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A Thesis

by

FLOYD MILTON JONES

Submitted to the Graduate College of the
Texas A&M University
partial fulfillment of the requirement for the degree of

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December 1972.

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CONTROL OF ANAPLASMOSIS AND BABESIOSIS IN YOUNG CATTLE

A Thesis

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ABSTRACT

Control of Anaplasmosis and Babesiosis in Young Cattle

(December 1972)

Floyd M. Jones, B.S., D.V.M., Texas A&M University

Directed by: Dr. L. C. Grumbles

A study was conducted on the control of anaplasmosis and babesiosis in young cattle in Colombia. Three groups of 10 calves were used at each of 3 different climatic and geographic areas. One group was vaccinated with an attenuated *Anaplasma marginale* vaccine and a killed *Babesia bigemina*, *Babesia argentina* vaccine. A second group was injected with infected *Anaplasma marginale*, *Babesia bigemina* and *Babesia argentina* blood that originated from donor cattle from the eastern plains. Five days post inoculation the induced infection was treated by injection of compounds 356C61 (alpha-ethoxyethylglyoxal dithiosemicarbazone) and 4A65 (3,3-Bis-(2-imidazolin-2-yl) carbanilide dihydrochloride). The third group of calves was used as a control. Calves selected for use at Monteria were not native to that area. All calves were subjected to natural exposure. Ticks were collected and identified at each site.

There was no apparent significant difference in weight gains and resistance to anaplasmosis and babesiosis between groups at any site. At Bugalagrande and Girardot the absence of death losses from anaplasmosis and babesiosis in the control groups indicates that the calves had a preexisting natural immunity, an acquired non-sterile

immunity at the beginning of the study, or no challenge during the study. At Monteria, it is apparent that the vaccinated and premunized calves did not develop resistance to anaplasmosis and babesiosis due to the use of antigenically different organisms; the simultaneous injection of the premunization drugs at 5 days post inoculation; the lack of sufficient sterile immunity to suppress tick-borne infection; or the inability of the very young calves to develop sufficient resistance.

The identification of Boophilus microplus ticks at all 3 sites confirms reports of this vector in anaplasmosis and babesiosis enzootic areas of Colombia. The significance of Anocentor nitens ticks on Anaplasma and Babesia spp. infected cattle is not apparent at this time.

As a result of this study, it is concluded that the control of bovine anaplasmosis and babesiosis in tropical areas is more complex than previously recognized. More investigation is needed to obtain information on strain antigenicity of Anaplasma marginale, Babesia bigemina and Babesia argentina; mechanisms of coinfectious immunity; sterile immunity; and the action of chemical compounds tested in this study.

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INTRODUCTION

A large amount of laboratory and field investigation has been erformed on control methods for anaplasmosis and babesiosis as eparate disease entities. However, in many subtropical and tropical reas of the world these diseases often occur concurrently, depending n the geographic distribution of arthropod vectors. In Peru, these iseases seriously limit national efforts to improve the cattle industry.[14,30] In Brazil and Argentina, a cattle disease commonly alled "Tristeza" has been studied extensively.[38] It is actually a ixed infection of babesiosis and anaplasmosis and has been described s an acute or chronic disease. In 1908, Lleras[62] reported on hemoarasites in Colombian domestic cattle. A survey of Colombian animal health records as well as personal observation has revealed the presence of extensive anaplasmosis and babesiosis enzootic areas.[37] According to Kuttler,[29] any ranching area less than 5000 feet elevation should generally be considered an anaplasmosis enzootic zone. Other surveys suggest that this is true for babesiosis as well.[57] Todorovic et al.[60] reinforced this view by a study of the distribution of the tick vectors for these diseases in Colombia.

The eradication of arthropod vectors is impossible in most areas of the world. This becomes evident when it is realized that although the United States cattle industry was suffering over $40

"The citations on the following pages follow the style of the American Journal of Veterinary Research."

million a year in livestock losses from bovine babesiosis, it took from 1906 until the early 1940's to eradicate the vector of this disease.[34] Geographic, economic, and cultural factors would prevent this approach in Colombia and in many other countries. Tick resistance to acaricides has become a recurring phenomenon over the past 20-30 years in South America, Australia, and Africa.[64]

The control of these diseases by means of chemotherapy and care of individual animals is costly,[30] and is not always applicable to ranch management practices in a developing country. Experience to date indicates that effective biological prophylaxis or premunization is probably the most practical and desirable procedure in the control of anaplasmosis and babesiosis.[28,30] Consequently, three different geographical areas and elevations were selected for this investigation with the following objectives: (1) to evaluate and monitor simultaneous anaplasmosis and babesiosis vaccination and premunization procedures under actual field conditions, (2) to evaluate the efficacy of two recently developed compounds in anaplasmosis and babesiosis premunization, and (3) to monitor and identify tick populations under varied conditions.

LITERATURE REVIEW

The hematozoan diseases of anaplasmosis and babesiosis are found worldwide and are of greater economic importance in cattle than in the other species of domesticated animals.[63] In the tropical and subtropical countries, these two diseases are responsible for great economic and productive loss. Although the indigenous cattle usually carry the infection without clinical signs, introduced animals are highly susceptible.[24,39,57,59] It seems reasonable to expect that cattle with an inapparent infection may suffer a decrease in meat and milk production.[59] Also, they are capable of infecting ticks and possibly other vectors thus propagating the disease cycle.[2,11,17,40,42,54,65]

In order to develop the present status of anaplasmosis and babesiosis control, these diseases, though similar in many ways, are discussed separately.

Anaplasmosis is an infectious hematozoan disease of cattle manifested by a rapidly progressing anemia with a syndrome divided into a prepatent period, a period of increasing anemia, maximal anemia, and a period of convalescence.[23,36,46]

The research developments in the field of anaplasmosis began in 1893 when Smith and Kilborne,[53] studying the erythrocytes of clinically sick cattle, observed "intracorpuscular bodies." They considered these coccoid-like bodies to be a stage in the life cycle of <u>Babesia bigemina</u>. Theiler in 1910 started a series of studies on

this organism which he correctly differentiated from Babesia organisms and named Anaplasma marginale.[63] Studies of this disease continued over the years, and in 1951 DeRobertis and Epstein[16] revealed that the "chromatin body" described by Theiler in 1910 was not a compact single entity. Instead, they described it as a number of smaller subunits.

Other significant findings have been made since the early 1950's. Two such developments were the complement fixation (CF) test for diagnosis of anaplasmosis[1,22,35] and that the tetracycline compounds inhibited the growth of Anaplasma organisms.[18,20,23] The CF test was the first reliable test for detecting carrier animals and it has greatly enhanced the study of the serological responses induced by A. marginale. The development of the capillary tube agglutination (CA) test in 1962 provided a relatively simple and effective means of diagnosing anaplasmosis.[44]

The specific staining of Anaplasma with fluorescein labeled antibodies identifies it as the causative agent to which the host produces specific antibodies.[43] Ristic,[46] in 1967, initiated a series of studies which showed that the subunit (initial body) of the marginal body was the primary infectious unit. Its ability to invade mature erythrocytes and multiply in them by binary fission led to the formation of the marginal body. Recently, Summers and Padgett[55] studied the morphologic features and reproduction of initial bodies of A. marginale by negative contrast (phosphotungstic acid) treatment of unfixed, dehemoglobinized, infected bovine erythrocytes. Their

observations indicate that the replication of initial bodies may be a process resembling budding rather than one of equal binary fission. In addition, these workers found that the internal structure of initial bodies could also be organized as a coiled, tubular, or ribbon-like structure. Previously, the internal structure had been described as an aggregation of finely granular material embedded in an electron lucid plasma.[25,46] They suggest that a combination of granular and ribbon-like states apparently indicates the occurrence of a change in organization during growth.[55]

The transmission of anaplasmosis occurs by both biological and mechanical means.[17,46] In biological transmission, the causal organism may persist for days or months in various stages of the tick vector. At least twenty species of ticks are capable of biologically transmitting anaplasmosis under experimental conditions.[54,63] Most ticks of cattle can also act as mechanical vectors.[2,54] Experimental and epizootiological evidence has incriminated horseflies as a significant mechanical insect vector.[65] Many other insects and mosquitoes are potential natural vectors and have been incriminated in experimental studies.[48] In addition to mechanical transmission by insects, transmission by syringe needles and surgical instruments is possible and is reported to have occurred.[17]

The knowledge of the mechanisms of immunity in bovine anaplasmosis is far from complete. Nevertheless, some basic findings concerning immunopathology have been made during the last twenty years. The principle states of immunity (resistance) in infections with A.

marginale are (1) innate species resistance, (2) natural resistance, (3) idiopathic resistance, and (4) acquired resistance or immunity.[46] This project is mainly concerned with acquired immunity since this includes premunization with an attenuated[28,30,45,56] and a virulent[5,18,19,38] Anaplasma organism for the control of this disease.

Acquired immunity in anaplasmosis was defined as the immune state of an animal resulting from exposure to Anaplasma antigens.[46] If Anaplasma antigens were used to infect an animal, and specific chemotherapy[18,20] was administered shortly thereafter to destroy the infectious organism, the residual immunity which persists after the hemoparasites have been eliminated was called "sterile immunity with previous infection." The second type of sterile immunity resulted from exposing a susceptible, Anaplasma-free animal to killed organisms[6,7] or their antigens and was called "sterile immunity without previous infection." The final type of acquired immunity depended on the persistance of a latent infection in animals which survived the initial infection.[19,24] This acquired immunity to reinfection was termed "co-infectious immunity, non-sterile immunity, or premunition."[5,23]

There are many reports of attempts to provide an immune response in susceptible cattle by inoculating them with antigens of killed Anaplasma preparations.[6,7,27] Some of these vaccines are currently on the commercial market. Although vaccinated animals showed some resistance,[23,28] practically all were infected following challenge exposure; a practical sterile immunity against A. marginale was not

produced by this agent.

Ristic[46] suggests that a lasting resistance to anaplasmosis could be established by providing for the constant maintenance of the immunopathological process associated with subclinical infections. This was best accomplished by an attenuated Anaplasma vaccine[28,30,45] or premunization[5,23,38,49] with virulent A. marginale organisms in susceptible animals. This study evaluated both of these procedures simultaneously with babesiosis premunization and vaccination.

Babesiosis, a tick-transmitted disease of animals, is associated with the appearance of the specific infecting protozoa in the erythrocytes. It occurs in many species, but this study was limited to B. bigemina and Babesia argentina which occur in cattle in Colombia.[57,61] Bovine babesiosis is highly pathogenic and may cause high mortality in susceptible cattle.[9] These blood parasites are generally responsible for severe hemolytic anemia.[39] The blocking of the vascular network of various organs by parasitized cells, cell debris, and free parasites may cause an accumulation of toxic metabolic products, local anoxia, and possible breakdown of the blood capillaries and cerebral vessels.[8]

The history of babesiosis dates from the discovery of Babesia bovis by Babes in 1888.[63] Smith and Kilborne[53] in 1893 recognized B. bigemina as the cause of Texas fever and proved that it was transmitted by the bovine tick Boophilus annulatus. Lignieres in 1901 discovered B. argentina and incriminated this organism in bovine babesiosis.[63] Both of the organisms are transmitted by the ixoid

tick Boophilus microplus.[11,40,42,57,60] Both B. bigemina and B. argentina pass from the infected adult female tick through the eggs to the larval ticks. Babesia argentina is transmitted to cattle by the larvae[42] of B. microplus; whereas, B. bigemina is transmitted by the nymphs and adult ticks.[40] Multiplication of the organism in the erythrocytes is usually by budding, giving rise to two to four daughter cells depending on the species.[39]

Immunity to babesiosis in cattle closely parallels that in anaplasmosis.[21,33,39,58] This study involved the use of premunization with virulent Babesia spp. organisms and vaccination with killed Babesia antigens to produce acquired immunity.

It has been shown that B. bigemina and B. argentina in the absence of reinfection usually persist for 10 to 12 months.[21,31] Severe relapses may occur at varying intervals within this period. Cattle which recover from clinically evident babesiosis develop a non-sterile immunity.[9,10,31,39] The duration of this "immune" or carrier state varies considerably, both with the species of Babesia and with the individual animals.[9,10,21] Although most of the resistance produced by an individual animal to babesiosis is the non-sterile immunity, there is some evidence of a graduation from a state of premunition to a state of sterile immunity.[9,15] Riek[39] reports on one animal infected with B. argentina that showed evidence of a sterile immunity. However, the duration of this sterile immunity has not yet been determined. It has become apparent that the state of equilibrium which develops between the host animal and the protozoan

parasite is not permanent.[39] The parasite is ultimately eliminated in the absence of continued reinfection by ticks, after which the animal is again susceptible if reinfection occurs after a certain lapse of time.[41] In a study of immunity to babesiosis, immunological differences in strains (isolates) have been demonstrated under laboratory conditions.[9] These differences also exist in the field, and the transfer of cattle from one enzootic area to another may be followed by a new attack.[10]

In a report on babesiosis in Australia, Todorovic[59] states that outbreaks of babesiosis may be seen under the following circumstances: (1) When susceptible cattle from "clean" areas are taken into, or allowed to pass through tick-infested (B. microplus) areas. (2) When the presence of ticks (B. microplus) is variable. (3) When the tick (B. microplus) population has been reduced to very small proportions by dipping. (4) When cattle are transferred from a "clean" part of a property to a tick-infested (B. microplus) part of the same property. (5) When conditions are especially favorable to tick life (B. microplus). (6) When cattle are removed from one tick-infested (B. microplus) area to another. (7) Following the passage of tick-infested (B. microplus) cattle through a property previously "clean" or only lightly tick-infested. (8) When carriers of Babesia suffer a relapse.

In nature Babesia is transmitted only by ticks.[40,42] The prevention of infection thus depends on the elimination of the tick vector. This is difficult in some countries, but dipping and spraying has worked well in certain areas and this practice has

widespread use.[64]

In most enzootic areas the eradication of the tick vector of babesiosis has given way to the control of this disease. Most of the killed vaccines[10,27,33,58] are of recent development, and more research is currently being done in this field. At the present, control of infection is carried out by the premunization of young or introduced stock. This procedure is also used to prevent outbreaks in marginal areas where tick infestation varies according to climate conditions. Premunization is accomplished by the injection of infected blood and controlling the subsequent infection with one of the babesicidal drugs.[3,4,12,38,50]

Both Anaplasma and Babesia exhibit immunological differences between strains (isolates) of the organisms. Florida and Oregon isolates of Anaplasma have been named as two antigenically distinct blood parasites.[25,26,56] This work was based on serologic, morphologic, immunogenic, and host-specificity experiments with these isolates. In a recent work on the comparison between 2 virulent strains and an attenuated strain, there was no detectable difference between the soluble antigens of the 3 A. marginale strains.[13] However, field studies recently conducted in Colombia evaluating the efficacy of an attenuated A. marginale vaccine in the control of anaplasmosis indicated that the attenuated vaccine may not protect calves against field challenge exposure.[28] In the United States and in Peru, older cattle inoculated with this attenuated vaccine withstood experimental and field exposure against a virulent isolate of A. marginale.[30,45]

The possibility of immunological differences between strains of *Babesia* has been investigated by Callow.[10] He inoculated calves with 1 of 2 strains of *B. bigemina* to produce a co-infectious and a sterile immunity. The calves were subsequently challenged with either the homologous or heterologous strain. His results showed that the calves with a co-infectious immunity were relatively resistance to superinfection with both the homologous and heterologous strains.

However, a greater degree of residual resistance was possessed by cattle challenged homologously. Those animals with sterile immunity subjected to heterologous challenge reacted severely and several died despite treatment.[9] Hence, it is concluded that in any attempt to produce an acquired immunity, the immunological differences between strains of organisms should be taken into consideration.[9,10,28,32,56]

The complexity of the interaction of the host, the blood parasite, and the tick vector suggests that more effective methods of prophylactic immunization are needed.

It logically follows that a more effective control of bovine anaplasmosis and babesiosis would help increase a country's protein food supply.

MATERIALS AND METHODS

General

This study was conducted at 3 different areas and elevations to evaluate bovine anaplasmosis and babesiosis control under actual field conditions. Vaccination and premunization procedures were used and tick populations were collected and identified. Thirty calves were selected at each experimental site and randomly divided into 3 groups of 10 animals each. Each animal was identified by an ear tattoo or by a metal ear tag.

The 3 groups were: Group 1, vaccination; Group 2, premunization; and Group 3, control.

Description of Sites, Animals, and Management Practices

Bugalagrande

A privately owned dairy operation located near Bugalagrande, Colombia in the Cauca River Valley was selected as one experimental site. It consisted of 2 ranches, Lucerna and San Marcos. Hacienda Lucerna was located adjacent to the town of Bugalagrande. The elevation was 955 meters (3132.4 ft.) and the average annual temperature was 24 C. (77.2 F.). The average annual rainfall measured 844 mm. (33.2 in.) per year and the heaviest rainfall occurred in March, April, and May. Hacienda San Marcos was situated 20 kilometers northeast of Bugalagrande at a higher elevation of 1500 meters

(4920 ft.) with an average temperature of 20 C. (68 F.). The highest rainfall occurred in April, May, and June and the yearly average was 750 mm. (29.5 in.).

The type of calves used at these haciendas was a new, unofficial, dual purpose breed developed in this area from the Holstein, Atun de Valle, and Milking Shorthorn breeds. This breed was named Lucerna by the breeders and owners who have been in the process of developing this type of animal for approximately 25 years. The breed was primarily red in color with some white and black spots. The calves used in this experiment ranged from 4 to 8 weeks and weighed from 22 to 41 kilograms. Thirty calves in groups of 10 were selected at random and included 28 females and 2 males.

Two or 3 days after birth, the calves selected for herd replacements were taken from the dams and placed in individual concrete stalls at Hacienda Lucerna. They were fed milk, with and without colostrum, and at 2 to 3 weeks of age the calves were started on a calf pellet concentrate. Calves that developed gastrointestinal problems were treated with sulfamethazine at a dosage of 3 gr./kg. orally. The calves were drenched with Thiabendazole* at a dosage of 100 mg./kg. on October 27, 1969. On December 20, 1969 the animals were moved from the individual concrete stalls to native grass pastures adjacent to the housing barns. On December 23, 1969 the calves were sprayed with Asuntol** for ectoparasites. The experimental animals were

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*Thibenzole, Merck & Company, Rahway, N. J.

**Coumaphos, Farbenfabriken Bayer AG, Leverkusen, Germany.

moved to Hacienda San Marcos on April 22, 1970 and placed on native pastures. The management agreed not to use insecticides on these animals until a sufficient number of ticks were present for field challenge exposure.

Girardot

Hacienda Valencia located in the Magdalena River Valley was 25 kilometers north of the city of Girardot, Colombia. This privately owned dairy and horse farm was at an elevation of 286 meters (936 ft.) where the average annual rainfall measured 1032 mm. (40.63 in.). The average annual temperature was 28 C. (82.4 F.). The highest rainfall was in September, October, November, and June.

The experimental animals used at Hacienda Valencia were calves derived from crossing Brown Swiss bulls on dams of Brown Swiss and Brahaman breeding. The 30 calves randomly selected included 20 females and 10 males, weighing from 29 to 93 kilograms. The animals ranged in age from 3 weeks to 4 months.

After birth, the calves were taken from the dams and placed in a large concrete corral. The calves were nursed daily on nurse cows until 4 weeks of age when they were placed on native pasture. After reaching one month of age, they were fed a ration, once daily, containing cotton seed hulls and a grain concentrate. The calves were treated on October 28, 1969 with Thiabendazole at the dosage of 100 mg./kg. They were sprayed with Asuntol at irregular intervals when the ranch managers considered the ectoparasite infestation sufficient-

ly heavy for treatment.

Monteria

The governmental experimental farm of Instituto Colombiano Agropecuario (ICA) - Turipana, located near Monteria, Colombia on the north coast was the site of one project. This site bordered on the Sinu River and had a tropical climate. The average rainfall was 1404 mm. (55.3 in.) and the average annual temperature was 27.1 C. (80.8 F.). The elevation of this area measured 13 meters (42.6 ft.). The months of highest precipitation were April, May, June, July, and August.*

Thirty Holstein calves from 8 to 12 weeks of age were purchased from a dairy in the Bogota area for use in this experiment. The 25 males and 5 females weighed from 57 to 120 kilograms at the beginning of the experiment.

These experimental animals were maintained on the farm in the Bogota savanna where they were born until they were 7 to 8 weeks of age. At this time, they were delivered to the government farm of ICA-Tibaitata, near Bogota. They were fed a ration containing ground whole corn and silage free choice. On January 9, 1970 the calves were drenched for internal parasites using Thiabendazole at a dosage of 100 mg./kg. of body weight. The 2 groups of animals randomly selected for premunization and vaccination were injected on January 16,

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*All climatic and geographical date from National Meterological Service, Bogota, Colombia and Atlas de Colombia, Instituto Geografico "Agustin Codazzi," Bogota, Colombia, 1969.

1970 and on February 13, 1970 all of the animals were flown to the government farm (Turipana) near Monteria, Colombia for field challenge exposure. The animals were placed on native pasture and fed ground whole corn and silage once a day.

Blood Collection

Blood samples from each animal at each experimental site were collected weekly in tubes under aseptic conditions. Sterile 12 ml. syringes and 18 gauge, 1-1/2 inch needles were used for the venipuncture. After withdrawing the blood from the external jugular vein, 4 ml. were placed into a tube containing disodium ethylenediamine tetraacetate (EDTA) as an anticoagulant and 8 ml. were infused into a tube containing no anticoagulant.

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. The blood tubes were processed for blood smears (slides), packed cell volumes, and serum no later than the day following collection.

Parasitemia

Thin blood smears were made using the Giemsa technique. The films were fixed in absolute methanol and stained with a 1 to 10 solution of Giemsa* for 20 minutes using phosphate buffered water

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*Giemsa Stain, Gradwohl Laboratories, 3514 Lucas Avenue, St. Louis, Mo. 63155.

(pH 7.0 - 7.1) to prepare the stain solution. Each prepared smear (slide) was examined under the oil immersion lens of a light microscope at 1000 X magnification. Twenty fields per slide were examined for parasitized red blood cells and the percentage of parasitemia calculated on the basis of the number of infected red blood cells in relation to the total number of cells in each field.

Packed Cell Volume

Packed cell volumes were determined by the microhematocrit methods as outlined by Schalm.[51] 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 5 minutes at 11,500 rpm and the packed cell volumes read directly from a calibrated tube reader.***

Serum Collection

Serum samples were collected from all calves weekly for the complement fixation (CF) test and microtiter procedures. Whole blood was collected as previously described, allowed to clot, and centri-

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*Capillary Tubes, I.D. 1.1 - 1.2 mm., Fisher Scientific Company, Pittsburg, Penn.

**International Micro-Capillary Centrifuge, Model MB, International Equipment Company, Needham Hts., Mass. 02194.

***International Micro-Capillary Reader, International Equipment Company, Needham Hts., Mass. 02194.

fuged* at 2,500 rpm for 20 minutes. Approximately 3 ml. of serum was decanted from the centrifuge tube into a 5 ml. screw cap vial. These vials were stored at -20 C. until the CF test and microtiter procedure were completed.

Serology

Serum samples collected and stored from each experimental calf were screened for the presence of complement fixing antibodies of *Anaplasma* and *Babesia*. The complement fixation test for *Anaplasma* was performed as recorded in the standard USDA procedures.[1] The CF test employed to test *Babesia spp.* antibodies was a modification by Todorovic *et al.*[61] of the original test by Mahoney.[31] *Anaplasma* and *Babesia spp.* CF serum antibody levels were determined by a microtiter procedure similar to the technique described by Hidalgo and Dimopoullus.[23]

Antigen used in the CF test for anaplasmosis was obtained from the United States Department of Agriculture.** *Babesia* CF antigen was obtained by the method described by Todorovic *et al.*[61] Six month old, splenectomized, Holstein calves were infected with pure strains of *B. bigemina* and *B. argentina*. When the parasitemias in these calves reached a high level, 3 passages were made through another group of

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*International Centrifuge, Size 2, Model K., International Equipment Company, Needham Hts., Mass. 02194.

**Laboratory Services, Animal Disease Eradication Division, Agricultural Research Service, Washington, D.C.

splenectomized calves. When the parasitemias reached approximately 50% for B. bigemina and 25% for B. argentina, 3 to 5 liters of infected blood was collected in flasks containing EDTA as an anticoagulant. The plasma was separated from the infected erythrocytes by centrifugation (2,500 rpm/20 minutes) and the erythrocytes were washed and centrifuged 2 times in normal saline solution. The washed erythrocytes were suspended in 10 volumes of veronal buffer (pH 7.2 - 7.4). A volume of 150 ml. of suspended erythrocytes was placed in an ice water bath (3-5 C.) on the sonifier* for 6 minutes at a power of 50 watts. The pooled, sonified erythrocytes were centrifuged** for 30 minutes at 15,000 rpm (27,000 g.). The supernatant (Supernatant I) was discarded and the sediment (Sediment I) was resuspended in veronal buffer. Sediment I was homogenized in a Ten Broech homogenizer*** for 3 minutes and recentrifuged in the Sorvall centrifuge. This supernatant (Supernatant II) was saved and used in the CF test as Babesia antigen A. Sediment II was resuspended, homogenized, and centrifuged as in the process above. This process was repeated to obtain antigens B, C, and D. These antigens were titrated according to the procedure described in Table #4 of the standard USDA procedures.[1] It was determined that antigenic fractions A, B, and C combined needed to be

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*Sonifier Cell Disruptor, Model W185, Heat Systems-Ultrasonics Inc., Plainview, N. Y.

**Sorvall Superspeed, RC2-B, Automatic Refrigerated Centrifuge, Ivan Sorvall Inc., Newtown, Conn.

***Crusher, Tissue Grinder, Ten Broeck Homogenizer, with Pour-out, Pyrex Brand, Fisher Scientific Company, Pittsburgh, Penn. 15219.

diluted 1 volume to 2.9 volumes of veronal buffer to contain 2 antigenic units per 0.5 ml. Antigen thus obtained was stored at -60 C. in 10 ml. ampules.

Weight of Calves

The weight of each experimental 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. Weights were recorded before vaccination and premunization and at monthly intervals for the first 3 months of the experiments. Thereafter, weights were recorded every 2 weeks until the termination of each experiment.

Collection and Identification of Ticks

Ticks were collected from the calves at each site at varying time intervals when the ectoparasite control program and the tick load permitted sufficient numbers to build up. Five calves were selected at random at Bugalagrande and Girardot and all of the ticks on each calf were collected. The same procedure was followed at Monteria except only approximately 10% of the ticks were collected from each calf.

The ticks were preserved by freezing to -54 C. in dry ice or by immersing in tubes of absolute alcohol. Identification was done at Universidad del Valle, Cali, Colombia, or at Universidad Nacional, Bogota, Colombia.

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*Metric calibrated spanish language cattle weighing tape, Nasco, Fort Atkinson, Wis.

Mortality and Necropsy

Because of the distances between experimental sites and the time involved in reaching these sites, it was impossible to necropsy every animal that died during the course of this study. However, clinical observations and necropsies on all but 3 of the dead animals were made by ICA veterinarians and by the Texas A&M staff veterinarians.

Experimental Organisms and Procedures

Vaccination Procedures

The experimental calves designated as Group 1 at each site were inoculated (injected) with an attenuated A. marginale vaccine[45] produced by Diamond Laboratories.* This vaccine had been stored in 25 ml. vials at -65 C. It was transported to the experimental sites in a frozen state with dry ice. The frozen vaccine was thawed by dipping the vials in lukewarm tap water for 4 to 6 minutes. The vial was then manually agitated and 5 ml. were administered subcutaneously in the neck of each calf in Group 1.

The same calves (Group 1) received two injections of a killed B. argentina and B. bigemina vaccine produced by Todorovic et al.[58] at the Laboratorio de Investigaciones Medicas Veterinarias (LIMV) facility at Bogota, Colombia. This vaccine was produced by the injection of pure strains of virulent B. bigemina and B. argentina into splenectomized calves. When the Babesia spp. parasitemia reached approximately

*DL - AMVC; Diamond Laboratories, Inc., Des Moines, Iowa.

40% in these calves, their blood was collected in flasks containing EDTA as an anticoagulant. The plasma was separated from the infected erythrocytes by centrifugation (2,500 rpm/20 minutes). The infected erythrocytes were pooled and washed 3 times in normal saline. These erythrocytes were lysed by freezing (-20 C.) and thawing 2 times. Twenty ml. of this lysate were injected intramuscularly into a splenectomized calf. It was found that the Babesia parasites were killed by the above procedure.

The vaccine was stored at -45 C. and then transported frozen with dry ice. At the farm, 10 ml. of Freund's complete adjuvant* were added to 30 ml. of vaccine and the vials were thawed by agitation under running tap water. Ten ml. of this vaccine-adjuvant mixture were injected intramuscularly in the hip of each calf in Group 1. The first injection of killed Babesia spp. vaccine was made at the same time as the single injection of attenuated A. marginale vaccine. On day 14 a second injection of this Babesia spp. vaccine was made in the same manner. This completed the vaccination procedure for the vaccinated calves at all locations.

Premunization Procedures

The organisms used for premunization in this project at each experimental site were isolates of A. marginale, B. argentina, and B. bigemina obtained from infected cattle in Los Llanos Orientales

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*Freund's Complete Adjuvant, Difco Company, Detroit, Mich. 48232

(the eastern plains) of Colombia. These isolates were maintained in intact carrier Holstein-Friesian bulls at LIMV in Bogota.

Infected blood from these carriers to be used for premunization was collected under aseptic conditions. It was placed in sealed vials containing 12% sodium citrate solution (0.5 ml. per 10 ml. blood) as an anticoagulant. This infected blood was either inoculated immediately after collection or cooled to 4 C. and held at this temperature until used. The elapsed time from collection to injection was no more than 6 hours at any of the experimental sites.

On day 0, Group 2 experimental calves were injected subcutaneously with 5 ml. of Anaplasma infected blood and 5 ml. of Babesia spp. infected blood. On day 5, following the injection of the infected blood, each Group 2 calf at each site was treated with Burroughs Wellcome drug* 356C61 (alpha-ethoxyethylglyoxal dithiosemicarbazone) intravenously at the dosage of 5 mg./kg. of body weight and Burroughs Wellcome drug 4A65 (3,3-Bis-(2-imidazolin-2-yl) carbanilide dihydrochloride) subcutaneously at the dosage of 1 mg./kg. of body weight.

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*Burroughs-Wellcome and Company, Raleigh, No. Ca.

Treatment Schedule

GROUPS	VACCINATION *Anaplasma marginale*	VACCINATION *Babesia spp.* (First)	VACCINATION *Babesia spp.* (Second)	PREMUNITION *Anaplasma* and *Babesia*	PREMUNITION TREATMENT 4A65 and 356C61
	Bugalagrande				
1	10-27-69	10-27-69	11-10-69	--------	--------
2	--------	--------	--------	10-27-69	11-1-69
3	--------	--------	--------	--------	--------
	Girardot				
1	11-18-69	11-18-69	12-1-69	--------	--------
2	--------	--------	--------	11-18-69	11-23-69
3	--------	--------	--------	--------	--------
	Monteria				
1	1-16-70	1-16-70	1-30-70	--------	--------
2	--------	--------	--------	1-16-70	1-21-70
3	--------	--------	--------	--------	--------

Challenge

A preliminary survey[29] suggested that any ranching area less than 5,000 feet elevation should generally be classified as an enzootic zone for anaplasmosis and probably for babesiosis also. All of the outlined experimental sites were located within this classification and it was decided to subject the experimental calves to field exposure. It was impossible to determine the exact date of challenge at each site as this depended on factors such as date moved to pasture, temperature, humidity, and vectors present. However, field challenge was possible when the calves were moved into an enzootic zone or when moved to native pasture. At Bugalagrande, the calves were moved from individual concrete pens to adjacent pasture on December 20, 1969. The calves near Girardot could be considered under possible field challenge from the time they were two weeks to one month of age. At Monteria, the calves were placed in an enzootic area with many vectors at the time they were unloaded from the airplane on February 13, 1970.

Duration of Experiments

All of the experiments were scheduled to continue for a duration of 6 months. It was thought that this would provide adequate time for field challenge exposure. The experiment at Bugalagrande was continued for an additional 4 months when only small numbers of arthropod vectors were noted during the first 6 months.

RESULTS

The injection date of the vaccines and infected blood is shown on each figure as week 0. Time of possible tick-borne (B. microplus) exposure is indicated on each figure by an arrow. Group averages and standard deviations are recorded for packed cell volumes (PCV) and weight gains. A logarithmic transformation of the reciprocals of the CF serum titers were averaged to determine group values. Anaplasma marginale, B. bigemina, and B. argentina parasitemias are recorded as the percentage of the animals within each group that show any parasites in the blood films (slides).

Bugalagrande

The results of the experiments conducted at Bugalagrande are shown in Figures 1 to 7 and in Table 1.

Two weeks prior to inoculation of the calves at this site, the packed cell volumes (PCV) of all 3 groups were approximately the same. The vaccinated calves (Group 1) showed an average PCV of 34% (±3); the premunized calves (Group 2), 37% (±4); the control calves (Group 3), 36% (±5). These differences were not statistically significant. After performing vaccination and premunization procedures, animals in all 3 groups developed a slight degree of anemia as indicated by the lower PCV. Eight weeks after inoculation all animals were exposed to field challenge by placing them on natural pasture infested with B. microplus. The calves in the control group had the lowest PCV (24%) at 35 to 40 weeks after beginning the experiment. Premunized and vaccinated

calves had an average PCV of 27% at this time. The differences were not statistically significant. Packed cell volume differences were statistically significant ($P<0.01$) at weeks 16, 18 and significant ($P<0.05$) at weeks 14, 15 (Fig. 2). Even though significant differences occurred between Groups 1 and 2, comparisons of these treated groups to the untreated control group were not significant.

One week before initiating the study, the calves in Group 1 weighed 53 kg. (±8), Group 2 weighed 46 kg. (±6), and Group 3 weighed 34 kg. (±6). Although these differences were significant, weight gains of the 3 groups after 39 weeks were essentially equal (Fig. 4).

The results of the CF test for anaplasmosis from week 13 to 25, with approximately 50% of the group reacting, showed that the premunized calves (Group 2) responded with a CF antibody titer of 1:14 at week 15 and 1:18 at week 25. However, the reacting calves in the group had an average CF titer of 1:190 at week 15. The vaccinated and control calves had very low antibody response during the course of the study (Fig. 5).

The premunized calves (Group 2) had the largest number of animals that showed parasitemia. From 12 to 18 weeks after premunization, these calves had 40% of the group with parasitemia. In comparison with this 20% of the vaccinated calves had parasitemia during this time and the control calves (Group 3) showed no parasitemia. At the end of the experiment, the vaccinated calves had 60% of the group with parasitemia, the premunized calves had 20%, and the control calves had 10% with parasitemia (Fig. 6). Using all of the calves in each group

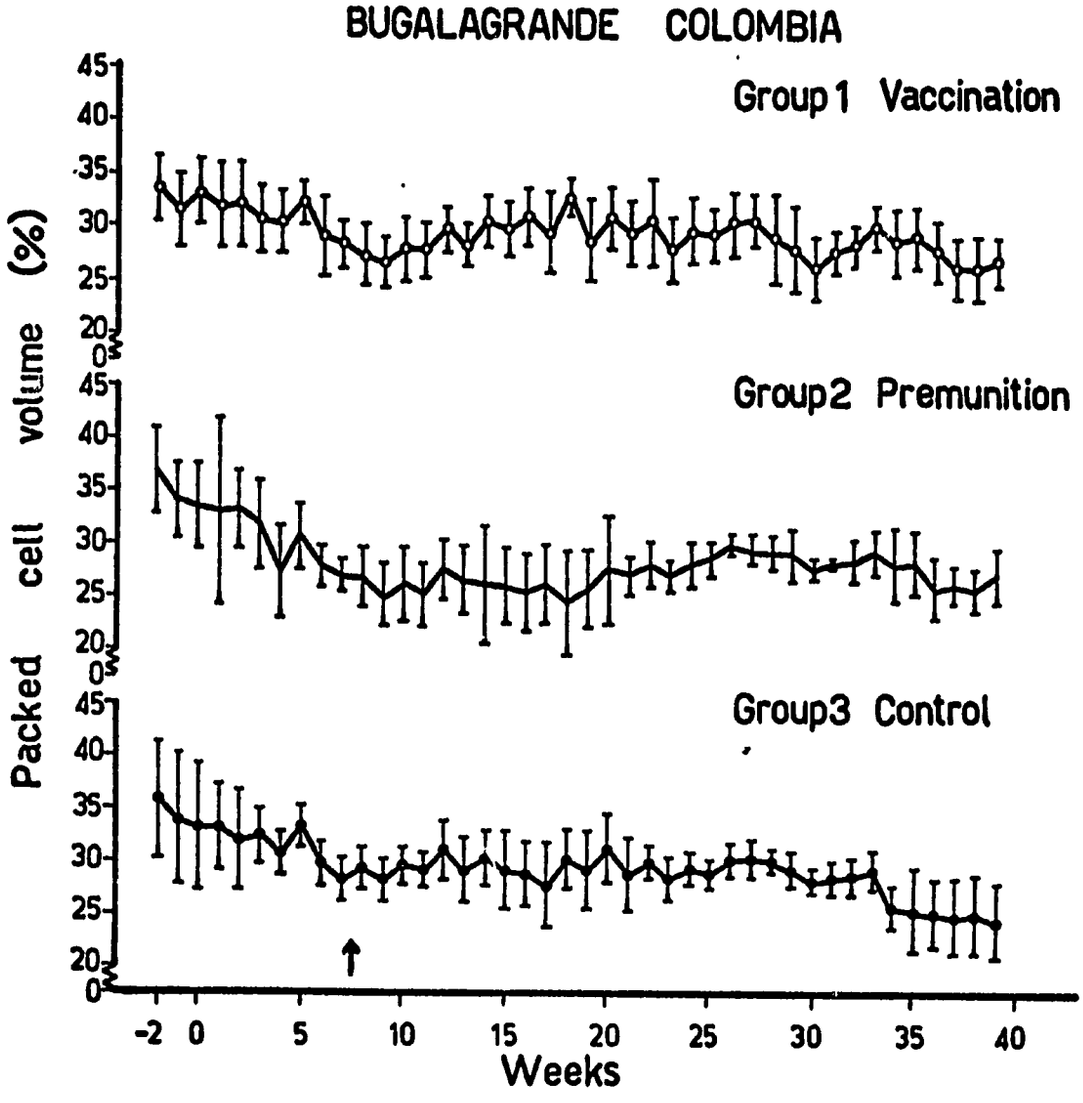

Fig. 1. Bugalagrande. Packed cell volumes by groups with standard deviations. Group means are presented and vertical lines represent ± standard deviation.

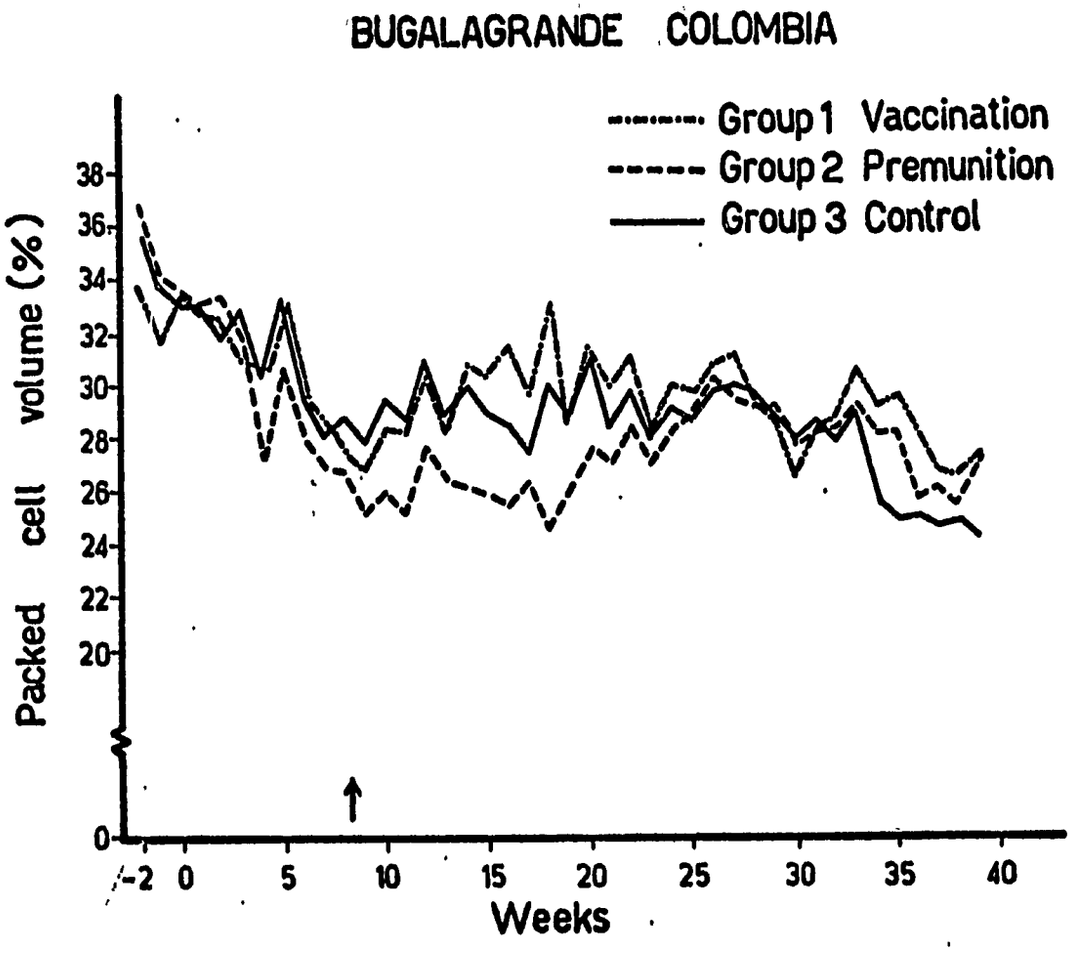

Fig. 2. Bugalagrande. Packed cell volumes by groups compared without standard deviation.

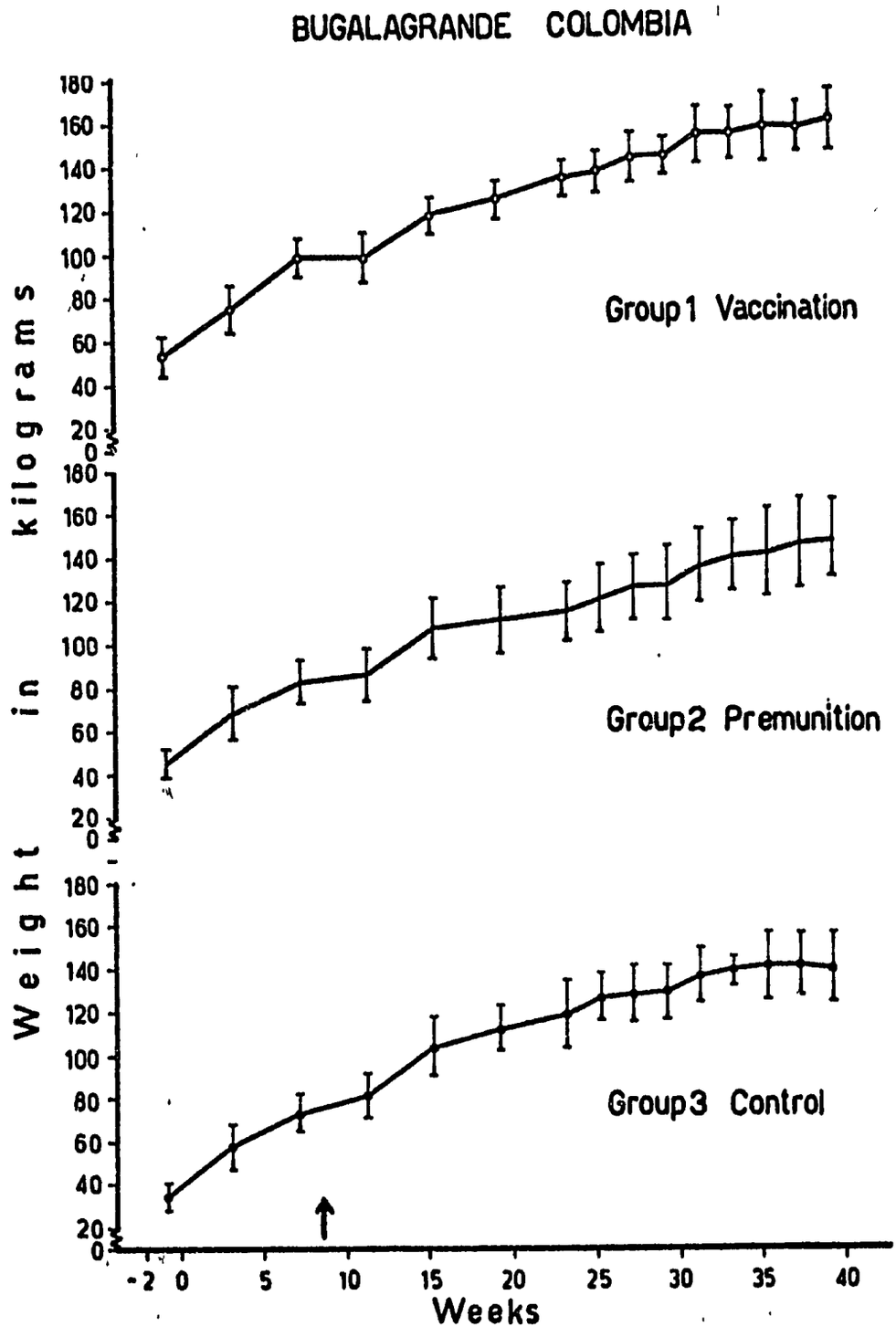

Fig. 3. Bugalagrande. Weight gains by groups with standard deviations. Group means are presented and vertical lines represent ± standard deviation.

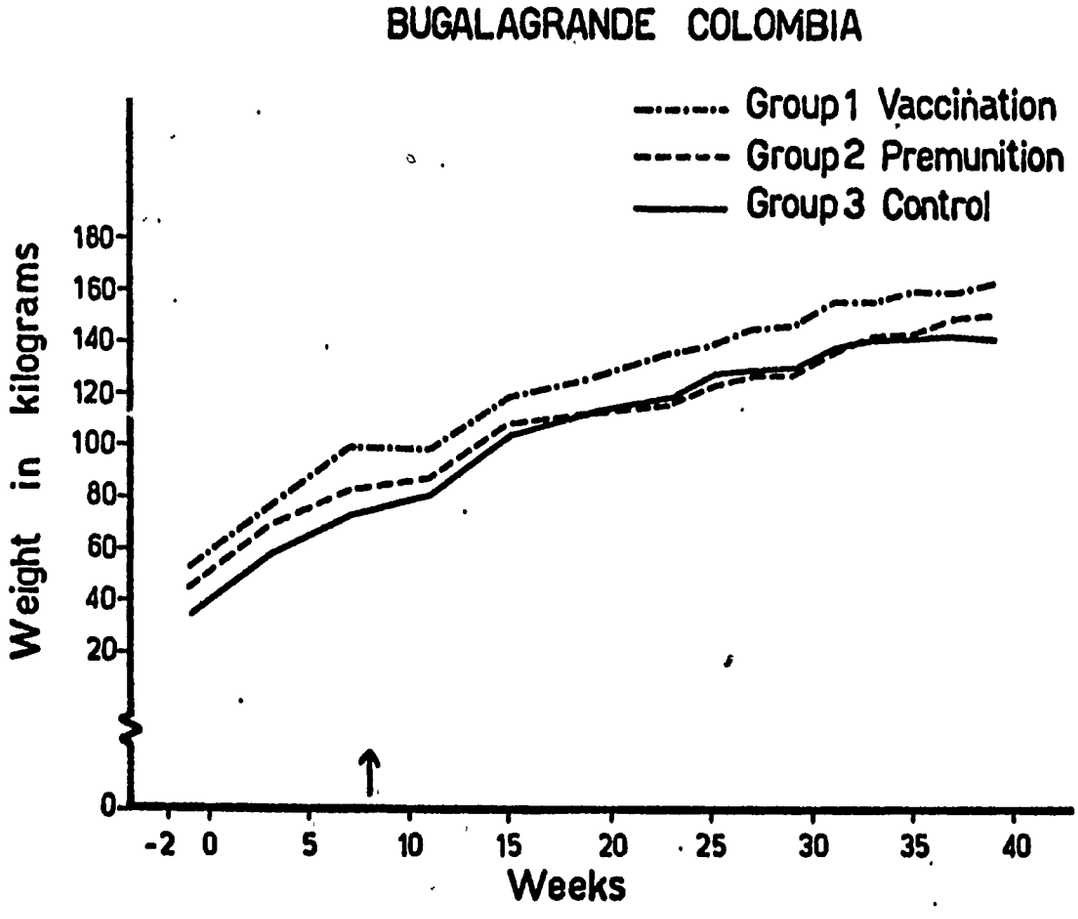

Fig. 4. Bugalagrande. Weight gains by groups compared without standard deviations.

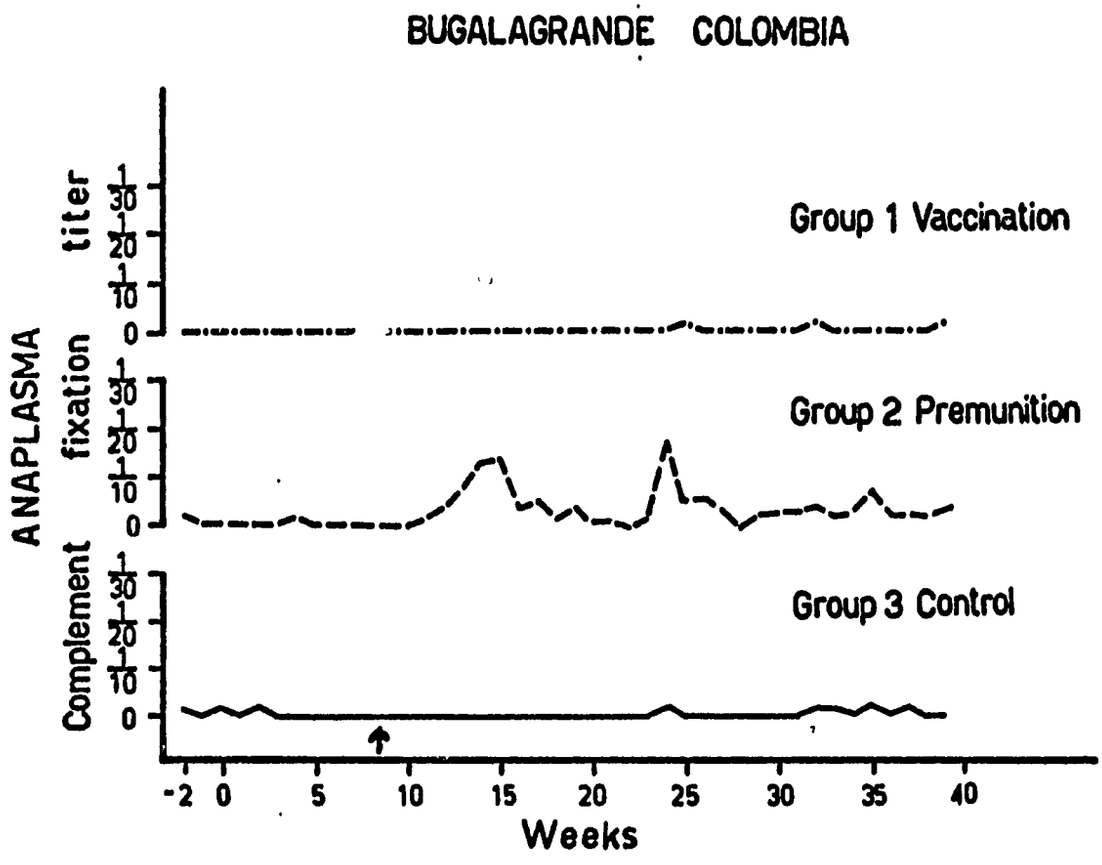

Fig. 5. Bugalagrande. Anaplasma complement fixing antibody titer response by groups.

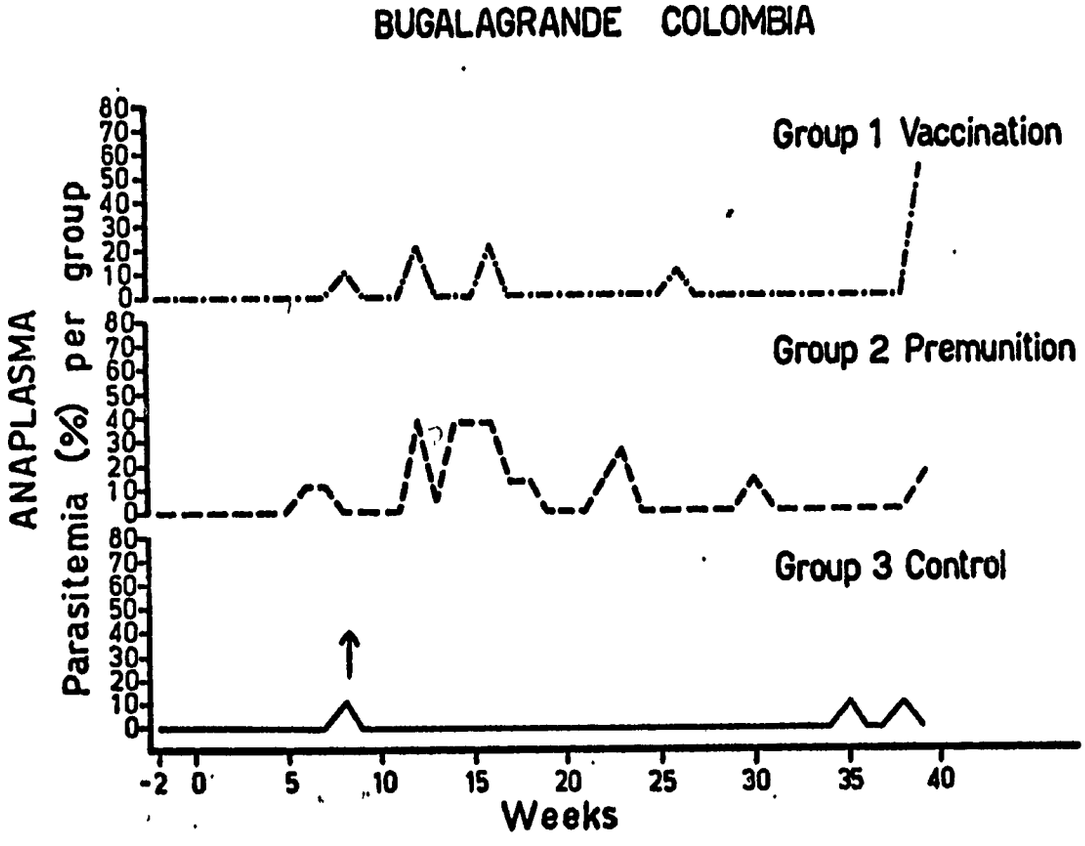

Fig. 6. Bugalagrande. Anaplasma parasitemia by groups.

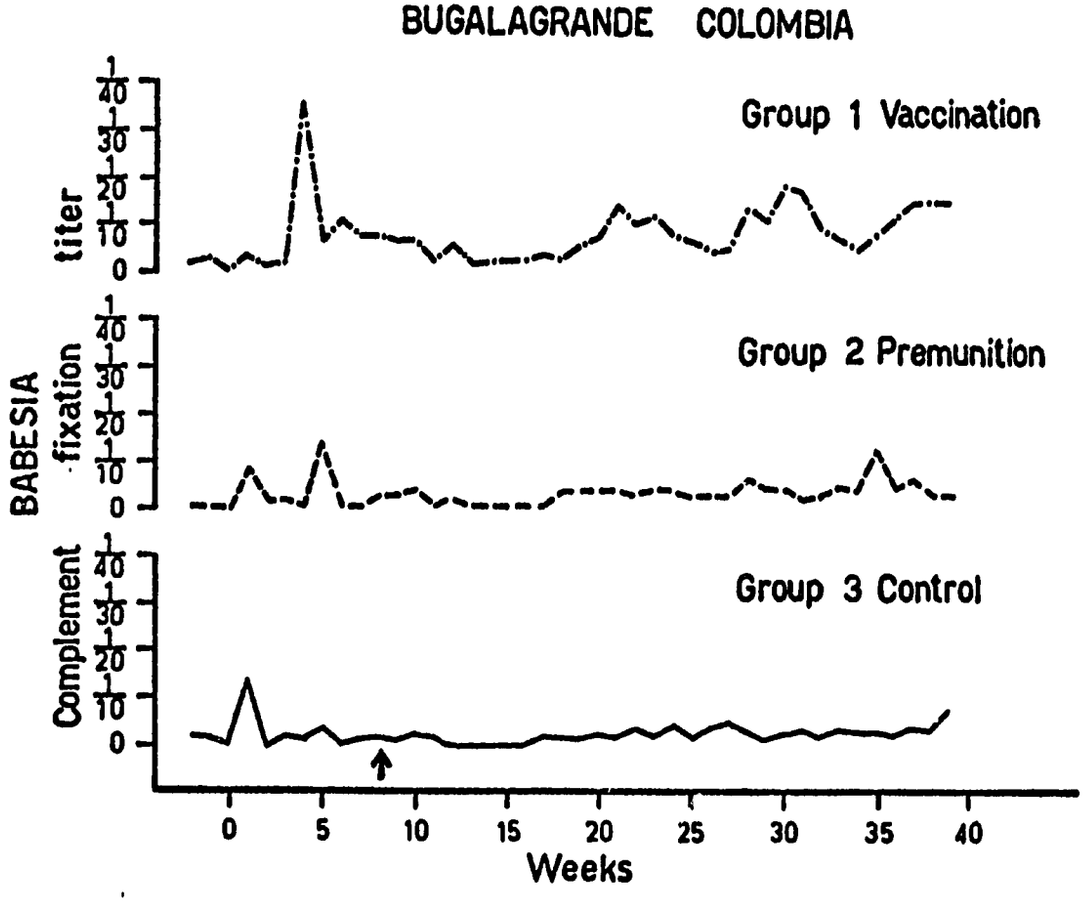

Fig. 7. Bugalagrande. Babesia spp. complement fixing antibody titer response by groups.

Table 1. Collection and Identification of Tick Species

Location	Date	*Boophilus microplus*				*Anocentor nitens*			
		Females	Males	Nymphs	Larvae	Females	Males	Nymphs	Larvae
Bugalagrande	1/14/70	12	4	6	0	0	0	0	0
	4/13/70	0	0	0	0	73	64	74	3
	6/22/70	25	30	14	0	42	36	52	0
Girardot	1/13/70	66	18	43	0	0	0	0	0
	1/19/70	381	17	6	0	0	0	0	0
	4/21/70	34	18	20	0	0	0	0	0
Monteria	3/19/70	55	116	233	0	0	0	0	0
	4/29/70	72	28	290	15	0	0	0	0

and considering the anticomplementary activity as negative, the average maximum CF antibody titer to Babesia spp. for the calves in Group 1 at week 5 was 1:35; for Group 2, 1:13; and for Group 3, 1:2. At the end of the study, the average CF titer was highest in the vaccinated calves at 1:14 (Fig. 7). Seven of 8 animals from the vaccinated calves (Group 1) reacting positively to the CF test had a titer of 1:66 four weeks after vaccination.

Two of the calves in the vaccinated group had a Babesia spp. parasitemia at week 35. At least one infected erythrocyte was observed on the Giemsa-stained slide from each of the 2 animals.

Boophilus microplus and Anocentor nitens were collected and identified from the experimental calves at Bugalagrande from January to June 1970 and the results are shown in Table 1.

Two calves from the premunized group died 2 weeks after starting this experiment. The cause of death in both animals was a gastrointestinal infection. No deaths occurred in the vaccinated or the control groups during the course of this study.

Girardot

The results of the experiments conducted at this site are shown in Figures 8 to 14 and in Table 1.

When measured 6 weeks prior to vaccination and premunization, the calves in Group 1 had an average PCV of 31% (±6); Group 2 had 33% (±6); and Group 3 had 30% (±6) (Fig. 8). Statistically significant differences in the average PCV values between Groups 1 and 2 calves

occurred on weeks 5, 12, 13, 16, and 17. On week 19 the average PCV of Group 1 calves was significantly lower than the control calves. At the termination of the study at week 25, there was no significant difference between the control calves and the vaccinated and premunized calves (Fig. 9).

The average weight of the vaccinated calves (Group 1) was 55 kg. (±17); the premunized calves (Group 2) weighed an average of 66 kg. (±21); and the control calves (Group 3) weighed 67 kg. (±25) at 5 weeks prior to initiating the study (Fig. 10). At the termination of the experiment, there was no statistically significant weight differences or evidence of altered weight gains between the groups (Fig. 11).

The average Anaplasma CF titer for the vaccinated calves (Group 1) was 1:124 at 2 weeks and 1:98 at 7 weeks after vaccination. The CF titer for the premunized calves (Group 2) was 1:82 one week before to 1:96 one week after premunization. The control calves had an Anaplasma CF titer of 1:103 one week before initiating the experiment. On week 1, the control calves (Group 3) had the highest CF titer of 1:640 and the CF titers were continually fluctuating during the course of the study (Fig. 12).

The vaccinated calves (Group 1) had an average percentage of Anaplasma infected animals with a detectable parasitemia of 40% to 55% from week 8 to week 12. Group 1 had a fluctuating parasitemia average of 20% to 55% throughout the rest of the experiment and the maximum average percentage of A. marginale parasitemia was 70% at 25 weeks. It is of interest to note that the premunized calves had a 30%

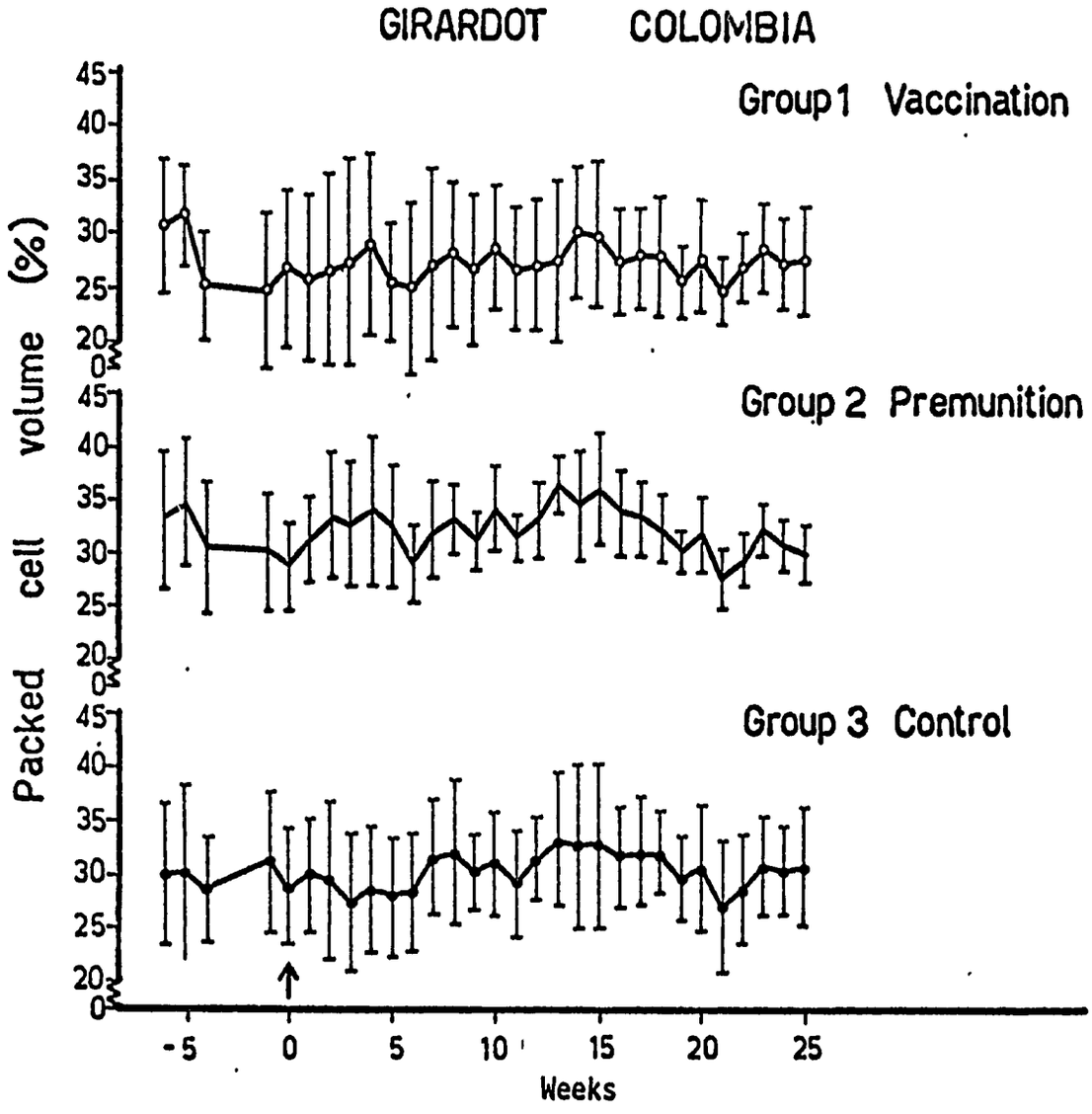

Fig. 8. Girardot. Packed cell volumes by groups with standard deviations. Group means are presented and vertical lines represent ± standard deviation.

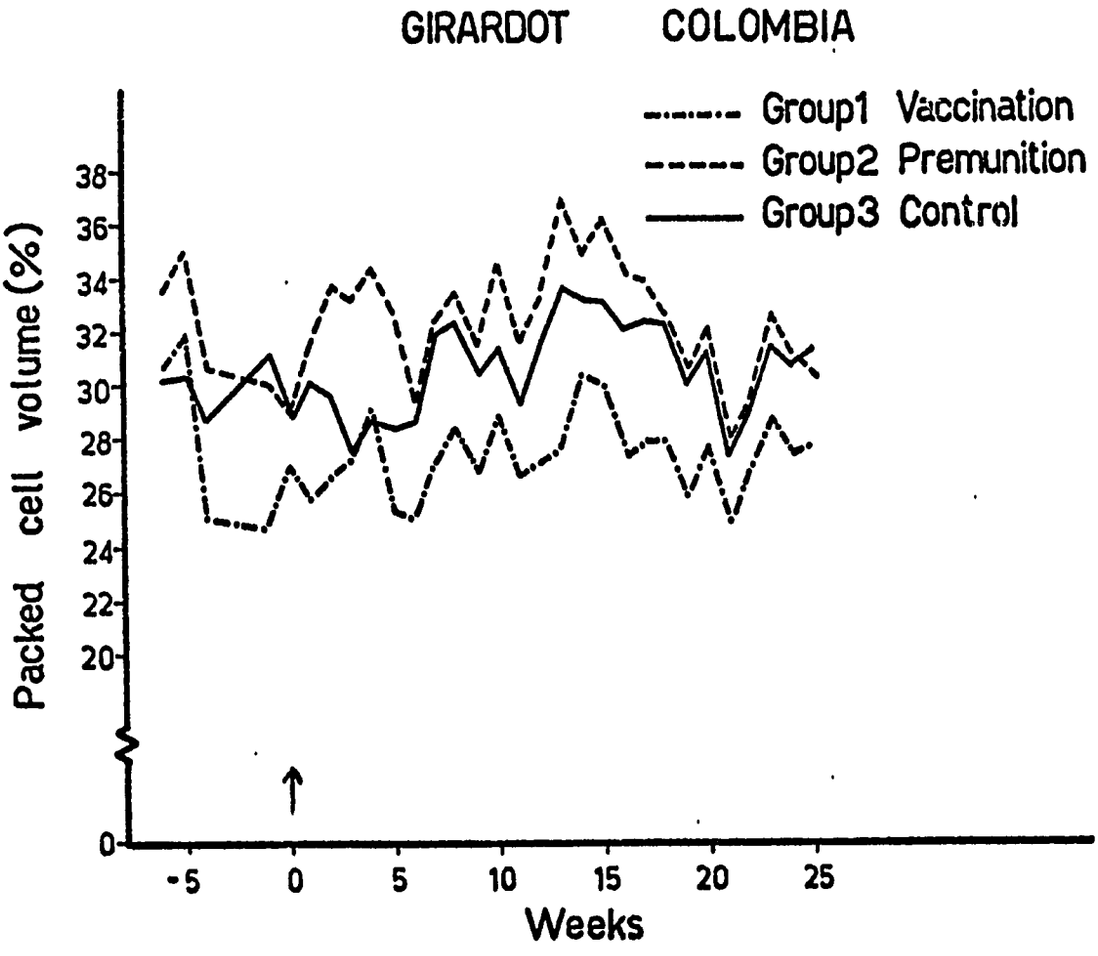

Fig. 9. Girardot. Packed cell volumes by groups compared without standard deviations.

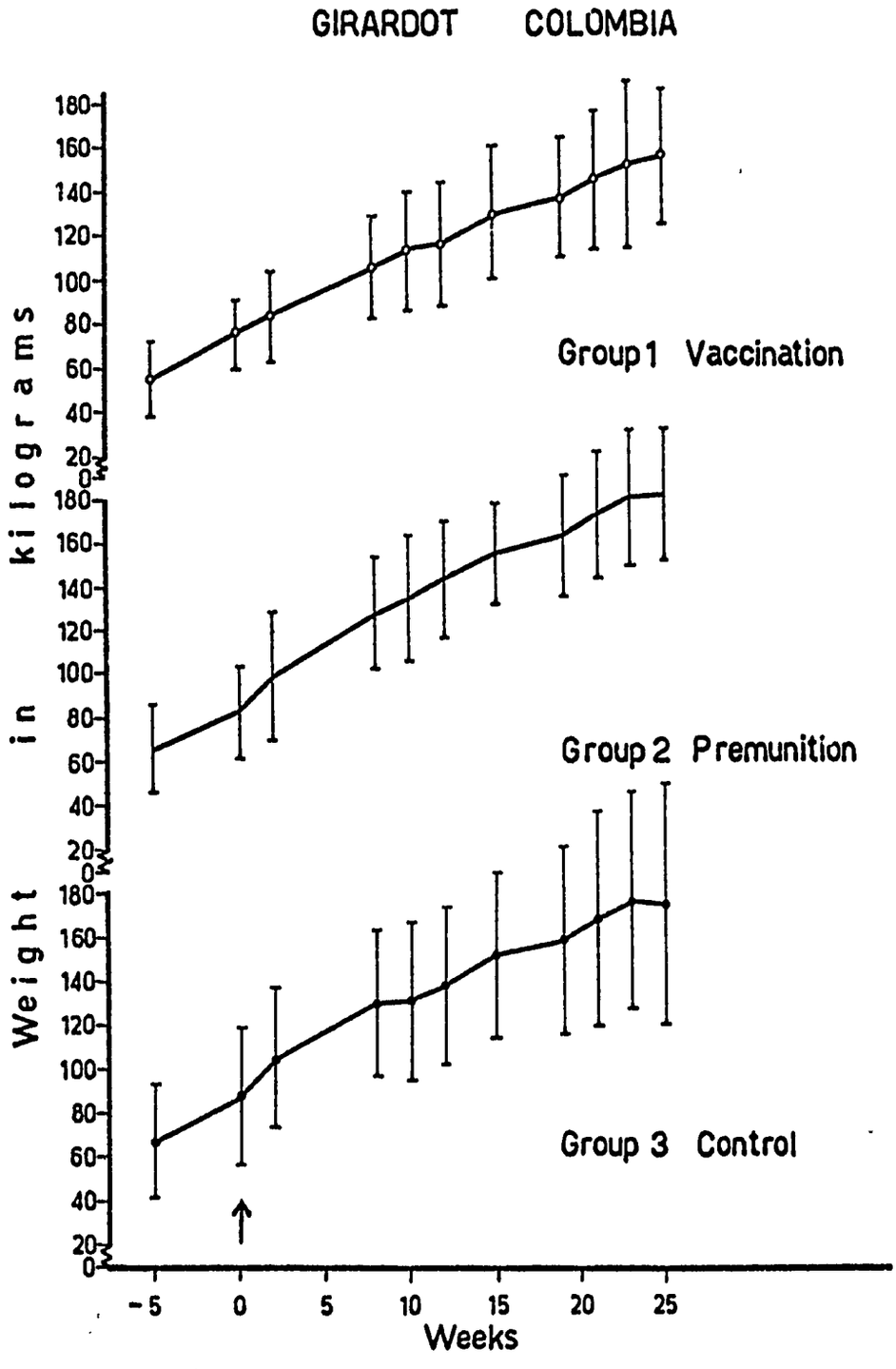

Fig. 10. Girardot. Weight gains by groups with standard deviations. Group means are presented and vertical lines represent ± standard deviation.

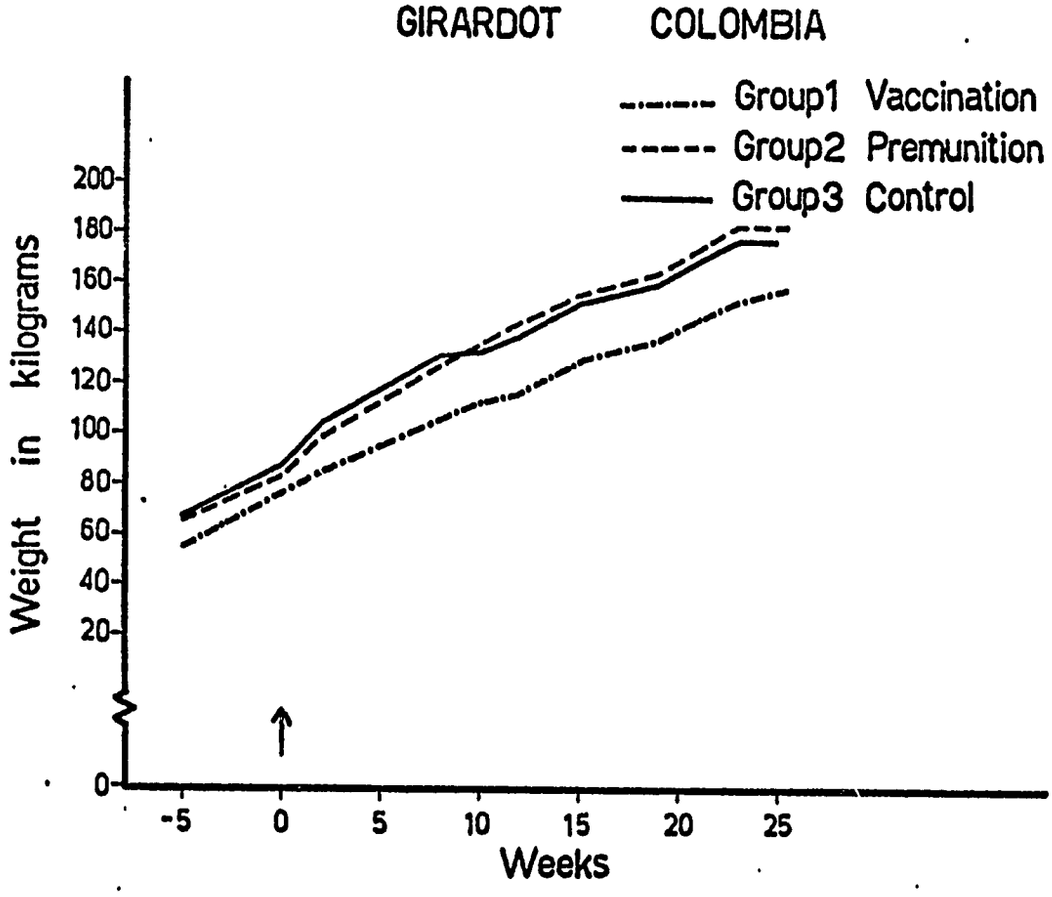

Fig. 11. Girardot. Weight gains by groups compared without standard deviations.

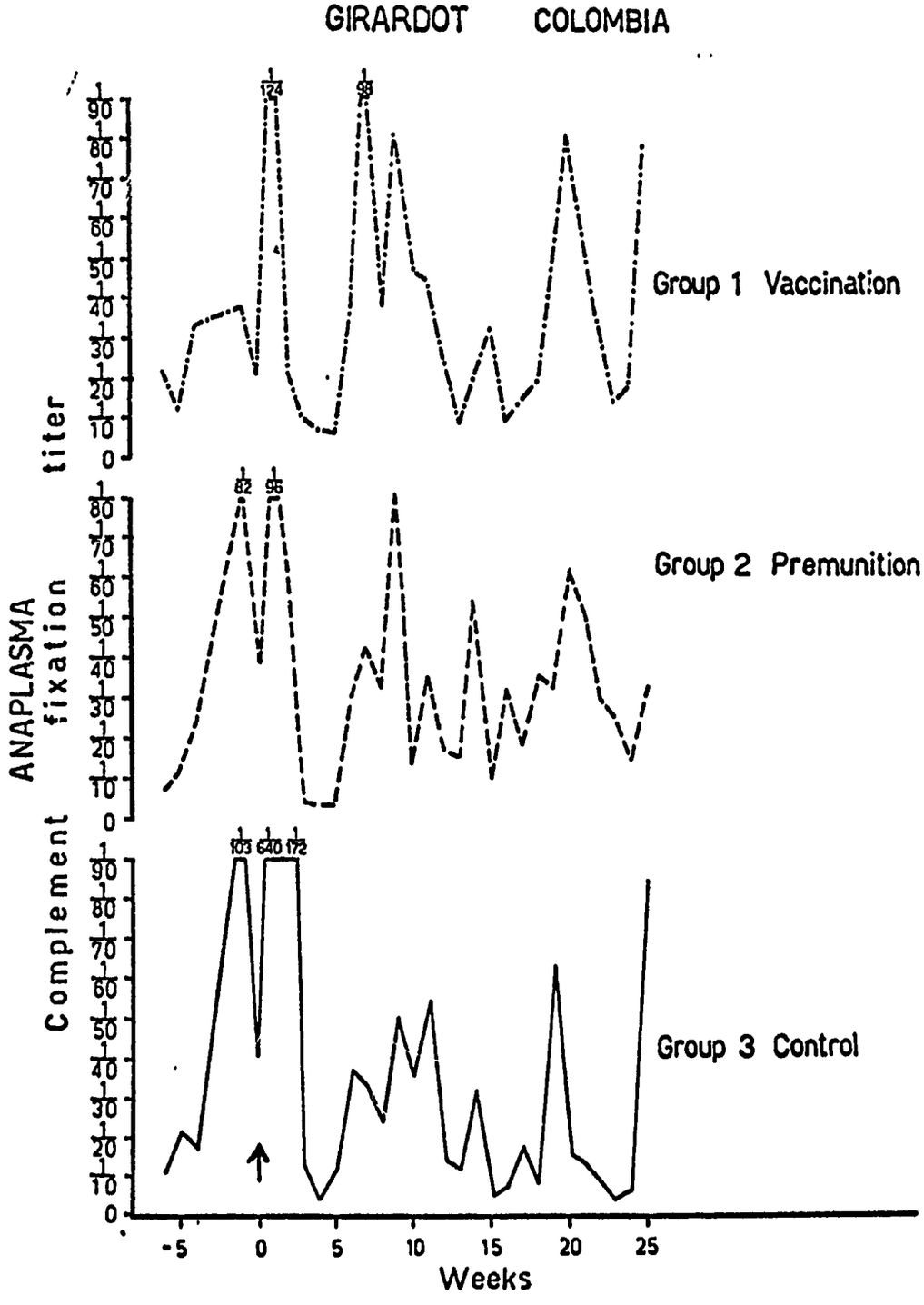

Fig. 12. Girardot. Anaplasma complement fixing antibody titer response by groups.

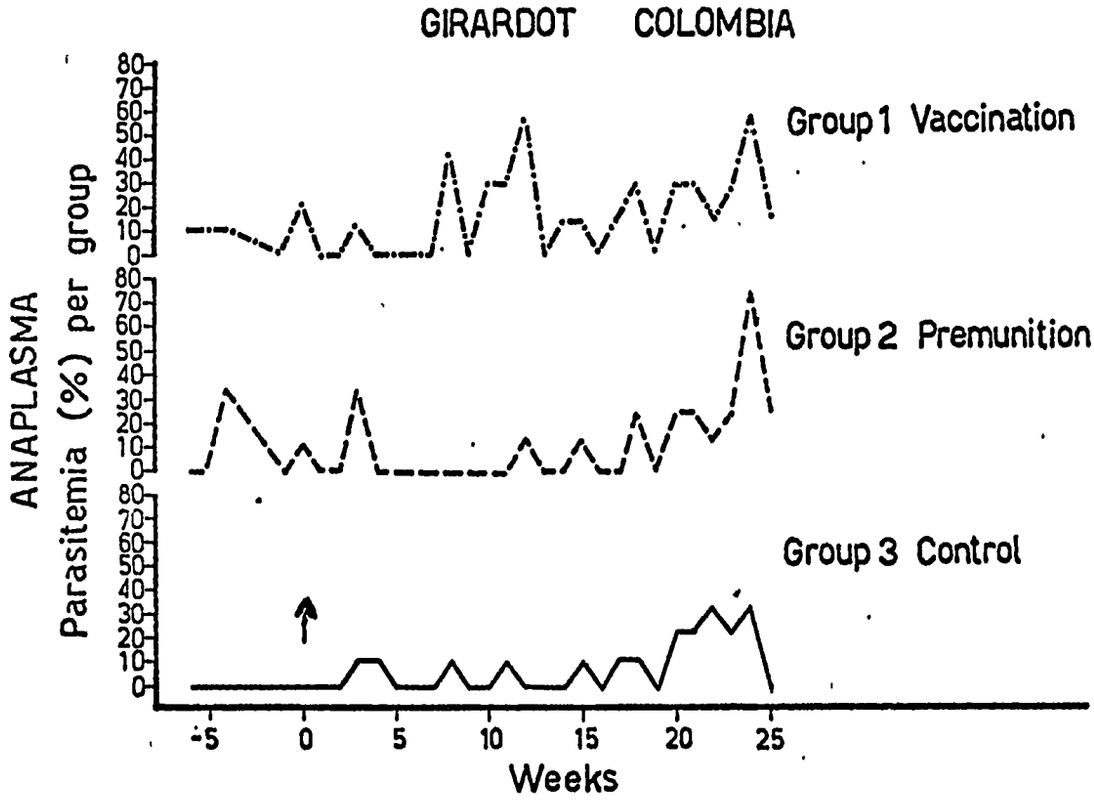

Fig. 13. Girardot. Anaplasma parasitemia by groups.

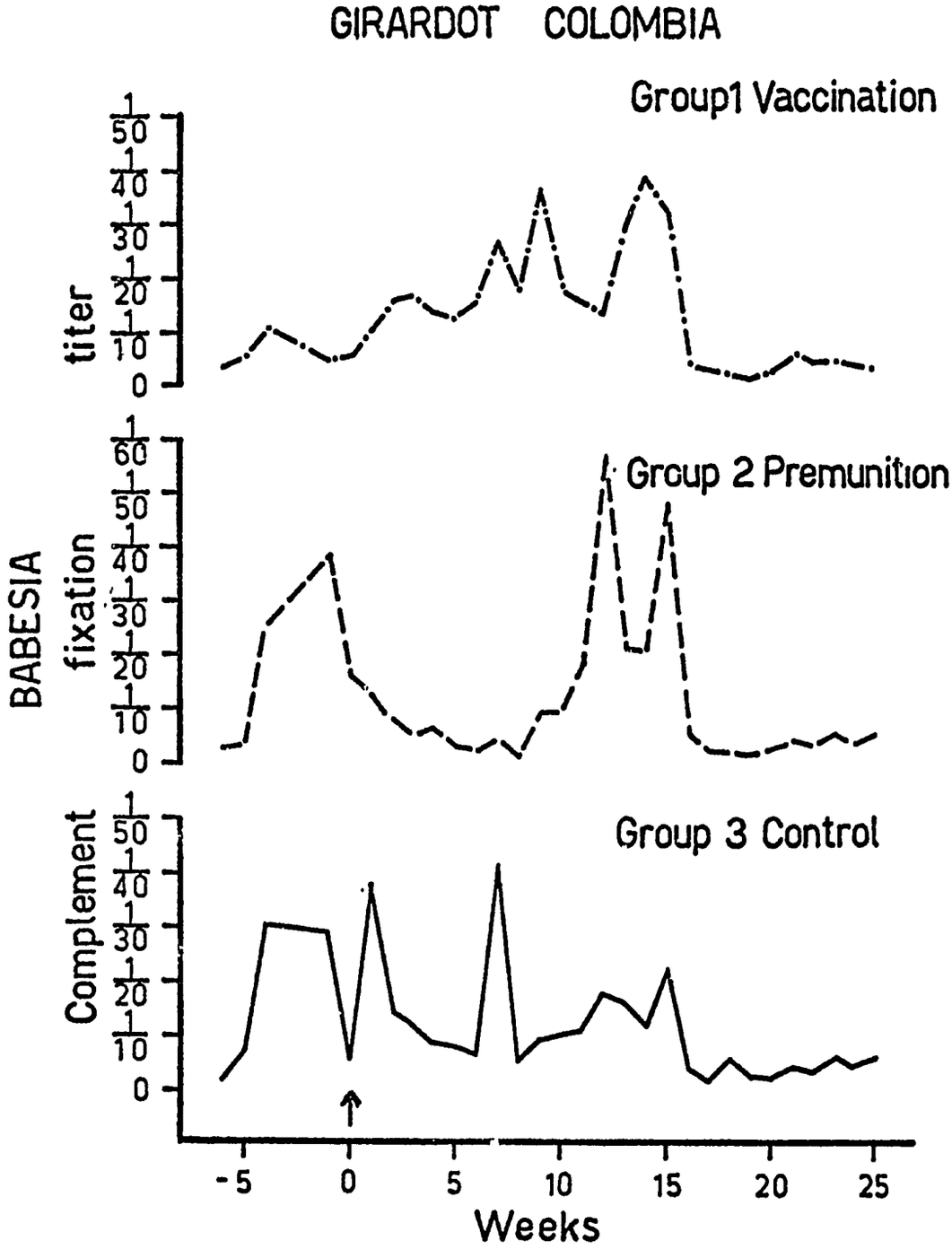

Fig. 14. Girardot. Babesia spp. complement fixing antibody titer response by groups.

parasitemia at 4 weeks before premunization. The control calves (Group 3) had 10 to 35% of the animals with parasitemias throughout the study (Fig. 13).

The average maximum Babesia spp. titers for the vaccinated calves (Group 1) were 1:35 at week 8 and 1:40 at week 13. The CF titer for the premunized calves (Group 2) was 1:40 at the time of the premunization, and thereafter the calves showed a descending CF titer for 8 weeks. At 15 weeks after premunization, the CF titers rose again to 1:55 in these calves (Group 2). The control calves (Group 3) developed a Babesia spp. CF titer of 1:30 four weeks before and a titer of 1:40 seven weeks after initiating the study. This group had an irregular decreasing CF titer to week 25 when the titer was 1:5 (Fig. 14).

No Babesia spp. parasitemias were seen in the calves at Girardot during the course of this study.

Three claves in the vaccinated group, 2 calves in the premunized group, and 1 calf in the control group died at approximately 4 weeks after premunization and vaccination. All deaths were caused by gastrointestinal infections.

All ticks collected from the calves at this site were identified as B. microplus. The largest number of female ticks were found in the month of January (Table 1).

Monteria

The results of the experiments conducted at Monteria are shown in

Figures 15 to 21 and in Table 1.

The average PCV values at day 0 for the vaccinated calves (Group 1) were 30% (±4); the premunized calves (Group 2), 33% (±4); and the control calves (Group 3), 39% (±4). The vaccinated group developed anemia 3 weeks after vaccination. This anemia persisted throughout the course of this study with the lowest values of 15% (±2) at week 10, and 12% (±1) at week 18. The premunized calves (Group 2) developed an anemia after premunization with the lowest value of 14% (±2) at week 10 and gradually recovered to 26% (±2) at week 16. The control calves (Group 3) developed an anemia after 5 weeks of field exposure. Low PCV values were found throughout the experiment. Group 3 had a PCV of 25% (±3) at week 28 when the study was terminated (Fig. 15).

Packed cell volume differences were statistically significant ($P<0.01$) at weeks 0, 1, 2, 3, 4, 5, 6, 7, 15, and 18; and also significant ($P<0.05$) at weeks 10, 11, 13, 14, and 17 (Fig. 16). At weeks 0, 1, 2, 3, 4, 5, 6, and 7, the significant difference was between the control calves and the treated calves. At weeks 10, 11, 13, 14, and 18, the difference was between the control calves and the premunized calves. At week 17, the differences occurred between all of the groups (Fig. 16).

The average weight at week 0 for the vaccinated calves (Group 1) was 88 kg. (±13); premunized calves (Group 2), 91 kg. (±9); and control calves (Group 3), 79 kg. (±12) (Fig. 17). The weight differences between the groups were not statistically significant at any time during

the experiment (Fig. 18).

The average Anaplasma CF antibody titers for the vaccinated calves (Group 1) was 1:70 at week 10 and 1:202 at week 13. The premunized calves (Group 2) had a CF titer of 1:50 at week 2. The Group 2 CF titer rose to a high of 1:254 at week 13, and maintained at least a level of 1:80 or more until week 26. The control calves (Group 3) had a rise in CF titer to 1:10 at week 10 and gradually increased to 1:226 at week 21; 1:1803 at week 22; but decreased to 1:80 at week 28 (Fig. 19).

The average percent of Anaplasma parasitemia for the vaccinated calves (Group 1) fluctuated from 10% at week 6 to 100% at week 10; the premunized calves (Group 2) from 40% at week 4 to 100% at weeks 11, 13, 14, and 20. The control calves (Group 3) had 55% of the animals with parasitemias at week 9. The percentage of the calves with detectable parasitemias fluctuated from 0% to 95% throughout the remainder of the experiment (Fig. 20).

The average Babesia spp. CF antibody titer response for the vaccinated calves (Group 1) was 1:226 at week 13, and decreased for the remainder of the 16-week period. The CF titer for the premunized calves (Group 2) was first detected 2 weeks following premunization and the CF titer increased to 1:127 at week 12 to 1:202 at week 13. It decreased to 1:101 at week 18 and increased again to 1:160 at week 22. The control calves (Group 3) had the first CF titer to Babesia spp. after field exposure at week 10 and the maximum titer of 1:145 at week 14. This CF titer gradually decreased to 1:20 at the termi-

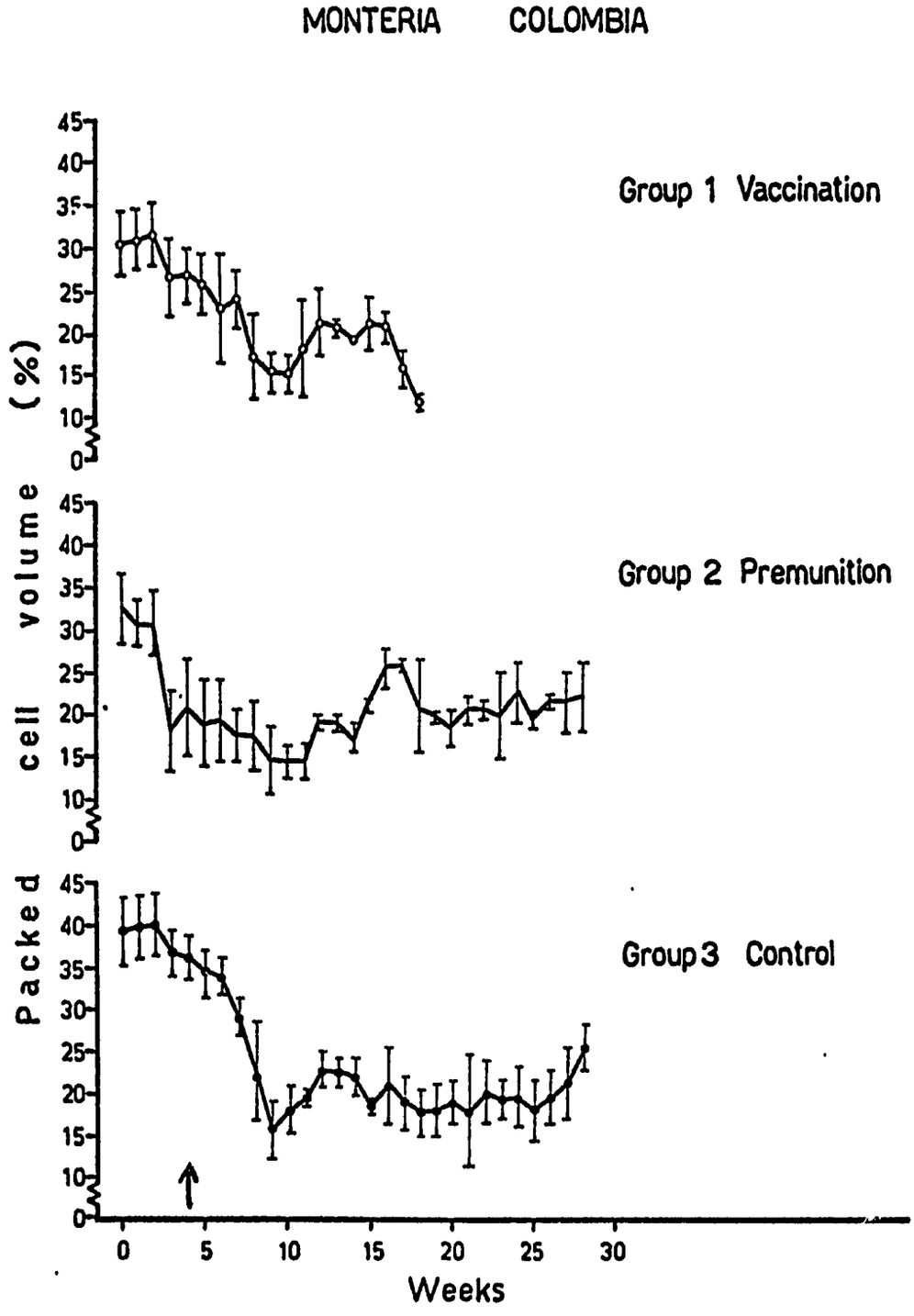

Fig. 15. Monteria. Packed cell volumes by groups with standard deviations. Group means are presented and vertical lines represent ± standard deviation.

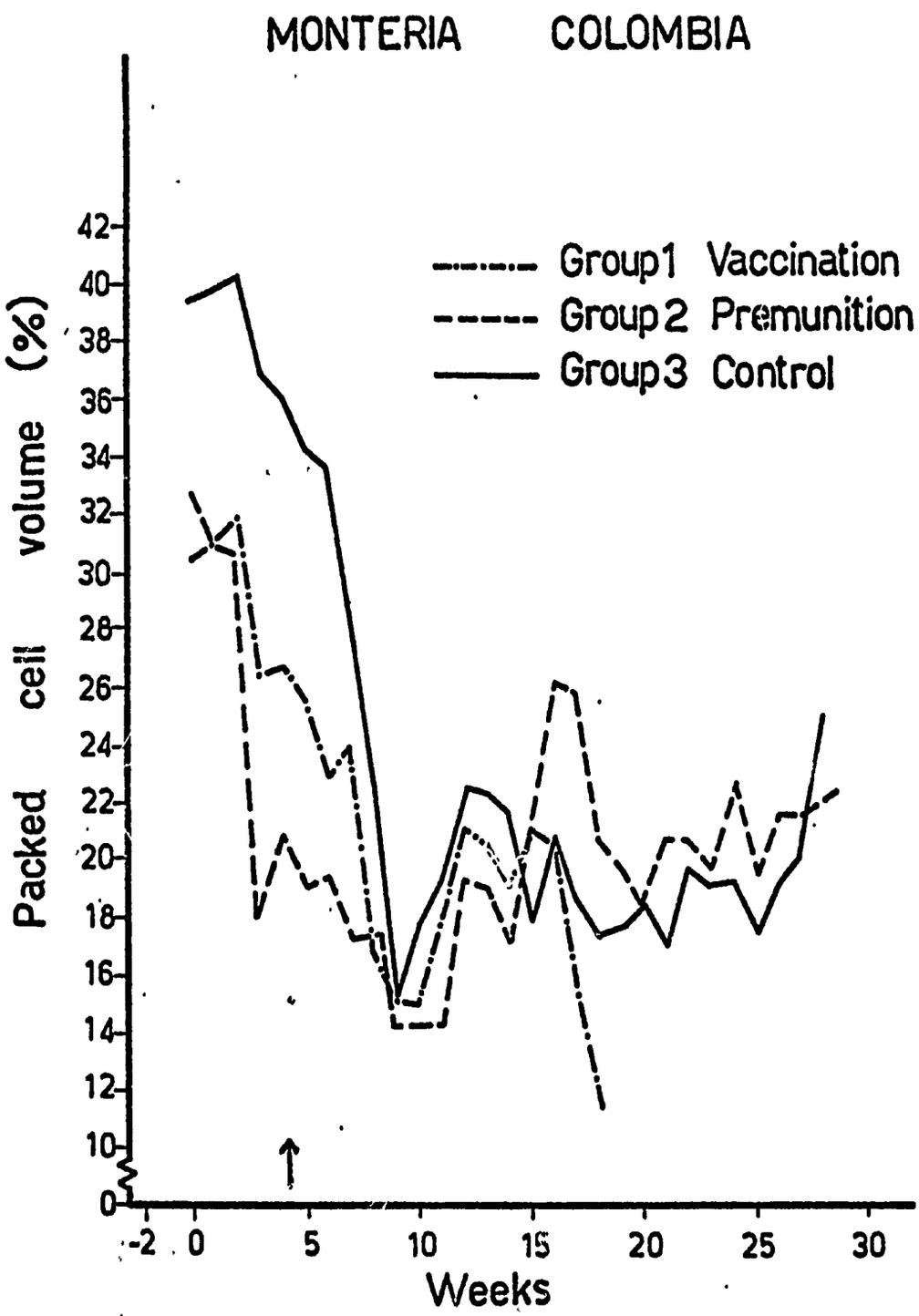

Fig. 16. Monteria. Packed cell volumes by groups compared without standard deviations.

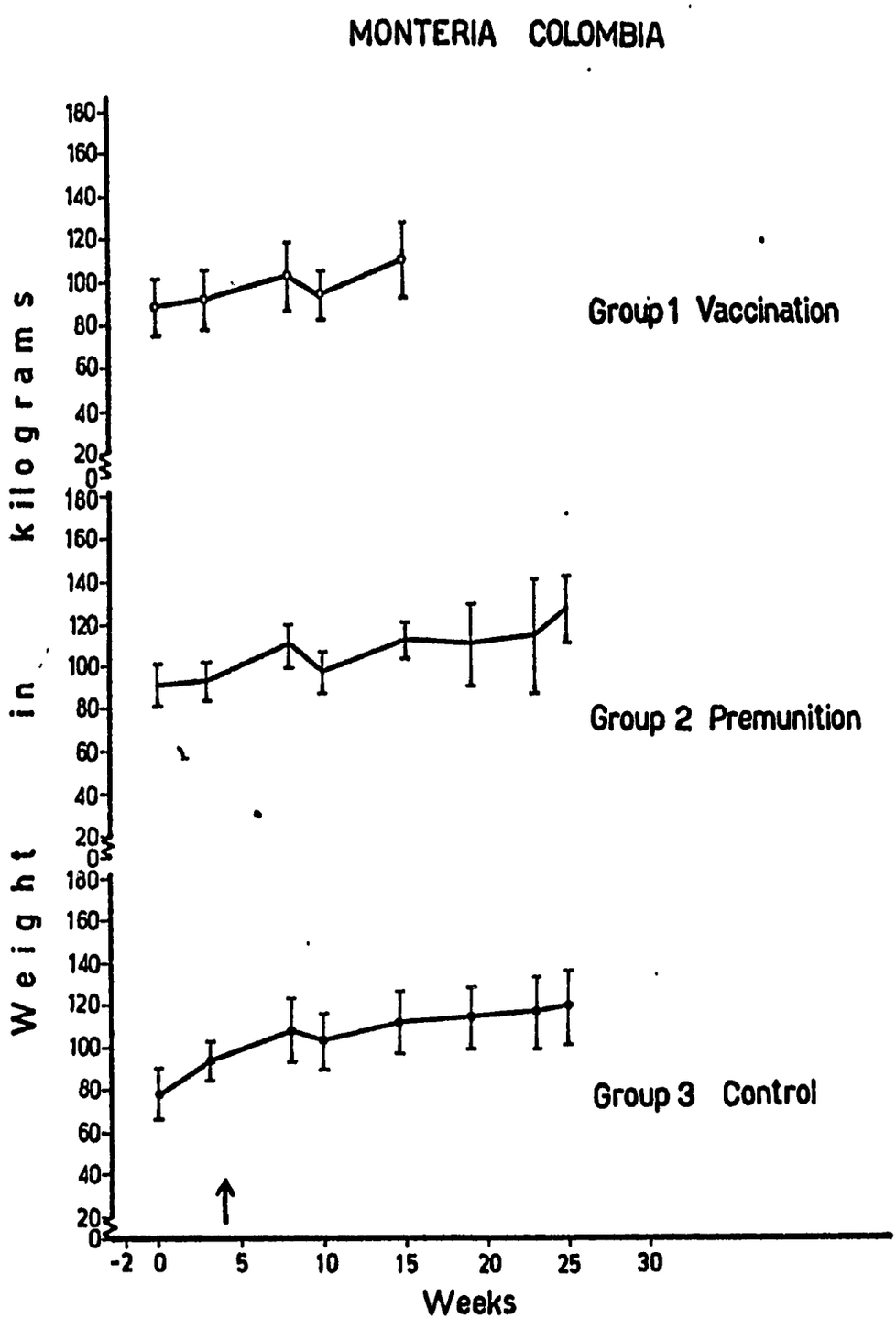

Fig. 17. Monteria. Weight gains by groups with standard deviations. Group means are presented and vertical lines represent ± standard deviation.

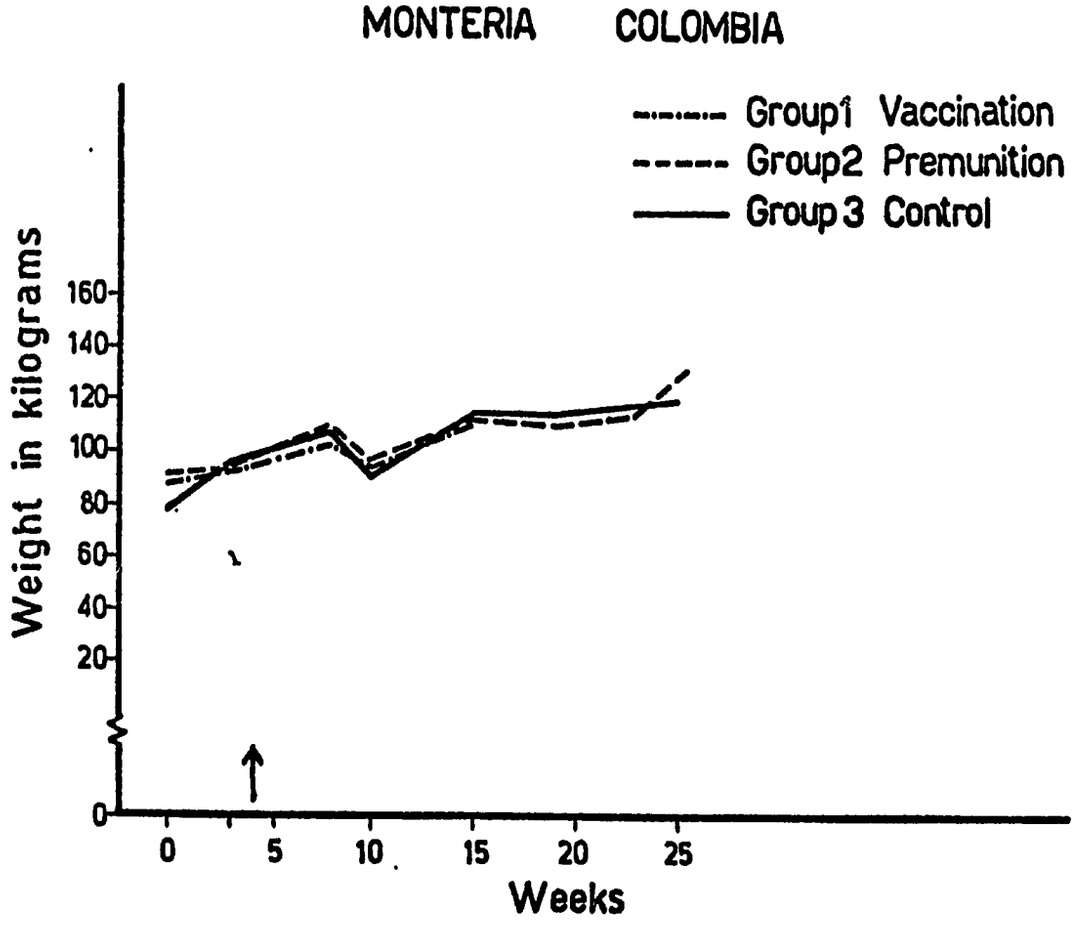

Fig. 18. Monteria. Weight gains by groups compared without standard deviations.

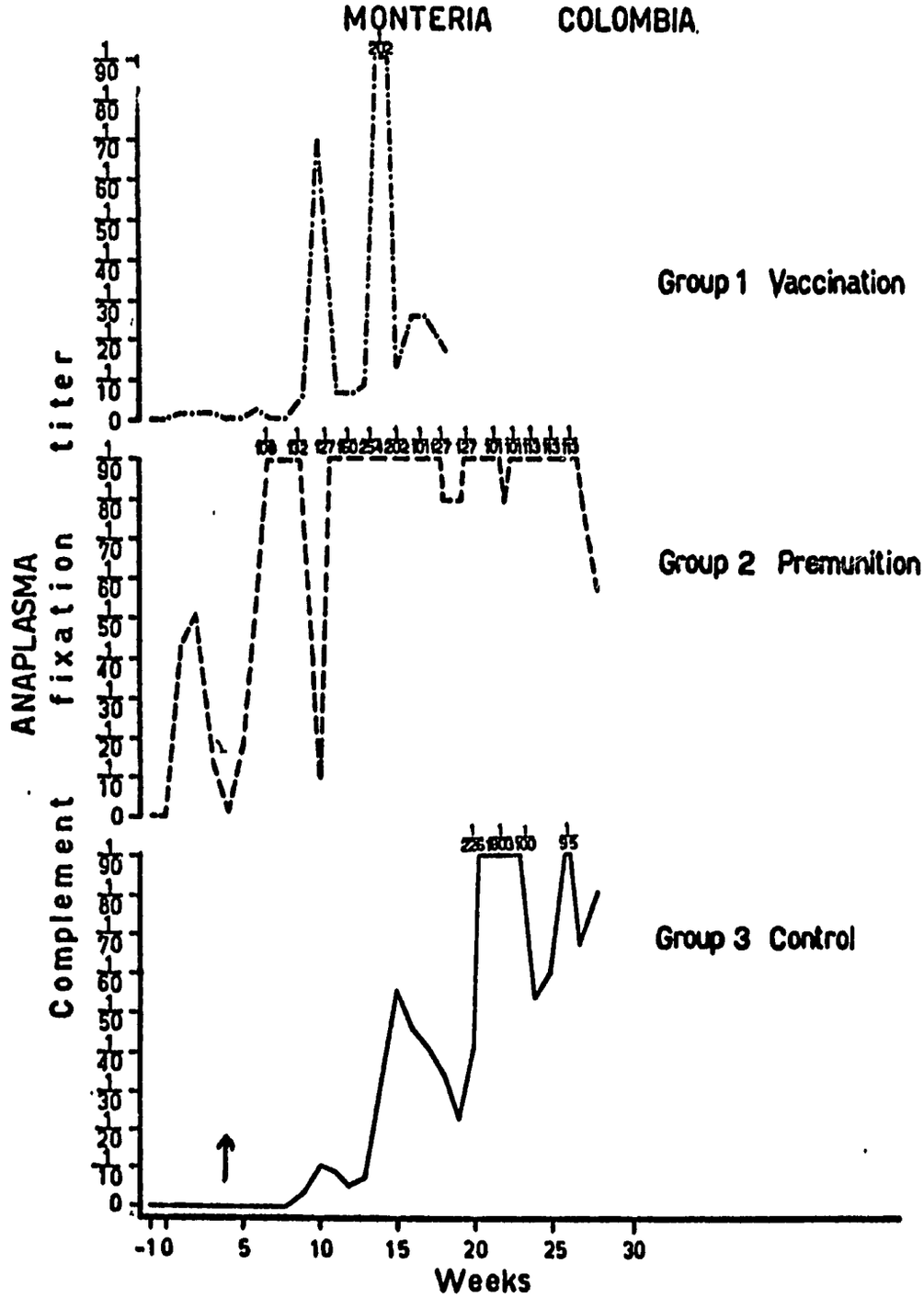

Fig. 19. Monteria. Anaplasma complement fixing antibody titer response by groups.

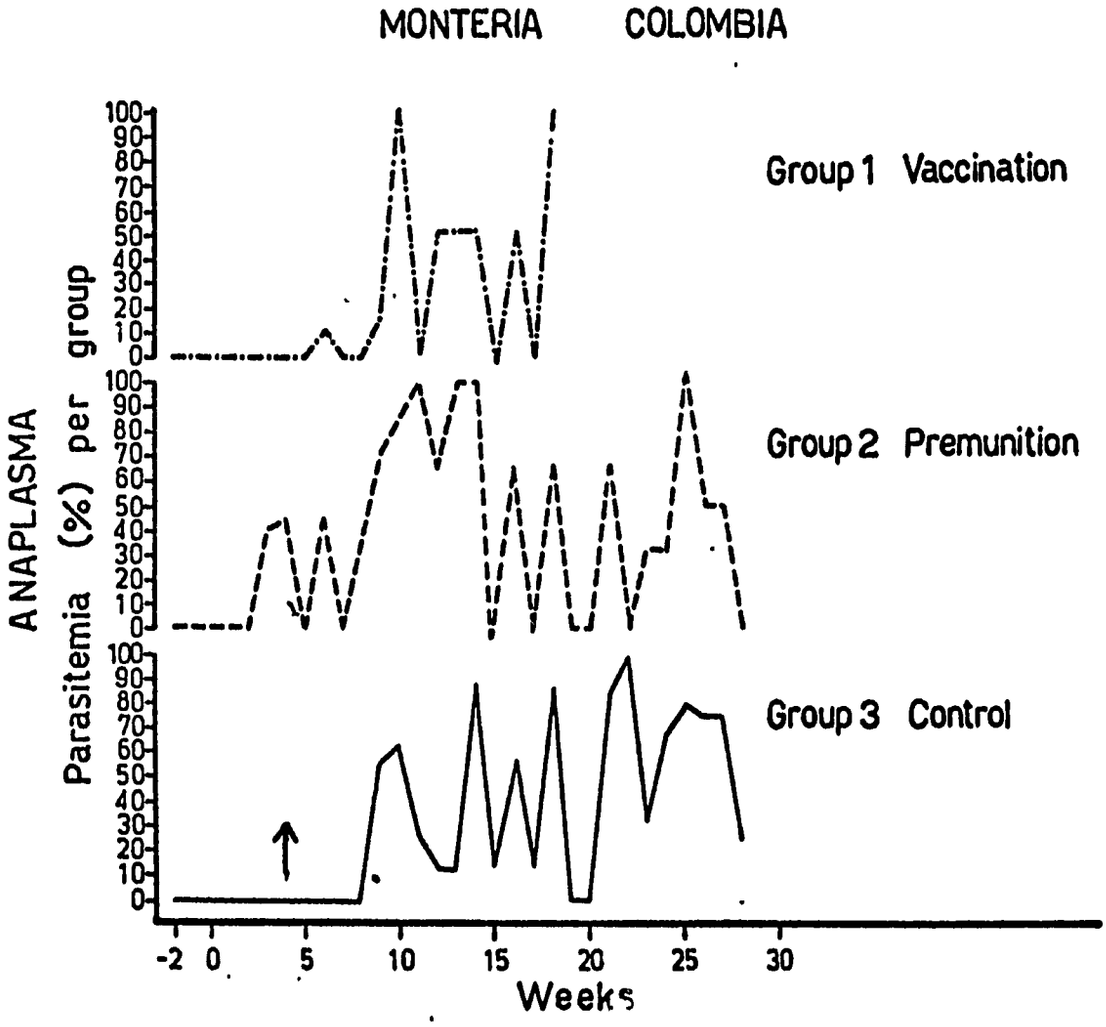

Fig. 20. Monteria. Anaplasma parasitemia by groups.

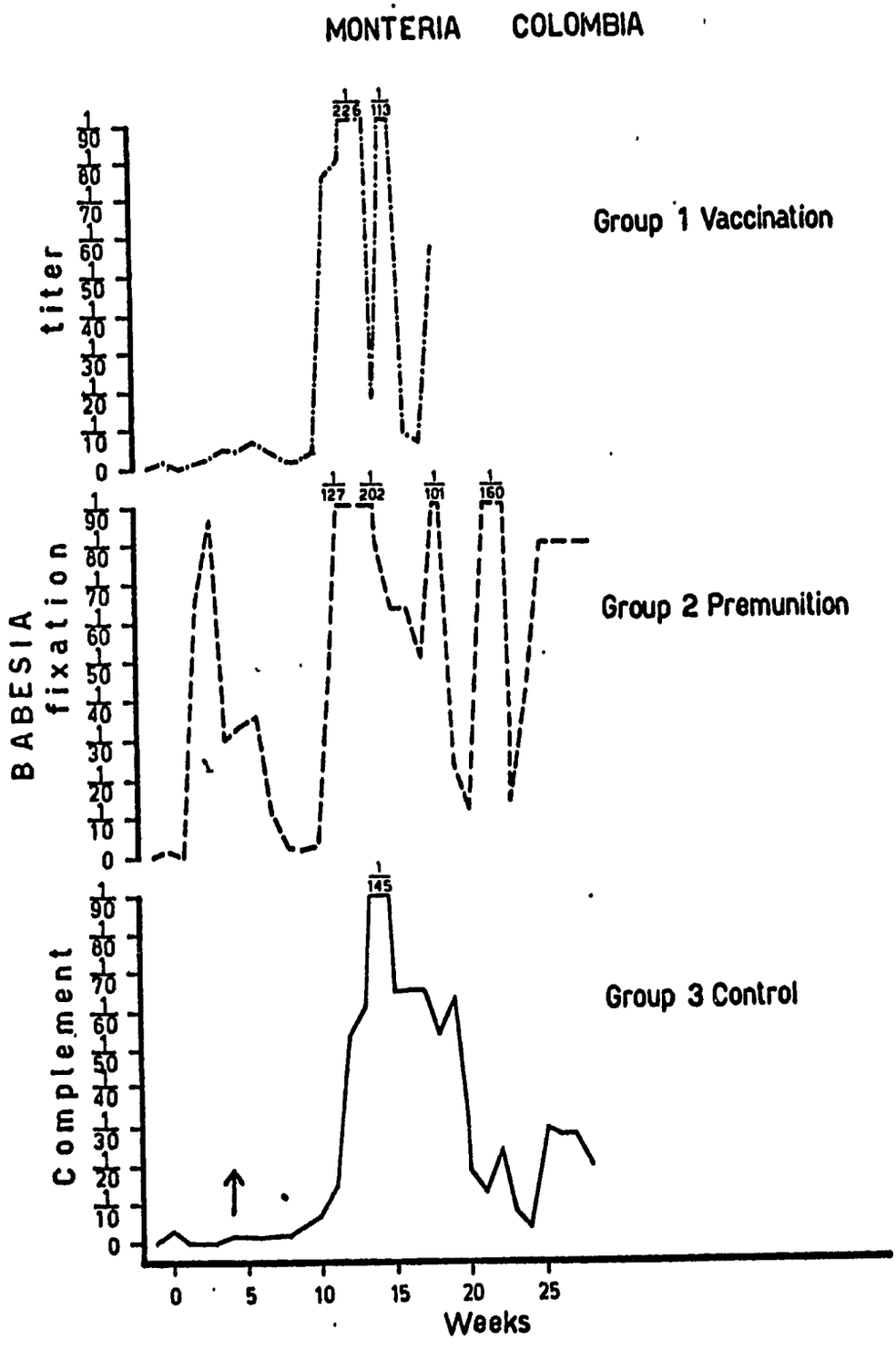

Fig. 21. Monteria. Babesia spp. complement fixing antibody titer response by groups.

nation of this study (Fig. 21).

Seventeen percent of the vaccinated calves (Group 1) had a Babesia spp. parasitemia at week 11, and the control calves (Group 3) had 22% of the animals with Babesia spp. parasitemia. Thereafter on week 12, 29% of the premunized calves (Group 2) and 39% of the control calves (Group 3) had Babesia spp. parasitemias. The control calves (Group 3) had 25% of the group with detectable Babesia parasites at week 14, and this percentage decreased to 14% at weeks 18, 20, and 21, when some of the calves died.

Three calves from the vaccinated animals (Group 1) died with signs of Clostridium infection on week 6. On week 10, 1 calf (Group 1) died with signs of babesiosis. Four calves (Group 1) died with signs of anaplasmosis. Two of these calves died 4 and 5 weeks after field exposure and 2 calves died 12 weeks after field exposure. Two calves (Group 1) died of indeterminate causes. One premunized calf (Group 2) died with pneumonitis before initiating the study. Two weeks after field exposure, 2 calves (Group 2) died with signs of Clostridium infection, and 6 weeks after field exposure 4 calves (Group 2) died with signs of acute anaplasmosis. In the control calves (Group 3), 1 calf died 2 weeks after field exposure with a Clostridium infection. Six animals (Group 3) died with signs of acute anaplasmosis and babesiosis 6 weeks after field exposure.

The ticks collected from all calves at Monteria were identified as B. microplus. This same species was persistent on these calves throughout this study (Table 1).

DISCUSSION AND CONCLUSIONS

The objective of this study was to evaluate methods to control bovine anaplasmosis and babesiosis under field conditions in 3 different climatic areas of Colombia. The locations selected were the Cauca River Valley, the Magdalene River Valley, and the north coast of Colombia. A previous study by Kuttler and Zaraza[29] in 1969 and Todorovic et al.[57] indicated that anaplasmosis and babesiosis were highly prevalent in these areas.

Based on the results of the vaccination and premunization at Bugalagrande with respect to packed cell volume (PCV) the following conclusions can be drawn. Calves in the vaccinated premunized groups had a temporary anemia as a result of the inoculations. The control calves did not show anemia at that time. However, during the 4-week period prior to the termination of the study, the control group suffered an anemia which was represented by a low PCV of 25%. In comparison, the vaccinated and premunized calves had resistance against bovine anaplasmosis and babesiosis as shown by a PCV of 29% (Fig. 2). At the end of the study which lasted 10 months, the average weight of the control calves was the lowest (149 kg.) in comparison with the vaccinated and premunized calves. The premunized calves had an average weight of 150 kg. and the highest average weight was 165 kg. in the vaccinated calves. Although these weights are different, the differences are not statistically significant. It should be remembered that these calves originated from the Cauca

River Valley with a high incidence of anaplasmosis and babesiosis with a resultant degree of natural and acquired immunity. The control calves would be expected to have some naturally acquired immunity through permanent exposure to Anaplasma and Babesia spp. infected B. microplus ticks. Riek[39] discussed this condition in which natural immunity develops and this appears to be the case here at Bugalagrande in the Cauca Valley. Here cattle are constantly infected because of the presence of B. microplus ticks (Table 1). In the absence of reinfection, anaplasmosis and babesiosis gradually disappear and the cattle again become susceptible.[59] Immunity to anaplasmosis and babesiosis in the absence of reinfection persists for varying periods. Young calves of immune mothers are protected by antibodies received in the colostrum milk, particularly Anaplasma antibodies.[39,46] This antibody response was shown by the CF titers in the control calves at the beginning of this study (Fig. 7).

Although 2 calves showed a slight parasitemia on 3 occasions (Fig. 6), no CF antibody titer response was shown (Fig. 5). It is suggested that errors occurred in the performance and reading of the CF test or in reading the slide. Generally, the detected parasitemias were very low at Bugalagrande and Girardot. Also, it is possible that the transient low level parasitemias seen in the vaccinated calves (Group 1) could have been a protracted response to the injected attenuated Anaplasma organisms.

The detectable parasitemias exhibited by the vaccinated and premunized calves after field exposure could have been due to the

antigenic differences of A. marginale and Babesia spp. found in this area. It must be remembered that the A. marginale and B. bigemina and B. argentina parasites used in the immunization procedures were isolated from donor cattle located on the eastern plains (Los Llanos Orientales) of Colombia. If this theory is true, it confirms the work of Callow[9,10] who found antigenic variations in Australian cattle especially after homologous and heterologous challenge.

At Bugalagrande, a higher rate of infection was expected than actually occurred. This expectation was based on the previous observations of Velasquez[62] and Todorovic et al.[57] Possibly these native cattle have become resistant through the years to anaplasmosis and babesiosis by natural selection and acquired immunity. This may explain the lack of deaths in the control group.

Finally, since this study had a time limit for observation, the possible beneficial effects of vaccination and premunization were not noticed at the termination of the 10-month period. The advantages of vaccination and premunization may become more obvious when these animals are older.[30,32,33]

Similar results were obtained from Girardot using the same techniques as at Bugalagrande. However, the response of vaccination and premunization imposed on existing infections is shown more clearly. The calves, evenly distributed into 3 groups, had complement fixing (CF) antibody titers for A. marginale, B. bigemina, and B. argentina before the study was initiated (Fig 12, Fig. 14). This finding was confirmed by demonstrable parasitemias and CF titers in these calves.

The outcome of vaccination and premunization on the weight gains of the Girardot calves was not significant probably because all had some acquired resistance to anaplasmosis and babesiosis. Boophilus microplus ticks found on these calves confirm earlier reports by Todorovic et al.[59] of the distribution of B. microplus in Colombia.

Due to the difficulties involved in locating and using privately owned calves in the Monteria area, calves were purchased from an anaplasmosis and babesiosis free area of Bogota. They were air transported to Monteria which has a true tropic climate and is an enzootic zone for anaplasmosis and babesiosis.

The calves selected for the experiment at Monteria were completely free from Anaplasma and Babesia spp. infection as evidenced by negative CF titers and the absence of parasitemias. Vaccination and premunization procedures were carried out 4 weeks prior to shipment to the north coast (Monteria) while the calves were located in the Bogota area.

The vaccinated and premunized calves developed a post inoculation anemia as demonstrated by the low PCV values of approximately 14 to 25% after arriving in Monteria. The calves continued to develop infection from anaplasmosis, babesiosis, and other infectious diseases of this area. These death losses indicate that the calves were not protected against these diseases. These non-native calves were also greatly stressed by the tropical climate at Monteria, especially during the first week of arrival.

Other factors may have contributed to the high death loss at Monteria. Since the incubation period for anaplasmosis and babesiosis differ considerably, the simultaneous injection of compounds 356C61 (alpha-ethoxyethylglyoxal dithiosemicarbazone) and 4A65 (3,3-Bis-(2-imidazolin-2-yl) carbanilide dihydrochloride) at five days post inoculation may not produce a premunized state in the animal against both diseases. The possibility exists that compound 4A65 had a chemotherapeutic effect against Anaplasma as well as Babesia spp. If this were true, the simultaneous injection of these drugs may interfere with the development of coinfectious immunity.[5,49] The calves at Monteria may have suffered a relapse for this reason.

Since older cattle inoculated with the attenuated Anaplasma vaccine successfully withstood experimental and field challenge with a virulent isolate,[30] the age of the animal at the time of vaccination may be important. The optimal age for vaccination is not known; however, there are some reports of fatal infections in young calves.[22,53] Hall[22] recorded the development of high fever and other manifestations of babesiosis in calves born of non-immune cows. They were inoculated at 15 to 55 days of age with infected Babesia blood or submitted to infestation with infected ticks. The progeny of "immune" cows, on the other hand, were less susceptible to infection with B. argentina, either by blood inoculation or by ticks. This may be the reason why some of the 4- to 6-month-old vaccinated and premunized calves suffered from anaplasmosis and babesiosis at Monteria.

It has been reported that sterile immunity exists to Babesia spp.

infection[9,10] but the degree of protection is not known. Mahoney[33] injected killed B. argentina vaccine into splenectomized calves and found some degree of protection when these calves were experimentally challenged by a homologous strain of organism. On the basis of the results obtained in this study with killed Babesia spp. vaccine tested under field conditions in Monteria, the degree of protection apparently was not sufficient to withstand the challenge of Babesia spp. infected ticks. It appears that a greater degree of protection could be produced by an attenuated Babesia vaccine of the same antigenic origin. This type of coinfectious immunity is known to be more protective than a sterile immunity under field conditions.

Antigenically different strains of Anaplasma and Babesia spp. organisms may have been present at the Monteria site.[9,10,56,57] According to Riek[39] it has frequently been observed that some cattle when moved from one locality to another within the anaplasmosis and babesiosis enzootic area show clinical signs of these diseases. Therefore, from the results at Monteria, it is suggested that different strains of hemoparasites differ immunologically or in virulence in Colombia.

The presence of B. microplus ticks in this anaplasmosis and babesiosis enzootic area confirms previous reports that this vector was probably involved in the epizootiology of these infections.[11,40,42,60] The significance of the presence of A. nitens on Babesia spp. infected cattle is not apparent (Table 1). It has been confirmed as a vector of equine babesiosis in the United States[47] but its role in

the transmission of bovine hematozoan diseases needs further study.

On the basis of the results obtained in this study, the control of these tropical maladies is very complex. More studies on the host-parasite relationship is needed especially in tropic zones. More information on strain antigenicity, mechanisms of coinfection, sterile immunity, and chemotherapy would be helpful toward development of more effective control systems under tropical conditions.

SUMMARY

A study was conducted on the control of anaplasmosis and babesiosis in young cattle in Colombia at 3 different geographical and climatic areas (Bugalagrande, Girardot, Monteria). Thirty calves at each site were randomly selected and divided into 3 groups of 10 animals each. Group 1 was vaccinated with an attenuated A. marginale vaccine and a killed B. argentina and B. bigemina vaccine. Group 2 was injected with infected A. marginale, B. argentina, and B. bigemina blood obtained from infected donor cattle native to the eastern plains of Colombia (Los Llanos Orientales). The resultant induced infection was followed by treatment 5 days later with compounds 356C61 (alpha-ethoxyethylglyoxal dithiosemicarbazone) given intravenously and 4A65 (3,3-Bis-(2-imidazolin-2-yl) carbanilide dihydrochloride) given intramuscularly. The third group was used as a control. The calves used in the study at Monteria were not native to that area. All of the experimental calves were subjected to natural exposure. Ticks were collected and identified at each site.

There was no apparent significant difference in resistance to anaplasmosis and babesiosis infection and in weight gains between the 3 groups at any of the 3 sites. No death losses from anaplasmosis and babesiosis occurred at Bugalagrande and Girardot but severe death losses resulted at Monteria from these diseases. Boophilus microplus and A. nitens ticks were collected and identified from the calves at Bugalagrande. Boophilus microplus ticks were collected and identified

at Girardot and Monteria.

The failure of the calves to develop resistance to anaplasmosis and babesiosis from the vaccination and premunization procedures could have been due to several factors.

At Bugalagrande and Girardot, the absence of death losses from anaplasmosis and babesiosis in the control groups indicates that the calves had a preexisting innate immunity, an acquired non-sterile immunity at the beginning of the study, or no challenge occurred during the study. At Monteria, it is apparent that the vaccinated and premunized calves did not develop resistance to anaplasmosis and babesiosis due to the use of antigenically different organisms; the simultaneous injection of the premunization drugs at 5 days post inoculation; the lack of sufficient sterile immunity to suppress tick-borne infection; or the inability of the very young calves to develop sufficient resistance.

The presence of B. microplus ticks at all 3 sites confirmed previous reports that this vector could be involved in the epizootiology of anaplasmosis and babesiosis in Colombia. The significance of the presence of A. nitens ticks on infected cattle and its role in the epizootiology of anaplasmosis and babesiosis is not apparent.

As a result of this study, it is concluded that the control of bovine anaplasmosis and babesiosis in tropical areas is more complex than previously recognized. More investigation is needed to obtain information on a strain antigenicity of A. marginale, B. bigemina, and B. argentina, mechanisms of coinfectious immunity, sterile immunity,

and the action of the chemical compounds tested in this study.

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VITA

Floyd Milton Jones, son of Charles Nunn Jones and Lula Mary Floyd, was born in Brazos County, Texas on October 21, 1928. He attended the public schools of Bryan, Texas and graduated from Stephen F. Austin High School as a member of the National Honor Society in May 1947.

Following graduation, he engaged for 3 years in ranching and dairy farming. He entered the U.S. Army in December 1950. He was honorably discharged from the Reserves in 1961 as a Captain of Artillery.

Immediately following discharge from active military service, he entered Texas A&M University and graduated in May 1959 with a B.S. in Animal Science and a Doctor of Veterinary Medicine.

In August of 1959 he began the general practice of Veterinary Medicine in Caldwell, Texas. Seven years later he moved to Bryan, Texas for 2 more years of practice.

In September 1968 he became an Institute of Tropical Veterinary Medicine post doctoral graduate student in Veterinary Microbiology at Texas A&M.

He was married on September 6, 1953 to Dolores Jeane Murray of Ft. Smith, Arkansas and is the father of a daughter, Mary Elizabeth, born on August 10, 1956 and a son, Stephen Murray, born on October 13, 1958.

The typist for this thesis was Mrs. Kathleen Christy.