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BOVINE BABESIOSIS: STERILE IMMUNITY TO *BABESIA BIGEMINA* AND *BABESIA ARGENTINA* INFECTIONS

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SUMMARY

Killed Babesia bigemina *and* Babesia argentina *vaccine was prepared from the infected erythrocytes and from the infected plasma (AG-S) collected from acutely infected calves with* bigemina *and* argentina. *The vaccine was tested in Colombian cattle under field conditions in the Cauca Valley. A total of* 40 *calves two and one half months of age received killed-*Babesia *vaccine, and* 5 *calves were not vaccinated; they served as controls. Vaccinated and non-vaccinated control calves were exposed to field-borne challenge with* Boophilus microplus *infected ticks. On the basis of the data obtained in these experiments, it was found that a high degree of sterile immunity to* Babesia bigemina *and* Babesia argentina *can be produced in calves injected with killed-*Babesia *vaccine. It appears that sterile immunity plays an important role in the mechanism of acquired immunity to babesiosis other than the well-known co-infectious immunity known as premunition.*

INTRODUCTION

Since the discovery of *Babesia bovis* (Babes, 1888) much work has been done on various aspects of immunity to bovine babesiosis, but, the pathogenic and immunogenic mechanisms in this and other haemotropic infections have not been elucidated. Among these processes is mechanism of acquired resistance to *Babesia bigemina* and *Babesia argentina* infections. It is thought that resistance to clinical infections is generally observed in cattle harbouring the parasites or being in state of immunity known as "premunition" (Sergent, *et al.* 1926). Recent evidence observed by the Australian investigators suggested that a sterile immunity exists and plays an important role in the development of acquired immunity to bovine babesiosis.

Sterile immunity was reported in cattle which had recovered from an infection with *Babesia bigemina*, however, this type of immunity was directed only against the homologous strain of *Babesia bigemina* (Callow, 1964). Attempts to produce a sterile immunity by injecting cattle with killed-*Babesia argentina* parasites had resulted in only partial protection (Mahoney, 1967). Recently, Mahoney and Goodger (1972) found that complete protection characterized by abrupt removal of *Babesia argentina* from the peripheral blood circulation was observed in the experiments in which the parasites used to challenge immunity were antigenically identical with those that produced the infected plasma used for immunization.

The following is a report on the immunizing properties of killed-*bigemina* and *argentina* of Colombian origin, produced by a freeze-dried technique to inactivate *Babesia* spp. parasites either in infected erythrocytes or plasma collected at an acute stage of infection.

MATERIALS AND METHODS

Location of Experiments: The Palmira experimental farm of the Colombian Institute of Agricultural Research (ICA) located in the Cauca Valley was used to carry on vaccination

trials. This farm is located in a subtropical zone of South America. The elevation was approximately 1,000 metres (3,195 ft.) and the average annual temperature was 26° C. (78.3°F). The average annual rainfall measured 849 mm (33.8 in.) per year and the heaviest rainfall occurred in March, April and May.

Experimental Animals: Forty-five male, Holstein × friesian calves weighing from 69 to 97 kg, born and raised on this farm, were used for evaluation of the vaccines against babesiosis. Two days after birth, the calves were taken from the dams and placed in individual concrete stalls under conditions that precluded accidental *Babesia* spp. infections. The calves were fed milk and calf pellet concentrate. All calves were assessed to be negative to *Babesia* infection by the complement-fixation test, blood transmission and blood examination of Giemsa stained films, calves were in good health and free of tick infestation prior to vaccination. At the age of 2½ months, they were used in the vaccination experiments. Calves were drenched with Thiabendazole* against intestinal parasites and they were bled weekly for samples for serological and haematological studies. Ten weeks after vaccination, the calves were kept on pasture.

The Parasites: Blood was collected from infected cattle located on this farm and injected into splenectomized calves at the Veterinary Laboratory of the Colombian Institute of Agricultural Research in Bogota. *B. bigemina* and *B. argentina* were isolated from the acutely infected cattle. *B. bigemina* and *B. argentina* were maintained in splenectomized calves and used for vaccine preparations. Disodium ethylenediamine tetraacetate (EDTA) 1.2/l of blood was used as anticoagulant. Blood was frozen using an empirical technique based on that described by Barnett (1964). Ten ml aliquots of infected blood in bijou bottles with 7.5 percent glycerol the cryoprotectant were slowly frozen to —79°C. at the rate of approximately 1° C. a minute. The aliquots were stored at a temperature of — 60 C. for period of up to 6 months. On resuscitation, the frozen material was thawed rapidly in a 37 C. water bath and passaged once through a splenectomized calf before being used for vaccine preparation. Standard infection of recently splenectomized calves gave rise to a 1 per cent parasitaemia in a mean prepatent period of 4.5 days.

Preparation of AG-E Vaccine: Blood was collected from a splenectomized calf infected with *B. bigemina* (25 per cent) and *B. argentina* (1 per cent) for preparation of the AG-E vaccine. Blood was collected in EDTA as anticoagulant by canulation of the carotid artery. Plasma was separated from the infected erythrocytes by centrifugation (2,500 rpm/20 minutes) and saved for further study. The infected erythrocytes were pooled and washed three times in 0.85 per cent physiological solution. These erythrocytes were lysed by freezing and thawing and lyophilized in Virtis† dry-freeze apparatus in 10 ml aliquots. The lyophilized lysate of infected erythrocytes with *B. bigemina* and *B. argentina* was termed vaccine AG-E. This vaccine was stored at —20 C.

Preparation of AG-S Vaccine: Plasma separated from the infected erythrocytes by centrifugation was frozen at —20° C. and recentrifuged for removal of fibrinogen at 3,000 rpm/30 minutes. Ten ml aliquots of this material was distributed in bijou bottles and lyophilized in Virtis apparatus. This material was termed AG-S vaccine, and stored at -- 20 C. until use for vaccination trials.

Vaccination Procedure: Vaccine prepared from the infected erythrocytes (AG-E) and from plasma (AG-S) was removed from the refrigerator and restored to one-third of the original volume with sterile distilled water and admixed with Freund's‡ complete adjuvant in equal amounts before injection. Vaccines with adjuvant or without were injected intramuscularly in the hip of calves at 14-day intervals. Four groups of calves (10 calves each, 2½ months of age) randomly distributed were divided in 4 groups. The experimental calves designated as Group A were intramuscularly inoculated with 5 ml of AG-S vaccine. The calves in Group B were inoculated with 5 ml of AG-S vaccine with equal amount of Freunds complete adjuvant. Ten calves in Group C were injected with 5 ml of AG-E vaccine. Ten calves in Group D were injected with 5 ml of AG-E vaccine with equal amount of Freunds

*Thiabenzole, Merck and Company, Rahway, N.J.
†Virtis Co., Inc., Gardiner, New York.
‡Freund's Complete Adjuvant, Difco Company, Detroit, Michigan.

complete adjuvant. Five calves of the same breed and origin kept under the same condition as principal calves were not vaccinated but used as controls.

Determination Assessment of Immune Responses of Vaccinated Calves: Ten weeks after vaccination all calves were exposed to natural field challenge with *Boophilus microplus* ticks naturally infected with *B. bigemina* and *B. argintina*. Blood samples from each animal were collected weekly in tubes under aseptic conditions. The blood tubes were refrigerated as soon as possible after collection by placing them in an ice chest and refrigeration was maintained until the blood samples were processed. Thin blood smears were made using the Giemsa technique described by Vizcaino *et al.* (1969). The films were fixed in absolute methanol and stained with Giemsa* for 20 minutes using phosphate buffered water pH 7.1 to prepare the stain solution. Each prepared smear was examined under the oil immersion lens of a light microscope at 1,000 X magnification. Twenty fields of slides were examined for parasitized erythrocytes and the percentage of parasitaemias calculated on the basis of the number of infected erythrocytes in relation to the total number of cells in each field.

Haematocrit values were determined by the microhaematocrit methods as outlined by Schalm (1965). Plain capillary tubes were filled to approximately 1 cm of the end with unclotted blood and the empty end of each tube was fused in the flame of a gas burner. The filled tubes were then centrifuged for five minutes at 11,500 rpm and the packed cell volume read directly from a calibrated tube reader. The complement-fixation test for *Babesia* spp. antibody detection was performed according to the technique described by Todorovic *et al.* (1971). Serum samples collected from each calf were screened for the presence of complement-fixing of *B. bigemina* and *B. argentina* infections.

The weight of each calf was determined by a Nasco** weight tape. Each animal was restrained in a normal standing position and the tape placed around the calf at the heart girth. The weight in kilograms was read directly from the tape.

Ticks were collected from each calf at weekly intervals and preserved by immersing them in tubes with absolute alcohol. Clinical observations and necropsies of the dead animals were performed by veterinarians working in the Pathology section of the Columbian Institute of Agriculture in Palmira.

The degree of protection in all animals vaccinated was assessed by comparison of the parameters measured with those of a comparable group of control non-vaccinated calves. The field challenge-was possible because all the calves were not included in any tick control program and the *Boophilus microplus* tick load permitted sufficient numbers to build up on animals.

RESULTS

Immune Responses of Calves to Vaccination

The results of haematological and serological responses of calves injected with killed-*Babesia* spp. AG-S vaccine (Groups A & B) with or without complete Freund adjuvant are shown in Fig. 1. The first injection of killed-*Babesia* spp. vaccine is indicated as week-10, and the second injection as week-8. The time of tick-borne (*Boophilus microplus*) challenge is indicated by an arrow as week 0. Prior to vaccination, the haematocrit values of 34 per cent were essentially the same for all Groups A & B. Two weeks after vaccination, calves in Groups A & B developed a slight temporary transient anaemia, which was evidenced by drop of haematocrit values to 29 per cent. This anemic state lasted for a week and all calves in Groups A & B recovered from anaemia by returning to the normal haematocrit values of 32 to 35 per cent per Group, at the time of tick exposure. The complement-fixing antibody titre response of calves in Groups A & B were not statistically significant. The daily weight gains of the cattle in Groups A & B at time of vaccination were essentially the same as in non-vaccinated control calves.

*Giemsa Stain, Gradwohl Laboratories, St. Louis, Missouri.
**Metric tape, Nasco, Fort Artkinson, Wisconsin.

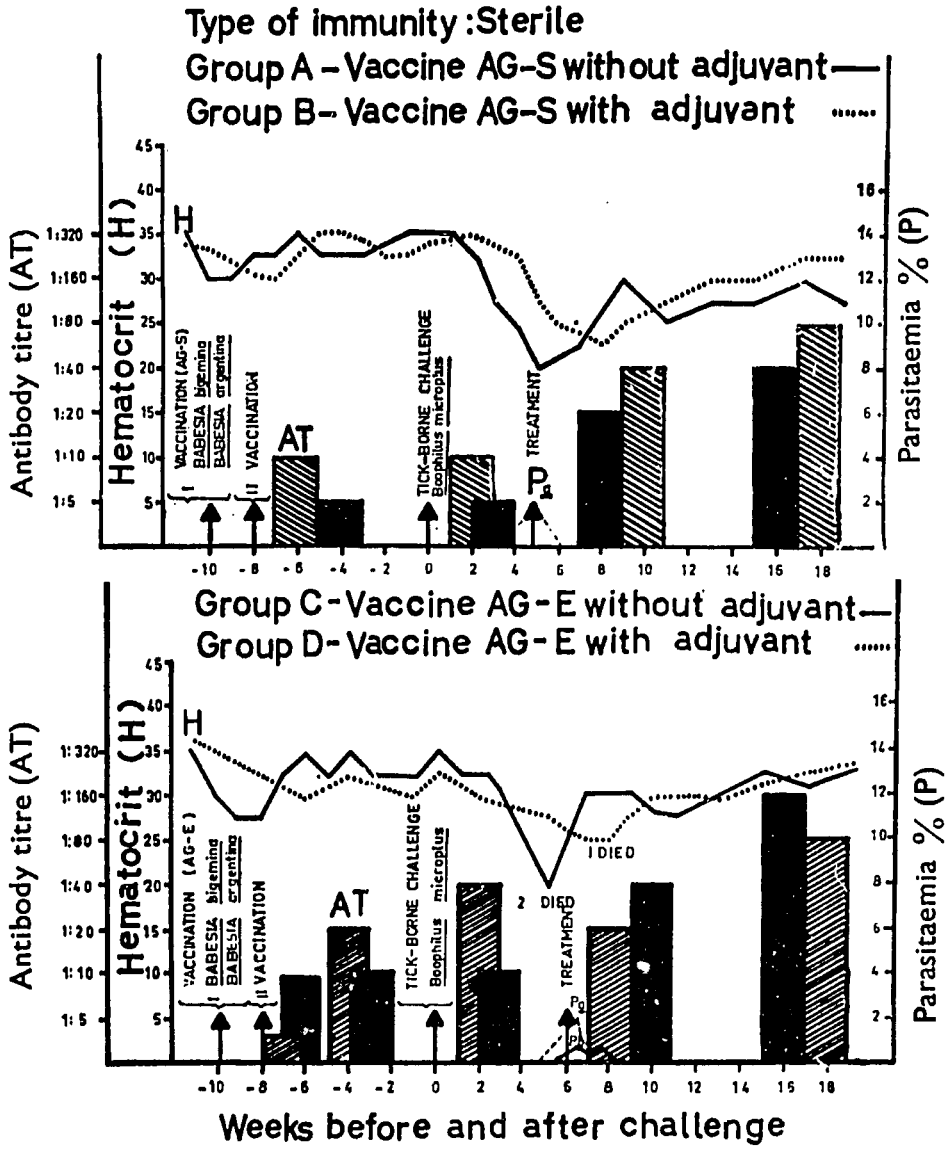

FIG. 1. Haematological and serological responses of cattle injected with killed *Babesia* spp. vaccine AG-S (infected plasma) Groups A & B; and AG-E (infected erythocytes) Groups C & D. Graphic representation of mean values for anaemia (H), parasitaemia (P), and antibody titre (AT) and their relationships to vaccination and tick-borne (*Boophilus microplus*) challenge.

The results of haematological and serological immune responses of calves injected with killed-*Babesia* spp. vaccine/AG-E injected with or without Freund's complete adjuvant is shown in Fig. 1 (Group C & D). The calves in Group C injected with killed-*Babesia* spp. AG-E vaccine developed a transient anaemia which was evident by drop of haematocrit values to 26 per cent at two weeks after vaccination. However, calves in Group D had delay in showing anaemia for one week. All calves recovered from this anaemic state at time of field exposure. The complement-fixing antibody titres and the daily average body weight gains were not statistically significant between the two Groups. All calves in Groups (A, B, C, D) were 5 months old at the time when they were exposed to *Boophilus microplus* ticks naturally infected with *B. bigemina* and *B. argentina*. Significant haematological and serological immune responses were not observed among different Groups of calves injected with various types of killed-*Babesia* spp. vaccines.

Immune Responses of Vaccinated Calves to Field-Challenge:

The results of haematological and serological immune responses of calves to tick-borne *Boophilus microplus* naturally infected ticks with *Babesia bigemina* and *B. argentina* at Palmira, Cauca Valley, are shown in Fig. 1.

The average of haematocrit values at the time of field exposure was essentially the same in all groups. Calves in Group A & B vaccinated with killed *Babesia* spp. AG-S vaccine with or without adjuvant developed resistance to clinical babesiosis, which was evidenced with negative blood examinations for *B. bigemina* and *B. argentina* parasites. In comparison with these groups, non-vaccinated control calves had parasitemias with *Babesia* spp. at two to three weeks after field exposure. Four to five weeks after field exposure, calves in control as well as in vaccinated groups developed signs of anaplasmosis, which was evidenced by an *Anaplasma marginale* parasitaemia,

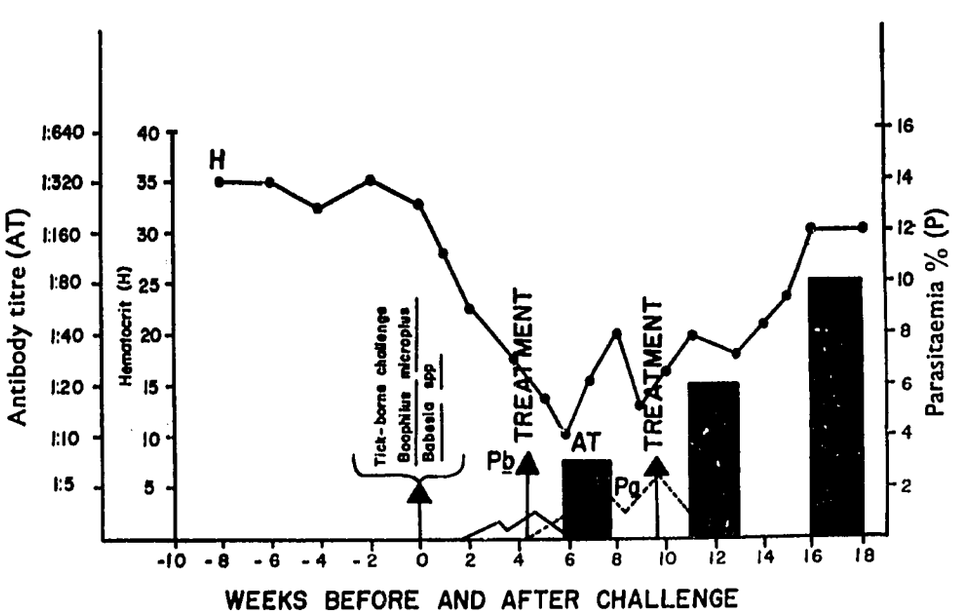

FIG. 2. Haematological and serological responses of five control calves exposed to *Boophilus microplus* ticks naturally infected with *Babesia bigemina* and *Babesia argentina*. Graphic representation of mean values for anaemia (H), parasitaemia (P), and antibody titre (AT) and their relationships to challenge and treatment.

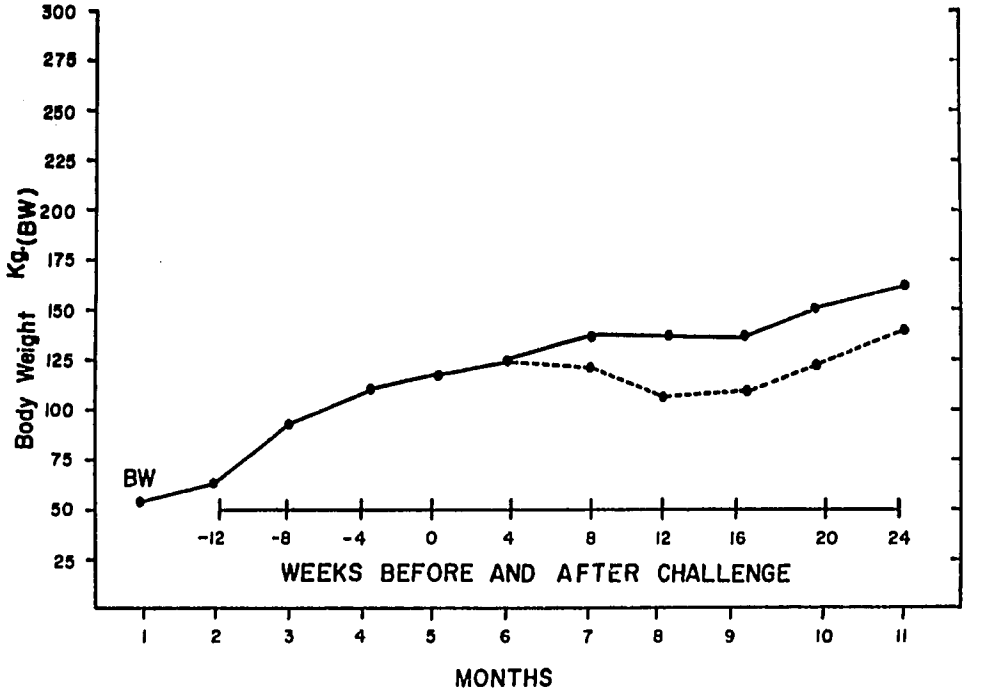

FIG. 3. Average body weight of vaccinated calves with killed-*Babesia* AG-S vaccine (continued line) in comparision with non-vaccinated control calves (dotetd line). in Time of tick-borne *Boophilus microplus* challenge is indicated as week 0. The vaccine was injected 10 weeks before tick-borne challenge. Age of calves is indicated by months on the abscissa.

and a drop of haematocrit values. In order to prevent further complications, all calves were treated with oxytetracycline against anaplasmosis infection. During 18 weeks of observation not a single calf had any evidence of babesiosis infection. No significant differences were observed in haematological or serological responses in calves vaccinated with AG-S adjuvant and non-adjuvant vaccines. Twenty to 60 ticks were found on each calf during field exposure, and these ticks were identified as *B. microplus* and *Dermacentor nitens*.

Five to six weeks after field challenge with *B. microplus* infected ticks, all calves in Group C and D also had signs of acute anaplasmosis infection which was evidenced by 2 per cent *A. marginale* parasitaemia, and only 1 calf (Group D) and 2 calves (Group C) had signs of babesiosis infection, which was evidenced with *B. bigemina* and *B. argentina* parasitemia. All calves (Group C and D) were treated against anaplasmosis with oxytetracycline to prevent losses; however, in spite of treatment two calves died from acute anaplasmosis which was confirmed by necropsy findings.

Complement-fixing antibody titre were not statistically significant among the four groups of vaccinated calves, in comparison with the non-vaccinated control group of calves. All animals responded with rising complement-fixing antibodies titres of 1 : to 1:160 and the titres persisted during the time of experimentation. Average daily weight gains of calves were only statistically significant from the control non-vaccinated calves for Groups A & B. All other Groups of calves did not have significant differences in daily weight gain from control calves at 10 months of age, when the experiments were terminated.

Immune Responses of Non-vaccinated Control Calves to Tick-Borne Challenge:

The results of haematological and serological immune responses of non-vaccinated control calves to tick-borne *Boophilus microplus* field challenge are shown in Fig. 2. At the time of tick-borne challenge all five calves were in good physical condition which was evident by normal haematocrit values of 33 per cent. However, after two to five weeks after exposure to naturally infected ticks with *B. bigemina* and *B. argentina* all calves were found infected with these organisms. Two calves died due to acute babesiosis and the further death losses were prevented by specific treatment with Ganaseg (Berenil). At the time of acute infection, the average haematocrit values were approximately 12 per cent. Two weeks after the babesiosis attack clinical signs of anaplasmosis were evident. *Anaplasma marginale* parasites were detected in blood smears stained by Giemsa, and the low haematocrit values were noted. Parasitaemia with *A. marginale* was 0.2 to 2 per cent average during four to eight weeks after field exposure. All animals were specifically treated with Oxytetracycline 12 mg/kg to prevent death losses due to anaplasmosis. Complement-fixing antibody responses were not statistically significant between control and four groups of vaccinated calves.

Responses of Vaccinated Calves in Body Weight

The effect of vaccination against bovine babesiosis with killed-*Babesia* spp. vaccines (AG-S) in body weight of vaccinated cattle is shown in Fig. 3. The first difference in body weight was evident after four weeks at field challenge between control and vaccinated calves. Thereafter, the average daily weight gains were significantly different as a result of resistance to *B. bigemina* and *B. argentina* infections. Vaccinated animals with AG-S vaccine gained 25 kg of body weight more than non-vaccinated control calves during the 11 months of observations.

DISCUSSION

Control of bovine babesiosis involves either control of the tick vectors by dipping or premunition of cattle by injecting them with infected blood followed by specific chemotherapy to prevent post-injection reactions. Premunition is somewhat hazardous in itself due to perpetual infection, and losses without aftercare might be considerable (Rick, 1968). This work introduces a new approach to control of bovine babesiosis in susceptible cattle with production of sterile immunity. This procedure might be of practical application in countries where babesiosis is enzootic or in the tropical zones of the world where cattle are at the permanent risk of exposure to babesiosis (Todorovic *et al.*, 1969). This type of immunity will protect them until they develop solid acquired resistance.

The theoretical concept for these experiments was based on the possibility that sterile immunity might be induced against bovine babesiosis (Callow, 1967; Mahoney, 1967; and Mahoney and Goodger, 1972). Although sterile immunity is not usually encountered in blood protozoan infections according to Neitz 1957), it does, however, occur naturally in *Theileria parva* infections. Sterile immunity was reported in malaria in rats which had recovered from an infection with *Plasmodium berghei* (Corradetti, 1950); however, attempts to produce a sterile immunity with killed *Plasmodium* parasites had resulted in only partial protection. Following immunization of rats with crude *P. berghei* antigens, Zuckerman *et al.* (1965) obtained a moderate degree of resistance; whereas, Corradetti *et al.* (1966) did not. The inconclusive results obtained with killed vaccines have been attributed to the loss or denaturation of a soluble antigenic fraction during the preparation of the vaccines. Furthermore,

the killed parasites may have been too rapidly destroyed in the host to elicit a discernible protective effect. Sinton (1939) postulated that the amount and the rate of development of immunity to malaria may depend upon the amount and duration of antigenic stimulation. It has also been demonstrated following experimental infection and cure in a number of mammalian and avian malaria (Corradetti, 1950). Some evidence shows that *P.berghei* infected mice cured by drug therapy become resistant to a challenge infection (Box and Gingrich, 1958).

Todorovic *et al.* (1967) reported sterile immunity in avian malaria after inoculating birds with ex-plasmodial antigens isolated from the serum collected from acutely infected birds with *Plasmodium gallinaceum*. Vaccinated birds developed a high degree of immunity and survived a high challenge dose of parasite, although these birds developed parasitaemias but not other pathological signs of disease. A disease comparable in some aspects with malaria is bovine babesiosis, so the experience obtained in developing a resistance against *Plasmodium* could be exploited in developing a resistance against *Babesia* infection since the mechanism of immunity in both blood infections is similar.

The principal facts of resistance immunity which are encountered in infection with *Babesia* spp. are: (1) natural resistance, (2) innate species resistance, (3) idiopathic resistance and (4) acquired resistance or immunity. The acquired immunity in bovine babesiosis can be defined as the immune state of an animal resulting from exposure to *Babesia* spp. parasites. If *Babesia* parasites are used to infect an animal and specific chemotherapy is administered shortly thereafter to destroy the infectious agent, the residual immunity which persists after the haemoparasite has been eliminated was called sterile immunity. The second type of sterile immunity results from exposing a susceptible *Babesia*-free animal to killed Babesia organisms or their antigens.

Not many *Babesia* spp. preparations have been investigated as vaccine materials in the past. In general, two sources of antigenic materials were reported: (1) the infected erythrocytes and (2) plasma collected from acute infection Ferris *et al.* (1968), Ristic *et al* (1971). The preparations were obtained by treatment of infected blood by a variety of physical and chemical process including among them, irradiation, lysed by saponin, freezing and thawing, heat inactivation, and from infected plasma by ammonium salts precipitation, column chromatography and differential centrifugation. The freeze-dried technique, one of the simplest procedures, appears to have been the least investigated.

Studies of the production of killed *Babesia* spp. vaccines have been complicated by the intracellular habitat of the *B. bigemina* and *B. argentina* parasites and the lack of an *in vitro* system for propagation and cultivation Gonzalez *et al.* (1971). The mechanism of immunity to *Babesia* infection is not clear, and it has not been definitely ascertained whether the host recognized the parasitized erythrocytes or the parasite alone. Although *Babesia* antigens have been detected free in the plasma of acutely infected animals and in the infected erythrocytes, the role of these antigens in production of sterile immunity has not been investigated.

The results of vaccination with killed *Babesia* spp. vaccine at Palmira, Colombia suggest the possibility of using it in zones where bovine babesiosis occur; however, conditions must be such that the introduction of infected ticks must be controlled so that the exposed animals with sterile immunity develop consequently co-infectious immunity. Results of experiments in Palmira indicated that calves vaccinated with killed *Babesia* spp. vaccine (AG-S) developed a resistance to field challenge with *B. microplus* ticks infected with *B. bigemina* and *B. argentina*, while non-vaccinated calves suffered a clinical babesiosis under the same field conditions. Vaccine AG-S

has proven to stimulate better protection than killed *Babesia* spp. vaccine (AG-E) produced from the infected erythrocytes. Vaccination with the killed *Babesia* AG-S vaccine, or without adjuvant, produced a characteristic complement-fixing antibody response with no parasitaemina, and a slight reduction in haematocrit values.

Following tick-borne challenge with *B. microplus* naturally infected with *Babesia* spp., no losses occurred in the vaccinated calves; however, non-vaccinated control calves suffered acute babesiosis and needed specific treatment to survive. Following field challenge, difficulties were encountered with *A. marginale* infection resulting in some death losses. Although some vaccinated calves with AG-E vaccine suffered clinical anaplasmosis and three died in these groups, the results of vaccination are somewhat inconclusive; however, evidence of resistance observed in calves vaccinated with killed *Babesia* spp. vaccines clearly indicates the existence of sterile immunity. The degree of resistance was different between calves which received the AG-S vaccine and those the AG-E vaccine. In calves injected with the AG-S vaccine, the degree of resistance was more pronounced. This was evidenced by the lack of detectable parasitaemias in all these animals.

The protection of cattle against *Babesia* infection produced in a response to killed *Babesia* vaccines demonstrated the importance of humoral factors in the mechanism of acquired immunity. Although bovine babesiosis is a disease in which immunity almost certainly depends on the presence of infection, the demonstration of the presence of protective antibodies to *B. bigemina* and *B. argentina* in the serum of infected cattle was regarded as evidence that the humoral mechanism was also an important factor in acquired immunity to babesiosis (Mahoney, 1967). The possibility of stimulating the production of protective antibody by vaccination is supported by this investigation. A suspension of infected erythrocytic lysate and plasma were freeze-dried and used as the vaccine preparations. Freund's complete adjuvant was used as a vehicle for the intramuscular administration of killed *Babesia* spp. vaccines to cattle, because in similar experiments with *B. argentina* a greater degree of protection had been stimulated (Mahoney, 1967). However, our results indicate that killed *Babesia* AG-S vaccine alone is highly immunogenic.

The freeze-dried inactivated *B. bigemina* and *B. argentina* as described here is non-infective and capable of imparting a level of protection against tick-borne babesiosis. These findings are similar to those obtained by Mahoney (1967) and Mahoney and Goodger (1972) involving immunization by killed *B. argentina* vaccine. The type and degree of protection against *B.bigemina* and *B.argentina* in cattle was essentially the same as that reported for calves following vaccination with killed *B. argentina* vaccine (Mahoney and Goodger, 1972).

In all experiments with intact, 5-month-old calves, the animals injected with killed *Babesia* spp. vaccine showed a higher degree of resistance to subsequent field challenge than the controls. This was particularly evident in the first (Group A) and second (Group B) experiments in which calves showed little or no general clinical signs after challenge. In contrast, the controls were obviously sick during the acute phase of the disease and three deaths occurred. The difference in the amount of erythrocytic destruction as indicated by reduction of haematocrit values between the immunizing and control groups was significant and this was the most reliable measure of protection.

None of the non-vaccinated cattle were protected against field challenge. All unprotected animals developed progressive severe diseases characterized by high parasitaemias and severe anaemias. The vaccine protection appears to be caused by

specific *Babesia* antigens, however; many of the factors involved in immunization against the blood stage of *Babesia* vaccination are still in their earliest stages of research. The studies described here promise to contribute much to these facets of *Babesia* vaccination technology and mechanism of immunity.

Degree of resistance produced by killed *Babesia* spp. vaccines is not the same mechanism as in co-infectious immunity, but the sterile immunity is sufficient to protect cattle from acute diseases until the animal's body develops solid immunity and maintains it by regular superinfection with infected ticks. The importance of frequent superinfection to promote adequate protective antibody levels is even necessary in cattle in artificially induced co-infectious immunity (Mahoney, 1967).

The protection afforded to cattle by injection of killed *Babesia* AG-S vaccine alone was the same as with the adjuvant vaccine. The successful immunization of intact calves (Group B) without adjuvant indicated that the killed *Babesia* spp. vaccine may be immunogenic alone when given by the intramuscular route. Mahoney and Goodger (1972) studied the immunological reactions of calves inoculated with plasma collected from acutely infected calves with *B. argentina*. Their study was directed to ascertain the general nature of the antigen contained and its possible role in the development of acquired immunity. The authors found that complete protection, which was characterized by an abrupt removal of parasites from peripheral blood, was found only in experiments in which the parasites used to artificially challenge immunity were antigenically identical with those that provided for immunization in the infected plasma. This finding was supported with data from our observation.

The results of experiments designed to ascertain the mechanism of sterile immunity to bovine babesiosis strongly suggest that immunity to *B. bigemina* and *B. argentina* in cattle can be produced by injecting cattle with killed *Babesia* spp. vaccines. The protection was more evident in calves injected with AG-S vaccine prepared from plasma collected during the acute stage of infection than from AG-E vaccine prepared from the infected erythrocytes with *B. bigemina* and *B. argentina*.

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Babesioses Bovines: Immunite Sterile aux Infections a Babesia bigemina *et* B. argentina.

Résumé—Des vaccins tués de *Babesia bigemina* et *B. argentina* ont été préparés à partir d'érythrocytes infectés et de plasma infecté récoltés chez des veaux présentant une infection élevée. Le vaccin a été testé, dans les conditions naturelles, chez du bétail colombien, dans la vallée Cauca. 40 veaux agés de 2 mois et demi ont reçu le vaccin tué, et 5 veaux, servant de témoins, n'ont pas été vaccinés. Les veaux vaccinés et les veaux témoins ont été exposés aux attaques de tiques (*Boophilus microplus*) infectées, nées sur le terrain. En se basant sur les résultats obtenus au cours de ces expériences, on constate qu'un degré élevé d'immunité stérile aux infections *a B. bigemina* et *B. argentina* peut être obtenu chez les veaux ayant reçu le vaccin tué. Il apparaît que l'immunité stérile joue un rôle important dans le mécanisme de l'immunité acquise, différent de l'immunité par infections légères res entretenues, telles que la prémunition.

Babesiosis Bovina: Inmunidad Esteril Para Infecciones de Babesia bigemina n Babesia argentina.

Sumario--Se prepararon vacunas con cepas muertas de *Babesia bigemina* y *Babesia argetina* obtenidas de eritiocitos infectados (AG-E) y de plasma infectado (AG-S) obtenidos de terneros agudomente infectados con *Babesia spp.* La vacina fue provada en ganado Colombiano bajo condiciones de campo en el departemento del Valle del Cauca. Se tomaron en total 40 terneros de 2-1/2 meses de edad los cuales recibieron vacinas de *Babesia* muertas, cinco terneros no vacunados sirvieron como controles. El terneros vacunados y no vacunados fueron expuestos a la accion de *Boophilus microplus* infectados. Teniendo en cuenta los resultados obtenidos en esos experimentos, se encontro que un alto grado de inmunidad esteril para *Babesia bigemina* y para *Babesia argentina* puede ser producido en terneros njectados ocn vacunas de *Babesia* muertas. Parece que la inmunidad esteril tiene uno mportancia similar a la inmunidad adquirido por infeccion llamada premunision.