serologically positive for E. platys only into a susceptible dog, an E. platys infection developed. Previous studies investigating cross-protective immunity against E. canis from infections with E. equi and a canine granulocytic ehrlichial agent found that cross-protective immunity did not develop.[64,74]

Attempted Blood Transmission of a Granulocytic Ehrlichia Species of Canine Origin to White Mice

Attempts to transmit a granulocytic Ehrlichia of canine origin to male mice were unsuccessful. Morulae of the canine agent were not detected in leucocytes of the peripheral blood of principals or controls and the mice remained healthy and active.

Attempts to transmit E. canis and E. equi to mice, rats, rabbits, and guinea pigs have also been unsuccessful.[21,48,49,65] E. risticii has been successfully transmitted to mice by intraperitoneal inoculation of rickettsemic equine blood elements.[50] The mice became lethargic and developed blepharophimosis, rough hair coats, and hunched backs. Morulae were demonstrated by electron

microscopy in mononuclear cells of the spleen. E. sennetsu has also been transmitted to mice through intraperitoneal inoculation of infective cell culture materials.[45,50] Similar clinical signs were observed and morulae were detected by light or electron microscopy in mononuclear cells of the spleen and peripheral blood. Results of the present preliminary studies indicate that mice are not in the host range of an Oklahoma isolate of a canine granulocytic Ehrlichia species and thus would not play a role in its epidemiology.

Attempted Blood Transmission of a Granulocytic Ehrlichia Species of Canine Origin to a Horses

An attempt to transmit a granulocytic Ehrlichia of canine origin to a horse was unsuccessful. Morulae were not detected in peripheral blood leucocytes of the recipient horse and no clinical manifestations of disease were detected. With the discovery of E. equi and successful transmission of E. equi from horses to dogs and from dogs to horses, it has been speculated that all granulocytic canine isolates from different geographic regions are in fact E. equi.[64,65,68] However, serologic findings revealed a closer relationship of some canine granulocytic isolates to E. canis than to E. equi.[5,9,24,102] Only one isolate from a dog in California which did not seroconvert to E. canis has been transmitted

from dog to horse with resultant clinical disease in the horse typical of equine ehrlichiosis.[68] The Oklahoma isolate of a canine granulocytic Ehrlichia used in the present experiment did not produce disease nor detectable infection in a susceptible horse; and it may represent a separate species of Ehrlichia.[24]

CHAPTER VI

SUMMARY AND CONCLUSIONS

Transmission of Ehrlichia canis and other species by intravenous or intraperitoneal inoculation of infective blood into a susceptible host has been well documented. The technique is employed to establish the vertebrate host range of ehrlichiae. In the present studies, eight successes among 8 attempts to transmit E. canis to dogs by intravenous inoculation of blood from a carrier dog confirms that the organism can be transmitted easily through whole blood transfusion to susceptible hosts. Five failures among 5 attempts to transmit E. canis to horses through intravenous and intraperitoneal inoculation of rickettsemic whole blood suggest that the horse is not a suitable host for the propagative development of E. canis. This finding is consistent with the commonly held opinion that E. canis is very host specific with infections being limited to canids and possibly primates. However, more research is needed to confirm that primates are susceptible to E. canis. Transmission of ehrlichial agents of human orgin to dogs should also be attempted.

Transstadial transmission of E. canis by adult D. variabilis exposed as nymphs probably occurred in one

instance in the present studies (dog 4, Trial 1). Results were equivocal, however, because of the accidental presence of R. sangiuneus, the known vector of E. canis. Unfortunately these extraneous ticks were found feeding on the dog at the same time as the experimentally exposed adult D. variabilis. This complication, coupled with failure to achieve transmission in Trial 2 and failure to demonstrate colonies of E. canis in exposed ticks of Trial 1, leaves us unable to conclude with confidence that transmission occurred in Trial 1. Given that these experiments have been repeated by S. A. Ewing and transmission did occur, it is considered very likely that transmission by D. variabilis was achieved in Trial 1.[25]

The use of anesthetic agents during Trial 2 raised the question that these drugs interfered with transmission; but an additional experiment established that they were not likely a factor contributing to the failure. The use of an organophosphate insecticide on experimental dogs (in Trial 2) two weeks prior to attachment and feeding of D. variabilis nymphs and adults did not inhibit attactment and feeding of ticks. Apparent failure to infect ticks in Trial 2 may have resulted from inappropriate timing; i.e., nymphal ticks may have been exposed at the wrong time in relation to exposure of the host dog to E. canis.

Although the vector potential of D. variabilis for E. canis may have been established, the role of this tick species in transmission of E. canis to dogs is probably

secondary to that of R. sanguineus, especially in the U.S.A. where the latter species feeds almost exclusively on canids during all developmental stages in its life cycle. D. variabilis feeds on dogs primarily as an adult tick. Larval and nymphal instars prefer to feed on small rodents such as meadow mice, although they may be found on dogs occasionally.[34] The major importance of establishing the potential role of D. variabilis in transmission of E. canis is related to its possible role in transmission of E. canis or a closely related organism to human beings. Future studies should investigate development of E. canis in D. variabilis and establish whether transovarial transmission occurs. Although not a part of the present studies, the vector potential of A. americanum, a three host tick which feeds on several host species including dogs and man in all stages of it life cycle, should be investigated.

Two pieces of evidence from the present studies suggest that horses are not susceptible to E. canis. One unsuccessful attempt was made to transmit E. canis to horse 6, Trial 1, by adult D. variabilis that were exposed as nymphs and may have been infected with the agent. Five attempts to transmit E. canis to horses through blood transfusion were unsuccessful. The unsuccessful attempts to transmit E. canis to horses 7 and 8, Trial 2 cannot be relied upon as evidence regarding susceptibility because the infective status of the ticks utilized in Trial 2 was not established.

The factors responsible for the apparent host specificity of E. canis, which contrasts with that of other Ehrlichia species, are not known. Although Nyindo studied the development of E. canis in canine monocytic cell culture and Smith et al. studied development of E. canis in R. sanguineus, the mechanism of entry into cells of the vertebrate and invertebrate hosts by elementary bodies has not been explored.[76,100] Immunological methods employing colloidial gold, perioxidase, or ferritin staining of timed sequential cell cultures and recombinant deoxyribonuclease studies have not been attempted. Investigations on the mode of infection of cells in both the vertebrate and invertebrate hosts and characterizations of the genomes and surface proteins of members of the Tribe Ehrlichieae may help to identify factors responsible for the host specificity of E. canis and to clarify taxonomic relationships among members of the Tribe Ehrlichieae.

Failure to demonstrate E. canis in selected tissues of experimentally exposed adult D. variabilis by light and electron microscopy may have resulted from the propagation and development of trypanosomes in the tick tissues. The source and identity of these trypanosomes could not be determined retrospectively with the materials and resources available. However, their presence, abundance, and disruption of the normal architecture of the tick tissues could have masked the presence of E. canis in the ticks. Their abundance implies a potential role for ticks in

transmission of trypanosomal organisms to human beings and domestic animals, especially in endemic areas where the reduviid and sand fly vectors are absent. Visceral leishmaniasis has been found in dogs from a kennel in Oklahoma and a research colony of English Foxhounds in Ohio.[3,103] The sand fly vectors of Leishmania species are not known to occur in either Oklahoma or Ohio. Although the origin of the infection in the Ohio dog was not determined, horizontal transmission was believed to be occurring in the research colony because 9 of 26 dogs in the colony had evidence of infection or exposure to Leishmania species.[103]

The incidental discovery of E. platys infections in four of thirteen dogs acquired from Oklahoma animal shelters establishes that the geographic range of E. platys includes Oklahoma as well as Florida and Louisiana. Whole blood transmission experiments confirmed that the organism can be transmitted during the acute rickettsemic phase of the infection, but indicated that a chronic carrier state does not develop. Prior infection with E. platys did not protect a dog from challenge exposure to E. canis, suggesting that cross protective immunity to E. canis does not develop. Further studies are needed to determine the longevity of E. platys infections and to establish whether protective immunity develops after recovery. It would also be interesting to know if simultaneous exposure of susceptible dogs to E. platys and other blood parasites

would have a potentiating effect on the severity of the disease produced by more virulent organisms. The natural mode of transmission for E. platys is not known, but circumstantial evidence suggest that ticks are involved. Future studies are needed to determine the natural mode of transmission for E. platys.

Attempted transmission of an Oklahoma isolate of a canine granulocytic Ehrlichia (CGE) to a horse and to mice by intraperitoneal inoculation of rickettsemic blood was unsuccessful. Failure to establish an infection in the horse and the horse's subsequent susceptibility to E. equi indicated that not all granulocytic isolates from dogs are E. equi.[24] The host range of this CGE isolate has not been determined, but preliminary results reported here indicate that mice are not susceptible and play no part in its epidemiology. The natural mode of transmission for this CGE has not been established and should be investigated. The pathogenic mechanism for the associated polyarthritis should be investigated and defined.

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APPENDIX

TABLE I

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF DOG 2 AFTER EXPOSURE TO EHRLICHIA CANIS BY INTRAVENOUS INOCULATION OF INFECTIVE CANINE WHOLE BLOOD

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	103.6	39.8	49.0	15,840	192.0
1	102.0	38.9	40.0		
2	102.4	39.1			
3	102.6	39.2			
4	102.2	39.0			
5	103.4	39.7			
6	103.8	39.9			
7[b]	104.0	40.0	36.5	11,110	43.0
8	104.6	40.3			
9	105.6	40.9			
10[a]	104.6	40.3			
11	106.0	41.1	34.0	6,710	61.0

[a]Morulae first detected in monocytic cells of the peripheral blood.
[b]Erythrophagocytosis and vacuolization of the cytoplasm of monocytic cells first detected on buffy coat smears.

TABLE II

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF DOG 3 AFTER EXPOSURE TO *EHRLICHIA CANIS* BY INTRAVENOUS INOCULATION OF INFECTIVE CANINE WHOLE BLOOD

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	103.0	39.4	36.0	12,540	240.0
1	103.0	39.4			
2	101.8	38.8			
3	103.6	39.8			
4	104.0	40.0			
5	104.0	40.0			
6	103.6	39.8			
7[b]	103.9	40.0	34.0	11,000	53.0
8	104.3	40.2			
9	105.2	40.7			
10	104.0	40.0			
11[a]	106.0	41.1	31.0	5,170	29.0

[a]Morulae first detected in monocytic cells of the peripheral blood.
[b]Erythrophagocytosis and vacuolization of the cytoplasm of monocytic cells first detected on buffy coat smears.

TABLE III

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF DOG 0924 AFTER EXPOSURE TO EHRLICHIA CANIS BY INTRAVENOUS INOCULATION OF INFECTIVE CANINE WHOLE BLOOD

Day	Body Temperature (^{o}F)	(^{o}C)	PCV (%)	WBC /µl	Platelet Count X10^{3}/µl
0	103.4	39.7	40.0	18,370	597.0
1	103.2	39.6			
2	102.8	39.3			
3	102.8	39.3			
4	103.2	39.6			
5	103.2	39.6			
6	102.4	39.1			
7	103.0	39.4	41.0		554.0
8	103.0	39.4			
9	102.0	38.9			
10	102.0	38.9			
11	103.0	39.4			
12[b]	102.0	38.9			
13	103.4	39.7			
14	103.2	39.6	36.0	9,570	85.0
15[a]	105.0	40.5			
16	104.2	40.1			

[a]Morulae first detected in monocytic cells of the peripheral blood.
[b]Erythrophagocytosis and vacuolization of the cytoplasm of monocytic cells on buffy coat smears.

TABLE IV

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF CONTROL DOG 1

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	102.0	38.9	37.0	9,570	165.0
1	102.6	39.2			
2	103.0	39.4			
3	102.6	39.2			
4	102.0	38.9			
5	103.0	39.4			
6	102.0	38.9			
7	104.0	40.0	37.5	13,970	319.0
8	103.2	39.6			
9	103.6	39.8			
10	103.0	39.4			
11	104.2	40.1	40.0	9,680	214.0

TABLE V

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF CONTROL DOG 0925

Day	Body Temperature (^{o}F)	(^{o}C)	PCV (%)	WBC /µl	Platelet Count X10^{3}/µl
0	103.2	39.6	42.0	15,070	405.0
1	103.6	39.8			
2	103.4	39.7			
3	103.4	39.7			
4	102.8	39.3			
5	102.4	39.1			
6	103.0	39.4			
7	103.2	39.6	40.0	17,600	498.0
8	103.0	39.4			
9	102.4	39.1			
10	102.4	39.1			
11	103.0	39.4			
12	101.4	38.6			
13	102.4	39.1			
14	102.4	39.1	39.0	15,180	536.0
15	103.0	39.4			
16	103.4	39.7			

TABLE VI

DAILY BODY TEMPERATURE, PCV, WBC, AND PLATELET COUNT VALUES OF PONY 1 AFTER EXPOSURE TO EHRLICHIA CANIS BY INTRAVENOUS INOCULATION OF RICKETTSEMIC CANINE WHOLE BLOOD

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	101.4	38.6	25.0	13,200	170.0
1	100.0	37.8	25.0	11,880	68.0
2	100.6	38.1	36.0	9,130	268.0
3	100.4	38.0	34.0	12,100	204.0
4	100.4	38.0	33.0	10,010	291.0
5	100.0	37.8	30.0	14,080	245.0
6	99.8	37.7	34.0	14,520	327.0
7	99.4	37.4	31.0	13,860	290.0
8	100.0	37.8	32.0	13,310	292.0
9	100.2	37.9	31.0	13,970	401.0
10	100.4	38.0	32.0	11,550	259.0
11	100.0	37.8	32.5	10,670	273.0
12	100.2	37.9	32.5	10,120	239.0
13	100.4	38.0	33.0	11,110	332.0
14	100.6	38.1	32.0	11,770	357.0
15	100.2	37.9	33.0	15,510	342.0
16	100.0	37.8	32.0	12,540	332.0
17	100.4	38.0	35.0	12,650	310.0
18	100.6	38.1	32.0	11,660	323.0
19	99.0	37.2	32.0	14,520	375.0
20	100.4	38.0	32.0	11,110	289.0
21	100.4	38.0	34.0	10,670	317.0
22	99.8	37.7	33.0	11,440	300.0
23	100.2	37.9	33.0	13,860	335.0
24	100.6	38.1	35.0	13,305	330.0
25	100.4	38.0	35.0	14,740	464.0
26	100.6	38.1	33.0	11,990	300.0
27	100.6	38.1	34.0	12,430	314.0
28	100.7	38.2	33.0	12,320	315.0
29	100.2	37.9	32.0	11,550	341.0
30	100.0	37.8	33.0	9,680	313.0

TABLE VII

DAILY BODY TEMPERATURE, PCV, WBC, AND PLATELET COUNT VALUES OF PONY 2 AFTER EXPOSURE TO EHRLICHIA CANIS BY INTRAVENOUS INOCULATION OF RICKETTSEMIC CANINE WHOLE BLOOD

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	100.6	38.1	31.0	12,870	200.0
1	102.0	38.9	33.0	15,180	261.0
2	100.6	38.1	32.0	14,850	261.0
3	100.4	38.0	30.0	19,580	228.0
4	100.0	37.8	33.0	17,820	282.0
5	100.4	38.0	33.0	17,600	235.0
6[a]	100.0	37.8	23.0	12,980	19.0
7	100.0	37.8	32.0	15,180	238.0
8	99.8	37.7	30.0	18,480	287.0
9	100.0	37.8	32.5	17,930	375.0
10	99.8	37.7	32.0	17,930	272.0
11	100.4	38.0	32.0	13,530	299.0
12	100.0	37.8	32.0	14,080	358.0
13	100.2	37.9	33.0	15,180	293.0
14	100.0	37.8	32.5	13,860	274.0
15	100.2	37.9	32.0	17,380	307.0
16	100.2	37.9	33.0	21,450	333.0
17	100.2	37.9	31.0	13,970	262.0
18	100.4	38.0	32.0	14,410	278.0
19	99.6	37,6	32.0	13,860	266.0
20	100.4	38.0	34.0	13,310	333.0
21	100.0	37.8	33.5	15,290	289.0
22	100.2	37.9	33.5	19,250	297.0
23	100.0	37.8	34.0	22,110	328.0
24	100.2	37.9	34.0	17,820	285.0
25	100.4	38.6	33.0	16,500	278.0
26	100.2	37.9	34.0	15,290	304.0
27	100.2	37.9	33.0	17,610	303.0
28	100.2	37.9	33.0	22,110	279.0
29	100.2	37.9	33.0	17,930	267.0
30	100.0	37.8	31.5	17,600	241.0

[a]Blood sample contained a small clot.

TABLE VIII

DAILY BODY TEMPERATURE, PCV, WBC, AND PLATELET COUNT VALUES OF PONY 3 AFTER EXPOSURE TO *EHRLICHIA CANIS* BY INTRAVENOUS INOCULATION OF RICKETTSEMIC CANINE WHOLE BLOOD

Day	Body Temperature (^{o}F)	(^{o}C)	PCV (%)	WBC /µl	Platelet Count X10^{3}/µl
0	102.4	39.1	35.0	17,600	214.0
1	101.0	38.3	38.0	15,180	235.0
2	100.6	38.1	39.0	12,100	308.0
3	101.0	38.3	36.0	14.080	201.0
4	100.6	38.1	34.0	13,420	240.0
5	100.2	37.9	35.0	12,980	189.0
6	100.6	38.1	35.5	11,440	249.0
7	100.0	37.8	34.5	11,770	303.0
8	100.6	38.1	37.0	18,700	289.0
9	100.0	37.8	35.0	12,650	274.0
10	99.8	37.7	35.0	13,420	389.0
11	100.2	37.9	36.0	14,300	310.0
12	100.6	38.1	34.0	11,000	246.0
13	101.0	38.3	38.0	11,880	300.0
14	101.0	38.3	37.0	9,020	310.0
15	100.6	38.1	35.0	11,990	318.0
16	100.0	37.8	37.0	9,460	301.0
17	100.4	38.0	37.0	10,450	296.0
18	100.6	38.1	37.0	11,660	322.0
19	99.6	37.6	37.0	11.100	242.0
20	99.8	37.7	37.0	14,520	362.0
21	100.0	37.8	38.0	15,400	319.0
22	100.6	38.1	35.0	15,180	265.0
23	100.6	38.1	37.0	11,880	297.0
24	100.6	38.1	38.0	11,550	297.0
25	100.8	38.2	34.0	13,420	303.0
26	100.4	38.0	36.0	13,090	256.0
27	100.4	38.0	37.0	12,210	311.0
28	100.8	38.2	37.0	15,730	261.0
29	100.4	38.0	33.0	14,630	290.0
30	100.4	38.0	36.0	13,420	286.0

TABLE IX

DAILY BODY TEMPERATURE, PCV, WBC, AND PLATELET COUNT VALUES OF PONY 4 AFTER EXPOSURE TO EHRLICHIA CANIS BY INTRAVENOUS INOCULATION OF RICKETTSEMIC CANINE WHOLE BLOOD

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	101.8	38.2	34.0	13,530	332.0
1	100.4	38.0	38.0	12,210	229.0
2	100.4	38.0	41.0	11,990	253.0
3	100.4	38.0	35.0	14,300	221.0
4	100.0	37.8	37.0	12,100	312.0
5	100.0	37.8	35.0	12,540	270.0
6	99.8	37.7	35.0	11,880	284,0
7	99.4	37.4	36.0	12,980	260.0
8	100.2	37.9	36.0	13,310	240.0
9	100.0	37.8	34.0	8,525	264.0
10	100.0	37.8	35.0	11,550	233.0
11	100.0	37.8	31.0	12,540	235.0
12	99.0	37.2	37.0	11,770	241.0
13	99.8	37.7	38.0	14,300	297.0
14	100.4	38.0	35.0	11,770	286.0
15	99.6	37.6	35.0	13,530	199.0
16	99.4	37.4	36.0	13,090	260.0
17	100.0	37.8	37.0	10,230	306.0
18	99.8	37.7	37.0	15,070	326.0
19	99.0	37.2	38.0	11,110	243.0
20	99.4	37.4	39.0	12,320	330.0
21	99.8	37.7	40.0	16,060	265.0
22	100.0	37.8	39.0	17,050	311.0
23	100.0	37.8	40.0	13,090	259.0
24	100.4	38.0	40.0	11,550	262.0
25	100.4	38.0	39.5	11,990	281.0
26	99.8	37.7	40.0	10,670	261.0
27	100.2	37.9		13,970	285.0
28	100.0	37.8	42.0	14,850	287.0
29	100.4	38.0	39.0	14,410	302.0
30	100.0	37.8	37.0	14,630	234.0

TABLE X

DAILY BODY TEMPERATURE, PCV, WBC, AND PLATELET COUNT VALUES OF PONY 9 AFTER EXPOSURE TO EHRLICHIA CANIS BY INTRAPERITONEAL INOCULATION OF WHOLE BLOOD FROM AN EHRLICHIA CANIS CARRIER DOG

Day	Body Temperature (^{o}F)	(^{o}C)	PCV (%)	WBC /µl	Platelet Count X10^{3}/µl
0	99.6	37.6	43.0	8,470	254.0
1	99.6	37.6	33.5	9,130	214.0
2	100.2	37.9	33.5	7,150	237.0
3	100.2	37.9	33.0	8,910	243.0
4	99.4	37.4	31.0	7,480	244.0
5	99.4	37.4	29.0	9,570	285.0
6	100.0	37.8	30.0	12,540	316.0
7	99.2	37.3	28.5	11,550	367.0
8	100.0	37.8	31.0	11,440	286.0
9	99.6	37.6	29.0	8,250	352.0
10	99.4	37.4	29.0	10,670	323.0
11	99.2	37.3	31.0	7,810	315.0
12	100.0	37.8	30.0	8,910	304.0
13	99.6	37.6	29.0	8,910	290.0
14	100.0	37.8	36.0	10,120	257.0
15	99.2	37.3	30.5	7,700	286.0
16	99.4	37.4	31.0	6,600	259.0
17	99.0	37.2	30.0	6,490	286.0
18	99.6	37.6	30.5	7,920	270.0
19	99.8	37.7	34.0	9,680	252.0
20	100.0	37.8	33.0	9,020	239.0
21[a]	99.0	37.2	32.0	7,150	273.0

[a]Found 120 day fetus during necropsy examination.

TABLE XI

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF DOG 7 AFTER ATTACHMENT AND REPLETE FEEDING OF NON-EXPOSED, ADULT *DERMACENTOR VARIABILIS*

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	102.4	39.1	43.0	16,270	295.0
1	102.4	39.1			
2	103.6	39.8			
3	102.6	39.2			
4	103.0	39.4			
5	103.0	39.4			
6	102.6	39.2			
7	103.2	39.6			
8	103.4	39.7	47.0	11,220	353.0
9	103.0	39.4			
10	103.0	39.4			
11	102.6	39.2			
12	102.0	38.9			
13	101.0	38.3			
14	101.8	38.8			
15	102.6	39.2	45.0	11,770	232.0
16	102.0	38.9			
17	102.4	39.1			
18	103.4	39.7			
19	101.4	38.6			
20	100.6	38.1			
21	101.4	38.6			
22	101.6	38.7			
23	101.0	38.3	41.0	11,220	152.0
24	102.2	39.0			
25	102.2	39.0			
26	101.8	38.8			
27	101.8	38.8			
28	101.0	38.3			
29	102.0	38.9			
30[a]	100.6	38.1	44.0	13,530	113.0

[a]Blood sample contained a small clot.

TABLE XII

DAILY BODY TEMPERATURE, and WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF DOG 8 AFTER ATTACHMENT AND REPLETE FEEDING OF ADULT DERMACENTOR VARIABILIS EXPOSED TO EHRLICHIA CANIS AS NYMPHS

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	101.4	38.6	39.0	23,210	440.0
1	101.0	38.3			
2	101.2	38.4			
3	101.0	38.3			
4	101.2	38.4			
5	101.0	38.3			
6	100.2	37.9			
7	101.0	38.3	35.0	35,970	410.0
8	100.8	38.2			
9	100.8	38.2			
10	101.2	38.4			
11	101.4	38.6			
12	101.0	38.3			
13	101.4	38.6			
14	101.0	38.3	39.0	22,550	545.0
15	101.0	38.3			
16	101.0	38.3			
17	101.0	38.3			
18	101.0	38.3			
19	100.6	38.1			
20	101.4	38.6			
21	101.4	38.6	33.0	28,840	459.0
22	101.0	38.3			
23	101.0	38.3			
24	100.0	38.1			
25	101.4	38.6			
26	99.0	37.2			
27[a]	102.0	38.9			
28	102.0	38.9	37.0	21,120	417.0
29	102.0	38.9			
30	102.0	38.9			

[a]Whelped a litter of 8 puppies.

TABLE XIII

DAILY BODY TEMPERATURE, PCV, WBC, AND PLATELET COUNT VALUES OF DOG 4 AFTER ATTACHMENT AND REPLETE FEEDING OF ADULT DERMACENTOR VARIABILIS EXPOSED TO EHRLICHIA CANIS AS NYMPHS

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	102.4	39.1	31.5	15,510	258.0
1	102.2	39.0	38.0	20,350	344.0
2	102.2	39.0	36.0	19,360	374.0
3	102.2	39.0	36.0	21,010	340.0
4	102.0	38.9	36.0	15,400	317.0
5	102.2	39.0	37.0	21,120	313.0
6	102.0	38.9	37.0	18,030	400.0
7	102.0	38.9	37.0	21,340	412.0
8	102.2	39.0	38.0	19,140	408.0
9	102.8	39.3	39.0	18,260	393.0
10	100.8	38.2	39.0	17,490	475.0
11	102.0	39.0	38.0	22,990	457.0
12	101.4	38.6	39.0	18,700	544.0
13	102.0	38.9	38.0	23,540	522.0
14	102.8	39.3	42.0	22,440	497.0
15[b]	102.8	39.3	42.0	18,040	350.0
16	102.8	39.3	40.0	18,480	377.0
17[a]	103.6	39.8	40.0	14,300	314.0
18	104.4	40.2	41.0	10,670	173.0
19	104.6	40.3	39.0	11,990	117.0
20	105.2	40.7	41.0	10,560	45.0
21	104.0	40.0	39.0	9,130	31.0
22	104.0	40.0	39.0	10,120	50.0
23	103.8	39.9	38.0	11,660	61.0
24	104.0	40.0	39.0	13,530	61.0

[a]Morulae first detected in monocytic cells of the peripheral blood.
[b]Erythrophagocytosis and vacuolization of the cytoplasm of monocytic cells first detected on buffy coat smears.

TABLE XIV

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF DOG 0962 AFTER ATTACHMENT AND REPLETE FEEDING OF ADULT *DERMACENTOR VARIABILIS* EXPOSED TO *EHRLICHIA CANIS* AS NYMPHS

Day	Body Temperature (°F)	Body Temperature (°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	103.6	39.8	41.0	36,410	280.0
1	102.8	39.3			
2	103.8	39.9			
3	103.6	39.8			
4	102.6	39.2			
5	103.0	39.4			
6	104.8	40.4			
7	104.6	40.3	46.0	21,010	387.0
8	104.2	40.1			
9	103.8	39.9			
10	104.4	40.2			
11	103.8	39.9			
12	103.0	39.4			
13	102.2	39.0			
14	103.8	39.9	47.0	15,620	376.0
15	103.0	39.4			
16	103.0	39.4			
17	102.0	38.9			
18	102.6	39.8			
19	102.0	38.6			
20	101.4	38.6			
21	103.4	39.7	54.0	7,910	329.0
22	103.8	39.9			
23	103.8	39.9			
24	102.4	39.1			
25	104.2	40.1			
26	102.4	39.1			
27	102.0	38.9			
28	101.8	38.8	55.0	11,770	351.0
29	102.6	39.2			
30	99.8	37.7			

TABLE XV

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF DOG 0926 AFTER ATTACHMENT AND REPLETE FEEDING OF ADULT *DERMACENTOR VARIABILIS* EXPOSED TO *EHRLICHIA CANIS* AS NYMPHS

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	103.6	39.8	56.0	23,100	412.0
1	102.2	39.0			
2	102.6	39.2			
3	102.2	39.0			
4	103.0	39.4			
5	102.0	38.9			
6	103.6	39.8			
7	102.4	39.1	54.0	11,550	347.0
8	101.8	38.8			
9	102.4	39.1			
10	102.0	38.9			
11	102.2	39.0			
12	102.0	38.9			
13	102.0	38.9			
14	101.8	38.8	50.0	14,080	320.0
15	102.8	39.3			
16	102.8	39.3			
17	102.6	39.2			
18	103.0	39.4			
19	102.6	39.2			
20	102.6	39.2			
21	103.6	39.8	56.0	11,330	408.0
22	102.6	39.2			
23	102.2	39.0			
24	102.8	39.3			
25	102.2	39.0			
26	102.0	38.9			
27	101.4	38.6			
28	102.4	39.1	55.0	14,190	317.0
29	101.4	38.6			
30	101.6	38.7			

TABLE XVI

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF DOG 0984 AFTER ATTACHMENT AND REPLETE FEEDING OF ADULT *DERMACENTOR VARIABILIS* EXPOSED TO *EHRLICHIA CANIS* AS NYMPHS

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	103.0	39.4	41.0	22,000	545.0
1	103.4	39.7			
2	102.4	39.1			
3	103.6	39.8			
4	103.0	39.4			
5	102.6	39.2			
6	101.4	38.6			
7	102.6	39.2	34.0	14,850	570.0
8	103.2	39.6			
9	103.2	39.6			
10	103.0	39.4			
11	103.4	39.7			
12	102.8	39.3			
13	103.4	39.7			
14	102.8	39.3	39.0	13,970	462.0
15	103.6	39.8			
16	103.4	39.7			
17	103.4	39.7			
18	103.8	39.9			
19	103.2	39.6			
20	104.6	40.3			
21	103.0	39.4	41.0	11,110	446.0
22	103.0	39.4			
23	103.0	39.4			
24	103.0	39.4			
25	103.4	39.7			
26	103.6	39.8			
27	103.2	39.6			
28	103.0	39.4	43.0	11,990	556.0
29	103.2	39.6			
30	103.2	39.6			

TABLE XVII

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF DOG 0983 AFTER ATTACHMENT AND REPLETE FEEDING OF NON-EXPOSED, ADULT *DERMACENTOR VARIABILIS*

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	103.0	39.4	36.5	18,810	516.0
1	103.6	39.8			
2	103.8	39.9			
3	103.6	39.8			
4	104.2	40.1			
5	104.6	40.3			
6	103.0	39.4			
7	104.2	40.1	38.0	23,430	582.0
8	103.4	39.7			
9	103.2	39.6			
10	103.2	39.6			
11	103.4	39.7			
12	103.0	39.4			
13	104.0	40.0			
14	104.0	40.0	40.0	18,100	414.0
15	104.0	40.0			
16	104.0	40.0			
17	103.6	39.8			
18	103.0	39.4			
19	104.6	40.3			
20	104.8	40.4			
21	103.2	39.6	38.0	15,840	504.0
22	102.4	39.1			
23	103.6	39.8			
24	103.4	39.7			
25	103.4	39.7			
26	102.4	39.1			
27	104.2	40.1			
28	104.6	40.3	42.0	16,060	531.0
29	104.2	40.1			
30	103.2	39.6			

TABLE XVIII

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF DOG 0925 AFTER INTRAVENOUS INOCULATION WITH TISSUES OF UNFED ADULT *DERMACENTOR VARIABILIS* EXPOSED TO *EHRLICHIA CANIS* AS NYMPHS

Day	Body Temperature (^{o}F)	(^{o}C)	PCV (%)	WBC /µl	Platelet Count X10^{3}/µl
0	102.4	39.1	43.0	19,690	572.0
1	103.6	39.8			
2	103.0	39.4			
3	103.2	39.6			
4	103.0	39.4			
5	102.4	39.1			
6	103.0	39.4			
7	104.0	40.0	44.0	17,820	453.0
8	103.8	39.9			
9	103.0	39.4			
10	104.0	40.0			
11	103.0	39.4			
12	102.0	38.9			
13	103.2	39.6			
14	102.6	39.2	46.0	24,200	474.0
15	103.8	39.9			
16	103.0	39.4			
17	102.6	39.2			
18	103.0	39.4			
19	103.2	39.6			
20	103.0	39.4			
21	103.8	39.9	47.0	19.690	517.0
22	103.4	39.7			
23	103.0	39.4			
24	103.2	39.6			
25	102.6	39.2			
26	104.0	40.0			
27	102.0	38.9			
28	104.0	40.0	47.0	17,380	454.0
29	103.4	39.7			
30	104.0	40.0			

TABLE XIX

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF DOG 0999 AFTER INTRAVENOUS INOCULATION WITH TISSUES OF INCUBATED, UNFED, ADULT *DERMACENTOR VARIABILIS* EXPOSED TO *EHRLICHIA CANIS* AS NYMPHS

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0[a]	101.6	38.7	45.0	10,890	129.0
1	101.4	38.6			
2	102.0	38.9			
3	101.0	38.3			
4	102.0	38.9			
5	102.2	39.0			
6	101.0	38.3			
7	100.4	38.0	46.0	12,430	105.0
8	100.8	38.2			
9	101.8	38.8			
10	102.0	38.9			
11	102.0	38.9			
12	102.4	39.1			
13	101.4	38.6			
14	101.8	38.8	45.0	11,220	13.0
15	101.8	38.8			
16	100.0	37.8			
17	101.0	38.3			
18	101.2	38.4			
19	100.0	37.8			
20	101.0	38.3			
21	101.6	38.7	48.0	13,750	133.0
22	102.4	39.1			
23	101.4	38.6			
24	102.6	39.2			
25	102.2	39.0			
26	102.4	39.1			
27	102.6	39.2			
28	102.2	39.0	48.0	13,970	17.0
29	102.0	38.9			
30	102.4	39.1			

[a]Morulae of *E. platys* first detected in platelets on peripheral blood and buffy coat smears.

TABLE XX

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF DOG 0933 AFTER INTRAVENOUS INOCULATION WITH TISSUES OF UNFED, NON-EXPOSED, ADULT *DERMACENTOR VARIABILIS*

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	102.0	38.9	44.0	23,650	225.0
1	101.4	38.6			
2	102.0	38.9			
3	101.6	38.7			
4	101.2	38.4			
5	101.0	38.3			
6	102.6	39.2			
7	102.0	38.9	43.0	18,700	326.0
8	102.4	39.1			
9	101.6	38.7			
10	102.4	39.1			
11	102.0	38.9			
12	102.2	39.0			
13	102.2	39.0			
14	102.4	39.1	43.0	17,160	326.0
15	102.6	39.2			
16	102.0	38.9			
17	102.0	38.9			
18	101.0	38.3			
19	101.6	38.7			
20	101.0	38.3			
21	102.0	38.9	45.0	13,860	256.0
22	101.0	38.3			
23	101.0	38.3			
24	101.4	38.6			
25	101.0	38.3			
26	101.0	38.3			
27	101.0	38.3			
28	101.8	38.8	43.0	14,410	263.0
29	101.4	38.6			
30	102.0	38.9			

TABLE XXI

DAILY BODY TEMPERATURE AND WEEKLY PCV, WBC, AND PLATELET COUNT VALUES OF DOG 0961 AFTER INTRAVENOUS INOCULATION WITH TISSUES OF UNFED, INCUBATED, NON-EXPOSED, ADULT *DERMACENTOR VARIABILIS*

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	102.2	39.0	46.0	11,660	233.0
1	101.6	38.7			
2	101.6	38.7			
3	102.6	39.2			
4	103.2	39.0			
5	102.2	39.0			
6	102.0	39.0			
7	101.0	38.3	44.0	14,960	250.0
8	101.6	38.7			
9	101.6	38.7			
10	102.0	38.9			
11	102.4	39.1			
12	103.6	39.8			
13	101.0	38.3			
14	101.4	38.6	47.0	12,870	278.0
15	101.2	38.4			
16	102.2	39.0			
17	102.6	39.2			
18	101.0	38.3			
19	102.8	39.3			
20	102.6	39.2			
21	102.4	39.1	48.0	11,550	278.0
22	103.0	39.4			
23	101.4	38.6			
24	101.6	38.7			
25	102.8	39.3			
26	101.8	38.8			
27	102.6	39.1			
28	102.0	38.9	47.0	12,760	271.0
29	102.4	39.1			
30	102.0	38.9			

TABLE XXII

DAILY BODY TEMPERATURE, PCV, WBC, AND PLATELET COUNT VALUES OF PONY 5 AFTER ATTACHMENT AND REPLETE FEEDING OF NON-EXPOSED, ADULT DERMACENTOR VARIABILIS

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	100.0	37.8	36.0	11,880	337.0
1	100.4	38.0	33.0	11,550	307.0
2	100.0	37.8	34.0	10.230	327.0
3	100.0	37.8	36.0	10,230	296.0
4	99.8	37.7	35.5	8,910	279.0
5	100.2	37.9	37.0	12,980	297.0
6	99.8	37.7	35.0	10,670	321.0
7	100.6	38.1	33.0	9,130	307.0
8	99.8	37.7	35.0	13,080	307.0
9	99.4	37.4	36.0	12,430	344.0
10	100.0	37.8	37.0	11,110	279.0
11	99.4	37.4	35.0	9,020	293.0
12	99.4	37.4	35.0	11,220	323.0
13	99.8	37.7	34.0	9,240	302.0
14	99.6	37.6	35.0	11,660	263.0
15	100.0	37.8	36.0	10,230	302.0
16	99.8	37.7	36.0	9,910	284.0
17	99.4	37.4	36.0	11,220	281.0
18	99.8	37.7	35.0	11,330	278.0
19	99.6	37.6	34.0	11,220	271.0
20	99.6	37.6	36.0	10,890	294.0
21	99.4	37.4	35.0	10,120	259.0
22	99.8	37.7	34.0	11,990	309.0
23	99.8	38.2	34.0	12,650	295.0
24	99.6	38.1	34.0	8,910	293.0
25	99.0	37.2	35.0	9,460	278.0
26	100.0	37.8	35.0	11,220	310.0
27	98.6	37.0	33.0	8,340	313.0
28	99.6	37.6	36.0	11,990	275.0
29	100.0	37.8	35.0	11,990	293.0
30	100.0	37.8	38.0	9,680	260.0

TABLE XXIII

DAILY BODY TEMPERATURE, PCV, WBC, AND PLATELET COUNT VALUES OF PONY 6 AFTER ATTACHMENT AND REPLETE FEEDING OF ADULT *DERMACENTOR VARIABILIS* EXPOSED TO *EHRLICHIA CANIS* AS NYMPHS

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	100.0	37.8	33.0	16,830	277.0
1	101.0	38.3	34.0	13,310	259.0
2	100.0	37.8	37.0	16,170	278.0
3	99.8	37.7	37.0	17,050	383.0
4	99.6	37.6	40.0	14,520	323.0
5	99.8	37.7	38.0	14,420	296.0
6	99.4	37.4	38.0	14,080	372.0
7	99.6	37.6	36.0	15,620	322.0
8	100.2	37.9	37.0	15,950	318.0
9	100.0	37.8	38.0	14,300	301.0
10	99.8	37.7	39.0	15,620	255.0
11	99.8	37.7	41.0	16,500	293.0
12	99.8	37.7	40.0	15,950	300.0
13	99.8	37.7	41.0	13,640	287.0
14	99.6	37.6	39.0	11,990	294.0
15	100.0	37.8	41.0	17,210	247.0
16	100.2	37.9	39.5	14,190	265.0
17	100.2	37.9	40.0	11,990	257.0
18	100.2	37.9	41.0	13,200	248.0
19	99.8	37.7	39.0	15,400	253.0
20	100.2	37.9	40.0	13,640	250.0
21	99.8	37.7	41.0	13,200	258.0
22	100.2	37.9	45.0	14,080	252.0
23	100.4	38.0	40.0	13,420	253.0
24	99.0	37.2	40.0	11,110	260.0
25	99.0	37.2	39.0	13,420	289.0
26	100.2	37.9	40.0	13,530	262.0
27	99.0	37.2	40.0	11,440	253.0
28	101.0	38.3	40.0	13,530	243.0
29	100.4	38.0	40.0	14,520	250.0
30	100.2	37.9	40.0	12,650	232.0

TABLE XXIV

DAILY BODY TEMPERATURE, PCV, WBC, AND PLATELET COUNT VALUES OF PONY 7 AFTER ATTACHMENT AND REPLETE FEEDING OF ADULT *DERMACENTOR VARIABILIS* EXPOSED TO *EHRLICHIA CANIS* AS NYMPHS

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	100.2	37.9	33.0	10,010	310.0
1	100.2	37.9	33.0	12,430	307.0
2	100.4	38.0	31.0	13,750	339.0
3	100.6	38.1	31.0	13,210	367.0
4	100.4	38.0	32.0	13,530	324.0
5	100.6	38.1	33.0	14,410	290.0
6	100.0	37.8	32.5	10,120	384.0
7	101.0	38.3	31.0	14,850	361.0
8	99.0	37.2	31.0	13,750	321.0
9	100.8	38.2	31.0	17,270	320.0
10	99.2	37.3	32.0	14,740	330.0
11	99.2	37.3	29.5	12,320	300.0
12	99.0	37.2	31.0	12,980	355.0
13	99.8	37.2	33.0	14,630	392.0
14	100.2	37.9	35.0	20,460	369.0
15	100.2	37.9	33.0	14,740	305.0
16	101.0	38.3	34.0	15,290	356.0
17	101.0	38.3	34.0	14,300	390.0
18	99.2	37.3	32.0	11,660	349.0
19	99.4	37.4	32.0	10,340	281.0
20	99.4	37.4	35.0	13,420	288.0
21	100.6	38.1	34.0	14,960	349.0
22	99.8	37.7	34.0	15,730	356.0
23	100.6	38.1	34.0	14,740	33.00
24	99.8	37.7	32.0	12,320	311.0
25	99.6	37.6	33.0	11,440	285.0
26	100.0	37.8	36.0	15,620	315.0
27	100.4	38.0	33.0	14,850	350.0
28	99.8	37.7	35.0	11,990	305.0
29	99.8	37.7	35.0	12,320	270.0
30	99.8	37.7	32.5	14,740	313.0

TABLE XXV

DAILY BODY TEMPERATURE, PCV, WBC, AND PLATELET COUNT VALUES OF PONY 8 AFTER ATTACHMENT AND REPLETE FEEDING OF ADULT *DERMACENTOR VARIABILIS* EXPOSED TO *EHRLICHIA CANIS* AS NYMPHS

Day	Body Temperature (°F)	(°C)	PCV (%)	WBC /µl	Platelet Count X10^3/µl
0	99.6	37.6	34.0	14,080	349.0
1	99.8	37.7	35.0	10,670	324.0
2	99.8	37.7	32.0	14,410	371.0
3	101.0	38.3	29.0	16,830	417.0
4	100.4	38.0	28.0	14,630	320.0
5	99.8	37.7	30.0	10,890	384.0
6	100.0	37.8	33.0	10,010	442.0
7	101.0	38.3	29.0	10,450	505.0
8	99.6	37.6	29.0	15,510	407.0
9	99.6	37.6	31.0	15,510	419.0
10	100.2	37.9	31.0	13,310	381.0
11	99.2	37.3	32.0	13,970	347.0
12	99.0	37.2	34.0	11,550	417.0
13	99.4	37.4	33.0	13,530	429.0
14	99.6	37.6	32.0	16.390	350.0
15	100.0	37.8	32.0	13,640	336.0
16	100.0	37.8	32.0	12,090	367.0
17	99.8	37.7	32.0	13,420	369.0
18	99.8	37.7	31.0	10,120	283.0
19	99.0	37.2	32.0	11,770	314.0
20	98.0	36.8	35.0	12,870	350.0
21	99.8	37.7	35.0	11,880	388.0
22	100.2	37.9	34.0	11,220	349.0
23	98.8	37.1	34.0	9,790	344.0
24	99.2	37.3	31.0	10,010	320.0
25	100.6	38.1	33.0	10,670	379.0
26	99.8	37.7	32.0	9,350	328.0
27	99.4	37.4	32.0	10,780	345.0
28	99.0	37.2	34.0	11,440	310.0
29	100.6	38.1	32.0	11,990	347.0
30	100.6	38.1	32.0	10,890	383.0

TABLE XXVI

PRE-EXPOSURE AND POST-EXPOSURE INDIRECT FLUORESCENT ANTIBODY TITERS TO *EHRLICHIA CANIS*, *EHRLICHIA EQUI*, *EHRLICHIA RISTICII*, AND *RICKETTSIA RICKETTSII* FOR THE DOGS OF TRIAL 1 AND TRIAL 2

Dog		*Ehrlichia canis*	*Ehrlichia equi*	*Ehrlichia risticii*	*Rickettsia rickettsii*
1	control	<1:10	<1:10	<1:10	<1:64
1	control	<1:10	<1:10	<1:10	<1:64
2	pre-exp	<1:10	<1:10	<1:10	<1:64
2	post-exp	≥1:1600	<1:10	<1:10	<1:64
3	pre-exp	<1:10	<1:10	<1:10	<1:64
3	post-exp	≥1:800	<1:10	<1:10	<1:64
4	pre-exp	<1:10	<1:10	<1:10	≥1:1024
4	post-exp	≥1:200	<1:10	<1:10	≥1:512
7	pre-exp	<1:10	<1:10	<1:10	≥1:128
7	post-exp	<1:10	<1:10	<1:10	≥1:64
8	pre-exp	<1:10	<1:10	<1:10	≥1:128
8	post-exp	<1:10	<1:10	<1:10	≥1:128
0924	pre-exp	<1:10	<1:10	<1:10	<1:64
0924	post-exp	≥1:20	<1:10	<1:10	>1:64
0925	control	<1:10	<1:10	<1:10	<1:64
0925	control	<1:10	<1:10	<1:10	<1:64
0925	pre-exp	<1:10	<1:10	<1:10	<1:64
0925	post-exp	<1:10	<1:10	<1:10	<1:64
0933	control	<1:10	<1:10	<1:10	≥1:2048
0933	control	<1:10	<1:10	<1:10	≥1:2048
0926	pre-exp	<1:10	<1:10	<1:10	<1:64
0926	post-exp	<1:10	<1:10	<1:10	<1:64
0962	pre-exp	<1:10	<1:10	<1:10	<1:64
0962	post-exp	<1:10	<1:10	<1:10	<1:64
0983	control	<1:10	<1:10	<1:10	<1:64
0983	control	<1:10	<1:10	<1:10	<1:64
0984	pre-exp	<1:10	<1:10	<1:10	<1:64
0984	post-exp	<1:10	<1:10	<1:10	<1:64
0961	pre-exp	<1:10	<1:10	<1:10	≥1:128
0961	post-exp	<1:10	<1:10	<1:10	≥1:128
0999	pre-exp	<1:10	≥1:10	<1:10	<1:64
0999	post-exp	<1:10	≥1:20	<1:10	<1:64

Table XXVII

PRE-EXPOSURE AND POST-EXPOSURE INDIRECT FLUORESCENT ANTIBODY TITERS TO *EHRLICHIA CANIS*, *EHRLICHIA EQUI*, *EHRLICHIA RISTICII*, AND *RICKETTSIA RICKETTSII* FOR PONIES OF TRIAL 1 AND TRIAL 2

Pony		*Ehrlichia canis*	*Ehrlichia equi*	*Ehrlichia risticii*	*Rickettsia rickettsii*
1	pre-exp	<1:10	<1:10	<1:10	<1:64
1	post-exp	<1:10	<1:10	<1:10	<1:64
2	pre-exp	<1:10	<1:10	<1:10	<1:64
2	post-exp	<1:10	<1:10	<1:10	<1:64
3	pre-exp	<1:10	<1:10	<1:10	<1:64
3	post-exp	<1:10	<1:10	<1:10	<1:64
4	pre-exp	<1:10	<1:10	<1:10	<1:64
4	post-exp	<1:10	<1:10	<1:10	<1:64
5	control	<1:10	<1:10	<1:10	<1:64
5	control	<1:10	<1:10	<1:10	<1:64
6	pre-exp	<1:10	<1:10	<1:10	<1:64
6	post-exp	<1:10	<1:10	<1:10	<1:64
7	pre-exp	<1:10	<1:10	<1:10	≥1:512
7	post-exp	<1:10	<1:10	<1:10	≥1:512
8	pre-exp	<1:10	<1:10	<1:10	≥1:128
8	post-exp	<1:10	<1:10	<1:10	≥1:128
9	pre-exp	<1:10	<1:10	<1:10	≥1:256
9	post-exp	<1:10	<1:10	<1:10	≥1:256

TABLE XXVIII

DAILY BODY TEMPERATURE AND PCV, AND WEEKLY WBC AND PLATELET COUNT VALUES OF HORSE 10 AFTER EXPOSURE TO A CANINE GRANULOCYTIC *EHRLICHIA* SPECIES BY INTRAPERITONEAL INOCULATION WITH RICKETTSEMIC CANINE WHOLE BLOOD

Day	Body Temperature (^{o}F)	(^{o}C)	PCV (%)	WBC /µl	Platelet Count X10^{3}/µl
0	100.8	38.2	33.0		
1	100.6	38.1	32.0		
2	100.4	37.4	30.0		
3	99.0	37.2	34.0		
4	99.2	37.3	29.0		
5	99.8	37.7	34.0		
6	100.0	37.8	32.0	9,570	219.0
7	100.2	37.9	30.0		
8	100.0	37.8	30.0		
9	99.8	37.7	29.0		
10	100.0	37.8	32.5	12,980	231.0
11	100.0	37.8	29.0		
12	100.2	37.9	30.0		
13	100.0	37.8	33.0		
14	99.4	37.4	33.0		
15	99.2	37.3	32.0		
16	100.0	37.8	32.0		
17	100.0	37.8	37.0	9,020	239.0
18	100.0	37.8	36.0		
19	100.0	37.8	32.0		
20	99.6	37.6	34.0		

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