EXPERIMENTAL INFECTION OF *BABESIA GIBSONI* IN COYOTES (*CANIS LATRANS*)

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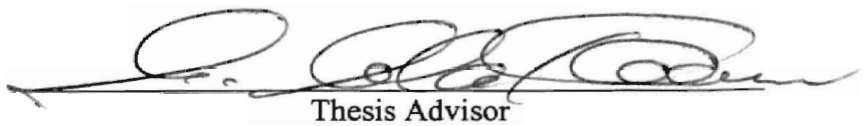

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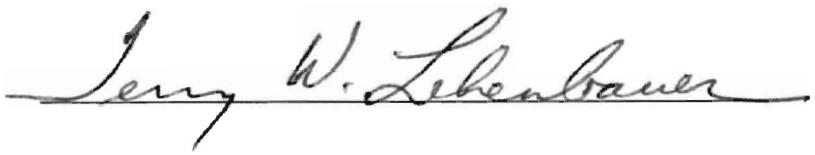

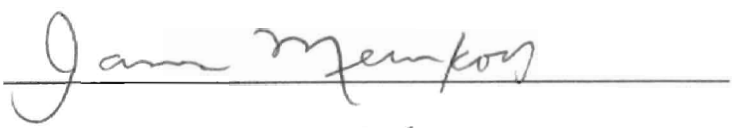

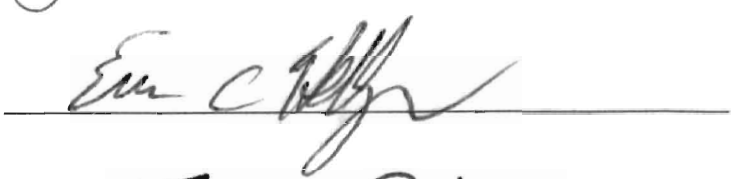

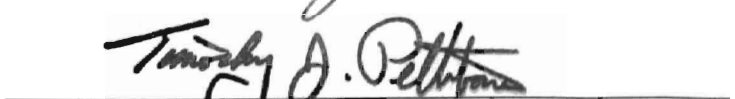

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CHAPTER I

Introduction

Babesia species are tick-transmitted protozoan blood parasites that infect numerous vertebrates worldwide. Infections are characterized by a regenerative hemolytic anemia, anorexia, depression, multi-organ dysfunction, and occasionally death. Throughout the world, canine babesiosis occurs as the result of infection with either the "large" *Babesia* spp., *B. canis*, or a "small" *Babesia* spp., *B. gibsoni*. In the United States, *B. canis* is the most frequently reported species. With a single exception (Anderson et al., 1979), *B. gibsoni* was not known to occur in North America until the 1990s when reports were documented from California (Conrad et al., 1991; Yamane et al., 1994; Wozniak et al., 1997) and various midwestern and eastern states (Birkenheuer et al., 1999; Irizarry-Rovira et al., 2001; Kocan et al., 2001; Macintire et al., 2002). Recent genetic analysis of the entire 18S nuclear subunit ribosomal RNA gene of all known isolates of *B. gibsoni* demonstrated that there are in fact not one, but three genetically distinct small canine *Babesia* spp. parasites, at least two of which occur in the United States (Kjemtrup et al., 2000; Zahler et al., 2000a; Zahler et al., 2000b). These three isolates are the originally discovered Asian *B. gibsoni* isolate found in the midwestern and eastern United States, an isolate found in California and an isolate found in Spain. In North America, *B. gibsoni sensu stricto* has been reported predominantly in the American Staffordshire Terrier (AST) and appears to be maintained within breeding colonies through mechanical blood transfer and possibly

vertical transmission (Abu et al., 1973; Correa, 1974; Irizarry-Rovira et al., 2001). Currently, there are no pharmacological agents available that are effective in clearing the parasite from infected dogs. It is speculated that the sudden increase of *B. gibsoni* cases in the United States is the result of importation of infected dogs from endemic locations outside of North America such as Okinawa, Japan (Farwell et al., 1982).

Infected domestic dogs remain carriers of *B. gibsoni*, therefore they not only increase the risk of spreading the disease to other dogs, but also pose a potential risk to wild canids where the impact of infection is unknown. Although naturally occurring *B. gibsoni* infections in wild canids have not been reported in the United States, it is known that interspecies interactions of domestic and wild canids occur through both natural and man-made conditions (Mengel, 1971; Freeman and Shaw, 1979; Schmitz and Kolenosky, 1985).

The purpose of this study was to determine if coyotes from Oklahoma were susceptible to experimental infection with *B. gibsoni* and to then compare the clinical course of disease in the coyotes to similar cases reported in domestic and wild dogs. Because a competent tick vector has not yet been identified (Sen, 1933; Datta, 1941; Yamane et al., 1993; Higuchi et al., 1995), this study also evaluated several commonly occurring ticks from Oklahoma for the ability to transstadially acquire *B. gibsoni*.

The objectives of this study were:

1. To evaluate the occurrence and extent of clinical disease in captive-raised coyotes following experimental infection via intravenous needle passage of whole blood infected with a canine *B. gibsoni* isolate from Oklahoma.

2. To compare the occurrence and clinical extent of disease produced by the experimental *B. gibsoni* infection in coyotes to the disease previously reported in mixed-breed dogs experimentally infected with the Oklahoma isolate, dogs naturally and experimentally infected with the California isolate, and coyotes and coydogs experimentally infected with an uncharacterized isolate from New England.

3. To evaluate the vector competence of three species of ticks (*Amblyomma americanum, Dermacentor variabilis,* and *Rhipicephalus sanguineus*) for transstadial acquisition of an Oklahoma isolate of *B. gibsoni* by microscopic examination of salivary glands.

CHAPTER II

Materials and Methods

Agent

The *B. gibsoni* isolate used in this study was obtained from a nine-month-old male American Staffordshire Terrier (AST) from Central Oklahoma (source dog) that had been naturally infected with *B. gibsoni*. Prior to initiating the experimental blood transfer study, blood from the source dog was tested by enzyme-linked immunosorbent assay (ELISA) for *Ehrlichia canis* and Rocky Mountain Spotted Fever (RMSF, *Rickettsia rickettsii*) antibodies at the Oklahoma Animal Disease Diagnostic Laboratory (Stillwater, Oklahoma, USA) via the Pan-bio INDX dipstick (Columbia, Maryland, USA). The blood was also tested for heartworm (*Dirofilaria immitis*) antigen via immunoassay (Snap® test, IDEXX, Westbrook, Maine, USA). Diff-Quik® (Dade Behring, Duluth, Georgia, USA) stained blood films were examined microscopically and the polymerase chain reaction (PCR) was performed to verify infection with *B. gibsoni*. For PCR, primers that amplify most of the 18S rRNA gene from the order Piroplasmorida were used (Kocan et al., 2001). DNA was extracted from 200µl of whole blood with the QIAamp Blood Kit (Qiagen Inc., Valencia, California, USA) according to manufacture's instructions. PCR amplification was performed as previously described by Kocan et al. (2001). The sequence of the forward primer (BH1-NSSRNA-) was 5'-GTCAGGATCCTGGTTGATCCTGCCAG-3' and that of the reverse primer (ER1-NSSRNA-3-2) was 5'-

GACTGAATTCGACTTCTCCTTCCTTTAAG-3'. The positive control reactions used DNA isolated from whole blood known to carry an Oklahoma isolate of *B. gibsoni*. The negative control was distilled, deionized water. PCR products were purified using the Qiagen, QIAquick PCR Purification Kit and sequenced at the Oklahoma State University Recombinant/DNA Protein Research Facility (Stillwater, OK, USA) using a 373 A automated DNA sequencer (Applied Biosystems, Foster City, California, USA).

Experimental Animals

Three male and 3 female wild-caught five-month-old coyotes were used in the present study. The coyotes were obtained at 3 weeks of age by United States Department of Agriculture Animal Control personnel and maintained in captivity. Animals were housed in shaded outdoor cement runs at the Wild Animal Research Facility, Oklahoma State University College of Veterinary Medicine (Stillwater, Oklahoma, USA) and observed daily for changes in attitude, appetite, and body condition. Coyotes were fed dry and canned commercial dog food and water was available *ad libitum*. Tick control was maintained through monthly application of fipronil (Frontline®, Merial, Iselin, New Jersey, USA). All coyotes were tested by ELISA (Pan-bio INDX, Columbia, Maryland, USA) for *E. canis* and RMSF and Diff-Quik® stained blood films were examined microscopically for blood-borne parasites.

Exposure of Coyotes to Babesia gibsoni

Whole blood was collected from the source dog via the jugular vein and stored in tubes containing potassium EDTA. Within 3 hours of the blood collection, the six coyotes were sedated via intramuscular injection with a combination of 4 mg/kg ketamine HCl (KetaVed, Vedco Inc., St. Joseph, Missouri, USA) and 2 mg/kg xylazine

(TranquiVed, Vedco Inc., St. Joseph, Missouri, USA) (Kreeger et al., 2002). During sedation, physical exams were performed and urine, whole blood and serum were collected from each coyote. Four coyotes (two male, two female) were then each inoculated intravenously with 3 ml of blood from the AST containing a total of approximately $1x10^6$ *B. gibsoni* organisms. The two remaining coyotes (one male, one female) served as uninfected controls and received no parasitemic blood.

Evaluation of Coyotes for Clinical Disease

Animals were sedated weekly for the first eight weeks and then monthly until 20 weeks post-inoculation in order to measure body mass, perform physical examinations, and collect urine, whole blood and serum. Complete blood counts (CBC) were performed on an automated hematology unit (CellDyn 3500, Abbott Diagnostics, Santa Clara, California, USA) validated for veterinary animal species and biochemistry panels consisting of 21 values were performed on a Vitros 950 analyzer (OrthoClinical Diagnostics, Rochester, New York, USA). Manual white blood cell differential counts were performed as were manual reticulocyte counts. Manual platelet counts were performed when the automated count was less than 100,000/µl. Infection status of *B. gibsoni* in coyotes was assessed using the PCR procedure described for the source dog as well as microscopic evaluation of Diff-Quik® stained blood films. PCR was performed once for each coyote at the peak of parasitemia, and stained blood films were examined from every sample of blood.

Evaluation of Ticks for Transstadial Acquisition of Babesia gibsoni

Amblyomma americanum, Dermacentor variabilis and *Rhipicephalus sanguineus* nymphs were obtained from the Oklahoma State University Department of

Entomology Tick Laboratory (Stillwater, Oklahoma, USA). Nymphs of each of the 3 tick species were placed under a stockinette between the shaved shoulder blades of a male coyote previously infected with *B. gibsoni*. To confine the ticks and minimize escape the stockinette was placed over the torso of the coyote and secured with one-inch porous tape. The stockinette was removed within two days. The coyote was housed in a stainless steel 1-m^2 cage that was placed over metal pans containing water and food and water were provided *ad libitum*. The edges of the pans were lined with duct tape to prevent escape of the feeding ticks as they dropped from the coyote. Replete nymphal ticks were collected daily for 2 weeks, transferred into paper cartons and maintained in a humidity chamber (90 to 98% relative humidity at 25 C with a 14-hour light-dark photophase) and allowed to molt.

The study was conducted in two phases. Phase one involved microscopic examination of tick salivary glands stained with methyl green pyronin for rapid evaluation with light microscopy for evidence of *B. gibsoni* replication as described by Walker et al. (1979). Phase two used light microscopy to further evaluate plastic-embedded thick sections of tick salivary glands (Blouin et al., 2002). Both of these procedures have been successful in detecting apicomplexan parasite development in tick salivary glands.

In phase one, 100 nymphs of each species (*A. americanum, D. variabilis* and *R. sanguineus)* were placed on the coyote. Eleven survived collection and molting (5 female and 1 male *A. americanum*, 1 female and 4 male *R. sanguineus*). In order to stimulate parasite development, molted ticks were placed in a stockinette cell attached to a shorn domestic sheep and allowed to feed for 5 days. The dorsal exoskeleton of

the ticks was removed with a razor blade and salivary glands were removed with fine forceps. Salivary glands were then stained with methyl green pyronin and examined under 1000X magnification with a light microscope.

In phase two, 150 nymphs of each tick species were placed on the coyote and a representative sample of those that underwent molting was evaluated (18 female and 5 male *A. americanum*, 1 female and 1 male *D. variabilis*, and 17 female and 6 male *R. sanguineus*). In order to stimulate parasite development, molted ticks were placed in a stockinette cell attached to a shorn domestic sheep and allowed to feed for 4 days. Ten adult males and females of each tick species that were not previously fed on coyotes served as negative controls and were also fed on the same sheep in a separate stockinette. The dorsal exoskeleton of the ticks was removed with a razor blade and salivary glands were removed with fine forceps. Salivary glands were immersed immediately in cold 2% glutaraldehyde (pH 7.4) in 0.1 M sodium cacodylate buffer (pH 7.2) and fixed at 4 C for 12 hours. Tissues were washed in buffer containing cacodylate, water and 10% sucrose, post-fixed in 2% cacodylate-buffered osmium tetroxide (pH 7.4), dehydrated in a graded series of increasing concentrations of ethyl alcohol, infiltrated with a 1:1 mixture of propylene oxide/DER 332 for 48 hours, and then embedded in 100% DER 332. Embedded samples were heated at 60 C for 48 hours and thick sections (approximately 1μm) were prepared, stained for 2 minutes at 60 C with Mallory's stain (Richardson et al., 1960) and examined with a light microscope under 1000X magnification.

CHAPTER III

Results

Evaluation of Experimental Babesia gibsoni *Infection*

All four experimentally inoculated coyotes became infected with *B. gibsoni* and infection was demonstrated both by microscopic detection of piroplasms in Diff-Quik® stained blood films and by PCR. Parasites were detectable on blood films as early as 1 week post-inoculation, and persisted for at least 20 weeks. Parasitemia peaked at 3-4 weeks (8-11%), but remained greater than 1% for at least 10 weeks (Fig. 1). All four experimentally infected coyotes developed a mild elevation in temperature (1-2 F, weeks 4-12), pale mucous membranes (weeks 4-8), and splenomegaly (weeks 3-20). A positive heme reaction in the urine was noted sporadically from 3 to 16 weeks post-inoculation. Mild depression and inappetance were recorded during weeks 3-4 in one male coyote. A regenerative anemia and thrombocytopenia of 4,500-21,000 plts/µl (reference range: 112,000-319,000 plts/µl) (Fig. 2) were detected and were inversely related to the parasitemia (Fig. 1). Packed cell volume (PCV) declined to 16.7-22.5% or 1.8-2.8x10^6 RBC/µl (reference range: 37-51% or 4.6-7.0x10^6 RBC/µl); reticulocyte numbers peaked at 454,300-590,800/µl (15.4-28.7%) at 4-5 weeks. Three of four animals also became consistently neutropenic from weeks 2-8 (1,520-2,950/µl, reference range: 3,900-9,600/µl). All but one biochemistry value remained within normal reference ranges for domestic dogs and similar to the uninfected control

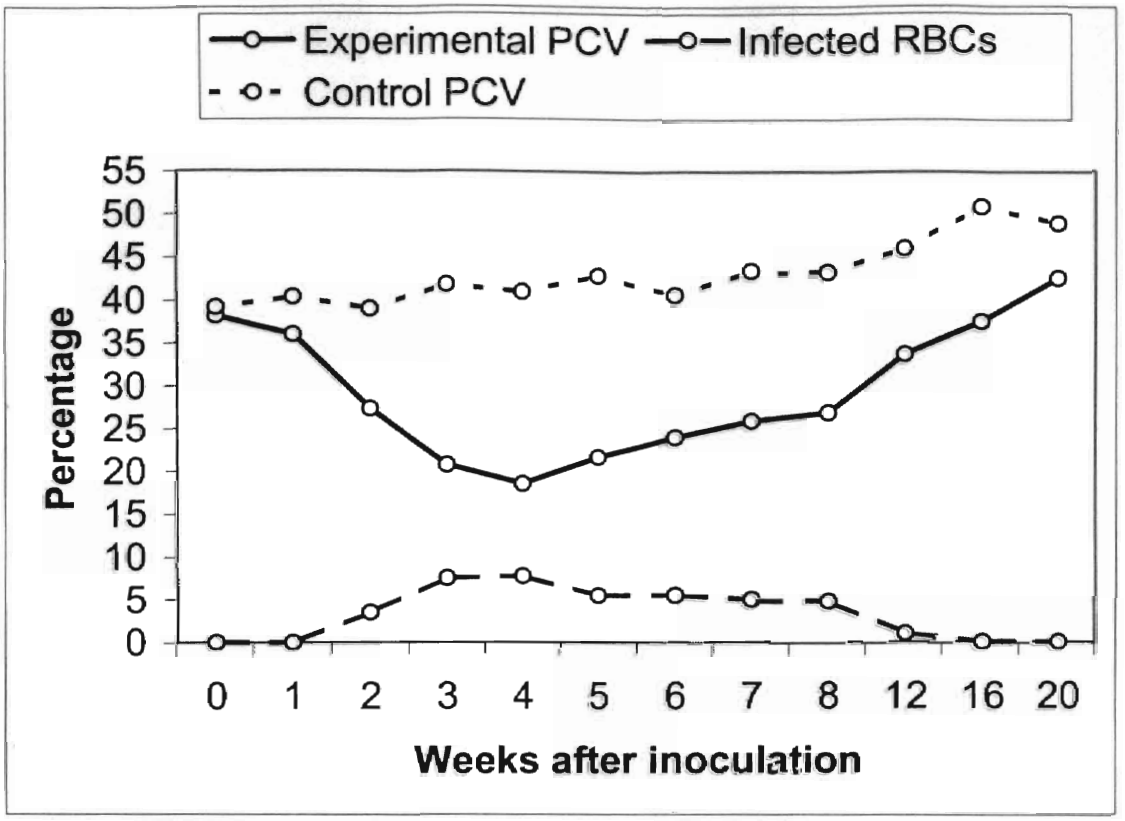

Figure 1. Average packed cell volume (PCV) and parasitemia (% RBC infected with *B. gibsoni*) for four coyotes experimentally infected with *B. gibsoni* and average PCV for two *B. gibsoni* uninfected control coyotes.

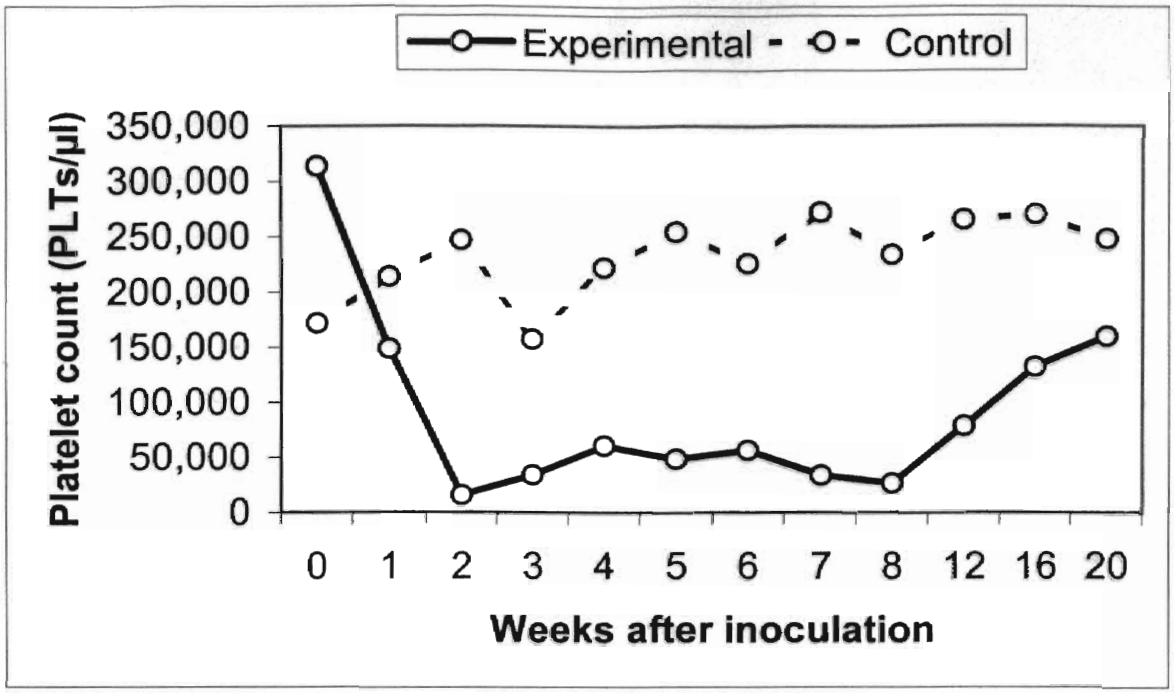

Figure 2. Average platelet count (platelets/µl) for four coyotes experimentally infected with *B. gibsoni* and two uninfected control coyotes.

coyotes. There was an elevation in globulins (GLOB) during weeks 4 to 16 and weeks 4 to 20 in two coyotes when compared to the uninfected control coyotes. The two uninfected coyotes remained negative for *B. gibsoni* and all evaluated hematologic and serum biochemical parameters remained within normal reference ranges for domestic dogs. Two exceptions were noted in GLOB and alkaline phosphatase (ALKP). Higher values were seen in the control coyotes. The reference range for domestic dog GLOB concentration is 2.5-3.4 gm/dl and the range for the uninfected coyotes was 2.6-4.1gm/dl. Values for ALKP for domestic dogs are 20-157 u/l while the range was 45-225 u/l in the uninfected coyotes.

Serum from the AST source dog obtained prior to the initiation of the experimental trials was negative for antigens to *D. immitis* and antibodies to *E. canis*. However a weakly reactive antibody titer (equivalent to 1:16-1:128 IFA titer) to RMSF was detected but was interpreted not to be indicative of active infection. Prior to experimental exposure, antibodies were not detected in the sera of the coyotes for *E. canis* or RMSF. Microscopic evaluation of Diff-Quik® stained blood films and PCR analysis demonstrated that the source dog was infected with *B. gibsoni* and that before the study the organism was not detected in the coyotes. Partial sequence analysis from purified PCR products demonstrated that the coyotes became infected with *B. gibsoni*.

Evaluation of Ticks for Transstadial Acquisition of Babesia gibsoni

Evaluation of salivary glands stained with methyl pyronin green with light microscopy showed possible evidence of replicating parasites, indicated by areas of diffuse staining within cells of salivary acini in 3 *A. americanum* and 1 *R. sanguineus*

female ticks (Fig. 3). Further evaluation by light microscopy of Mallory's stained plastic-embedded salivary glands of the 3 species of ticks showed no evidence of replicating parasites within the salivary glands (Fig. 4).

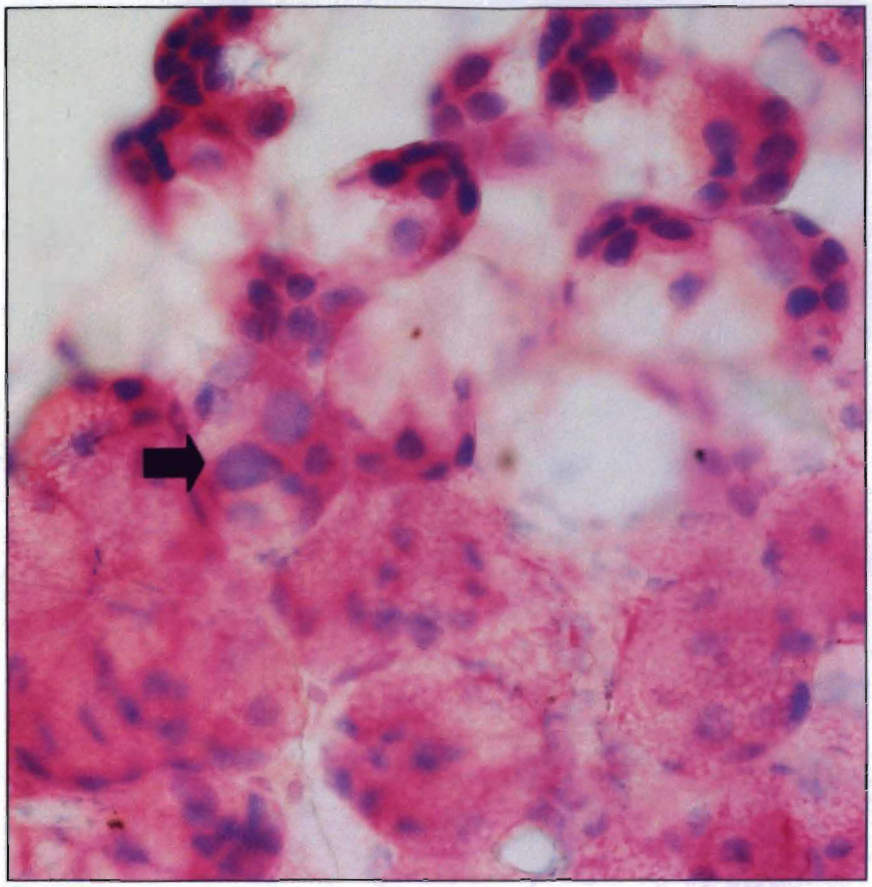

Figure 3. Photomicrograph of methyl green pyronin stained salivary glands from an adult female *R. sanguineus* tick previously fed on a *B. gibsoni*-infected coyote. The arrow indicates a suspicious area inside a salivary acinus. Magnification = 400X.

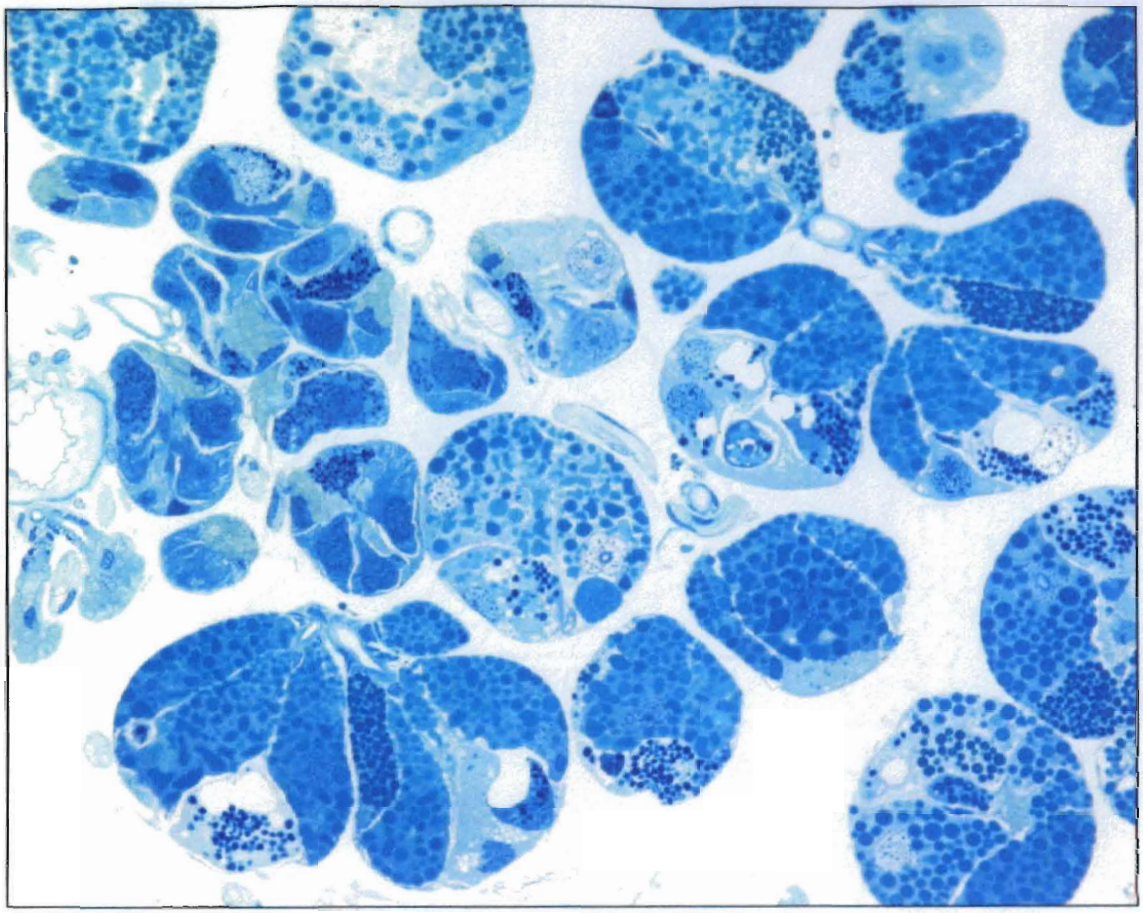

Figure 4. Photomicrograph of Mallory's stained salivary glands from an adult female *R. sanguineus* tick previously fed on a *B. gibsoni*-infected coyote. Various stages of cellular development and disintegration were observed within salivary acini. No *B. gibsoni* organisms were present. Magnification = 400X.

CHAPTER IV

Discussion

The results of the present study demonstrated that coyotes were susceptible to experimental infection with *B. gibsoni* via blood transfer from a naturally infected domestic dog. Although hematological evidence of hemolytic disease was observed, mild clinical disease and lack of mortality indicated that experimental infection with *B. gibsoni* did not have a significant adverse affect the health of these coyotes. Additionally, the long duration and high level of parasitemia suggested that coyotes could serve as potential reservoirs for *B. gibsoni*. Although *B. gibsoni* is a tick-borne parasite, in North America the tick vector(s) for it or any other small piroplasms of canids have yet to be identified. Although suspicious areas were observed in salivary acini of several ticks of different species by light microscopic evaluation of methyl green pyronin stained salivary glands (Fig. 3), the more specific procedure of evaluating plastic-embedded glands showed that these were likely areas of cells in various stages of development and disintegration (Fig. 4). These findings suggested that *A. americanum* and *R. sanguineus* may not be suitable vectors for *B. gibsoni* and support the hypothesis that a suitable tick vector may not be present in North America. Because only two *D. variabilis* ticks were evaluated, no conclusion can be drawn regarding this tick. Additional studies involving additional tick species and transmission trials are warranted to further investigate these findings.

In North America, the maintenance of the disease almost exclusively in AST colonies suggests that mechanical blood transfer and vertical transmission may be the methods for maintaining this parasite (Abu et al., 1973; Correa, 1974; Irizarry-Rovira et al., 2001). The finding that coyotes were susceptible to infection and that they became subclinical while maintaining detectable parasites indicated that even without a tick vector, interspecies interactions could result in spread of *B. gibsoni* between wild and domestic canids. It has been previously reported in Oklahoma that 10-14% of 252 wild canids studied contained both domestic dog (*Canis familiaris*) and coyote traits and 5.6-10.7% contained both red wolf (*Canis rufus*) and coyote traits (Freeman and Shaw, 1979). While these numbers give some indication of the frequency of interspecies breeding, it is likely that fighting and other opportunities for blood transfer between species also occurs.

An exact comparison of the clinical, hematologic and serum biochemical parameters evaluated in this study and in other studies of *B. gibsoni*-like parasites in North America was difficult for several reasons. The age of the animal, concentration of parasite, source and amount of blood and route of inoculation all varied between reports, as did the length of time the animals were observed. An additional complication was that the same parameters were not evaluated in all animals in all studies. For example, concentrations of organisms were either not known (Roher et al., 1985; Conrad et al., 1991) or varied from 30-530 x 10^6 parasites (Wozniak et al., 1997; Meinkoth et al., 2002). The source of the parasite-isolate varied from unknown, to cryopreserved passaged blood to inoculates collected directly from an infected dog. Additionally, in some cases, the source dog had been treated for the infection and in

other cases it had not. Volume of blood inoculated ranged from 0.5-5.0 ml or was unknown. Routes of infection were either not known for the natural infections or were intravenous, subcutaneous, intraperitoneal or a combination of methods. With the exception of the naturally infected dogs for which length of observation varies, experimentally infected nonsplenectomized canines were studied from 3-28 weeks.

Generally, the clinical response of coyotes to *B. gibsoni* infection in the present study was similar to the disease produced in mixed-breed dogs (Meinkoth et al., 2002) and coyotes and coydogs (Roher et al., 1985) but relatively mild in comparison to that reported in dogs infected with the California *B. gibsoni*-like organism (Conrad et al., 1991; Wozniak et al., 1997). The present study and the four previously conducted studies all reported a regenerative response to the hemolytic anemia due to *B. gibsoni* infection. Evidence of a regenerative response was found by observing some or all of the following: reticulocytosis, polychromasia, anisocytosis, or nucleated red blood cells. With the exception of the California isolate, platelet counts declined within the first 2 weeks, then parasitemia peaked and PCV declined 3-5 weeks post-inoculation. It is difficult to obtain time-related data from the California isolate studies because Wozniak et al. (1997) only reported parasitemia at 21 and 23 days, and the study by Conrad et al. (1991) discussed natural infections for which time of infection was unknown.

Mixed-breed dogs experimentally infected with *B. gibsoni* and monitored for 28 weeks exhibited clinical signs of anorexia, depression, fever and pale mucous membranes (Meinkoth et al., 2002). Parasitemia ranged from 1.9-6.0% and remained at or above 1% for 2-3 weeks. A nadir in PCV occurred at 4-5 weeks and was within

normal reference range by 14 weeks. The dogs initially developed a thrombocytopenia returned to normal limits by 18 weeks post-infection. A neutropenia developed in the 6-month-old mixed-breed dogs, but not in the adults.

Although the 2 adult mixed-breed dogs experimentally infected with the California *B. gibsoni*-like organism in Wozniak et al. (1997) were only followed for 22 days before being splenectomized, 2 dogs naturally infected were also included in the study. Both of these dogs were euthanized due to severe illness. Wozniak reported splenomegaly and hemoglobinuria, as well as a decreased mean PCV (19%) and thrombocytopenia (32,000/µl). The 2 experimentally infected dogs had peak parasitemias at day 21 (0.9%) and day 23 (3.7%) post-inoculation. Neutrophil counts remained within normal limits, and elevations in alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine phosphatase (ALT) and bilirubin were noted. Hypoalbuminemia also occurred.

Eleven dogs naturally infected with the California *B. gibsoni*-like isolate developed a regenerative response to the anemia caused by the parasite, but many developed severe illness (Conrad et al., 1991). Clinical symptoms were complicated by the disease affecting other organs such as the liver or kidneys. Vomiting and icterus were described and recrudescence of the disease was observed. Parasitemia peaked at 40% (5-40%), and a decreased PCV (9-16%) and thrombocytopenia (all < 80,000/µl) also occurred. Neutropenia was observed in some young dogs while neutrophilia occurred in some adult dogs. Dogs were followed various lengths of time and some were lost to follow-up. Six of the 11 cases in Conrad's study died or were euthanized.

The higher level of parasitemia and recrudescence of the disease, coupled with the mortality and morbidity indicate that the California organism may be more virulent.

Experimental infection of 6 adult coyotes and 2 adult coydogs inoculated with the uncharacterized *Babesia* spp. isolate from New England (Roher et al., 1985), resulted in anorexia, splenomegaly and a parasitemia that ranged from 1-11.5%. A nadir in PCV (16%) and peak parasitemia (11.5%) occurred at 4 weeks, and elevations in bilirubin and globulins were noted. Dark, red-brown urine was also reported. Neutrophil counts remained within normal limits. The coyotes and coydogs were followed anywhere from 3.5 to 16 weeks.

CHAPTER V

Summary and Conclusions

The discovery that coyotes, a widely distributed and commonly occurring wild canid, are susceptible to infection with *B. gibsoni* coupled with the recent entry and establishment of this parasite in domestic dogs in North America demonstrates the role of coyotes as a potential wildlife reservoir for this parasite. The observed clinical response suggested that coyotes would likely be a competent wildlife reservoir if *B. gibsoni* were to enter wild canids because they maintained a high parasitemia for a long duration while developing a mild clinical disease with no mortality. These findings also support the need for more stringent evaluation of canines entering into and traveling between states in the United States. Although quarantine periods are beneficial, complete physical exams and microscopic evaluation of stained blood films should also be performed to reduce the risk of this or another foreign animal disease entering the United States.

In the present study, the clinical disease produced by *B. gibsoni* in coyotes was similar to the disease previously reported in mixed-breed dogs, coyotes and coydogs experimentally infected with the same organism and an uncharacterized isolate from New England. In contrast, the disease that was reported in naturally and experimentally infected mixed-breed dogs with a California *B. gibsoni*-like isolate was more severe. We were unable to confirm that any of three common North American ticks (*A. americanum, D. variabilis* and *R. sanguineus)* could acquire *B. gibsoni* following

feeding on an infected coyote, but further studies are warranted to determine the potential role of ticks in maintaining and transmitting this parasite in North America.

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Appendix 1-Selected hematologic and urologic parameters for coyotes.

#188: Experimentally infected coyote; intact male

Parameter	(units)	9/25/2001 DAY 0	10/2/2001 1 WK	10/9/2001 2 WK	10/16/2001 3 WK	10/23/2001 4 WK	10/30/2001 5 WK	11/6/2001 6 WK	11/13/2001 7 WK	11/20/2001 8 WK	12/18/2001 12 WK	1/15/2002 16 WK	2/12/2002 20 WK
RBC	($x10^6$/µL)	4.74	4.93	2.75	2.12	1.93	2.15	2.76	2.95	3.2	4.89	4.51	5.23
HGB	(g/dL)	11.1	11.5	6.48	5.37	5.14	5.74	6.93	7.26	7.67	11.3	10.9	12.4
PCV	(%)	35.5	35.7	19.8	17.2	16.7	19.2	23.3	24.2	25.4	35.8	34.2	38.4
PLT	(plt/µL)	236,000	137,000	4,500	28,500	72,500	38,000	24,000	30,500	24,500	42,500	111,000	52,500
RETIC	(%)	2.1	2.7	2.4	13.7	16.6	16.7	15.5	15.4	6	1.7	3.8	0.6
RETIC	(retic/µL)	99,540	133,110	66,000	290,440	320,380	359,050	427,800	454,300	192,000	83,130	171,380	31,380
nRBC	(nRBC/100 WBC)	0	0	1	12	6	6	18	18	3	3	6	0
PARASITEMIA	(%)	0	<1	4	10	8	6	6	5	4	1	<1	<1
WBC	(WBC/µL)	11,500	7,340	4,770	8,880	3,440	4,550	3,300	3,330	3,320	10,800	6,890	10,300
NEUT	(neut/µL)	6,120	3,700	2,190	2,160	1,600	2,800	1,520	2,460	1,560	4,230	4,400	4,620
BUN	(mg/dL)	8	15	15	10	11	15	21	15	12	13	16	20
LDH	(U/L)	1227	43	690	555	733	441	334	568	817	271	160	182
BILI	(mg/dL)	0.2	0.3	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
PROT	(gm/dL)	6.1	5.8	5.3	5.9	6.1	6.1	6	6.2	6	5.7	5.8	6.5
GLOB	(gm/dL)	3.8	3.5	3.3	3.8	3.9	3.8	3.7	3.8	3.7	3	3	2.8
ALKP	(U/L)	147	156	90	75	66	78	87	73	76	85	54	57
ALT	(U/L)	23	26	13	14	14	16	12	16	26	33	34	39
ALB	(gm/dL)	2.3	2.3	2	2.1	2.2	2.3	0.6	0.6	2.3	2.7	2.8	2.8
Heme in urine	(mg/dL HGB or mGB or 5-10 RBC/µL)	nd	nd	nd	mod-lg	nd	nd	sm-mod	mod	nd	mod	trace	nd

nd = no data

Appendix 1-Selected hematologic and urologic parameters for coyotes.

#191: Experimentally infected coyote; intact male

Parameter	(units)	9/25/2001 DAY 0	10/2/2001 1 WK	10/9/2001 2 WK	10/16/2001 3 WK	10/23/2001 4 WK	10/30/2001 5 WK	11/6/2001 6 WK	11/13/2001 7 WK	11/20/2001 8 WK	12/18/2001 12 WK	1/15/2002 16 WK	2/12/2002 20 WK
RBC	($x10^6$/µL)	5.65	5.17	5.15	3.13	2.8	3.11	3.15	3.55	3.4	4.01	4.8	5.41
HGB	(g/dL)	12.9	12.2	12	7.7	7.14	7.91	8.11	9.01	8.51	9.6	11.4	12.9
PCV	(%)	41.5	37.2	37.1	23.7	22.5	25.9	25.9	29	27.5	30.3	36.7	40.4
PLT	(plt/µL)	234,000	82,400	13,100	36,500	48,500	58,500	32,500	22,500	17,500	27,000	84,500	103,000
RETIC	(%)	1.1	2.6	3.1	7.4	21.1	13.1	13.8	20.7	10.2	3.2	2	3.9
RETIC	(retic/µL)	62,150	134,420	159,650	231,620	590,800	407,410	434,700	734,850	346,800	128,320	96,000	210,990
nRBC	(nRBC/100 WBC)	0	0	1	2	36	74	27	35	34	7	6	1
PARASITEMIA	(%)	0	<1	2	3	11	5	6	5	5	1	<1	<1
WBC	(WBC/µL)	8,730	7,730	6,890	4,810	6,270	6,120	5,670	6,980	11,200	7,880	7,530	11,200
NEUT	(neut/µL)	4,980	3,930	4,190	2,950	3,500	3,990	3,470	4,660	4,310	5,260	4,440	7,480
BUN	(mg/dL)	6	12	13	7	9	11	25	17	11	15	9	10
LDH	(U/L)	1278	522	477	318	515	531	305	339	616	322	175	199
BILI	(mg/dL)	0.2	0.1	0.4	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.1	0.1
PROT	(gm/dL)	6.4	6	6.2	5.7	6.3	6.3	6.3	6.6	6.2	5.9	6.1	6.6
GLOB	(gm/dL)	3.9	3.6	3.5	3.6	3.9	4	3.8	4.1	3.9	3.5	3.7	4.1
ALKP	(U/L)	142	141	121	83	83	68	65	63	63	72	55	42
ALT	(U/L)	25	35	20	20	16	18	13	16	23	16	21	28
ALB	(gm/dL)	2.5	2.3	2.7	2.1	2.4	2.3	2.4	2.5	2.3	2.4	2.4	2.4
Heme in urine	(mg/dL HGB or MGB or 5-10 RBC/µL)	nd	nd	nd	tr	nd	nd	mod	sm-mod	lg	sm-mod	mod	No

nd = no data

Appendix 1-Selected hematologic and urologic parameters for coyotes.

#193: Experimentally infected coyote; intact female

Parameter	(units)	9/25/2001 DAY 0	10/2/2001 1 WK	10/9/2001 2 WK	10/16/2001 3 WK	10/23/2001 4 WK	10/30/2001 5 WK	11/6/2001 6 WK	11/13/2001 7 WK	11/20/2001 8 WK	12/18/2001 12 WK	1/15/2002 16 WK	2/12/2002 20 WK
RBC	($x10^6$ /µL)	5	5.02	3.42	2.78	1.98	2.03	2.35	2.68	3.14	4.73	5.66	6.16
HGB	(g/dL)	12.3	12.6	8.54	7.26	5.63	5.7	6.34	6.89	7.64	10.9	12.9	14.3
PCV	(%)	38.2	37.9	25.7	23	18.2	18.9	21.3	23.1	26	34.7	40.5	43.8
PLT	(plt/µL)	424,000	226,000	23,500	45,500	44,500	52,500	88,000	39,500	27,000	80,000	157,000	122,000
RETIC	(%)	1.6	1.3	1.9	12.4	24.4	28.7	16.2	13.8	11.1	3.1	1.4	1.7
RETIC	(retic/µL)	79,200	65,260	64,980	344,720	483,120	582,610	380,070	369,840	348,540	146,630	79,240	104,720
nRBC	(nRBC/100 WBC)	0	0	3	20	6	11	8	4	4	2	0	0
PARASITEMIA	(%)	0	<1	4	9	9	7	6	6	6	1	<1	<1
WBC	(WBC/µL)	14,300	8,660	6,040	4,310	4,270	4,180	4,770	6,010	5,510	7,800	11,000	12,900
NEUT	(neut/µL)	8,380	4,600	2,980	2,080	2,190	2,340	2,160	3,010	2,850	3,790	5,880	6,920
BUN	(mg/dL)	8	14	15	9	13	10	16	21	15	18	12	9
LDH	(U/L)	934	460	418	426	904	442	376	367	547	353	259	234
BILI	(mg/dL)	0.1	0.3	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1
PROT	(gm/dL)	6.5	5.7	5.6	6.1	6.6	7.1	7.7	7.3	7.6	7	7.1	7.1
GLOB	(gm/dL)	4.1	3.4	3.3	3.9	4.3	4.7	5.2	4.8	5.1	4.3	4.3	4.3
ALKP	(U/L)	162	149	101	81	60	66	63	57	60	65	55	58
ALT	(U/L)	24	25	17	20	15	14	9	13	17	19	29	35
ALB	(gm/dL)	2.4	2.3	2.3	2.2	2.3	2.5	2.5	2.5	2.5	2.8	2.8	2.7
Heme in urine	(mg/dL HGB or MGB or 5-10 RBC/µL)	nd	nd	nd	mod-lg	nd	lg	lg	lg	nd	mod	No	IN HEAT

nd = no data

Appendix 1-Selected hematologic and urologic parameters for coyotes.

#194: Experimentally infected coyote; intact female

Parameter	(units)	9/25/2001 DAY 0	10/2/2001 1 WK	10/9/2001 2 WK	10/16/2001 3 WK	10/23/2001 4 WK	10/30/2001 5 WK	11/6/2001 6 WK	11/13/2001 7 WK	11/20/2001 8 WK	12/18/2001 12 WK	1/15/2002 16 WK	2/12/2002 20 WK
RBC	($x10^6$/µL)	4.74	4.25	3.46	2.32	1.84	2.51	2.88	3.24	3.42	4.48	5.06	6.37
HGB	(g/dL)	11.9	10.7	8.61	6.02	5.39	6.79	7.6	8.11	8.44	10.5	12.1	15.1
PCV	(%)	37.3	33	26.4	18.9	16.7	22.5	25.1	26.8	28	33.3	37.8	46.5
PLT	(plt/µL)	361,000	148,000	21,000	25,000	77,000	44,500	81,500	42,000	37,000	165,000	177,000	361,000
RETIC	(%)	1.3	2.5	2.6	5.7	12.5	18.9	11.5	8.2	7.9	2.3	2	1.3
RETIC	(retic/µL)	61,620	106,250	89,960	132,240	230,000	474,390	331,200	265,680	270,180	103,040	101,200	82,810
nRBC	(nRBC/100 WBC)	0	0	0	16	8	13	13	8	8	2	1	2
PARASITEMIA	(%)	0	<1	4	8	3	4	4	4	4	1	<1	<1
WBC	(WBC/µL)	17,600	10,700	6,360	5,260	6,640	4,330	4,790	4,680	4,870	14,000	10,000	14,300
NEUT	(neut/µL)	11,500	6,080	3,470	2,590	3,370	2,200	2,360	2,460	2,780	8,850	5,400	9,340
BUN	(mg/dL)	13	10	16	9	10	10	31	19	17	10	11	11
LDH	(U/L)	621	355	359	356	1418	356	247	312	575	312	161	181
BILI	(mg/dL)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
PROT	(gm/dL)	7.2	6.2	6.1	5.8	6.2	6.4	6.4	6.5	6.6	6	6.7	5.8
GLOB	(gm/dL)	4.8	4.1	3.7	3.8	4.3	4.4	4.3	4.2	4.2	4.5	4.2	3.2
ALKP	(U/L)	171	138	90	85	71	91	94	83	73	84	56	38
ALT	(U/L)	57	52	32	28	20	20	14	24	31	28	31	29
ALB	(gm/dL)	2.4	2.1	2.4	2	2	2.1	2.2	2.3	2.4	2.6	2.6	2.6
Heme in urine	(mg/dL HGB or MGB or 5-10 RBC/µL)	nd	nd	nd	nd	nd	sm	mod	nd	sm	tr-sm	IN HEAT	IN HEAT

nd = no data

Appendix 1-Selected hematologic and urologic parameters for coyotes.

#189: Uninfected control coyote; intact female

Parameter	(units)	9/25/2001 DAY 0	10/2/2001 1 WK	10/9/2001 2 WK	10/16/2001 3 WK	10/23/2001 4 WK	10/30/2001 5 WK	11/6/2001 6 WK	11/13/2001 7 WK	11/20/2001 8 WK	12/18/2001 12 WK	1/15/2002 16 WK	2/12/2002 20 WK
RBC	($x10^6$/µL)	5.49	5.6	5.69	6.02	5.89	6.08	6.07	6.13	6.34	6.9	7	6.6
HGB	(g/dL)	12.8	13.4	13.4	14.2	14	14.5	14.4	14.6	15	16.3	17	15.6
PCV	(%)	40.7	40.8	41	43.7	43.1	44.2	44	44.4	46.3	50.3	51.1	48.1
PLT	(plt/µL)	307,000	315,000	315,000	135,000	282,000	316,000	277,000	297,000	264,000	296,000	291,000	319,000
RETIC	(%)	1.7	1.1	2.1	1.3	1	0.5	0.4	0.7	0.4	0.2	0.6	1
RETIC	(retic/µL)	93,330	61,600	119,490	78,260	58,900	30,400	24,280	42,910	25,360	13,800	42,000	66,000
nRBC	(nRBC/100 WBC)	0	0	0	0	0	0	2	0	6	0	0	0
PARASITEMIA	(%)	0	0	0	0	0	0	0	0	0	0	0	0
WBC	(WBC/µL)	9,220	10,500	9,050	7,880	7,540	7,190	8,990	9,320	8,010	7,810	6,060	8,230
NEUT	(neut/µL)	4,970	7,310	5,770	5,100	4,670	4,260	5,170	5,260	5,100	5,470	3,920	5,630
BUN	(mg/dL)	12	13	11	10	12	14	15	19	11	14	14	14
LDH	(U/L)	868	773	332	338	447	352	180	392	883	284	263	229
BILI	(mg/dL)	0.1	0.3	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2
PROT	(gm/dL)	6.4	6.3	6	6.1	6.1	6.2	6.1	6.1	6.1	6.1	5.4	5.8
GLOB	(gm/dL)	3.7	3.7	3.2	3.3	3.3	3.3	3.2	3.2	3.2	3.2	2.6	3.1
ALKP	(U/L)	143	150	114	108	103	109	89	83	74	56	45	46
ALT	(U/L)	23	21	22	31	30	27	24	24	33	29	45	39
ALB	(gm/dL)	2.7	2.6	2.7	2.8	2.8	2.9	2.9	2.9	2.9	2.9	2.8	2.7
Heme in urine	(mg/dL HGB or MGB or 5-10 RBC/µL)	nd	nd	nd	No	nd	nd	No	No	No	No	No	No

nd = no data

Appendix 1-Selected hematologic and urologic parameters for coyotes.

#190: Uninfected control coyote; intact male

Parameter	(units)	9/25/2001 DAY 0	10/2/2001 1 WK	10/9/2001 2 WK	10/16/2001 3 WK	10/23/2001 4 WK	10/30/2001 5 WK	11/6/2001 6 WK	11/13/2001 7 WK	11/20/2001 8 WK	12/18/2001 12 WK	1/15/2002 16 WK	2/12/2002 20 WK
RBC	($x10^6$/µL)	4.56	4.98	4.68	4.98	4.89	5.3	4.76	5.42	5.11	5.39	6.4	6.3
HGB	(g/dL)	11.5	12.6	12	12.4	12.3	13.5	12	13.7	12.9	13.6	16.1	16.1
PCV	(%)	37.6	39.8	36.7	39.7	38.6	41.2	36.9	42	39.8	41.3	50.1	49.1
PLT	(plt/µL)	36,000	112,000	179,000	179,000	162,000	192,000	175,000	247,000	204,000	235,000	250,000	177,000
RETIC	(%)	1.9	1.3	2	1.1	1.4	2.2	1.5	1.2	2.4	1.2	0.9	1.1
RETIC	(retic/µL)	86,640	64,740	93,600	54,780	68,460	116,600	71,400	65,040	122,640	64,680	57,600	69,300
nRBC	(nRBC/100 WBC)	0	0	0	0	0	0	1	0	0	0	0	0
PARASITEMIA	(%)	0	0	0	0	0	0	0	0	0	0	0	0
WBC	(WBC/µL)	8,710	12,300	11,000	6,640	9,820	8,600	7,370	9,330	9,690	10,000	8,710	10,900
NEUT	(neut/µL)	5,520	9,560	8,110	4,510	6,500	6,080	4,310	6,680	6,840	6,510	5,950	7,230
BUN	(mg/dL)	8	17	19	17	13	20	19	26	17	15	24	21
LDH	(U/L)	555	912	656	328	376	301	709	478	519	264	258	427
BILI	(mg/dL)	0.1	0.5	0.3	0.1	0.3	0.1	0.1	0.2	0.1	0.1	0.1	0.3
PROT	(gm/dL)	6.5	6.5	6.3	5.9	5.8	6	5.6	5.9	5.7	5.4	5.6	5.9
GLOB	(gm/dL)	4.1	4	3.4	3.2	3	3.1	3	3.1	3	2.8	2.7	2.9
ALKP	(U/L)	194	225	194	197	177	177	133	140	132	108	91	71
ALT	(U/L)	25	28	22	31	26	29	27	32	33	33	33	34
ALB	(gm/dL)	2.4	2.5	2.9	2.7	2.8	2.9	2.6	2.8	2.8	2.6	2.9	3
Heme in urine	(mg/dL HGB or MGB or 5-10 RBC/µL)	nd	nd	nd	No	nd	nd	No	No	No	No	No	No

nd = no data

2.

VITA

Holly V. Evers

Candidate for the Degree of

Master of Science

Thesis: EXPERIMENTAL INFECTION OF *BABESIA GIBSONI* IN COYOTES (*CANIS LATRANS*)

Major Field: Veterinary Biomedical Sciences

Biographical:

Education: Received Bachelor of Science in Zoology from North Carolina State University in December 1995; received Doctor of Veterinary Medicine from the Oklahoma State University College of Veterinary Medicine in May 2001; completed the requirements for the Master of Science degree at Oklahoma State University in August, 2003.

Professional Experience: Relief Veterinarian, Stillwater and Oklahoma City, Oklahoma, May 2001-December 2002; Self-employed mobile veterinary care, Guthrie and Stillwater, Oklahoma, May 2001-Present; Graduate Teaching Assistant, Department of Pathobiology, Oklahoma State University College of Veterinary Medicine, July 2001-Present.

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