Bovine Babesiasis: Its Diagnosis and Control

R. A. Todorovic, DVM, PhD

SUMMARY

The investigation was conducted to develop new systems and to evaluate existing ones for diagnosis and control of bovine babesiasis in Colombia, South America. Antigens of *Babesia bigemina* and *Babesia argentina* were isolated and were used in complement-fixation (CF) and rapid agglutination (RA) tests for the diagnosis of babesiasis in calves.

Three systems were evaluated for control of bovine babesiasis: (1) vaccination of calves with killed *Babesia* spp vaccine to produce resistance (based on sterile immunity), (2) premunition of calves with virulent *Babesia* spp and then administration of a chemotherapeutic drug to produce resistance (based on coinfectious immunity), and (3) chemoprophylaxis, using a babesiacide having long residual activity. The 3 systems controlled bovine babesiasis under the conditions of the present experiments.

Epizootiologic conditions in enzootic areas, however, will indicate which system is preferable. In zones having a high population of the tick *Boophilus microplus*, premunition (system 2) is indicated; in areas where the tick population is controlled or in areas where cattle are at constant risk of tick exposure, vaccination with killed *Babesia* spp (system 1) or chemoprophylaxis (system 3) are indicated.

Although bovine babesiasis has been recognized as a serious economic disease for more than 80 years,[2] practicable methods for diagnosis and control programs have not yet been obtained.[35] This hemoprotozoan malady causes considerable production and death losses of cattle, especially in lowlands of tropical zones of the world where there are ticks.[34,37,a] Identification of acutely infected cattle has not presented a problem because *Babesia* spp can be readily detected in Giemsa-stained blood films.[10,31] However, if cattle are latently infected, diagnosis presents a difficult problem since samples of blood from these animals do not contain enough *Babesia* spp parasites upon which to base the diagnosis.[31,34,35] Therefore, interest has been directed toward developing serodiagnostic techniques.

In the last decade, several serologic tests have been developed for the detection of specific *Babesia* spp antibodies in infected cattle: CF,[25,27,48] hemagglutination,[13,18] capillary[24] and slide agglutination,[12] fluorescent antibody,[4,5,14,16,21,38,49] and precipitation in gel.[48] These tests showed a high degree of specificity for each *Babesia* spp tested, and due to the sensitivity differences of each technique, antibodies can be detected for various periods after infection, depending on the test used.[30] The CF test, which has the lowest sensitivity, may reliably detect antibodies to *B argentina* for several months and to *B bigemina* for 4 months in cattle after a single infection.[30] With indirect fluorescent antibody and passive hemagglutination techniques, serodiagnostic periods in excess of a year have been reported.[18,20,38] Because reinfection will occur under natural field conditions, detectable antibodies should almost always be present after the first infection has been established.[30] All of these techniques are laboratory tests; they are tedious to perform, and considerable skill and equipment are required. Therefore, there is an evident need for a rapid, simple serologic test which can be applied under field and laboratory conditions for the diagnosis of bovine babesiasis.

Control of bovine babesiasis remains a problem, especially in the tropical areas of the world where this blood disease is most prevalent.[11,17,30,32,33,34,37,a] Since the natural transmission of *Babesia* spp is dependent on certain species of ticks, infection can be prevented by maintaining an adequate tick control program to keep cattle free from tick infestation.[34,36] This can be accomplished by regular dipping of cattle, a method which has resulted in the eradication of bovine babesiasis in the United States.[32] However, in the lowlands of subtropical and tropical areas of the world, a vector control program as a measure of *Babesia* spp control, while appealing, is virtually impossible to maintain, for various reasons.[45] Since ticks occur abundantly in permanent grasslands of the tropics and they feed regularly on both wild and domesticated animals, it is difficult to eradicate them. At present, in countries where a tick control program is not possible to maintain, the only measure used to prevent production and death losses and to control the disease is a vaccination or a chemoprophylactic program.[6,10,26,29,34,43,44,46,47]

A series of investigations were undertaken to test and evaluate 3 control systems for bovine babesiasis in tropical zones of Colombia, South America, where babesiasis is most prevalent. These systems included (1)

Received for publication Sept 19, 1973.

From the Institute of Tropical Veterinary Medicine and the Department of Microbiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843.

Presented at the Section on Hemotropic Diseases, 7th Panamerican Congress of Veterinary Medicine and Zootechnics, Bogotá, Colombia, July 23-28, 1973.

Acknowledgment is made of the assistance given by the Rockefeller Foundation, the US Agency for International Development, the Centro Internacional de Agricultura Tropical, the Instituto Colombiano Agropecuario (ICA), and Texas A&M University.

[a] Todorovic, R. A., Adams, L. G., and Roberts, E. D.: A Study of Bovine Babesiosis in Colombia, South America (abstr). JAVMA, 154, (June 1, 1969): 1399.

vaccination of susceptible calves with killed *Babesia* spp vaccine to produce a resistance to the disease (based on sterile immunity)[7,26,28,29] and (2) premunition of susceptible calves with virulent *Babesia* spp isolated from cattle acutely infected with these parasites. Postpremunition reactions were controlled by instituting specific chemotherapy. After the calves had recovered from the premunition reactions, they developed a resistance (based on coinfectious immunity).[7,10,11,20,34,40,42]

Additional experiments were directed at the control of bovine babesiasis with chemoprophylaxis (system 3). This system was based on a resistance against natural infection produced by a babesiacide having long residual activity.[9] The protective period lasts for a few weeks, and then the drug concentration gradually decreases to the point when *Babesia* spp can establish a mild infection. As a result of the interaction between drug and *Babesia* spp parasites, chemoprophylactically treated calves develop a resistance against babesiasis based on coinfectious immunity known as premunition.[9,19,47]

This is a report of a more practicable serodiagnostic test for identifying cattle infected with *Babesia* spp and the results of evaluations of 3 systems for controlling bovine babesiasis under tropical conditions in Colombia, South America.

Materials and Methods

Serologic Diagnosis of Bovine Babesiasis—Complement-Fixation (CF) Technique—Serums from experimentally and naturally infected cattle were subjected to the CF test for more than 3 years. These serum samples were obtained from cattle in enzootic areas in the Cauca River Valley, the Magdalena River Valley, the Llanos, and the northern coast of Montería. Also, tests were made of serum samples from cattle having histories of experimentally induced babesiasis at our laboratory. Samples of serum were obtained from cattle before and after tick-borne or blood-borne infections.

Experiments were done to evaluate serologic techniques for detecting *Babesia* spp antibodies in calves and to develop a serodiagnostic test for babesiasis. The CF test used to detect *Babesia* spp antibodies in the serum of naturally and experimentally infected calves was modified from the procedures reported by Mahoney.[25,27] Techniques consisting of isolation, purification, and titration of *B bigemina* and *B argentina* antigens; preparation of the hemolytic system; complement titration; and performance of the test in tubes and in the microtitration system are described in detail by Todorovic and Adams[41] and Todorovic et al.[43]

Rapid Agglutination (RA) Technique—Babesia bigemina and *B argentina* were used for infecting calves and for producing the respective antigens. *Babesia bigemina* and *B argentina* antigens were prepared from blood in which at least 20% of the erythrocytes were infected. High percentages of parasitemia were developed by serial passage of the parasites through 2 to 4 splenectomized calves. To enhance development of parasitemia, 9-α-fluoro-16-α-methylprednisolone[b] (0.5 mg/kg) was injected intramuscularly (IM) for 3 days prior to infection. Following blood passage, thin and thick[31] blood films of peripheral blood were prepared at 3- to 4-hour intervals to determine the percentage of parasitized erythrocytes. When the parasitemia remained constant during successive examinations, the organisms were passed to the next animal. After the 2nd or 3rd passage with *B bigemina* and *B argentina*, as many as 20% of the erythrocytes were parasitized.

Blood for the preparation of antigen was drawn into flasks containing ethylenediaminetetraacetic acid (EDTA; 1.2 g/L) from calves with 20% or more parasitemia. Plasma was removed by centrifugation at 1,085 g for 30 minutes at 4 C. The erythrocytes were washed twice in 0.85% NaCl solution. The last wash was in antibiotic saline solution containing 100 units of penicillin and 100 μg of streptomycin[c]/1 ml of saline solution. Then, 5 volumes of sterile 0.35% NaCl was used to lyse the infected erythrocytes for 1 hour at 4 C.[19,27] The lysate was centrifuged at 1,085 g for 30 minutes and resuspended in an equal volume of antibiotic saline solution. *Babesia* spp suspensions (35 ml) were passed through the French pressure cell[d] at 1,200 psi and centrifuged at 10,000 g at 3 C for 30 minutes. The supernatant fluid was discarded, and the concentrated parasites were suspended in equal amounts of antibiotic solution. Suspensions were homogenized in TenBroeck tissue grinders. Fast green dye[e] (1%) was added at the rate of 0.1 ml/25 ml of parasite suspension; this was mixed by shaking and was passed through the French pressure cell at 1,200 psi. Excess dye was removed from the antigen by decanting the supernatant fluid after centrifugation at 10,000 g for 30 minutes. After centrifugation and decantation of the supernatant fluid, the antigen pellets were suspended in equal volumes of antibiotic saline solution, homogenized in TenBroeck grinders, sonified in a cell disruptor[f] for 3 minutes (using a 70-cycle power source), and tested for antigenic activity. Serial dilutions of antigen and drops of antigen (about 0.025 ml) were mixed with equal amounts of serially diluted serum samples. Reference samples of positive and negative serums for evaluating *Babesia* spp antigen reactivity and stability were obtained from 10 calves in a carrier phase of *Babesia* spp infection and from several normal calves originating from an area free from babesiasis. The test, done in disposable plastic trays, was similar to that used for the diagnosis of brucellosis. In addition, Brewer diagnostic cards[g] were used to mix 2 drops of serum with 1 drop of *Babesia* spp card antigen, and were rotated for 5 minutes.

Vaccination of Cattle with Killed Babesia spp Vaccine (System 1)—Vaccination experiments were done at the Palmira experimental station of the Colombian Institute of Agricultural Research (ICA) in the Cauca River Valley. Blood was collected from a splenectomized infected calf (20% of erythrocytes parasitized with *Babesia* spp) for preparation of killed *Babesia* spp vaccine. Blood was collected in EDTA (used as anticoagulant) by cannulation of the carotid artery. Blood was lyophilized in a freeze dryer[h] in 20-ml portions and stored at −20 C. Before use, the vaccine was restored to 1/3 of the original volume with sterile distilled water, and 5 ml of this solution was injected IM into each calf.

Preparation of the killed *Babesia* spp vaccines was described in detail by Todorovic et al.[43] The degree of protection in 10 vaccinated calves was assessed by packed cell volume (PCV) percentage of parasitemia, antibody titer, body weight, and number of deaths and their comparison with those of a comparable group of 5 nonvaccinated con-

[b] Dexamethasone, Schering Corporation, Bloomfield, NJ.

[c] Gibco Biological Company, Grand Island, NY.

[d] American Instrument Company, Silver Springs, MD.

[e] Fisher Scientific Company, Houston, TX.

[f] Heath Systems Ultrasonic, Inc, Plainview, LI, NY.

[g] Hynson, Westcott and Dunning, Inc, Baltimore, MD.

[h] The Virtis Company, Inc, Gardiner, NY.

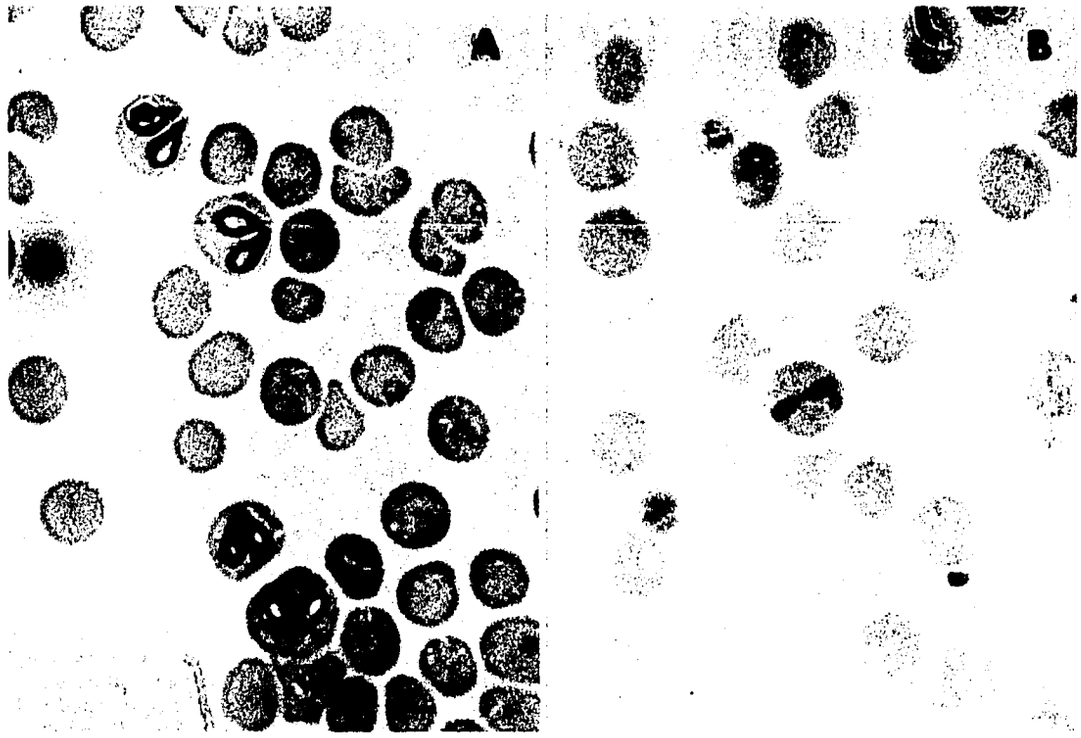

Fig 1—*Babesia bigemina* (A) and *Babesia argentina* (B) isolated from naturally infected cattle in Colombia and used for preparation of antigens for serologic tests and inoculums for premunition. Giemsa stain; × 1,000.

trol calves following field challenge. The field challenge was possible because none of these calves were subjected to any tick control program and thus were exposed to *B microplus*.

Premunition of Cattle with Virulent Babesia spp (System 2)—*Babesia bigemina* and *B argentina* were isolated from naturally infected calves in the Cauca River Valley.[44] These isolates were frozen, using an empirical technique similar to that described by Barnett.[3] The frozen material was thawed rapidly in a water bath (37 C) and passaged once through a splenectomized calf before it was used in premunition experiments. Infection of recently splenectomized calves resulted in 1% *Babesia* spp parasitemia within a mean prepatent period of 4½ days. Control of postpremunition reaction and determination of immune responses to premunition and challenge exposure to infected *B microplus* were described in detail by Todorovic et al[42,44] and Todorovic and Tellez.[46]

Blood (5 ml) containing approximately 10^7 *Babesia* spp parasites/ml was inoculated IM into each of 10 calves. A new experimental compound, imidocarb[1] (3,3′-bis-(2-imidazolin-2-yl) -carbanilide dihydrochloride), at a dosage of 0.75 mg of salt/kg of body weight was injected IM to control postpremunition reaction in the experiment. After all inoculations were done, the calves (5 controls and 10 principals) were examined twice each week for the presence of *B bigemina* and *B argentina* parasitemia by the use of thin and thick[21] blood film technique, PCV,[29] and CF technique.[46] Rectal temperature and body weight changes were also recorded. The immune responses of all calves were studied for 8 months. During field exposure, ticks were collected from all calves, preserved in absolute alcohol, and then identified.

Chemoprophylaxis (System 3)—The experimental compound imidocarb was used; the drug, received as a water-soluble salt, was dissolved in sterilized distilled water as a 10% solution and used within 24 hours after preparation. This solution was administered IM at the dose level of 3 mg/kg of body weight.

A total of 5 calves was used in system 3—4 treated with imidocarb and 1 nontreated control. Four weeks after imidocarb inoculation, the 5 calves were inoculated with 100 ml of blood containing parasitized erythrocytes, 2% *B bigemina* and 0.1% *B argentina*. The prophylactic efficacy of imidocarb was determined by resistance of the calves to artificial and natural challenge exposure to *Babesia* spp.[47] The therapeutic efficacy of imidocarb was determined by recovery of the calves from acute infection after they were treated.[1] Blood (500 ml) from the 4 treated calves was inoculated into susceptible splenectomized calves to determine whether the 4 treated calves had *Babesia* spp infection before and after treatment. Treated calves were observed for signs of drug toxicity within 24 hours after treatment.

Results

Serologic Diagnosis of Bovine Babesiasis—Complement-Fixation Test—By the CF test, specific *Babesia* spp antibodies were detected on the 8th day after infection. *Babesia bigemina* and *B argentina* in blood films stained by Giemsa were observed microscopically 3 to 8 days after experimental infection (Fig 1). The specific *Babesia* spp antibodies were detected an average of 3 to 5 days after the peak of parasitemia was reached. The peak of parasitemia with *Babesia* spp usually coincided with anemia and signs of acute babesiasis. Approximately 8 to 10 days after infection, the first CF titer was detected, and the titer persisted until the calf died. On postinfection day 10, a 2-plus reaction was observed in the microsystem, with a titer of 1:10. In the following 2 days, the sample usually gave a 4-plus reaction with the titer 1:40 (Fig 2). Good agreement existed between the CF reaction and the histories of calves having artificial and natural infections. Approximately 96% of serum samples from cattle having known babesiasis

[1] Imidocarb, Burroughs Wellcome Company, Inc, Raleigh, NC.

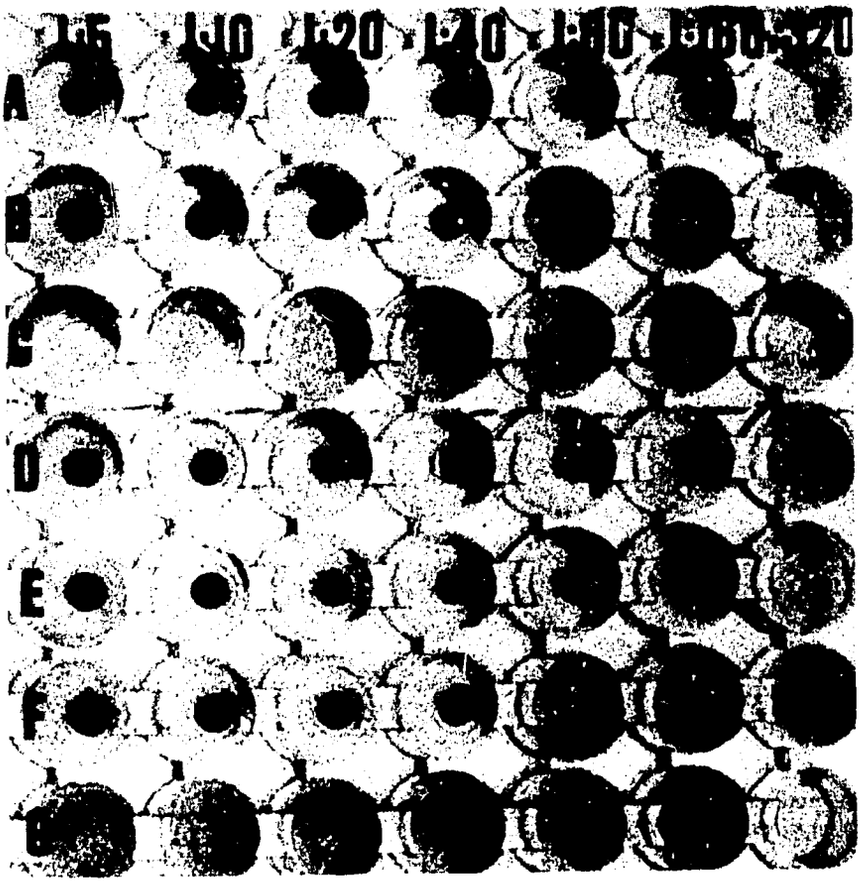

Fig 2—Complement-fixation reaction in microtitration system for detection of specific *Babesia* spp antibodies. Row A contained serum from a cow infected with babesiasis with a titer of 1:160. Rows B and F show a titer of 1:40. Row D shows a titer of 1:320. Row E shows a titer of 1:80. Row C had serum from a noninfected animal. Row G had a known negative serum with a negative reaction.

gave CF reactions; approximately 4% gave discordant reactions. The latter were especially seen in serum samples from several of the carrier calves.

Rapid Agglutination Test—Lysis of *B bigemina*- and *B argentina*-infected erythrocytes with sterile 0.35% NaCl yielded an antigen composed of concentrated *Babesia* spp parasites. After they were passaged through the French pressure cell, *Babesia* spp parasites were ruptured, and the unstained *Babesia* spp antigen was grayish white and free of visible hemoglobin. Fine small particles of *Babesia* spp parasites were stained with fast green dye; this mixture was stable and uniform. Preliminary studies with this antigen revealed that a degree of specific agglutination reaction occurred with the serum from *Babesia* spp-infected cattle (Fig 3). The reaction was visible during a period from 3 to 10 minutes of plate agitation. However, nonspecific agglutination occurred with serums prepared from cattle without history of babesiasis, especially when stored card antigen was used.

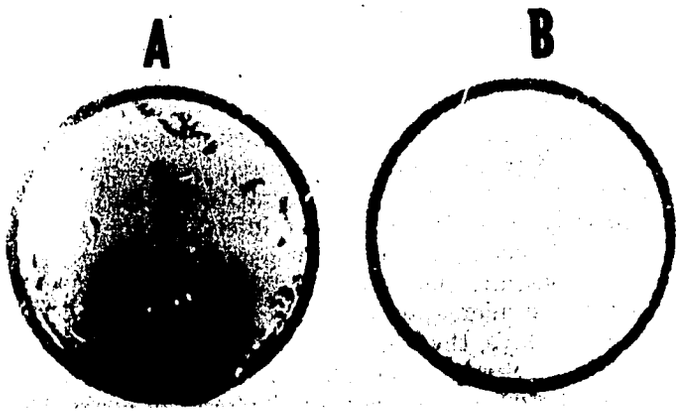

Fig 3—Pattern of agglutination reaction in the rapid agglutination test for diagnosis of bovine babesiasis. Positive (A) and negative (B).

Sterile Immunity (System 1)—Hematologic and serologic responses of calves injected with killed *Babesia* spp vaccine and exposed to infected *B microplus* are shown (Fig 4). The 1st injection of killed *Babesia* spp

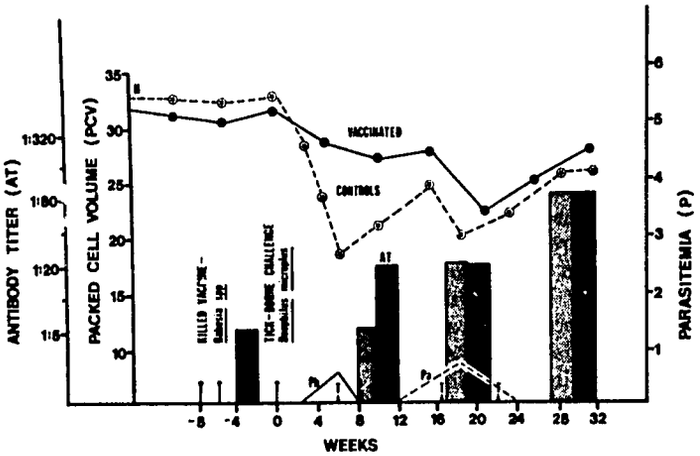

Fig 4—Hematologic and serologic responses of calves injected with killed *Babesia* spp vaccine 8 weeks before tick-borne *(Boophilus microplus)* challenge exposure. Graphic representation of mean values for anemia (PCV = packed cell volume), parasitemia (Pb = *Babesia* spp; Pa = *Anaplasma marginale*), and *Babesia* spp CF antibody titer (AT) and their relationships to the vaccination and the treatment (T).

vaccine is indicated at week −8, and the 2nd injection at week −6. The tick-borne challenge is indicated by an arrow at week 0. Before vaccination, nonvaccinated control calves had PCV of 33% and vaccinated calves had PCV of 32%. After vaccination, PCV values remained in normal limits. The CF titers of 1:5 were detected in all vaccinated calves 4 days after vaccination.

At the time of field exposure to *B microplus*, all calves had approximately the same PCV (33%) values. Resistance to clinical babesiasis in calves given the killed *Babesia* spp vaccine was indicated by negative results of blood examinations for *B bigemina* and *B argentina*.

Nonvaccinated control calves had 0.5% *Babesia* spp parasitemia at 3 to 4 weeks after field exposure. At the time of *Babesia* spp parasitemia, the calves developed anemia with low PCV value of 18% and the calves were given specific treatment against babesiasis to prevent production and death losses. Eleven to 12 weeks after field exposure, all calves (vaccinated and nonvaccinated) developed clinical signs of anaplasmosis evidenced by *Anaplasma marginale* parasitemia and the PCV value decreasing to 20%. At this time all calves were treated with specific chemotherapy against anaplasmosis to prevent death losses. Differences between complement-fixing titers for *Babesia* spp in vaccinated and nonvaccinated calves were not statistically significant. All calves responded with rising CF titers of 1:5 to 1:80, and the titers persisted during the period of observation.

Coinfectious Immunity (System 2)—Hematologic and serologic responses of calves simultaneously injected with blood of calves acutely infected with *B bigemina* and *B argentina* and subsequently treated with imidocarb (0.75 mg/kg) to moderate the postpremunition reaction are shown (Fig 5). All premunized calves re-

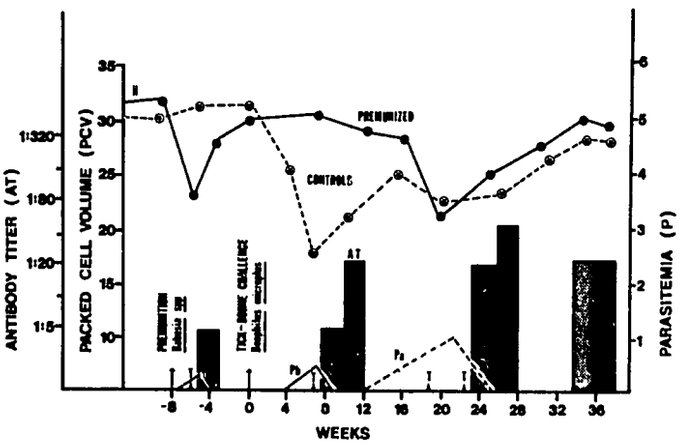

Fig 5—Hematologic and serologic responses of calves premunized with *B bigemina* and *B argentina* and 8 weeks later exposed to tick-borne challenge. Graphic representation of mean values for anemia (PCV = packed cell volume), parasitemia (Pb = *Babesia* spp; Pa = *A marginale*), and *Babesia* spp antibody titer (AT) and their relationships to premunition, field exposure to *Boophilus microplus* ticks, and treatment (T).

sponded severely to blood inoculation. *Babesia bigemina* and *B argentina* were evident on day 4, and a maximum parasitemia of 0.3% was reached on postpremunition day 9. After calves were given imidocarb, *Babesia* spp parasitemia disappeared the day after treatment. During the *Babesia* spp parasitemia, all inoculated calves developed anemia with a PCV of 23%. After drug treatment, all calves recovered from anemia, with PCV values returning to preinfection values of 30%. At the time of field exposure to *B microplus* ticks, all calves (control and premunized) had PCV values of 32%.

After calves were challenge-exposed to *B microplus* ticks naturally infected with *Babesia* spp, all premunized calves had a high degree of resistance to field exposure. *Babesia bigemina* and *B argentina* were not detected in stained thick and thin blood films, and PCV values were in normal limits (28–30%). The nonpremunized control calves developed severe clinical signs of babesiasis at 4 to 8 weeks following field exposure—0.5% *Babesia* spp parasitemia and low PCV values (17%). All infected calves were treated against babesiasis to prevent production and death losses. Twelve weeks following field exposure, all calves (premunized and control) developed clinical anaplasmosis, characterized by *A marginale* parasitemia, and they were given specific chemotherapy against anaplasmosis. Following this treatment, calves recovered from anemia and *A marginale* parasitemia. The CF titers to *Babesia* spp were first detected on postpremunition day 13, and these persisted with increasing levels of 1:20 to 1:80 during the experiment.

Chemoprophylaxis (System 3)—Results of chemoprophylactic treatment with imidocarb against bovine babesiasis and subsequent blood-borne challenge inoculation are shown (Fig 6). Within 3 hours after they

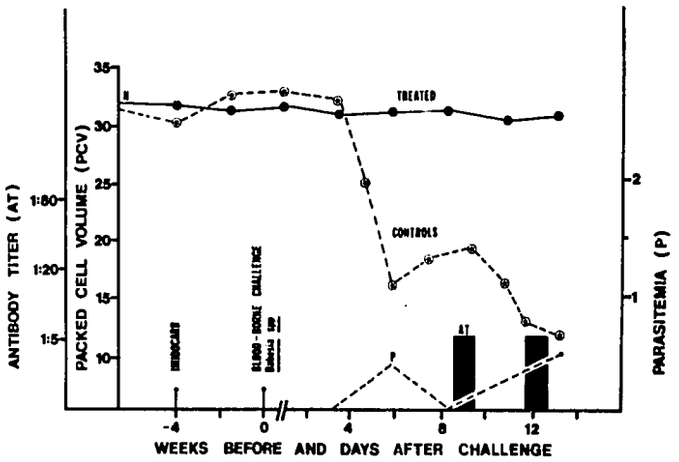

Fig 6—Hematologic responses of calves treated with imidocarb 4 weeks before blood-borne challenge exposure to *B bigemina* and *B argentina*. Graphic representation of anemia (PCV = packed cell volume), parasitemia (P), antibody titer (AT), and their relationships to prophylactic effects of drug and blood-borne challenge exposure.

were treated, all 4 treated calves showed salivation, lacrimation, coughing, and difficulty in breathing. At day 0, or 28 days following drug inoculation, all calves, including the control calf, were inoculated intravenously (IV) with *Babesia* spp-infected blood. At this time all 5 calves had PCV values of 32%. Three days after blood-borne challenge inoculation, the control calf not treated with imidocarb had 0.1% *B bigemina* and on day 5,

TABLE 1—Progress in the Serodiagnosis of Bovine Babesiasis

Author(s)	Serologic technique	Species
Mahoney (1962)[26]	Complement-fixation	*Babesia bigemina; Babesia argentina*
Mahoney (1967)[27]	Complement-fixation	*B bigemina; B argentina*
Curnow and Curnow (1967)[13]	Indirect hemagglutination	*B argentina*
Ross and Lohr (1968)[38]	Capillary agglutination	*B argentina*
Zwart et al (1968)[49]	Indirect fluorescent antibody	*B bigemina; Babesia major*
Lohr and Ross (1969)[24]	Indirect fluorescent antibody	*B bigemina*
Todorovic et al (1971)[48]	Complement fixation	*B bigemina; B argentina*
Goodger (1971)[18]	Indirect hemagglutination	*B bigemina; B argentina*
Brocklesby et al (1971)[4]	Indirect fluorescent antibody	*B major*
Goldman et al (1972)[16]	Fluorescent antibody	*B bigemina; Babesia berbera*
Oonnelly et al (1972)[14]	Indirect fluorescent antibody	*Babesia divergens*
Joyner et al (1972)[21]	Indirect fluorescent antibody	*B divergens; B major*
Burridge et al (1973)[5]	Indirect fluorescent antibody	*B bigemina*
Curnow (1973)[13]	Slide agglutination	*B bigemina*

0.01% *B argentina* parasitemia. *Babesia* spp parasitemia persisted until day 13 when the calf died with signs of acute babesiasis and with low PCV values (12%). The 4 calves treated with imidocarb 28 days before blood-borne challenge inoculation did not have any detectable *Babesia* spp, and their PCV values remained at a 31% level during the period of observations. Only the control calf showed a CF antibody response (a titer of 1:5) on day 8 following challenge with *Babesia* spp. parasitemia.

Discussion

Serologic Diagnosis of Bovine Babesiasis—A characteristic feature of *Babesia* spp infection is that cattle which recover from acute infection become carriers of the respective hemoparasites. In these cattle, diagnosis cannot be made by examining blood films prepared with any of the contemporary staining methods.[17,19,25,27,48] Thus, to identify a carrier animal, serologic methods are needed that will detect specific antibodies rather than the *Babesia* spp parasites themselves (Table 1). The CF technique was one of the earliest tests for the diagnosis of bovine babesiasis.[25] A specific reaction for *Babesia* spp parasites has been found useful in detecting CF antibodies in the serum of cattle with latent babesiasis; however, the practicable application of the CF test was not conclusively demonstrated. The CF antigen used in our laboratory was obtained from the infected erythrocytes of cattle affected with acute babesiasis. Because of the impurities of these antigens, they can be used only in the CF test; under these circumstances, the variables and the difficulties of the CF technique become more pronounced and the dependability of the CF test lessens.[27] Obviously, technical and mechanical errors which are inherent in other CF procedures also apply to our technique. However, when the test is carefully conducted, using an antigen prepared from cattle having high percentages of *B. bigemina* or *B argentina* parasitemia, a minimum of error in test accuracy and specificity should occur.

The preliminary results of the RA test for detection of specific agglutinins in serums of cattle infected with *Babesia* spp are encouraging, and methods of eliminating some inherent problems of specificity of the agglutination reaction are being studied.[12] In adjusting the agglutination reaction to detect a small concentration of *Babesia* spp antibodies, care is taken so that the agglutinating antigen is not overly sensitive, causing it to be reactive with serum samples from noninfected cattle. Studies are underway to better evaluate the methods of isolation of the card agglutinating antigen as well as to determine specificity of the card agglutination reaction. In addition, a problem with the preservation of *Babesia* spp agglutination antigen was found, and different techniques for its preservation are being tested.

Methods devised to date for serologic diagnosis of bovine babesiasis obviously remain imperfect. Improvements must be sought and serologic procedures standardized. Nevertheless, the admittedly imperfect techniques reported by several investigators (Table 1) now available are already capable of diagnosing bovine babesiasis. As a result of the investigations and observations by others,[30] it is apparent that more research is needed for the perfection of a serodiagnostic technique for bovine babesiasis.

Sterile Immunity—The theoretical concept for these experiments was based on the possibility that sterile immunity might be induced.[7,15] Although sterile immunity is not usually encountered in blood protozoan infections, it does occur naturally in *Theileria parva* infections.[32] Mahoney[28] reported that the species-specific babesia antigen required for cross-protection immunity between different strains of *Babesia* spp parasites was found in the infective plasma during the acute phase of infection. Todorovic et al[43] found that different *Babesia* spp preparations had different immunogenic properties. Protection was more evident in cattle injected with a vaccine prepared from plasma rather than from infected erythrocytes. Results of experiments reported here indicate that cattle inoculated with blood containing killed *Babesia* spp developed a resistance to field exposure to infected *B microplus* ticks, whereas, nonvaccinated cattle kept under the same field conditions developed clinical babesiasis. Following field challenge, new difficulties were encountered with *A marginale* infection, causing severe anemia in experimental cattle. This indicates that control of babesiasis alone is not economical under most tropical conditions.[44,46]

The freeze-dried inactivated *B bigemina* and *B argentina* vaccines as described here would seem to be capable of imparting some protection against babesiasis. The sterile immunity reported in the present study appears more effective than the form of immunity reported by Australian workers.[26,28,29] This difference may be attributable to the fact that intact calves were used in the present experiments, and the killed *Babesia* spp vaccine was prepared from blood. Mahoney and Goodger[29] studied the immunogenic reactions of splenectomized calves inoculated with plasma harvested from calves with acute infections and they found protection, characterized by a sudden disappearance of *B argentina* from peripheral blood of the calves, only in the experiments in which the challenge inoculum (parasites) was antigenically

identical with the infective plasma used for immunization. Similar results were reported by Todorovic et al,[43] who studied the degree of protection produced by 2 preparations—plasma and infected erythrocytes. Seemingly, the immunogenic material was present mainly in the plasma collected during the acute stage of infection, especially at the peak of *Babesia* spp parasitemia, rather than in the infected erythrocytes. The complete protection observed in cattle vaccinated with both preparations can be explained by differences reported by other investigators.[26,28] Results of experiments designed to ascertain the mechanism of sterile immunity to bovine babesiasis strongly indicate this form of immunity to *B bigemina* and *B argentina* exists and has an important role in the development of acquired immunity to bovine babesiasis.

Coinfectious Immunity—The coinfectious immunity in bovine babesiasis is referred to as a premunition,[40] labile infection, latent infection, or immunitas non sterilizans.[32] The immunity persists so long as the animal remains a carrier of the infection. Several variables must be solved before premunition can be recommended as a method of immunizing cattle on a large scale for the control of babesiasis.[6,8,11,20,42,44] These variables include (1) volume of blood used as inoculum; (2) number, infectivity, and history of *Babesia* spp parasites; (3) phase of infection when blood is collected from cattle either recovered from, or reacting to, *Babesia* spp; (4) storage of infected blood from time of collection to time of inoculation; (5) age and breed of animals used for premunition; and (6) drugs used for moderating the postpremunition reaction. These factors, associated with premunition of Colombian cattle and to limit the number of variables, were discussed.[42–44,46] In the present work, infected blood used as premunition inoculum was always obtained from a splenectomized calf acutely infected with *Babesia* spp; the infective inoculum contained approximately the same number of *Babesia* spp parasites (10^7/ml). Callow and Tammemagi[11] reported that low infectivity for *B argentina* was the cause of vaccination failures in the field. According to them, 1/3 of cattle inoculated with 5 ml of blood from 6 different carriers of *B argentina* between 1 and 6 months after primary infection failed to become infected. In the present experiments, a splenectomized calf was used to produce adequate infection, so that the blood inoculum taken from this calf contained enough *Babesia* spp parasites to accomplish premunization. The second obstacle against an effective premunition program is severity of the postpremunition reaction. In view of the effectiveness of imidocarb[1,9,47] against *B bigemina* and *B argentina*, this problem would not be serious except that cattle are made susceptible if the compound is used in excess and sterilizes the infection.

Plata[33] reported on the common distribution of bovine babesiasis and anaplasmosis in Colombia and the high frequency of concurrent *Babesia* spp and *A marginale* infections in cattle. Findings in the present work confirmed his observations and indicated the need to develop simultaneous control programs for babesiasis and anaplasmosis in tropical zones of Colombia.[23,44]

The mechanism of acquired immunity to bovine babesiasis is complex and is not completely understood. In a study of immunity to babesiasis, it became apparent that the state of equilibrium which develops between the bovine host and the hemoprotozoan parasite is not permanent.[30] The parasite will ultimately be eliminated in the absence of continued infection by ticks, and the animal may become susceptible if reinfection occurs after a certain lapse of time.[32] In enzootic areas in Colombia, due to repeated reinfection from infected ticks, it is not unusual for cattle to retain immunity for several years.

Chemoprophylaxis—There is an obvious need for drugs that can be given prophylactically to susceptible cattle at risk of exposure to *Babesia* spp. In Australia, Callow and McGregor[9] reported that cattle treated with 2 mg of imidocarb/kg of body weight were refractory to infection with *B bigemina* and *B argentina*. Toudic et al[47] confirmed their observations on the prophylactic effect of imidocarb against *Babesia* spp of Colombian origin. Cattle were protected as long as several weeks against blood- or tick-borne babesiasis. In addition, imidocarb was highly efficacious against acute infection with *B bigemina* and *B argentina*.[1,9,10,22,47] The mechanism of imidocarb action is not clearly understood. Severe toxic reactions appeared in cattle injected with imidocarb[47]; salivation, lacrimation, labored breathing, coughing, and even death were observed if the drug was administered IV at dose level of 3 mg/kg. These effects were less severe when imidocarb was administered IM or subcutaneously.

Potential use of imidocarb as a prophylactic drug is indicated in a number of circumstances.[9,19,47] To protect susceptible cattle moved from a tick- and *Babesia*-free country through a tick-infested area, imidocarb can be used to replace hazardous premunition procedures with virulent *Babesia* spp. Furthermore, administration of imidocarb and exposure of cattle to ticks infected with *Babesia* spp might help in the development of natural coinfectious immunity without severe postpremunition reactions. The prophylactic effect of imidocarb was effective for a longer period than any babesiacide tested. Severe toxic effects were found which might limit its use, but these need to be studied.[47]

The actual epizootiologic conditions determine which of the 3 control systems should be used. In an area where the tick population is not effectively controlled, the system of premunition is indicated. In tick-free zones or areas where cattle are at a constant risk of tick exposure and babesiasis, cattle either could be vaccinated with killed *Babesia* spp vaccine or could be treated chemoprophylactically.

References

1. Adams, L. G., and Todorovic, R. A.: The Chemotherapeutic Efficacy of Imidocarb Dihydrochloride on Concurrent Bovine Anaplasmosis and Babesiosis. I. The Effects of Single Treatment. Trop Anim Health & Prod, 6, (1974): 171–17[illegible]
2. Babes, V.: Sur l'Hémogobinurie Bacteriénne des Bœufs. Compt rend Acad sci, 107, (1888): 6[illegible]–698.
3. Barnett, S. F.: The Preservation of *Babesia bigemina*, *Anaplasma centrale* and *Anaplasma marginale* by Deep Freezing. Vet Rec, 76, (1964): [illegible]–8.
4. Brocklesby, D. W., Zwart, D., and Perie, N. M.: Serologic Evidence for the Identification of *Babesia major* in Britain. Res Vet Sci, 12, (1971): 285–287.
5. Burridge, M. J., Kimbor, C. D., and McHardy, N.: De-

tection of Antibodies to *Babesia bigemina* in Dried Blood Samples Using the Indirect Fluorescent Antibody Test. Ann trop Med, 67, (1973): 191–195.

6. Callow, L. L.: The Control of Babesiosis with a Highly Infective Attenuated Vaccine. In *Proceedings*. XIX World Vet Congr, Mexico City, Mexico, Aug 15–22, 1971, (1971): 357–359.

7. Callow, L. L.: Sterile Immunity, Coinfectious Immunity and Differences in *Babesia bigemina* Infections. Parasitology, 57, (1967): 455–465.

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

9. Callow, L. L., and McGregor, W.: The Effect of Imidocarb Against *Babesia argentina* and *Babesia bigemina* Infection of Cattle. Austral Vet J, 46, (1970): 195–200.

10. 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.

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

12. Curnow, J. A.: The Use of a Slide Agglutination Test To Demonstrate Antigenic Differences Between *Babesia bigemina* Parasites. Austral Vet J, 49, (1973): 290–293.

13. Curnow, J. A., and Curnow, B. A.: An Indirect Haemagglutination Test for the Diagnosis of *Babesia argentina* Infection in Cattle. Austral Vet J, 43, (1967): 286–290.

14. Donnelly, J., Joyner, L. P., and Crossman, P. J.: The Incidence of *Babesia divergens* Infection in a Herd of Cattle as Measured by the Indirect Immunofluorescence Antibody Test. Res Vet Sci, 13, (1972): 511–514.

15. Ferris, D. H., Todorovic, R. A., and Ristic, M.: Significance and Application of Babesial and Plasmodial Ectoantigens to Comparative Medicine. JAVMA, 153, (Dec 15, 1968): 1888–1896.

16. Goldman, M., Pipano, E., and Rosenberg, A. S.: Fluorescent Antibody Tests for *Babesia bigemina* and *B. berbera*. Res Vet Sci, 13, (1972): 77–81.

17. Gonzalez, E. F., Todorovic, R. A., and Adams, L. G.: Ultraestructura de *Babesia bigemina*. Rev Inst Colombiano Agropecuario, 6, (1971): 89–112.

18. Goodger, B. V.: Preparation and Preliminary Assessment of Purified Antigens in the Passive Haemagglutination Test for Bovine Babesiosis. Austral Vet J, 47, (1971): 251–255.

19. Holbrook, A. A., and Roby, T. O.: Research Advances in Babesiosis and Anaplasmosis. In *Proceedings*. XIX World Vet Congr, Mexico City, Mexico, Aug 15–22, 1971, (1971): 365–367.

20. 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.

21. Joyner, L. P., Donnelly, J., and Payne, R.: The Indirect Fluorescent Antibody Test for the Differentiation of Infections with *Babesia divergens* or *Babesia major*. Res Vet Sci, 13, (1972): 515–518.

22. Kuttler, K. L.: Review: Current Status of Control and Treatment of Anaplasmosis with Drugs. In *Proceedings*. VI Nat Anaplasmosis Meeting, Las Vegas, NV, March 19–20, 1973, (March, 1973): 93–97.

23. Kuttler, K. L., and Todorovic, R. A.: Techniques of Premunition for Control of Anaplasmosis. In *Proceedings*. VI Nat Anaplasmosis Meeting, Las Vegas, NV, March 19–20, 1973, (March, 1973): 106–112.

24. Lohr, K. F., and Ross, J. P. J.: Capillary Tube Agglutination Test for the Demonstration of *Babesia bigemina* Antibody. Ztschr Tropenmed u Parasitol, 20, (1969): 287–292.

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

26. Mahoney, D. F.: Bovine Babesiosis: The Immunization of Cattle with Killed *Babesia argentina*. Exptl Parasitol, 20, (1967): 125–129.

27. Mahoney, D. F.: Bovine Babesiosis: Preparation and Assessment of Complement Fixing Antigen. Exptl Parasitol, 20, (1967): 232–241.

28. Mahoney, D. F.: Immunization Against *Babesia argentina*. In *Proceedings*. XIX World Vet Congr, Mexico City, Mexico, Aug 15–22, 1971, (1971): 351–355.

29. Mahoney, D. F., and Goodger, B. V.: *Babesia argentina*: Immunogenicity of Plasma from Infected Animals. Exptl Parasitol, 32, (1972): 71–85.

30. Mahoney, D. F., and Ross, D. R.: Epizootiological Factors in the Control of Bovine Babesiosis. Austral Vet J, 48, (1972): 292–298.

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

32. Neitz, W. O.: Classification, Transmission, and Biology of Piroplasms of Domestic Animals. Ann New York Acad Sci, 64, (1956): 56–111.

33. Plata, R. G.: Contribucion al Estudio de las Piroplasmosis Bovinas en Colombia. Rev Fac Med Vet Zoot, National University, Bogotá, Colombia, 21, (1956): 427–434.

34. Riek, R. F.: Babesiosis. In *Infectious Blood Diseases of Man and Animals*. Edited by D. Weinman and M. Ristic. Academic Press Inc, New York, NY (1968): 219–268.

35. Ristic, M.: Protozoal Diseases. In *Bovine Medicine and Surgery*. Edited by W. J. Gibbons, E. J. Catcott, and J. F. Smithcors. American Veterinary Publications, Wheaton, IL (1970): 208–219.

36. Ristic, M.: The Vertebrate Development Cycle of *Babesia* and *Theileria*. In *Biology of Parasites*. Edited by E. J. L. Soulsby. Academic Press Inc, New York, NY (1966): 127–141.

37. Rogers, R. J.: An Evaluation of Tick Fever Outbreaks in Northern Queensland in Recent Years. Austral Vet J, 47, (1971): 415–417.

38. Ross, J. P. J., and Lohr, K. F.: Serologic Diagnosis of *Babesia bigemina* Infection in Cattle by the Indirect Fluorescent Antibody Test. Res Vet Sci, 9, (1968): 557–562.

39. Schalm, O. W.: Veterinary Hematology. Lea & Febiger, Philadelphia, PA (1965).

40. Sergent, E., Parrot, L., and Donatien, A.: Une Question de Terminologie: Immunizer et Prémunir. Bull Soc path exot, 17, (1924): 37–38.

41. Todorovic, R. A., and Adams, L. G.: Serologic Diagnosis of Babesiosis. In *Proceedings*. XIX World Vet Congr, Mexico City, Mexico, Aug 15–22, 1971, (1971): 1114–1116.

42. Todorovic, R. A., Gonzalez, E. F., and Adams, L. G.: Bovine Babesiosis: Co-Infectious Immunity to *Babesia bigemina* and *Babesia argentina* Infections. Exptl Parasitol, 35, (1974): 351–358.

43. Todorovic, R. A., Gonzalez, E. F., and Adams, L. G.: Bovine Babesiosis: Sterile Immunity to *Babesia bigemina* and *Babesia argentina* Infections. Trop Anim Health & Prod, 5, (1974): 234–245.

44. Todorovic, R. A., Lopez, L. A., Lopez, A. G., and Gonzalez, E. F.: Bovine Babesiosis and Anaplasmosis: Control by Premunition and Chemoprophylaxis. Exptl Parasitol, 35, (1974): 675–678.

45. Todorovic, R. A., Luque, F., and Adams, L. G.: Contribucion al Estudio de la Distribucion de la Garrapatas en Colombia, Sur America. Rev Fac Med Vet Zoot, 34, (1972): 63–67.

46. Todorovic, R. A., and Tellez, E. H.: The Premunition of Adult Cattle Against Babesiosis and Anaplasmosis. Trop Anim Health & Prod, 6, (1974): 211–217.

47. Todorovic, R. A., Vizcaino, O. G., and Adams, L. G.: Chemoprophylaxis (Imidocarb) Against *Babesia bigemina* and *Babesia argentina* Infections. Am J Vet Res, 34, (Sept, 1973): 1153–1161.

48. Todorovic, R. A., Vizcaino, O. G., and Adams, L. G.: Determinacion de Anticuerpos de *Babesia* por la Tecnica de la Fijacion del Complemento. Rev Inst Colombiano Agropecuario, 6, (1971): 213–233.

49. Zwart, Van Den Ende, M.C. Kouwenhoven, B., and Buys, J.: The Differences Between *B. bigemina* and a Dutch Strain of *B. major*. Tijdschr diergeneesk, 93, (1968): 126–140.