STUDIES ON THE TRANSMISSION OF *CYTAUXZOON FELIS* Kier, 1979 BETWEEN BOBCATS AND DOMESTIC CATS BY IXODID TICKS

BY

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CHAPTER I

INTRODUCTION

Cytauxzoon spp.

In March of 1943, a two-year-old gray duiker (*Sylvicaprae grimmia*) was brought to the Veterinary Research Institute at Pretoria, South Africa for examination. The animal died within a day. Examination of blood smears revealed intraerythrocytic piroplasms that resembled *Theileria* spp. Histological examination also revealed large multinucleated masses in cells lining the blood vessels of many organs. These infected cells were identified as histiocytes and the parasitic stages within them were determined to be schizonts. These findings differed from those seen in typical infections with *Theileria* sp. in which the schizonts occur in lymphocytes. At the time of the initial discovery, it was not clear whether piroplasms and histiocytic schizonts were different stages of the same organism or if there was a mixed infection of a *Theileria* sp. and another organism. It was assumed that the piroplasms and the histiocytic schizonts were related. Based on the cell type in which schizogony occurred, Nietz and Thomas (1948) named the organism *Cytauxzoon sylvicaprae* sp. n. The new genus and species designation was proposed

in order to separate the newly discovered organism from Theileria spp. Nietz (1957) described a second member of this genus, Cytauxzoon strepsicerosi from a fatally ill greater kudu (Tragelaphus strepsiceros strepsiceros) from the Transvall area of South Africa. A third species, C. taurotragi was described by Martin and Brocklesby (1960) from a fatal infection in an eland (Taurotragus oryx pattersonianus) in Kenya. In this species, the large schizonts were observed in macrophages and smaller schizonts were observed within parenchymal cells of the liver. Brocklesby (1962) felt that it was possible that all three described members of this genus might be the same species but due to the rarity of the organism and the variety of hosts, it was more convenient to refer to them as separate species. Since these earlier reportings, there have been subsequent reports of Cytauxzoon sp. from other African ungulates including a fatal infection in a giraffe in Zululand by McCully et al.(1970) and in roan and sable antelope in the Transvall (Wilson et al., 1974). A fourth species of Cytauxzoon was described by Haiba (1962) from an Egyptian Nile fish (Clarias lazera), but Levine (1971) reclassified this as Haemohormidium clariae (Family Dactylosomatidae).

Barnett and Brocklesby (1968) have indicated that Theileria-like piroplasms are very common in wild African ungulates. Because most of these have not been well characterized, it is probable that infections with species

of the genus Cytauxzoon are more common than reported.

A piroplasm was described in a young Sudanese wildcat (Felis ocreata) by Davis (1929) and named Babesia felis. The first report of Cytauxzoon sp. in the Felidae and cytauxzoonosis outside of Africa was by Wagner (1975). He described two fatal cases attributed to this organism in two domestic cats in Missouri. In 1976, Wagner elaborated on this first report and included two additional cases. The latter report described fatalities among the four cats, each from a separate area of southwestern Missouri. All of the cats involved were from homes or farms in heavily wooded areas. In one case, the cat was the last of approximately 20 to have died on the premises during the summer. The etiological agent of the disease was described as a Cytauxzoon-like organism.

Since the initial report in Missouri, Cytauxzoon the organism has been reported in cats from Arkansas, Texas, Georgia, Mississippi, Oklahoma, Florida and Louisiana (Bendele et al., 1976; Hauck et al, 1982; Kier et al, 1979 and Glenn and Stair, 1984). All reported cases in domestic cats were fatal and apparently from rural areas. To date there have been over 100 reported cases in the United States (Stair, 1985).

Opinions vary regarding the taxonomic status of Cytauxzoon. Following a succession of earlier revisions, Levine (1971) proposed a classification of the piroplasms in which Cytauxzoon is placed in the genus Theileria. The

major reason given for combining the two genera was a report by Shaeffler (1962) indicating that schizonts of Theileria cervi from a white-tailed deer were found in undifferentiated mesenchymal cells and primative erythrocytic cells of the bone marrow. Levine reasoned that a new genus would have to be established for T. cervi if, as in Cytauxzoon, the location where schizogony occurs is sufficient reason for establishing a generic designation. Additionally, since the erythrocytic forms of these two organisms, and other unnamed piroplasms are all indistinguishable, they should be considered synonymous with Theileria until further studies are conducted Levine (1985) listed the organism of domestic cats in the United States as Theileria felis. Barnett (1977) proposed a classification of the piroplasms that retained the genus Cytauxzoon in the Family Theileridae. Brocklesby (1978) felt that the host cell in which schizogony occured was sufficient reason to retain the genus Cytauxzoon. In addition,he noted that schizonts of Cytauxzoon occur multiply and in aggregations within host cells while those of the genus Theileria occur singly or in small aggregations within lymphocytes.

Evidence to support separation of these two genera was proposed by Stagg et al (1976), who reported the establishment of five cell lines from an eland infected with Theileria sp. Four cell lines were derived from a lymph node biopsy and the fifth from peripheral mononuclear leucocytes. In both cell types, shizonts developed that were described

as typical theilerial schizonts. However, in the monocytic cell line, occasional large syncytial cells were found that contained multiple schizonts resembling cytomeres of Cytauxzoon. The authors felt that this might indicate that two organisms (Cytauxzoon and Theileria) were present in the eland or that one species could undergo schizogony in both lymphoblastoid cells and monocytic cells.

In experimental infections of domestic cats caused by inoculation with fresh or frozen homogenates of tissues from naturally occurring cases of C. felis infection, Wagner et al (1980) noted that the most frequently observed clinical signs included pyrexia, depression, anorexia and dehydration. Beginning on the sixth to eighth days post inoculation, a temperature rise of 0.5-1 C per day occurred and it usually peaked at approximately 41 C (but occasionally went as high as 41.8 C). During the febrile period, depression was profound and anorexia complete, after which anemia, icterus and dehydration developed rapidly (Whiteman, 1978). At about the time of the temperature increase, piroplasms were seen in erythrocytes. Erythrocytic stages were not observed in all cats but some had a parasitemia of as high as 25% by the time of death. Most often, parasitemias were generally near 1%. Following the peak temperature rise there is a decline in temperature for 1 to 5 days (often falling to subnormal levels) after which cats became lethargic, displayed abdominal breathing, dehydration, and pale mucus membranes. Finally they lapsed

into a coma and died. Death generally occured 2 to 3 days after the peak body temperature. Duration of the disease was 14 to 20 days post- inoculation and the course of clinical disease was generally under one week (Kier et al, 1977). Because the early signs tend to be nonspecific and the duration of clinical disease usually short, most cats that had been brought into clinics have been moribund or dead (Whiteman, 1978).

Kier et al. (1979) described the gross pathological findings in experimentally infected cats. At necropsy, pallor and icterus were commonly seen. Veins in the abdominal cavity were distended (especially the splenic, mesenteric and renal veins). The spleen was usually dark and markedly enlarged the lungs usually contained petechial hemorrhages. In addition, the pericardial sac in some animals was distented with gelatinous and icteric fluid, accompanied by petechial and ecchymatic hemorrhaging of the epicardium. The major microscopic lesion reported by Kier et al (1979) was the accumulation of large numbers of parasitized mononuclear phagocytes filled with schizonts within the lumen of veins and venous channels of the lungs, liver, lymph nodes and spleen. They were also found in vessels of other organs but to a lesser extent. After these studies on the pathological changes of feline cytauxzoonosis were completed, Kier (1979) named the etiological agent Cytauxzoon felis.

While studying the clinical disease in cats, treatment

of domestic cats was also investigated. Kier et al. (1979) reported that supportive treatments were successful only in prolonging life several days. The disease has thus far been uniformly fatal. Ferris (1979) reported data from Plum Island Animal Disease Center on a single cat that was immunized using parasites attenuated on VERO cells. The cat had been challenged 12 times and appeared resistent. He also reported on the unsuccessful use of numerous antibiotics and antimalarial drugs against the organism.

Because of the uniformly fatal nature of Cytauxzoon felis infection in domestic cats and its relationship to African cytauxzoonosis and theileriosis, there was a concern for the potential of infecting other species of North American animals. Studies at the University of Missouri examined the potential of interspecies transmission of Cytauxzoon felis. Combinations of freshly collected blood, spleen or lymph node from cats euthanized during acute disease were parenterally inoculated into 4 species of domestic animals (cattle, sheep, goats and swine), 17 wildlife species and 9 species of laboratory animals (Kier et al, 1982b). Of these animals, two bobcats became infected. Data on the development of persistent but nonclinical parasitemia in two sheep was also reported. A Florida bobcat (Lynx rufus floridanus) developed clinical signs of cytauxzoonosis and died two weeks post inoculation. Piroplasms were found in blood smears and schizont-laden macrophages in tissues. An eastern bobcat (Lynx rufus

rufus) developed a persistent parasitemia without clinical signs.

Blood and\or spleen homogenate from a fatally infected bobcat was inoculated into domestic cats (Kier et al.,1982a). All of these cats died of cytauxzoonosis within two weeks post- inoculation. Blood and tissue from an eastern bobcat was also inoculated into domestic cats. These cats developed a parasitemia but no clinical signs. No evidence of Cytauxzoon was found in the tissues of any of the other animals inoculated.

It is of interest to note that Uilenberg (1981) produced evidence that sheep were susceptible to Theileria (Cytauxzoon) taurotragi. However, Nietz and Thomas (1948) inoculated sheep with blood from a duiker containing Cytauxzoon sylvicaprae without producing a parasitemia or disease. Nietz (1957) also inoculated sheep and calves with C. strepsicerosi from a kudu and failed to produce disease or a detectable parasitemia.

Kier et al. (1982b) stated that susceptibility of bobcats to both a persistent parasitemia and clinical disease was evidence that that they may serve as a reservoir for C. felis. This theory was supported by the fact that all naturally occurring cases of cytauxzoonosis were in cats from rural, wooded areas where bobcats were likely to inhabit (Kier et al., 1979).

Blood samples were taken and blood smears from 26 free-ranging bobcats were examined during a routine field

survey involving the ecology of bobcats in Oklahoma. Several of the cats were found to harbor piroplasms morphologically indistinguishable from Cytauxzoon felis. Glenn et al. (1982) reported that 13 of 26 bobcats examined from Oklahoma harbored a Cytauxzoon-like piroplasm. Bobcats with piroplasms were found in three counties (Osage, Pawnee and Leflore). Parasitemias of the cats ranged from 0.5% to 5%. Since only erythrocytic forms were observed, it could not be definitively identified as Cytauxzoon. Glenn et al. (1983) inoculated blood from 4 wild-trapped bobcats with naturally occurring piroplasms into domestic cats. All but one of the cats developed a parasitemia without clinical signs of disease and one domestic cat died from cytauxzoonosis. Schizonts were found in macrophages of this cat and clinical signs and lesions were consistent with those described in domestic cats with natural and experimentally-induced cytauxzoonosis. Schizonts were not observed in tissues from any of the bobcats. These results established a relationship between the naturally occuring bobcat piroplasm and Cytauxzoon felis in domestic cats. This relationship could be confirmed with the biological transmission of the bobcat piroplasm to domestic cats. While the vector of C. felis is unknown, there is sufficient information on related organisms to suggest that ticks are a probable vector.

Tick Transmission of Cytauxzoon

Koch (1906) first discussed the development of Theileria parva in ixodid ticks. Gonder (1911) suggested that a sequence of development that was likely to occur in Rhipicephalus appendiculatus. Cowdry and Ham (1932) confirmed the stages of T. parva in R. appendiculatus. These studies implicated ticks as vectors of Theileria spp. Young et al. (1980) described the development of Cytauxzoon taurotragi in R. appendiculatus, identifying stages in gut epithelium and salivary gland cells. These studies confirmed the presence of a piroplasm in a vector but did not establish an association between the two hosts.

Brocklesby (1962) collected R. appendiculatus and R. pulchellus from an eland that died from infection with C. taurotragi. These ticks were allowed to molt and then fed on rabbits. When ticks were collected on successive days of feeding, five (including both species) were found to contain Theileria-like stages. Other newly molted ticks were fed on cattle. One cow fed on by R. pulchellus adults developed an increased temperature, and lymph node smears revealed schizonts of Theileria sp. Although tick transmission of a piroplasm was successful, the presence of a Cytauxzoon sp. was not confirmed. Brocklesby (1982) suggested that two organisms might be present in the eland (Theileria sp. and Cytauxzoon sp.). The experiments of Stagg et al (1976) mentioned earlier, produced both Theileria-like and Cytauxzoon-like schizonts from a single eland and appear to support this idea.

Experimental tick transmission of C. felis was attempted by Whiteman (1978). Nymphal Dermacentor variabilis and Amblyomma americanum were fed on experimentally infected domestic cats and a bobcat. Newly molted adults were then fed on uninfected domestic cats. No clinical signs of cytauxzoonosis were observed in the recipient cats and piroplasms were not found in blood smears. Subsequent challenge of these cats with cryopreserved Cytauxzoon resulted in fatal cytauxzoonosis. These results were not interpreted to mean that ticks were unsuitable vectors of feline cytauxzoonosis but rather that further investigations were necessary.

The Research Problem

This present study will attempt to characterize the Cytauxzoon-like piroplasm found naturally occuring in bobcats in Oklahoma and determine its relationship to Cytauxzoon felis in domestic cats. Selected species of ixodid ticks will be used to attempt the biological transmission of the bobcat origin piroplasm to domestic cats. Ixodid ticks will also be used to attempt the transmission of the bobcat-origin piroplasm to uninfected bobcats. The behavior and life cycle of this organism in bobcats and domestic cats following transmission will be compared. Tick transmission between experimentally infected domestic cats and uninfected domestic cats will also be investigated.

Attempts will also be made to identify the developmental stages of the bobcat origin piroplasm in the potential tick vectors using light microscopic staining, fluorescent antibody techniques and electron microscopic evaluation. The ultrastructure of the erythrocytic stage will also be investigated.

CHAPTER II

Materials and Methods

Experimental Animals

The bobcats used in this study were obtained from several sources. The Oklahoma Department of Wildlife Conservation, Game Division issued the permit to keep wild-caught bobcats and aided in the capture of many of these cats. Other bobcats were obtained from the Animal Damage Control Unit of the U.S. Fish and Wildlife Service. Several cats were also obtained from private trappers. Bobcats were trapped using steel live traps or steel leg-hold traps. Captured animals were transferred to the Wild Animal Disease Laboratory at Oklahoma State University. The cats were anaesthetized using 1-2 cc of ketamine hydrochloride (Ketaset, Bristol Laboratories, Syracuse, New York) and blood samples were taken and examined for piroplasms. Several bobcats (infected and uninfected) were retained for long term study in large outdoor pens. Several naturally infected bobcats were examined for schizogenous stages in tissues. Food for the bobcats was provided by the OSU Department of Veterinary Pathology.

Domestic cats used in the study were obtained from private individuals. They were maintained in stainless steel

cages by the OSU Laboratory Animal Resources Unit. They were fed a standard dry ration ad libitum.

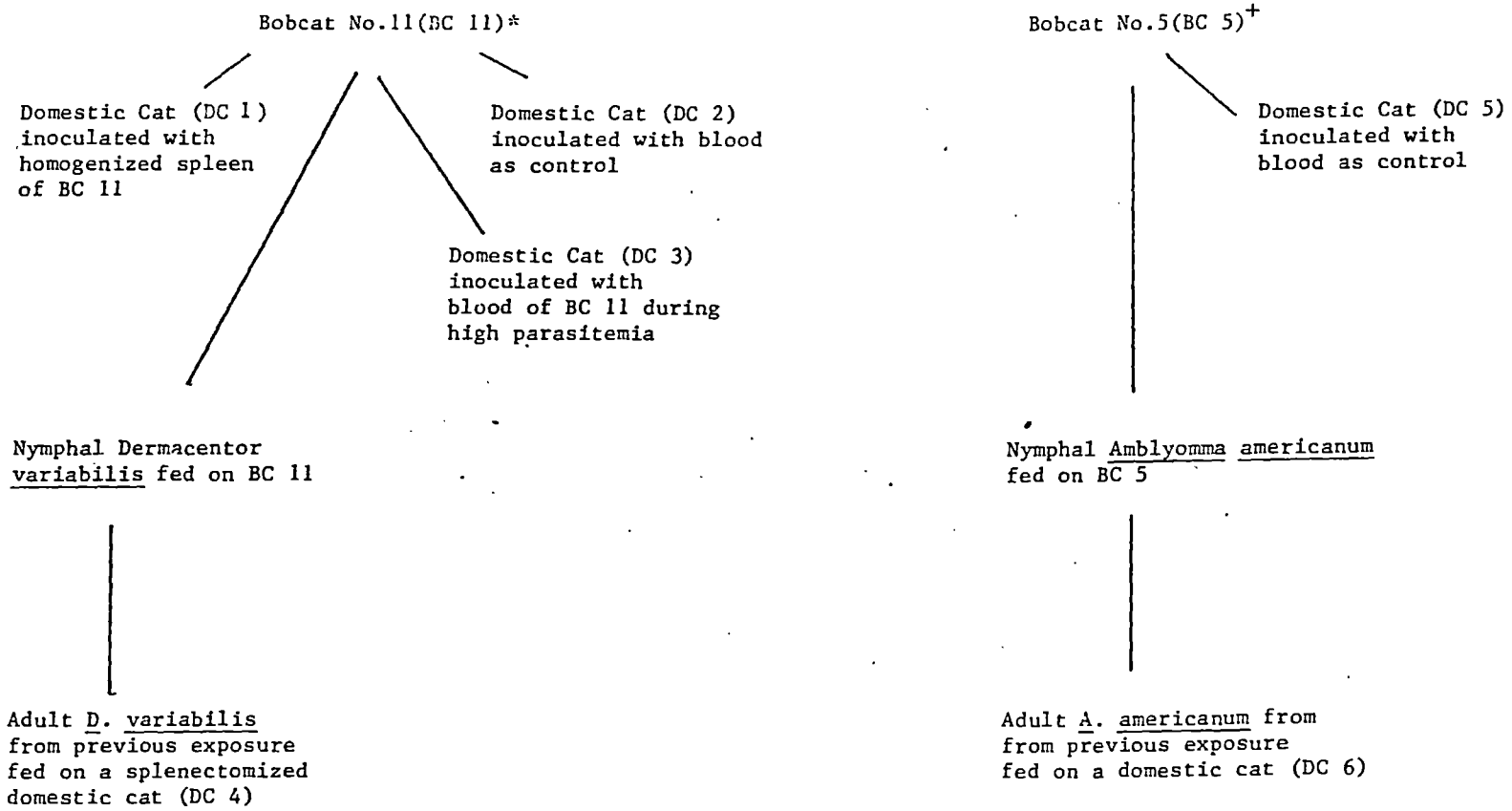
Tick species used in these studies were reared and maintained at the OSU Department of Entomology Tick Research Laboratory. Larval stages were fed on rabbits to facilitate the development to the nymphal stage.

Transmission Studies

Transmission from Bobcats to Domestic Cats.

The transmission studies outlined in this section are illustrated in Fig.1. A bobcat (BC 11) with a naturally occurring infection of the Cytauxzoon-like organism was bled and 2cc of blood was parenterally inoculated into an uninfected domestic cat. This served as a control to show that direct inoculation of infected blood will cause only a nonclinical parasitemia. Approximately 2cc of blood was also inoculated into a second uninfected bobcat (BC 5). Both bobcats were then splenectomized in an attempt to increase the parasitemia. Part of the spleen homogenate from BC 11 was inoculated into an uninfected domestic cat. Each of the bobcats was placed into a plywood box measuring 24 by 24 by 24 inches. The cover of each box had a fine mesh screen for ventilation. A container with approximately 2000 nymphal Dermacentor variabilis was opened and added to one of the boxes. A container with approximately 2000 nymphal Amblyomma americanum was added to the second box. A small container of

Figure 1. Summary of Cytauxzoon felis transmission studies between bobcats and domestic cats



*=Naturally infected with piroplasms
+=Received infected bloob from BC 11

water was placed in each box and the covers were screwed onto the boxes. The entire periphery of the top of each box was lined with adhesive tape. The bobcats were left in the boxes for 24 hours, until most ticks had attached to the cats.

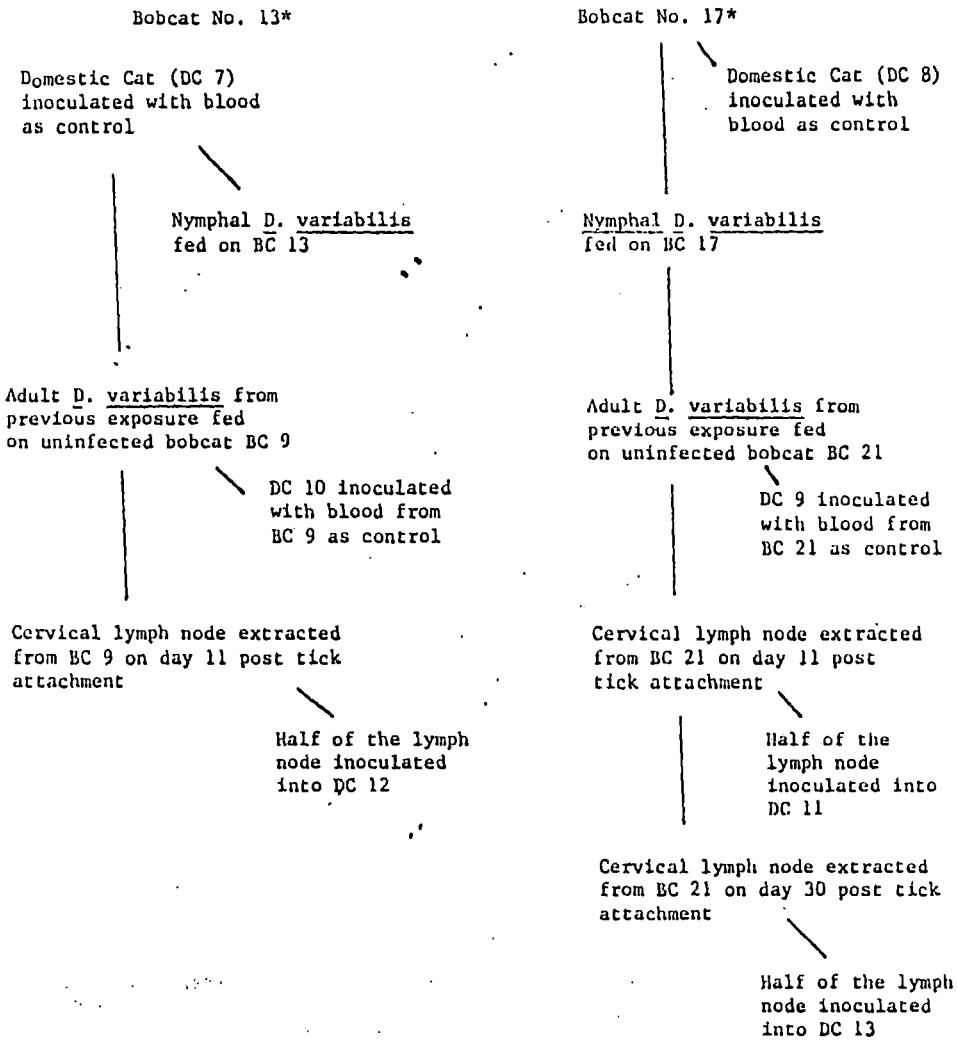
After 24 hours, each bobcat was removed from the wooden box and placed in a steel cage measuring 35 by 25 by 31 inches. The cage rested in a large collecting pan. The pan was approximately six inches wider than the cage on all sides. The cage rested approximately four inches above the base of the pan. The pans were lined with paper and the edges of the entire pan were lined with adhesive tape. Ticks from each cat became replete and fell into the pan. Ticks were collected daily and stored in a humidity chamber (90-98% relative humidity) at 25 C with a 14 hour photophase (Kocan et al., 1981) and allowed to molt.

After the ticks had molted to the adult stage, approximately 300 of each species were placed into similar plywood boxes with an uninfected domestic cat and left for 24 hours. At the end of this time the domestic cats were placed in small animal cages and closely monitored for clinical signs of cytauxzoonosis.

Transmission Between Bobcats

The procedures for the exposure of bobcats in this trial are outlined in Fig.2. Another naturally infected bobcat (BC 13) was obtained. Two cc of infective blood was inoculated into an uninfected domestic cat as a control to determine

Figure 2. Summary of Cytauxzoon felis transmission studies between bobcats



* Naturally infected with piroplasms

whether transmission of fatal cytauxzoonosis occurs by blood inoculation. The bobcat was splenectomized to increase the parasitemia and on day 15 post-splenectomy it was put into a plywood box for 24 hours with approximately 2000 nymphal Dermacentor variabilis. At the end of this time the bobcat was removed to a large steel cage suspended over a collecting pan. As ticks became replete, they were transferred to the humidity chamber and allowed to molt.

A bobcat (BC 9), determined to be negative for the presence of piroplasms by the examination of blood smears, was obtained and two cc of its blood was inoculated into a domestic cat as control. This second bobcat was then placed into a plywood box with approximately 400 of the newly molted adult D. variabilis ticks for 24 hours. The bobcat was removed to a small animal cage and monitored. On day 11 post tick attachment, a superficial, prescapular lymph node was removed from this second bobcat. The lymph node was cut into two pieces. One piece was used to make impression smears and fixed in neutral buffered formalin for histological evaluation. The smears were stained with Diff-Quik stain and examined with a light microscope. The second half of the lymph node was homogenized in RPMI media and inoculated parenterally into an uninfected domestic cat. The domestic cat was monitored for clinical signs of cytauxzoonosis by taking daily temperatures and direct observations. A second superficial, prescapular lymph node was removed on day 30 post tick attachment. This lymph node was processed in the

same manner as the first (including cat inoculation). The bobcat was also monitored during the 30- day period for any clinical signs of cytauxzoonosis.

Transmission Between Domestic Cats

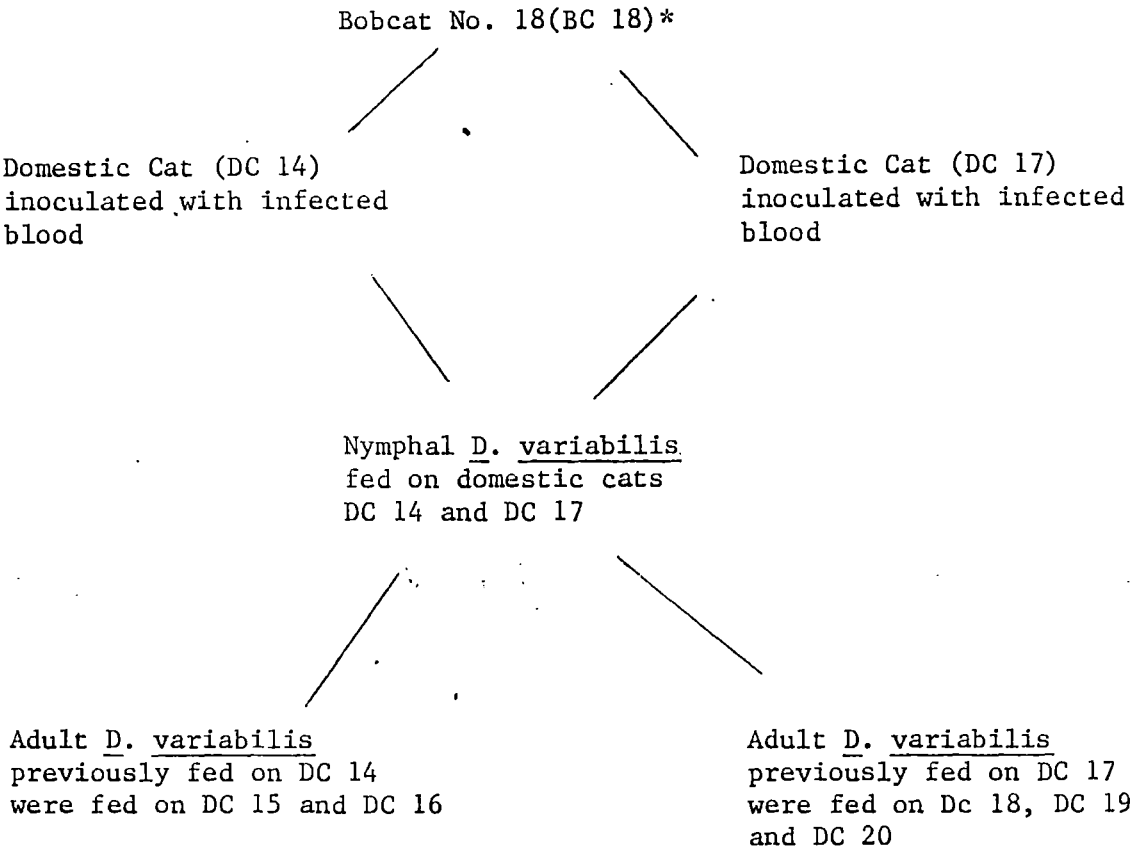
The procedure for exposure of domestic cats in this trial are outlined in Fig.3. An uninfected domestic cat was parenterally inoculated with blood from a naturally infected bobcat (BC 3). When a parasitemia was established in the domestic cat, it was placed in the plywood box with approximately 1000 nymphal *D. variabilis* and left for 24 hours. The cat was moved to a small animal cage and as ticks engorged and fell into the collecting pan they were removed to a humidity chamber. Newly molted *D. variabilis* adults were then placed into a plywood box with an uninfected domestic cat for 24 hours. At the end of this time the second cat was removed to a small animal cage and monitored for clinical signs of cytauxzoonosis.

Studies on the Developmental Stages of *Cytauxzoon felis* in the Salivary Glands of *Dermacentor variabilis*

Collection of Salivary Glands

An outline for the collection and processing of salivary glands is presented in TABLE I. A bobcat with a naturally occurring parasitemia was splenectomized. When the

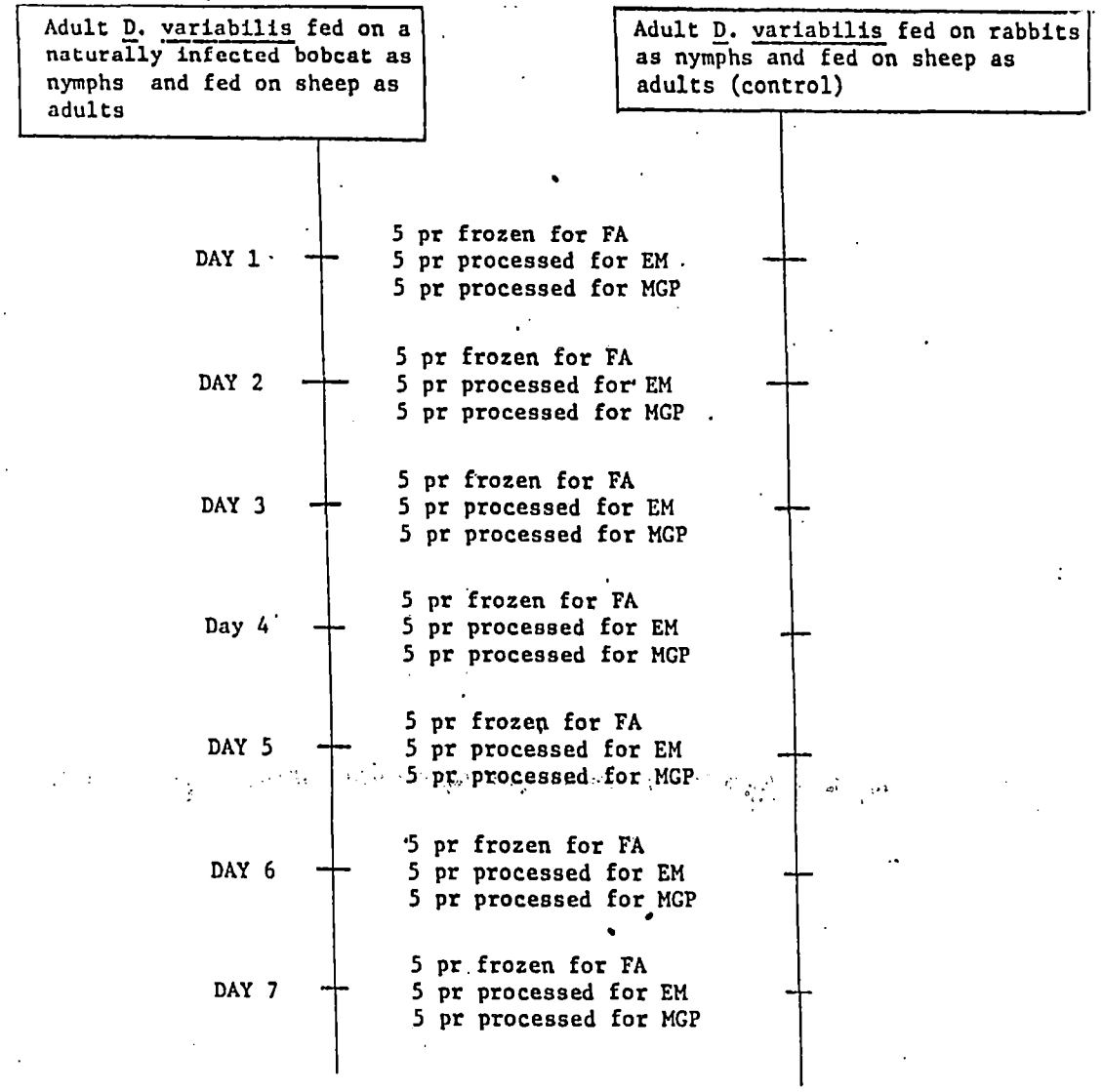
Figure 3. Summary of the *Cytauxzoon felis* transmission studies between domestic cats



* Naturally infected with piroplasms

TABLE I

SUMMARY OF THE COLLECTION AND PROCESSING PROCEDURES OF SALIVARY GLANDS FROM DERMACENTOR VARIABILIS FOR THE EVALUATION OF DEVELOPMENTAL STAGES OF CYTAUXZOON FELIS



FA= Florescent antibody
EM= Electron microscopy
MGP= Methyl green/pyronin stain

parasitemia was found to be increasing by monitoring daily blood films, the bobcat was placed in a wooden box with approximately 500 nymphal Dermacentor variabilis as described previously. Engorged nymphs were collected and allowed to molt in a humidity chamber. At one week post-molting, adult ticks were placed in four orthopedic stockinettes approximately 12 inches long that were glued to one side of a freshly cleaned and shorn sheep. Each stockinette covered a feeding surface of approximately six inches in diameter. Thirty pairs of adult ticks that had fed as nymphs (exposed group) on a naturally infected bobcat were added to each stockinette. The open end of each stockinette was folded over and tightly closed with a rubber band. On the opposite side of the sheep four additional stockinettes were attached and 30 pairs of adult ticks (unexposed group) were added to each to serve as controls. After 24 hours, unattached ticks were removed and discarded. Beginning on day 2 post attachment, 15 pairs of ticks were removed from both the exposed and control group on each day. The ticks were dissected in RPMI Medium 1640 (Gibco Laboratories, Chagrin Falls, Ohio) and salivary glands were removed for processing as described in the following sections. Ticks were collected until day seven post attachment.

Processing Salivary Glands of Dermacentor variabilis for Electron Microscopy

A portion of the salivary glands from each tick was immediately placed into cold 2% glutaraldehyde in 0.2M sodium cacodylate buffer (pH 7.4) for up to 48 hours. The tissues were then placed in three consecutive washes in 0.2 sodium cacodylate buffer for 10 minutes each. Tissues were then post-fixed for one hour in osmium tetroxide mixed 1:1 with 0.2M sodium cacodylate buffer followed by three 10 minute washes in 0.2M sodium cacodylate buffer. Tissues were washed twice in maleate buffer (10 minutes each) and were enbloc stained in 1% uranyl acetate in maleate buffer for one hour. The staining was followed by three 10-minute washes in maleate buffer and two 10 minute washes in triple distilled water. The glands were dehydrated beginning with two quick washes in 50% ETOH followed by one 10 minute wash in graded ethanols. Propylene oxide was used as a transitional solvent and the tissues were transferred to 1:1 mixture of propylene oxide and Dow Epoxy Resin (DER). The vials were capped and left for 48 hours, uncapped and after 12 hours embedded in DER. Thick sections were stained with Mallory's stain. Thin sections were prepared and stained with 5% uranyl acetate followed by lead citrate. Sections were cut on a Sorvall MT 5000 ultramicrotome and examined on a JOEL 100CXII electron microscope.

Processing of Salivary Glands for Light Microscopy

Following the procedure of Walker et al (1979), a portion of the salivary glands from each control and exposed tick was teased onto a microscope slide in RPMI 1640 medium and allowed to air dry. These tissues were fixed for five minutes in Carnoy's fixative. Slides were placed in 70% ethanol(ETOH) for two minutes and then rinsed in distilled water for two minutes. The slides were then placed directly into methyl green/pyronin stain for seven minutes, rinsed in distilled water and air dried (Walker et al, 1979). The glands were then covered with Permount (synthetic mounting medium) and covered with a coverslip. After allowing the medium to settle, the slides were examined for infected acini.

Processing of Salivary Glands for Fluorescent Antibodies

All remaining salivary glands were spread onto microscope slides and air dried. The glands were fixed in cold acetone for five minutes, air dried and transferred to slide holders. The slides were stored in a deep freezer at -20 C. Serum was obtained from naturally infected bobcats. The serum IgG was purified and labeled with fluorescein isothiocynate following the procedures of Stelos (1967) and Oberst et al. (1981) and stored in vials. The fluorescein-labeled antibody was diluted 1:15 with distilled water and applied to each of the slides. Slides were incubated in a warm, dark incubator for 90 minutes at 37 C,

rinsed in 0.25M phosphate buffer (pH 7.3), air dried and coverslipped with 10% glycerol in distilled water. Slides were then examined with a fluorescent microscope with epillumination.

Studies on the Developmental Stages of *Cytauxzoon felis* in Feline Red Blood Cells

Blood samples were collected from bobcats and domestic cats during periods of increasing parasitemia. Smears were made and stained with Diff-Quik stain (American Scientific Products, McGaw Park, Ill.) and the morphology of piroplasms was observed under the light microscope.

Blood samples taken from cats with high or increasing parasitemias were also prepared for study with the electron microscopy. Whole blood was collected in EDTA tubes and samples were drawn into microhematocrit tubes. Tubes were spun in a microhematocrit centrifuge for five minutes, scored just above the buffy coat with a small file and broken. A small metal rod was inserted into the sealed end of the tube and the concentrated red cells were pushed directly into cold 2% glutaraldehyde in 0.2M sodium cacodylate buffer. After one hour the buffy coat was removed from the mass of red cells with a razor blade. The red cells were then processed for electron microscopy as described previously.

CHAPTER III

RESULTS

Bobcat Survey

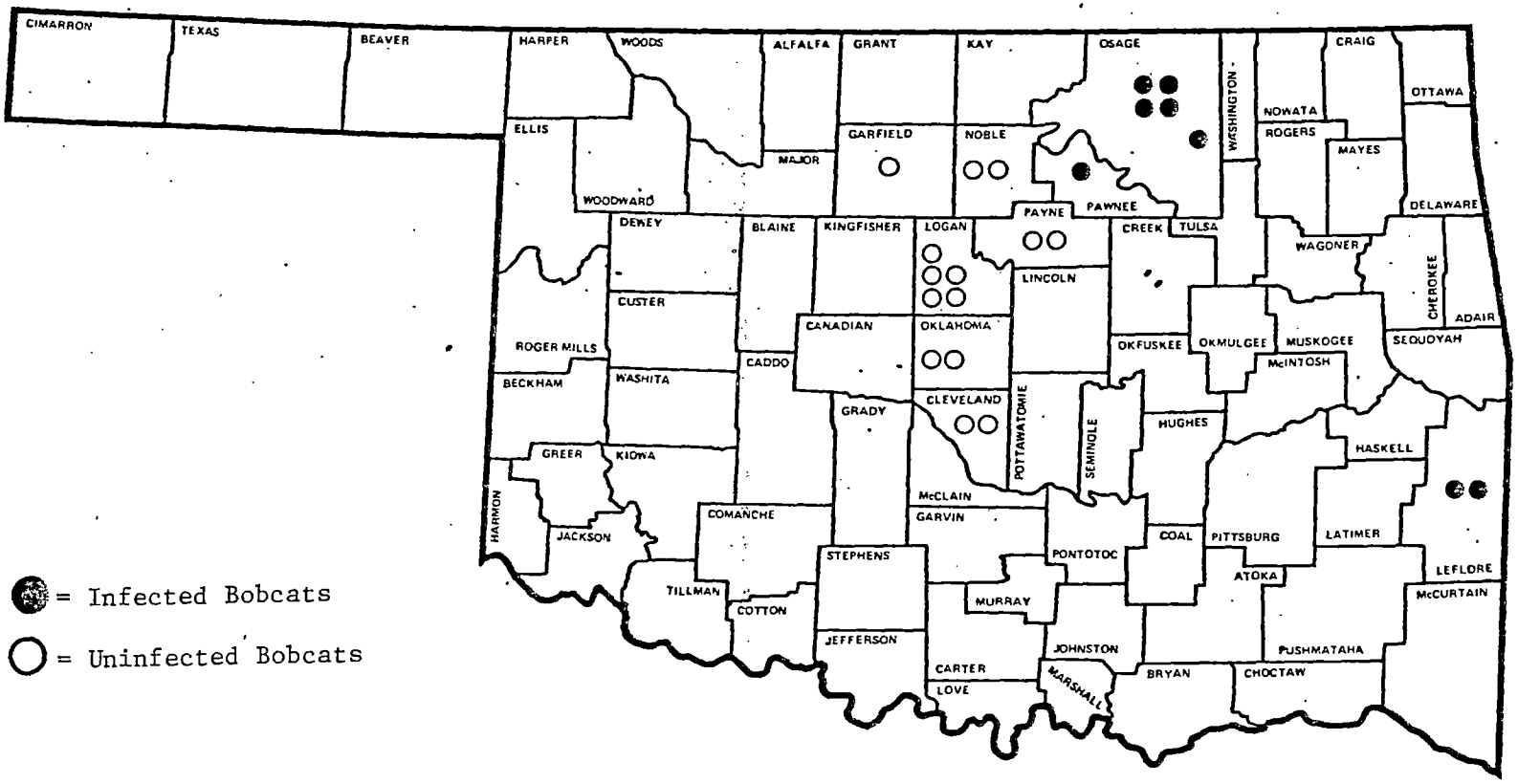
During the initial part of this study, a total of 22 bobcats were examined for the presence of piroplasms. Of these, eight were found to harbor piroplasms (TABLE II). The naturally occurring parasitemias were found to be 1% or less in most animals. One bobcat (BC 18) had a parasitemia of 3-4%. The bobcats were obtained from nine Oklahoma counties (Fig. 4). The origin of Bobcat No.17 was imprecise and was identified as "northeastern Oklahoma" rather than by county. Infected bobcats were found in three counties. These counties were in the northeastern part of the state and all of the bobcats examined from these counties were infected. Bobcats Nos. 3, 4 and 5 were necropsied and histological evaluation of various tissues failed to reveal schizogonous stages.

Transmission Studies

Transmission from Bobcats to Domestic Cats

Bobcat No.11 had an initial parasitemia of 0.8%. The 2 cc of blood inoculated into the uninfected domestic cat (DC

Figure 4. Distribution by county of bobcats examined for piroplasms in Oklahoma



● = Infected Bobcats

○ = Uninfected Bobcats

TABLE II

SUMMARY OF THE PIROPLASM SURVEY FOR BOBCATS FROM OKLAHOMA, 1982-1984

Bobcat No.	Location/County where trapped	Age	Sex	Presence of Piroplasms
1	Payne	A	M	N
2	Payne	A	F	N
3	Leflore	A	M	P
4	Leflore	A	M	P
5	Garfield	J	M	N
6	Logan	A	F	N
7	Logan	A	F	N
8	Noble	J	M	N
9	Logan	J	F	N
10	Logan	A	F	N
11	Osage	A	F	P
12	Osage	A	F	P
13	Osage	A	M	P
14	Logan	A	F	N
15	Noble	A	F	N
16	Osage	A	M	P
17	NE Okla.	J	F	P
18	Pawnee	A	M	P
19	Cleveland	A	F	N
20	Cleveland	A	F	N
21	Oklahoma	A	F	N
22	Oklahoma	A	M	N

A= Adult
J= Juvenile

N= Negative
P= Positive

M= Male
F= Female

Total No. Examined-22

Total No. Positive-8

1) resulted in a nonclinical, low level parasitemia (Fig.1). The blood inoculated into the second bobcat (BC 5) produced a low level parasitemia. The spleen homogenate from BC 11 resulted in a low level parasitemia in the domestic cat (DC 2) (Fig.1). Histological evaluation of tissues from both domestic cats failed to reveal tissue stages of the organism. Six days following the splenectomy of BC 11, the parasitemia had risen to 4% and by day 18 post splenectomy had reached 17%. During this time the bobcat's temperature did rise slightly to 102.2 F. The parasitemia had increased to approximately 45% by day 24 post-splenectomy and subsequently began to decrease. Domestic cat No.3 developed only a low level parasitemia (Fig.1).

Bobcat No.5 (which received blood from BC 11) developed a low level parasitemia 7 days post inoculation. Infected blood from BC 5 produced a low level infection when inoculated into the control domestic cat (DC 5) (Fig.1). The parasitemia of BC 5 continued to rise until it reached approximately 40% on day 55 post-inoculation. Since the parasitemia continued to increase, the splenectomy was not performed on BC 5.

When the parasitemia of both bobcats was approaching 40%, an increasing number of immature erythrocyes was seen in blood films. The bobcats became sluggish at this time, appeared anemic and hematocrits were low. Temperatures did not increase significantly during this time.

When both bobcats were placed into the tick boxes, the

parasitemia of BC 11 had decreased to 18% while BC 5 was still at approximately 40%. Of the 2000 nymphal ticks placed into the box with each bobcat, approximately 600 engorged Dermacentor variabilis were recovered from BC 11 and approximately 900 engorged Amblyomma americanum were recovered from BC 5 (TABLE III). The first engorged D. variabilis nymphs were recovered from BC 11 at 5 days post attachment and the first engorged Amblyomma americanum nymphs were recoverd from BC 5 at 3 days post attachment. Dermacentor nymphs were collected for 7 days and Amblyomma nymphs were collected for 9 days. After all the ticks had fallen off BC 11, the parasitemia had decreased to 5% but the parasitemia of BC 5 had risen to 48%. Two months after the tick recovery, BC 5 died. Necropsy and histological evaluation of tissues failed to reveal any indication of fatal cytauxzoonosis and the cause of death was undetermined.

After molting to the adult stage, adult ticks of both species exposed as nymphs were used to attempt to transmit the infection. Following 24 hours of tick exposure, approximately 20 adult Dermacentor variabilis ticks attached to to domestic cat No.4(DC 4) and 5 Amblyomma americanum adults had attached to domestic cat No.6(DC 6). On day 9 post-tick attachment, DC 4 had a temperature of 102.8 F. On day 13, the temperature had risen to 103.8 F. The cat appeared sluggish, had stopped eating and was slightly icteric. Piroplasms were also observed in blood smears. On the morning of day 14 post tick attachment, DC 4 was ataxic,

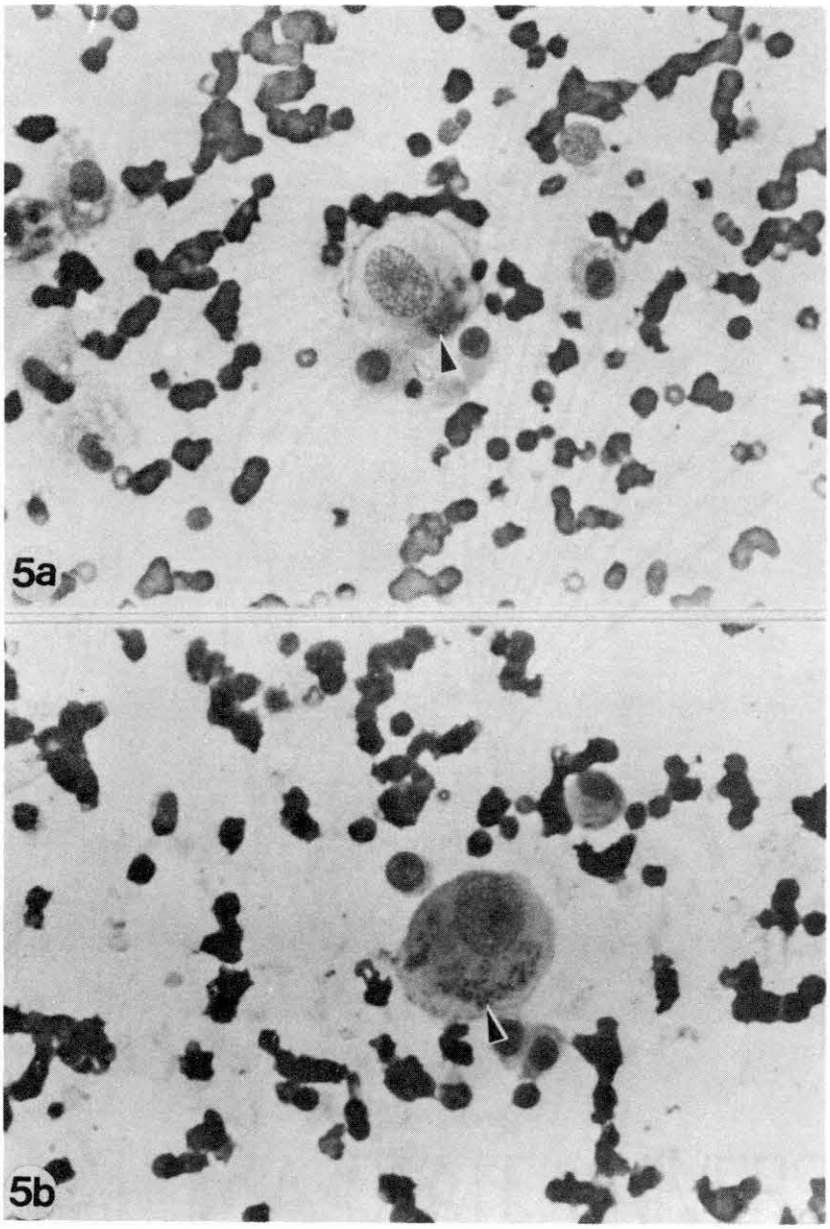
TABLE III

COLLECTING AND FEEDING DATA OF TICKS FROM INFECTED BOBCATS FROM OKLAHOMA

Bobcat No.	11	5
Prefeeding Parasitemia	18%	40%
Tick Species	Dermacentor variabilis	Amblyomma americanum
No. of Nymphal Ticks Fed	2000	2000
No. of Ticks Recovered	600	900
1st Collecting Day	5 DPA*	3 DPA
Last Collecting Day	11 DPA	11 DPA
Post Feeding Parasitemia	5%	48%

* Days Post Attachment

Figure 5. Impressionn smear of lung from domestic cat no.4 (Diff-Quik). (a) Macrophage with an early schizont (arrow; X400. (b) A progressively larger schizont (arrow); X400.


5a
5b

began to exhibit labored respiration and died shortly thereafter. Gross pathological findings at necropsy included heavy petechial hemorrhaging of the lungs and to a lesser extent the heart. The pericardial sac was filled with a dark yellow fluid. At the time of death, six ticks were removed from the cat (three males and three females). Blood smears revealed a low parasitemia (less than 1%). Impression smears of lungs, bone marrow and lymph nodes revealed schizonts similar to Cytauxzoon felis in macrophages. Schizonts appeared to be in different stages of maturity. Some host cells contained early schizonts which took up only a small portion of host cell cytoplasm (Fig.5a). Other cells contained schizonts which occupied progressively larger amounts of host cell cytoplasm (Fig.5b and 6a). Some cells were completely filled with parasitic stages (Fig.6b). In these later stage schizonts, the parasite chromatin appeared as small and numerous dark staining bodies. In earlier stages the chromatin appeared as larger bodies and were fewer in number. The later stage schizonts appeared to be distending the host cell membrane so that macrophages with fully developed schizonts were considerably larger than those with earlier stages or uninfected ones. Post mortem blood smears (from pooled cardiac blood) revealed infected macrophages. Histological evaluation of tissues revealed large macrophages in the endothelial lining of blood vessels in many organs (Fig.7a) that were most noticeable in the lungs where endothelial-associated macrophages completely

Figure 6. Impression smear of lung from domestic cat no.4 (Diff-Quik). (a) A schizont (S) occupying much of cytoplasm of a macrophage and host cell nucleus (N) at the periphery; X1000. (b) A macrophage completely filled by a schizont (S) and with a small remnant of host cell nuclear material (N); X1000.

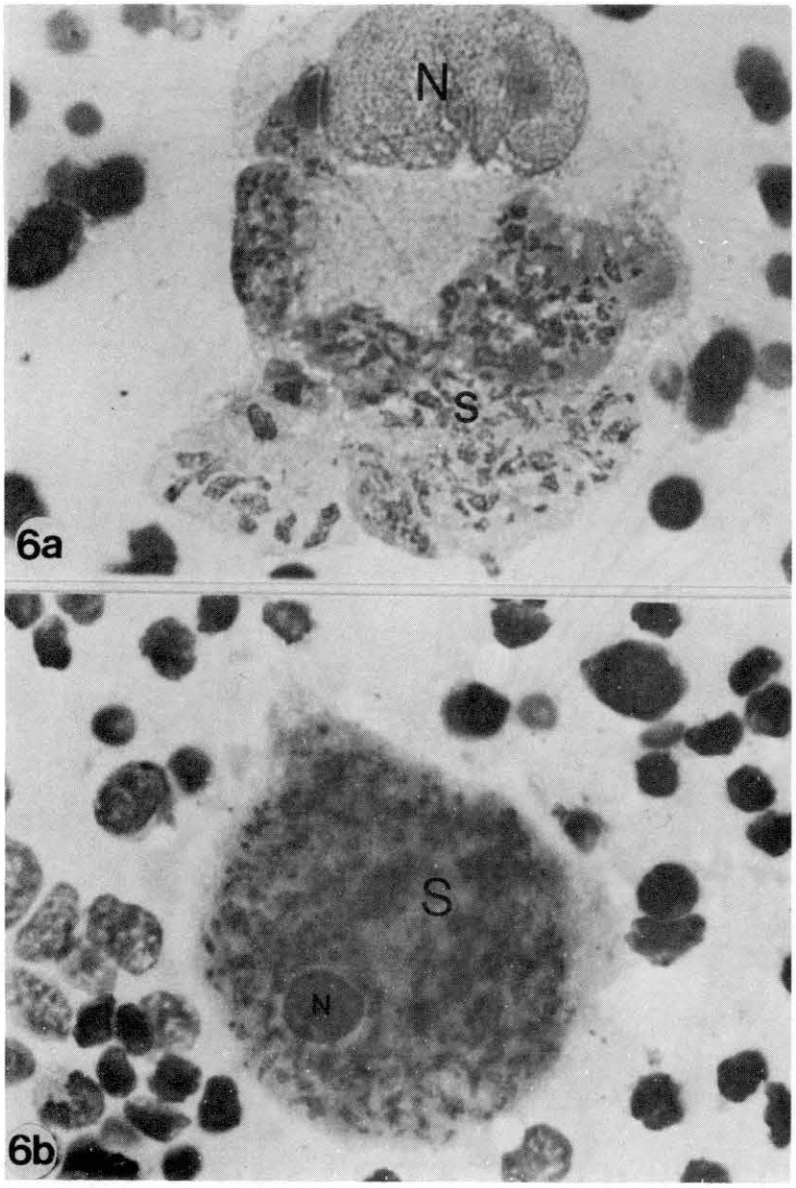
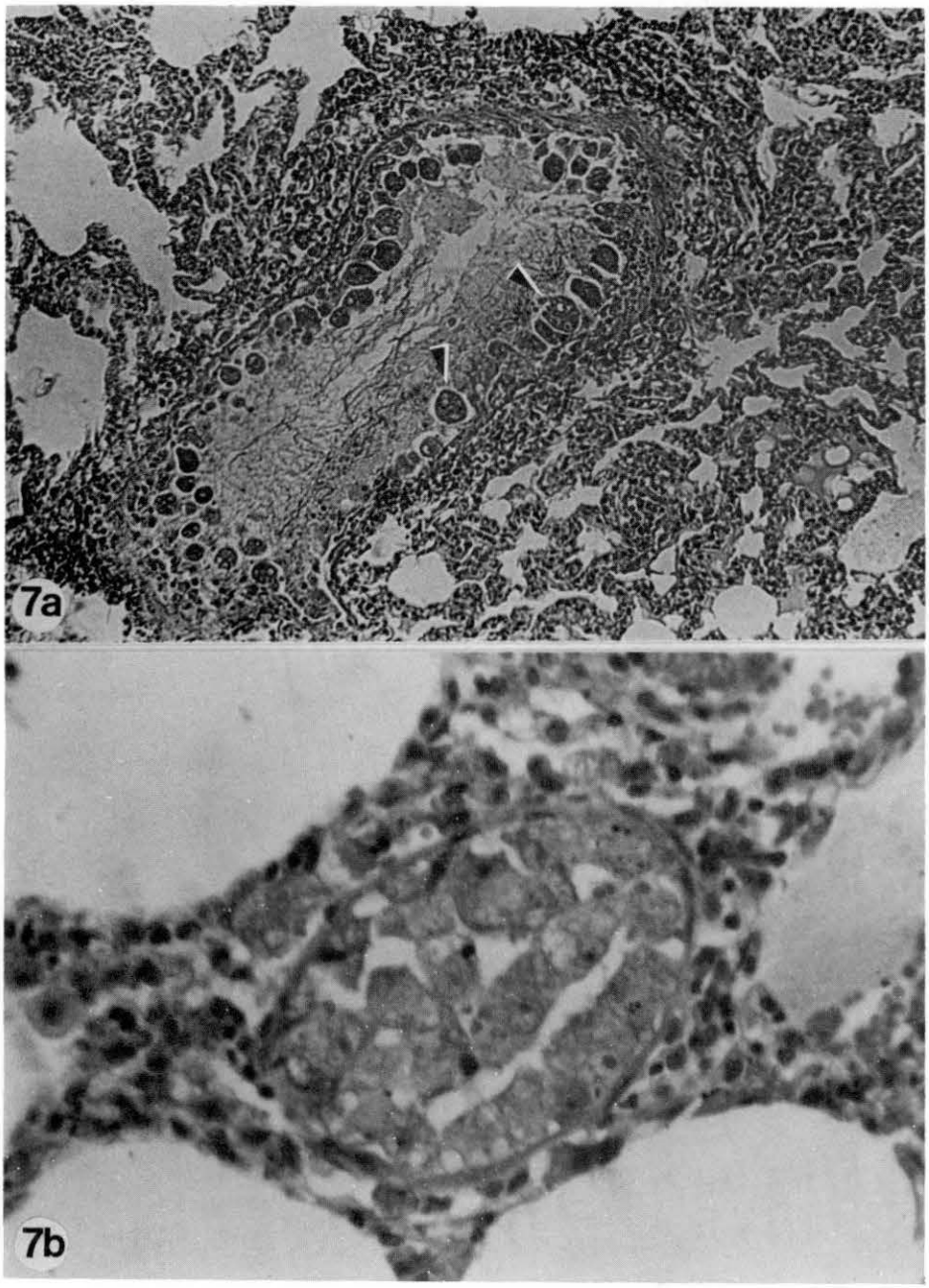

N
S
6a
S
N
6b

Figure 7. Histological sections of lung from domestic cat no. 4 (Hematoxylin and Eosin). (a) A large blood vessel lined with enlarged macrophages (arrow); X100. (b) A smaller vessel completely occluded by enlarged macrophages; X400.


7a
7b

occluded some vessels (Fig.7b).

Domestic cat No.6 (Fig.1) had an increased body temperature of 103.8 F on day 9 post-tick attachment but returned to normal the next day. The temperature rose again on day 15 to 103.2 F but returned to normal on day 16. Blood smears were examined on day 15 and found to be negative. No ticks were observed on the cat at this time. The cat was monitored for 28 days and no further clinical changes were observed. Additional blood films failed to reveal piroplasms.

Transmission Between Bobcats

Bobcat No.13 developed a steadily increasing parasitemia following splenectomy. The parasitemia peaked at 30% two weeks post-splenectomy. Approximately 500 replete Dermacentor variabilis nymphs were recoverd and the parasitemia on the final day of tick feeding was 12-13%. The blood from BC 13 inoculated into DC 7 produced only a low level parasitemia in the domestic cat (Fig.2).

Approximately 30 of the adult D. variabilis (fed on BC 13) attached to BC 9. The domestic cat (DC 10) that received blood from BC 9 developed neither a parasitemia nor clinical signs of disease (Fig.2). The cervical lymph node preparation inoculated into into DC 12 on day 11 post-tick attachment produced a fatal cytauxzoonosis in the domestic cat with all of the typical clinical signs mentioned previously. Lymph node impressions from from day 11 had

numerous schizonts in reticulo-endothelial macrophages (Fig.8). Most of these appeared to be in early stages of development.

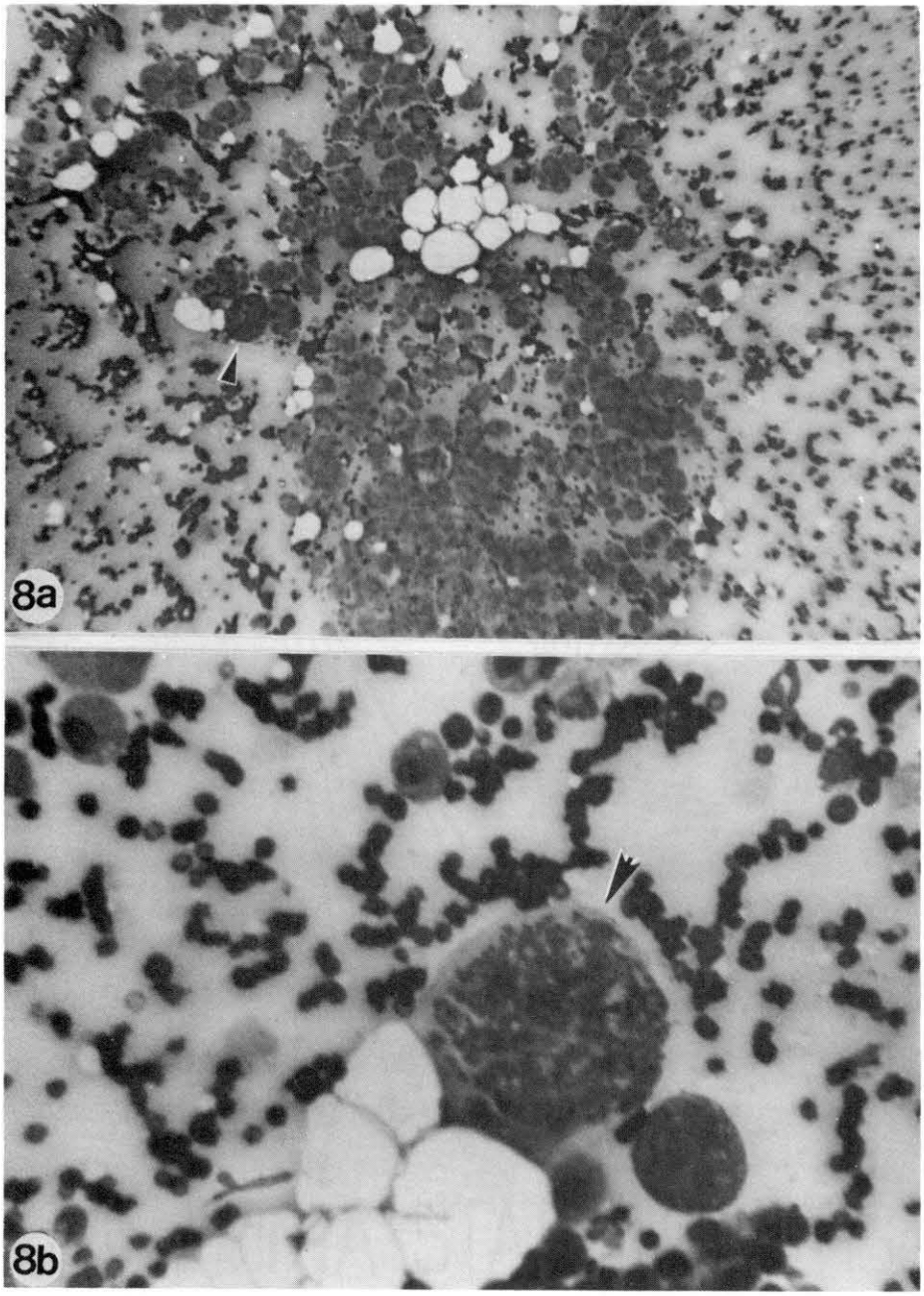
Bobcat No.9 died on day 19 post-tick attachment and, therefore, the second planned lymphadenectomy could not be performed. Post mortem examination and histological evaluation of tissues revealed that the bobcat had died of cytauxzoonosis.

In a repeat of this experiment, BC 17 developed a 50% parasitemia following splenectomy. Domestic cat No.8 (Fig.2), which served as a control, developed a low level, nonclinical parasitemia. Approximately 1500 engorged D. variabilis nymphs were recovered from BC 17 and its parasitemia on the final day of tick feeding was approximately 30%.

Approximately 50 adult D. variabilis (fed on BC 17) attached to the uninfected bobcat (BC 23). Blood from BC 23 produced neither a parasitemia nor clinical disease in DC 9. The lymph node preparation from BC 21 on day 11 post tick attachment produced fatal cytauxzoonosis when inoculated into DC 11. The lymph node impressions from day 11 revealed schizonts in macrophages.

The cervical lymph node extracted from BC 21 on day 30 post-tick attachment was removed and inoculated into DC 13 but failed to produce any clinical signs of cytauxzoonosis.The domestic cat was observed for 21 days post inoculation and euthanized. Histological examination of

Figure 8. Lymph node impression from bobcat no.4. on day 11 magnification of a schizont-laden macrophage (arrow); X100. (b) Higher magnification of a shizont-laden macrophage (arrow); X400.


8a
8b

tissues failed to reveal any parasite stages but a low-level parasitemia was observed. Examination of lymph node impressions and histological sections of the second lymph node from BC 21 failed to reveal any stages of the parasite. The parasitemia of BC 21 at 30 days post tick attachment was 15%.

Transmission Between Domestic Cats

Domestic cat No.14 developed a parasitemia of 68% following splenectomy and inoculation with blood from BC 18 (Fig.3). Approximately 150 engorged D. variabilis nymphs were recovered from DC 14. When a domestic cat (DC 15) was placed in the plywood box with 60 of the newly molted adult ticks, none was found to have attached by 24 hours. An attempt to infect a second domestic cat (DC 16) with 50 adult ticks resulted in two attaching. The cat was monitored for several weeks and no clinical signs of cytauxzoonosis were observed.

A second domestic cat (DC 17), inoculated with blood from BC 18 developed a 10% parasitemia following splenectomy. Approximately 200 engorged D. variabilis nymphs were recovered from DC 17. Attempts to infest three different domestic cats with newly molted adults were unsuccessful with two cats (DC 18 and DC 19). Only one tick attached to the third cat (DC 20). The third cat was observed for several weeks and no clinical signs of cytauxzoonosis were seen.

Studies on the Developmental Stages of *Cytauxzoon felis* in the Salivary Glands of *Dermacentor variabilis*

Collection of Salivary Glands

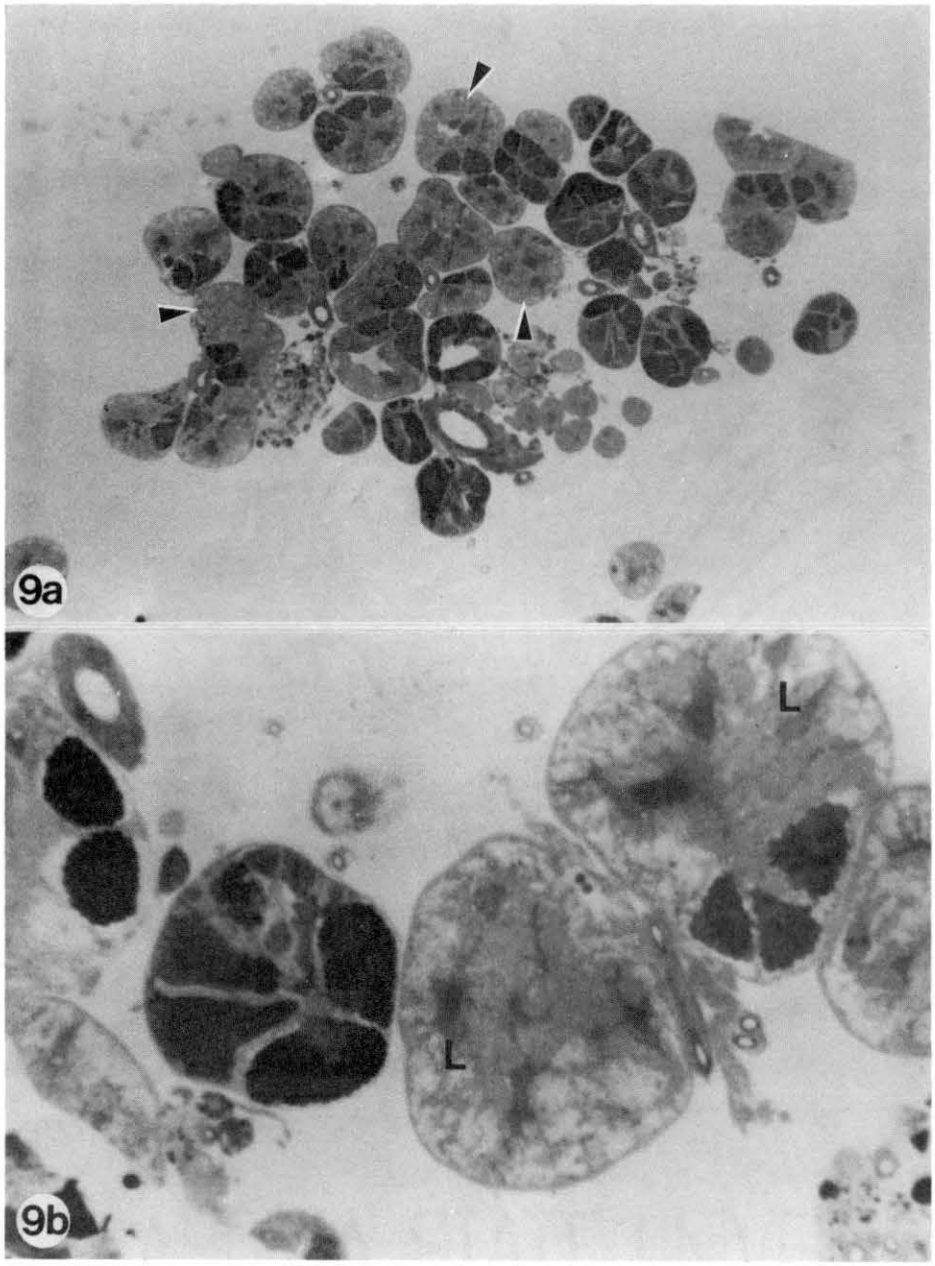
In those cases in which attachment occured, it was observed that the majority of adult ticks attached within several hours. There was a gradual increase in the size of salivary glands from days two through five. There was not a noticeable difference in size or other gross morphologic features between control and infected ticks.Most of the adult ticks were found to have detached (fully engorged) by 6.5 days post attachment.

Evaluation of Salivary Glands

Examination of thick sections stained with Mallory's stain allowed for the differentiation of acinar cell types as well as individual cell types within the acinus. No distinct differences could be noted in the salivary glands from ticks in the control and infected groups. In salivary glands of both control and exposed group ticks which had fed for four and five days, there appeared to be a labyrinth in E-cells of Type III acini (Fig.9). However, nothing resembling a schizogonous stage could be identified in these areas.

Examination of Types I, II and III acini in electron micrographs failed to reveal any obvious parasitic forms.

Figure 9. Histological section of salivary gland from infected Dermacentor variabilis adult ticks which had fed on a sheep for four days (Mallory's stain). (a) Low magnification of acini with labyrinth-like open areas (arrows); X100. (b) Higher magnification of the labyrinth-like area; X400.

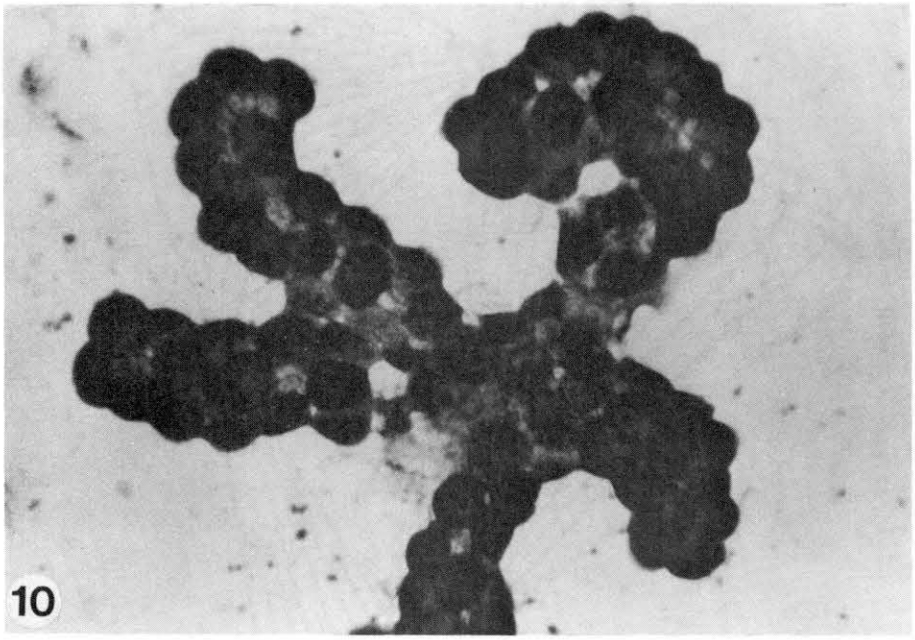

9a
9b
L
L

Particular attention was given to the E-cells of the Type III acinus. The labyrinyh observed in these cells in light micrographs was closely examined and in the salivary glands of ticks on later days of feeding nothing resembling a schizogonous stage could be seen. The labyrinth was observed to be mostly acellular matrix and remnants of host cell cytoplasm and organelles. None of the tissues examined appeared to have cells which were disproportionately larger than others in the acinus.

Light Microscopy of Salivary Glands

In the salivary glands stained with methyl green/pyronin, nuclear material stained light blue while the remainder of individual cells stained pink. Glands from ticks feeding only a short time stained more intensely than those from longer feeding ticks. On slides with well spread glands, the position from which individual acini arose could be determined. Individual acinar types could not be determined however (Fig,10). The various secretory granules were not obvious in acini of salivary glands from ticks in either the control or exposed groups. At each successive day of feeding the cytoplasm appeared more diffuse. Blue staining particles (other than host cell nuclei) could not be found in any of the salivary glands from the exposed group ticks. There did not appear to be any abnormally distended acini as seen in glands infected with Theileia spp (Melhorn and Schein, 1984).

Figure 10. Methyl Green/Pyronin stained salivary glands from a Dermacentor variabilis adult ; X100.


10

Fluorescent Antibody Studies

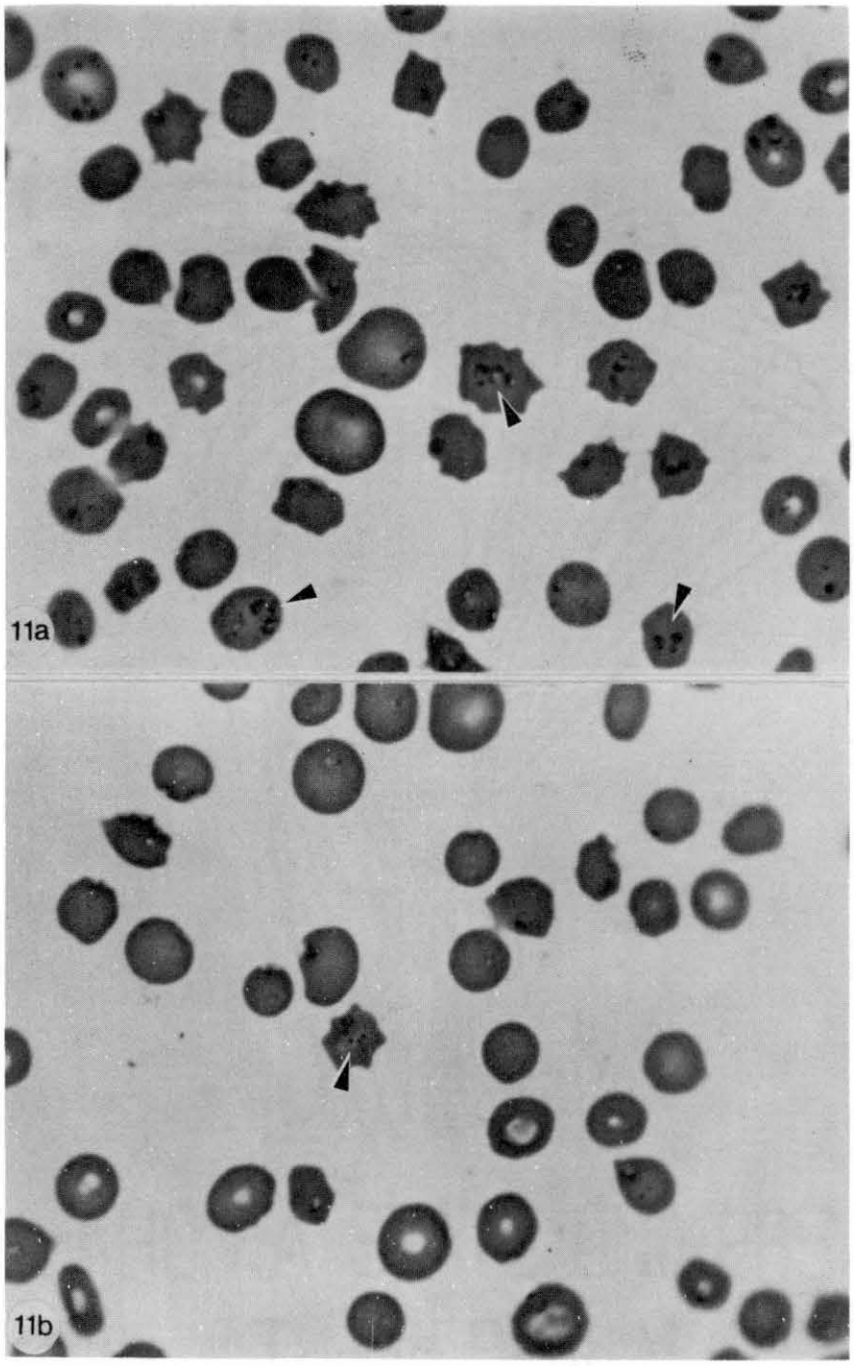
Two slides of salivary glands from each feeding day were prepared from ticks in both control and exposed group. When the fluorescein-labeled antibody (diluted 1:15) was applied to the fixed slides, salivary glands from both groups of ticks exhibited a high degree of fluorescence. Cells from both control and exposed group ticks did exhibit large areas of intense fluorescence. Because of the high degree of fluorescence on both sets of slides it was determined that the dilution of antibody was not low enough and that there was too much nonspecific binding to detect parasitic stages. Since no additional ticks were available for use at this time the test was not repeated.

Developmental Stages of *Cytauxzoon felis* in Feline Red Blood Cells

Light Microscopy

Following splenectomy, the infected bobcats began to show an increase in parasitemia. Regular blood films revealed a wide variation in size and morphology of piroplasms (Fig.11a). Many red blood cells had multiple infections (five or six in some cells). Both very large and small piroplasms were found in the same cells. Many of the larger organisms appeared more irregular in shape and contained a large amount of cytoplasm. In addition, numerous

Figure 11. Blood films from a splenectomized bobcat naturally infected with piroplasms (Diff-Quik). (a) Multiply infected erythrocytes with piroplasms of various sizes (arrows); X1000. (b) "Maltese Cross" formation which appears to be pulling apart (arrows); X1000.


11a
11b

"Maltese cross" formations were observed (Fig.11b) and in some of these an occasional member of the association appeared to be pulling away from the rest (although they all appeared to be connected by cytoplasm). The nuclear material tended to be more diffuse in dividing forms but more compact and peripheral in single piroplasms.

In bobcats with a low parasitemia, the piroplasms appeared to be roundish and of uniform size. There was generally one organism per cell. The nucleus was dense and appeared to be restricted to one end of the cell.

Electron Microscopy

Sections of red blood cells revealed a number of electron dense organelles in piroplasms. Most of these corresponded to the structures identified by Simpson et al. (1985) in C. felis. In most piroplasms the nucleus was granular and large and tended to be polarized (Fig.12a). The nuclear membrane of all piroplasms was not clear (Figs. 12 and 13). Large organelles that were more electron dense than any of the other organelles were found in many piroplasms (Fig.12b). The number of these organelles varied from two to three in smaller single cells to seven or eight in dividing forms. Their morphology varied from round to pyriform and usually appeared polarized (opposite the nucleus). Similar structures were identified as food vacuoles by Simpson et al. (1985) but some of these had the appearance of rhoptries (Fig.12c).

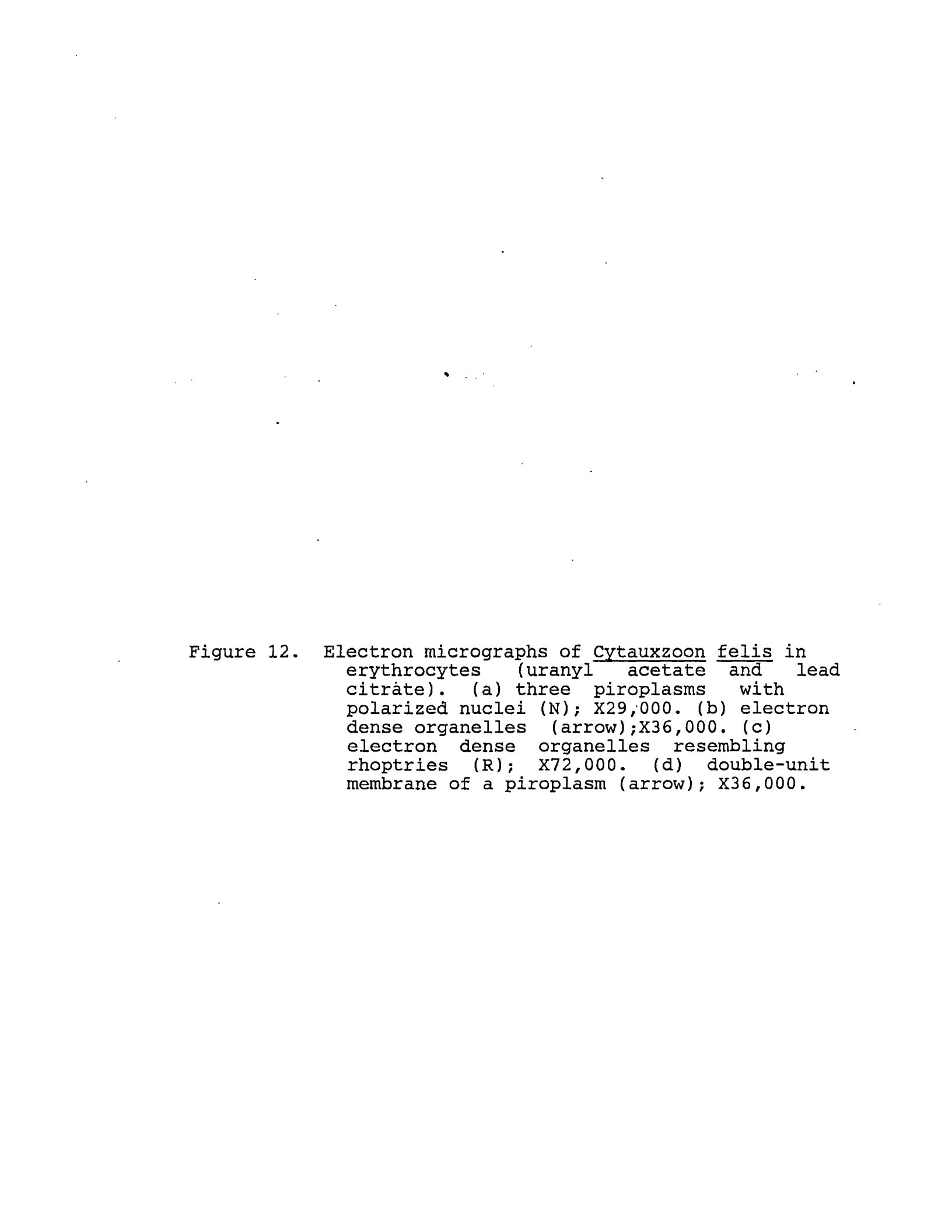
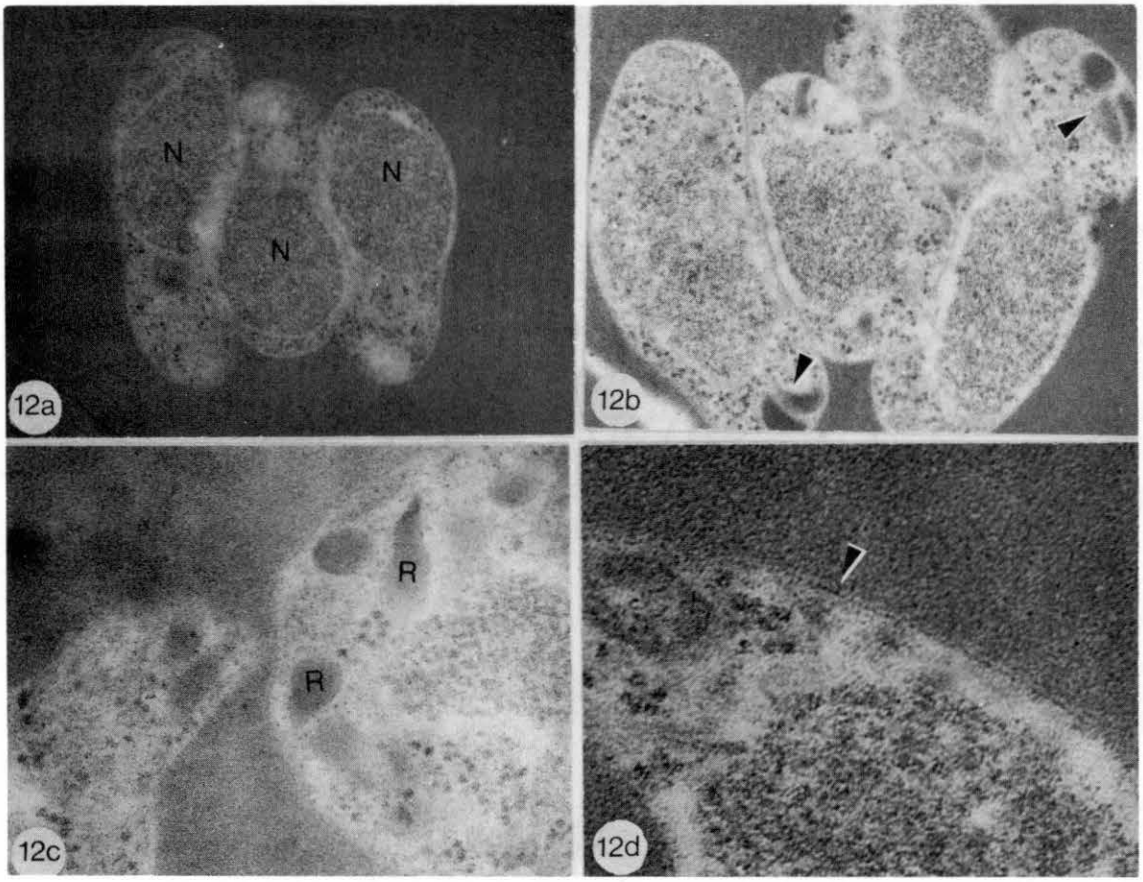


Figure 12. Electron micrographs of Cytauxzoon felis in erythrocytes (uranyl acetate and lead citrate). (a) three piroplasms with polarized nuclei (N); X29,000. (b) electron dense organelles (arrow);X36,000. (c) electron dense organelles resembling rhoptries (R); X72,000. (d) double-unit membrane of a piroplasm (arrow); X36,000.


N
N
N
12a
12b
R
R
12c
12d

Piroplasms appear to be limited by a double-unit membrane in very close apposition to the host cell cytoplasm (Fig. 12d). The double unit-membrane was not visible on all piroplasms and only portions of the wall were visible on some. No parasitophorous vacuoles were observed.

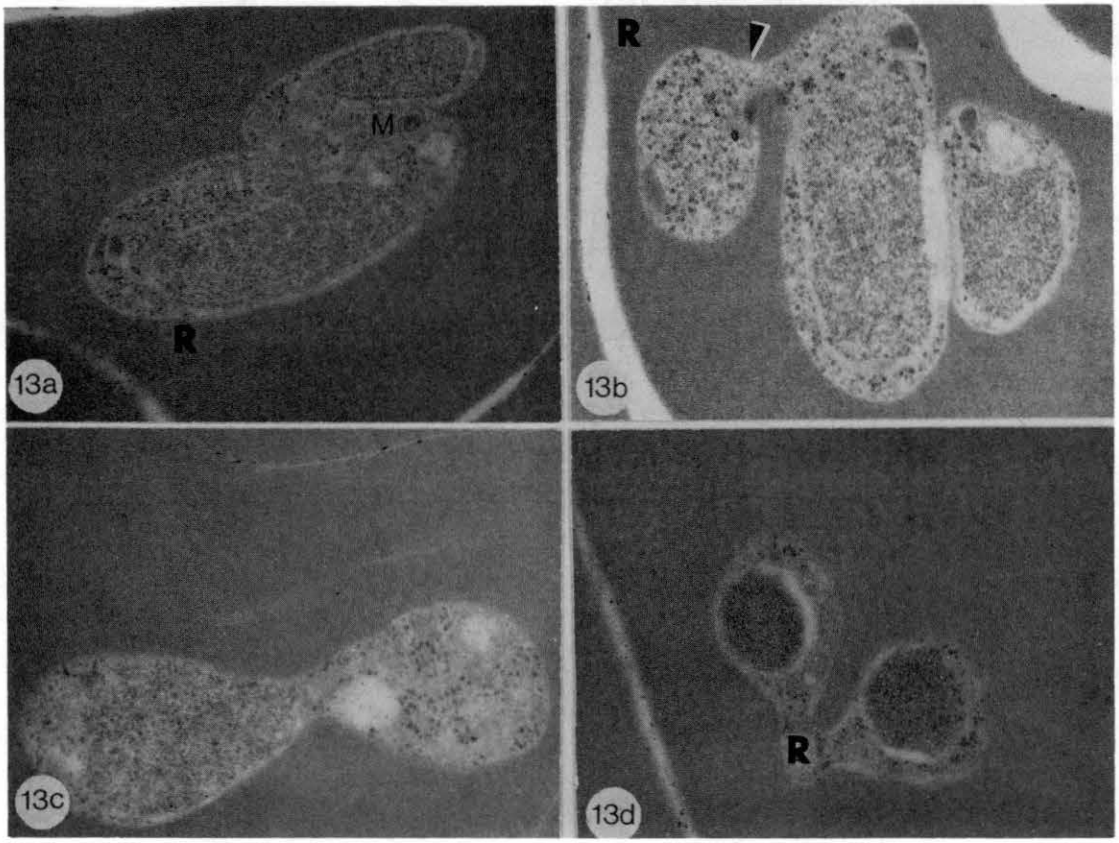
A structure that resembles a micropore was observed in several piroplasms (Fig.13a). It had a dense center surrounded by a lighter area and these appeared to be bounded by a double unit-membrane.

Several forms of division were observed. The first appeared to be a longitudinal binary fission. Erythrocytes containing two, three or four organisms in close apposition were observed (Figs.12a, 12b and 13a). There appeared to be a polarized distribution of organelles with the nucleus at one end and smaller organelles at the other. Daughter cells had an opposite arrangement of organelles. Figure 12b shows a red cell with four piroplasms that appear to be pulling away from each other. This division does not appear to be synchronous. This type of fission may represent what is actually seen in the "Maltese cross" formation.

A second type of division was observed in which cells appear to be in the process of budding off small pieces of cytoplasm (Fig.13b). This budding was seen consistently in different samples. Still others appear to be pinching off in the middle (Fig.13c).

A type of division interpreted to be binary fission in which the piroplasm is dividing in a Y-formation was observed

Figure 13. Electron micrographs of Cytauxzoon felis in erythrocytes (uranyl acetate and lead citrate). (a) stuctures resembling a micropore (M) and a residual body (R); X29,000. (b) a piroplasm budding off a portion of cytoplasm (arrow) and a residual body (R); X36,000. (c) a small piroplasm pinching off in the middle; X36,000. (d) division in a Y-form with a residual body (R) at the point of division; X29,000.


M
R
13a
R
13b
13c
R
13d

in one red cell (Fig.13d). The two daughter cells appear to be attached to a residual body. Small structures which are thought to be a residual body following this type of fission (Figs.13a and 13b), were observed in many infected erythrocytes.

CHAPTER IV

DISCUSSION

Bobcat Survey

The results of the survey suggest that certain sampling areas are more likely to have infected bobcats than others. This observation supports observations of Glenn et al. (1982) regarding 26 bobcats. In that study 20 of the bobcats were from LeFlore county and 12 of these were positive.

Despite the high incidence of infection in bobcats there have been no cases of cytauxzoonosis reported in domestic cats from either Osage or LeFlore counties. Four counties from which fatal cases in domestic cats have been reported (Hughes, Seminole, Pontotoc and Carter) have not been surveyed. It is likely that there are other areas within the state where the prevalence among bobcats is high. Since the total number of reported cases of feline cytauxzoonosis throughout Oklahoma and the county is small, it is not surprising that there have been no cases reported in areas where infection rates in bobcats are high. It is likely however, that a correlation exists between local infectivity rates in bobcats and cytauxzoonosis in domestic cats. This is difficult to determine however, due to the rural nature of the transmission cycle.

Transmission Between Bobcats and Domestic Cats

Kier et al. (1982) established a basis for suspecting the bobcat as a susceptible host for Cytauxzoon felis by establishing infection in a bobcat following parenteral inoculation of infected blood from a domestic cat. One of the major purposes of the present study was to confirm the association between the piroplasm found naturally occurring in the bobcat and that found in domestic cats with fatal cytauxzoonosis. This association has been confirmed with the successful transmission of the organism to two domestic cats by Dermacentor variabilis ticks. The disease produced in domestic cats was identical to that seen in natural cases of feline cytauxzoonosis. The ticks used in the study were laboratory reared and pathogen free at the time of their feeding on bobcats. It is therefore concluded that the piroplasm found naturally occurring in bobcats in Oklahoma is Cytauxzoon felis and that D. variabilis can serve as a transstadial vector. Based on the large number of infected bobcats found and the relative lack of clinical disease in these cats, it is also concluded that bobcats are the natural host of C. felis. Since it has been shown that the organism multiplies asexually in erythrocytes, it is likely that bobcats can serve as asymptomatic carriers of the piroplasm for life.

Although D. variabilis did act as a transstadial,

biological vector for C. felis, it is not necessarily the primary natural vector.

The increased parasitemia following splenectomy probably enhanced transmission of the organism. Naturally occurring parasitemias were low in bobcats, and it would be expected that infection rates of ticks in the wild would be low. Low infection rates in wild ticks would likely result in low transmission rates to domestic cats which are probably accidental hosts. In addition, transmission among bobcats would tend to be low and somewhat restricted to bobcats within a defined area. This is consistent with the survey data which indicated that positive bobcats tended to concentrated within distict areas. Transmission among bobcats is likely to occur most frequently among related bobcats as in family groups. This may account for the clustering of infected bobcats in Osage and Leflore counties.

Transmission Between Bobcats

The results of this study have shown that the developmental cycle of C. felis is similar in both domestic cats and bobcats. The death of bobcat No. 9 from cytauxzoonosis indicates that they may succumb to clinical disease under some conditions. In preliminary work for the present study, two bobcats that were inoculated with spleen homogenate from a domestic cat that died of cytauxzoonosis developed mild clinical signs including a rise in body

temperature and temporary loss of appetite. Kier et al (1982) produced fatal cytauxzoonosis in one bobcat and a nonclinical parasitemia in another following inoculation of tissue homogenate from a domestic cat that died of cytauxzoonosis. It is concluded that C. felis may occasionally produce fatal disease in bobcats, especially animals that are stressed. The stress associated with capture, confinement and surgery might all contribute to the development of fatal disease. All bobcats probably undergo a minor clinical syndrome associated with the cycle of schizogony in tissue macrophages. The temporary increase in body temperature and development of anorexia are probably a manifestiation of mild cytauxzoonosis. Fatal cytauxzoonosis in bobcats is rare.

It is also concluded that bobcats are the natural host of C. felis. The completion of the life cycle with minimal clinical signs in the bobcat and subsequent low level parasitemia would account for the large number of asymptomatic, naturally infected bobcats encountered in the study. Because there are no schizogoenous tissue stages detectable after approximately two weeks following exposure to sporozoites, inoculation of infected bobcat blood into a domestic cat after this time should result in only a nonclinical parasitemia in the domestic cat. If blood from an infected bobcat were taken at the end of the schizogonous cycle, schizonts sloughed from the walls of blood vessels might end up in the peripheral circulation. Inoculation of

this blood into a domestic cat might result in a fatal case of cytauxzoonosis in the domestic cat.

Blood films from infected bobcats revealed that organisms multiply in erythrocytes. As indicated previously, this long-standing reproductive activity probably leads to a persistent parasitemia and thus these bobcats can serve as reservoirs for the infection of other bobcats or an occasional domestic cat.

Transmission Between Domestic Cats

It seems likely that clinical disease would have developed in domestic cats had the transmission attempts been successful. Although two ticks attached to one of the domestic cats and one tick attached to a second cat, these ticks apparently did not initiate infection. The number of ticks that attach would be especially important if *D. variabilis* were not the primary vector of *C. felis*. In that case infection rates in the ticks might be lower and the likelihood of an individual tick containing infective stages would also be reduced.

The poor attachment rates to the cats might be attributed to the larger size of the adult ticks and grooming habits of domestic cats. Attachment might be enhanced by increasing exposure time or by keeping cats and ticks in a more confined area. It may also be necessary to restrict the grooming activities of cats perhaps by the use of Elizabethan collars.

In any case, it is concluded that transmission of C. felis between domestic cats would rarely occur in natural situations. All naturally occurring cases in domestic cats have been fatal and the parasitemia at the time of death is very low or not present at all. Organisms do not usually appear in the blood until the last few days of the clinical syndrome. At this time, there are fluctuations in the body temperature of the cat and it becomes subnormal just before death. These changes in body temperature oare likely to affect the feeding behavior of attached ticks. Ticks may even begin detaching before piroplasms are present in the blood. Small numbers of organisms in the blood, paucity of potential vectors feeding, clinical changes in sick cats and the uniformely fatal nature of the disease all probably contribute to the likelihood that transmission between domestic cats would not occur.

Studies on Cytauxzoon felis in Tick Salivary Glands

The staining of salivary glands with methyl-green pyronin appeared to provide good contrast between nuclei and cytoplasm. An identical staining procedure has been used to identify the schizonts of Theileria cervi in the salivary glands of Amblyomma americanum. In the case of T. cervi, the parasites were clearly differentiated within host cells. Since the stain and procedure were identical, it was concluded that procedures were sufficient to identify

parasites had they been present.

There are several possible explanations as to why parasites were not observed in salivary glands. It is possible that C. felis does not develop in salivary glands of the ticks. This organism is, however, closely related taxonomically to Theileria spp. which have developmental stages in the salivary glands of their tick vectors (Cowdry and Ham, 1932). Babesia spp. also have developmental stages in their tick vectors (Melhorn and Schein, 1984). The biology and systematic position of C. felis make it seem likely that this organism has a similar developmental cycle. Another possibility is that none of the ticks examined were infected. If D. variabilis is not a good vector, infection rates might be expected to be low and transmission rates would be even lower than in a good vector.

A third possibility lies with the morphology of the developmental stages of C. felis. It has been assumed that the schizogonous stage is similar in appearance to that of Theileria sp. As such, large schizonts which greatly enlarge salivary gland cells would be expected. It is possible that the developmental stages of C. felis are significantly smaller and may not be as obvious, especially in light microscopy.

After many years of study of the developmental stages of Theileria parva in Rhipicephalus appendiculatus, optimum conditions for achieving successful infection of ticks have been determined. Morzaria (1985) indicated that feeding

ticks at a time when the parasitemia in a cow is increasing at a significant rate will result in increased infection rates in ticks. When the parasitemia begins to stabilize and starts to decrease, infectivity may be significantly reduced. This may have been a contributing factor in this study, particularily if Dermacentor variabilis is not an efficient vector.

A final factor to consider is the age of infected adult ticks. If infected ticks are allowed to remain unfed for too long, starvation may have an adverse effect on the longevity of the parasite in the ticks. Optimum feeding time might be one week after the ticks have molted to adult stage, just after the mouthparts have hardened (Morzaria, 1985).

All of the structures noted in the electron micrographs of salivary glands are thought to be related to processes within the cells of the gland. In salivary glands of Rhipicephalus appendiculatus infected with Theileria parva, Fawcett et al(1982) report a type of labyrinth within infected E-cells. This labyrinth is the result of the parasite expanding throughout the host cell and parasite organelles can be distinguished from those of the host cell. The labyrinth observed in cells of D. variabilis appear to be devoid of either host cell or parasite cytoplasm. In addition, none of the glands examined exhibited any degree of host cell hypertrophy. Even with the decrease in host cell content during normal feeding processes, it might be expected that sporozoite formation would result in some distention of

the host cell as the schizont develops. It was concluded that there were no parasites in the tissues examined.

Although no parasites were identified in the ticks examined, a subsequent study should focus primarily on finding the developmental stages. Other possible tick hosts should be evaluated and they should be fed when the parasitemia is increasing. Using a larger number of ticks would enhance the chances of finding the organism. In addition, identification of the organisms should first be made at the light microscopic level.

Dermacentor variabilis did transmit C. felis and it seems likely that the organism undergoes a developmental cycle in the salivary glands. It is also felt that the schizogonous stages of C. felis are similar to those of Theileria sp in tick salivary glands. Although D. variabilis has been shown to be a biological vector for C. felis it will be necessary to identify the organism in the tick to satisfy the prevailing criteria for positively incriminating a vector.

Developmental Stages of Cytauxzoon felis in Feline Red Blood Cells

The results of this survey were generally similar to those of Simpson et al.(1985). The double unit membrane was not observed in all of the piroplasms and was thought to br due to the angle at which the section of tissue was cut. No parasitophorus vacuole or red cell membrane was observed and

this is consistent with Theileria sp. in which the red cell membrane disintegrates soon after penetration by the piroplasm. The single remaining membrane is often not visible (Melhorn and Eberhard, 1984).

The organelle thought to be a micropore had a thickened neck and resembles those seen in Theileria sp. Simpson et al.(1985) described a micropore in C. felis but did not indicate its abundance. In any case it would seem that a micropore would not be essential to parsite nutrition in view of the parasite's close apposition to the host cell cytoplasm.

Based on light microscopy the only divisional stages that could be seen were the "Maltese cross" formations. The bipolar arrangement of organelles in some of the dividing forms seen in the electron micrographs indicate that a longitudinal binary fission is a common form of asexual division. This division may result in two, three, four or more daughter cells. There were no "Maltese cross" formations seen in the electron micrographs. There were, however, numerous examples of four organisms in close apposition which had just divided by binary fission. Given the bipolar arrangement of nuclei in these cells, this grouping of daughter cells may be what is viewed as the "Maltese cross" formation in light microscopy.

The presence of what appear to be buds is consistent with the findings of Simpson et al. (1985). This budding was seen in many samples and does not appear to be an

artifact of tissue preparation. Budding has not been observed in other piroplasms. It is likely that these forms became more numerous as a result of the increased asexual division that followed splenectomy and the resultant stress. This form of division might provide an additional taxonomic criterion for differentiating Cytauxzoon from Theileria.

The Y-form of asexual division illustrated in Fig.13d has not been described previously in C. felis. The two daughter cells appear to be separating and leaving a residual body. Although this form was observed only once, the structures identified as residual bodies were seen in many infected red cells and are believed to be a result of the same divisional process.

The variety in the asexual division of C. felis in erythrocytes is thought to be unique among the piroplasms. If the observations are borne out in future studies, it may be a further means of justifying the generic status of Cytauxzoon.

SUMMARY

Free ranging bobcats from several locations within the state of Oklahoma were surveyed for the presence of a Cytauxzoon-like piroplasm. Eight of 22 bobcats examined were found to be infected with a piroplasm morphologically indistinguishable from Cytauxzoon felis. Infected bobcats were found primarily in northeastern Oklahoma. Laboratory reared Dermacentor variabliis ticks were fed on an infected bobcat as nymphs. Newly molted adults were fed on uninfected, splenectomized domestic cats. Two domestic cats died approximately 14 days post tick attachment from cytauxzoonosis. This demonstrated that the organism found naturally occuring in bobcats in Oklahoma was C. felis and that D. variabilis can serve as a biological, transstadial vector for the organism.

Nymphal D. variabilis ticks were fed on another naturally infected bobcat and the newly molted adults were fed on an uninfected bobcat. A superficial, prescapular lymph node was removed from the second bobcat on day 14 post tick attachment. Schizonts of C. felis were identified in this lymph node. Schizonts were not found in a lymph node taken on day 30 post tick attachment but a low level parasitemia was present. The development of the organism in the bobcat produced only minimal clinical signs. This

demonstrated that C. felis does undergo a cycle of schizogony in the bobcat and that bobcats do not usually succumb to clinical disease. The absence of obvious disease among a large number of naturally infected bobcats is evidence that bobcats are the natural host of C. felis in Oklahoma.

Domestic cats with an experimentally induced parasitemia but without clinical signs were exposed to nymphal D. variabilis. Attempts were made to feed the newly molted adults on several domestic cats but these attempts were unsuccessful

Adult D. variabilis which had fed on an infected cat as nymphs were fed on a sheep. Ticks were removed on success days and the salivary glands were removed. Efforts were made to identify the developmental stages of C. felis in the salivary glands at both the light and electron microscopic level. No parasitic stages could be identified.

Electron microscopic evaluation of infected red blood cells revealed two forms of asexual division. A form of budding, distinct among the piroplasms, was noted. The second form was a longitudinal binary fission involving a bipolar arrangement of organelles. In one cell a binary fission in Y-form, previously undescribed in Cytauxzoon felis was seen. A residual body was left behind as daughter cells divided.

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VITA 2

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