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INFECTIVITY AND CROSS IMMUNITY STUDIES OF COLOMBIAN BOVINE *BABESIA* SPECIES

A Thesis

by

THOMAS MCKIE CRAIG

Submitted to the Graduate College of
Texas A&M University in
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ABSTRACT

Infectivity and Cross Immunity Studies
of Colombian Bovine *Babesia* Species
(December 1973)
Thomas McKie Craig, B.S., D.V.M., Colorado State University
Directed by: Dr. L. G. Adams and Dr. K. L. Kuttler

Colonies of bovine hemotropic disease free *Boophilus microplus* ticks were established. Adult *B. microplus* females and eggs were incubated at 28 to 30° C. at a relative humidity of from 70 to 80%. Larvae were maintained at 24 to 28° C. and a relative humidity of 60 to 80% for maximal survival.

A colony of *B. microplus* infected with *Babesia argentina* was established by allowing non-infected ticks to feed on normal cattle for 10 to 11 days, at which time a stabilate of *B. argentina* was inoculated into the cattle subcutaneously. This resulted in a parasitemia at the time of final tick engorgement. The organism was maintained in ticks by allowing non-infected ticks to feed on a calf which was later infected by the release of infected larvae 11 to 13 days after the non-infected larvae commenced feeding. Diagnosis of *Babesia spp.* in ticks was done by examination of hemolymph.

Diagnosis and estimation of the effects of *Babesia spp.* infections in cattle were made on the basis of thick and thin blood films, packed cell volumes, rectal temperatures, body weights,

cerebral biopsies, complement fixation titers and clinical signs.

Boophilus microplus eggs, larvae and nymphs infected with *B. argentina* were disrupted by several methods and the resulting material inoculated subcutaneously into splenectomized calves. None of the calves showed signs of infection and proved to be fully susceptible when challenged with *B. argentina*.

Babesia bigemina was isolated from other bovine hemotropic agents by rapid serial passage through splenectomized calves. This isolate was compared with a laboratory strain previously isolated from a different geographic region of Colombia. Two groups of 8 calves each were inoculated subcutaneously with 10^9 *B. bigemina* organisms of each isolate. A third group of 8 calves remained as untreated controls. Twenty-eight days later, 4 calves in each of the 3 groups were challenged with 2×10^{10} *B. bigemina* organisms of each isolate. The challenge groups were homologous, heterologous and control. Both homologous and heterologous groups demonstrated immunity to challenge. No differences in the virulence of the two isolates were demonstrated.

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INTRODUCTION

The frequency of occurrence of hemotropic diseases, anaplasmosis, babesiosis, theileriosis and trypanosomosis has been a contributory factor to limiting beef cattle production throughout the world. Babesiosis is endemic to many global areas and remains one of the most costly afflictions of cattle in the world.

The development of a reliable program designed to immunize cattle against babesiosis has perplexed scientists for decades. Premunition of susceptible cattle by natural or controlled exposure to viable infectious Babesia organisms usually results in resistance to future exposure to homologous strains, but perpetuates the disease by creating carriers of the infection.[4,16,19,25,49,69,77] To premunize susceptible cattle, it is usually necessary to inject whole blood or blood fractions which contain the infective, viable Babesia organisms. Because whole blood or blood products may contain viable and infectious adventitious microorganisms, premunition remains a potentially dangerous procedure. Neonatal hemolytic anemia in the offspring of cows inoculated with blood products has been encountered and represents a potential problem.[22,39]

The only known non-bovine tissue in which growth and development of Babesia occurs is the tick. This study was attempted to provide data to ascertain the feasibility of utilizing macerated

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The citations in this paper follow the style of the American Journal of Veterinary Research.

ticks, infected with Babesia argentina, as a source of infectious material.

Repeated failure to protect cattle against babesiosis by premunition has also been attributed to antigenic variations inherent in different strains of Babesia.[9,15,30,75] There is no evidence that antigenic variation persists between various strains of Babesia found in Colombia. However, evidence directed to this consideration could explain variations in susceptibility which are evident in premunized cattle when these animals are geographically relocated. Data relative to the premunizing efficiency of inocula was evaluated to determine if antigenic variation is evident between isolates of Babesia bigemina from two widely separated geographic areas of Colombia.

LITERATURE REVIEW

Immunity to *Babesia* Infections

Immunity from hemotropic disease is generally dependent upon premunition.[41] Premunition using *Babesia* organisms was described before 1900.[25,67] Premunition was established by the inoculation of blood from carrier cattle or by the injection of macerated ticks which had engorged on infected cattle. Attempts to attenuate the organisms by cold storage of blood was used.[77] Trypan blue was used to regulate clinical response to *Babesia* infections by preventing death losses in cattle but not sterilizing the infection.[78] Blood from latent carriers has been considered a safe inoculum for the induction of premunition. However, the blood from latent carriers sometimes contained a sub-infective number of organisms.[71]

Immunity against *Babesia argentina* (*B. argentina*) has been established with a vaccine consisting of an inoculum of 10^7 to 10^8 organisms.[14] This vaccine has been tested under field conditions with satisfactory results.[19,49] The organisms were grown in splenectomized calves, diluted with blood from non-infected cattle and chilled to a temperature of 1 to 5° C. A loss of approximately 1 log of infectivity occurred for each week of storage at that temperature.[14]

Apparent strain differences have been observed in both blood and tick passaged *Babesia bigemina* (*B. bigemina*),[8] with isolates increasing[37] or decreasing in virulence. One strain of blood

passaged B. argentina lost its infectivity for ticks.[54] The organism having lost its capacity to infect ticks cannot be transmitted by natural means. Premunition with such an organism could not be sustained under field conditions.[54]

Infection with bovine Babesia spp. usually elicits protection against homologous challenge and partial protection against heterologous strains of the same Babesia species.[9,30,64,75] Therefore, the strain content of an effective vaccine should be selected on the basis of the challenge likely to be encountered.[9]

The inoculation of tissues has the inherent danger of introducing pathogenic adventitious microorganisms into recipient animals. A greater danger exists with tissues from the same or closely related species than from unrelated donor tissues.[62] The inoculation of bovine blood or blood products to protect cattle against hemotropic disease has led to the occurrence of neonatal hemolytic disease.[22,39]

Tick Tissues as an Agent for Infecting Cattle

Engorged female ticks, ground in mortar and pestle, were injected into cattle to induce Babesia infections.[23,66,77]

Several protozoan, viral and rickettsial diseases have been transmitted through the inoculation of infected tick tissues into susceptible animals.[51] Heartwater, a disease caused by a rickettsia, Cowdria ruminatium, has been transmitted through a tissue

suspension of nymphal Amblyomma haebreum.[70] Theileria parva, the causative agent of East Coast Fever, was transmitted by the injection of a suspension of ground infected Rhipicephalus appendiculatus,[17,70,76] or salivary secretions of the infected adult tick.[56] Theileria annulata schizonts were seen in calves after the inoculation of tissues from infected Hyalomma dromedarii.[47]

Although the mechanism is unknown, cattle can acquire resistance to larval Boophilus microplus (B. microplus) ticks.[57] Precipitating antibodies developed in cattle inoculated with eggs and larvae of B. microplus; however, the antibody response had no correlation to tick resistance by the cattle.[28]

Boophilus microplus and Babesia spp. Transmission

Boophilus ticks are the only known vectors of bovine babesiosis in the western hemisphere.[61] Boophilus microplus shows minor morphological differences in various parts of the world but is considered to be one species.[63]

Pre-oviposition and oviposition periods are influenced mostly by temperature, with the onset of ovipositing occurring earlier at higher temperatures.[27,32] In Australia, B. microplus females and eggs are incubated at 29.4° C. and a relative humidity (RH) of 96% to produce greater numbers of viable larvae in the shortest time.[12,32] Unfed larvae survived longest when kept in darkness at 23.9° C. at 90 to 100% RH.[32]

Feeding and moulting times of the parasitic stages were: larvae, 4.5 to 13.1 days with a mean of 5.5 days, nymphs, 11.9 to 20.3 days following larval release with a mean of 15.4 days. Adult females detached from the host in 18.9 to 35.5 days, with a mean of 21.9 days following release of larvae.[31]

Variation in tick infestation rates was noted to be dependent upon breed and age of host, and the time of the year. The nutritional status of the host is important. Fewer ticks feed to repletion on a well nourished host.[53]

Most Boophilus are infected with Babesia during the last few days of attachment, with the greatest number becoming infected during the last 24 hours of feeding.[10,12,61] Another report indicated that the ticks may become infected as larvae and transmit Babesia through their progeny.[58] High mortalities have been noted in female ticks feeding on cattle with a Babesia parasitemia of 5% or higher.[60]

Babesia argentina was transmitted by the larvae as early as 1 to 2 days following attachment.[45] The infective stage of B. argentina was found in the salivary glands of B. microplus larvae 2 to 3 days following attachment to the host. Evidence of infection has been difficult to demonstrate in unfed larvae, and it has been impossible to find evidence of infection in larvae which had fed for longer than 3 days.[60]

Although the adult tick may be capable of transmitting the organism, B. bigemina is transmitted primarily by the nymph.

Infection of cattle with B. bigemina occurred 8 or 9 days following infestation with infected larvae. The prepatent period to parasitemia was 13 to 14 days following larval release.[12,33]

Isolation and Preservation of Babesia

Babesia argentina was isolated after releasing a splenectomized calf in a pasture containing infected ticks for 6 days and spraying the calf with ixodicides as soon as the first larvae were seen to moult.[73] Another method for isolation of B. argentina involved the use of trypan blue to suppress B. bigemina.[11]

Babesia bigemina was separated from B. argentina and Theileria mutans by rapid passage of blood through 5 splenectomized calves.[5,11]

Attempts were made to eliminate Eperythrozoon spp. by cooling blood to 21° C. for 24 hours prior to inoculation.[11] Treatment of infected cattle with neoarsphenamine[11] or sodium (2-di-(B, gamma, dioxipropil)-aminophenol-(4 arseno 5)-B-(benzoxasolil-(2)-mercapto) propionate[a,1] may eliminate Eperythrozoon parasitemias.

Stabilates, which are defined as "A population whose reproduction has been arrested by viable preservation on a unique occasion," [40] have been made of Babesia spp. by low temperature preservation. Various anticoagulants were successfully used in the preparation of stabilates including: heparin,[3] sodium citrate[55,74] and disodium ethylenediaminetetraacetate (EDTA).[26] Glycerol at 7 to 15%[3,55,74]

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[a]Spirotrypan-fuerte, Farbwerke Hoechst, Frankfurt am Main, Germany.

and 4 M dimethylsulfoxide (DMSO)[20] have been successfully used as cryoprotective agents.

A greater viability of organisms preserved at temperatures less than -60° C. has been observed.[3] Rapid thawing bringing the stabilate to body temperature is thought to reduce loss of viability.[21] Stabilates prepared with DMSO can be administered either intravenously or subcutaneously with equal efficacy; those preparations containing glycerol should be inoculated subcutaneously for maximum efficiency.[21]

MATERIALS AND METHODS

Part I: Macerated *Boophilus microplus* Experiments

Experimental Cattle

The cattle used in the experiments originated from 1 of 2 farms in the Sabana de Bogota (3,000 meters in altitude).

Boophilus ticks have not been found on farms in the Sabana. The Holstein-Friesian calves, primarily bulls, were purchased at 3 to 5 months of age. Prior to arrival at the research facilities the cattle were vaccinated with *Clostridium chauvoei*, *Clostridium septicum* and *Pasteurella multocida* bacterin. A formalin killed foot and mouth disease vaccine, types A and O, was given every 4 months. These calves were fed a single ration (Table I) throughout the trials.

The cattle were housed in screened and moated barns at the Centro Internacional de Agricultura Tropical (CIAT), El Porvenir research farm, Palmira, Valle, Colombia.

All cattle were bled, rectal temperatures taken and body weights determined every 2 weeks unless clinical signs indicated that more frequent sampling was necessary. Twenty calves were splenectomized.[50] These calves were bled and temperatures taken twice weekly for 45 days following splenectomy.

Fecal samples were taken on arrival of the calves at the CIAT facilities. When fecal samplings indicated a parasite burden, the

TABLE I

Ration for Experimental Cattle*

Concentrate Mix

Maize or Sorghum, Ground	430 Kg.
Soybean or Cotton Seed Meal	300 Kg.
Wheat or Rice Bran	230 Kg.
Iodized Salt	10 Kg.
Vitamin Premix**	10 Kg.
Mineral Premix***	10 Kg.
	1000 Kg.

Three volumes of ground maize cobs were mixed with 2 volumes of concentrate and fed at a rate of approximately 3% of body weight daily.

In addition, the cattle were provided clean water and 1:1 iodized salt: bone meal mineral mix ad libitum.

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*Based on the 1966 National Research Council recommendations.

**Vitamin Premix

Maize or Sorghum, Ground	10 Kg.
Roche A-D	
(Vit A 750,000 i.u. Gm.	
Vit D 150,000 i.u. Gm.)	8 or 10 Gm.
	10 Kg.

8 Gm Roche A-D with Maize diet
10 Gm Roche A-D with Sorghum diet

***Mineral Premix

Copper Sulfate	195 Gm.
Iron Sulfate	747 Gm.
Zinc Oxide	124 Gm.
Magnesium Sulfate	309 Gm.
Potassium Iodide	7 Gm.
Cobalt Sulfate	20 Gm.
Maize or Sorghum, Ground	8.598 Kg.
	10 Kg.

calves were treated with Thiabendazole[b] for gastrointestinal parasites and a combination of Isoniazide[c] and Diethylcarbamizine citrate[d] for Dictyocaulus viviparus infections.

Calves infested with Linognathus vituli were sprayed twice at a 14 day interval with Carbaryl.[e]

The calves were 5 to 10 months of age at the time of the trials.

Establishment and Maintenance of Boophilus microplus Colonies

Replete female B. microplus ticks were gathered at the Instituto Colombiano Agropecuario (ICA) Turipana experimental station located in the Sinu Valley, near Monteria, Cordoba, on the Caribbean coast. These ticks were collected from dairy cattle not showing signs of hemoparasitic infection. Another group of ticks was obtained from the commercial beef herd of (CIAT) El Porvenir experimental farm. This herd had a low level of tick infestation and hemoparasitic disease was not a problem.

Engorged female ticks were placed in individual glass vials with cloth stoppers.[f] At the completion of ovipositing, the ticks

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[b]Omnizole, Merck, Sharp & Dohme, Rahway, N.J.

[c]Luvorem, E. R. Squibb & Sons, Inc., New York, N.Y.

[d]Franozan 40%, Cooper, McDougall & Robertson, London, England.

[e]Sevin, Union Carbide S. A., Yumbo, Valle, Colombia.

[f]Wright, J. E., USDA Vet. Tox. & Ento. Res. Lab., College Station, Texas: Pers. Comm., 1971.

were removed and the eggs incubated at room temperature 23 to 31° C. and relative humidity 40 to 85%. Two to 6 weeks following emergence, larvae of each isolate were placed on splenectomized calves. Replete female ticks, gathered 20 to 25 days following larval release, were placed in glass vials.

The calves were bled daily for 30 days following larval release and twice weekly for another 40 days. Blood samples from each of the 3 calves were taken 70 days after larval release, pooled and injected into another splenectomized calf. The calves into which the pooled blood was injected were bled on a similar schedule for 60 days.

Other generations of ticks were released and collected in a like manner from splenectomized cattle, except that the eggs were pooled into 1 Gm. (approximately 20,000 eggs), 0.5 Gm. or 0.25 Gm. lots. Ticks were collected from the floor or from the calf.

Later generations of pre-ovipositing females and eggs were incubated at 28 to 30° C. in a bacteriological incubator[g] in which a pan of wet sand was placed to increase the RH to 70 to 80%, as measured by a hygrometer.

Larvae were raised under 3 environmental conditions: at room temperatures of 21 to 34° C. and 40 to 80% RH; at 28 to 30° C. and 70 to 80% RH; and at 24 to 28° C. and 60 to 80% RH.

Attempts were made to overcome fluid loss in larval ticks by

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[g]Thelco, Precision Scientific Co., Chicago, Ill.

placing drops of distilled water or filtered, fetal calf serum against the cloth stoppers, in an effort to induce drinking by the larvae.[65]

Establishment of Singularly Infected Tick Colonies

Progeny of ticks which for 2 generations did not transmit hemotropic disease to splenectomized calves were placed on splenectomized and non-splenectomized calves. Ten and 11 days following release of larvae, a stabilate of B. argentina was injected subcutaneously into the host calf in an effort to coincide parasitemia with the final engorgement of the adult female tick. In subsequent generations, the release of hemoparasite free larvae was followed in 11 to 13 days by the release of B. argentina infected larvae. By these means infected adult ticks were obtained.

Methods of Evaluation

Thick blood films were prepared using a wire loopful of blood. Approximately 1.4 mm.[3] of blood were placed near one end of a microscopic slide. The drop of blood was smeared on the slide with the loop in a circle approximately 6 mm. in diameter.[46] The blood was air dried and placed in an incubator at 65° C. for 45 minutes.[6]

Thin blood films were made on the opposite end of the slide, air dried and fixed in absolute methanol.

The blood film was stained in a 1:50 Giemsa solution[h] diluted

[h]Giemsa Stain, Gradwohl Labs., St. Louis, Mo.

with phosphate buffered water (pH 6.9-7.1) containing 0.01% alkyl-aryl polyether alcohol.[i] The blood film was stained for 45 minutes. Excess stain was gently washed from the slide using phosphate buffered water.[6]

Packed cell volume (PCV) was determined by the micro-hematocrit method on blood collected in tubes containing 2 drops of a 10% solution of disodium EDTA per 5 ml. blood as an anticoagulant.

Rectal temperatures were generally taken before 9 a.m. each day. Thermometers, scaled in degrees Celsius, were inserted for one minute.

Body weights were estimated with weigh tapes[j] applied around the heart girth.

Cerebral biopsies were done with local anesthesia following the technique described by Johnston and Callow.[35] The incision site was determined by an imaginary line drawn from the lateral canthus of the left eye to the base of the right horn, intersecting with a line parallel to the median saggital plane 3 cm. left of the midline. A stainless steel 1/8 inch bit in a bone drill was used to penetrate the frontal sinus and calvarium. A 14 gage 3 inch cannula was introduced into the brain, until the hub of the cannula or bone on the opposite side of the brain prevented further penetration. The cannula was rotated and negative pressure applied with a syringe. The contents of the cannula were emptied into 0.85% saline.

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[i]Triton X 100, J. T. Baker Chemical Co., Phillipsburg, N.J.

[j]Nasco, Ft. Atkinson, Wis.

The core of cerebral material was separated into 0.5 X 2 mm. segments which were placed between 2 glass slides, squashed and drawn across one slide. The slides were fixed with absolute methanol for 5 minutes and stained with Giemsa as for blood slides.

Two to 3 days after the onset of ovipositing, 1 leg was removed from each tick with a small pair of scissors, as close to the body as possible. The tick's body was gently squeezed and a drop of hemolymph was placed on a slide. The dried hemolymph was fixed in absolute methanol for 5 minutes and stained with Giemsa as for blood slides.

Determination of complement fixing (CF) antibody titers was accomplished by a microtiter method.[42] Blood samples were collected on all cattle at least every 14 days. Serum was separated, clarified by centrifugation and stored in glass vials at -20° C. Serum, inactivated for 35 minutes at 56° C. in a water bath, was used in the microtiter test.[2]

Sheep, obtained from high mountain flocks presumed free of hemotropic diseases, were used as erythrocyte donors. The blood was collected in Alsever's solution.[2]

Commercially obtained hemolysin (rabbit anti-sheep erythrocyte serum)[k] was standardized by titration, using 2 units of complement and a 2% washed sheep erythrocyte suspension in veronal buffer. One unit of hemolysin was regarded as the highest dilution producing complete hemolysis in 45 minutes at 37° C.[2]

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[k]Sheep Cell Hemolysin, The Sylvana Co., Millburn, N.J.

Complement (guinea pig serum) was obtained commercially[m] and titrated using a hemolytic system composed of 2 units of hemolysin and 2% sheep erythrocyte suspension. A unit of complement was regarded as the highest dilution of complement, producing complete hemolysis in 45 minutes at 37° C.[2]

Antigen was prepared from the parasitized erythrocytes of 3 calves having parasitemias of 15, 17 and 21%. One hundred ml. of a stabilate of B. argentina were injected subcutaneously into a splenectomized calf which was treated with 30 mg. Triamaciolone acetate[n] prior to injection of the stabilate.[13] When a moderate (2.5%) parasitemia was detected, 500 ml. of blood were passaged intravenously to a second calf, which was treated with 30 mg. Triamaciolone every second day from 10 days prior to inoculation and continued until death. At the time of maximum parasitemia (21%), the calf was exsanguinated by carotid canulation and 500 ml. blood passaged to each of 2 other Triamaciolone treated splenectomized calves.

The infected blood used for antigen production was collected by vacuum into filtration flasks which contained 1.2 Gm./liter disodium EDTA as a 10% solution. Plasma was separated at a relative centrifugal force (RCF) of 1,020 for 20 minutes and removed by suction. The erythrocytes were washed twice in sterile physiological saline. The non-infected erythrocytes were selectively lysed

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[m]Dehydrated Complement, Texas Biological Labs., Inc., Ft. Worth, Texas.

[n]Vetalog, E. R. Squibb & Sons, Inc., New York, N.Y.

in a 0.35% sodium chloride solution. This lysis relies on differences in osmotic fragility between parasitized and non-parasitized erythrocytes.[44] The remaining sediment was washed once in physiological saline, then lysed in 10 volumes of distilled water and centrifuged.

The remaining sediment was subjected to lysis in 2 volumes of distilled water and mechanical disruption in a Ten Broeck tissue grinder. Each fraction was separated at a RCF of 5,000 for 30 minutes, the supernatant retained and the remaining sediment resuspended in distilled water. To increase complement fixing activity, the supernatant was centrifuged at a RCF of 27,000 for 1 hour, and the resulting sediment resuspended in an equal volume of distilled water.[44] The antigen was stored at -60° C. Antigen was titrated as described, using 1 negative and 3 or 4 positive serums.[42]

The microtiter procedure was done in plastic trays,[o] into which 0.025 ml. of veronal buffer was added to each well via a dropper pipette.[p] One drop or 0.025 ml. of inactivated serum, diluted with buffer 1:2.5, was added to the first well in each row. The serum was serially diluted from 1:5 to 1:640 with a micro loop.[q] Antigen, 0.025 ml. (2 units/ml.), and 0.025 ml. of complement (2 units/ml.) were then added and allowed to react for 1 hour at 37° C.

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[o]Disposable Microtiter Plates, Cooke Engineering Co., Alexandria, Va.

[p]Microtiter Pipette Dropper, Cooke Engineering Co., Alexandria, Va.

[q]Microtiter Loops, Cooke Engineering Co., Alexandria, Va.

The hemolytic system, 0.05 ml., consisting of equal volumes of 2 units of hemolysin and 2% sheep erythrocytes, was added to each well. The trays were incubated at 37° C. for 45 minutes, then placed in a refrigerator overnight. The titer of the serum was considered to be the highest dilution having complete fixation of complement. Controls in the form of positive and negative serums, antigen control and complement control were prepared.

Experimental Preparations Using *Babesia argentina* Infected *Boophilus microplus* Tissues

Minimal essential media (Eagle) with Earle's salts (MEM)[r] was prepared in double distilled, deionized water, to which was added bovine serum albumin (BSA) crystals[s] at 1.05 and 3.5% v/v, then adjusted to pH 7.5.[17] Sterilization was accomplished by filtration through a 0.22 μ Millipore filter.[t] After filtration the pH of the 1.05% and 3.5% batches was 7.35 and 7.3, respectively. The media were refrigerated at 4° C. in 500 ml. bottles.

Four hundred twenty-five ml. of fetal bovine serum (FBS) were obtained from abatoirs in Bogota and were mixed with 15 ml. glycerol, sterilized by filtration through a Millipore 0.45 μ filter and stored in 250 ml. bottles at -20° C.[17]

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[r]MEM (Eagle) with Earle's Salts, Grand Island Biological Co., Grand Island, N.Y.

[s]Bovine Serum Albumin, Fract. V, Armour Pharmaceutical Co., Chicago, Ill.

[t]Millipore Corp., Bedford, Mass.

The dorsal midline of the calves was clipped and shaved. A cloth bag covering an area of approximately 600 square centimeters was attached to the skin with glue.[u] A flexible adhesive[v] was used to attach the cloth bags to the last 4 calves. Tick larvae were introduced through a hole in the bag after which the hole was resealed. The bag was protected by a burlap cover.

Preparation 1: Approximately 6.25 Gm. of B. argentina infected B. microplus larvae were released on susceptible splenectomized calf 352. Subsequently calf 352 became infected with B. argentina and died as a result of the infection.

Larvae and nymphs were removed from calf 352, 6 days after larval release. Approximately 4 Gm. of larvae and nymphs were washed 3 times in physiological saline solution and centrifuged at 1200 r.p.m. for 10 minutes. The ticks were then suspended in 50 ml. of MEM with 3.5% BSA, and macerated for 1 hour with ground glass fragments in a mortar with a pestle. After sedimenting for 105 minutes, 20 ml. of supernatant were injected into calf 341 subcutaneously. An additional 25 ml. were decanted and mixed with an equal volume of FBS-glycerol, dispensed in 4 ml. vials and frozen in a dry ice cabinet.

The same day an additional 6 Gm. of larvae and nymphs were scraped from calf 352, washed twice in physiological saline solution,

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[u]Weldwood, U.S. Plywood, Kalamazoo, Mich.

[v]Duratite, DAP Inc., Dayton, Ohio.

suspended in 150 ml. MEM with 1.05% BSA, macerated, as before, for 1 hour and sedimented for 30 minutes. Forty-five ml. of supernatant were injected into calf 341 subcutaneously, and 47 ml. were decanted and mixed with an equal volume of FBS-glycerol, dispensed in 4 ml. vials and frozen in a dry ice cabinet.

Calf 341 was bled and temperatures were taken daily for 15 days and twice weekly for an additional 33 days. A cerebral biopsy, to determine if infection with B. argentina was present, was performed on day 35 post inoculation. Approximately 0.25 Gm. B. argentina infected larvae was released on calf 341 on day 48 post inoculation to establish the susceptibility of calf 341.

Preparation 2: Approximately 2 Gm. of B. argentina infected B. microplus larvae were released on susceptible splenectomized calf 348. Subsequently calf 348 became infected with B. argentina and died as a result of the infection.

Larvae were removed from calf 348, 48 hours after larval release. Approximately 1 Gm. of larvae from calf 348 was suspended in 16 ml. of MEM with 3.5% BSA and macerated for 30 minutes as previously described. After sedimenting for 1 hour, 10 ml. of supernatant were injected into calf 243 subcutaneously. An additional 2 ml. were decanted and mixed with an equal volume of FBS-glycerol, dispensed in a 4 ml. vial and frozen in a dry ice cabinet.

Calf 243 was bled and temperatures were taken daily for 15 days and twice weekly for an additional 23 days. On day 35 post

inoculation, a cerebral biopsy was performed. On the same day, approximately 0.25 Gm. B. argentina infected larvae was released to establish the susceptibility of calf 243.

Preparation 3: Approximately 20 Gm. of larvae (from the same source as used in preparations 1, 2 and 4 and presumed to be infected with B. argentina), 20 days after emergence from the egg, were suspended in 15 ml. of MEM with 3.5% BSA and macerated in a glass Ten Broeck tissue grinder. After sedimenting for 45 minutes, 5 ml. of supernatant were injected into calf 247 subcutaneously. An additional 8 ml. of supernatant were decanted and mixed with an equal volume of FBS-glycerol, dispensed in 4 ml. vials and frozen in a dry ice cabinet.

Calf 247 was bled and temperatures were taken daily for 15 days and twice weekly for an additional 10 days. On day 25 post inoculation, a cerebral biopsy was performed. On the same day, approximately 0.25 Gm. B. argentina infected larvae was released to establish the susceptibility of calf 247.

Preparation 4: Approximately 1 Gm. of B. argentina infected B. microplus larvae was released on susceptible splenectomized calf 338. Subsequently calf 338 became infected with B. argentina and died as a result of the infection.

Larvae were removed from calf 338, 30 hours after release. Approximately 1 Gm. of larvae was washed in a 1:50 solution of chlorhexadine[w] and rinsed in distilled water. The larvae were

[w]Nolvasan, Fort Dodge Labs., Ft. Dodge, Iowa.

suspended in 6 ml. of MEM with 3.5% BSA and macerated in a Ten Broeck tissue grinder. After sedimenting for 30 minutes, 5 ml. of supernatant were injected into calf 266 subcutaneously. An additional 4 ml. were decanted and mixed with an equal volume of FBS-glycerol, dispensed in 4 ml. vials and frozen in a dry ice cabinet.

Calf 266 was bled and temperatures were taken daily for 30 days and twice weekly for an additional 25 days. On day 55 post inoculation, approximately 0.25 Gm. B. argentina infected larvae was released to establish the susceptibility of calf 266.

Preparation 5: One Gm. of B. argentina infected B. microplus eggs, obtained from females which had fed on a calf with a tick induced B. argentina infection, was suspended in 10 ml. of MEM with 3.5% BSA and 10 ml. FBS-glycerol. Using silicone[x] coated glassware, the suspended larvae were macerated in a Ten Broeck tissue grinder.[72] After sedimenting for 30 minutes, 8 ml. of supernatant were injected into calf 248 subcutaneously. An additional 12 ml. were decanted, dispensed in 4 ml. vials and frozen in a dry ice cabinet.

Calf 248 was bled and temperatures were taken daily for 15 days and twice weekly for an additional 36 days. On day 34 post inoculation, a cerebral biopsy was performed. On day 51 post inoculation, 5 ml. of a stabilate containing approximately 1.35×10^9 B. argentina organisms were injected intravenously to establish the susceptibility of calf 248.

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[x]Siliclad, Clay-Adams, Parsippany, N.J.

Preparation 6: Approximately 2 Gm. unfed larvae from the egg pool used in preparation 5 were combined with 0.5 Gm. larvae which had fed on susceptible splenectomized calf 338 for 48 hours.

The larvae were suspended in a solution of 10 ml. of MEM with 3.5% BSA and 10 ml. of FBS-glycerol. Using silicone coated glassware, the suspended larvae were macerated in a Ten Broeck tissue grinder, and the resulting mixture injected into calf 286 subcutaneously.

Calf 286 was bled and temperatures were taken daily for 17 days and twice weekly for an additional 10 days. On day 14 post inoculation, a cerebral biopsy was performed. On day 27 post inoculation, approximately 0.25 Gm. B. argentina infected larvae was released to establish the susceptibility of calf 286.

Preparation 7: Approximately 5 Gm. of B. argentina infected B. microplus larvae, 17 days after emergence from the egg, were suspended in 50 ml. of MEM with 3.5% BSA and 50 ml. of FBS-glycerol and macerated in a silicone coated Ten Broeck tissue grinder. After sedimenting for 30 minutes, 10 ml. of supernatant were injected into calf 295 subcutaneously. An additional 48 ml. of supernatant were decanted, dispensed in 4 ml. vials and frozen in a dry ice cabinet.

Calf 295 was bled and temperatures were taken daily for 14 days and twice weekly for an additional 16 days. On day 14 post inoculation, a cerebral biopsy was performed. On day 30 post inoculation, 5 ml. of a stabilate containing approximately 1.35×10^9 B. argentina organisms were injected intravenously to establish the

susceptibility of calf 295.

Part II: Strain Immunity Experiment

Twenty-four, 6 to 8 month Holstein-Friesian calves, 23 bulls and 1 heifer, were purchased from one of the same farms as previously indicated. The calves were managed in a similar manner as previously described, except they were housed in a non-screened barn. The calves were divided into 6 groups of 4 calves each.

Replete B. microplus were collected from cattle at the ICA experimental station Carimagua, Meta in the Colombian Llanos. The ticks, eggs and larvae were incubated at 28 to 30° C. with 70 to 80% RH. Two weeks after emergence, larvae were released on a splenectomized calf. Daily temperatures and blood samples were taken. Upon the detection of a B. bigemina parasitemia, 5 ml. of blood were passed by intravenous inoculation into a second splenectomized calf which in turn was examined until the onset of parasitemia. One hundred ml. of blood were passaged to a third calf and 12 ml. into a fourth calf in a similar manner. A stabilate was prepared from the blood of the fourth calf. Shortly after death, each calf was subjected to necropsy and organ smears were examined for evidence of B. argentina.

A stabilate of B. bigemina originating from Monteria, Cordoba on the northern Colombian coast was available in the laboratory.[5]

Babesia bigemina isolates were preserved in a manner similar

to those previously described.[21,26,55,74] Blood was collected from calves with a parasitemia greater than 5% via jugular venapuncture. Collection was into flasks with 12.5 ml. of 10% sterile disodium EDTA per liter of blood. Glycerol 11.6% v/v was added to whole blood, placed on a magnetic stirrer for equilibration at 4° C. for 30 minutes. Aliquots of 5, 50 and 100 ml. were placed in plastic bottles and frozen in an insulated dry ice cabinet where they were stored. Stabilates were thawed in a water bath at 37° C. with gentle agitation. The calves were inoculated subcutaneously.

The estimated number of infected erythrocytes was based on erythrocyte counts and percentage of parasitemia. Counts and parasitemias were done in triplicate and the average used for calculation; an example is given as follows:

4.6×10^6 erythrocytes/mm.^{3} determined by hemocytometer
3.5% parasitemia determined by direct count on a thin blood film
4.6×10^6 erythrocytes/mm.^{3} - 11.6% glycerol displacement =
4.07×10^6 erythrocytes/mm.^{3}
4.07×10^6 erythrocytes/mm.^{3} $\times 10^3 = 4.07 \times 10^9$ erythrocytes/ml.
4.07×10^9 erythrocytes/ml. X 3.5% parasitemia =
1.42×10^8 organisms/ml.
1.42×10^8 organisms/ml. X 5 ml. volume =
7.1×10^8 organisms/stabilate

Two isolates of B. bigemina were inoculated into splenectomized calves. These stabilates, from calves 61 and 265, were frozen for 22 and 31 days respectively.

The day of primary inoculation was defined as day 0, and prior to this time, temperatures, weights, blood and serum samples were

taken to determine an approximate base line for each calf. The blood and serum were examined for evidence of hemotropic disease, by examination of blood films and CF tests.

The Llanos stabilate (calf 265) was calculated to have approximately 3.2×10^8 B. bigemina/ml. prior to freezing, and the Monteria stabilate (calf 61) approximately 10^9 B. bigemina/ml.

On day 0, 8 calves were inoculated subcutaneously with 1 ml. of stabilate 61 Monteria isolate (10^9) organisms; another 8 calves were inoculated with 3 ml. of stabilate 265 Llanos isolate (10^9) organisms; the remaining 8 calves were not inoculated and served as controls. Half of the calves of each group were inoculated subcutaneously on day 28 with 20 ml. of stabilate 61 containing approximately 2×10^{10} organisms, and the other half of each group of calves inoculated subcutaneously with 60 ml. of stabilate 265 containing 2×10^{10} organisms. The groups were designated as to homologous, heterologous and control challenges.

Samples were taken as follows: daily temperatures for the duration of the experiment, daily blood samples for parasitemia and packed cell volume (PCV) for 15 days, then every other day until day of challenge on day 28. Following challenge, blood samples were taken daily until day 45, then every other day until the end of the experiment on day 48. Serums for CF tests were taken approximately every 5 days. Weight was estimated every 2 weeks with a measuring tape, at which time the average weight on 2 successive

days was recorded.

For the purpose of this study, a negative parasitemia occurred when the examination of 50 fields of a thick blood film at 1000 X failed to disclose any Babesia organisms. Positive parasitemias of <0.01% occurred when at least one Babesia organism could be found in 50 fields of a thick blood film. Higher percent parasitemias were calculated by counting the number of parasitized cells in 50 fields of a thin blood film, when each field contained approximately 200 erythrocytes. Statistical methods used, including standard deviation and an analysis of variance, to establish significance are described by Snedecor.[68]

Prior to the commencement of the experiment, 8 calves were identified as having Eperythrozoon spp. On day -5, all calves were treated with 20 ml. Spirotrypan-fuerte intravenously.[1]

RESULTS

Part I: Macerated *Boophilus microplus* Experiments

Experimental Cattle

Generally good health and satisfactory weight gains were maintained by the cattle, although a variety of infections were encountered during the first few weeks following arrival at the research facilities. Some of the calves were treated for digestive disturbances, pneumonia, purulent arthritis and pododermatitis.

Eperythrozoon spp. were encountered in both splenectomized and intact cattle. A mild anemia and transient temperature rise accompanied most infections. *Eperythrozoon* infections were treated with 20 ml. of Spirotrypan-fuerte intravenously.[1]

Establishment and Maintenance of *Boophilus microplus* Colonies

The splenectomized calves, on which *B. microplus* larvae were released, did not develop hemoparasitic infections, as evidenced by negative blood smears, lack of temperature response, negative complement fixing antibody titers and lack of clinical signs.

The tick strains were arbitrarily chosen from the progeny of a single female (Figure 1) of each isolate and maintained for 4 generations without causing hemoparasitic disease in any of the splenectomized calves on which they were fed.

The pre-ovipositing period was shortened from 5 or 6 days at

FIGURE 1: Engorged female Boophilus microplus ventral view 14 X.

room temperature and RH to 2 to 3 days at 28 to 30° C. with a 70 to 80% RH. The incubation period of eggs was shortened from 39 days to 20 to 22 days.

Larvae survived approximately 1 month at room temperature and RH; 2 months at 28 to 30° C. and 70 to 80% RH; and 3 months at 24 to 28° C. and 60 to 80% RH. Considerable variation in longevity of individual groups of larvae was observed. Some groups of Babesia infected larvae died within 2 weeks of emergence under optimal conditions.

Methods of Evaluation

When parasitemias were low, Babesia were more easily detected in thick blood films. Thin blood films were more useful in studying morphology and to determine percentage parasitemia (Figures 2, 3, 4 & 5).

The PCV of normal calves ranged from 30 to 35% when first purchased but decreased to 26 to 32% after remaining at the experimental facility. The PCV of calves infected with B. argentina decreased to 15 to 25% but seldom fell below 15% even in fatal cases.

Considerable variation in normal temperature was observed; rectal temperatures of 39.5° C. or higher were considered indicative of a febrile response. Febrile response usually preceded the appearance of parasitemia in B. argentina infections. On several occasions the calves had a febrile response with conditions other than babesiosis.

FIGURE 2: *Babesia argentina* in a thick bovine blood film, Giemsa, approximately 1400 X.

FIGURE 3: *Babesia argentina* in a thin bovine blood film, Giemsa, approximately 1400 X.

FIGURE 4: Babesia bigemina in a thick bovine blood film, Giemsa, approximately 1400 X.

FIGURE 5: Babesia bigemina in a thin bovine blood film, Giemsa approximately 1400 X.

Cerebral biopsy was performed on 13 calves, including 1 calf on 2 occasions. Most of the calves showed no deleterious effects due to the biopsy and were eating within one-half hour of completion. The presence of B. argentina in the capillaries of the cerebrum were readily observed (Figure 6).

Many vermiculi were found in the hemolymph of female ticks feeding on cattle with a high Babesia parasitemia. A lower percentage of ticks were found to be infected if they fed on cattle with lower parasitemias. The B. argentina vermiculi tended to be slightly larger than those of B. bigemina, with enlarged heads and curled tails (Figure 7). Babesia bigemina vermiculi tend to be cigar shaped (Figure 8). More than 800 hemolymph samples were examined with the number of vermiculi positive samples varying from group to group.

Positive complement fixation titers, of 1:10 or more,[44] first appeared 5 to 10 days after the exposure to Babesia spp. Some B. argentina infected splenectomized calves died without exhibiting CF titers.

Experimental Preparations

None of the calves inoculated with B. argentina infected tick material had any evidence of babesiosis as a result of the inoculations, nor was there evidence of induced protective immunity. All calves proved to be fully susceptible to a B. argentina challenge (Table II).

FIGURE 6: Cerebral Babesia argentina from a fatal case of bovine cerebral babesiosis, Giemsa, approximately 2000 X.

FIGURE 7: Babesia argentina vermiculi from the hemolymph of a replete female Boophilus microplus, Giemsa, approximately 2500 X.

FIGURE 8: Babesia bigemina vermiculi from the hemolymph of a replete female Boophilus microplus, Giemsa, approximately 2500 X.

TABLE II

Inoculation of Various Preparations of *Babesia argentina* Infected *Boophilus microplus* Eggs, Larvae and Nymphs into Susceptible Splenectomized Cattle

Calf No.	Inoculum[1]	Response to Inoculum	Challenge	Prepatent Period Following Challenge (Days)
341	Prep. 1: Larvae & Nymphs fed on 352[2]	none	1/4 Gm. infected Larvae	12
243	Prep. 2: Larvae fed 48 hours on 348[3]	none	1/4 Gm. infected Larvae	12
247	Prep. 3: Unfed infected Larvae	40.1° C. on day following inoculation	1/4 Gm. infected Larvae	13
266	Prep. 4: Larvae fed 30 hours on 338[4]	none	1/4 Gm. infected Larvae	9
248	Prep. 5: Infected Eggs	none	1.35×10^9 *B. argentina* Stabilate 286[5]	9
286	Prep. 6: Unfed & Larvae fed 48 hours on 338	40.3° C. on day 2 following inoculation	1/4 Gm. infected Larvae	9
295	Prep. 7: Unfed infected Larvae	none	1.35×10^9 *B. argentina* Stabilate 286	9

[1]Preparations as listed in Materials and Methods.
[2]352 died with cerebral *B. argentina* 12 days following larval release.
[3]348 died with cerebral *B. argentina* 14 days following larval release.
[4]338 died with cerebral *B. argentina* 12 days following larval release.
[5]Stabilate 286 *B. argentina* preserved in dry ice.

Part II: Strain Immunity Experiment

Seventeen days after release of larvae of Llanos origin, B. bigemina was found in blood smears. On the following day, 5 ml. of blood was passaged by intravenous inoculation to a second calf. The first calf died on day 18 with a mixed B. argentina and B. bigemina parasitemia. Twenty-seven hours following inoculation of the second calf, blood was inoculated intravenously into a third splenectomized calf. Blood was passaged intravenously to a fourth calf 50 hours following inoculation. A stabilate was prepared from the fourth calf as shown in Table III. All of the calves in the isolation process died as a result of the B. bigemina infections, except the first calf which had a dual infection. No evidence of B. argentina was found in the other calves.

TABLE III

Isolation of Babesia bigemina

Calf No.	Method of Infection	Elapsed Time From Exposure to Passage	Passaged to Calf No.
345	0.15 Gm. B. microplus larvae released	18 days	284
284	5 ml. blood I.V.	27 hours	288
288	100 ml. blood I.V.	50 hours	237
237	12 ml. blood I.V.	4 days	Stabilate

Results of the cross immunity experiment are recorded in Figures 9, 10, 11a, 11b, 12a and 12b. Average prepatent period to parasitemia, average maximal temperature, average maximal CF titer, average minimal percentage of PCV and average daily gain are recorded (Table IV).

At the time of the first inoculation, there was an increase in the temperature of control as well as inoculated calves. During the febrile period, the calves were coughing, lacrimating, anorectic and 1 calf had signs of pneumonia. Attempts were made to isolate viruses or pathogenic bacteria from whole blood, serum and nasal swabs. No known pathogens were isolated. A similar temperature response did not accompany the challenge inoculation.

One calf in the Monteria X Llanos group failed to exhibit parasitemia or CF titer as a result of the premunizing inoculation. This calf proved to be fully susceptible to challenge; data gathered on this calf is not presented in the graphs and tables.

Significant differences in the severity of disease between the Llanos and Monteria isolates were not demonstrated except for a shorter prepatent period to parasitemia by the Llanos isolate (Table V). The response to homologous and heterologous challenge indicated no significant differences between the isolates, but the control calves demonstrated a significantly higher average temperature response and lower packed cell volumes than the premunized calves (Table VI).

FIGURE 9: Daily morning rectal mean and range temperatures in degrees Celsius. I = day of "premunizing inoculation." C = day of challenge.

*Llanos X Llanos = Group of calves inoculated and challenged with Llanos isolate.

Llanos X Monteria = Group of calves inoculated with Llanos isolate and challenged with Monteria isolate.

Llanos = Group of calves challenged with Llanos isolate.

Monteria X Llanos = Group of calves inoculated with Monteria isolate and challenged with Llanos isolate.

Monteria X Monteria = Group of calves inoculated and challenged with Monteria isolate.

Monteria = Group of calves challenged with Monteria isolate.

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*Groups of calves so designated above are used throughout the paper.

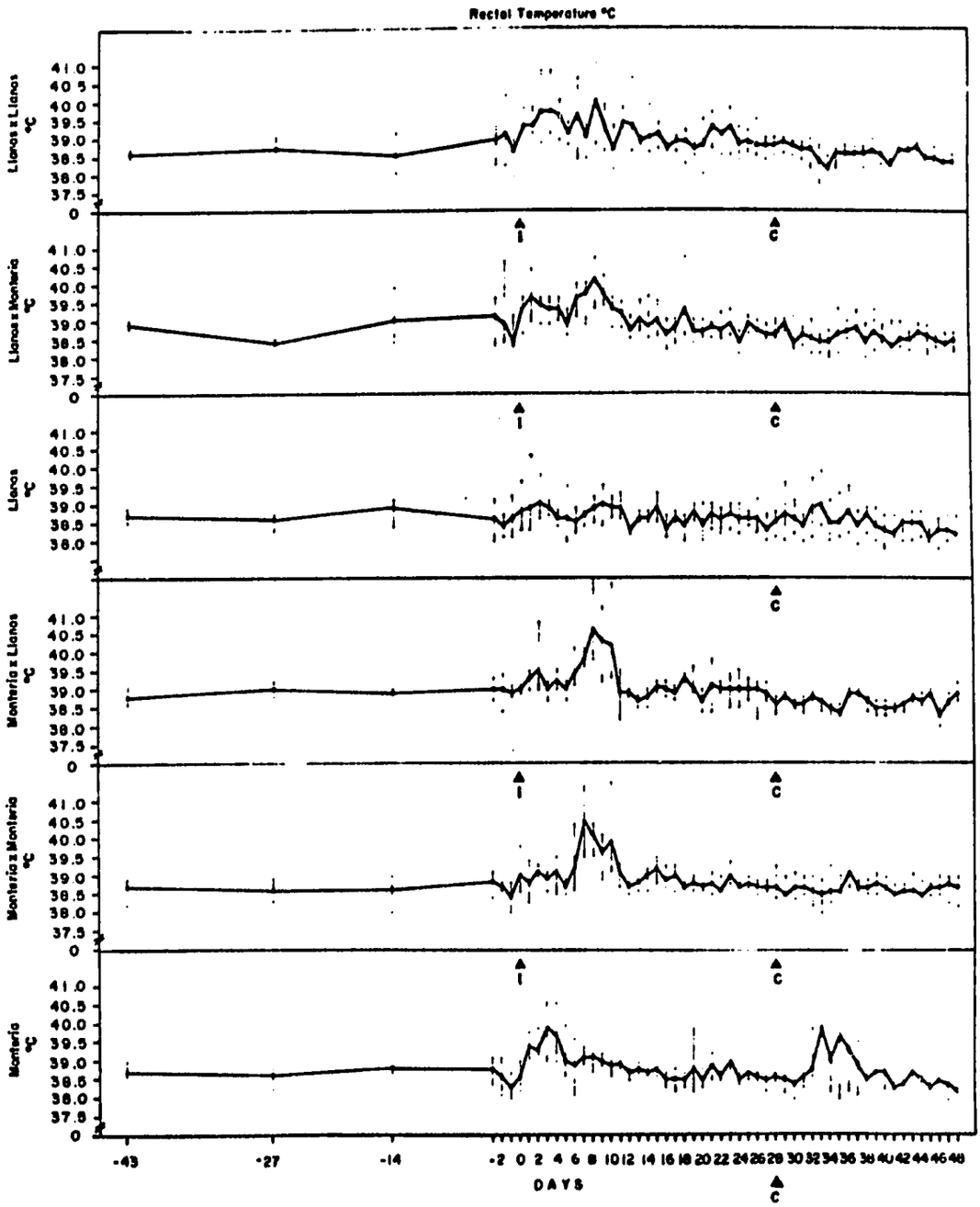
Rectal Temperature °C
Llanos x Llanos
Llanos x Monteria
Llanos
Monteria x Llanos
Monteria x Monteria
Monteria
°C
41.0
40.5
40.0
39.5
39.0
38.5
38.0
37.5
0
I
C
-43
-27
-14
-2 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48
DAYS

FIGURE 10: The mean and range of the percentage of packed cell volumes. I = day of "premunizing inoculation." C = day of challenge.

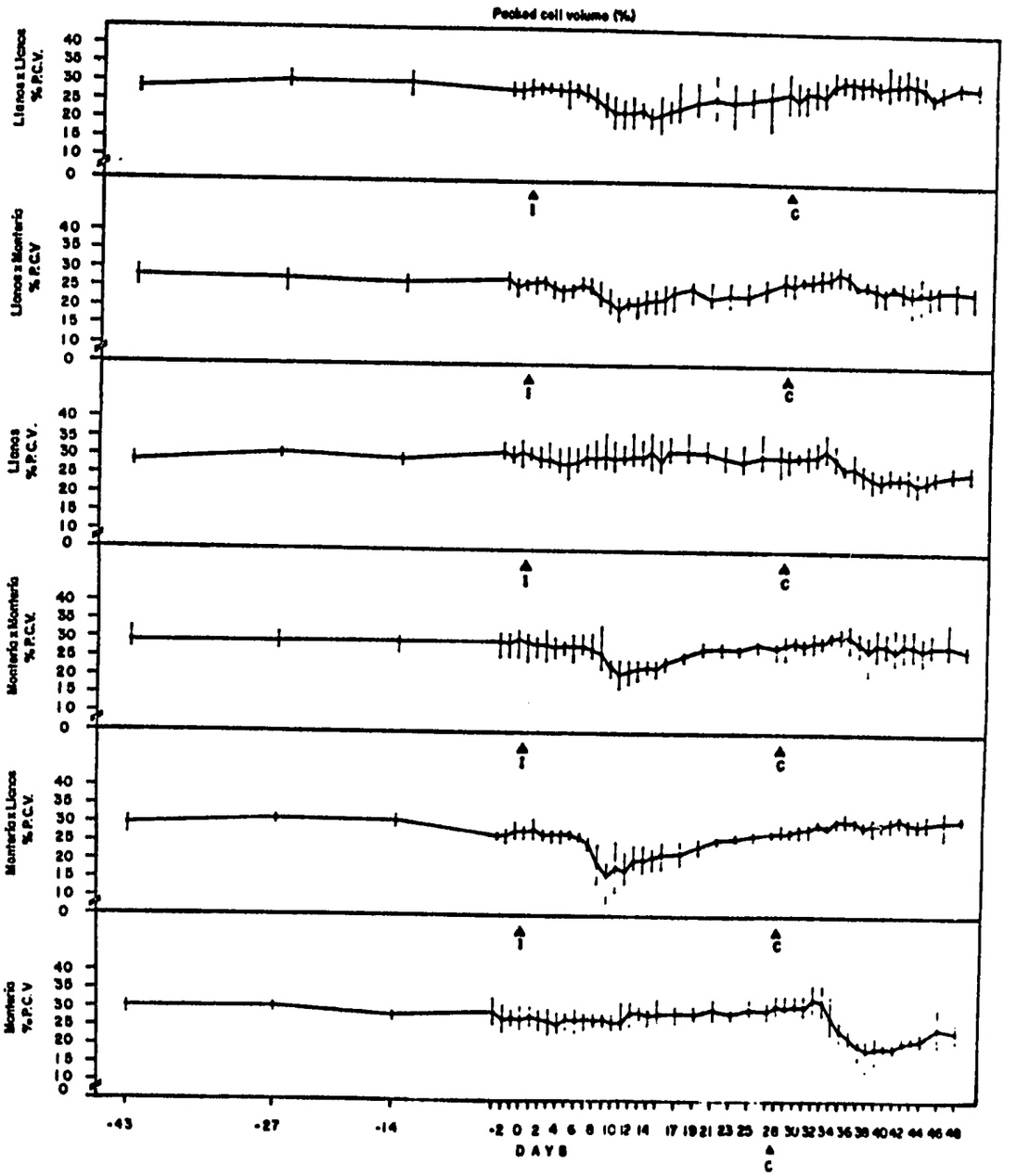
Packed cell volume (%)
Llanos x Llanos % P.C.V.
Llanos x Monteria % P.C.V
Llanos % P.C.V.
Monteria x Monteria % P.C.V.
Monteria x Llanos % P.C.V.
Monteria % P.C.V
I
C
-43
-27
-14
-2 0 2 4 6 8 10 12 14 17 19 21 23 25 28 30 32 34 36 38 40 42 44 46 48
DAYS

FIGURE 11a: Percentage of *Babesia bigemina* parasitemia from thick and thin bovine blood films, with individual parasitemias indicated. I = day of "premunizing inoculation." C = day of challenge.

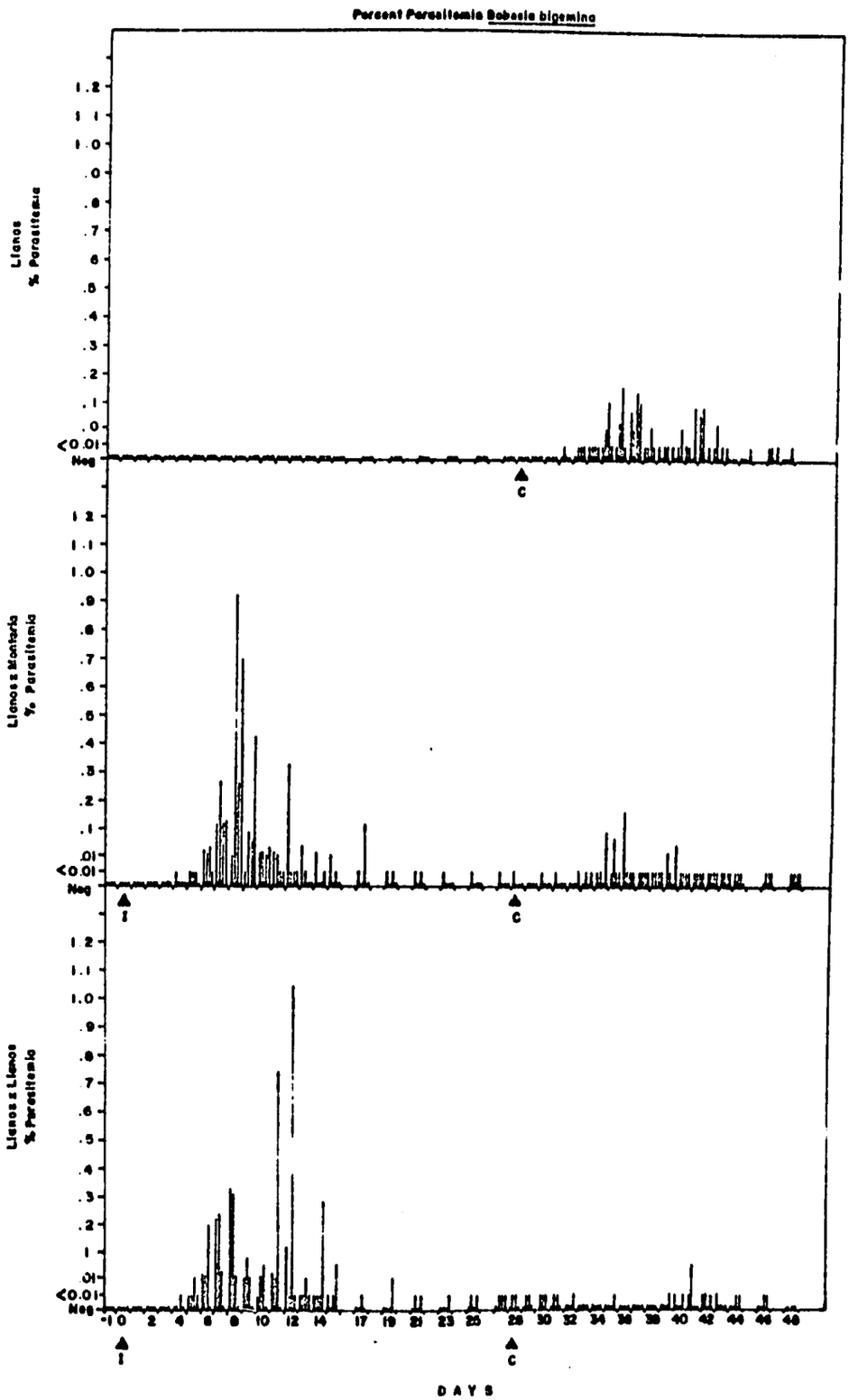
Percent Parasitemia Babesia bigemina
Llanos
% Parasitemia
Llanos x Montaria
% Parasitemia
Llanos x Llanos
% Parasitemia
<0.01
Neg
I
C
DAYS

FIGURE 11b: Percentage of *Babesia bigemina* parasitemia from thick and thin bovine blood films, with individual parasitemias indicated. I = day of "premunizing inoculation." C = day of challenge.

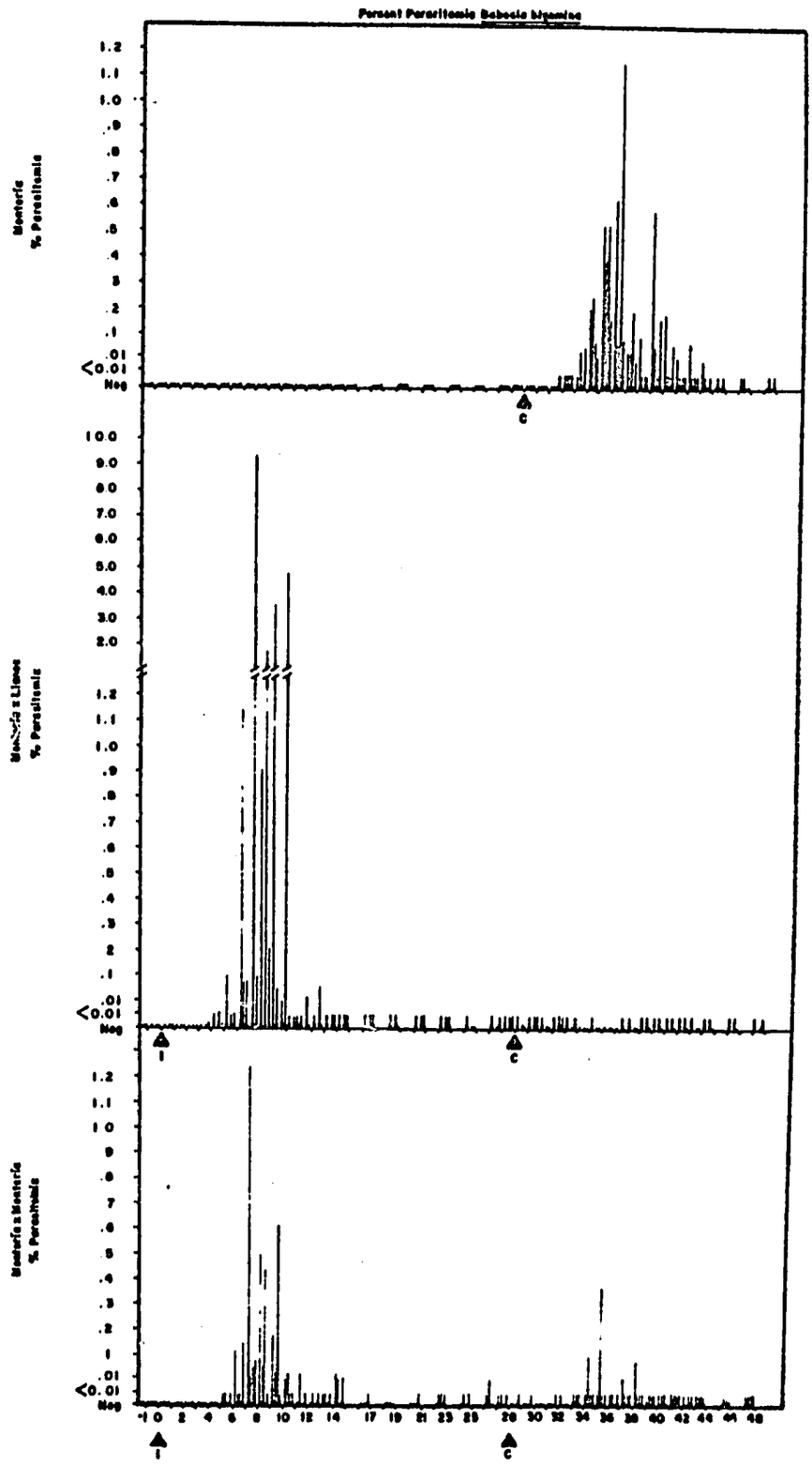
Percent Parasitemia Babesia bigemina
Bovteria % Parasitemia
Bovteria x Lionex % Parasitemia
Bovteria x Bovteria % Parasitemia
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
.01
<0.01
Neg
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
I
C
-1 0 2 4 6 8 10 12 14 17 19 21 23 25 28 30 32 34 36 38 40 42 44 46 48
DAYS

FIGURE 12a: Individual complement fixation titers for *Babesia bigemina*. I = day of "premunizing inoculation." C = day of challenge.

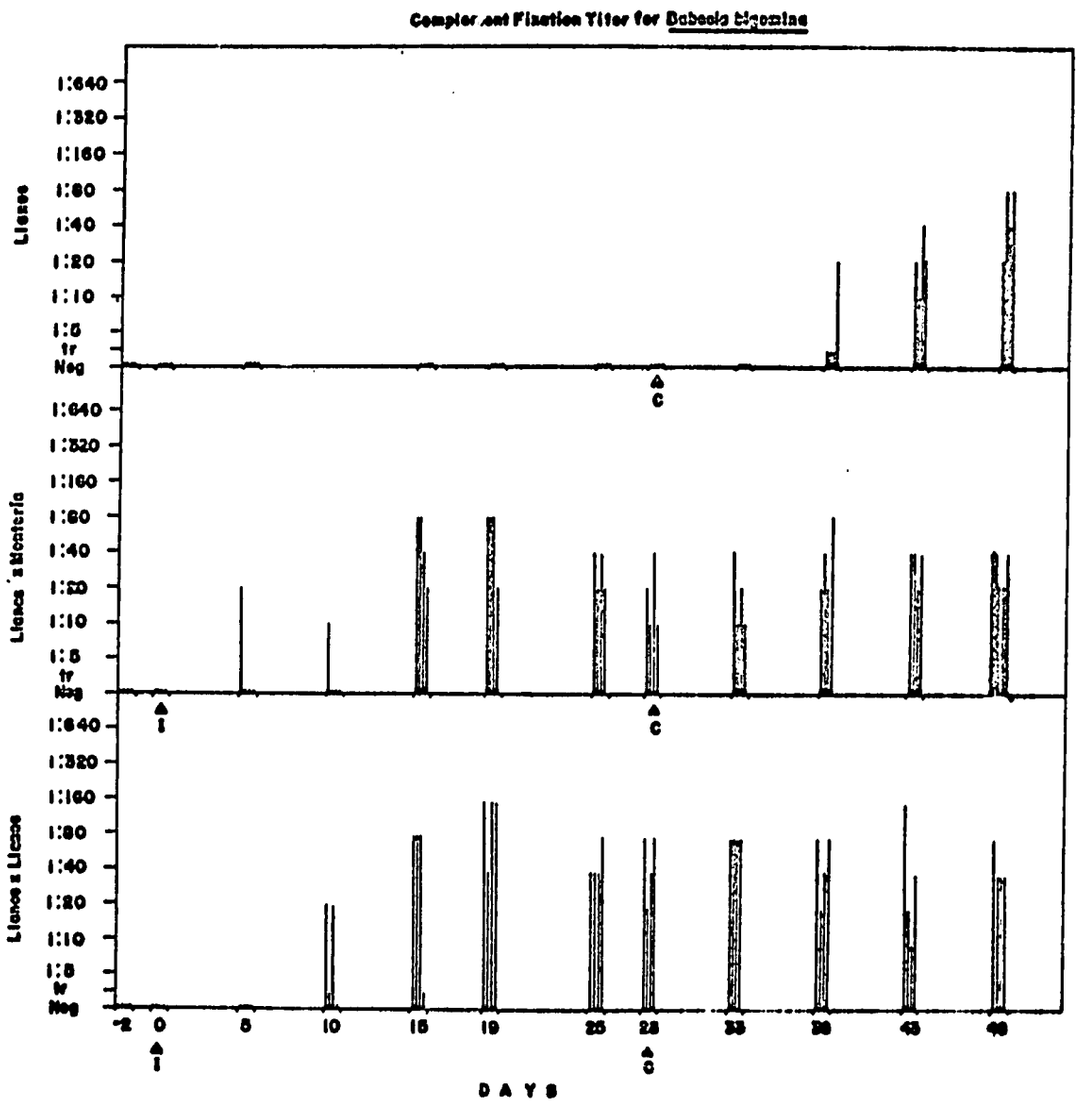
Complement Fixation Titer for Babesia bigemina
Liesse
Liesse x Monteria
Liesse x Liesse
1:640
1:320
1:160
1:80
1:40
1:20
1:10
1:5
tr
Neg
I
C
-2
0
5
10
15
19
25
28
33
38
43
48
DAYS

FIGURE 12b: Individual complement fixation titers for *Babesia bigemina*. I = day of "premunizing inoculation." C = day of challenge.

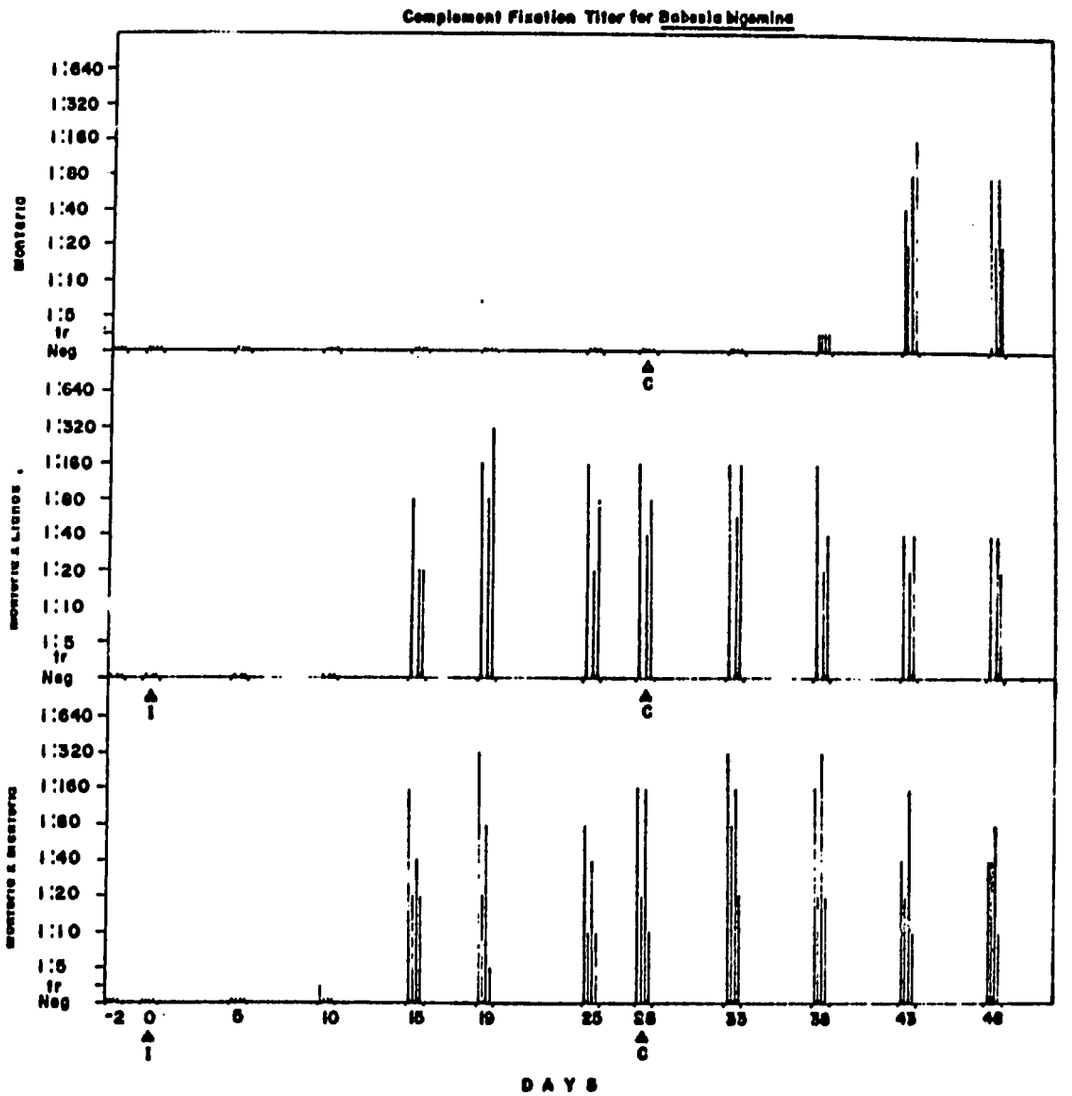
Complement Fixation Titer for Babesia bigemina
1:640
1:320
1:160
1:80
1:40
1:20
1:10
1:5
tr
Neg
C
I
-2
0
5
10
15
19
25
28
35
38
43
46
DAYS

TABLE IV. STRAIN IMMUNITY OF *BABESIA BIGEMINA*

EXPERIMENTAL GROUP	NO. OF ANIMALS	SIZE[1] & ORIGIN OF INOCULUM	PREPATENT PERIOD TO PARASITEMIA	MAXIMUM % PARASITEMIA	MAXIMUM TEMPERATURE °C	MINIMUM % PCV[2]	MAXIMUM CF[3] TITER	SIZE[1] & ORIGIN OF CHALLENGE	PREPATENT PERIOD TO PARASITEMIA	MAXIMUM % PARASITEMIA[4]	MAXIMUM TEMPERATURE °C	MINIMUM PCV %	MAXIMUM CF TITER	AVERAGE DAILY RATE OF GAIN KG/DAY
LLANOS X LLANOS	4	1×10^9 Llanos	4.75[5] (4-5)	0.49 (.29-1.04)	40.8 (40.6-41.0)	19.75 (19-21)	1/140 (1/80-1/160)	2×10^{10} Llanos	4.00 (1-12)	0.015+ (+ - 0.16)	39.0 (38.8-39.1)	28.50 (27-30)	1/100 (1/80-1/160)	.667 (.531-.802)
LLANOS X MONTERIA	4	1×10^9 Llanos	5.00 (4-6)	0.49 (.26-.92)	40.4 (39.5-40.7)	19.5 (18-21)	1/65 (1/20-1/80)	2×10^{10} Monteria	4.25 (1-7)	0.08+ (+ - 0.16)	39.0 (38.8-39.3)	24.25 (20-26)	1/45 (1/20-1/80)	.391 (.198-.438)
CONTROL X LLANOS	4	—	—	—	39.5 (39.0-40.3)	26.75 (25-28)	—	2×10^{10} Llanos	4.00 (3-5)	0.125 (0.07-0.16)	39.6 (39.4-39.9)	22.50 (21-25)	1/55 (1/20-1/80)	.658 (292-708)
MONTERIA X MONTERIA	4	1×10^9 Monteria	6.25 (6-7)	0.52 (.06-1.24)	40.7 (39.5-41.4)	20.5 (18-24)	1/90 (1/20-1/160)	2×10^{10} Monteria	4.50 (1-6)	0.135+ (+ - 0.37)	39.2 (39.0-39.5)	26.25 (22-30)	1/205 (1/20-1/320)	.607 (.563-.708)
MONTERIA X LLANOS	3	1×10^9 Monteria	5.33 (5-6)	3.56 (0.22-9.4)	41.4 (40.3-42.0)	14.67 (10-19)	1/153 (1/80-1/320)	2×10^{10} Llanos	1.67 (1-2)	+ (+)	39.0 (39.0)	28.67 (28-30)	1/120 (1/40-1/160)	.496 (.406-.677)
CONTROL X MONTERIA	4	—	—	—	40.1 (39.3-40.6)	26.25 (24-30)	—	2×10^{10} Monteria	3.75 (3-6)	0.55 (0.17-1.15)	40.3 (39.9-40.7)	19.50 (15-21)	1/85 (1/20-1/160)	.679 (.156-.792)

[1] APPROXIMATE NUMBER OF ORGANISMS

[2] PACKED CELL VOLUME

[3] COMPLEMENT FIXATION

[4] + POSITIVE PARASITEMIA <0.01%

[5] MEAN AND RANGE OF PARAMETERS

TABLE V

Relative Severity of *Babesia bigemina* Isolates in Intact Calves Prior to Challenge

Isolate	No. of Calves	Average Prepatent Period (days)	Average Maximum Parasitemia (%)	Average Maximum Temperature (°C)	Average Minimum PCV (%)	Average Maximum CF Titer (log mean)	Average Daily Weight Gain (Gm./day)
Llanos	8	4.9 ±0.6	0.49 ±0.31	40.6 ±0.4	19.6 ±1.1	1:98	1000 ±350
Monteria	7	5.9 ±0.7	2.33 ±3.50	41.0 ±0.9	18.0 ±4.5	1:87	730 ±650
Significance		P<0.02	NS	NS	NS	NS	NS

±: Standard deviation from the mean

NS: Not significant

DISCUSSION

The presence of Eperythrozoon spp. in both intact and splenectomized cattle was a cause for concern;[24,33] however, parasitemias of Babesia spp. appeared simultaneously with that of Eperythrozoon spp. The influence of Eperythrozoon spp. on the level of the parasitemia or prepatent period of Babesia is unknown. The Babesia parasitemias in this study did not appear to be influenced by the presence of Eperythrozoon spp. Treatment with Spirotrypanfuerte eliminated a demonstrable parasitemia within 24 hours on nearly every occasion it was used. However, blood from treated calves was infective when inoculated into other calves.

The parasitic period of B. microplus agrees very closely with that found in Australia.[31] The ovipositing and incubation environment of 28 to 30° C. with a 70 to 80% RH seemed to be quite satisfactory, because it was impossible to achieve the higher humidities considered optimal elsewhere.[12,32] It was also impossible to achieve the low temperatures reported as being ideal for survival of larvae;[32] however, larvae kept in the dark at 24 to 28° C. with a 60 to 80% RH survived for 3 months. Lower humidities probably helped in curtailing the problem of fungal growth on eggs and larvae.[y]

The observation was made that ticks singularly infected with Babesia had a higher percentage of infection when infected via a tick-cow-tick rather than blood-cow-tick transmission. This

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[y]Wright, J. E., USDA Vet. Tox. & Ento. Res. Lab., College Station, Texas: Pers. Comm., 1971.

finding supports previous observations on the infectivity of blood for ticks.[54,61] The maintenance of infected ticks on splenectomized calves resulted in a higher percentage of infected ticks than those maintained on intact calves.

Combination thick and thin blood films were a useful diagnostic tool;[6,35,46] it was possible to detect parasitemias 1 to 2 days earlier by examination of thick blood films. The thin blood film provided excellent visualization of parasites; however, it was not always possible to differentiate Babesia species by morphology alone.[34]

The use of a weight tape to estimate weight allowed considerable chance for error; however, its use on a rather homogenous group of cattle (age, sex, breed) improved relative accuracy. One advantage the use of the weight tape had was that it was not necessary to withhold feed and water to establish shrink.

The technique of cerebral biopsy was quite satisfactory and one of the most useful techniques in the diagnosis of B. argentina infections.[34,35] The use of cerebral or renal impressions were useful adjuncts in necropsy procedures in cattle with suspected B. argentina infections.

Examination of hemolymph from Boophilus ticks was simple and identification of Babesia spp. was relatively easy; however, negative findings were of little value.[45,59,60,64]

The infective particles of B. argentina apparently must be

excreted from the larval salivary glands.[61] It appears that vermiculi are not infective for cattle, and evidence to support this concept might be gained by harvesting vermiculi from hemolymph. The vermiculi present in hemolymph would be intact and not disrupted as many in this experiment apparently were. Silicone coated capillary tubes with an anticoagulant might facilitate this approach.

Experiments using B. bigemina rather than B. argentina may be more rewarding, because the percentage of infected female ticks, eggs and larvae seemed to be significantly higher.[45] Because B. bigemina is transmitted at the nymphal rather than the larval stage, as with B. argentina, dissection rather than disruption techniques might be used to harvest infective particles.

Failure to infect the cattle was possibly due to collecting insufficient numbers of mature infective particles.[61] Serial collections of larvae from 30 to 60 hours post release might give some insight as to the optimum time of collection.

Previous attempts to transmit Babesia spp. with tick tissues involved the engorged female which was in fact only a package for the transportation of bovine blood.[23,66,77] Tick borne diseases have been successfully transmitted by disruption of the tick and injection of the resulting material into susceptible hosts.[51,70] Of the agents transmitted by this method, Theileria spp. are the most similar to Babesia. Adult ticks are disrupted to infect

cattle with Theileria spp.[17,47,76] The relative amount of chitin is less with adult ticks than larvae, and the size of the salivary glands is larger in adults. The number of infective particles necessary to establish an infection in cattle may be greater with Babesia than with Theileria.

No significant differences in the virulence of the 2 isolates of B. bigemina could be ascertained, with the exception of the prepatent period which was shorter with the Llanos isolate (Table V). Because samples were taken at 24 hour intervals the prepatent period should not be considered to be of great value in assessing differences between isolates.

The minimal infective dose of B. bigemina for cattle was calculated as 10^3 organisms.[8] Data obtained in other experiments indicated that 2.55×10^4 B. bigemina were an infective dose but 9.5×10^4 organisms were not.[38] This experiment utilized several donor cattle and the recipients were of varying ages, so a minimal infective dose could vary with the source of organisms or with variations in the cattle receiving the inocula. The Australian vaccine for B. argentina contains 10^7 to 10^8 organisms to insure its infectivity for cattle. This vaccine is calculated to lose one log of infectivity per week of storage at 1 to 5° C.[14] The loss of infectivity for frozen B. bigemina is not known, but the stress on the erythrocytes and organisms by preparation, freezing and thawing surely would be significant. Therefore, 10^9 organisms

were arbitrarily decided as a dose which would insure infectivity to most susceptible cattle and would probably not kill.

The challenge dose of 2 X 10^{10} organisms was arbitrarily chosen as likely to be high enough to overcome natural resistance and to cause disease in control and non-protected cattle. The reason for the generally mild response to the challenge by the control calves is unknown. The literature indicates there is a direct relationship between inoculation dose and prepatent period,[38] and the severity of the clinical signs.[15,25,38] The febrile response of unknown origin at the time of the primary inoculation may have made the Babesia seem more virulent. The virulence of the smaller dose may have been due to the volume of the containers used in preservation. Stabilates used for the initial inoculation were stored in 10 ml. bottles, the challenge dose in 60 ml. containers. The contents of the smaller containers may have frozen and thawed more rapidly, causing less damage to the organisms, so that more viable Babesia were inoculated with the smaller volume.[3]

The severity of the disease, as a result of the premunizing inoculation, on this group of well fed and housed calves was such that death losses due to B. bigemina in field stressed calves might be expected with the dose given. In all likelihood the experimental calf with a 10% PCV would have succumbed under field conditions.

One calf did not become infected with the premunizing inoculation but later proved to be fully susceptible. The injection of

the stabilate into this and all of the calves was carefully done. One possible explanation is that the stabilate was deposited into an area which did not absorb the stabilate into the body rapidly. Extra-erythrocytic Babesia probably do not survive long.[33] The injection of only 1 ml. of stabilate increases the likelihood of an error being made in the inoculation. A minimum dosage of 2 ml. using normal bovine tissue fluids as a diluent should reduce possible vaccination mistakes.[14]

Thick blood films, used to determine parasitemia, are more sensitive than thin blood films.[6]

Packed cell volume seemed to be the single best criteria for observing the progress of the B. bigemina infection. The usual pattern of PCV during an infection was that of a slight increase at the onset of parasitemia, then a rapid decrease followed by a slow steady recovery. Parasitemias following challenge were not accompanied by the same decrease in PCV as occurred during the initial infection and some PCV continued to increase despite an increase in parasitemia.

No demonstrable differences were detected in previously premunized cattle to both homologous and heterologous challenges (Figures 9, 10 & 11; Tables IV & VI). Strain differences in Babesia spp. are reported[18,19,29,51] with varying degrees of resistance to heterologous challenge.[9,30,36,61] Callow[7,8] indicated that cattle premunized to B. bigemina were resistant to reinfection to either

a homologous or heterologous strain. While in cattle challenged after loss of infection, immunity was more pronounced with homologous than heterologous strains of Babesia. The method of determining the presence or absence of Babesia organisms was by splenectomy or by the passage of blood to susceptible calves.[8] Determination of the presence or absence of Babesia spp. in cattle by the passage of blood, even in large quantities, may be misleading. In some instances infections have been detected in cattle apparently free of infection by this method.[43] This finding makes it very difficult to state with absolute certainty that a cow who has at any time been exposed to Babesia is now free of infection.

Six of the 15 calves challenged in this experiment did not have a detectable parasitemia at the time of challenge. No differences were detected between these calves and those who were challenged when a parasitemia was present (Table VII). Challenge 6 months after the initial inoculation may have shown differences in the isolates not apparent at 1 month.

Response to challenge was based on the criteria recorded; PCV, parasitemia, temperature and CF titers as well as clinical evaluation, i.e. anorexia, lethargy, hemoglobinuria etc. On the basis of response to challenge (Table VI), it would seem that cattle premunized with B. bigemina could be moved from the Monteria area of the Caribbean coast to the Carimagua area of the Llanos with little danger of dying as a result of B. bigemina infection acquired

TABLE VII

Comparative Response to Challenge of Calves
With and Without Parasitemia on Day of Challenge

Group	No. of Calves	Average Maximum Parasitemia (%)	Average Maximum Temperature (°C)	Average Minimum PCV (%)	Average Maximum CF Titer (log mean)
With Parasitemia	9	0.09 ±0.12	39.1 ±0.2	26.9 ±3.7	1:80
Without Parasitemia	6	0.03 ±0.04	39.0 ±0.2	26.7 ±1.2	1:80
Significance		NS	NS	NS	NS

±: Standard deviation from the mean

NS: Not significant

in the area to which they were moved.

Cross immunity was demonstrated in this experiment (Tables IV & VI), as shown by the mild response to the homologous and heterologous challenges. The control calves showed a significant response to the challenge inoculum, as far as temperature and PCV were concerned. No significant differences were seen with parasitemias; this may have been due to concurrent challenge parasitemias and recrudescence of the premunizing parasitemia occurring in the

homologous and heterologous groups.

The response to the premunizing inoculation was considered to be too severe for field use. A smaller number of organisms to establish premunition would probably be sufficient to cause infection.

SUMMARY

Experiments were conducted at the Centro Internacional de Agricultura Tropical (CIAT) El Porvenir research station, Palmira, Valle, Colombia using Holstein-Friesian calves obtained in *Boophilus* free areas. The cattle were maintained in such a way as to eliminate chances of natural tick infestation.

Colonies of *Boophilus microplus* infected with *Babesia argentina* were established. Attempts were made to transmit *B. argentina* to susceptible calves by artificial means using progeny of infected ticks. Tick eggs, unfed larvae, larvae which had fed for 30 hours or 48 hours, and larvae and nymphs which had fed for 6 days on non-infected calves were used in these experiments. The eggs, larvae and nymphs were macerated using a mortar with pestle and ground glass fragments or a Ten Broeck tissue grinder. Glassware was siliconized for several trials. The resulting supernatant fluid was injected into splenectomized calves subcutaneously. None of the calves showed indications of infection and proved fully susceptible when challenged with *B. argentina*.

Immunologic and pathogenic characteristics of a *Babesia bigemina* isolated from the eastern Colombian Llanos were compared with an isolate obtained near Monteria, Cordoba on the Caribbean coast. Eight calves were inoculated with the Llanos isolate and a second group of 8 calves was inoculated with the Monteria isolate; a third group of 8 calves remained as untreated controls.

Twenty-eight days later, 4 calves in each of the three groups were challenged with the Llanos isolate and the other 4 calves of each group were challenged with the Monteria isolate. Parameters recorded were rectal temperature, PCV, percent parasitemia, CF titers and rate of gain. No significant differences in the pathogenicity of the two isolates were detected. Both homologous and heterologous challenge groups demonstrated a significant resistance to challenge when compared to the response of control calves. No differences could be ascertained in the response of Babesia carrier calves to challenge by the Llanos or Monteria isolates.

REFERENCES

1. Adams, L. G., Craig, T. M., Platt, K. B., and Wyss, J. H.: Eperythrozoonosis in Colombia. Revista ICA, (1973): in press.

2. Anon: A Manual for Conducting the CF Test for Anaplasmosis. Lab Services ADE, ARS, USDA, Washington, D.C., (1958).

3. Barnett, S. F.: The Preservation of Babesia bigemina, Anaplasma centrale and A. marginale by Deep Freezing. Vet. Rec., 76, (1964): 4-8.

4. Bazurto, G. R.: Bovine Premunition in Bullfight Breed Cattle. Proc. 5th Panamerican Cong. Vet. Med. Zootech., Caracas, (1966): 212-217; abstr. in Vet. Bull., 38, (1968): 4502.

5. Bishop, J. P.: Immune Response of Cattle Inoculated With Irradiated Babesia bigemina. Ph.D. Dissertation, Texas A&M University, (1972).

6. Bishop, J. P., and Adams, L. G.: Combination Thin and Thick Blood Films for the Detection of Babesia Parasitemia. Am. J. Vet. Res., 34, (1973): 1213-1214.

7. Callow, L. L.: Strain Immunity in Babesiosis. Nature, 204, (1964): 1213-1214.

8. Callow, L. L.: Sterile Immunity, Coinfectious Immunity and Strain Differences in Babesia bigemina Infections. Parasitol., 57, (1967): 455-465.

9. Callow, L. L.: A Note on Homologous Strain Immunity in Babesia argentina Infections. Austral. Vet. J., 44, (1968): 268-269.

10. Callow, L. L.: The Infection of Boophilus microplus with Babesia bigemina. Parasitol., 58, (1968): 663-670.

11. Callow, L. L., and Hoyte, H. M. D.: The Separation of Babesia bigemina from B. argentina and Theilera mutans. Austral. Vet. J., 37, (1961): 66-70.

12. Callow, L. L., and Hoyte, H. M. D.: Transmission Experiments Using Babesia bigemina, Theilera mutans, Borrelia sp. and the Cattle Tick Boophilus microplus. Austral. Vet. J., 37, (1961): 381-390.

13. Callow, L. L., and Parker, R. J.: Cortisone-Induced Relapses in Babesia argentina Infections of Cattle. Austral. Vet. J., 45, (1969): 103-104.

14. Callow, L. L., and Mellors, L. T.: A New Vaccine for Babesia argentina Infection Prepared in Splenectomized Calves. Austral. Vet. J., 42, (1966): 464-465.

15. Callow, L. L., and Tammemagi, L.: Vaccination Against Bovine Babesiosis. Infectivity and Virulence of Blood from Animals Either Recovered or Reacting to Babesia argentina. Austral. Vet. J., 43, (1967): 249-256.

16. Cordier, G., and Menager, J.: Four Years of Experience of Premunition Against Piroplasmosis in Tunis. Pub. Serv. Elevage Tunis, (1935); abstr. in Vet. Bull., 6, (1936): 505.

17. Cunningham, M., Brown, C. G. D., Burridge, M., Irvin, A., and Kimber, C.: Personal comm., FAO Tick Borne Disease Project, East African Research Org., Muguga, P. O. Kabete, Kenya, (1971).

18. Curnow, J. A.: In Vitro Agglutination of Bovine Erythrocytes Infected With Babesia argentina. Nature, 217, (1968): 267-268.

19. Dalgliesh, R. J.: Field Observations on Babesia argentina Vaccination in Queensland. Austral. Vet. J., 44, (1968): 103-104.

20. Dalgliesh, R. J.: Dimethyl Sulphoxide in the Low-Temperature Preservation of Babesia bigemina. Res. Vet. Sci., 12, (1971): 469-471.

21. Dalgliesh, R. J.: Theoretical and Practical Aspects of Freezing Parasitic Protozoa. Austral. Vet. J., 48, (1972): 233-239.

22. Dennis, R. A., O'Hara, P. J., Young, M. F., and Dorris, K. D.: Neonatal Immunohemolytic Anemia and Icterus of Calves. J.A.V.M.A., 156, (1970): 1861-1869.

23. Dodson, W. R.: Immunization Against Texas Fever by Blood Inoculation. II. Ticks as a Source of Blood for Inoculating Cattle to Produce Immunity. Bull. Ag. Exp. Sta. Louisiana, 57, (1899): 141-185.

24. Foote, L. E., Levy, H. E., Torbert, B. J., and Oglesby, W. T.: Interference Between Anaplasmosis and Eperythrozoonosis in Splenectomized Cattle. Am. J. Vet. Res., 18, (1957): 556-559.

25. Francis, M., and Connaway, J. W.: Texas Fever. Texas Ag. Exp. Sta. Bull., 53, (1899): 55-106.

26. Frerichs, W. M., Johnson, A. J., and Holbrook, A. A.: Storage of Babesia caballi and Babesia equi in Liquid Nitrogen. J. Parasitol., 54, (1968): 541.

27. Gregson, J. D.: Ticks. Insect Colonization and Mass Production. Smith, C. N., ed. Academic Press, New York & London, (1966): 49-72.

28. Grillo Torrado, J. M., and Gutierrez, R. O.: Serological Response of Cattle Inoculated With Suspensions of Developing Forms of Boophilus microplus and its Immunological Interpretation. Revista Invest. Agropec. Ser. 4, no. 5, (1967): 45-64; abstr. in Vet. Bull., 38, (1968): 1454.

29. Hall, W. T. K.: The Immunity of Calves to Tick-Transmitted Babesia argentina Infection. Austral. Vet. J., 39, (1963): 386-389.

30. Hall, W. T. K., Tammemagi, L., and Johnston, L. A. Y.: Bovine Babesiosis: The Immunity of Calves Babesia bigemina Infection. Austral. Vet. J., 44, (1968): 259-264.

31. Hitchcock, L. F.: Studies on the Parasitic Stages of the Cattle Tick Boophilus microplus (Canestrini) (Acarina:Ixodidae). Austral. J. Zool., 3, (1955): 145-155.

32. Hitchcock, L. F.: Studies of the Non-Parasitic Stages of the Cattle Tick Boophilus microplus (Canestrini) (Acarina: Ixodidae). Austral. J. Zool., 3, (1955): 295-311.

33. Hoyte, H. M. D.: Initial Development of Infections With Babesia bigemina. J. Protozool., 8, (1961): 462-466.

34. Hoyte, H. M. D.: Differential Diagnosis of Babesia argentina and Babesia bigemina Infections in Cattle Using Thin Blood Smears and Brain Smears. Austral. Vet. J., 47, (1971): 248-250.

35. Johnston, L. A. Y., and Callow, L. L.: Intracerebral Inoculation and Brain Biopsy in Cattle. Austral. Vet. J., 39, (1963): 22-24.

36. Johnston, L. A. Y., and Tammemagi, L.: Bovine Babesiosis: Duration of Latent Infection and Immunity to Babesia argentina. Austral. Vet. J., 45, (1969): 445-449.

37. Kemron, A., Pipano, E., Hadani, A., Neuman, M., and Egyed, M.: Studies on Bovine Piroplasmosis Caused by Babesia bigemina. I. The Enhancement of the Virulence of Babesia bigemina by Serial Passage in Non-Splenectomized Calves Using Blood Collected at the Acute Stage of the Disease. Refuah Vet., 19, (1962): 124-122.

38. Kemron, A., Hadani, A., Egyed, M., Pipano, E., and Neuman, M.: 'Studies on Bovine Piroplasmosis Caused by Babesia bigemina. III. The Relationship Between the Number of Parasites in the Inoculum and the Severity of the Response. Refuah Vet., 21, (1966): 112-108.

39. Langford, G., Knott, S. G., Dimmock, C. K., and Derrington, P.: Haemolytic Disease of Newborn Calves in a Dairy Herd in Queensland. Austral. Vet. J., 47, (1971): 1-4.

40. Lumsden, W. H. R., and Wells, E. A.: Infectious Blood Diseases of Man and Animals. Vol. II. Weinman, D. and Ristic, M., eds. Academic Press, New York, (1968): 341.

41. Maekelt, G. A.: Immunity Mechanisms to Protozoa. Biology of Parasites. Soulsby, E. J. L., ed. Academic Press, New York, (1966): 321-334.

42. Mahoney, D. F.: Bovine Babesiosis: Diagnosis of Infection by a Complement Fixation Test. Austral. Vet. J., 38, (1962): 48-52.

43. Mahoney, D. F.: Bovine Babesiosis: An Assessment of the Significance of Complement Fixing Antibody Based Upon Experimental Infection. Austral. Vet. J., 40, (1964): 369-375.

44. Mahoney, D. F.: Bovine Babesiosis: Preparation and Assessment of Complement Fixing Antigens. Exptl. Parasitol., 20, (1967): 232-241.

45. Mahoney, D. F., and Mirre, G. B.: Bovine Babesiosis: Estimation of Infection Rates in the Tick Vector Boophilus microplus (Canestrini). Ann. Trop. Med. Parasitol., 65, (1971): 309-317.

46. Mahoney, D. F., and Saal, J. R.: Bovine Babesiosis: Thick Blood Films for the Detection of Parasitemia. Austral. Vet. J., 37, (1961): 44-47.

47. Mazlum, Z.: Transmission of Theileria annulata by the Crushed Infected Unfed Hyalomma dromedarii. Parasitol., 59, (1969): 597-600.

48. Maurer, F. D., Newberne, P. M., and Poppensiek, G. C.: Protein for Tomorrows Faces of Hunger. Cong. Rec., 116, (1970): 169.

49. Meehan, J. F.: Field Proof for Tick Fever Vaccine. Qd. Agric. J., 95, (1969): 768-770.

50. Miessner, H.: Piroplasmosis and Splenectomy. Arch. Wiss. Prakt. Tierhlk., 63, (1931): 78-90; abstr. in Vet. Bull., 1, (1931): 210.

51. Neitz, W. O.: A Consolidation of Our Knowledge of the Transmission of Tick-Borne Diseases. Onderstpt. J. Vet. Res., 27, (1956): 115-163.

52. Neitz, W. O.: Observations on the Duration of Premunity Following Administration of the Bivalent Redwater Vaccine. J. So. Afric. Vet. Med. Assoc., 40, (1969): 419-420.

53. O'Kelly, J. C., and Seifert, G. W.: The Effects of Tick (Boophilus microplus) Infestations on the Blood Composition of Shorthorn X Hereford Cattle on High and Low Planes of Nutrition. Austral. J. Biol. Sci., 23, (1970): 681-690.

54. O'Sullivan, P. J., and Callow, L. L.: Loss of Infectivity of a Vaccine Strain of Babesia argentina for Boophilus microplus. Austral. Vet. J., 42, (1966): 252-254.

55. Pipano, E., and Senft, Z.: Storage of Babesia bigemina, Babesiella berbera, Anaplasma marginale and Anaplasma centrale in the Deep Freeze. J. Protozool., 13 Supp., (1966): 34.

56. Purnell, R. E.: Infection of the Tick Rhipicephalus appendiculatus with Theileria parva Using an Artificial Feeding Technique. Res. Vet. Sci., 11, (1970): 403-405.

57. Riek, R. F.: Studies on the Reactions of Animals to Infestation With Ticks. VI. Resistance of Cattle to Infestation With the Tick Boophilus microplus (Canestrini). Austral. J. Ag. Res., 12, (1961): 532-549.

58. Riek, R. F.: Bovine Babesiosis. Appendix F, Report 2nd Meeting FAO/OIE Expert Panel on Tick-Borne Diseases of Livestock, (1962): 37-40.

59. Riek, R. F.: The Life Cycle of Babesia bigemina (Smith & Kilbourne, 1893) in the Tick Vector Boophilus microplus (Canestrini). Austral. J. Ag. Res., 15, (1964): 802-821.

60. Riek, R. F.: The Life Cycle of Babesia argentina and B. bigemina in Boophilus microplus. Proc. 2nd Int. Cong. Acarology, (1967): 625.

61. Riek, R. F.: Babesiosis: Infectious Blood Diseases of Man and Animals. Vol. II. Weinman, D. and Ristic, M., eds. Academic Press, New York, (1968): 219-268.

62. Ristic, M.: Anaplasmosis: Infectious Blood Diseases of Man and Animals. Vol. II. Weinman, D. and Ristic, M., eds. Academic Press, New York, (1968): 473-542.

63. Roberts, F. H. S.: The Taxonomic Status of the Species of the Genera Rhipicephalus Kock and Boophilus Curtice (Acarina: Ixodidae) Occurring in Australia. Austral. J. Zool., 13, (1965): 491-523.

64. Rogers, R. J.: The Acquired Resistance to Babesia argentina of Cattle Exposed to Light Infestation With Cattle Tick (Boophilus microplus). Austral. Vet. J., 47, (1971): 237-241.

65. Schuntner, C. A., and Tatchell, R. J.: Drinking by Larval Cattle Ticks Boophilus microplus (Acarina:Ixodidae). J. Parasitol., 56, (1970): 1239-1247.

66. Smith, T., and Kilborne, F. L.: Texas Fever. Bur. Ani. Ind. Bull., 1, (1893): 7-114.

67. Smith, T., Kilborne, F. L., and Schroeder, E. C.: Additional Observations on Texas Cattle Fever. Bur. Ani. Ind. Bull., 3, (1893): 67-72.

68. Snedecor, G. W.: Statistical Methods. 5th ed., Iowa State Press, Ames, Iowa, (1956).

69. Tchernomoretz, I.: Blocking of the Brain Capillaries by Parasitized Red Blood-Cells in Babesiella berbera Infections in Cattle. Ann. Trop. Med. Parasitol., 37, (1943): 77-79.

70. Theiler, A., and DuToit, P. J.: The Transmission of Tick-Borne Diseases by the Intrajugular Injection of the Emulsified Intermediary Host Itself. 13th and 14th Director Vet. Educ. Res. Report Union So. Africa, (1928): 17-44.

71. Tsur, I.: Immunization Trials Against Bovine Babesiosis. I. Vaccination With Blood From Latent Carriers. Refuah Vet., 18, (1961): 110-103.

72. Vanderberg, J. P., Nussenweig, R. S., and Most, H.: Further Studies on the Plasmodium berghei-Anopheles stephensi-Rodent System of Mammalian Malaria. J. Parasitol., 54, (1968): 1009-1016.

73. Vizcaino, O. G.: Immunoserological Characterization of the Antigens of Babesia bigemina and Babesia argentina by Complement Fixation, Gel Precipitation and Immunoelectrophoresis. Masters Thesis, National University of Colombia, Bogota, (1973).

74. Waddell, A. H.: Deep Freeze Storage of Babesia bigemina. Austral. Vet. J., 39, (1963): 400.

75. Walker, J.: Some Observations in Connection With the Immunization of Cattle Against South African Redwater and Genuine Gallsickness (Anaplasmosis). Report So. Afr., Dept. Ag., Div. Vet. Res., 3-4, (1915): 503-526.

76. Wilde, J. K. H., Brown, C. G. D., Hulliger, L., Gall, D., and MacLeod, W. G.: East Coast Fever: Experiments With the Tissues of Infected Ticks. Brit. Vet. J., 124, (1968): 196-207.

77. Yakimoff, W. L., Markoff-Pétraschewsky, E. N., Loukanoff, W. L., Woitzekhowsky, M., Rastegaieff, E. F., and Gniedine, A. N.: Immunization Against Babesiellosis in Bovines. Ann. Inst. Pasteur, 42, (1928): 282-319.

VITA

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