HEMATOLOGICAL AND SEROLOGICAL RESPONSES OF

DIFFERENT AGE GROUPS OF BEAGLE DOGS

TO EXPERIMENTAL BABESIA CANIS

INFECTION

By

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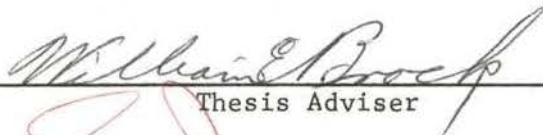Fort Collins, Colorado
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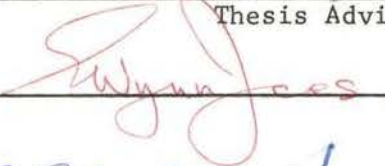Fort Collins, Colorado
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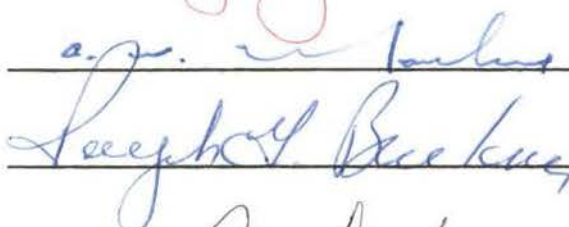MASTER OF SCIENCE
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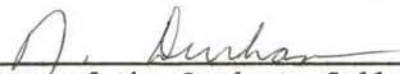
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CHAPTER I

INTRODUCTION

Babesidae is a family of non-pigmented parasites of mammalian
erythrocytes. These parasites reproduce within erythrocytes by division
into two or four daughter individuals. Transmission from host to host
is via ticks belonging to the family Ixodidae (40). While members of
the Babesidae are species specific, cross infections, including human
infection, have been reported (6, 14, 42).

Diagnosis depends upon detection of the organism in the erythro-
cytes of the host or in the erythrocytes of a subinoculated animal (47).

Babesia canis, a hemoprotozoon of the family Babesidae, affects
erythrocytes of species of Canidae. Babesia vogeli and Babesia gibsoni
also infect the erythrocytes of Canidae (40). This study deals only
with the Babesia canis organism.

History of the Disease

Piana and Galli-Valerio first reported canine biliary fever in
Italy in 1895. The causative organism was later designated as Babesia
canis (54). The organism and/or the disease was subsequently reported
from France, Russia, England, Africa, India, China, and the Phillipines
(26). In 1914, Martinez (36) recognized canine babesiosis in Puerto
Rico and in 1918 Clark (54) reported it from the Panama Canal Zone.

The canine babesiosis syndrome was first recognized in the United

States by Eaton (13) and the organism was subsequently identified by Mayne in a dog from Jacksonville, Florida in 1934. Since that time there have been sporadic reports of the disease from various states including Texas (37), Virginia (3), Florida (19), Arizona (45), and Oklahoma (5).

In 1966, _Babesia canis_ was first reported in Australia (22).

Incidence in the United States

There has been no evidence that this disease has reached epidemic proportions in the United States. It is possibly endemic in warmer regions of this country. Arizona, for instance, reports an estimated incidence of 4-5% (44).

Since disease transmission is accomplished primarily by Ixodides ticks, _Rhipicephalus sanguineus_ being incriminated in the United States, it follows that the spread of the disease might be correlated with the habitat of this tick (31). However, mechanical transmission is possible, and all dogs used as blood donors should be carefully screened for this disease (44). Hirsh (24) has reported an outbreak of _B. canis_ in animals used for chronic surgical research.

Characteristics of the Disease

Susceptibility

Neitz (40) and Maegraith (33) have reported that puppies are highly susceptible to babesiosis. Hill (22) described the disease in pet shop puppies. However, it has also been reported that young puppies in an endemic area are naturally resistant or exhibit infection immunity (premunition) (26). Dogs from areas relatively free from _Babesia canis_

are said to be equally susceptible regardless of age (18).

Clinical Syndrome

Clinically affected dogs have a hemolytic anemia, the erythrocyte (RBC) count, the hemoglobin (Hb), and the packed cell volume (PCV) all being reduced (47). Should the hemolysis be exceptionally rapid, bilirubin and hemoglobin can be detected in the plasma, and hemoglobinuria is observed. As a result of the anemia younger RBCs, normoblasts, polychromatophilic macrocytes and reticulocytes can be detected in peripheral blood smears. Careful measurements can detect these changes even in cases of mild anemia (18, 52).

Listlessness, malaise, and dyspnea result from hemolytic infection and inappetence of varying degrees follows (18, 35).

Canine babesiosis may be peracute, acute, or chronic. The peracute and acute syndromes are usually fatal within a few days. The chronic cases may have acute recrudescences, become debilitated and succumb to the infection, or may recover. The recovered animal remains a carrier for several months or years, during which time he is immune to reinfection (premunition) (42). Sterile immunity to canine babesiosis has not been reported.

Although many clinical cases of canine babesiosis have been reported, the symptoms and course have been quite variable and, in some cases, the disease has been complicated by coincident infections. Malherbe (35) and Rokey (44) have both reported that babesiosis may easily be confused with such diseases of the dog as distemper, leptospirosis, viral hepatitis, and rickettsiosis.

Hemolytic Anemia

The mechanism by which the anemia is induced has not been entirely

established. Intravascular and/or extravascular hemolysis may occur. Maturation of the parasites after binary fission and their liberation into the plasma is thought to lead to destruction of the parasitized erythrocyte (33). In Simian malaria and human hemobartonellosis it is believed that the intraerythrocytic parasite induces hemolysis by increasing erythrocytic osmotic and mechanical fragility (52). Removal of red blood cells by the large mononuclear cells, and occasionally by neutrophils, takes place in the blood stream (33).

Extravascular hemolysis is brought about by the cells of the reticulo-endothelial system, primarily of the spleen. Altered red blood cells are sequestered and phagocytized by the RE cells (21). However, in canine babesiosis, as in certain other hemotropic infections, seemingly normal, as well as parasitized erythrocytes, are removed from the circulation (42).

The anemia occuring in babesiosis does not always correlate with the degree of parasitemia. In animals with a high parasitemia, anemia is due to alteration of the cell by the parasite, but in low parasitemia the anemia must be due to other factors (33).

It is possible that an antigen-antibody reaction, due to autoantigens, with sites on the red blood cell causes erythrocyte destruction (49). Investigators have shown that soluble antigens induce anemia as well as resistance to infection (58).

Studies are being conducted to determine if opsonins might stimulate in vivo erythrophagocytosis as has been shown in vitro (58). Perhaps hemaglutinins and opsonins present in small quantities in normal animals aid in the disposal of aged erythrocytes. If these antibodies

are increased in quantity during the course of the infection, they could possibly contribute to the observed anemia (49).

Toxins or end products have been eliminated as causes of hemolysis in babesiosis. Substantiation of this early theory could not be demonstrated experimentally, as the hemolytic anemia is at the peak of the parasitemia, thus, the time sequence is not related to toxicity reaction (49).

Steiner is quoted as saying that "injury resulting from the reaction to an infectious agent may be more severe than the injury produced by the agent itself" (49).

Serum Proteins

The plasma of the blood provides a transport media for proteins produced by hepatic and reticulo-endothelial (RE) cells. These circulating proteins serve as a source of nutrition for the tissues and are in a state of dynamic equilibrium with the tissue proteins (56).

Serum proteins are separated electrophoretically into albumin and alpha, beta, and gamma globulin fractions. The alpha and beta fractions are further separated into $alpha_1$ and $alpha_2$, and $beta_1$ and $beta_2$. Kosma (30) has established average values for these serum proteins in a group of male and female Beagle dogs. Nearly all of his dogs had a third beta fraction. Irfan (27) has also established electrophoretic patterns on normal dogs.

The main function of albumin is that of exerting osmotic pressure, thus maintaining normal distribution of water. In addition, albumin forms reversible complexes with dyes, toxins, and antibiotics, thereby aiding their transport and excretion. The globulins transport enzymes

and participate in immune mechanisms (55).

Examination of the serum or plasma proteins may indicate the general status of protein metabolism in the body. Since much of the synthesis of protein occurs in the liver, variations in serum protein concentrations may be related to functional damage of the liver. It is, therefore, advantageous to determine serum protein concentrations when studying disease mechanisms.

An increase in the total serum protein is seldom encountered except in cases of shock, dehydration, or certain neoplasms. A decrease in total protein is usually associated with a decrease in the albumin fraction, since this fraction predominates. Decreases in albumin are attributed to deficient intake, e.g. malnutrition or malabsorption, decreased synthesis, e.g. liver cirrhosis, excessive breakdown as in infections, and excessive loss, e.g. burns, hemorrhage, ascites, or nephrotic syndrome. Decreases in albumin are usually offset by a rise in the globulin fraction, primarily gamma globulin which increases in response to infectious and parasitic diseases (39).

A fall in albumin which sometimes occurs in babesiosis may be associated with hepatic damage. The heavy proteinuria which may also occur is another factor (33).

Maegraith (33) observed a reversal of the albumin:globulin ration more consistently than a definitive drop in total protein values in Babesia canis infection in young dogs.

Haptoglobin may comprise as much as 25% of the alpha$_2$ globulin fraction. A decline in this fraction could be brought about by a greatly increased intravascular hemolysis, the haptoglobin binding to hemoglobin and being removed by the reticulo-endothelial system (9).

Complement-Fixing Antibodies

The complement-fixation (CF) test is recognized as a sensitive test
to detect a specific antibody response (7). The rise and fall of CF
antibody reflect the amount of circulating antibody present in the
animal. The CF test has been used for years to detect the carrier state
of animals with anaplasmosis (1). Hirato (23) and Holbrook (25) have
found use for the CF test in studies of equine babesiosis, and Mahoney
(35) has found it to be of value in the evaluation of bovine babesiosis
in Australia.

Previous Studies and Experimentation

Babesia canis has been studied extensively in South Africa where it
is a major disease and almost every dog sooner or later acquires the
infection (35). Typical and atypical manifestations of the disease have
been reported; clinical pathology, symptomatology, and treatment being
emphasized. Most of the reports from the United States and Australia
have been individual case reports describing symptoms, diagnosis, and
treatment.

Ewing (16), however, compared hematological changes of two dogs
infected with Babesia canis with groups of dogs infected with Ehrlichia
canis, a combined Ehrlichia and Babesia infection, and two normal con-
trols. He reported a hemolytic anemia with a consequent erythropoeitic
response.

In eight dogs ranging in age from six months to four years, Dorner
(11) reported initial hemolytic anemia incommensurate with the parasi-
temia, reticulocytosis, and lymphocytic leukocytosis. His investigations
also included clinical and morphological studies (12).

Maegraith (33) examined and compared some of the physiological and pathological changes of babesiosis in young dogs with similar processes in malaria and found many similarities in the pathogenesis of the two diseases.

CHAPTER II

EXPERIMENTAL DESIGN AND METHODS

This experiment was designed to compare the hematological and sero-
logical responses of three age groups of dogs to _Babesia_ _canis_ infection.
Those dogs which survived this infection were studied for a maximum of
six weeks.

Experimental Animals

Each group comprised six purebred Beagle dogs raised in tick free
animal quarters isolated from other animals. The newborn puppies, two
females and four males, were whelped in isolation and kept with their
dam throughout the experiment. The young adult group was a litter of
four males and two females of non-hemophiliacs obtained from the hemo-
philic colony at Oklahoma State University, College of Veterinary
Medicine. They were six months of age at the time of the experiment.
The mature group comprised six bitches ranging in age from three to six
years which were obtained from TRI-CO Research, Kalamozoo, Michigan.[+]

All of the dogs in the two older groups were vaccinated for canine
distemper and infectious canine hepatitis and maintained in the dog
research facilities. The dogs were exercised twice daily and fed a
commercial dry dog food. Water was available free choice throughout

[+] Now, TRI-CO Research Projects, Inc., Galesburg, Michigan.

the experimental period.

Organism and Method of Infection

The _Babesia_ _canis_ organism was obtained through the courtesy of Dr. Miodrag Ristic of the University of Illinois. It was necessary to take a splenectomized dog to Illinois where he was inoculated intraperitoneally with frozen blood collected from a case of acute babesial infection. A number of dogs were subinoculated in order to maintain the isolate (Fig. 16, Appendix). The number of passages prior to use in this experiment is unknown.

Each experimental dog was inoculated intravenously with 1.0 milliliter (ml) of whole blood from a dog having patent parasitemia. Each group received blood from the same carrier on the same day. The newborn puppies were infected at one day of age.

Collection of Blood and Serum

One-half ml of blood was collected daily via jugular venapuncture from the newborn puppies. The packed cell volume (PCV) and a blood smear were the only hematological examinations made on these samples.

Dipotassium ethylene diamine tetra acetate (EDTA), 0.1 ml per ml of blood, was used as an anticoagulant for daily samples obtained by either radial or jugular venapuncture from each dog in the older age groups.

Five ml of whole blood taken from the jugular vein on alternate days was allowed to clot in a dry test tube. After centrifugation, the serum was aspirated into a clean test tube. Total protein determinations were made immediately and the samples frozen at -5° C until

termination of the experiment. The complement-fixation (CF) test and

electrophoretic separation were done on all serum samples from one dog

at one time.

<p style="text-align:center">Laboratory Procedures</p>

The data presented in this study were obtained by recognized

laboratory procedures (8, 38, 47, 57).

Hematology

Total erythrocyte and leukocyte counts were performed using an

electronic grid particle counter[+] with a 100u aperture tube. To reduce

the coincidence factor, the standard erythrocyte dilutions were doubled.

Saponin was used to lyse the erythrocytes prior to leukocyte enumera-

tion.

Hemoglobin (Hb) determinations were made colorimetrically using

the cyanomethemoglobin method. Drabkins solution was the diluent and

the samples were read using a Spectronic 20[++] at a wave length of 540u.

The percentage transmission was converted to grams percent by use of a

previously prepared standard curve.

Packed cell volume (PCV) was determined by the microcapillary tube

method using a Clay-Adams microhematocrit centrifuge.[*] The samples were

measured on an International rotary reading unit.[**] Heparinized

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[+] Coulter Counter, Model B, Coulter Electronic Co., Hileah, Fla.

[++] Bausch and Lomb, Rochester, N. Y.

[*] Clay-Adams Co., New York, N. Y.

[**] International Electric Co., Evanston, Ill.

capillary tubes were used when determining the PCV of the newborn puppies.

Blood smears were made using the coverslip technique and stained with Wright's Romanowsky stain using a phosphate buffer having a pH of 6.8.

Reticulocyte counts were made on coverslip smears stained with brilliant cresyl blue and counterstained with Wright's stain. The percentage of reticulocytes was estimated after counting 500 erythrocytes.

The percentage of erythrocytes containing trophozoites was estimated after counting the infected cells among 10,000 erythrocytes on the stained smear. Counting was facilitated by the enumeration of 40 high power fields with an average of 250 cells or the enumeration of 200 oil immersion fields with an average of 50 red cells. Trophozoites were recorded as being present or absent when the counts were less than 0.1%.

The mean corpuscular volume was calculated using the PCV and the erythrocyte count, and the mean corpuscular hemoglobin concentration was calculated from the PCV and Hb values.

Serology

The following serological examinations were performed on samples from dogs in the two older age groups.

The refractometric method using the Bausch and Lomb serum protein meter[+] was used to measure the total protein directly in grams per hundred ml of blood (8).

[+]Bausch and Lomb, Rochester, N. Y.

Electrophoretic separation of the protein components of serum was completed using the Beckman microzone electrophoresis chamber[+] with the Beckham cellulose acetate support medium. The barbital buffer had an ionic strength of 0.075 and was adjusted to a pH of 8.6. The serum was applied to the strips with a microzone serum applicator that picks up a 0.25 microliter sample. The power (250 volts) was applied for 20 minutes. The strips were stained with Ponceau S fixative dye. After clearing and drying, the strips were scanned using the Beckman Analytrol.[+]

Complement-fixing antibody titer was obtained using a method similar to that established for anaplasmosis (1). Babesia antigen for the test was prepared by the following method established by the author and her adviser (15).

The Babesia organism was passaged through splenectomized dogs using massive doses of infected blood, until high parasitemia was obtained. At this time the dog was anesthetized and exsanguinated by means of cardiac puncture, collecting the blood in ACD solution.

The cells were washed by centrifugation three times (30 minutes at 5,000 RPM) and reconstituted to approximately one half the original volume. Ultrasonification was used to rupture the cells, which were again centrifuged (30 minutes at 6,500 RPM). The small amount of sonicated sediment in the bottom of the centrifuge tube was saved and reconstituted with 50 ml of saline. This was purified using a 10-40% sucrose gradient and centrifuging for 15 minutes at 10,000 RPM.

Sediment from the gradient was aspirated and washed twice in

[+]Beckman Instruments, Inc., Fullerton, California

saline, then frozen at -5° C until titered for antigenicity. Sediment
from the lowest level of the gradient tube contained the antigenic
material. This was aspirated and washed twice in normal saline. The
antigen was stored at -5°C until titered for antigenicity.

CHAPTER III

RESULTS

The data in this study are reported in tabular and graphic forms with the aid of the following designations:

CF titer — complement-fixing antibody titer, recorded as Log_{10} of the reciprocal of dilution,

Day 0 — first day of patent parasitemia,

Hb — hemoglobin, gms per 100 ml whole blood,

MCHC — mean corpuscular hemoglobin concentration, percent,

MCV — mean corpuscular volume, cubic microns,

PCV — packed cell volume, volumes percent,

PP — patent parasitemia, the organism is observed in the erythrocytes,

RBC — total erythrocyte count, x 10^6 per mm^3,

Reticulocyte — immature red blood cell containing nuclear remnants, recorded as percent of total erythrocyte count,

Trophozoite — asexual stage of B. canis organism seen in the erythrocyte, recorded as percent of total erythrocyte count or, if less than 0.1%, as present or absent,

TP — total serum protein, gms per 100 ml blood,

WBC — total leukocyte count, x 10^3 per mm^3,

± — mean value ± one standard deviation.

The albumin and globulin serum fractions are so designated and are

reported as percentages of the total protein.

The graphs presented were plotted in relation to the duration of the syndrome, all data being adjusted to coincide with Day 0. Established normal values and pretreatment means are given in the Appendix (Tables XXIV, XXV).

It should be noted that all neonates died during the experiment. Four had erythrocytes infected with Babesia trophozoites; one of these neonates died from causes other than babesiosis, as did two other neonates which were not observed to have infected erythrocytes.

All of the dogs in the two older age groups had patent parasitemia and no treatment of any kind was administered.

Hematological Results

Packed Cell Volume

All three groups had a rapid linear fall in the packed cell volume (Figure 1). The low value for the mature group was 23.6% ± 3.8 on day 8.0 ± 2.8 following Day 0. In the young adult group the lowest PCV was 20.6% ± 2.8 on day 4.2 ± 1.2 after Day 0, and the newborn group had a PCV of 14.7% ± 1.3 on day 2.1 ± 0.6 following Day 0 (Table I).

The recovery period of the two older groups was again linear, but occurred more slowly than the initial blood loss (Figure 1). The mature group reached pretreatment mean on day 28.8 ± 0.98 after Day 0 and the young adult group reached this value on day 25.3 ± 5.06 after Day 0 (Table II).
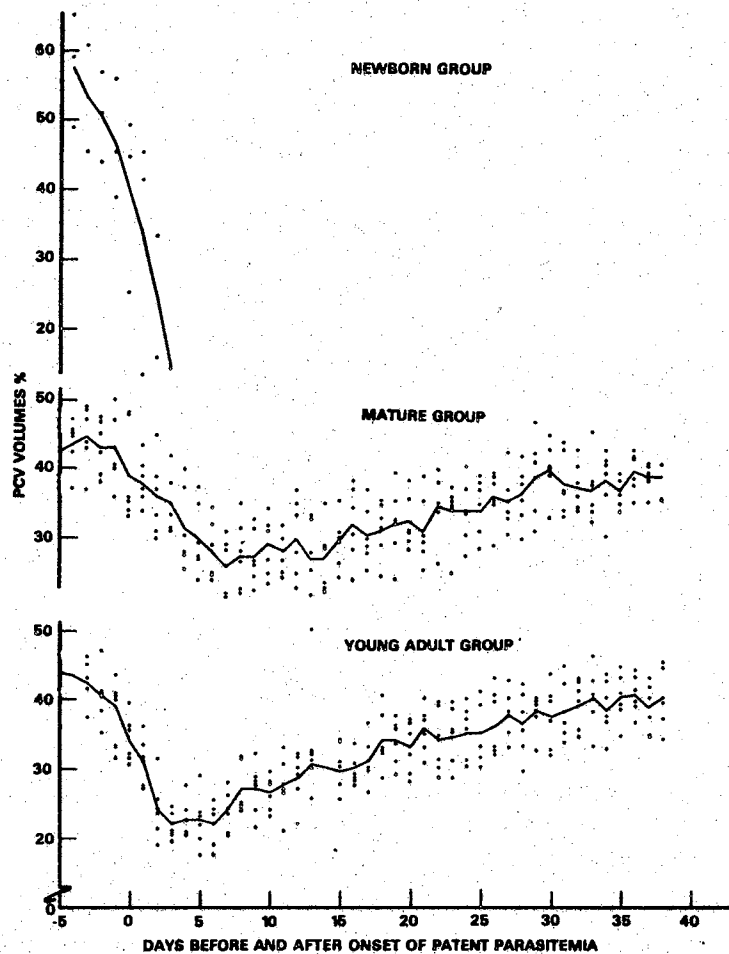
Figure 1. Percent Packed Cell Volume in newborn,
          mature (3-6 year old), and young
          adult (6 month old) Beagles during
          prepatent and patent Babesia canis
          infection. Included in the figure
          are the mean and individual obser-
          vation points.

Hemoglobin

Figure 2 compares the hemoglobin loss in the two older age

groups. The fall in hemoglobin was again linear, the older age

group appeared to lose hemoglobin more slowly. In the mature

group the lowest hemoglobin concentration (7.7 gms % ± 1.4) was

observed on day 8.2 ± 2.6 after Day 0. The lowest hemoglobin concen-

tration of the young adult group was 6.1 gms % ± 0.97 on day 4.3 ± 1.5

following Day 0 (Table III).

Figure 2. Grams of hemoglobin in mature (3-6
          year old) and young adult (6 month
          old) Beagles during prepatent and
          patent Babesia canis infection.
          Included in the figure are the
          mean and individual observation
          points.

Total Erythrocyte Counts

The pattern of the fall in erythrocytes corresponded with that of the packed cell volume and hemoglobin in the two older groups (Figure 3). In the mature group the lowest erythrocyte count of 3.0×10^6 per $mm^3 \pm 0.53$ occurred on day 8.2 ± 3.2 after Day 0, whereas, the lowest erythrocyte count of the young adult group (2.6×10^6 per $mm^3 \pm 0.33$) occurred on day 3.7 ± 0.77 following Day 0 (Table IV). The subsequent return to normal erythrocyte counts corresponded with that of the blood values reported previously.

Reticulocytes

The reticulocyte response varied considerably, but graphically the mature group appeared to have a more consistant rise ($6.2\% \pm 2.1$ on day 15.0 ± 4.3 after Day 0) as shown in Figure 4. The young adult group had a similar peak reticulocytosis of $6.7\% \pm 2.2$ on day 16.7 ± 8.1 following Day 0 (Table V).

Mean Corpuscular Volume and Mean Corpuscular Hemoglobin Concentration

In Figure 5 it can be seen that the MCV increased from a pretreatment mean in both groups, reached maximal values 10 to 20 days after the onset of patent disease, and maintained these values up to 30 days after Day 0. The increase in MCV from the pretreatment mean was approximately 5.7% in the mature group and 16.1% in the young adults (Table VI). Concomitant with this macrocytic response the MCHC was decreased (Figure 6), the percent decline of the MCHC being 6.1% in both groups (Table VII).

Figure 3. Erythrocyte count of mature (3-6 year
old) and young adult (6 month old)
Beagles during prepatent and patent
<u>Babesia</u> <u>canis</u>. Included in the fig-
ure are the mean and individual
observation points.
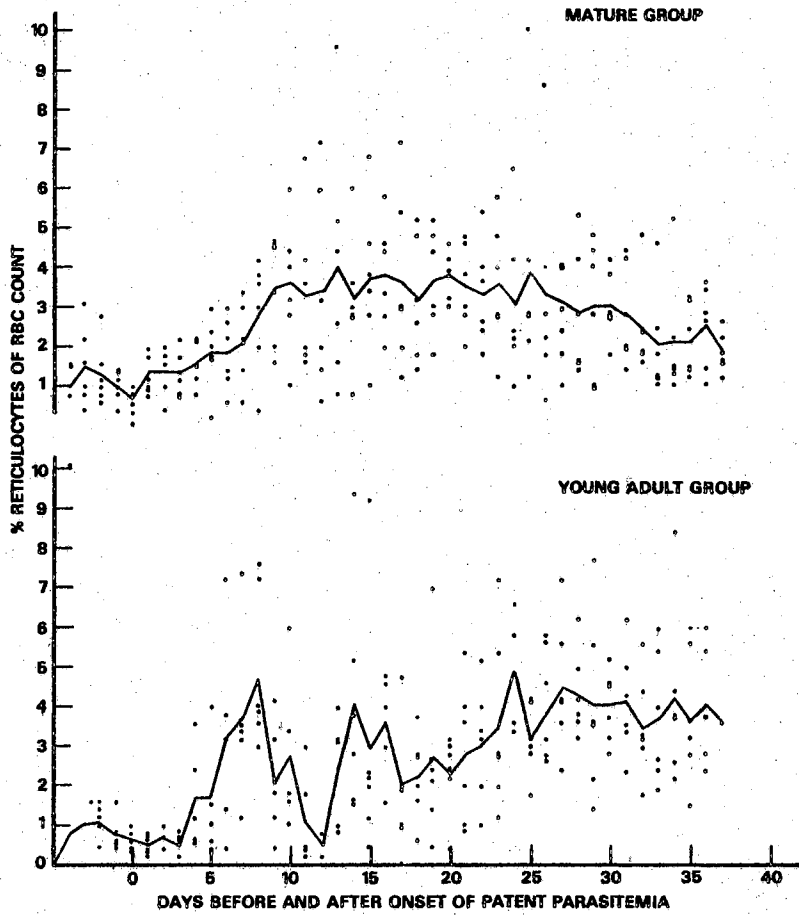
Figure 4. Percent reticulocytes of erythrocyte
count in mature (3-6 year old) and
young adult (6 month old) Beagles
during prepatent and patent *Babesia
canis* infection. Included in the
figure are the mean and individual
observation points.
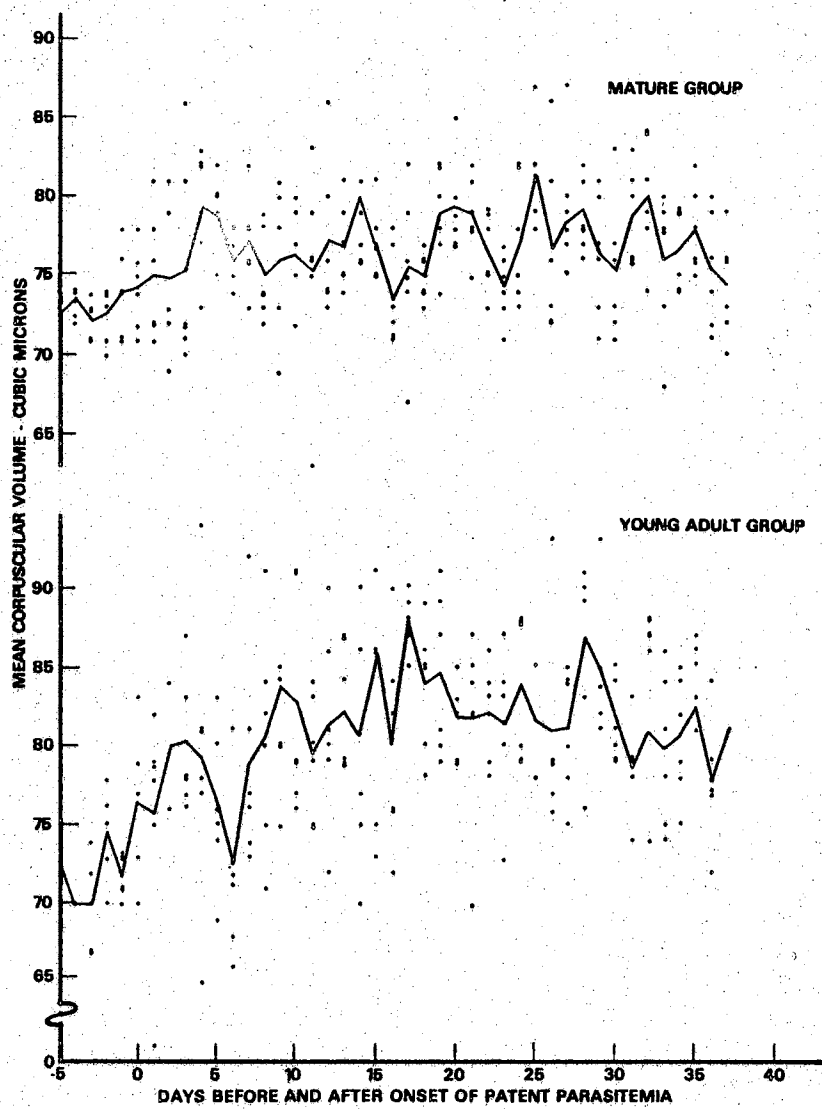
Figure 5. Mean corpuscular volume of mature (3-6
year old) and young adult (6 month
old) Beagles during prepatent and
patent _Babesia_ _canis_ infection.
Included in the figure are the mean
and individual observation points.
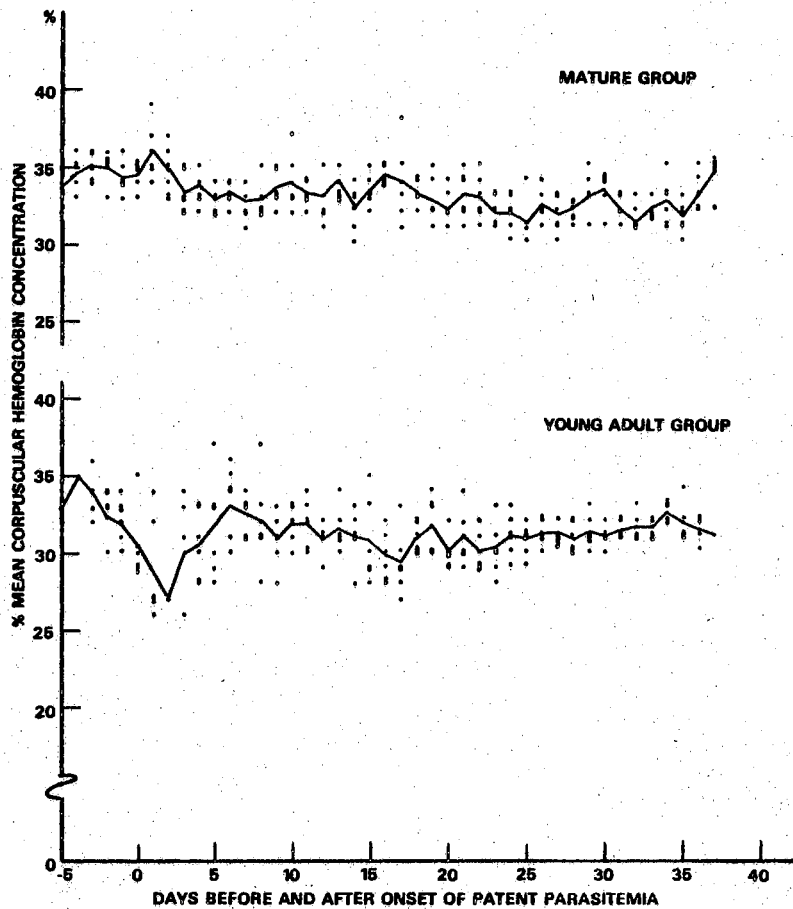
Figure 6. Mean corpuscular hemoglobin concentration of mature (3-6 year old) and young adult (6 month old) Beagles during prepatent and patent _Babesia_ _canis_ infection. Included in the figure are the mean and individual observation points.

Total Leukocyte Counts

After an initial pre-parasitemic leukocytosis following inoculation,
leukocyte counts decreased rapidly and then increased once again
(Figure 7). Only the leukopenia and subsequent leukocytosis were
included in this data. It can also be seen that the variation as demon-
strated by standard deviations was considerable. The day on which the
leukopenia was maximal was 1.8 ± 2.1 after Day 0 in the mature group
and 1.76 ± 0.45 after Day 0 for the young adult group. The mature dogs
had a low count of 7,418 ± 3534 and the young adult group had a mean low
count of 5,145 ± 685 (Table VIII).

The total leukocyte count then rose to 15,822 ± 6497 on day 15.7 ±
6.7 after Day 0 in the mature group and to a mean of 14,163 ± 2896 on
day 11.3 ± 3.3 after Day 0 in the young adults (Table IX).

Parasitemia

The periods of patent parasitemia are shown in Table X. Initially,
the appearance of trophozoites was rather uniform, but the variation
was greater as the disease progressed. In the event that less than 0.1%
trophozoites were observed, they were recorded only as present or
absent.

The first day of patent parasitemia occurred on day 3.6 ± 0.52
following infection in the mature group. The young adult group had
post-inoculation parasitemia first on day 3.2 ± 0.98, and the newborn
group on day 5.2 ± 0.5 following inoculation (Table XI).

The mean length of the first period of patent parasitemia was
longer in the mature group (9.8 days ± 1.5) than that of the young
adults (6.0 days ± 2.0) as shown in Table XII.

Figure 7. Blood leukocyte count of mature
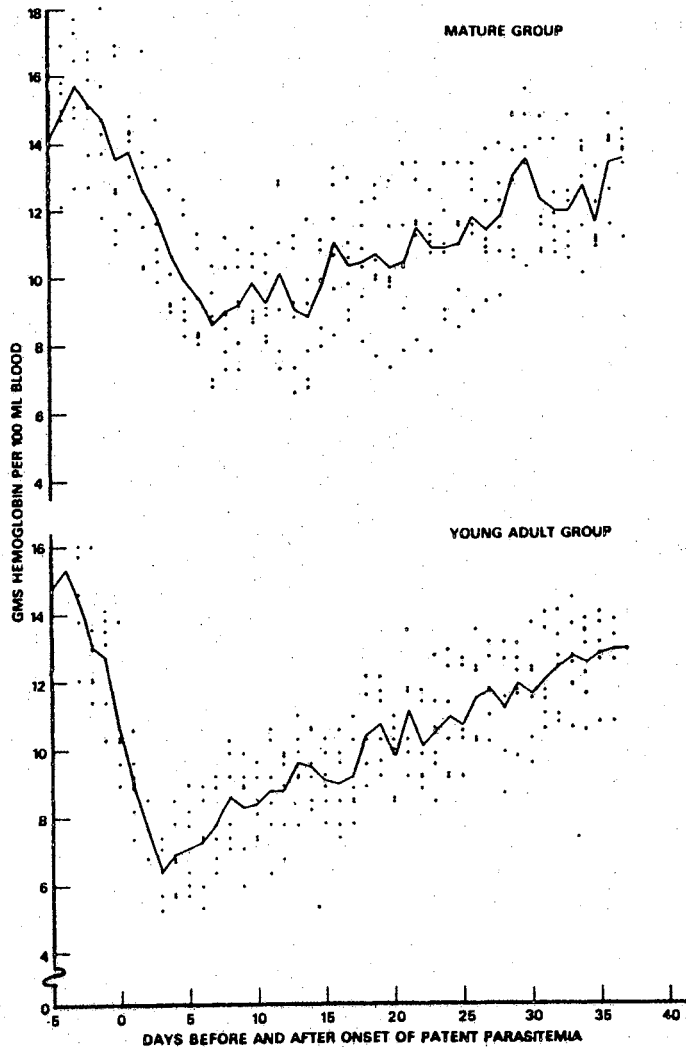   (3-6 year old) and young adult
           (6 month old) Beagles during
           prepatent and patent _Babesia_
           canis infection. Included in
           the figure are the mean and
           individual observation points.

The peak parasitemia of the mature group was 0.3% ± 0.14 on day 3.2 ± 1.8 after Day 0, the young adult group 0.8% ± 0.67 on day 1.7 ± 0.51 after Day 0, and of the newborn group 9.1% ± 12.4 on day 1.7 ± 0.59 after Day 0 (Table XIII).

Serological Results

Complement-Fixing Antibody Titer

The CF antibody responses are illustrated in Figure 8 and analyzed in Tables XIV, XV, and XVI.

Both older groups had distinct increases in antibody titers, the mature group having the most rapid increase and highest titer. The day following inoculation on which CF antibodies were first detected was 7.3 ± 1.4 in the mature group and 8.3 ± 2.2 in the young adults. When the CF response was related to Day 0, CF antibodies were first detected day 3.7 ± 1.9 after Day 0 in the mature group and on day 5.2 ± 2.0 following Day 0 in the young adult group.

Peak titers were 2.706 ± 0.245 on day 10.0 ± 5.6 after Day 0 for the mature group, and 2.254 ± 0.614 on day 22.8 ± 11.4 after Day 0 for the young adult group. The antibody response in this latter group was very variable.

Each group achieved a similar plateau of CF antibody at a similar stage of the disease (Mature group 1.878 ± 0.466 and young adult group 1.995 ± 0.566 during the last 10 days of the experiment).

Total Protein

In both groups Total Protein values increased after an initial decrease (Figure 9). During this response the mature group had a low

Figure 8. Serum complement-fixing antibody titer
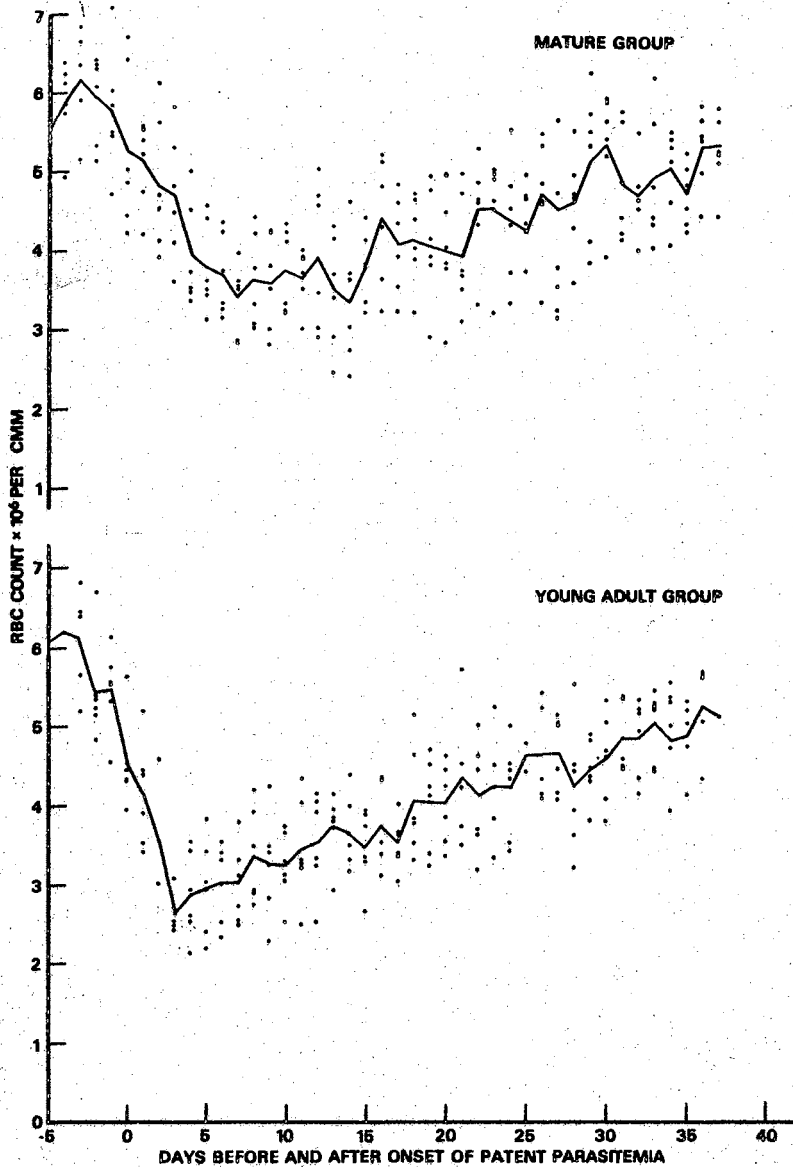of mature (3-6 year old) and young
adult (6 month old) Beagles during
prepatent and patent _Babesia canis_
infection. Included in the figure
are the mean and individual obser-
vation points.

of 5.3 gms $\pm$ 0.46 on day 4.2 $\pm$ 1.33 after Day 0, and the young adult

group had a low of 4.5 gms $\pm$ 0.33 on day 2.3 $\pm$ 1.51 following Day 0

(Table XVII). The subsequent increase peaked at 8.1 gms $\pm$ 0.61 on day

33.0 ± 9.80 for the mature group and 6.8 gms ± 0.27 on day 20.7 ± 7.34

for the young adult group (Table XVIII).

Figure 9. Total serum proteins of mature (3-6 year old) and young adult (6 month old) Beagles during prepatent and patent *Babesia canis* infection. Included in the figure are the mean and individual observation points.

Albumin

Albumin values declined in both groups at varying rates. In the mature group a low of 29.0% $\pm$ 2.2 occurred on day 13.7 $\pm$ 7.4 following Day 0. The young adult group had a low of 34.2% $\pm$ 3.2 on day 20.7 $\pm$ 4.7 after Day 0 (Figure 10 and Table XIX).

Alpha$_1$ Globulin

The response of the Alpha$_1$ globulins differed in each group. The mature group increased to a high of 6.3% $\pm$ 0.81 on day 8.0 $\pm$ 8.2 after Day 0, whereas, the young adult group decreased to a low of 3.7% $\pm$ 0.4 on day 13.7 $\pm$ 5.6 after Day 0 (Figure 11 and Table XX). This data shows a 50% increase from pretreatment mean in the mature group and a 35.1% decrease from pretreatment mean in the young adult group (Table XXIII).

Alpha$_2$ Globulin

In each group the Alpha$_2$ globulins had a small rise followed by a distinct decline (Figure 12). The mature group decreased to a low of 5.7% $\pm$ 1.2 on day 16.3 $\pm$ 3.7 following Day 0 and the young adult group had a low of 10.5% $\pm$ 3.7 on day 18.7 $\pm$ 11.4 after Day 0 (Table XX). The percentage decrease from pretreatment means were 45.7% and 26.6% for the mature and young adult groups respectively (Table XXIII).

Beta$_1$ Globulin

Beta$_1$ globulin concentrations increased in each group but was quite variable especially in the mature group. A high of 21.7% $\pm$ 6.8 on day 4.5 $\pm$ 10.2 after Day 0 occurred in the mature group, while the young adult group had a high of 13.2% $\pm$ 1.0 on day 14.3 $\pm$ 10.5 following Day 0
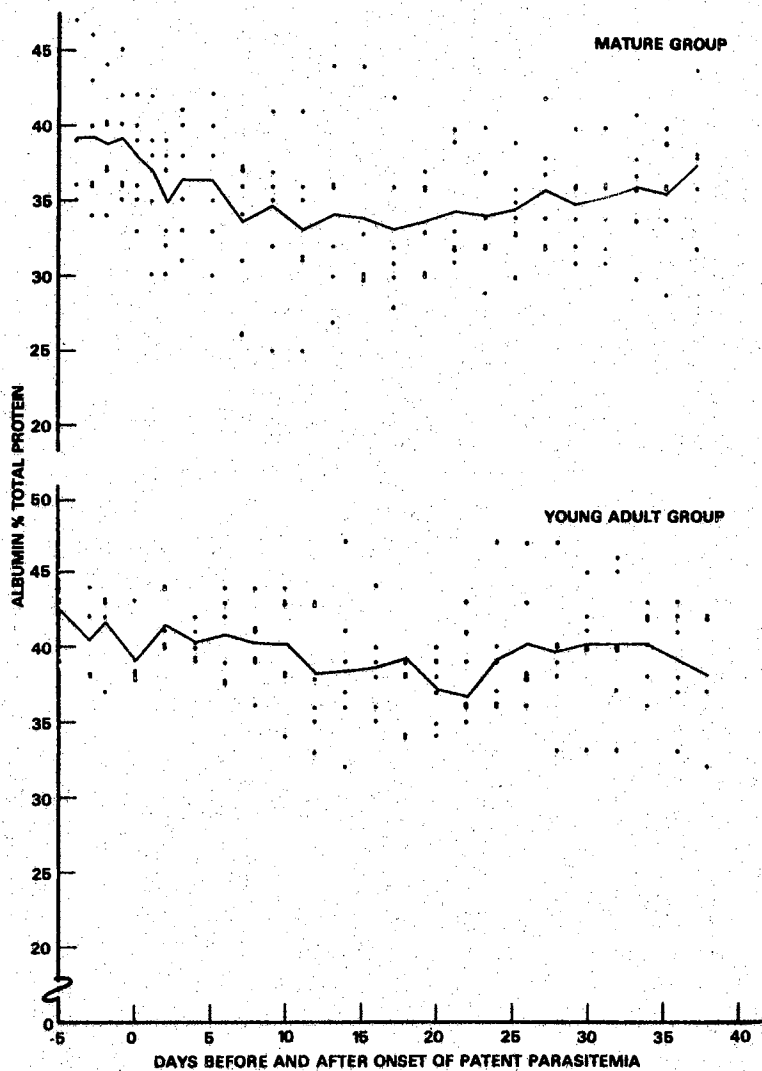
Figure 10.   Serum albumin of mature (3-6 year
             old) and young adult (6 month
             old) Beagles during prepatent
             and patent Babesia canis infec-
             tion. Included in the figure
             are the mean and individual
             observation points.
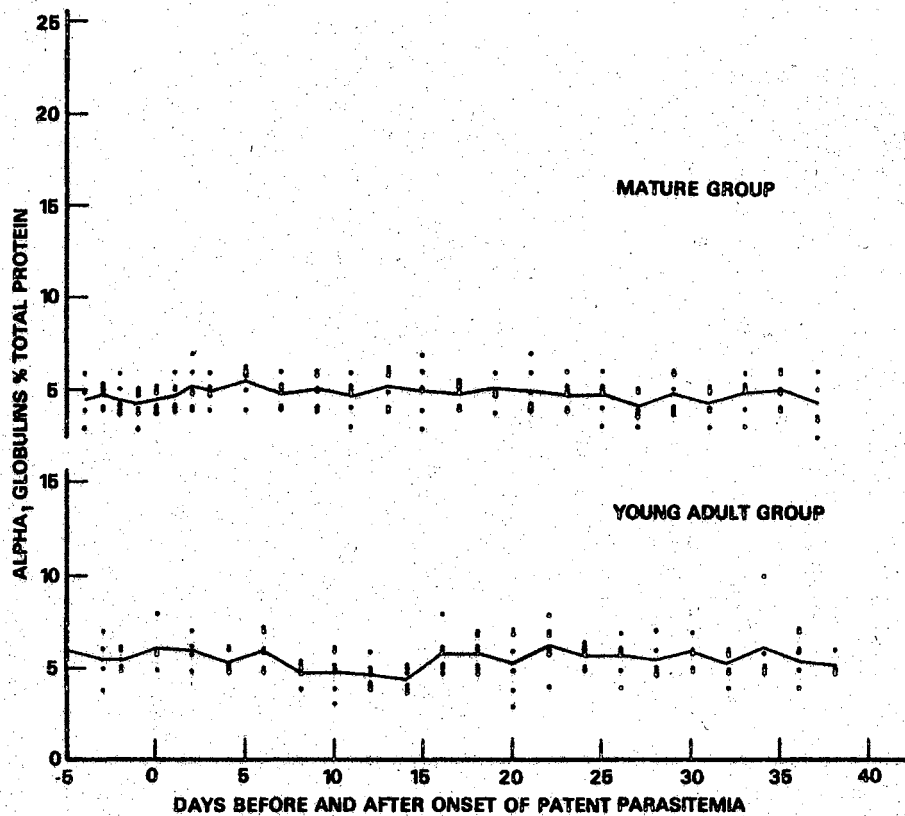
Figure 11. Serum alpha$_1$ globulins of mature (3-6 year
old) and young adult (6 month old)
Beagles during prepatent and patent
Babesia canis infection. Included in
the figure are the mean and individual
observation points.
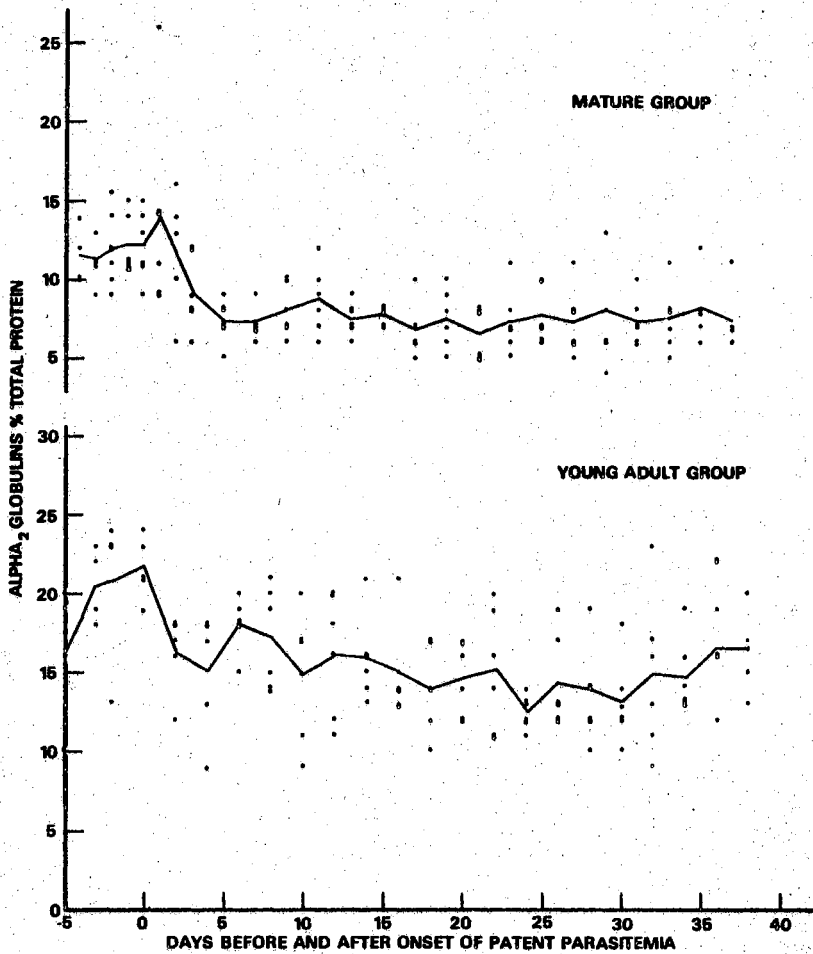
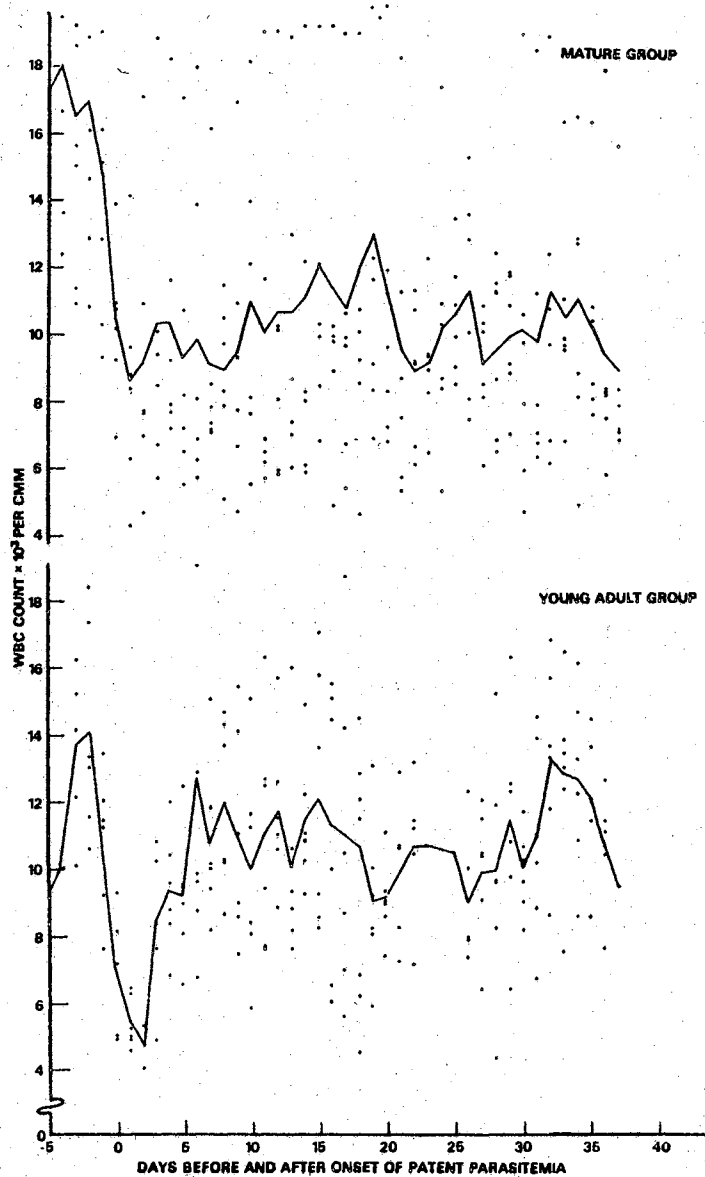Figure 12. Serum alpha$_2$ globulins of mature (3-6 year old) and young adult (6 month old) Beagles during prepatent and patent _Babesia canis_ infection. Included in the figure are the mean and individual observation points.

(Figure 13 and Table XXI). The increase from pretreatment mean was 40.0% in the mature group and 28.0% in the young adult group (Table XXIII).

Beta$_2$ Globulin

Beta$_2$ globulin concentrations increased to a high of 20.0% $\pm$ 2.9 for the mature group on day 8.0 $\pm$ 2.9 after Day 0, and to a high of 19.2% $\pm$ 1.9 on day 16.7 $\pm$ 10.0 after Day 0 for the young adults (Figure 14 and Table XXI). This represents a 17.6% increase from pretreatment mean in the mature group and a 30.6% increase in the young adult group (Table XXIII).

Gamma Globulin

Gamma globulins increased in both groups, the mature group had an earlier increase and higher peak value. The mature group had a high of 26.2% $\pm$ 4.6 on day 17.0 $\pm$ 2.2 after Day 0 and the young adult group had a high of 19.2% $\pm$ 3.3 on day 16.3 $\pm$ 6.8 following Day 0 (Figure 15 and Table XXII). A rise of 109.6% from pretreatment mean occurred in the mature group, whereas, the young adults had a 62.7% increase (Table XXIII).
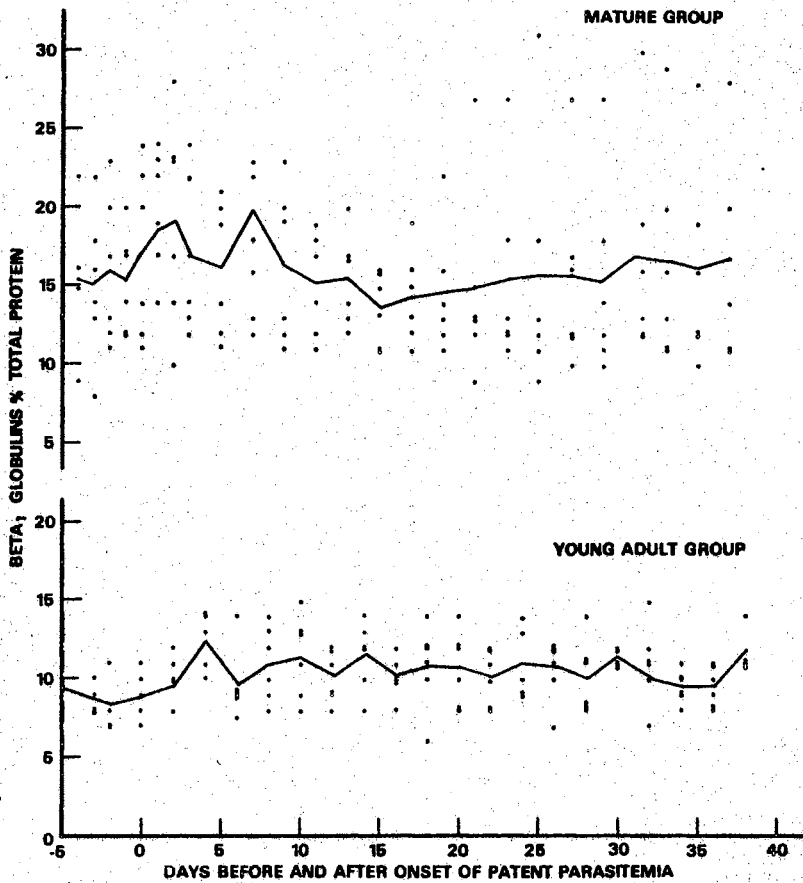
Figure 13,   Serum beta$_1$ globulins of mature (3-6
                year old) and young adult (6 month
                old) Beagles during prepatent and
                patent Babesia canis infection.
                Included in the figure are the
                mean and individual observation
                points.
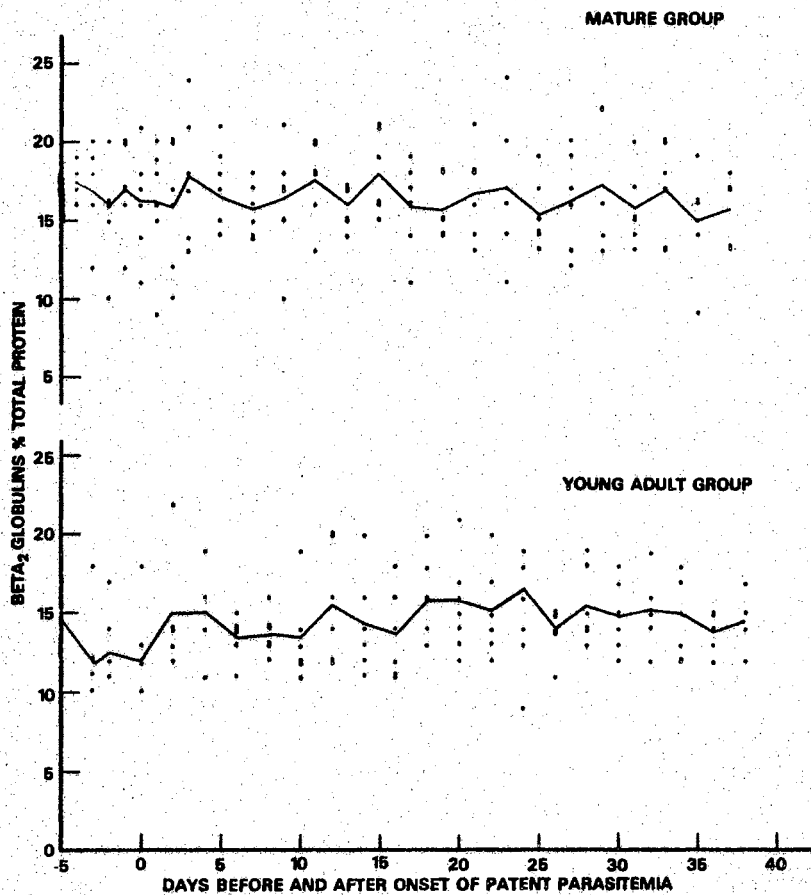
Figure 14. Serum beta$_2$ globulins of mature (3-6
              year old) and young adult (6 month
              old) Beagles during prepatent and
              patent *Babesia* *canis* infection.
              Included in the figure are the mean
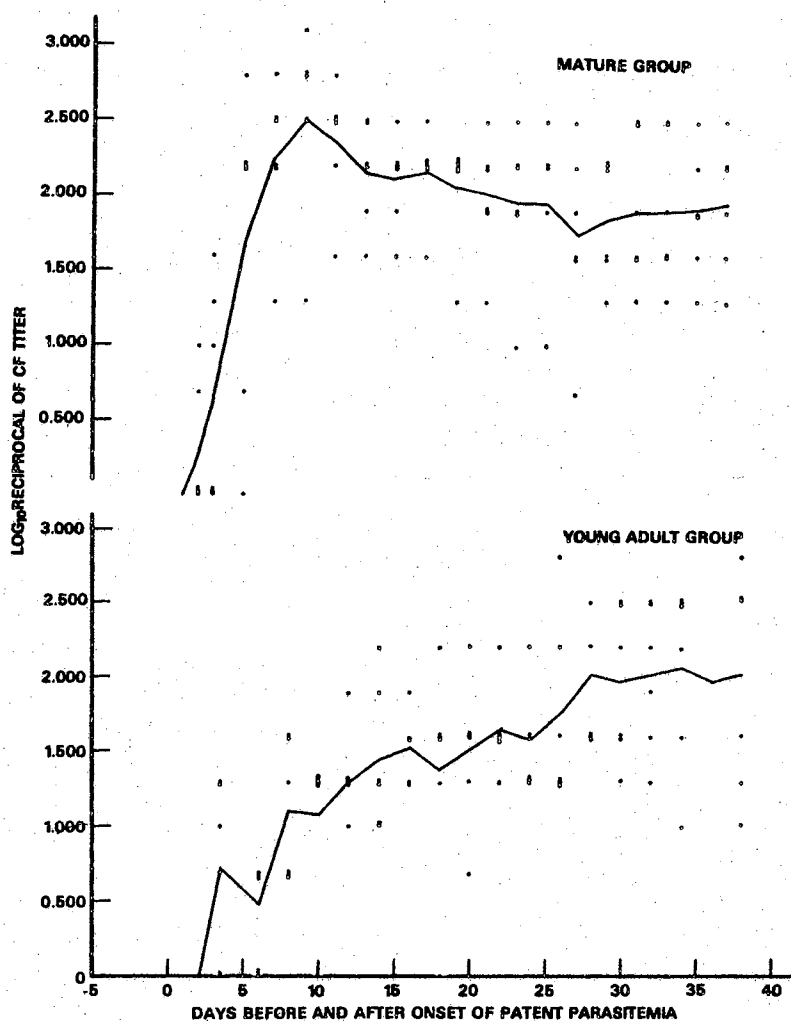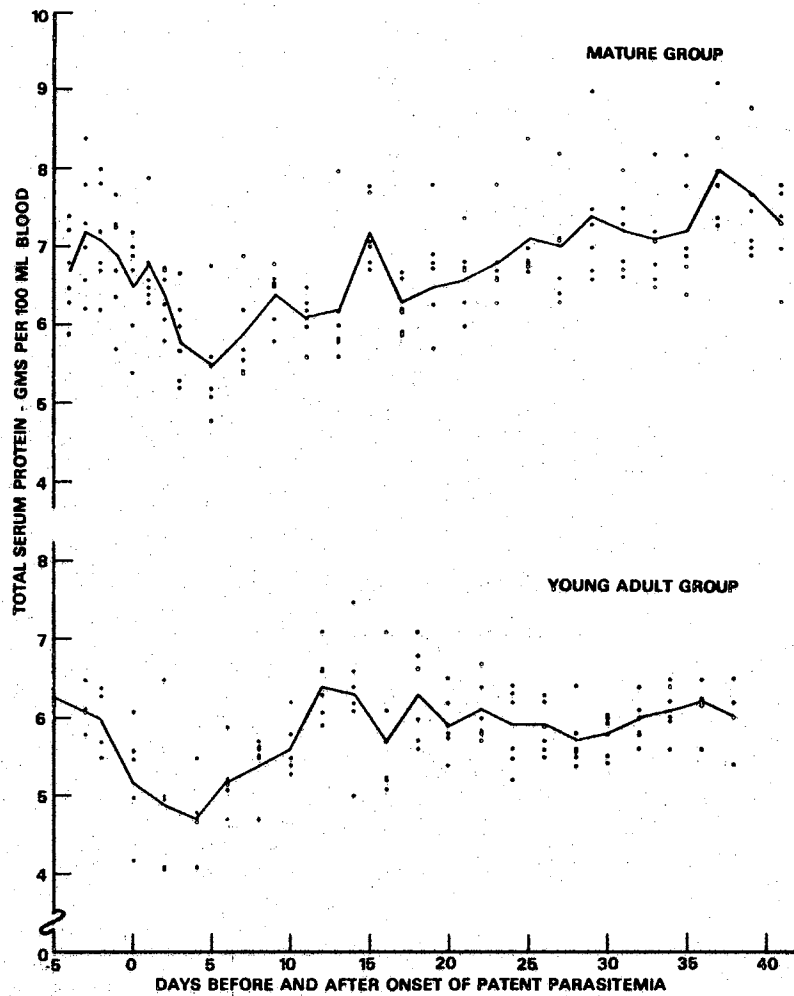   and individual observation points.
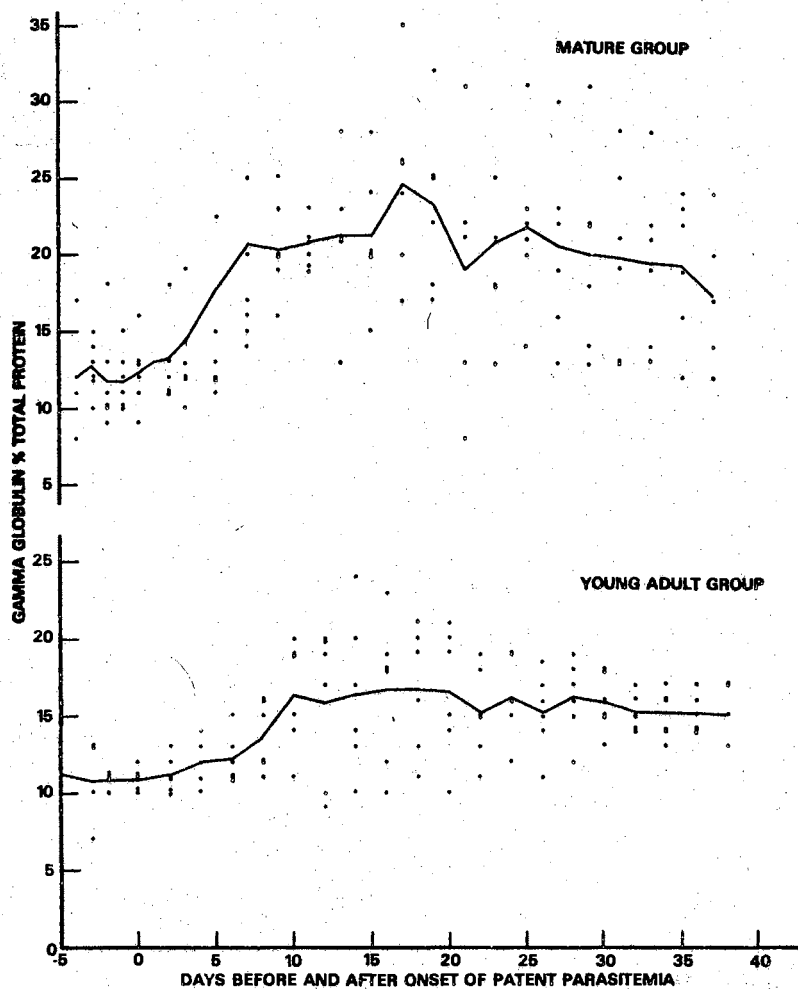
Figure 15. Serum gamma globulins of mature (3-6 year old) and young adult (6 month old) Beagles during prepatent and patent _Babesia_ _canis_ infection. Included in the figure are the mean and individual observation points.

TABLE I

LOWEST PACKED CELL VOLUME AND TIME
OF OCCURRENCE RELATIVE TO DAY 0

GROUP	NO. ANIMALS	LOW %	DAY
Mature	6	23.6 ± 3.8	8.0 ± 2.8
Young Adult	6	20.6 ± 2.8	4.2 ± 1.2
Newborn	3	14.7 ± 1.3	2.1 ± 0.6

TABLE II

TIME OF RECOVERY OF PACKED CELL VOLUME TO
PRETREATMENT MEAN IN RELATION TO DAY 0

GROUP	PRETREATMENT (PCV%)	DAY
Mature	41.3 ± 3.92	28.8 ± 0.98
Young Adult	40.1 ± 3.21	25.3 ± 5.06

TABLE III

LOWEST HEMOGLOBIN VALUE AND TIME OF
OCCURRENCE RELATIVE TO DAY 0

GROUP	NO. ANIMALS	LOW GMS %	DAY
Mature	6	7.7 ± 1.42	8.2 ± 2.6
Young Adult	6	6.1 ± 0.97	4.3 ± 1.5

TABLE IV

LOWEST ERYTHROCYTE COUNT AND TIME OF
OCCURRENCE RELATIVE TO DAY 0

GROUP	NO. ANIMALS	LOW x 10^6 per mm^3	DAY
Mature	6	3.0 ± 0.53	8.2 ± 3.1
Young Adult	6	2.6 ± 0.33	3.7 ± 0.77

TABLE V

PEAK PERCENTAGE RETICULOCYTE COUNT AND TIME
OF OCCURRENCE RELATIVE TO DAY 0

GROUP	NO. ANIMALS	HIGH %	DAY
Mature	6	6.2 ± 2.1	15.0 ± 4.3
Young Adult	6	6.7 ± 2.2	16.7 ± 8.1

TABLE VI

CHANGES IN MEAN CORPUSCULAR VOLUME[+]
RELATIVE TO DAY 0

GROUP	PRETREAT.	0-10 DAY	10-20 DAY	20-30 DAY
Mature	73.2 ± 0.2	76.4 ± 1.6	77.0 ± 2.2	77.4 ± 2.1
Young Adult	71.2 ± 1.8	78.9 ± 3.3	82.7 ± 2.8	82.4 ± 2.0

[+] See note next page.

TABLE VII

CHANGES IN MEAN CORPUSCULAR HEMOGLOBIN[+] CONCENTRATION RELATIVE TO DAY 0

GROUP	PRETREAT.	0-10 DAY	10-20 DAY	20-30 DAY
Mature	34.4 ± 0.65	33.7 ± 1.2	33.1 ± 0.68	32.3 ± 0.67
Young Adult	32.9 ± 1.40	30.8 ± 1.8	30.7 ± 0.83	30.8 ± 0.44

TABLE VIII

LOWEST LEUKOCYTE COUNT AND TIME OF OCCURRENCE RELATIVE TO DAY 0

GROUP	NO. ANIMALS	LOW	DAY
Mature	6	7418 ± 3534	1.8 ± 2.1
Young Adult	6	5145 ± 685	1.1 ± 0.4

TABLE IX

PEAK LEUKOCYTE COUNT AND TIME OF OCCURRENCE RELATIVE TO DAY 0

GROUP	NO. ANIMALS	HIGH	DAY
Mature	6	15,822 ± 6,496	15.7 ± 6.7
Young Adult	6	14,163 ± 2,896	11.3 ± 3.3

[+]The pretreatment mean for each dog was determined and the pretreatment mean for each group then calculated. The period following the first day of patent parasitemia was divided into ten day periods and the mean value for each group calculated in the same manner and compared with the pretreatment.

TABLE X

OBSERVABLE TROPHOZOITE PATTERN

The number of days preceeding a patent parasitemia and the number of days of each patent parasitemia for four cycles are tabulated for the three age groups. Information is based on presence or absence of trophozoites.

Dog No.	No. Days to 1st P	Duration of 1st P	No. Days to 2nd P	Duration of 2nd P	No. Days to 3rd P	Duration of 3rd P	No. Days to 4th P	Duration of 4th P
GROUP - Mature								
48	4	9	2	2	10	1	3	1
49	4	8	2	2	16	1	-	-
50	3	10	1	1	1	1	3	1
51	4	9	3	1	5	1	13	1
52	4	11	1	1	1	1	20	2
53	3	12	1	1	24	1	-	-
GROUP - Young Adults								
6	2	4	3	1	2	3	15	1
7	3	4	2	2	18	2	1	1
8	3	5	1	3	21	2	1	1
9	5	7	41	1	-	-	-	-
10	3	7	19	1	5	1	3	1
12	3	9	6	3	10	1	2	1
GROUP - Newborn								
101	5,5	1 died						
102	5.5	3 died						
104	5.5	4 died						
106	4,5	3 died						

TABLE XI

DURATION OF PREPATENT PERIOD

GROUP	NO. ANIMALS	DAYS
Mature	6	3.6 ± 0.52
Young Adult	6	3.2 ± 0.98
Newborn	4	5.2 ± 0.50

TABLE XII

DURATION OF FIRST PERIOD OF PATENT PARASITEMIA

GROUP	NO. ANIMALS	DAYS
Mature	6	9.8 ± 1.5
Young Adult	6	6.0 ± 2.0
Newborn	3	3.3 ± 0.9[+]

TABLE XIII

PEAK PERCENTAGE PARASITEMIA AND TIME
OF OCCURRENCE RELATIVE TO DAY 0

GROUP	NO. ANIMALS	HIGH %	DAY
Mature	6	0.3 ± 0.14	3.2 ± 1.8
Young Adult	6	0.8 ± 0.67	1.7 ± 0.51
Newborn	3	9.1 ± 12.4	1.7 ± 0.59[+]

[+]All animals in group died.

TABLE XIV

DAY COMPLEMENT-FIXING ANTIBODIES FIRST DETECTED
RELATIVE TO TIME OF INFECTION AND DAY 0

GROUP	NO. ANIMALS	DAY OF FIRST TITER POST INOC.	DAY OF FIRST TITER AFTER DAY 0
Mature	6	7.3 ± 1.36	3.7 ± 1.9
Young Adult	6	8.3 ± 2.15	5.2 ± 2.0

TABLE XV

PEAK COMPLEMENT-FIXING TITER AND TIME
OF OCCURRENCE RELATIVE TO DAY 0

GROUP	NO. ANIMALS	HIGH TITER	DAY HIGH TITER
Mature	6	2.706 ± 0.254	10.0 ± 5.6
Young Adult	6	2.254 ± 0.614	22.8 ± 11.4

TABLE XVI

MEAN CF TITER FOR LAST TEN DAYS OF EXPERIMENT[+]

GROUP	NO. ANIMALS	MEAN TITER FOR LAST 10 DAYS
Mature	6	1.878 ± 0.466
Young Adult	6	1.995 ± 0.556

[+]The mean for the last ten days of each dog was calculated. From
this the mean titer for the group was established.

TABLE XVII

LOWEST TOTAL PROTEIN AND TIME OF
OCCURRENCE RELATIVE TO DAY 0

GROUP	NO. ANIMALS	MEAN LOW GMS	MEAN DAY PP
Mature	6	5.3 ± 0.46	4.2 ± 1.33
Young Adult	6	4.5 ± 0.33	2.3 ± 1.51

TABLE XVIII

HIGHEST TOTAL PROTEIN AND TIME OF
OCCURRENCE RELATIVE TO DAY 0

GROUP	NO. ANIMALS	HIGH GMS	DAY
Mature	6	8.1 ± 0.61	33.0 ± 9.80
Young Adult	6	6.8 ± 0.27	20.7 ± 7.34

TABLE XIX

LOWEST ALBUMIN CONCENTRATION AND TIME
OF OCCURRENCE RELATIVE TO DAY 0

GROUP	NO. ANIMALS	LOW AMOUNT	DAY
Mature	6	29.0 ± 2.2	13.7 ± 7.4
Young Adult	6	34.2 ± 3.2	20.7 ± 4.7

TABLE XX

LOWEST AND HIGHEST ALPHA GLOBULIN CONCENTRATIONS
AND TIME OF OCCURRENCE RELATIVE TO DAY 0

| GROUP | ALPHA$_1$ | | ALPHA$_2$ | |
	AMOUNT	DAY	AMOUNT	DAY
Mature	6.3 $\pm$ 0.8 (high)	8.0 $\pm$ 8.2	5.7 $\pm$ 1.2	16.3 $\pm$ 3.7
Young Adult	3.7 $\pm$ 0.4 (low)	13.7 $\pm$ 5.6	10.5 $\pm$ 3.7	18.7 $\pm$ 11.4

TABLE XXI

PEAK BETA GLOBULIN CONCENTRATIONS AND TIME
OF OCCURRENCE RELATIVE TO DAY 0

| GROUP | BETA$_1$ | | BETA$_2$ | |
	AMOUNT	DAY	AMOUNT	DAY
Mature	21.7 $\pm$ 6.8	4.5 $\pm$ 10.2	20.0 $\pm$ 2.9	8.0 $\pm$ 2.9
Young Adult	13.2 $\pm$ 1.0	14.3 $\pm$ 10.5	19.2 $\pm$ 1.9	16.7 $\pm$ 10.0

TABLE XXII

PEAK GAMMA GLOBULIN CONCENTRATIONS AND TIME
OF OCCURRENCE RELATIVE TO DAY 0

GROUP	AMOUNT	DAY
Mature	26.2 $\pm$ 4.6	17.0 $\pm$ 2.2
Young Adult	19.2 $\pm$ 3.3	16.3 $\pm$ 6.8

TABLE XXIII

MAXIMAL DEVIATION OF SERUM PROTEINS FROM PRETREATMENT MEAN

DETERMINATION	PRETREATMENT MEAN	MAXIMAL DEVIATION	PERCENT CHANGE
	MATURE GROUP		
Total Protein	6.5 ± 0.44	$- 5.3 \pm 0.46$	$- 18.5 \%$
Albumin	40.2 ± 4.96	$- 29.0 \pm 2.20$	$- 27.9 \%$
Alpha$_1$ Globulin	4.2 ± 4.96	$+ 6.3 \pm 0.77$	$+ 50.0 \%$
Alpha$_2$ Globulin	10.5 ± 1.61	$- 5.7 \pm 1.20$	$- 45.7 \%$
Beta$_1$ Globulin	15.5 ± 4.49	$+ 21.7 \pm 6.80$	$+ 40.0 \%$
Beta$_2$ Globulin	17.0 ± 3.03	$+ 20.0 \pm 2.90$	$+ 17.6 \%$
Gamma Globulin	12.5 ± 3.03	$+ 26.2 \pm 4.60$	$+ 109.6 \%$
	YOUNG ADULT GROUP		
Total Protein	6.2 ± 0.26	$- 4.5 \pm 0.33$	$- 27.4 \%$
Albumin	42.7 ± 4.49	$- 34.2 \pm 3.20$	$- 19.9 \%$
Alpha$_1$ Globulin	5.7 ± 1.52	$- 3.7 \pm 0.45$	$- 35.1 \%$
Alpha$_2$ Globulin	14.3 ± 2.93	$- 10.5 \pm 3.70$	$- 26.6 \%$
Beta$_1$ Globulin	10.7 ± 1.22	$+ 13.2 \pm 1.00$	$+ 28.0 \%$
Beta$_2$ Globulin	14.7 ± 1.22	$+ 19.2 \pm 1.90$	$+ 30.6 \%$
Gamma Globulin	11.8 ± 1.95	$+ 19.2 \pm 3.30$	$+ 62.7 \%$

CHAPTER IV

DISCUSSION

The literature has been quite vague regarding the pathogenesis of
Babesia _canis_ infection in various ages of dogs. Canine babesiosis
investigators have used groups of animals of mixed ages. Maegraith (33)
studied sixty dogs of various ages and breeds divided into only "puppy"
and "adult" groups. Dorner's (11) group consisted of mongrel dogs from
six months to six years of age. Ewing's (16) observations on Babesiosis
involved only two siblings. Some discrepancy arises regarding the sus-
ceptibility of the puppy. Maegraith (33), being the only investigator
to report age groups, declared that the disease in puppies is almost
invariably fatal within a few days of the onset of symptoms. The full
grown dogs in his experiments did not always die, although some had
severe hemolysis.

Other species affected with Babesia or other hemotropic parasites
also exhibit variations in resistance. Babesiosis is known to affect
older cattle and horses more severely, however, clinical cases in
younger animals have been reported (43).

Weinman (53) reported that 5-7 day old rats showed a transitory
appearance of _Bartonella_ _muris_ with moderate anemia, but those inocu-
lated at three weeks of age were more susceptible and had a fatal
course. Adult rats usually had a transient bartonellosis with or with-
out anemia. He states that "In the albino rat, clinical resistance is

marked, immunity to infection probably non-existent." Hemobartonella canis affects younger dogs more acutely and with a higher mortality rate than older animals (53). However, young calves infected with Anaplasmosis have a greater resistance to the disease than older cattle (29).

Although this experiment concerns but few animals the controlled conditions and the use of one isolate of Babesia canis should permit valid comparisons between the three age groups of Beagles.

According to Leavell "anemia occurs whenever a decreased survival time of erythrocytes is not balanced by increased erythropoiesis." Since the bone marrow has the capacity to increase its production of erythrocytes sixfold, decrease in erythrocyte survival time to less than twenty days may be necessary to produce anemia when the bone marrow is normal. The term "hemolytic anemia" is used to refer to patients having severe hyperhemolysis (32).

The PCV, Hb, and RBC values recorded in each group of beagles were typical of hemolytic anemia. Shock or acute respiratory failure, as described by Maegraith, from the extreme hemolytic crisis following patent parasitemia may have caused death in the newborn group. Although PCV, Hb, and RBC values decreased more rapidly in the young adult group than in the mature group, these values did not approach the lethal range of the newborn group. In fact, critical illness was not observed in either of the older groups.

In the mature group there appeared to be a slight lag in the development of the anemia subsequent to the onset of the patent parasitemia. Perhaps the erythrocytes were more resistant to the parasite or the clearance of infected cells was delayed. Although there is a slight difference in the degree and development of the anemia between the older

groups, the overall results compare closely with the reports of Ewing (16) and Dorner (11).

Clinical diagnosis of anemia without laboratory support would have been difficult in the two older groups for the mucous membranes, although slightly paler than normal, were neither blanched nor icteric.

The reticulocyte response was more variable in the young adult group, but very similar in degree to that of the mature group. This appears to coincide with the normal response of the erythron, indicating that the bone marrow was equally stimulated. Maegraith (33), however, reported reticulocyte responses from 15-25% in his sixty dogs of various ages, breeds, and degrees of parasitemia. This might be caused by a variation in the virulence of the organism.

In his studies of Babesia, Ewing (16) described a normocytic, normochromic anemia. Dorner (11) classified the anemia, in his work, as "normocytic, normochromic with a tendancy toward macrocytic in the recovery phase." The data of each group in this study had a definite rise in MCV values. The mature group, while increasing only 5.7% more than the pretreatment mean, slightly exceeded the established normal range. The anemia of the young adult group was definitely macrocytic.

The early drop in the MCHC seen graphically in the young adult group was due to sampling error (only two animals were recorded that day). Both young adult and mature groups had the same percent decline from pretreatment means. Once again, the mature group approximated the normal limits while the young adult group had a hypochromic response.

Coles (8) considers a macrocytic, hypochromic anemia as a "transitory condition occurring during the active phase of erythrogenic regeneration following acute blood loss or erythrocyte destruction." The

young adult group conforms to this definition, whereas, the mature group "tends" toward a macrocytic, hypochromic classification.

The small initial rise in WBC prior to Day 0 was attributed to fever and hemoconcentration (47). The similarity between the two older groups after Day 0 of the rapid initial leukopenia and subsequent leukocytosis was very striking. There were, however, some distinct individual differences. For example, dog 49 in the mature group had a vaginal discharge and a high WBC throughout most of the experiment, but did follow the same pattern of the group.

Although most of the leukocyte counts were within normal limits, graphically each group had a leukopenia followed by a leukocytosis. Some authors report this initial leukopenia (33, 46), however, most only describe the leukocytosis (3, 44). Maegraith (33), in reporting the leukocyte counts on 10 of his animals noted an early leukocytosis in 8, some reaching a high of 30 thousand. The other two had a leukopenia. The absolute cell increase has been reported to be both neutrophilic (3, 44), and lymphocytic (10, 47). Differential determinations were not included in this experiment.

Only one newborn puppy had a high parasitemia. The anemia in this puppy was, however, very similar to that of puppies having a low parasitemia, an observation which agrees with the report that anemia can be intense without intense parasitemia (33). The two older groups had similar degrees of parasitemia, the young adults had a shorter duration of patent parasitemia but a greater intensity. In this study, the peak parasitemia occurred in both groups during the first period of parasitemia. Both Ewing (16) and Dorner (11) observed similar patterns and intensity of parasitemia. The parasitemia in Maegraith's (33) studies,

although having a higher percent in some cases had a similar onset.

It is concluded that the newborn had a more severe hemolytic disease than the older groups in which recrudescences tended to induce chronic disease.

Although individual variation existed, the graphic pattern of the CF antibody response indicated that the older dogs had a seemingly more competent antibody producing system than the young adults. When correlating the titer rises with the changes in the protein fractions the differences become more apparent. In serum proteins in bovine anaplasmosis, Dimopoullus (10) has found early CF antibodies in the alpha and beta fractions. These fractions are at their highest concentration during peak parasitemia.

Schindler and Dennig (48) reported the appearance of CF antibodies from 11 to 34 days following inoculation in their Babesia canis studies. This data indicates the antibody response to be earlier (7.3 $\pm$ 1.36 in the mature dogs and 8.3 $\pm$ 2.15 in the young adults).

Although the initial CF antibody response was related to the first patent parasitemia, subsequent parasitemic recrudescences did not appear to elicit any additional immune response.

The decreased total protein values corresponded well with the low values of other blood constituents. Albumin declined as usual in an infectious process, being replaced by the globulin protein fractions, primarily that of gamma globulin. A continued low albumin value would indicate hepatic damage resulting in poor or diminished protein synthesis. Although the mature group had a mean percent total protein decrease of 8.5% less than that of the young adult group, the albumin loss was 9.4% higher. It is reported that metalbumin is formed in Blackwater

Disease (malaria) in man causing a fall in albumin, however, this has

not been reported in dogs, nor was it included in this report (55);

neither was the examination for proteinuria part of this experiment.

Out of six cases, Maegraith (33) found a drop of total protein in only

one, but observed a reversal of A/G ratio in four.

Alpha$_1$ globulin proteins had the most radical change between the

two age groups. Graphically the rise in the alpha$_1$ globulins appears

slight in the older group, but it actually rose 50%. On the other hand,

the younger group had a decrease of 35.1% before rising. The theory

that early CF antibodies are present in the alpha fraction could perhaps

be substantiated in the older group.

The rapid intravascular hemolysis with formation of haptoglobin-

hemoglobin complex perhaps caused the decline of alpha$_2$ globulin. The

young adult dogs would, however, be expected to have a greater loss than

exhibited, because of their lower blood values during the anemia. Some

interpret decreased albumin and increased alpha globulins to stress and

tissue injury and destruction caused by disease (41).

The initial rise of beta$_1$ globulin correlates with the initial CF

titer in the mature group. Perhaps this fraction also contains early

CF antibodies. The young adult group had no early rise in CF titer nor

in beta$_1$ globulin. The initial fall in the beta$_2$ fraction in each group

is unexplainable as is the rest of the pattern which was characterized by

a rise from pre-treatment means. Maegraith (33) theorized that synthe-

sis of plasma globulins might occur, hemoglobin from destroyed erythro-

cytes perhaps being the principle source. The beta globulin fraction

contains the larger lipoproteins and changes in this fraction may

reflect disturbances in lipid metabolism. Beta globulins are reported

to rise in chronic infections (39). A serum antigen prepared from dogs with B. canis infection has been reported to be associated with the beta globulin serum fraction (51).

The gamma globulin, although having a similar graphic pattern, but with considerable individual variation, appears to illustrate the greater capacity of the older age group to form early antibodies.

Polsin and Malherbe (41) reported on changes in serum proteins in peracute cerebral, and untreated acute cases of Babesia canis. Albumin was decreased in all cases, gamma globulin was normal to increased, beta globulin remained normal, and alpha globulin values increased by varying degrees.

The hemolytic and serological changes in the three age groups of dogs infected with Babesia canis have been recorded and compared, and the resistance of the different ages under these experimental conditions must be noted. With the results presented here and by other investigators there are still many unanswered questions concerning babesiosis in dogs. These questions include factors that might change the virulence of the organism, the host-parasite relationship, and the variable parasitemias and anemias. Does the virulence of the organism depend on the isolate, the cycle through the tick and/or the immune status of the host? Under the conditions of this experiment the differences and similarities are apparent, but by varying any of the above factors different results might be obtained.

Application of this Study

Clinical Application

With the rapid transportation of dogs via air and the spread of the

Brown Dog Tick, _Rhipicephalus sanguineus_, to new areas of the country, babesiosis may well spread to other areas of the United States. It might be noted that with the number of military personnel and other Americans living abroad with their pets, other species of Babesia can and are being introduced into the country (18). No matter how remote there is always the possibility of zoonosis.

Due to possible misdiagnosis during patent infection, and the lack of clinical signs in the carrier state, canine babesiosis could easily be overlooked. In this stage of modern medicine, it is disconcerting to think that a disease might be propagating in our midst without recognition.

Experimental Application

Babesia canis, being a hemotropic parasite, might well be a model for the study of other similar diseases in man and animals, e.g., the equine and bovine. Malherbe (35) states that the pathogenesis of Babesia is so similar in the various species that for practical purposes knowledge gained in one species can be applied to all species.

Frerich (17) has tried to infect dogs, among other laboratory animals, with _Babesia equi_ in order to provide a more usable laboratory animal with which to study equine babesiosis.

Although the life cycles of plasmodium and babesia species differ, Malherbe (35) reports many similarities in the pathogenesis of the resulting infections.

The study of this disease might well be applied to the study of immunological responses, therapy, and possible prevention of hemoprotozoan diseases in many species.

Summary and Conclusions

Three age groups of Beagle dogs were infected with the same isolate of *Babesia canis*. The two older groups had similar hemolytic anemias. The PCV reached a low of 23.6% $\pm$ 3.8 in the mature group 8.0 $\pm$ 2.8 days following patent parasitemia and the young adult group had a low PCV of 20.6% $\pm$ 2.8 in 4.2 $\pm$ 1.2 days. These animals recovered and their PCV values returned to normal. The organism, however, persisted in their blood throughout the experiment. The newborn puppies had severe hemolytic crises, the PCV dropped to 14.7% $\pm$ 1.3 in 2.1 days after patent parasitemia, and the disease terminated fatally.

Other hematologic data correlated with the PCV values and was consistent with hemolytic anemia.

In 3.7 $\pm$ 1.9 days after the first day of patent parasitemia the mature group had a CF antibody response, whereas, this response occurred 5.2 $\pm$ 2.0 days after patent parasitemia in the young adult group. The mature group reached a slightly higher peak than the young adults. The CF antibody response plateaued in each group at 1.878 $\pm$ 0.466 and 1.995 $\pm$ 0.556 $\log_{10}$ reciprocal of CF titer respectively for the last ten days of the experiment.

Serum protein electrophorograms varied, but both older groups had decreased total protein and albumin and increased $alpha_2$ and gamma globulin values.

The development of the *Babesia canis* CF antigen used in this study might facilitate field diagnosis of the disease.

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APPENDIX

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 STORED
(frozen)
ILLINOIS
 ISOLATE
    |
    v
   DOG
   E-5
    |
    v
   DOG        ------------>    Blood used to infect mature dogs
   E-8                         Nos.  48  49  50  51  52  53
    |
    v
   DOG        ------------>    Blood used to infect newborn puppies
   820                        Nos.  101  102  103  104  105  106
    |
    v
   DOG        ------------>    Blood used to infect young adult dogs
   50A                        Nos.  B-6  B-7  B-8  B-9  B-10  B-12
```

Figure 16. Origin of infected blood used for inoculation,

TABLE XXIV

ESTABLISHED NORMAL VALUES

HEMATOLOGY[+]

	PCV	Hb	RBC	MCV	MCHC	WBC	TP
Range	37-55	12-18	5.5-8.5	60-77	32-36	6.0-7.0	6.0-7.5
Average	45	15	6.8	70	34	11.5	6.75

SERUM PROTEINS

	Albumin	$Alpha_1$	$Alpha_2$	$Beta_1$	$Beta_2$	$Beta_3$	Gamma	Total Protein
Males[++]	55.9	3.9	6.6	7.5	7.8	10.7	7.7	4.6-6.5
Females[++]	52.2	7.5	9.8	6.9	7.8	8.1	7.8	4.6-6.7
Unknown Sexes*	53.8	4.4	8.9	---- 19.9	Total Beta	----	13.0	6.64

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TABLE XXV

PRETREATMENT MEAN VALUES

DETERMINATION	MATURE GROUP	YOUNG ADULT GROUP
PCV	41.3 ± 3.92	40.1 ± 3.21
Hb	14.0 ± 2.48	13.2 ± 0.68
RBC	5.86 ± 0.67	5.64 ± 0.71
WBC	16,294 ± 6916	10,992 ± 3016
MCV	73.2 ± 0.23	71.2 ± 1.79
MCHC	34.4 ± 0.65	32.7 ± 1.34
TP	6.5 ± 0.44	6.2 ± 0.26
Albumin	40.2 ± 4.96	42.7 ± 4.49
Alpha$_1$	4.2 ± 0.77	5.7 ± 1.52
Alpha$_2$	10.5 ± 1.61	14.3 ± 2.93
Beta$_1$	15.5 ± 4.49	10.7 ± 1.22
Beta$_2$	17.0 ± 3.03	14.7 ± 1.22
Gamma	12.5 ± 3.03	11.8 ± 1.95

NEWBORN GROUP
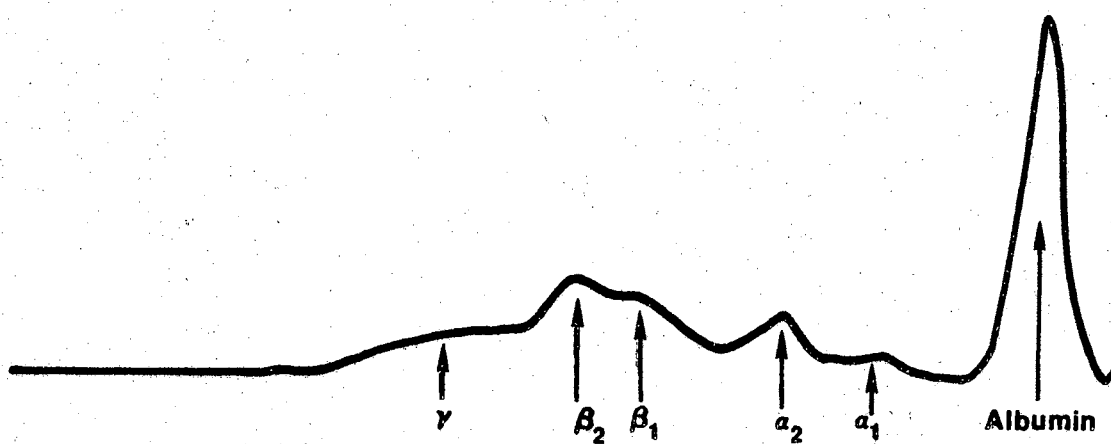
PCV	60.17 ± 4.39

Figure 17. Serum Electrophorogram of Normal Dog

VITA

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Master of Science

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