AGENCY FOR INTERNATIONAL DEVELOPMENT
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BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY
BATCH 47

1. SUBJECT CLASSIFICATION

A. PRIMARY: Food production and nutrition — AL72-0000-0000

B. SECONDARY: Pests of animals

2. TITLE AND SUBTITLE

Babesia bigemina,Babesia argentina,and Anaplasma marginale: coinfectious immunity in bovines

3. AUTHOR(S)

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4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1975	14p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS

Tex.A&M

8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability)

(In Experimental parasitology,v.37,p.179-192)

9. ABSTRACT

Results of experiments on the effects of chemotherapy on cattle infected with B. bigemina, B. argentina, and A. marginale. Forty-eight intact and eight splenectomized cattle were used to evaluate different systems of coinfectious immunization against these infections. Coinfectious immunity was induced by two methods: (1) blood of cattle acutely infected with the three organisms was used as the source of inoculum and the post-vaccination reactions were controlled with Imidocarb, Ganaseg, Gloxazone, and Liquamycin; (2) by artificially inducing babesiosis with the blood of carrier cattle with chronic infections of B. bigemina, and B. argentina without chemotherapy. The degree of resistance was determined by blood-borne and tick-borne challenges. Ticks were collected from cattle and identified as Boophilus microplus and Dermacentor nitens. Vaccinated cattle demonstrated a high degree of resistance to babesiosis and anaplasmosis; however, cattle without coinfectious immunity were treated chemotherapeutically to prevent death losses.

10. CONTROL NUMBER
PN-AAC-941

11. PRICE OF DOCUMENT

12. DESCRIPTORS

Anaplasmosis
Babesiasis
Cattle
Chemotherapy
Complement fixation
Immunization technics
Tick infestations
Vaccine therapy

13. PROJECT NUMBER

14. CONTRACT NUMBER
CSD-1947 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

Tex. Ag. 4 CSU 1947 Cos
PN-AAC-941

EXPERIMENTAL PARASITOLOGY 37, 179–192 (1975)

Babesia bigemina, Babesia argentina, and *Anaplasma marginale:* Coinfectious Immunity in Bovines

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(Submitted for publication March 26, 1973)

TODOROVIC, R. A., GONZALEZ, E. F., AND ADAMS, L. G. 1975. *Babesia bigemina, Babesia argentina,* and *Anaplasma marginale:* Coinfectious immunity in bovines. *Experimental Parasitology* 37, 179–192. Forty-eight intact and eight splenectomized cattle were used to evaluate different systems of coinfectious immunization against *Babesia bigemina, Babesia argentina,* and *Anaplasma marginale.* Coinfectious immunity was induced by two methods: (1) blood of cattle acutely infected with *B. bigemina, B. argentina* and *A. marginale* was used as the source of inoculum and the post vaccination reactions were chemotherapeutically controlled with Imidocarb, Ganaseg, Cloxazone, and Liquamycin, and (2) by artificially inducing babesiosis with the blood of carrier cattle with chronic infections of *B. bigemina* and *B. argentina* without chemotherapy. The degree of resistance was determined by blood-borne and tick-borne challenges. Ticks were collected from cattle and identified as *Boophilus microplus* and *Dermacentor nitens.* Vaccinated cattle demonstrated a high degree of resistance to babesiosis and anaplasmosis; however, cattle without coinfectious immunity were treated chemotherapeutically to prevent death losses.

INDEX DESCRIPTORS: *Babesia bigemina; Babesia argentina; Anaplasma marginale;* Hemotropic diseases; Cattle; Vaccination; Coinfectious immunity; Ticks; *Boophilus microplus; Dermacentor nitens;* Complement-fixation test; Splenectomy; Chemotherapy.

Knowledge of the mechanism of immunity to bovine babesiosis and anaplasmosis is far from complete (Rick 1968; Ristic 1968). Nevertheless, some basic findings concerning the immunopathology of hemotropic infections have been made during the last 20 years (Todorovic *et al.* 1974). Bovine babesiosis and anaplasmosis still exist, particularly in the tropical and subtropical areas of the world where ticks are abundant. In Colombia, South America, these hemotropic diseases cause great losses in susceptible cattle and the incidence of infection appears to be related to the occurrence and activities of tick vectors at various altitudes (Velasquez 1938; Roman 1945; Plata 1956; Todorovic *et al.* 1969; Gonzalez *et al.* 1971). Disease incidence can be reduced by an adequate tick control program to keep cattle free of tick infestations. In Colombia, a vector control program as a measure of control, while appealing, is practically impossible for various reasons (Todorovic 1974); therefore, another measure which can be used to prevent losses and control disease is an effective vaccination program (Todorovic *et al.* 1970, 1971, 1973, 1974) in conjunction with tick

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control. Because bovine babesiosis and anaplasmosis occur as concurrent infections in Colombia (Zaraza *et al.* 1969; Todorovic *et al.* 1973), attempts were made to produce coinfectious immunity against both diseases. A series of investigations were undertaken to study the mechanism of coinfectious immunity in cattle simultaneously vaccinated against *Babesia spp.* and *Anaplasma marginale,* so that the results of vaccination could be elucidated and better methods of immunization and control devised. The experiments were carried out to obtain information on (1) simultaneous vaccination of cattle with *Babesia spp.* and *A. marginale,* and (2) their responses to vaccination and tick- or blood-borne challenge. In addition, experiments were conducted to (3) obtain information on the infectivity and virulence of blood from cattle undergoing primary reaction with *Babesia spp.* and *A. marginale* or from latent infection, and (4) the effect of chemotherapy on the development of coinfectious immunity. The results of these experiments are reported in this paper.

Materials and Methods

Vaccination and Blood-Borne Challenge

Experiment 1. Ten male Holstein–Friesian cattle eight months of age located at Tibaitata, outside of Bogota (altitude 2600 m) were inoculated intramuscularly (im) with 5 ml of infected blood containing 1% *Babesia bigemina,* 0.1% *Babesia argentina,* and 2% *A. marginale.* All cattle were simultaneously treated against babesiosis with Imidocarb (3,3′-bis-(2-imidazolin-2-yl)carbanilide dihydrochloride, Burroughs Wellcome & Co., Tuckahoe, N.Y.) at a dosage of 0.75 mg/kg of body weight given im, and against anaplasmosis with Gloxazone (α-ethoxyethylglyoxal dithiosemicarbazone, Burroughs Wellcome and Co., Tuckahoe, N.Y.) at a dosage of 4 mg/kg of body weight given intravenously (iv) seven days after vaccination. Five cattle (Group B) were additionally treated against anaplasmosis with Gloxazone at a dosage of 4 mg/kg on Days 28, 30, and 32 after vaccination. Nine weeks after vaccination all cattle were injected im with 5 ml of infected blood collected from a calf acutely infected with homologous parasites used for vaccination. The cattle were transported to Putumayo, a tropical zone of Colombia, 12 and 14 weeks after vaccination.

Experiment 2. One Brown Swiss and three Holstein–Friesian bulls two years of age located at Bogota (altitude 2600 m) were used for this experiment. All bulls were subcutaneously (sc) injected with 5 ml of infected blood containing 2% *B. bigemina,* 0.01% *B. argentina,* and 5% *A. marginale* parasitemias. After vaccination, all bulls were treated against *Babesia spp.* with Ganaseg (Squibb, Cali, Colombia) at a dosage of 1 mg/kg of body weight on Days 6 and 8, and against anaplasmosis with Gloxazone at a dosage of 4 mg/kg of body weight given iv on Days 22 and 24 and with Liquamycin (Chas. Pfizer and Co., N.Y.) at a dosage of 12 mg/kg of body weight on Day 28. On Day 36 all animals were injected with the original inoculum used for vaccination.

Vaccination and Tick-Borne (Boophilus microplus) Challenge

Experiment 3. Two groups of Holstein–Friesian male cattle four months of age were used for this experiment, conducted at Palmira in the Cauca Valley (altitude 1000 m). The first group (A), consisting of 15 cattle, was inoculated with 5 ml of blood infected with *B. bigemina* and *B. argentina* isolated from naturally infected cattle. Seventy-five days after vaccination, cattle were exposed to tick-borne challenge. Five cattle of the same age and breed were not vaccinated and were used as controls (Group B).

Experiment 4. Two groups of Holstein–Friesian male cattle eight months of age located at Palmira in the Cauca Valley

were used for this experiment. The first group (Group A), consisting of five cattle, was inoculated with 5 ml of blood from the splenectomized calf used as a donor of *B. bigemina* and *B. argentina* infected blood for cattle in Expt. 3. Ten weeks after vaccination, all cattle were exposed to tick-borne challenge. The second group of cattle (Group B), consisting of five cattle, was injected with the same blood as Group A, but this group of cattle was vaccinated three months previously against anaplasmosis. All cattle were exposed to tick-borne challenge at 10 weeks after vaccination against babesiosis.

Nonvaccinated Control Cattle

Two groups of control cattle were used to evaluate the efficacy of the vaccination techniques used in this study. All control nonvaccinated cattle were raised on the same farm and they were of the same breed and age as the principal animals. The first group (Group A) of five control intact cattle was exposed to ticks on the same day as the vaccinated animals. After exposure they were treated with specific therapy against babesiosis and anaplasmosis. The second group of control cattle (Group B) consisted of two cattle of the same age and breed as the vaccinated cattle. After tick-borne challenge, they were not treated with specific chemotherapy against anaplasmosis and babesiosis.

Control of Infectivity of Blood Used for Vaccination

Eight splenectomized cattle were used to evaluate the pathogenicity of the *B. bigemina, B. argentina,* and *A. marginale* parasites used for the vaccination trials. Cattle were bought from a babesiosis- and tick-free area in Bogota. These cattle were splenectomized and maintained at the Laboratorio de Investigaciones Medicas Veterinarias, Bogota, for these trials. The cattle were used for isolation of *Babesia spp.* and for the evaluation of the pathogenicity of *Babesia spp.* parasites from the patent carriers; blood from these cattle was used for preparation of vaccination inocula.

Preparation of Blood Inocula for Vaccination

Three splenectomized cattle were used as a source of acute infected blood with *B. bigemina, B. argentina,* and *A. marginale,* respectively, for vaccination trials in Bogota. These cattle were inoculated with blood of naturally infected cases isolated from the northern coast of Colombia (Monteria) used in Expt 1, and Rio Sumapaz, used in Expt 2. At the time of primary reaction, blood was collected in EDTA (1.2 g/liter) as anticoagulant, stored at 5 C, and used for vaccination experiments in Bogota. The infective dose was 5×10^7 of *Babesia spp.* or *A. marginale* parasites, and it was injected on the same day of collection.

Two intact Holstein–Friesian male cattle six months of age were injected with *B. bigemina* and *B. argentina* infected blood which originated from the Cauca Valley. When they recovered from primary infection, approximately two months thereafter, they were reinoculated twice with the same organisms. Blood from these cattle was pooled and injected into a splenectomized calf. When this splenectomized calf developed a 0.5% *B. bigemina* and 0.01% *B. argentina* parasitemia, blood was collected and used for vaccination Expts 3 and 4 in Palmira.

Determination of Immune Responses to Vaccination and Challenges

After vaccination, the cattle were examined daily and then twice a week for the presence of erythrocytes parasitized with *B. bigemina, B. argentina,* or *A. marginale* parasitemias (*P*) by the use of thin and thick blood smears described by Mahoney (1962). Packed cell volumes (*PCV*) were determined by the micro-

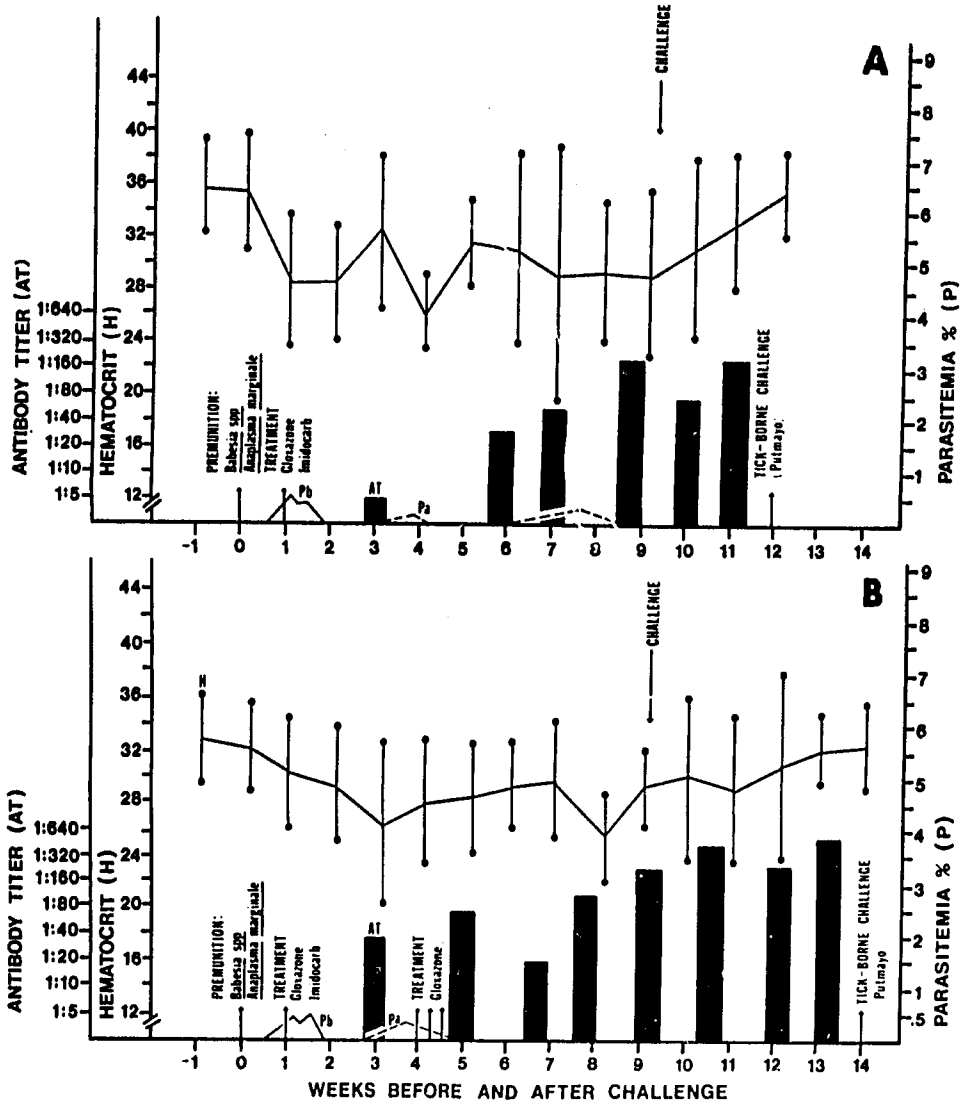

FIG. 1. Immune response of cattle simultaneously vaccinated against babesiosis and anaplasmosis and subsequently treated with Imidocarb and Gloxazone to moderate post vaccination reaction. Graphic representation of mean values for anemia (H), parasitemia (Pb = *Babesia spp.*; Pa = *Anaplasma marginale*), antibody titer (AT) and their relationship to vaccination and single treatment (Group A) in comparison with repeated treatments (Group B).

hematocrit method, and biweekly complement-fixation tests (*AT*) were performed on collected samples according to the method described by Todorovic *et al.* (1971). Rectal temperature (*T*) and weekly body weight (*BW*) changes were also recorded. All control and vaccinated cattle were examined in the period of one year for immune responses during vaccination and challenge. Ticks were collected from all animals and were preserved in absolute alcohol for identification purposes.

RESULTS

Experiment 1

Results of immune responses of cattle injected with blood acutely infected simul-

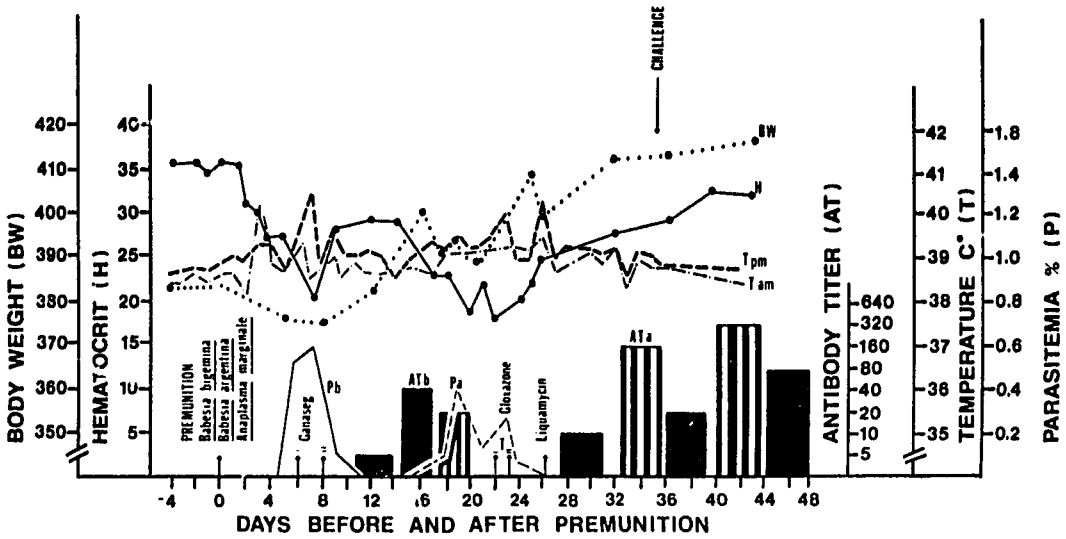

FIG. 2. Immune response of cattle simultaneously vaccinated against babesiosis and anaplasmosis and subsequently treated with Ganaseg, Gloxazone, and Liquamycin to moderate postvaccination reaction. Graphic representation of mean values for anemia (H), parasitemia (Pb = *Babesia spp.*, Pa = *Anaplasma marginale*), antibody titer (AT), temperature (Tam, Tpm), body weight (BW) and their relationship to vaccination, treatment, and challenge.

taneously with *B. bigemina, B. argentina,* and *A. marginale* and subsequently treated against babesiosis with Imidocarb and against anaplasmosis with Gloxazone are shown in Fig. 1. All cattle in Group A, treated once on Day 7 postinoculation (PI), responded severely to vaccination. The *B. bigemina* and *B. argentina* parasitemias were evident on Day 3 and reached a maximum on Day 8 PI. The degree of parasitemia in a given calf varied from 0 to 0.1% until completely disappearing on Day 13 PI. The infected cattle became anemic on Day 8 PI as a result of *Babesia spp.* infection. After treatment against babesiosis, hematocrit values increased temporarily, but started to decrease again due to an *A. marginale* parasitemia which was evident at three weeks PI. Four weeks after vaccination, all cattle except one recovered from anemia which was manifested with a return to normal hematocrit values. Only one calf suffered a prolonged anemic state and finally recovered after 11 weeks PI.

The second group of cattle (Group B) was treated against anaplasmosis on Days 28, 30, and 32 and responded more uniformly to the *A. marginale* vaccination. At nine weeks PI all cattle in Groups A and B were challenged with the original inoculum of *Babesia spp.* and *A. marginale* and did not show any reactions; all had resistance to challenge. All cattle had complement-fixing antibody titers to *Babesia spp.* and *A. marginale* with titers gradually increasing during the vaccination period. At 12 and 14 weeks PI cattle in Groups A and B were exposed to tick-borne challenge when they were transported to Putumayo and did not require treatment against babesiosis or anaplasmosis during the three months of observations.

Experiment 2

Results of immune responses of cattle injected with blood acutely infected simultaneously with *B. bigemina, B. argentina,* and *A. marginale,* and subsequently treated against babesiosis with Ganaseg on Days 6 PI and 7 PI and against anaplasmosis with Gloxazone on Days 22 and 24 PI and Liquamycin on Day 26 PI are shown in

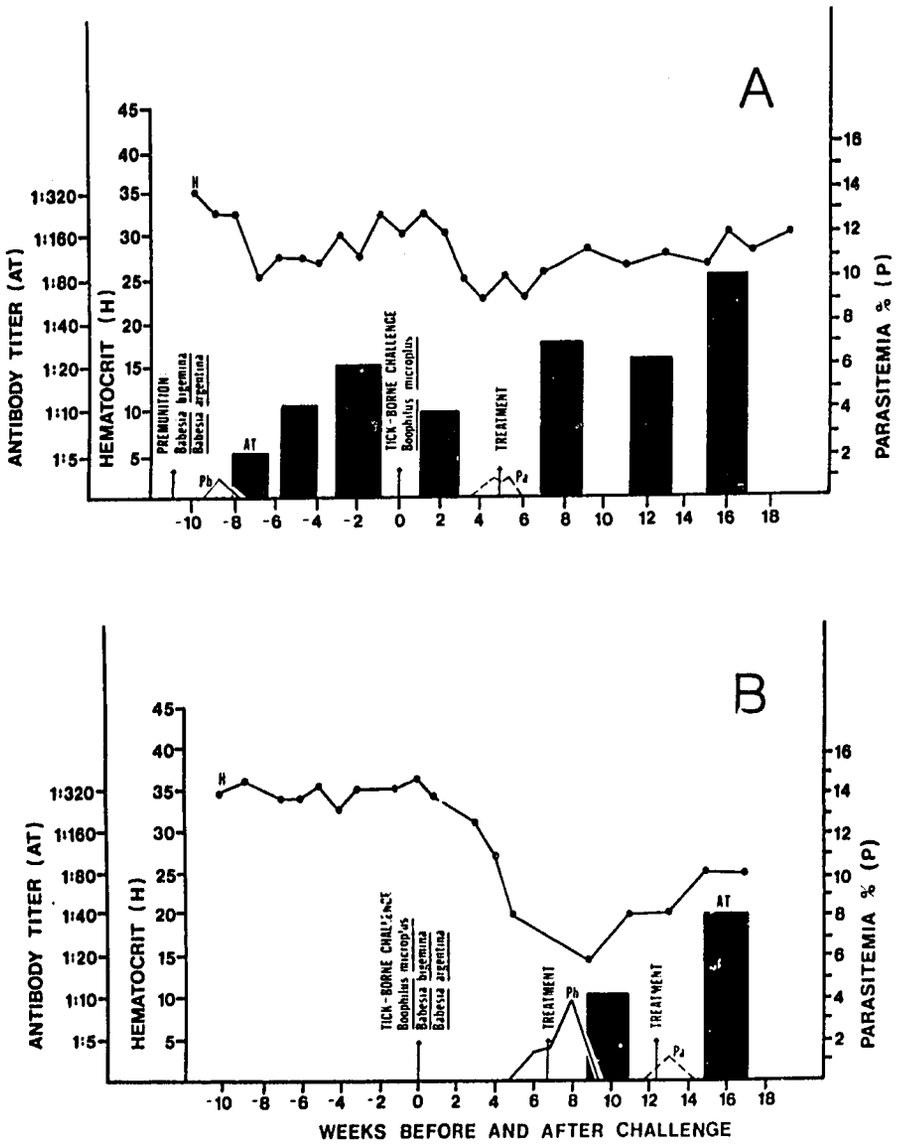

FIG. 3. Immune response of cattle vaccinated against babesiosis and 75 days later exposed to *Boophilus microplus* ticks (Group A top). Graphic representation of mean values for anemia (H), parasitemia (Pb = *Babesia spp.*, Pa = *Anaplasma marginale*), and antibody titer (AT) and their relationship to vaccination, challenge, and treatments in comparison with nonvaccinated cattle (Group B bottom).

Fig. 2. The parasitemia with *B. bigemina* and *B. argentina* became evident on Day 5 PI and reached a maximum on Day 6 PI when the first injection of Ganaseg was given. After the second treatment with Ganaseg, *Babesia spp.* parasitemia decreased and completely disappeared on Day 11 PI. *Anaplasma marginale* parasitemia became evident on Day 17 and reached a maximum on Day 19 PI. After treatment, the degree of parasitemia decreased and disappeared from peripheral circulation on Day 27 PI. At the time of *B. bigemina, B. argentina,* and *A. marginale* parasitemias all cattle had a fever and a loss of body weight, with an anemic

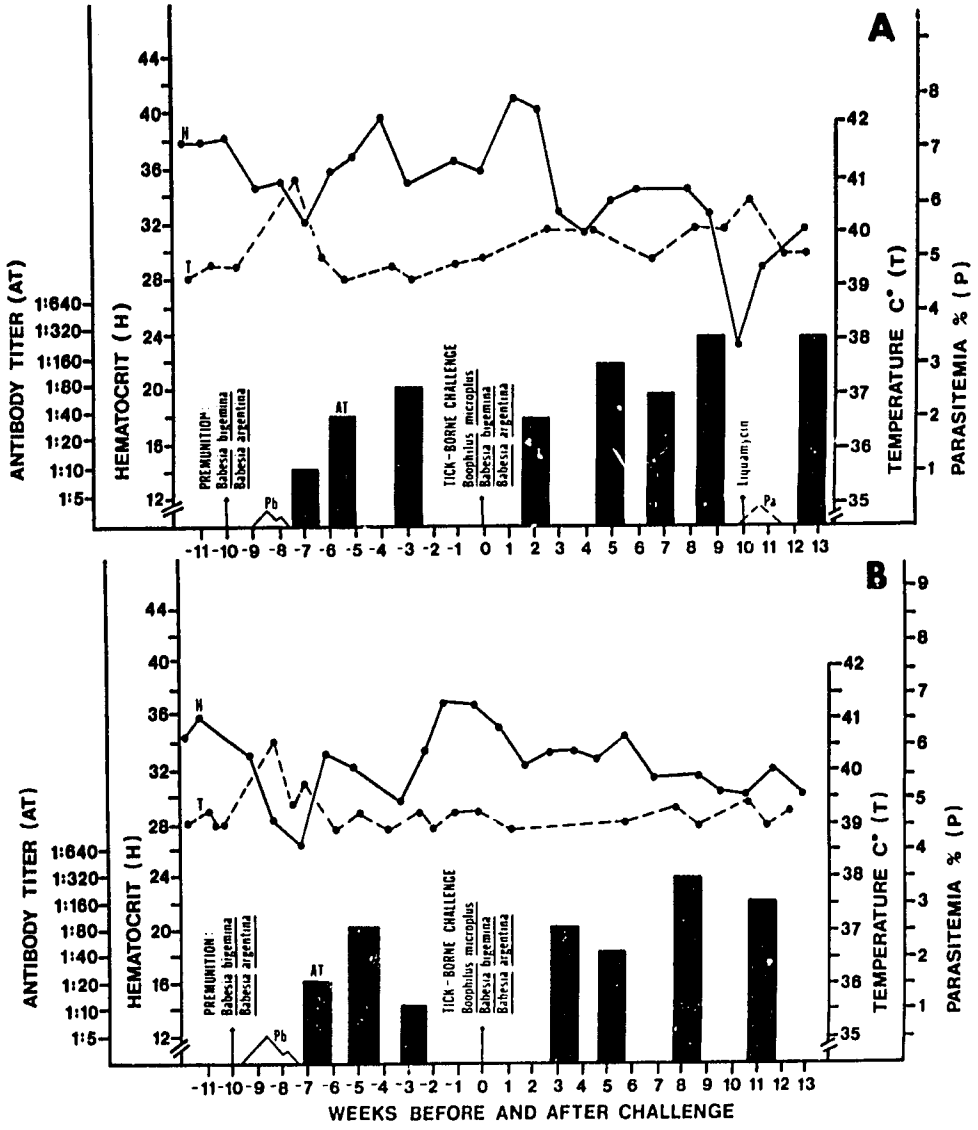

FIG. 4. Immune response of cattle vaccinated against babesiosis alone (Group A). Cattle in Group B were vaccinated against anaplasmosis three months before vaccination against babesiosis. Graphic representation of mean values for anemia (H), parasitemia (Pb = *Babesia spp.*, Pa = *Anaplasma marginale*), and antibody titer (AT) and their relationship to vaccination and challenge.

state characterized by low hematocrit values. A lowest hematocrit value of 18% was recorded in some cattle after appearance of an *A. marginale* parasitemia. All cattle recovered from the vaccination reaction and had normal parameters by Day 30 PI. The first appearance of detectable complement-fixing antibodies was on Day 12 PI for *Babesia spp.* and on Day 16 PI for *A. marginale*. At 36 days PI all cattle were challenged with the original inoculum and were found resistant. Cattle were exposed to tick-borne *B. microplus* challenge on Day 90 PI; they were found resistant to babesiosis and anaplasmosis and in good health after field exposure.

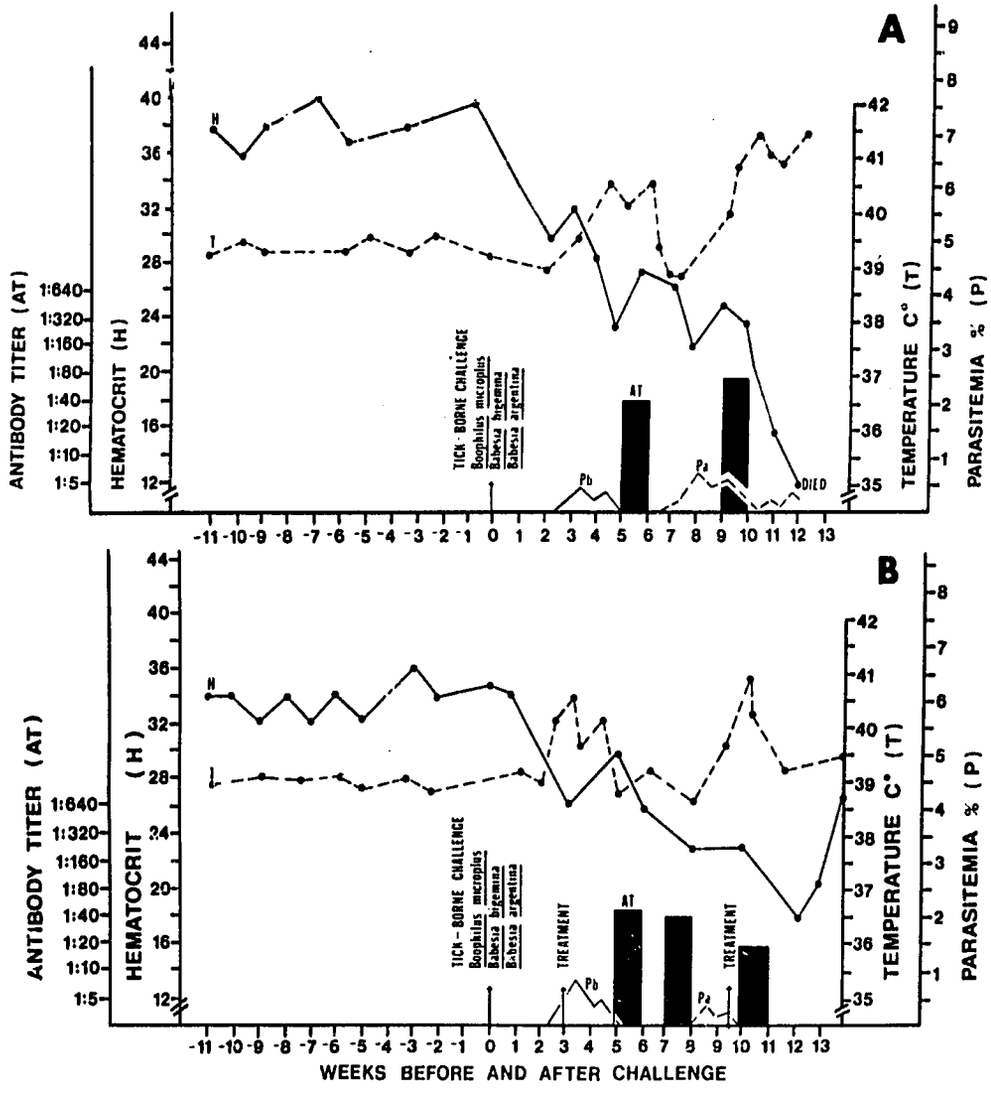

FIG. 5. Controls. Nonvaccinated cattle were exposed to *Boophilus microplus* ticks at the same time as vaccinated animals. Cattle in Group A were not treated against babesiosis and anaplasmosis; cattle in Group B were treated. Graphic representation of mean values for anemia (*H*), parasitemia (Pb = *Babesia spp.*, Pa = *Anaplasma marginale*), and temperature (*T*) and their relationship to *B. microplus* challenge, treatment, and mortality.

Experiment 3

Results of immune responses of cattle injected with blood collected from patent carriers of *B. bigemina* and *B. argentina* are shown in Figs. 3 and 4A. The parasitemia with *B. bigemina* and *B. argentina* became evident on Day 8 PI, reached a maximum on Day 12, gradually decreased on Day 16 PI, and thereafter was no longer detectable in the peripheral circulation. All cattle developed an anemia which was characterized with low hematocrit values averaging 25% on Day 14 PI. At the time of field exposure to *B. microplus* ticks naturally infected with *Babesia spp.*, all cattle had hematocrit values averaging 33%. After tick-borne challenge, *B. bigemina* and *B. argentina* were not detected in the periph-

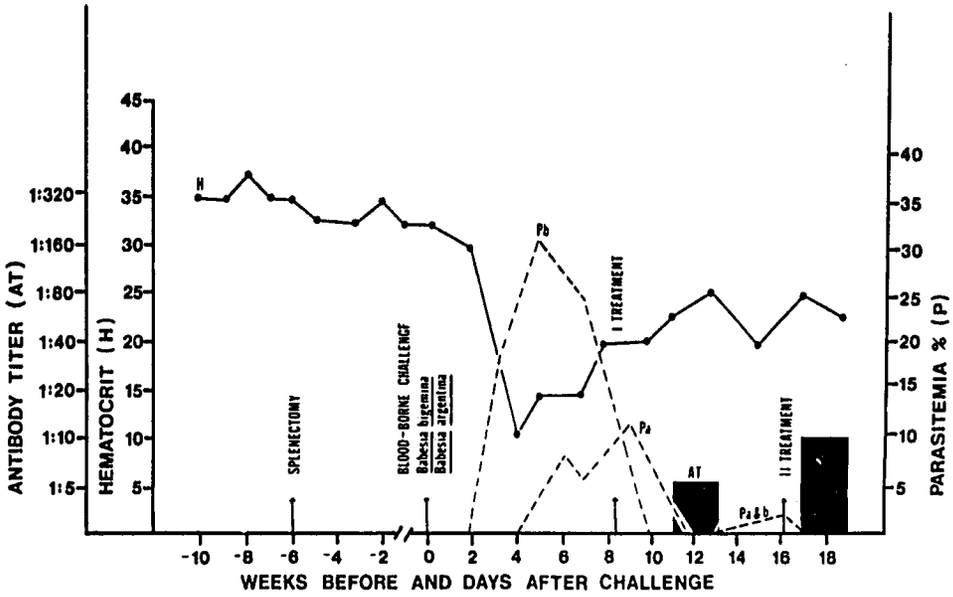

FIG. 6. Controls. Infectivity. Graphic representation of mean values for anemia (*H*), parasitemia (Pb = *Babesia bigemina*, Pa = *Babesia argentina*), and antibody titer (*AT*) of splenectomized cattle used to evaluate pathogenicity of *Babesia spp.* parasites for preparation of vaccines.

eral blood films; however, cattle suffered clinical anaplasmosis and *A. marginale* parasitemias were demonstrated in blood films four and six weeks after challenge. After specific chemotherapy against anaplasmosis, cattle recovered from anemia and the *A. marginale* parasitemia. Complement-fixing antibody titers were first detected on Day 10 PI and they persisted with increasing titers during the period of challenge. Control cattle not vaccinated developed clinical babesiosis, and they needed treatment to prevent production and death losses. Two cattle not treated died with acute babesiosis. These control cattle also suffered clinical anaplasmosis and they were treated to prevent death losses.

Experiment 4

Results of immune responses of cattle (Group B) vaccinated against anaplasmosis and three months later injected with blood from patent carriers of *B. bigemina* and *B. argentina* are shown in Fig. 4B. The parasitemia with *B. bigemina* and *B. argentina* became eviden five days after vaccination, reached a maximum on Day 10 PI, and gradually decreased and disappeared by Day 18 PI. At the time of *B. bigemina* and *B. argentina* parasitemias all cattle became anemic but recovered from vaccination without treatment on Day 24 PI with a return to normal hematocrit values. After tick-borne challenge all vaccinated cattle had a high degree of resistance to field challenge. *Babesia bigemina, B. argentina,* and *A. marginale* parasites were not detected in blood films. Temperatures and hematocrit values were in the normal range. Complement-fixing antibody titers were first detected on Day 21 PI; they persisted with increased titers during the period of 13 weeks of field exposure to *B. microplus* ticks infected with *B. bigemina* and *B. argentina*. All cattle had a fever at the time of *B. bigemina* and *B. argentina* postvaccination reactions.

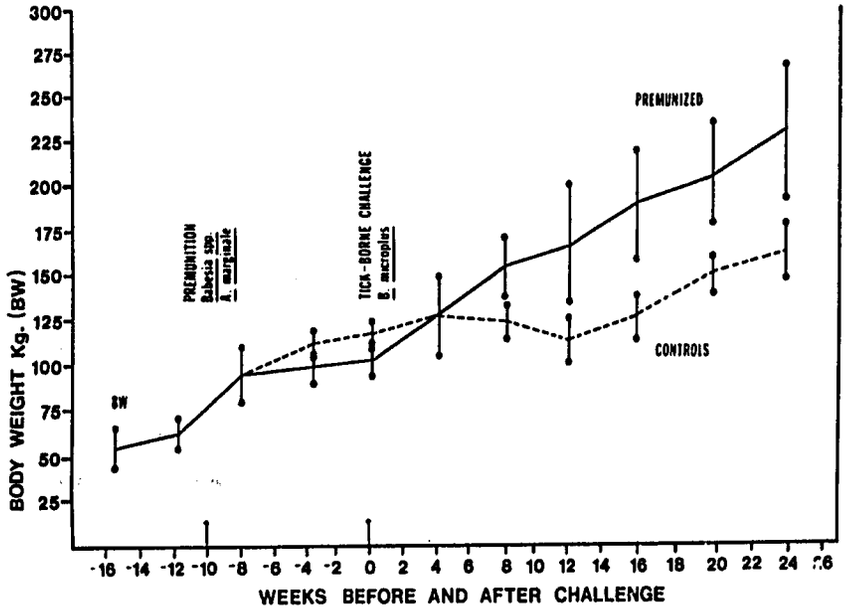

FIG. 7. Average body weight of vaccinated and nonvaccinated cattle and their relationship to vaccination and *Boophilus microplus* challenge.

Responses of Nonvaccinated Control Cattle

Results of hematologic and serologic examinations of intact and splenectomized control cattle used to determine the degree of resistance of vaccinated cattle to anaplasmosis and babesiosis and the pathogenicity of *B. bigemina* and *B. argentina* parasites are shown in Figs. 3B, 5A, 5B, and 6. All splenectomized cattle inoculated with *B. bigemina* and *B. argentina* blood collected from acute infections were severely infected (Fig. 6). Intact cattle inoculated with blood collected from patent carriers developed a slight infection. Nonvaccinated cattle exposed to field challenge with naturally infected *B. microplus* ticks suffered clinical babesiosis and anaplasmosis and were treated to prevent death losses (Figs. 3B and 5B); nontreated cattle died with signs of acute babesiosis and anaplasmosis (Fig. 5A).

Responses of Vaccinated Cattle in Body Weight

The effect of vaccination against bovine babesiosis and anaplasmosis in body weight of vaccinated cattle is shown in Fig. 7. At the time of vaccination against babesiosis, all cattle temporarily lost body weight in comparison with nonvaccinated control cattle; however, after field exposure to *B. microplus* ticks naturally infected with *B. bigemina, B. argentina,* and *A. marginale,* significant differences in body weight were found between vaccinated and nonvaccinated cattle (Expt 4, Group B). As a result of resistance to *B. bigemina, B. argentina,* and *A. marginale,* vaccinated cattle gained 53 kg more in body weight during the 11 months of observations than the nonvaccinated control cattle.

DISCUSSION

This work was undertaken to determine immune responses of Colombian cattle vaccinated against *B. bigemina, B. argentina,* and *A. marginale,* their relationships to chemotherapy, the infectivity of blood from acute and patent infections, and simultaneous vaccination against bovine babesiosis and anaplasmosis in comparison with unilateral control of either one of these tropical maladies.

The classical concept of coinfectious immunity was first introduced by Sergent *et al.* (1924) and was based on the observation that immunity to babesiosis persists as long as the animal remains a latent carrier of *Babesia spp.* parasites. If parasites disappeared as a result of autosterilization or effective chemotherapy, the animal became fully susceptible; therefore, immunity against babesiosis and anaplasmosis can be achieved by inoculating cattle with small doses of blood containing *Babesia spp.* and *A. marginale* parasites. This method will usually accomplish the purpose of coinfectious immunity although the method is, of course, somewhat hazardous in itself (Todorovic 1970). According to Australian investigators (Callow and Tammemagi 1967; Legg 1939) who practiced vaccination against babesiosis alone, losses can be considerable. If fully susceptible cattle are vaccinated without aftercare, losses of approximately 1–5% may occur. Vaccinated cattle must be observed very closely in case severe reactions develop. Legg (1939) reported two instances in which one-third and two-thirds, respectively, of vaccinated cattle in two groups died from severe *B. argentina* postvaccination reactions.

In early reports on the postvaccination reactions of both diseases, it was evident that many uncontrolled variables were responsible for the inconsistent results (Callow and Tammemagi 1967; Johnston and Tammemagi 1969; Kemron *et al.* 1962, 1964; Legg 1939; Rick 1968; Ristic 1968; Todorovic 1974). These variables included: (1) volume of blood used as inoculum; (2) number of *Babesia spp.* and *A. marginale* parasites; (3) infectivity and virulence of *Babesia spp.* and *A. marginale* parasites; (4) pathogenicity of parasites; (5) infectivity and virulence of blood collected from cattle either recovered from or reacting to *Babesia spp.* and *A. marginale* parasites; (6) storage of infected blood from time of collection to time of vaccination; (7) age and breed of animals used for vaccination; and (8) chemical (type and dosages) used to monitor postvaccination reactions. These variables must be solved before vaccination as a method can be recommended on a large scale for the control of bovine babesiosis and anaplasmosis. The present study was designed to limit the number of variables to as few as possible.

Zaraza *et al.* (1969) reported on the common distribution of babesiosis and anaplasmosis in Colombia, the high frequency of concurrent *Babesia spp.* and *A. marginale* infections in cattle, and the need for a control program against both diseases. This work confirmed their observation and demonstrated that unilateral control of hemotropic diseases is not practical (Todorovic *et al.*, 1973). Our data indicate vaccination against *B. bigemina, B. argentina,* and *A. marginale* can be accomplished simultaneously without severe postvaccination reactions if chemotherapeutic treatment of cattle is performed properly. The second approach, to vaccinate against anaplasmosis and after cattle completely recover to vaccinate against babesiosis, might also be practical, as our data indicate (Fig. 4B). Observations showed that clinical and pathological manifestations of concurrent infection with *B. bigemina, B. argentina,* and *A. marginale* were more severe than those observed during infection with either of the hemotropic parasites alone, and were attributed to the concurrent infection being additive in nature (Todorovic *et al.* 1971). Simultaneous vaccination against babesiosis and anaplasmosis would be more practical, but in some cases when it is not possible, vaccination of an individual disease is recommended.

Callow and Tammemagi (1967) reported that low infectivity for *B. argentina* was the cause of vaccination failures in Australia. One-third of cattle vaccinated with 5 ml of blood from six different carriers of *B. argentina,* between one and six months after primary infection, failed to become immunized. In order to avoid this obstacle,

a splenectomized calf was injected with carrier blood to be sure the inoculum would contain an adequate number of *B. argentina* parasites to accomplish vaccination. The present work also demonstrated that when a splenectomized calf in Colombia was inoculated with blood from a patent carrier, postvaccination reactions were usually mild, which indicates the attenuated nature of the *Babesia spp.* parasites. This was confirmed by the fact that vaccinated cattle at Palmira did not require treatment (Figs. 3A, 4A, and 4B).

Another obstacle to an effective vaccination program against babesiosis and anaplasmosis in Colombia is the severity of the post vaccination reaction in cases where blood from naturally infected cattle was collected during the acute stage of disease (Figs. 1A and 1B). In view of the effectiveness of various chemicals against *B. bigemina, B. argentina,* and *A. marginale,* this problem would not be serious, except that cattle are made susceptible again if drugs are used in excess and sterilize the infection (Callow and McGregor 1970). In our work, and in results reported by Callow and McGregor (1970) and Roby *et al.* (1968), new drugs (Imidocarb, Gloxazone) and commercially available compounds (Ganaseg, Liquamycin) were found satisfactory to monitor postvaccination reactions in cattle inoculated with virulent blood collected from cattle acutely infected with *Babesia spp.* and *A. marginale.*

The volume of inoculum used for vaccination, including the challenged doses when applicable, were standardized at 5 ml of blood (10^7 parasites per ml). The blood samples used as inoculum were obtained during the initial acute phase of *B. bigemina, B. argentina,* and *A. marginale* infections. Blood used as vaccination inoculum was obtained when *B. bigemina* parasitemia was 1%, *B. argentina* between 0.1 and 1%, and *A. marginale* 2–5%. The source of blood used for vaccination was from a splenectomized calf either injected with blood from patent carriers or from cattle acutely infected with *Babesia spp.* or *A. marginale.* This method gave us an advantage because the infective inoculum contained approximately the same number of *Babesia spp.* and *A. marginale* parasites. The criterion for infectivity and virulence of *B. bigemina* and *B. argentina* parasites was determined as a percent of the parasitemia of splenectomized cattle, degree of anemia, and the mortality rate. It was established that demonstration of *B. bigemina* or *B. argentina* parasites in the erythrocytes is the necessary criterion of infections (Fig. 6).

On the basis of results obtained in Palmira, it appears that cattle vaccinated with blood from patent carriers maintained shortly into a splenectomized calf had mild postvaccination reactions, manifested with a transient parasitemia, without clinical signs; treatment was not necessary to monitor such reactions. The complete resistance of these cattle was observed upon field exposure to ticks naturally infected with *B. bigemina* and *B. argentina.* The mechanism of immunity was based on the fact that cattle were resistant to superinfection and they never showed any signs of *Babesia spp.* infection (Figs. 3A and 4).

The acquired immunity of cattle to *B. bigemina, B. argentina,* and *A. marginale* was investigated by challenging cattle either by blood- or tick-borne homologous challenges. Our results indicate that complete immunity was produced in cattle against homologous challenges. These results are in accordance with data reported by Callow (1967) that homologous immunity develops quickly and is sufficient to prevent parasitemias in cattle exposed to field challenge, in comparison with heterologous challenge where a slight degree of parasitemia is noted without severe clinical diseases. In all cattle vaccinated at Bogota and Palmira, the homologous immunity was developed in all instances and

was sufficiently solid to prevent any signs of disease at a time when controls died from the same challenge (Figs. 5A and 5B).

The mechanism of simultaneously induced immunity to babesiosis and anaplasmosis is complex in nature and is not quite elucidated. In a study of immunity to these diseases, it becomes apparent that the state of equilibrium which develops between the host and the protozoan parasites is not permanent. The parasite will ultimately be eliminated in the absence of continued infection by ticks, and the animal may become susceptible to a clinical attack if reinfection occurs after a certain lapse of time (Mahoney 1962). In endemic areas of Colombia, due to the repeated reinfection from infected ticks, it is not unusual for cattle to retain their immunity for a considerable period of time. It has been shown that *B. bigemina* and *B. argentina* in the absence of reinfection usually persist for 10–12 months and that severe relapses may occur at varying intervals within this period. Cattle which recover from clinically evident babesiosis develop a coinfectious immunity which may last four to 12 months (Mahoney 1962). However, it should be noted that the duration of this resistance varies considerably both with the species of *Babesia* and *A. marginale* and with individual animals (Rick 1968; Ristic 1968).

The results obtained from our work in Colombia on the development of simultaneous coinfectious immunity in cattle indicate that the lasting immunity to babesiosis and anaplasmosis can be established by providing for the constant maintenance of the immunopathologic process associated with subclinical infection monitored by chemotherapy. This process activates the host's anti-*Babesia* and *A. marginale* humoral and cellular defenses, and finally produces resistance to natural infection. Until better methods are developed, simultaneous vaccination against bovine babesiosis and anaplasmosis is practical and feasible in Colombia's tropical environment.

Acknowledgments

This work was supported by grants from the Rockefeller Foundation and the U.S. Agency for International Development to the Institute of Tropical Veterinary Medicine, Texas A&M University, College Station, Texas 77843. The Instituto Colombiana Agropecuario and Laboratorio de Investigaciones Medicas Veterinarias, where the work was conducted, contributed space, laboratories, and the use of equipment.

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