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IMMUNE RESPONSE OF CATTLE VACCINATED AGAINST BABESIOSIS IN COLOMBIA, SOUTH AMERICA

by

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SUMMARY

Attempts to produce co-infectious and sterile immunity in cattle against Babesia infections have been carried out by vaccinating animals with live or killed-Babesia vaccines at Palmira, Valle del Cauca, Colombia (altitude 1000 meters). Immune responses of the vaccinated animals were evaluated by several immuno-serologic methods. The degree of resistance to tick-borne challenge (Boophilus microplus naturally infected with Babesia spp.) was determined by the percentage of recovery to normal parameters used in this study.

According to the experimental design used, a total of 110 animals was divided in 5 experimental groups to ascertain the immunologic responses. The first group consisted of 20 male, 85 kg., Holstein, 3 month-old calves which were premunized with Babesia bigemina, Babesia argentina, and 4 weeks later were exposed to tick-borne (Boophilus microplus) challenge. The second group consisted of 20 male, 95 kg., Holstein, 4 month-old calves subdivided into 4 groups and vaccinated with a killed-Babesia vaccine derived from the erythrocytes and plasma, respectively, of animals acutely

infected with Babesia bigemina and Babesia argentina. The animals were inoculated with vaccine with or without Bacto-Adjuvant Complete H 37 Ra. The third group of 40 male, 80 Kg., Holstein, 3 month-old calves was divided into two sub-groups. The first sub-group consisted of 20 animals which were premunized with Babesia bigemina and Babesia argentina and 8 days later were treated with a new experimental babesiacidal drug. The second sub-group which consisted of 20 animals was simultaneously premunized with Babesia spp. and Anaplasma marginale and later treated with their respective drugs. The fourth group consisted of 20 female, 75 kg., Holstein, 3 month-old calves prophylactically treated with drug No. 4A65 and 3 weeks later exposed to Boophilus microplus naturally infected with Babesia bigemina and Babesia argentina. The fifth group consisted of 10 animals used as controls. Responses to vaccination and tick-borne challenge were evaluated by packed cell volumes, percentage of parasitemia, body temperatures, body weight, complement fixing antibody titers, general physical conditions and percent recoveries after tick-borne challenge. Results in general indicate that resistance to babesiosis can be produced by co-infectious or sterile immunity. Experiments in prophylaxis, based on residual action of the babesiacidal drug, have given consistent and satisfactory results. In the future, it may be possible to develop control programs against bovine babesiosis based on these observations. The present status of these studies will be described.

INTRODUCTION

Bovine babesiosis (Babesia bigemina)was first reported in Colombia by Carrasquilla (1899) and was later recognized as a serious tick-borne disease by Lleras (1908). In 1931, Velásquez identified Babesia argentina and Babesia major for the first time in Colombia. Bovine babesiosis was reported to have a high mortality and widespread incidence in Colombia which depended on the presence and distribution of tick vectors at various altitudes. The mortality was specially high among the imported cattle (Guerrero and Patiño 1933, Virviescas 1934, Velásquez 1938, Román 1945 and Guerrero 1956). At the present, however the exact prevalence is difficult to estimate (Todorovic, Adams and Roberts, 1969).

This paper describes the research carried out at Laboratorio de Investigaciones Médicas Veterinarias directed mainly toward the control of bovine babesiosis in Colombia with special emphasis on the problems encountered in this type of research.

MATERIALS AND METHODS

A total of 110 Holstein-Friesian, male calves with an average age of 150 ± 20 days were used in this study. The experiments were carried out a the Palmira-ICA experimental farm in Valle del Cauca (altitude 1,000 meters). Randomly selected calves, were divided into five groups, according to the experimental design used to evaluate the immunoserological responses. The first group consisted of 20 male, 85 kg., Holstein-Friesian, 3 month-old calves which were premunized with Babesia bigemina and Babesia argentina and 10 weeks later were exposed to tick-borne challenge, (Boophilus microplus naturally infected with B. bigemina and B. argentina). The second group consisted of 20 male, 95 kg., Holstein-Friesian calves, 4month-old which were subdivided into four groups A, B, C, D. Four intact calves in each group were vaccinated with respective vaccines and one splenectomized calf was not vaccinated and used as control. Calves from group A and B were vaccinated with vaccine AG-S with and without Adjuvant Complete-Freund (Todorovic et al, 1968) and calves from group C and D were vaccinated with vaccine AG-E with and without Adjuvant Complete-Freund (Mahoney, 1967), respectively. The third group of 40 male, 80 kg., Holstein, 3 month-old calves was divided into two sub-group. The first sub-group consisted of 20 animals which were premunized with Babesia bigemina and Babesia argentina and 8 days later were treated with a new experimental babesiacidal drug. The second sub-group which consisted of 20 animals was simultaneously premunized with Babesia spp. and Anaplasma marginale and later treated with their respective drugs. The fourth group consisted of 20 female, 75 kg., Holstein, 3 month-old calves prophylactically treated with drug No. 4A65 and 3 weeks later exposed to Boophilus microplus naturally infected with Babesia bigemina and Babesia argentina. The fifth group consisted of 10 animals used as controls. Furthermore, several experiments were conducted to evaluate the prophylactic and therapeutic effects, dosage, route of injection, toxicity and responses of the animals injected with a new Burroughs-Wellcome* babesiacidal drug No. 4A65.

The percentage of parasitemia (P), packed cell volume (PCV), complement fixing antibody titer (AT), body temperature (T) and percentage of mortality (M) were used as the basis for comparing the reaction produced after vaccination and tick-borne challenge. In addition, experiments were carried out to identify the existing Babesia species occurring in Colombia by morphologic and immunoserologic techniques. Attempts were made to develop a sensitive and practical serologic test for detecting Babesia serum antibodies.

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* Burroughs-Wellcome Co., Tuckahoe, N. Y.

Furthemore, experiments were carried out to evaluate complement fixation techniques described by Mahoney (1962) and tube latex agglutination test (Todorovic et al, 1968) for diagnosis of bovine babesiosis.

Some attempts were made to survey the tick population in certain parts of Colombia, particularly where the prevalence of babesiosis is high. Animals infected with Babesia bigemina and Babesia argentina were used as a source of the tick collections from Valle del Cauca, Rio Magdalena and Costa Norte. Ticks were collected from different parts of the animals body and preserved in absolute alcohol. Microscopic examination of the peripheral blood smears from animals infected with Babesia was made to establish infection. The collection of ticks was made throughout the year. Adults, nymphs, and larvae of both sexes were collected for identification purposes.

RESULTS AND DISCUSSION

Animals premunized with blood collected from patent carriers of Babesia bigemina and Babesia argentina developed slight post-inoculation reactions which were manifested by parasitemia and anemia (Fig. 1). After 3 weeks, these animals recovered from the anemia, and parasitemia was not detectable. When the animals were exposed to tick-borne challenge, Boophilus microplus naturally infected with Babesia bigemina and Babesia argentina, they were highly resistant to babesiosis but all of these were infected with Anaplasma marginale. Ticks collected from these animals were identified as Boophilus microplus and Dermacentor nitens.

A control group of non-premunized intact animals was exposed to tick-borne challenge at the same time as the premunized group. These control animals became infected with babesiosis and anaplasmosis after 4 to 10 weeks exposure and 25% of them died (Fig.2). Further losses were prevented by treatment against anaplasmosis and babesiosis. The conclusion can be made that the ticks present carriers of both Babesia spp. and Anaplasma marginale and that effective control of tick-borne disease at this location cannot be made unilaterally.

A second control group of splenectomized animals, used to test the infectivity of the blood used in the premunization experiment, developed acute babesiosis from 2 to 10 days after blood-borne challenge. As a result, 90% of these animals died (Fig.3). Further losses were prevented by 2 additional treatments againsts babesiosis. The data of this experiment show that the blood used for premunization was highly infective. Additional experimentation is needed to determine the minimum amount of blood sufficient to produce infection without severe post-premunization reactions. Experiments designed to answer these questions are now in progress.

Results of the experiments to ascertain the mechanism of sterile immunity, demonstrate the possibility of producing this type of immunity by killed-Babesia vaccine, (Fig. 4 and 5). Because some animals in this experiment died from Anaplasma and helminthic parasites, results are inconclusive and specific conclusions cannot be made at this time. Part of this experiment is currently being repeated. However, results of the experiment with AG-S vaccine plus adjuvant demonstrated a high degree of protection against tick-borne challenge. No losses occurred in the vaccinated animals, however, non-vaccinated control animals died.

On the basis of the observations made with these experiments, conclusions can be drawn that Babesia antigens produce some degree of sterile immunity. To understan the exact mechanism of this type of immunity, more works needs to be done. Degree of resistance and duration of the immunity in relationship to different environmental conditions, strains, pathogenicity of Babesia organisms and quality of tick-borne challenge needs to be determined. Susceptibility and resistance of the host also plays an important role in the tropical areas and needs further study.

At the present, some of the experiments in this report are still in progress in regard to the evaluation of the new experimental drug No. 4A65. Some preliminary results are recorded in Figures 6 and 7. However, on the basis of the data of the experiments terminated, some conclusions can be drawn. The new experimental drug No. 4A65 has prophylactic activity but has also been shown to be toxic. Animals injected with 4A65 up to 46 days before challenge are highly resistant to blood-borne challenge with Babesia spp. Non-injected control animals died when exposed to this same challenge. It was found that toxic symptoms were most pronounced when 4A65 was injected intravenously at a dosage of 3 mg/kg. Two animals of 5 injected died after injection with 3 mg/kg with the following signs of reaction: excessive salivation, lacrimination, difficulty in breathing, urination and defecation. These signs were observed one minute following injection and fatal results occurred within 2 to 10 minutes.

When dosage was reduced to 1 to 2 mg/kg and the route of injection changed from I.V. to I.M., the reaction to 4A65 were much less severe. Since all of the experiments are not completed, conclusions cannot be made as to the specific dosage to be used and to the duration of protection. The experiments now in progress should answer these questions when they are terminated. Animals injected with drug No. 4A65 prophylactically,

show resistance to babesiosis, but not to anaplasmosis. It is concluded that drug No. 4A65 is highly effective against bovine babesiosis.

In January, 1969, ticks were collected from 10 animals at the ICA farm in Palmira. These animals were known to be infected with Babesia bigemina and Babesia argentina. The species of ticks (7 males and 31 females) were identified at the Parasitology Laboratory at the National University in Bogotá as Boophilus microplus. During February and March, 1969, the following ticks were collected from the same animals: Boophilus microplus: 10 females, 10 males and 57 nymphs; Dermancentor nitens: 21 females, 15 males and 29 nymphs. In May, 1969, on 2 private farms in the Magdalena River Valley, 4 female Amblyomma cajennense and 3 female and 2 nymphs of Boophilus microplus were found.

On ICA and private farms in Valle del Cauca, the following species of ticks were identified during September, 1969: 35 females, 6 males and 17 nymphs of Boophilus microplus; 49 females, 72 males and 18 nymphs of Dermacentor nitens. On October, 1969, on the ICA experimental farm in Palmira there were 109 females, 146 males and 161 nymphs of Dermacentor nitens. On the Magdalena River Valley 3 female of Boophilus microplus were found in November, 1969 and in the Fusagasugá area in the same month 2 female identified as Dermacentor nitens were found. Also in November, 1969, at Bugalagrande, Valle del Cauca, 19 males, 18 females and 62 nymphs of Dermacentor nitens, and 2 females of of Boophilus microplus were identified from Hacienda San Marcos. During the month of December, collections were again made near Girardot and Hacienda Lucerna and 75 males, 380 females and 155 nymphs of Boophilus microplus were identified.

From this survey, very interesting observations can be made. Boophilus microplus were present in the enzootic areas of babesiosis and anaplasmosis. This confirms previously published reports that this vector was involved in the epizootiology of infection of Babesia spp. in Colombia. However, the fact that Dermacentor nitens were found on animals known to be infected with babesiosis and anaplasmosis is a very interesting finding and the significance of this is not evident. It is well known that Dermacentor nitens has as its hos the horse, and it has been reported as a vector of equine babesiosis in the United States. The question remains is the bovine an accident host of Dermacentor nitens and, if not what is the role of this tick in the transmission of bovine babesiosis? These questions must be answered by additional experimental work to clarify this relationship before a control program of tick vectors can be designed.

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