IN VIVO AND IN VITRO STUDIES OF ANAPLASMA MARGINALE IN NON-BOVINE CELLS

By

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PREFACE

Anaplasma marginale is a Rickettsia-like organism that has been studied extensively since 1893. This research project was designed to investigate in vitro transmission of marginal bodies from infected bovine erythrocytes to uninfected cervid erythrocytes and from infected cervid erythrocytes to uninfected bovine erythrocytes. Four experiments were conducted to help illustrate this transmission phenomena. In addition, an experiment involving infected splenectomized, uninfected splenectomized, infected intact and uninfected intact white-tailed deer was designed to help illustrate parasitemia percentages, hematocrit values, complement-fixation titers and the course of infection in each experimental white-tailed deer model.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Anaplasmosis is an infectious, noncontagious, parasitic disease of domestic cattle and certain wild ruminants. The incidence of anaplasmosis among domestic cattle in the beef and dairy industries is of utmost concern because of the severity of the clinical symptoms and the poor prognosis involved. The nature of this disease warrants an efficient eradication and vaccination control program.

In addition to the fact that domestic bovines are known to become infected with Anaplasma marginale, numerous species of wild ruminants have also been shown to be susceptible. Experimental infection and evaluation of serologic tests have shown that elk (Cervus canadensis canadensis), bighorn sheep (Ovis canadensis canadensis), pronghorn antelope (Atilocapra americana americana), and mule deer (Odocoileus hemionus hemionus) are susceptible to infection (Howe et al., 1964). Numerous studies involving white-tailed deer (Odocoileus virginianus) and Columbian black tailed deer (Odocoileus hemionus columbianus) have also shown these species to be susceptible to infection, under certain conditions (Roberts and Lancaster, 1963; Christensen et al., 1958, 1960; Kreier and Ristic, 1963; Boynton and Woods, 1933). Boynton and Woods (1939, 1940) were successful in developing clinical anaplasmosis in a recipient cow which had been injected with blood pooled from seven Columbian black-tailed deer collected in the Mount Hamilton, California,

area. Christensen, Osebold, and Rosen (1958) were able to produce experimental infection in four male Columbian black-tailed deer following subcutaneous inoculations of blood from bovine carriers of A. marginale. Anaplasma infections were readily detected in the deer by microscopic examination of blood films and by a rise in the complement-fixation titers. Acute, fatal anaplasmosis was produced in two splenectomized calves following inoculation of blood from two of the deer. These studies indicate that specific species of deer can serve as reservoir hosts for Anaplasma marginale infections and that transmission between cattle and deer may occur.

Neitz and Du Toit (1932) discovered that the blesbuck (Damaliscus albifrons) and duiker (Sylvicapra grimmi) are susceptible to infection with A. marginale. Transmission was also accomplished when blood from these experimentally infected antelope was inoculated into additional susceptible cattle. Neitz (1935) also reported the susceptibility of a third species of African antelope, the black wildebeest (Conochaetes gnu), to infection with A. marginale. Osebold et al. (1962) was able to demonstrate, in two of five attempts, biological transmission of anaplasmosis to splenectomized calves when ticks removed from wild Columbian black-tailed deer were allowed to feed on calves within twelve days following collection. According to Renshaw et al. (1976) acute anaplasmosis occurred in two of three calves that received inoculations of pooled blood from eighty-seven Rocky Mountain mule deer. Likewise, blood from three inoculated calves produced anaplasmosis when subsequently inoculated into adult non-splenectomized cattle.

Roberts and Lancaster (1963) utilized three white-tailed deer in

a susceptibility study. A splenectomy was performed on one deer. One hundred cc of inoculum containing A. marginale was administered subcutaneously to both the splenectomized deer and a non-splenectomized deer. The third deer, intact, was used as a uninoculated control. Blood samples were drawn, after thirty-one days (post-inoculation), from all three deer and inoculated subcutaneously into splenectomized calves. Clinical cases of the disease were produced in the calves which received blood inoculations from the two infected deer, but not with blood from the control deer. This was confirmed by increases in complement-fixation test titers. This work demonstrated that white-tailed deer are susceptible to anaplasmosis and that the disease is transmissible from intact and splenectomized white-tailed deer back to cattle by laboratory methods.

Since deer and cattle often inhabit the same rangelands, it is of epidemiologic importance to understand the course of infection of A. marginale in the numerous species of susceptible cervids. It should be noted, however, that clinical anaplasmosis in non-splenectomized wild ruminants has been reported only in black-tailed deer experimentally inoculated with A. marginale (Christensen et al., 1958).

Recent advancements in cell culture techniques have focused attention on the in vitro cultivation of many intracellular parasites. Trager and Jensen (1977) have been able to maintain Plasmodium falciparum in continuous culture. Trager and Jensen's "Candle Jar Method" has been utilized in the cultivation of other intracellular parasites including Babesia bovis (Erp et al., 1978) and A. marginale (Kessler et al. (1979) also demonstrated in vitro transmission of A. marginale

from infected ovine cells to normal bovine cells using this culture system. Marble and Hanks (1973) were able to maintain A. marginale in culture utilizing primary rabbit bone marrow cells. Mazzola et al. (1976) was able to successfully maintain A. marginale in vitro with the use of bovine lymph nodes in explant cultures using the Raft Technique and the use of lymph node organ cultures in roller culture bottles with and without antibiotics. Hidlago (1975) also propagated A. marginale in a bovine lymph node cell culture. Davis et al. (1978) demonstrated the synthesis of DNA and protein by A. marginale in bovine erythrocytes during short term culture. The cultures were placed in a growth chamber containing 5% CO_2 and air (7% CO_2, 83% N_2 and 10% O_2), and incubated at 37^{o}C for one to five days. RPMI 1640 media was also used and tested in this experiment. Hruska, Kliewer and Brock (1965) utilized a procedure designed to indicate multiplication of A. marginale. The procedure depended on the dilution of the original infective inoculum by serially inoculated tissue cultures sufficiently to reduce the chance that viable organisms in the absence of multiplication would be carried into later cultures. Cell types used in these experiments were bone marrow from an adult cow and bovine embryonic kidney shown to be negative to anaplasmosis.

Since numerous cervid hosts are known to be susceptible to infection with A. marginale, an evaluation of the susceptibility of non-bovine cells under both in vitro and in vivo conditions is warranted.

The objective of the in vivo study was to determine susceptibility, parasitemia, hematocrit values and complement-fixation titers in both intact and splenectomized white-tailed deer.

The second phase of this research project dealt with the in vitro

maintenance, maturation, replication and transmissibility of A. marginale in a non-bovine culture system. Bovine blood (infected with A. marginale and non-infected blood), was used in the culture system as well as infected and non-infected white-tailed deer blood, Rocky Mountain mule deer blood, and elk blood. Determination of infection was based on microscopic evaluation of cells and the complement-fixation test titers.

CHAPTER II

MATERIALS AND METHODS

Deer Holding Facility

The Oklahoma State University Deer Health Research Facility is a cooperative effort between Oklahoma State University and the Oklahoma Department of Wildlife Conservation. The facility includes five enclosed acres of land, a research laboratory, six experimental runs (10' X 60') and three 12' X 12' isolation stalls.

Experimental Animals

A captive herd of forty-five white-tailed deer, one mule deer and one elk are housed at the facility and maintained on a commercially prepared free-choice feed source. All animals were acquired at less than two weeks of age, bottle-raised and pre-tested and shown to be negative to intestinal parasites, anaplasmosis, bluetongue, epizoitic hemorrhagic disease and leptospirosis. Five one year old white-tailed deer were splenectomized for susceptibility studies utilizing routine surgery procedures.

In Vivo Susceptibility

Experiment I

The objective of this experiment was to determine the

susceptibility of splenectomized and intact adult white-tailed deer to bovine blood parasitized with A. marginale. Infected bovine blood was used to infect six of the white-tailed deer. The complement-fixation titer of the carrier cow at the time the blood was drawn was 20 (1:5), and the parasitemia at this time was below 1%. The infected bovine blood was treated with neoarsphenamine (2.5 mg/ml of whole blood) at 5°C for twenty-four hours to prevent possible Eperythrozoon wenyonii infection.

Experimental Groups. Group I consisted of three splenectomized deer: #200, #203 and #206. These three adult, splenectomized white-tailed deer received 5cc each of infected bovine blood intravenously.

Group II consisted of the two uninfected splenectomized controls: #215 and #201.

Group III consisted of three intact white-tailed deer. Deer #111 and #208 received 5cc each of carrier blood intravenously. Deer #001 received the 4cc of carrier blood intravenously and 4cc subcutaneously.

Group IV consisted of two uninfected, intact white-tailed deer: #230 and #006.

Sample Procedures. Blood was drawn three times a week for forty-four days. Thin blood films were made at each bleeding, stained with Wright's stain and examined microscopically. Micro-hematocrit values for each deer were measured and recorded as the percentage of packed cells for each bleeding time. Sera were separated from clotted blood, placed in tubes and frozen for later use in complement-fixation tests. Serologic evaluations were determined by personnel of the Oklahoma Animal Disease Diagnostic Laboratory utilizing complement-fixation

test procedures.

In Vitro Cultivation

Experiment II

The "Candle Jar Method" developed by Trager and Jensen (1977) was used in this study. The objective of this experiment was to determine the susceptibility of non-bovine erythrocytes to infection with A. marginale under the Trager culture system.

Source of Infective Blood. The infected bovine blood was obtained from a donor cow (#719). The blood was extracted in a 60cc heparin coated syringe. When the blood was collected, the cow had a parasitemia count of 38.6% and a hematocrit value of 15.5%. Non-bovine cell sources included: elk, intact white-tailed deer, splenectomized white-tailed deer and mule deer. Blood from all non-bovine sources was collected in sterile heparinized tubes. Sera was separated from the clotted blood, placed in sterile tubes and stored in a freezer at -1^{o}C and 30% humidity.

Blood Processing Procedures. Following collection, all blood was centrifuged at 300 X g for fifteen minutes. The plasma and buffy coat layers were removed from the tubes. Ten ml of RPMI 1640 (Grand Island Biological Company) (RPMI) media was added. Blood cells were washed twice with 10 ml of RPMI at 300 X g for twenty minutes. The third wash was with 25 ml of RPMI, 4 ml of fetal calf serum and 1 ml of 5000 Units of Penicillin G Potassium (E.R. Squibb and Sons, Inc., Princeton, N.J.) and 50 mg of Streptomycin Sulfate (Pfizer, Inc.,

New York, New York) (Pen-Strep). Media was stored in a refrigerator at 10°C in 30 ml tissue culture flasks with: 25 ml RPMI, 4 ml of fetal calf serum and 1 ml of Pen-Strep. This preparation was used daily, (warmed in a water bath to room temperature), to replenish the cultures with new media. The infected bovine, uninfected intact mule deer, uninfected intact white-tailed deer, uninfected splenectomized white-tailed deer and uninfected elk blood were all prepared by this method.

Culture Groups. Each mixed blood group consisted of five petri dishes per experimental group. In one experimental group, 0.5 ml of uninfected intact white-tailed deer blood was mixed gently by swirling with 0.5 ml of infected bovine blood plus 1.5 ml of media. In the experimentally infected bovine-splenectomized deer mixed blood group 0.3 ml of uninfected splenectomized white-tailed deer blood was added to 0.3 ml of infected bovine blood, plus 1.5 ml of media. In the second mixed blood group, 0.4 ml of uninfected intact mule deer blood and 0.4 ml of infected bovine blood was combined in addition to 1.5 ml of media. In the third mixed blood group, 0.5 ml of uninfected intact elk blood was combined with 0.5 ml of infected bovine blood and 1.5 ml of media. In the five control groups, 0.1 ml of each type of blood was placed in separate 35 mm petri dishes with 1.5 ml of media. The cultures were incubated in a sterile stopcock dessicator for twelve days. Each day when fresh media was added to each petri dish a small portion of blood was removed and placed in microtitier plates. Blood samples from replicate culture groups were pooled into one microtiter well. Thin films were made from the pooled blood and

stained with Wright's stain. Blood cultures were incubated in a sterile stopcock dessicator, oxygen removed with a lighted candle and kept in an incubator at 37°C. New media was added daily under a safety hood.

Counting Procedures. Blood slides from the control and mixed cell groups were examined microscopically under 100X magnification for the presence of marginal bodies. In each case, 500 cells were randomly examined to determine percent parasitemia count (PPC). The number of parasitized cells was divided by five to obtain the PPC. In the case of the mixed cell cultures, bovine blood was easily differentiated microscopically from white-tailed deer erythrocytes, because white-tailed deer erythrocytes characteristically "sickle" under conditions of low oxygen tension. Mule deer and elk erythrocytes do not characteristically sickle like white-tailed deer erythrocytes *in vitro*. To obtain PPCs for mixed cultures of mule deer and elk blood, 500 random cells were examined in the same manner as described above, but these cervid cells could not be differentiated from the bovine erythrocytes as they lack the sickling characteristic.

Experiment III

This experiment was a replicate of the bovine and intact white-tailed deer mixed blood group in Experiment II. In this experiment infected bovine blood was also used as the control. Other controls included uninfected bovine blood and uninfected intact white-tailed deer blood. The experimental blood group consisted of a mixture of infected bovine blood and uninfected, intact white-tailed deer blood.

Source of Infective Blood. The infected bovine blood was obtained in a sterile 60cc heparin coated syringe from cow #123. The PPC was 46.4, hematocrit value was 16.0% and the complement-fixation titer was R=20. The uninfected intact bovine blood donor was cow #113 with a negative complement-fixation titer. The unifected, intact white-tailed deer donor #209 had a negative complement-fixation titer and no observable marginal bodies.

Blood Processing Procedures. All blood was first centrifuged at 300 X g for fifteen minutes. The plasma and buffy coat layers were removed as in Experiment II. The first two washings were done with 10 ml of phosphate buffered saline. The third wash was with 25 ml RPMI, 4 ml calf sera, and 1 ml Pen-Strep.

Culture Groups. The uninfected, intact deer blood control group consisted of one 35 mm petri dish with 0.1 ml of deer erythrocytes plus 1.5 ml media. The infected bovine blood control group consisted of three 35 mm petri dishes containing 0.3 ml of infected bovine blood and 1.5 ml of media. The mixed experimental blood group consisted of two 35 mm petri dishes of 0.5 ml infected bovine blood, 0.5 ml uninfected, intact white-tailed deer blood plus 1.5 ml of media.

Counting Procedures. The cultures were maintained for six days, with 1.5 ml of fresh media replaced daily. Thin blood films were prepared daily as in Experiment II. The PPCs for each experimental group were obtained as described in Experiment II.

Experiment IV

The objective of this experiment was an in vitro study of the susceptibility of bovine blood to A. marginale when combined with infected, splenectomized white-tailed deer blood in culture.

Source of Infective Blood. Deer #200 served as the infected, splenectomized white-tailed deer donor. On day #24 of Experiment I, #200 had a PPC of two and a negative complement-fixation titer. Ten cc of blood was obtained from deer #200 on day #28. The blood was collected in sterile heparinized and non-heparinized tubes. Cow #852 served as the uninfected bovine blood donor. The complement-fixation titer of cow #852 was negative and no parasitemia was detected.

Blood Processing Procedures. All blood was washed twice with 10 ml of phosphate buffered saline. The third wash was with 25 ml of RPMI, 4 ml calf sera and 1 ml Pen-Strep.

Culture Groups. The infected control group consisted of three 35 mm petri dishes containing 0.2 ml of infected deer blood plus 1.5 ml of media per petri dish.

The experimental group consisted of three 35 mm petri dishes containing 0.4 ml of infected, splenectomized white-tailed deer blood and 0.4 ml of uninfected bovine blood and 1.5 ml of media per petri dish. The bovine uninfected control group consisted of three 35 mm petri dishes containing 0.2 ml of uninfected bovine blood plus 1.5 ml of media.

Counting Procedures. The cultures were maintained for five days.

Each day fresh media was added to each petri dish and thin blood films were made and stained with Wright's stain for microscopic examination in the same manner as Experiment II and III.

Experiment V

This experiment was a replicate of Experiment IV.

Source of Infective Blood. Uninfected splenectomized deer #215 was inoculated with twenty cc of bovine blood infected with A. marginale. At the time of inoculation, the bovine blood (cow #123), had a parasitemia of 38.6%. On day twenty-one post-infection, twenty cc of blood was aseptically drawn from deer #215 and placed into sterile tubes to be used as the infective blood source for experimental cultures. The parasitemia level at the time the blood was drawn was 1.6%.

Blood from uninfected cow #157 served as the bovine control for this experiment. It was complement-fixation negative and had a 0.0% parasitemia.

Blood Processing Procedures. All blood was washed twice with 10 ml of phosphate buffered saline. The third was with 25.0 ml RPMI, 4.0 ml calf sera and 1.0 ml Pen-Strep.

Culture Groups. The infected deer control group consisted of two 35 mm petri dishes containing 0.3 ml of infected deer blood and 1.5 ml of media.

The uninfected bovine control group consisted of two 35 mm petri dishes containing 0.5 ml of uninfected bovine blood and 1.5 ml of media.

The experimental group consisting of infected deer blood mixed with uninfected bovine blood contained five 35 mm petri dishes with 0.5 ml of infected deer blood, 0.5 ml of uninfected bovine blood and 1.5 ml of media.

Counting Procedures. All cultures were maintained for eight days. Each day fresh media was added to each petri dish. Thin blood films were made daily and stained with Wright's stain for microscopic examination in the same manner as in Experiments II, III and IV.

CHAPTER III

RESULTS AND DISCUSSION

In Vivo Susceptibility

In Experiment I, the prepatent period for splenectomized white-tailed deer given intravenous inoculations of bovine blood infected with A. marginale was seventeen days (Table III, Appendix). Only one deer in Group I (#206, splenectomized-infected), produced a positive complement-fixation (CF) reaction. The rest of the seven week observation period, the deer displayed a negative CF. The parasitemia level of 0.8% was detected in deer #200 on day seventeen and increased to 8.0% by day twenty-one. A peripheral blood parasitemia occurred in two other infected, splenectomized white-tailed deer in Group I (#203 and #206), between days seventeen and forty-four post-infection. Deer #206 had a detectable CF titer on days twenty-eight and thirty-five. None of the three splenectomized, infected white-tailed deer showed signs of depression, anemia or anorexia. Significant decreases in hematocrit values were not detected in any of the infected, splenectomized white-tailed deer.

Due to the quality of the blood films, it is questionable whether or not the infected, intact white-tailed deer actually demonstrated marginal bodies. All of the deer (#001, #111 and #208) exemplified various CF titers during the forty-four day period (Table IV,

Appendix). Complement-fixation titers varied from day three through forty-four post-infection. On day forty-four, deer #111 was found to be CF reactive. All of the infected, intact white-tailed deer hematocrit values remained within normal ranges throughout the experiment (Table III, Appendix).

The results of Experiment I indicate that the infected, splenectomized and intact white-tailed deer do not show significant clinical signs when experimentally infected with A. marginale. The viability of the A. marginale organisms was not checked by inoculation of organisms into a splenectomized, uninfected calf.

The splenectomized white-tailed deer, experimentally infected with A. marginale did not develop significant CF titers although low level parasitemia were demonstrated (Table IV, Appendix). Suspicious and positive CF titers readily developed in the experimentally infected, intact white-tailed deer. It is not clear from this study if marginal bodies were present in the erythrocytes of the infected, intact white-tailed deer as they were not found. However, other investigators (Roberts and Lancaster, 1963) were successful in detecting marginal bodies in an experimentally infected intact white-tailed deer. Also, marginal bodies have been seen in blood from other non-bovine animal species (Howe et al., 1964).

In Vitro Susceptibility

Using the method of in vitro cultivation developed by Trager and Jensen (1977), various investigators have been able to study certain intracellular parasites in more detail. Such experiments, however, do not guarantee that the parasites will behave in vitro in the same

manner as they do *in vivo*, but the culture systems are useful tools in trying to comprehend the complex life processes of such organisms.

Experiments II through V explored the processes of maturation, replication and transmissibility of *A. marginale* in bovine and non-bovine blood culture systems.

The economic importance of many parasites has prompted several investigators to concentrate their studies in areas of *in vitro* cultivation. Kessler et at., 1977 and 1979 conducted numerous *in vitro* cultivation experiments involving *A. marginale* utilizing a modification of the Trager and Jensen "Candle Jar Method". They found that the number of parasitized erythrocytes increased three times during an eight-day period, and ten times during a fourteen-day period. The viability of the cultured organisms was verified by inoculation of susceptible animals using inoculum from thirteen and thirty-three day cultures.

Kessler et al. (1979) also demonstrated *in vitro* transmission of *A. marginale* from infected ovine erythrocytes to normal bovine erythrocytes which was verified by using a fluorescent antibody method to differentiate erythrocytes. Kreier and Ristic (1961) took blood from *Anaplasma*-free white-tailed deer and introduced it into two calves with acute *A. marginale* infections. By the use of histochemical and immunofluorescent staining techniques, it was demonstrated that Anaplasma organisms entered into the introduced mature deer erythrocytes.

Experiment II in this study evaluated the susceptibility of non-bovine cells to infection with *A. marginale* when placed in culture when infected bovine erythrocytes. The infected bovine control PPC

was 38.6 when the cultures were set up. By day four the bovine cell PPC had dropped to 4.0, but between days six and nine there was a three fold increase (See Figure 1). Microscopic examination revealed that the marginal bodies became enlarged and more distinct as the bovine erythrocytes grow older.

In the mixed culture group containing bovine erythrocytes and uninfected, intact white-tailed deer erythrocytes (Table V, Appendix) it was noted that on day two, the deer erythrocytes had reached a PPC of 3.0 and the infected bovine erythrocytes were at a PPC of 4.0. Marginal bodies were seen in both cell types and there were appreciable increases in the number of parasitized bovine erythrocytes.

In the infected bovine and uninfected, splenectomized deer culture group (Experiment II), the infected bovine erythrocytes had a PPC of 4.0 and the deer erythrocytes also had reached a PPC of 4.0 on day 6 (See Figure 2).

Both the mixed culture groups containing infected bovine erythrocytes and splenectomized deer erythrocytes and infected bovine and intact deer erythrocytes, showed a marked decrease in the parasitemia from day one to day two, and similar parasitemias through ten days under culture conditions. These results suggest that marginal bodies matured in the cultured infected bovine erythrocytes and transferred to uninfected intact and uninfected splenectomized white-tailed deer erythrocytes.

It is difficult to interpret the results in mixed cultures containing mule deer or elk erythrocytes. Differentiation of erythrocytes stained with Wrights stain was not possible because neither the mule

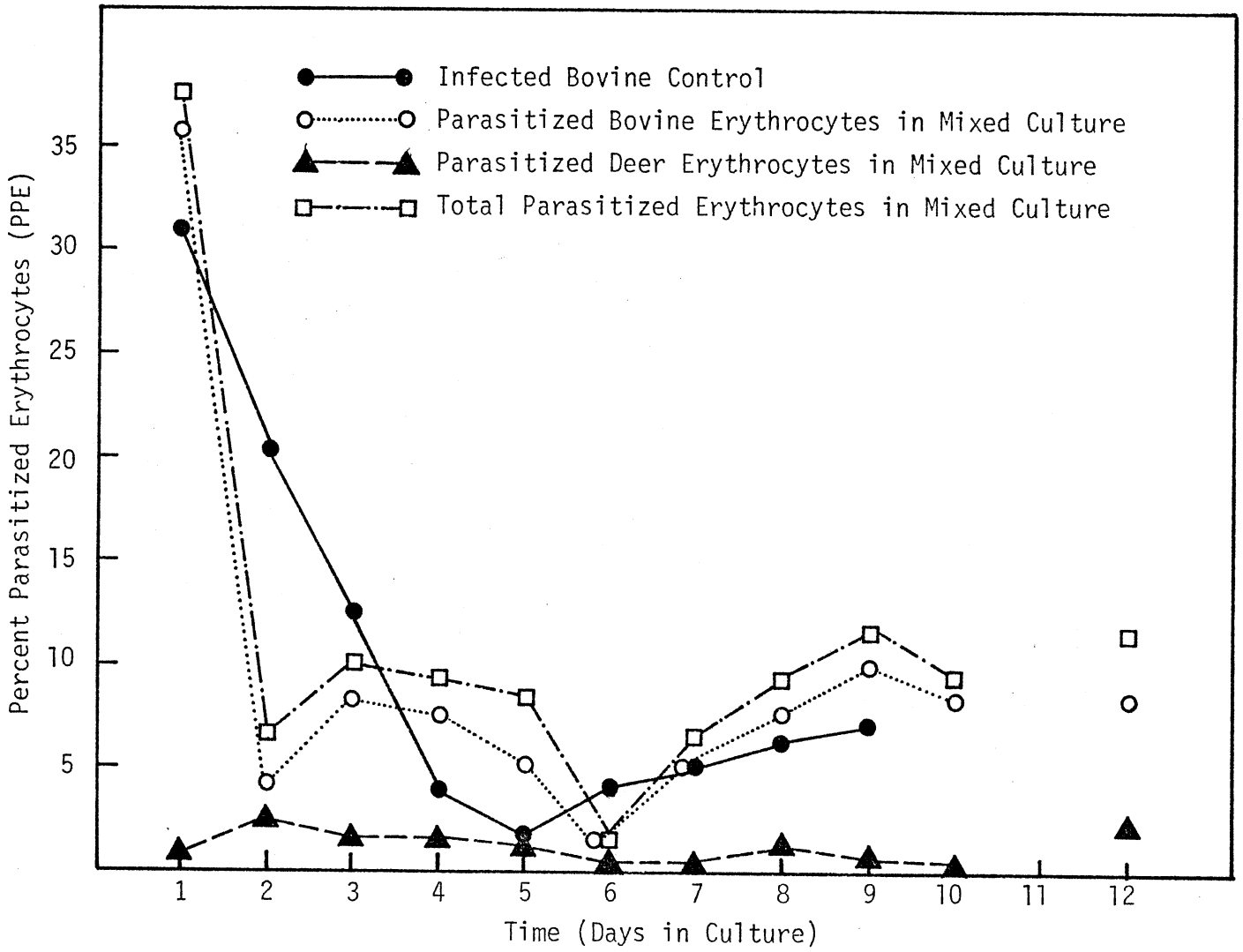

Figure 1. Experiment II: Percent Parasitized Erythrocytes from In Vitro Cultures of Mixed Infected Bovine and Uninfected, Intact White-Tailed Deer Erythrocytes During a Twelve-Day Culture Period

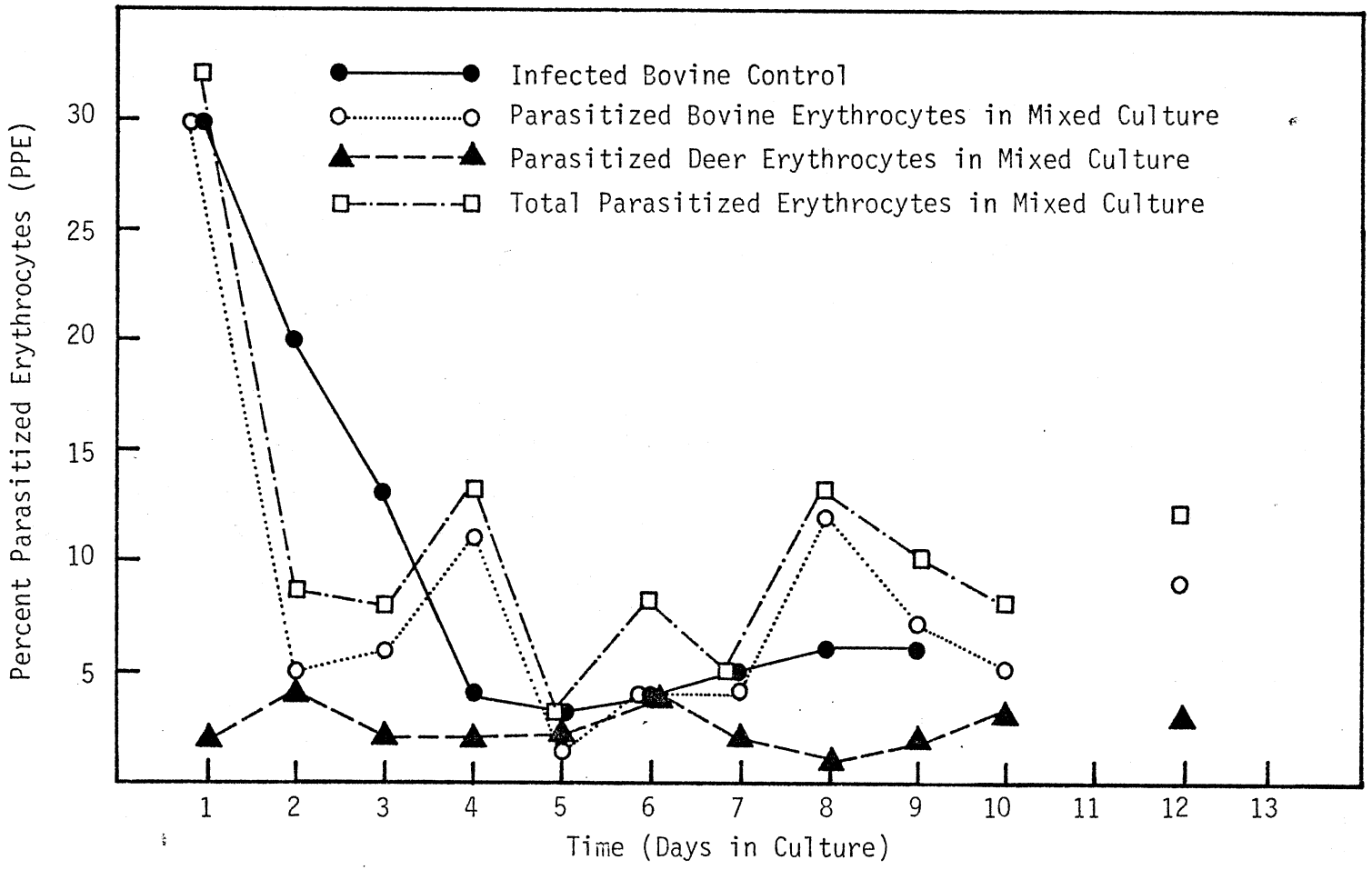

Figure 2. Experiment II: Percent Parasitized Erythrocytes From In Vitro Cultures of Mixed Infected Bovine and Uninfected, Splenectomized White-Tailed Erythrocytes During a Twelve-Day Culture Period

deer or elk erythrocytes exhibit the "sickling" characteristic of white-tailed deer erythrocytes. The parasitemia count in both the bovine-mule deer and bovine-elk groups were comparable to those observed in the bovine-white-tailed deer groups (Table VI, Appendix and Figure 3 and 4).

Experiment III was a duplicate of Experiment II. The initial PPC of the donor cow was 46.4 when the blood was extracted for cultures. At day one, the PPC in the bovine control culture had dropped to 8.6 (See Figure 5). Subsequently, there were two marked increases in the parasitemia counts during the next six days. On day two in the infected bovine-uninfected intact white-tailed deer mixed blood culture group, both the deer and bovine erythrocytes had reached a PPC of 4.0. These results indicate that the number of parasitized bovine erythrocytes increased during the six-day culture period and that the uninfected white-tailed deer erythrocytes became infected (Table I).

Experiments IV and V were designed to determine the susceptibility of uninfected bovine erythrocytes to infection of A. marginale when placed in culture with infected splenectomized white-tailed deer erythrocytes. In Experiment IV the culture groups became contaminated with a yeast and made accurate parasitemia counts impossible. The only significant finding was that by day two, the bovine erythrocytes had reached a PPC of 2.4 (Table II and Figure 6).

In Experiment V, the infected splenectomized white-tailed deer controls showed three consecutive increases in parasitemia levels during a three-day period. The bovine erythrocytes showed more increases in the PPC than the infected, splenectomized white-tailed deer erythrocytes. Marginal bodies were demonstrated in both cell

TABLE I

PERCENT PARASITIZED ERYTHROCYTES (PPE) FROM IN VITRO CULTURES OF MIXED INFECTED BOVINE AND UNINFECTED, INTACT WHITE-TAILED ERYTHROCYTES DURING A SIX-DAY CULTURE PERIOD*

Day of Culture Period	PPE		
		Mixed Culture	
	Bovine Control	Infected Bovine	Uninfected, Intact Deer
1	8.6	11.6	UND**
2	13.0	4.8	3.6
3	9.8	15.0	7.4
4	16.8	17.2	13.0
5	11.4	8.2	7.8
6	9.8	4.2	1.4

*Experiment III

UND** unable to differentiate erythrocyte genera

TABLE II

PERCENT PARASITIZED ERYTHROCYTES (PPE) FROM IN VITRO CULTURES OF MIXED INFECTED, SPLENECTOMIZED WHITE-TAILED DEER AND UNINFECTED BOVINE DURING AN EIGHT-DAY CULTURE PERIOD*

Day of Culture Period	PPE		
		Mixed Culture	
	Deer Control	Infected Deer	Uninfected Bovine
1	3.4	3.4	1.2
2	5.0	1.2	4.4
3	5.2	4.6	5.4
4	2.0	3.6	2.4
5	.6	2.0	2.2
6	--**	.6	5.0
7	--	1.2	3.2
8	--	.6	1.4

*Experiment V

**Erythrocytes not distinct enough to make an accurate percent parasitemia count

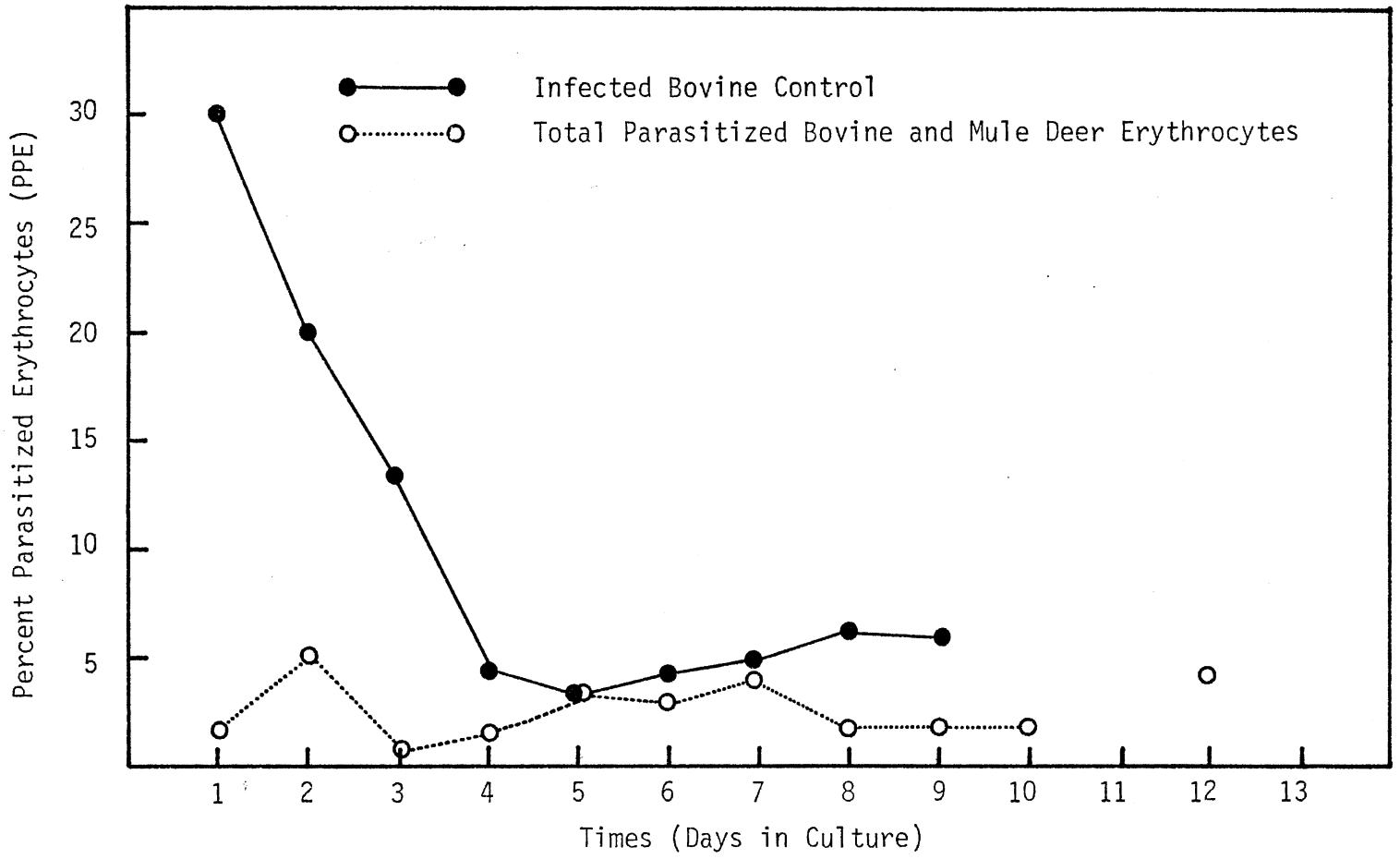

Figure 3. Experiment II: Percent Parasitized Erythrocytes From In Vitro Culture of Mixed Infected Bovine and Uninfected, Intact Rocky Mountain Mule Deer Erythrocytes over a Twelve-Day Culture Period

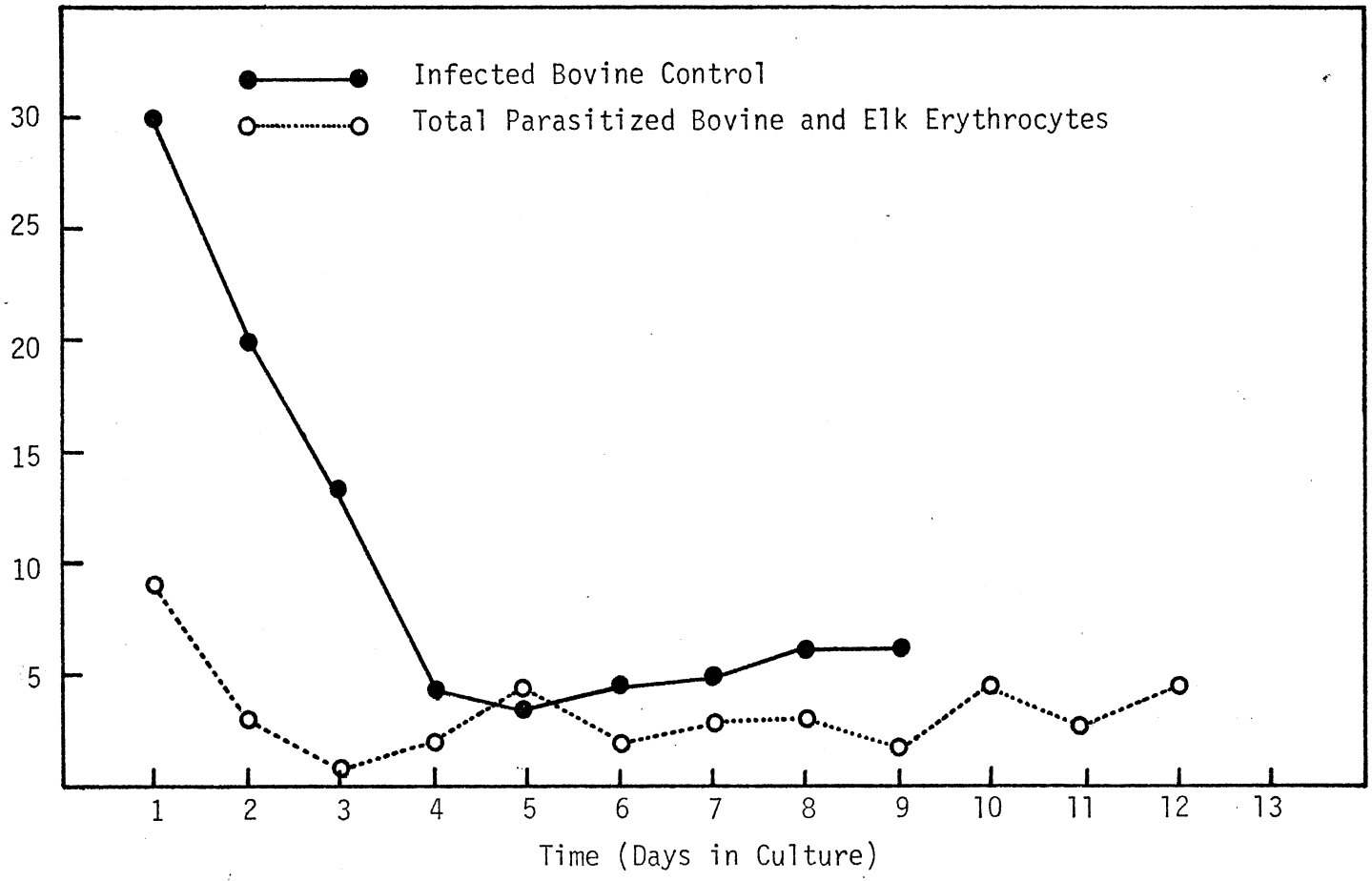

Figure 4: Experiment II: Percent Parasitized Erythrocytes from In Vitro Cultures of Infected Bovine and Uninfected Elk Erythrocytes over a Twelve-Day Culture Period

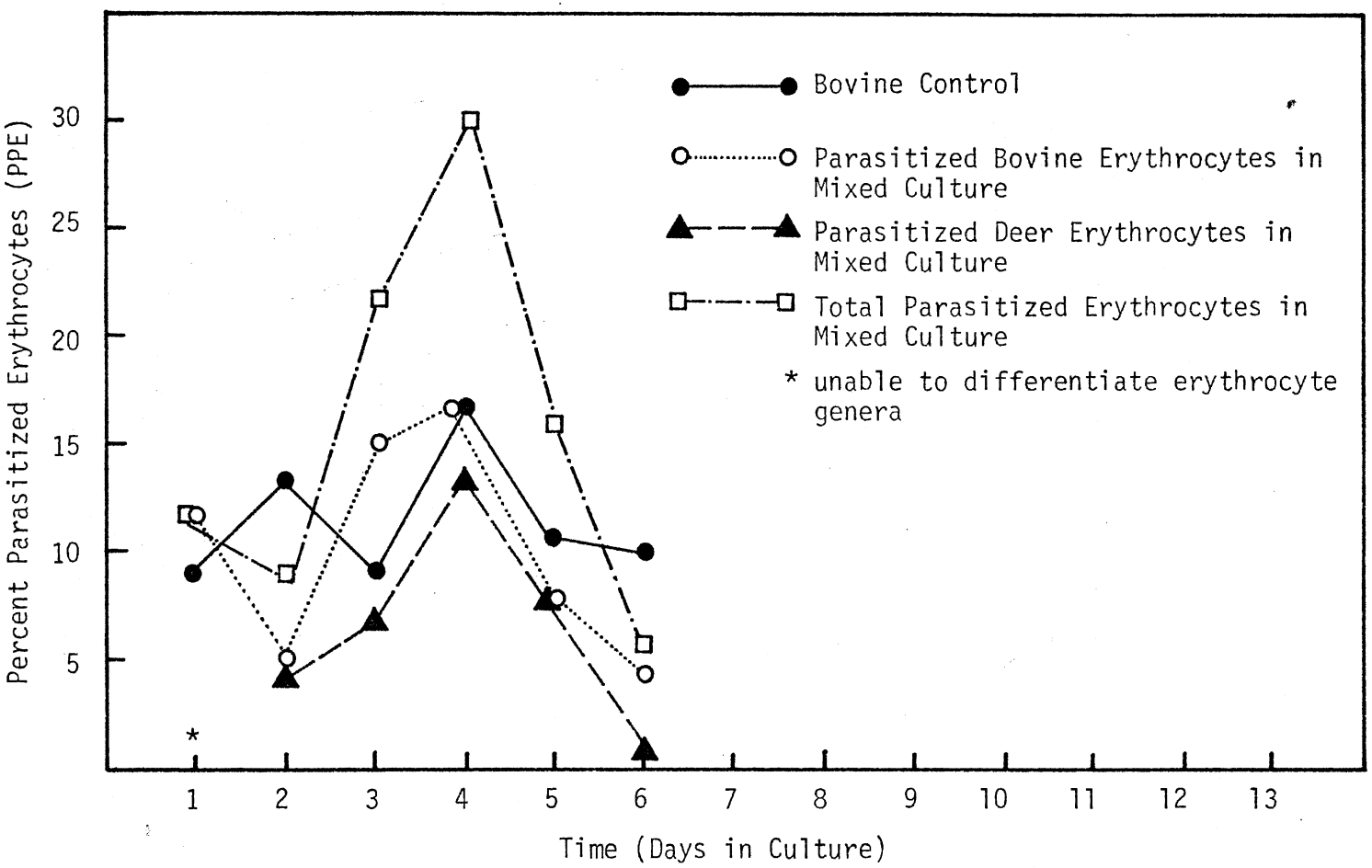

Figure 5. Experiment III: Percent Parasitized Erythrocytes from In Vitro Cultures of Mixed Infected Bovine and Uninfected Intact White-Tailed Deer over a Six-Day Culture Period

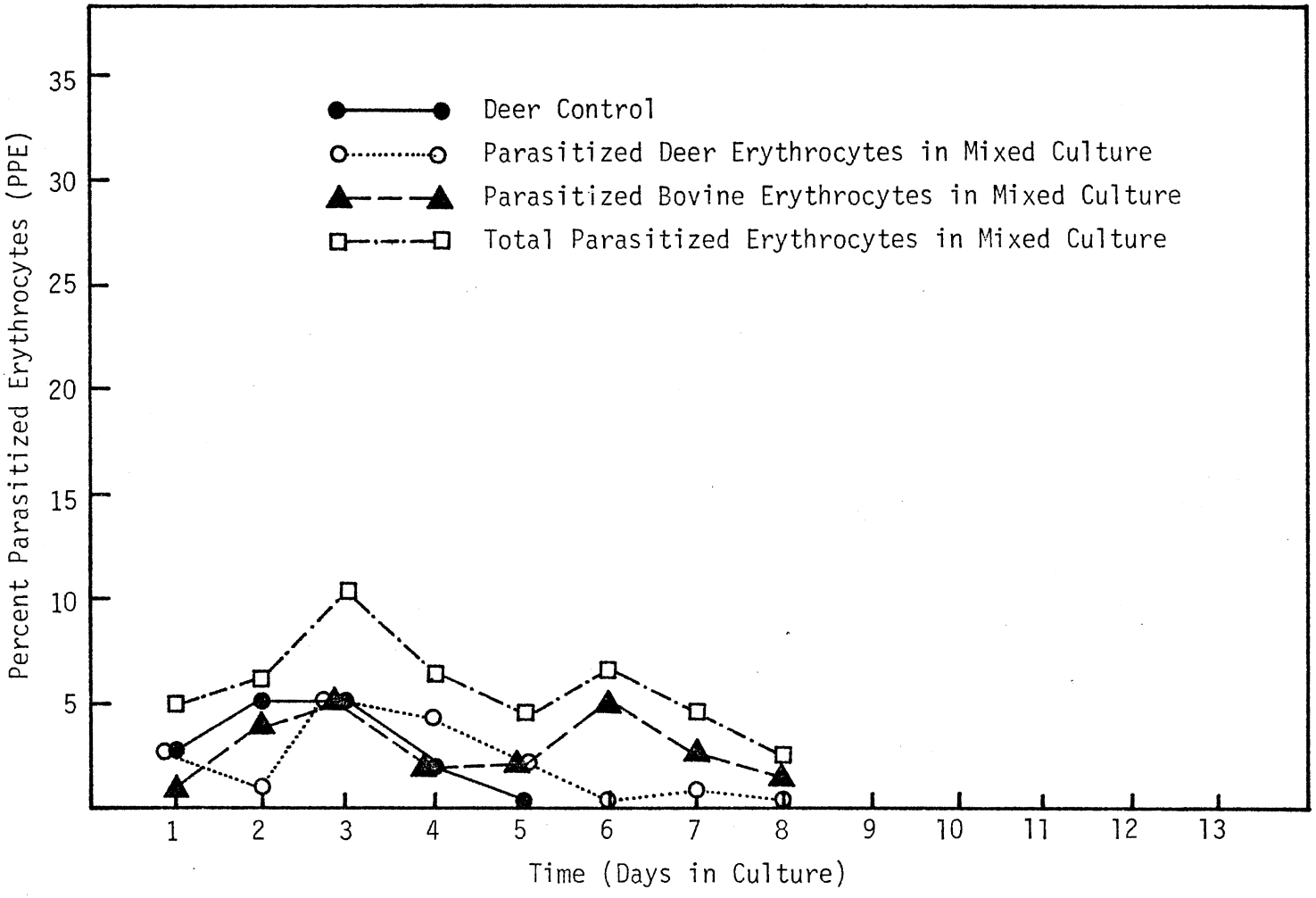

Figure 6. Experiment V: Percent Parasitized Erythrocytes from In Vitro Cultures of Mixed Infected, Splenectomized White-Tailed Deer and Uninfected Bovine Erythrocytes over an Eight-Day Culture Period

types by microscopic evaluation. The results of Experiment I indicate that the infected splenectomized and intact white-tailed deer do not show clinical signs when experimentally infected with A. marginale. Since these deer do not exhibit significant clinical signs, they can possibly serve as models for future susceptibility studies. The rangelands of many of these non-bovine animals are known to overlap with those of domestic cattle. An evaluation of the susceptibility of these host cell types is of epidemiological importance in understanding the host-parasite interactions of Anaplasma infections.

The in vitro aspect of this study focused on the maturation, replication and transmissibility of marginal bodies from one cell type to another. The findings of these studies indicate that A. marginale organisms will infect non-bovine cells when they are mixed with infected bovine cells. Transmissibility was also demonstrated when uninfected bovine cells were mixed with infected non-bovine cells under the culture conditions utilized.

Such experiments, however, do not guarantee that the parasites will behave in vitro in the same manner as they do in vivo, but the culture systems are useful tools in trying to comprehend the complex life processes of such organisms.

CHAPTER IV

SUMMARY

Experiment I evaluated the susceptibility of splenectomized and intact white-tailed deer to experimental infection with Anaplasma marginale. The results of Experiment I indicate that the infected splenectomized and intact white-tailed deer do not show significant clinical signs when experimentally infected with A. marginale. Suspicious and positive complement-fixation titers readily developed in the experimentally infected intact white-tailed deer. It is not clear from this study if marginal bodies occurred in the erythrocytes of the experimentally infected, intact white-tailed deer. In Experiment I, the viability of the A. marginale organisms was unknown since inoculation of the organism into a splenectomized calf was not performed. Among the splenectomized white-tailed deer experimentally infected with A. marginale, complement-fixation titers did not develop, but low level parasitemias were demonstrated. None of the infected intact or splenectomized white-tailed deer exhibited significant hematocrit changes or signs of anemia, depression or anorexia.

Experiments II through V attempt to demonstrate development of A. marginale in non-bovine blood culture systems. Experiments II and III evaluated the susceptibility of non-bovine cells to infection with A. marginale when placed in culture with infected bovine erythrocytes. Microscopic examination indicated that the marginal bodies became

enlarged and more distinct as the bovine erythrocytes grew older. There was a definite evidence of marginal bodies in both cell types as well as increases in parasitemia percentages in the bovine erythrocytes. These results indicate that maturation of the marginal body occurs in the infected bovine cells as well as transfer of infection to uninfected, intact and splenectomized white-tailed deer erythrocytes in culture. The parasitemia levels in both the bovine-mule deer and bovine-elk groups was comparable to those observed in the bovine-white-tailed deer groups. Differentiation of cell types was not possible in the bovine-mule deer and bovine-elk groups, therefore infections could not be positively documented for either of these cell types.

Experiments IV and V were designed to determine the susceptibility of uninfected bovine erythrocytes to infection with A. marginale when placed in culture with infected splenectomized white-tailed deer erythrocytes. Marginal bodies were demonstrated in both cell types by microscopic evaluations. Such experiments, however, do not guarantee that the parasites will behave in vitro in the same manner as they do in vivo, but the culture systems are useful tools in trying to comprehend the complex life processes of such organisms.

Since experimentally infected white-tailed deer do not exhibit significant clinical signs, they can possibly serve as models for future susceptibility studies. The rangelands of many of these non-bovine animals are known to overlap with those of domestic cattle. An evaluation of the susceptibility of these host cell types is of epidemiological importance in understanding the host-parasite interactions of Anaplasma infections.

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APPENDIX

TABLE III

HEMATOCRIT VALUES (HV), PERCENT PARASITEMIA COUNTS (PPC), AND COMPLEMENT-FIXATION TITERS (CF) OF SPLENECTOMIZED WHITE-TAILED DEER OVER A FORTY-FOUR DAY PERIOD

Number of Days Post-Infection	Infected*			Uninfected Control **		
	HV	PPC	CF	HV	PPC	CF
1	--	0.0	---***	29	---	---
	33	0.0	---			
	29	0.0	---	29	---	---
3	27	0.0	Negative	30	---	---
	30	0.0	Negative			
	29	0.0	Negative	30	---	---
7	26	0.0	Negative	28	---	---
	29	0.0	Negative			
	29	0.0	Negative	29	---	---
10	26	0.0	---	26	---	---
	30	0.0	---			
	29	0.0	---	24	---	---
14	24	0.0	Negative	26	---	---
	30	0.0				
	27	0.0		17	---	---
17	26	.2	---	25	---	---
	27	.8	Negative			
	27	.4	Negative	12	---	---
21	26	8.0	Negative	25	---	---
	28	---	Negative			
	28	.4	Negative	17	---	---
24	26	2.0	---	25	---	---
	31	2.0	---			
	32	2.4	---	21	---	---
28	32	---	Negative	26	---	---
	35	---	Negative			
	30	---	Suspicious[a]	19	---	---
31	---	---	---	29	---	---
	(#203 died of pneumonia on day #32)					
	34	2.5	---	28	---	---

TABLE III (Continued)

Number of Days Post-Infection	Infected* HV	PPC	CF	Uninfected Control** HV	PPC	CF
35	28	0.0	---	29	---	---
	33	1.4	Suspicious	20	---	---
38	29	---	Negative	29	---	---
(#206 died of pneumonia on day #36)				29	---	---
44	30	1.2	Negative	29	---	---
				27	---	---

[a]Partial fixation of complement at a 1:5 dilution of serum
*Group I - Experiment I (infected, splenectomized deer: #200, #203 and #206)
**Group II - Experiment I (uninfected control deer: #215 and #201)
***No results

TABLE IV

HEMATOCRIT VALUES (HV), PERCENT PARASITEMIA COUNTS (PPC) AND COMPLEMENT-FIXATION TITERS (CF) OF INFECTED, INTACT WHITE-TAILED DEER OVER A FORTY-FOUR DAY PERIOD

Number of Days Post-Infection	Infected*			Uninfected Control**		
	HV	PPC	CF	HV	PPC	CF
1	29	---	---	30	---	---
	30	---	---			
	41	---	---	30	---	---
3	26	---	Negative	31	---	---
	34	---	Negative			
	34	---	Suspicious[a]	42	---	---
7	27	---	Negative	26	---	---
	29	---	Suspicious			
	28	---	Suspicious	27	---	---
10	23	---	---	25	---	---
	31	---	---			
	30	---	---	28	---	---
14	27	---	---	23	---	---
	30	---	---			
	34	---	---	33	---	---
17	30	QMB[b]	Negative	25	---	---
	28	QMB	Suspicious			
	36	QMB	Negative	20	---	---
21	38	QMB	Suspicious	30	---	---
	29	QMB	Suspicious			
	36	QMB	Suspicious	35	---	---
24	31	QMB	---	#006 had infected hooves		
	46	QMB	---	and was euthanized day #22		
	39	QMB	---	38	---	---
28	31	QMB	Suspicious			
	41	QMB	Suspicious	44	---	---
	43	QMB	Suspicious			
31	--	---	---			
	29	---	---	42	---	---
	--	---	---			

TABLE IV (Continued)

Number of Days Post-Infection	HV	PPC	CF	HV	PPC	CF
35	43	QMB	---			
	31	QMB	Negative	43	---	---
	52	---	Suspicious			
38	43	QMB	Suspicious			
	31	---	---	44	---	---
	52	---	---			
44	43	---	Suspicious			
	42	---	Reactive (titer of 80 at a 1:80 dilution)	43	---	---
	51	---	Suspicious			

[a]Partial fixation of complement at a 1:5 dilution of serum
*Group III - Experiment I (infected, intact deer: 001, 111 and 208)
**Group IV - Experiment I (uninfected controls: 006 and 230)
***No Results
QMB[b]-Questionable Marginale Bodies

TABLE V

PERCENT PARASITIZED ERYTHROCYTES (PPE) FROM IN VITRO CULTURES OF MIXED INFECTED BOVINE AND UNINFECTED WHITE-TAILED DEER ERYTHROCYTES DURING A TWELVE-DAY CULTURE PERIOD**

Day of Culture Period	PPE				
		Mixed Culture		Mixed Culture	
	Bovine Control	Infected Bovine	Uninf. Spl. Deer	Inf. Bov.	Uninf. Intact Deer
1	30.6	30.4	1.8	36.0	1.0
2	19.8	5.0	3.6	3.8	2.6
3	12.8	6.0	2.0	8.0	1.8
4	4.0	11.0	1.8	7.0	2.0
5	2.6	1.4	2.0	5.4	2.2
6	4.0	4.0	4.0	2.0	0.2
7	5.0	4.0	1.8	5.0	0.6
8	5.6	12.0	1.4	7.0	1.8
9	6.2	7.2	2.4	10.4	1.4
10	--*	4.8	3.0	8.2	0.8
11	--	--	--	--	--
12	--	9.0	2.8	8.2	2.6

*Erythrocytes not distinct enough to make accurate Percent Parasitemia Count.
**Part of Experiment II.

TABLE VI

PERCENT PARASITIZED ERYTHROCYTES (PPE) FROM IN VITRO CULTURES OF MIXED INFECTED BOVINE AND UNINFECTED, INTACT ROCKY MOUNTAIN MULE DEER AND ELK ERYTHROCYTES DURING A TWELVE-DAY CULTURE PERIOD*

	PPE		
Day of Culture Period	Bovine Control	Mixed Culture Infected Bovine and Uninfected Mule Deer	Mixed Culture Infected Bovine and Uninfected Elk
1	30.6	2.4	9.2
2	19.8	5.4	2.8
3	12.8	1.4	1.0
4	4.0	2.0	2.0
5	2.6	3.2	4.2
6	4.0	2.8	1.6
7	5.0	4.2	2.8
8	5.6	1.6	3.0
9	6.2	1.6	2.2
10	--**	2.2	3.6
11	--	--	2.8
12	--	4.4	4.0

*Part of Experiment II

**Erythrocytes not distinct enough to make accurate Percent Parasitemia Count

VITA[2]

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