AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY
BATCH #16

1. SUBJECT CLASSIFICATION

A. PRIMARY: Serials

Y-AL72-0000-0000

B. SECONDARY: Agriculture--Pests of animals

2. TITLE AND SUBTITLE
Colombian annual report of hemotropic disease research and training,March 1970 to May 1971

3. AUTHOR(S)
(101) Tex.A&M Univ. Institute of Tropical Veterinary Medicine

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1971	290p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS
Tex.A&M

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*
(Research summary)

9. ABSTRACT

10. CONTROL NUMBER	11. PRICE OF DOCUMENT
PN-RAA-790	
12. DESCRIPTORS	**13. PROJECT NUMBER**
Colombia Livestock Protozoan infections	**14. CONTRACT NUMBER** CSD-1947 Res.
	15. TYPE OF DOCUMENT

AID 590-1 (4-74)

COLOMBIAN ANNUAL REPORT OF HEMOTROPIC DISEASE RESEARCH AND TRAINING

March, 1970 to May, 1971

Institute of Tropical Veterinary
Medicine
Texas A&M University Group
in Colombia

Instituto Colombiano Agropecuario
Laboratorio de Investigaciones Médicas
Veterinarias
Ciudad Universitaria, Bogotá, D. E.,
Colombia

Centro Internacional de Agricultura Tropical
Cali, Colombia

Edited and Compiled by:

L. G. Adams
R. A. Todorovic

Including the Research and Training Accomplished by:

L. G. Adams
J. P. Bishop
D. E. Corrier
C. A. Daley
E. F. González
M. G. Guerrero
F. M. Jones
F. Lozano
A. G. López
L. A. López
J. G. Mateus
L. A. Serrano
C. H. Téllez
K. C. Thompson
R. A. Todorovic
O. G. Vizcaíno
H. O. Zaraza

I Preface and Acknowledgements 1

II Introduction 3

III Anaplasmosis

A. Projects in Progress

1. Isolation and Purfication of *Anaplasma marginale* from Cattle in the Valle del Sinú 5

2. Low Temperature Preservation of *Anaplasma marginale* 8

B. Future Projects

1. *In vitro* Cultivation of *Anaplasma marginale* in Bovine Splenic, Lymph Node, Blood Leucocytes and Bone Marrow Tissue Culture Systems

2. *In vitro* Cultivation of *Anaplasma marginale* in *Boophilus microplus* Tissue Culture Systems

3. Irradiated *Anaplasma marginale* Vaccine

4. Preliminary Investigation of the Use of *Anaplasma centrale* in the Premunition of Cattle

5. Development and Titration of a Standard Inoculum of *Anaplasma marginale* from Low

Preservation of Ground Up Tick Supernatant and Infected Blood

IV. Babesiosis

A. Projects in Progress

1. A Study of the Pathogenesis of Babesiosis in Intact Calves; Including Serological, Clinical Pathological and Histopathological Techniques 11

2. The Isolation, Separation, and Preservation of *Babesia bigemina* 56

3. Serodiagnosis of Babesiosis 66

4. Immunoserological Characterization of the Antigens of *Babesia bigemina* and *Babesia argentina* for Complement Fixation, Gel Precipitation and Immunoelectrophoresis 99

5. Immune Response of Cattle Inoculated with Irradiated *Babesia bigemina* 113

6. Isolation, Separation and Preservation of *Babesia argentina* from Cattle in Valle del Sinú 120

7. Establishment and Maintenance of a Non-Infected *Boophilus microplus* Colony 125

8. Combination Thin and Thick Blood Films for the Detection of *Babesia* Parasitemia 128

B. Future Projects

1. Effect of *Melinis minutiflora* (Molasses Grass) Pasture on the Development of *Boophilus microplus* and the Incidence of Bovine Babesiosis in Carimagua, Los Llanos Orientales

2. Development of a Rapid Card Agglutination Test for the Diagnosis of Bovine Babesiosis

3. Strain Immunity to Bovine Babesiosis in Colombia

4. Ultrastructure of *Babesia argentina*

5. Antigenic Analysis of *Babesia bigemina* Antigens Isolated from Acute and Chronic Tick-Borne and Blood-Borne Infections

6. Antigenic Analysis of *Babesia bigemina* and *Babesia argentina* and their Application in Serodiagnosis

7. Establishment of *Boophilus microplus* Colonies Infected with *Babesia bigemina* Only.

8. Establishment of *Boophilus microplus* Colonies Infected with *Babesia argen-*

tina Only

9. *In vitro* Cultivation of *Babesia bigemina* and/or *Babesia argentina* in Bovine and *Boophilus microplus* Tissue Culture Systems

10. A Study of the Cross Immunity and Premunition of Cattle with a Frozen Ground Up Tick Supernatant and Blood Borne Infection of *Babesia bigemina* and *Babesia argentina*

V Anaplasmosis and Babesiosis

A. Projects in Progress

1. A Clinical, Pathological and Serological Study of a Concurrent Infection of Anaplasmosis and Babesiosis in Experimentally Infected Calves 135

2. Control of Anaplasmosis and Babesiosis Simultaneously in Young Cattle

3. Simultaneous Control of Helminths, Anaplasmosis and Babesiosis in Cattle 145

4. Premunization Against Bovine Babesiosis and Anaplasmosis of Holstein Calves in Riopaila, Valle del Cauca 164

5. Evaluation of the Compounds 4A65 and 356-C-61 as Chemoprophylactic Agents Against Bovine Babesiosis and Anaplasmosis under Field Conditions at San Juanito, Valle del Cauca 170

6. Premunition Against Bovine Babesiosis and Anaplasmosis in Brown Swiss Cattle in Aguasalada, Valle del Cauca 175

7. A Study of the Problem of Anaplasmosis and Babesiosis and other Infections on the Farm San Felipe, Tolima 182

8. Premunization of Adult Cattle Against Babesiosis and Anaplasmosis with 4A65 and Oxytetracycline in Tibaitatá 187

9. Premunization Against Babesiosis and Anaplasmosis in Adult Cattle with Ganaseg, 356-C-61 and Oxytracycline in Tibaitatá 192

10. Simultaneous Treatment of Bovine Babesiosis and Anaplasmosis with the Compound 4A65 197

11. Simultaneous Sterilization of Bovine Babesiosis and Anaplasmosis with Compound 4A65 208

12. A Study of the Use of an Integrated Control Program for Hemo, Endo and Ectoparasites of Cattle on the North Coast of Colombia 213

13. Evaluation of Different Dosages of Ganaseg and Oxytetracycline for the Premunition of Cattle Against Anaplasmosis and Babesiosis in Turipaná 224

B. Future Projects

1. Investigation of Bovine Hemotropic Infections in Norte de Santander

2. Premunition Against Bovine Anaplasmosis and Babesiosis with Oral Treatment

3. To study the Pathogenesis of a Triple Simultaneous Infection with *Anaplasma marginale*, *Babesia argentina* and *Trypanosoma vivax* Utilizing Serological, Clinical Pathological, Histopathological and Immunofluorescent Techniques

VI Trypanosomiasis

A. Projects in Progress

1. A Sequential Study of the Pathogenesis of Disease Caused by *Trypanosoma vivax*

in Experimentally Infected Calves, Utilizing Clinical, Pathological, Histopathological and Immunofluorescent Techniques 240

B. Future Projects

1. Development and Use of the Indirect Fluorescent Antibody Test for Diagnosis of *Trypanosoma vivax*

2. Ultrastructure of *Trypanosoma vivax*

3. A Study of the Prevalence of *Trypanosoma vivax* Infections in Colombian Cattle

4. The Use of an Irradiated Vaccine for the Protection Against Bovine Trypanosomiasis

VII Training in Hemotropic Diseases and Related Areas

A. Participation in and Direction of Post-Graduate Degree Theses 274

B. Attendance and Participation in Scientific Meetings 275

I Preface and Acknowledgements

The research was executed at Laboratorio de Investigaciones Médicas Veterinarias and Centros Nacionales de Investigaciones Agropecuarias del ICA, in Tibaitatá, Palmira and Turipaná, as well as on private farms located in Valles del Cauca and Magdalena. Acknowledgements are gratefully given to each one of the directors and his staff of the experiment stations as well as to the owners of the private farms for providing facilities, use of animals and administrative support as the case may be.

Acknowledgements are also gratefully given to Drs. U. J. Grant, N. S. Raun, E. D. Roberts, Jorge Ortiz Méndez, Jaime Estupiñán, Eduardo Aycardi, Guillermo Mateus, Gustavo Riveros, R. K. Waugh, Nels Konnerup and John Pino for their professional guidance, encouragement and administrative support that have been invaluable for the completion of projects involved.

The Texas A&M University group is grateful for the technical assistance given by Mr. Luis E. Ramírez, Miss Nelly Girón, Miss Beatrice Roitman and Miss Nubia Carvajal as well as to Miss Lidia Hincapié for her secretarial assistance.

The Texas A&M University group acknowledges the financial assistance afforded by the United States Agency for International Development, Rockefeller Foundation, Instituto Colombiano Agropecuario, Centro Internacional de Agricultura Tropical, and Texas A&M University without which none of the projects could have

been achieved.

The annual report is to be considered as a document of the progress that has been in hemotropic diseases and as a guide for discussion of future research areas to be probed. The data of this report is not to be published or otherwise communicated without permission of the authors.

Bogota, Colombia

May, 1971

II Introduction

The Texas A&M University Group continued to collaborate with with the ICA-National Program of Veterinary Parasitology and Entomology which is directed by Dr. Guillermo Mateus and was responsible for conducting the hemotropic disease research and training area of the program. The relationship between the 2 entities was mutually satisfactory in that both were equally benefited.

In general, the research and investigation were primarily concerned with premunition, chemotherapy, serodiagnosis, prevalence, irradiated vaccines, concurrent infections, vectors, low temperature preservation, isolation and purification of organisms, and pathogenesis as well as other facets of host-parasite-vector relationships.

Training was mostly involved in the direction of thesis problems of North American and Colombian graduate students in the field of veterinary medical science, as related to hemotropic diseases.

In regard to North American personnel, Drs. Adams and Todorovic have been in charge of the project during the last year and worked together to execute the research projects involved. Dr. K. C. Thompson arrived in July, 1970 to begin his orientation and commence the preparation for the North Coast Premunition Project . He moved to Montería, Córdoba in December, 1970 to begin the North

Coast Premunition Project as well as laying the foundation for his dissertation. Drs. C. A. Daley and F. M. Jones completed the experimental portion of theses after one year of work and departed for the USA in August, 1970. Drs. J. P. Bishop and D. E. Corrier arrived in August, 1970 and started their thesis projects.

The Texas A&M University Group in Colombia is supported by three laboratory technicians, Mr. L. E. Ramírez, Miss Nelly Girón and Miss Nubia Carvajal, and one bilingual secretary, Miss Lidia Hincapié.

III. A. 1. The Isolation and Purification of *Anaplasma marginale* from Cattle in the Sinú Valley

Introduction

The experimental infection of cattle with anaplasmosis, for research purposes, has required that pure isolates of *Anaplasma* uncontaminated by other hemoparasites, be available for use. In all areas thus far investigated in Colombia *Anaplasma* exists as a mixed infection with *Babesia bigemina*, *Babesia argentina*, and occasionally with *Trypanosoma sp.* The isolation of *Anaplasma* as a pure isolate has thus meant that the *Babesia* and *Trypanosoma* species present be eliminated.

The chemotherapeutic purification of isolates for experimental purposes has been accomplished by using a drug which is specific for the undesirable organisms present and also has no effect on the organism to be isolated. The isolation of a pure isolate of *A. marginale* from cattle in the Sinú Valley of Colombia was accomplished by the use of a specific chemotherapeutic agent to eliminate contaminating hemoparasites.

Materials and Methods

A pure isolate of *Anaplasma marginale* was obtained by tick transmission of this organism to susceptible calves under field

conditions. Two intact, and 2 splenectomized Holstein calves, 4 months of age with a known history of no exposure to tick, vectors, negative to the complement fixation test for anaplasmosis, and with negative blood smears for Anaplasma organisms, were transported by airplane from the Savanna of Bogotá to the North Coast of Colombia and placed in tick infested pastures at the ICA-Turipaná experimental station near Montería. Blood samples were collected from these calves each day and hematocrit levels, and body temperatures ascertained. Giemsa stained blood smears were also examined daily for the presence of hemoparasites. A mixed infection of Anaplasma marginale, B. bigemina and B. argentina was observed in an intact calf following 32 days exposure to tick vectors. One-hundred mls of blood was collected from this calf by intravenous puncture of the jugular vein, using ethylene diamine tetraacetic acid (Na_2) as an anticoagulant. The blood was transported to Bogotá by airplane and immediately upon arrival inoculated intravenously into a splenectomized calf. This splenectomized calf was treated at the time of inoculation and for 2 consecutive days thereafter with 4,4'-diaminidino diazoamino-benzene diaceturate* at a sterilizing dose reported for Babesia

---

* Ganaseg; E. R. Squibb, Cali, Colombia

of 3.5 mg/kg of body weight. Hematocrit levels and stained blood smears were examined from this calf 3 times each week for 8 weeks following inoculation to observe the clinical progression of anaplasmosis and to ensure that all Babesia had been eliminated by treatment.

Results

A patent parasitemia of A. marginale was observed in this calf 18 days following inoculation. The percentage parasitemia continued to increase to a maximum of 18%. The development of a severe anemic condition indicated the necessity of treatment to prevent death due to acute clinical anaplasmosis. The calf was thus treated on day 40 and day 41 post inoculation with oxytetracycline. The continued observation of this calf to day 56 failed to indicate the presence of Babesia parasites. Inocula containing pure A. marginale was prepared from the infected blood of this splenectomized calf.

III. A. 2. Low Temperature Preservation of *Anaplasma marginale*

Introduction

The experimental infection of cattle with *Anaplasma* or with *Babesia* hemoparasites has, in most cases, been accomplished by the transfer of infected blood from acute or chronically infected cattle to susceptible experimental animals. This procedure has required that infected animals, free from infection with other disease agents, be available as blood donors. A more economical and convenient method for producing experimental infections has been reported to be by the separation of inocula which can be conveniently stored and used as needed. The technique to be described is a modification of the method reported by Barnett, Vet. Res., 76, (1964): 4-8.

Materials and Methods

Blood was collected from a splenectomized calf having a pure patent infection of *Anaplasma marginale*. Collection was by intravenous puncture, using heparin 4 units/ml as an anticoagulant, utilizing sterile technique. Sterile, 95.8% glycerol was added to the infected blood to produce a final glycerol concentration of 8.2% by volume. The blood-glycerol components were thoroughly mixed and placed in a refrigerator at 4°C for 2 hours. Following

refrigeration 5 ml aliquots of the blood-glycerol mixture was dispensed into 7 ml glass tubes with rubber stoppers. These tubes were then placed in an absolute alcohol bath which had been precooled to 4°C. The temperature of this alcohol bath was gradually reduced by the addition of solid carbon dioxide. Temperature fall was regulated to 1°C per minute to -16°C, at which time the temperature was lowered from -16°C to -79°C over a 5 minute period. The tubes of frozen blood were then placed in a dry ice chest at -79°C and stored there for later use as inocula. Prior to use as inocula, the tubes of frozen blood were removed from the dry ice chest used for storage and thawed by immersion for 1 minute in a warm water bath at 38°C.

Results

A total of 20, 7 month old, intact, Holstein calves have thus far been experimentally infected with A. marginale utilizing inocula prepared by the above described technique. All calves inoculated received 5 ml of the thawed inoculum subcutaneously. Inoculation procedures were completed in each case within 30 minutes after thawing of the frozen inocula. Twelve calves were inoculated 5 days following preparation and storage of the inocula. All 12 calves became infected with Anaplasma with an average incubation period of 21.5 days. Percentage of infected erythrocytes varied

from a maximum of 0.9% in one calf to a maximum of 2.5% in another calf. All calves of this group developed acute clinical anaplasmosis.

On day 37 following preparation and storage of the inocula, an addition group of 8 calves were inoculated. All calves of this group became infected with Anaplasma with an average incubation period of 23.5 days. Maximum parasitemias observed varied from 0.7% in one calf of the group to 2.8% in another member of the group. All of the calves of this group developed acute clinical anaplasmosis.

IV. A. 1. A Study of the Pathogenesis of Babesiosis in Intact Calves; Including Serological, Clinical Pathological and Histopathological Techniques.

Introducción

Durante muchos años la especie bovina ha sido de gran interés en la economía de muchos países, así como factor nutritivo en el alimento diario de la población. En la última década, se han reconocido numerosas enfermedades que han venido mermando el desarrollo de esta especie como industria y como fuentes de proteínas. Dentro de estas enfermedades, la babesiosis es considerada como uno de los azotes para el ganado de los climas tropicales y subtropicales.

En Colombia esta enfermedad es producida por unos protozoarios Babesia argentina, Babesia bigemina y Babesia mayor, los cuales se introducen dentro del estroma del glóbulo rojo ocasionando sus destrucciones conduciendo a la anemia; lo cual más tarde se traduce en atraso en el desarrollo ocasionando grandes pérdidas económicas.

Estos hemoparásitos realizan la mayor parte de sus ciclos evolutivos dentro de un artrópodo de la familia IXODIDE cuyo género es Boophilus microplus, y el resto de sus ciclos los llevan a cabo dentro de los eritrocitos del huésped intermediario.

La babesia ingerida por el artrópodo, pasa al intestino,

La Babesia ingerida por el artrópodo, pasa al intestino, atraviesa la pared intestinal, va al ovario, infecta los óvulos y en la progenie se desarrolla dentro de los músculos y las glándulas salivares. Esta última localización facilita la transmissión del protozoario al bovino cuando la larva se alimenta del huésped intermediario. Una vez dentro del bovino, parasita los eritrocitos, se reproduce y va a atacar nuevas células rojas. Esta situación ocurre en las fases agudas de la enfermedad. Sin embargo, durante los períodos crónicos, la Babesia desaparece de la circulación sanguínea periférica y hasta ahora se desconoce su presencia en los tejidos y las alteraciones que pueden determinar durante dichos períodos crónicos.

Esta es la inquietud que precisa un pronto estudio de la patogenesis de la babesiosis en los bovinos, con fines de establecer este proceso, así como las alteraciones hematológicas en los diversos tejidos del organismo animal dentro de las diferentes enfermedades.

Este trabajo está encaminado a elucidar las alteraciones bioquímicas, inmunológicas y morfológicas que ocurren durante las fases prepatentes y patentes (agudo, subagudo y crónico) de esta importante enfermedad, mediante la utilización de métodos y técnicas de laboratorio adecuadas y modernas que más tarde sean de utilidad en la práctica veterinaria profesional.

Materiales y Métodos

Animales Experimentales

Este experimento fue realizado en el Laboratorio de Investigaciones Médicas Veterinarias (LIMV) dependencia del Instituto Colombiano Agropecuario (ICA). Se utilizaron 15 terneros Holstein Friesian, machos, castrados, intactos, cuyas edades fueron 4 meses y 6 días, comprados en la Sabana de Bogotá, una zona aparentemente libre de hemoparásitos. Todos los animales fueron concentrados en un mismo establo, en la dependencia antes citada, el agua y la comida la cual consistió en silaje de avena forrajera y concentrado de maíz, salvado de trigo y torta de algodón, permanecieron a disposición. Los terneros demostraron estar libres de anaplasmosis, babesiosis y tripanosomiasis por medio de las pruebas de anatest, fijación del complemento y por inoculación de terneros esplenectomizados.

En la Tabla No. 1 se anuncian los diferentes exámenes llevados a cabo en el presente trabajo.

Material de Inoculación

Organismo experimental: La sangre (50 ml) para la inoculación fue tomada de un animal portador crónico de *Babesia bigemina* el cual había sido inoculado con una cepa estudiada por el Dr. Mahoney. Se inocularon alicuotas de 5 ml a cada animal por vía subcutánea en la tabla del cuello. Este inóculo no presentó babesias en el

frotis sanguíneo delgado coloreado con Giemsa. Antes de ser sometidos a la inoculación los animales fueron objeto de los siguientes exámenes:

Observaciones clínicas. Se realizó un exámen clínico general siguiendo las siguientes indicaciones descritas por Blood y Henderson. En este examen se incluyó la temperatura corporal que fue determinada dos veces diarias (7:00 am y 5:00 pm) durante todo el experimento.

Patología Clínica. Hematología: Se tomaron muestras de sangre de la vena yugular con y sin anticoagulante (EDTA), estas muestras fueron recibidas en tubos de 10 ml y se utilizaron en frotis sanguíneos delgados, los cuales fueron coloreados con Giemsa y Wright, el primero para determinar el porcentaje de parasitemia y el segundo para realizar el recuento leucocitario diferencial. Para la determinación del hematocrito se utilizó la microtécnica, en la cual se empleó una microcentrífuga. La determinación de la hemoglobina se realizó con la técnica para cianometahemoglobina, su lectura fue realizada en un espectrómetro a 540 mU. El recuento leucocitario total y diferencial fue realizado por la técnica descrita por Maxine Benjamín y los frotis fueron coloreados con Wright. El día anterior a la inoculación se practicó a todos los animales biopsias de medula ósea, ésta fue realizada en la costilla No. 12 y se utilizó la técnica descrita por Coles. La muestra fue extendida

sobre los cubre-objetos para luego ser coloreada con Wright.

Química sanguínea: Las proteínas séricas totales fueron determinadas por el refractómetro de Goldber. La glucosa sanguínea fue evaluada por el método de Somogi-Nelson modificado y la lectura se realizó a 580mU. La concentración de la transaminasa glutámica fue hallada según la técnica de los laboratorios Sigma y leída a 505 mU. La dehidrogenasa de sorbitol fue determinada por el método de Secchi y su lectura se realizó a 340 mU. El análisis de la bilirrubina tanto directo como indirecto se realizó según la técnica de Van den Berg y la lectura se realizó a 335 mU. Para el análisis del fósforo se utilizó el método de Fiske y Subbarow y fue leído a 640 mU. Para la fosfatasa alcalina se empleó el método de Fiske y Subbarow y Bodansky y la lectura se realizó en un espectrofotómetro a 640 mU.

Las lecturas de hemoglobina, glucosa y bilirrubina, fueron llevadas a cabo en espectronic 20, las de transaminasa oxalacética glutámica y dehidrogenasa de sorbitol fueron realizadas en espectrofotómetro Beckman.

Las electroforesis de las proteínas séricas fue realizada en bandas de poliacetato de celulosa, estas bandas fueron colocadas en una cámara, dicha cámara contenía barbital buffer con una concentración de iones de 0.05 M y de pH 8.6. La electroforesis fue continuada durante 45 minutos a un voltaje directo y constante de 350

voltios y 12 miliamperios los cuales fueron mantenidos por una fuente suplente de poder. Las bandas de poliacetato de celulosa fueron coloreadas durante 5 minutos en una solución al 0.2% Ponceau S con 3% de ácido tricloroacético, el exceso de colorante fue eliminado por medio de una solución de ácido acético al 5%, posteriormente las bandas fueron aclaradas en una solución que contenía ácido acético glacial al 10% y metanol al 90%, se secaron sobre una lámina de vidrio de 60°C durante 20 minutos. La lectura de las diferentes fracciones proteínicas fue realizada en un analizador automático.

Serología: La prueba de fijación del complemento fue realizada según la técnica descrita para dicho análisis en anaplasmosis. El antígeno utilizado en la prueba de fijación del complemento fue elaborado por los Dres. R. A. Todorovic, L. G. Adams y O. G. Vizcaíno.

Urianálisis: El día anterior a la inoculación se tomaron muestras de orina a todos los terneros con el fin de establecer el estado del funcionamiento renal. Se realizaron tanto pruebas físicas como determinación del color y gravedad específica, como pruebas químicas, dentro de las cuales tenemos; pH, albúmina, glucosa, acetona, sangre oculta, bilirrubina y uribilinógeno. Todas las pruebas químicas así también como el estudio del sedimento urinario fueron realizados según la técnica de los laboratorios Ames. Durante la necropsia se tomó orina con el fin de estudiar los cambios posibles.

Parasitologia.

Todos los animales fueron sometidos a un exámen coprológico antes de ser inoculados con el fin de establecer el grado de infestación parasitaria gastrointestinal y pulmonar. En vista de que las infestaciones de Haemonchus sp., Trichostrongylus sp., Ostertagia sp., Cooperia sp., Oesophagostomum sp., y Eimeria sp., oscilaron entre ligera y moderada se procedió a tratar a los animales con Thiabendazole y Amprolsol. Una semana después se realizó un nuevo examen coprológico y se observó una infestación parasitaria baja.

Observaciones Post-mortem.

Patología Macroscópica: Cada tercer día a partir de la fecha de inoculación se sacrificó un animal, se utilizó el sistema de electrocutación, la descarga aplicada fue de 120 voltios de corriente alterna, se tomaron los siguientes órganos; cerebro, cerebelo, médula oblonga, puente cerebral, medula espinal, ganglio semilunar, ileon, colon, glándula mandibular, páncreas, hígado, vesícula biliar, corazón, aorta, diafragma, músculo semitendinoso, médula ósea, riñón, vejiga urinaria, pene, mucosa nasal, tráquea, pulmón, bazo, timo, amígdala faríngea y los siguientes nódulos linfáticos: parotídeo, suprafaríngeo mandibular, preescapular, mediastínico, hepático, esplénico, meséntérico, prefemoral y renal (algunas veces).

Patología microscopica: Estos órganos se fijaron en formalina

buferada neutra (fosfato de potasio monobásico 4.0 gr% y fosfato de potasio dibásico 6.5 gr%, por cada 1000 ml de agua destilada) para después ser incluído en parafina. Posteriormente fueron procesados en autotechnicon y cortados en un micrótomo a 4 micras de espesor.

Los cortes fueron coloreados con hematoxilina y eosina (H.E.) y luego montados con Permount. Se utilizó la coloración de Gomori para determinar la presencia de hierro en hemosiderina y hemoglobina en los nódulos linfáticos y túbulos proximales del riñón respectivamente. Se colorearon tejidos con la técnica de May-Grunwald-Giemsa para tratar de visualizar el hemoparásito en los diferentes tejidos corporales. Se utilizó la coloración de floxina descrita por Mallory para visualizar sustancias hialinas.

El equipo de microscopía utilizado para el estudio morfológico fue American Optical.

Los promedios, varianzas y desviaciones estandard se realizaron según las indicaciones de Calzada.

Resultados

Observaciones Clínicas.

Durante el tiempo comprendido entre los días 4 y 16, los terneros presentaron decaimiento, se mostraron apáticos, pero no se observaron pérdidas de apetito ni síntomas nerviosos, unicamente

en 4 de estos se presentó coloración amarillenta de las mucosas visibles. Después del tercer día de inoculación los terneros presentaron elevación moderada de la temperatura tomada en la mañana (Figura 1)* Esta siguió subiendo progresivamente obteniéndose su valor máximo de 40°C el día 13, luego se observó un descenso paulatino siendo su valor final de 38.07°C. La temperatura leída en la tarde (Figura 2) mostró un aumento moderado a partir del día 14 obteniéndose su máximo valor el día 7 que fue de 40.2°C, después se observó un descenso lento obteniéndose su valor mínimo de 39.7°C, al final de experimento.

Patología Clínica.

Hematología: En el frotis sanguíneo delgado se observaron parásitos endo y extraglobulares en las diversas fases de reproducción. El hemoparásito (Figura 1) se hizo evidente el día 4 obteniéndose el máximo porcentaje de eritrocitos parasitados el día 8 y su valor fue de 0.7%. En algunos animales se presentaron parasitemias del 1.50%, la *B. bigemina* fue observada durante todo el experimento (Figura 1).

Se observó un descenso marcado del hematocrito desde el

---

* Todos los valores representados en las gráficas fueron logrados al promediar los resultados obtenidos de los terneros en ese día para cada una de las pruebas realizadas.

segundo día, este descenso se acentuó a nivel del día 4, siendo su valor mínimo de 18.6% el día 13, luego se observó un ascenso progresivo alcanzando un valor final del 24.0% (Figura 4). La lectura de la hemoglobina presentó bajas desde el segundo día acentuándose este descenso a partir del cuarto día, siendo su valor mínimo de 6.3gm el día 12, luego se observó un ascenso moderado, su valor al final del experimento fue de 8.2% gm (Figura 4). El recuento leucocitario total presentó (Figura 5) un aumento moderado (leucocitosis) a partir del día de inoculación, el cuarto día se observó un descenso moderado (leucopenia), el valor mínimo se obtuvo el día 8, siendo de 8,000 leucocitos por mm^3; a partir de este día se observó una leucocitosis moderada, obteniéndose al final del experimento una lectura de 13.500 leucocitos por mm^3. En el recuento leucocitario diferencial los neutrófilos representaron un ascenso ligero a partir del día de inoculación, el recuento máximo fue 37%, hallado el día 10, después se presentó un ascenso relativo continuo en el recuento y el porcentaje final fue de 32.0%, (Figura 5). A partir del día 16 se observaron abundantes formas celulares jóvenes (bandas) en la sangre periférica. Los eosinófilos (Figura 5) permanecieron bajos durante todo el experimento, especialmente al final del mismo. Los monocitos (Figura 6) presentaron un aumento desde el día de inoculación hasta el día 12, en el cual, el recuento relativo descendió notablemente hasta 0.3% para luego subir, siendo al final del experi-

mento de 6%. Los linfocitos (Figura 6) al comienzo del estudio presentaron un descenso relativo ligero, luego se presentó un ascenso moderado y su valor el día 10 fue de 8.2%, después del recuento descendió lentamente para tener al final del trabajo un valor de 61%

Medula Osea.

Linea eritroide: Los rubriblastos (Tabla 2) presentaron un aumento ligero a partir del día 8 y se mantuvieron casi constantes durante la investigación. Los prorubrocitos (Tabla 2) presentaron un aumento a partir del día 6, éste aumento se hizo más notorio al final del experimanto. Los rubrocitos (Tabla 2) mostraron un aumento desde el día 4, éste se hizo prominente el día 14, luego se observó un descenso ligero y finalmente ascendieron. Los metarrubrocitos (Tabla 2) presentaron un aumento desde el día 4, el cual fue más notorio el día 18, luego se presentó un descenso para luego ascender nuevamente al final. Tanto los rubrocitos como los metarrubrocitos fueron observados en la sangre perificada a partir del día 16.

Línea mieloide: En los mieloblastos (Tabla 3) se observó un aumento los días 6, 8, 10, 12 y 14, luego se presentó un descenso en el recuento, para nuevamente aumentar desde el día 20 hasta el final. Los promielocitos (Tabla 3) presentaron un aumento durante los día 4, 6,8 y 10, luego se observó una caída, más tarde se presentó un ascenso desde el día 22 hasta el final del experimento. En los mielocitos neutrofilícos (Tabla 3) su recuento se incrementó

durante los días 2 y 10, luego se vió un ligero descenso, para aumentar moderadamente hacia el final del estudio. Los metamielocitos neutrofílicos (Tabla 3) presentaron variaciones similares a las observadas en los mielocitos neutrofílicos. En las bandas neutrofílicas se observó un aumento el día 8, luego se presentó un descenso durante los días 10, 12, 14, 16 y 18, más tarde se obtuvo un aumento marcado, al final su valor se encontró un poco más bajo de lo normal. Todos los granulocitos basofílicos (Tabla 5) presentaron variaciones similares, aumentaron en su recuento los días 6, 8 y 10 luego se observó un descenso ligero hasta el final del experimento, este descenso fue interrumpido por un ascenso moderado el día 22. Los linfocitos (Tabla 3) mostraron incremento en su recuento durante los días 4 y 20, durante los otros días se observaron pocas variaciones. Se observó un aumento en el número de plasmocitos (Tabla 3) durante los días 6, 20, 22 y 25, al final su recuento descendió. Los monocitos (Tabla 3) se encontraron aumentados durante los días 6, 8, 10, 22 y 24, su recuento al final fue bajo.

En general se observó un aumento en las diferentes células de la línea mieloide durante los días 6, 8 y 10, un aumento similar fue notado durante los días 20, 22 y 24. Al final del experimento se encontraron en el recuento valores inferiores a los obtenidos en los animales sanos.

<u>Química sanguínea:</u> Las proteínas séricas totales (Figura 8)

comenzaron a decrecer desde el segundo día, observándose luego un ascenso que no llegó a alcanzar su valor normal, mas tarde se vió una caída y tenemos para el día 22 su valor mínimo que fué de 5.33%, posteriormente se observó un aumento en su lectura, llegando a valores cercanos a los normales en la parte final del experimento.

La albúmina (Figura 8) se mantuvo casi constante durante el experimento, sus variaciones estuvieron dentro de lo normal. La alfaglobulina (Figura 12) presentó un aumento relativo moderado y progresivo durante todo el experimento. En la globulina beta (Figura 12) se observó un aumento relativo similar al presentado por las globulinas alfa. Las globulinas gama (Figura 12) presentaron un aumento relativo moderado, el día 12, luego se observó un descenso marcado, para luego subir nuevamente al final del experimento.

En general la albúmina y las globulinas séricas presentaron ligeras variaciones durante todo el estudio, igual cosa sucede con la relación A/G (albúmina-globulinas) (Figura 8) la cual mantuvo dentro dentro de los límites normales para los bovinos. La glucosa sanguínea (Figura 9) en estos animales se observó aumentada, tanto antes como después de la inoculación; su valor mínimo fue observado el día 18 y la lectura nos dió 95 mg%, al final del experimento se obtuvo 86 mg%. La transaminasa oxaloacética glutámica (Figura 10)

presentó aumento al comienzo del experimento, a partir del día de inoculación se observó un descenso, presentándose un aumento en su nivel sérico durante los días 20 y 22, en los cuales su concentración fue igual a la observada durante el tiempo de preinoculación, 101 unidades Frenkel. La lectura obtenida al final del experimento fue de 85 unidades Frenkel. Durante el dia 2 de inoculación se observó un aumento moderado en la dehidrogenasa de sorbitol (Figura 10) obteniéndose 1.040 unidades Sigma/ml, éste es el valor máximo hallado, luego se presentó un descenso paulatino, registrándose al final del experimento el valor mínimo 330 unidades Sigma/ml.

Al comienzo del estudio el valor de la bilirrubina total (Figura 11) se encontró disminuído, pero a partir del cuarto día se presentó un ascenso, obteniéndose su lectura máxima de 0.83 mg/101 ml el día 12, después de este día se observó un descenso muy marcado, su valor al final del experimento fue de 0.2 mg/101 ml. Los resultados de fósforo y fosfatasa alcalina obtenidos durante todo el experimento, presentaron ligeras fluctuaciones, las cuales no sobrepasaron los valores normales establecidos para estos animales.

Serología: La fijación del complemento (Figura 4) nos indicó que los animales presentaron anticuerpos fijadores del complemento en el suero sanguíneo, desde el día 8, los títulos más altos fueron obtenidos los días 14, 18, 24, 26, 28, 30 y su lectura fue 1:80, a partir de esa fecha el título se mantuvo constante hasta el final.

Urianálisis: El análisis de la orina tomada durante la necropsia, no mostró cambio alguno en la coloración (durante el experimento no se observó ninguna variación en el color de la orina). En algunos casos se presentó un descenso de la gravedad específica. El pH se observó ligeramente ácido en la mayoría de los animales y su lectura promedio fue de pH 5.9. La determinación de proteínas en la orina nos demostró la presencia de "trazas", especialmente al final del experimento. La glucosa y la acetona nunca fueron detectadas, iguales resultados se obtuvieron con los análisis de sangre oculta, bilirrubina y urobilinógeno. La observación del sedimento nos dió de 4 a 6 leucocitos durante los días 18 y 20; células renales de 2-4 durante los días 14, 16 y 18,; células vesicales de 2-4 durante los días 14, 16, 18 y 20; células pélvicas de 2-4 durante los días 14, 16, 18 y 20; cilindros granulosos de 1-3 desde el día 8 hasta el final del experimento, todos estos resultados fueron obtenidos por campo microscópico (400X).

Observaciones Post-Mortem.

Patología macroscópica: A partir del día de inoculación (día 0) cada tercer día se sacrificó un animal, luego se realizó una necropsia completa y se observaron los siguientes cambios macroscópicos: Coloración amarillenta de todas las fascias y aponeurosis, a los 8-10 y 12 días de inoculación, hemorragias petequiales subpleurales difusas, exceso de líquido pericárdico el cual presentó

colores que oscilaron desde el ámbar hasta el rojo sangüinolento, en casos más avanzados se observó abundante fibrina, esto se observó a los 6, 10, 12, 14 y 16 días. Las cavidades pleural y peritoneal presentaron líquido en cantidad mayor de lo normal con las características antes mencionadas.

Los animales sacrificados los días 4, 10, 12, 16, 18, 20, 22 y 24 presentaron los nódulos linfáticos parotídeo, mandibular, suprafaríngeo, preescapular, mediastínico, hepático, esplénico, mesentérico, prefemoral y renal (algunas veces) aumento de tamaño, aspecto jugoso y al corte rezumaban líquido, los vasos linfáticos se observaron muy distendidos. Los nódulos hemolinfáticos de los animales sacrificados durante los días 6, 8, 10 y 12 se hallaron más grandes de lo normal.

Los animales sacrificados los días 6 y 12 presentaron coloración roja intensa de la medula renal, en el animal sacrificado a los 10 dias, se encontró coloración amarilla intensa de la grasa perirenal.

El bazo de los animales sacrificados los días 6, 8, 10, 12, 14, y 16 se encontró aumentado de tamaño y el sacrificado el día 12 presentó un aumento de 3 veces el volumen normal; en este mismo animal se encontró hepatomegalia; en el sacrificado el día 20 se encontró edema y hemorragia petequiales en la vesícula biliar.

En los pulmones de los animales sacrificados los días 4, 8 y 10

hepático desde el día 10 hasta el día 12 post-inoculación, el nódulo mandibular presentó edema y hemorragias focales desde el día 4 hasta el día 20, en el mediastínico se presentaron estos cambios desde el día 10 hasta el 30, el edema y las hemorragias focales se presentaron en el nódulo preescapular a partir del día 16 y duraron hasta el día 30, en el prefemoral este edema y estas hemorragias fueron observadas a partir del día 12 y persistieron hasta el día 22, el edema y las hemorragias focales se hicieron presentes en los nódulos mesentéricos desde el día 12 y duraron hasta el día 22, degeneración hialina de las trabéculas e hiperplasia reticular (Tabla 5). Se halló dilatación de vasos linfáticos, algunos centros germinales, presentaron degeneración hialina (Figura 20).

Los nódulos hemolinfáticos de los animales sacrificados los días 10, 12, y 14 presentaron congestión vascular, edema, arteriolas con degeneración hialina y los 26 y 28 días, hiperplasia endotelial en algunos vasos, eritrofagocitosis y hemosiderina a los 24, 26 y 28 días de inoculación. El bazo presentó hemorragias focales en la cápsula, congestión moderada, marcada y muy marcadade la pulpa roja durante los días 6,8, 10, 12, 14 y 16. Tambíen el bazo presentó hipertrofia del músculo liso de las arterias centrales de los corpúsculos, esto fue observado a partir del día 14 y persistió durante el resto del experimento, eritrofagocitosis y hemosiderina fueron observadas desde el día 18 hasta el 24. En algunos corpúsculos esplénicos

se encontró a los 20 y 22 días una corona de células reticulares rodeando el centro germinal. En el último bazo se presentó infiltraciones de eosinófilos y neutrófilos en todo el parénquima, durant e todo el experimento se encontró congestión y hemorragias focales marginación de leucocitos, edemas, pequeños e hiperplasia endotelial. En todos los casos la tonsila presentó material caseoso en el fondo de las criptas, la marginación leucocitaria e hiperplasia endotelial fueron vistas desde el día 14 hasta el final del experimento.

En el sistema digestivo, se observó congestión moderada en el labio, durante los días 4, 6 y 8 pero en el paladar no se observó ningún cambio. En la glándula salival submaxilar sólo se observó congestión vascular. En algunos cortes de la lengua se hallaron quistes de sarcosporidios (sarcoquistes), congestión vascular y un megacariocito en la luz de un vaso sanguíneo. En el esófago se encontró marginación leucocitaria durante los días 12 y 14 y quistes de sarcosporidios. Se halló hiperplasia endotelial en los vasos de las capas musculares del abomaso, a los 18 días post-inoculación. En algunos cortes del retículo se observaron pequeñas úlceras en el epitelio en los días 2, 4 y 16. En el rumen no se encontró lesión alguna. En el duodeno se observaron dilataciones quísticas en las glándulas de Brhunner a los 6 y 8 días de inoculación, hemorragias pequeñas en la mucosa, dilatación de algunos conductos quilíferos del animal sacrificado el día 12, se encontró hiperplasia

endotelial y macrófagos con hemosiderina en la mucosa durante los días 14, 16, 18, 20 y 22 de inoculación. En el páncreas se encontró congestión vascular, hiperplasia endotelial y degeneración grasa en algunas células parenquimatosas.

En las vellosidades de la mucosa del ileon se presentaron, en algunos casos, esquizontes de Eimeria bovis, hiperplasia endotelial en los vasos de la submucosa; en la mucosa se observaron algunos conductos quilíferos dilatados, estos dos últimos cambios fueron hallados desde el día 14 de inoculación . En el colon se presentó congestión de mucosa y submucosa, hiperplasia endotelial degeneración hialina en algunas arteriolas. El hígado fue uno de los órganos con más alteraciones, este presentó la hiperplasia de las células de Kupffer que evolucionó desde ligera hasta muy marcada. Esto fue visto desde el día 6 hasta el final del experimento. También se encontró durante los días 24, 26, 28 y 30 hemosiderina fagocitada por macrófagos en las áreas portales, dilatación sinusoidal y a los 6 días de inoculación se observó un foco necrótico. Desde el día 8 hasta el 16 las venas hepáticas terminales presentaron marginación leucocitaria la cual varió de moderada a muy marcada. Marginación leucocitaria en las arterias hepáticas. Algunos hepatocitos presentaron variaciones en su coloración y cariorrexis los días 18 a 24, otros presentaron degeneración grasa especialmente en zona 3 infiltración linfocitaria peritorial. En la vesícula biliar

se observó infiltración linfocitaria en la submucosa desde el día 14 hasta el 26, marginación leucocitaria endotelial desde el día 12 hasta el 28 y edema en la submucosa.

Se comprobaron numerosas hemorragias subepicárdicas, infiltración linfocitaria perivascular y congestión vascular en el corazón. En la aorta no se encontraron lesiones.

En el diafragma y en el músculo semitendinoso se encontraron quistes de sarcosporidios.

Tanto en la sustancia blanca como en la gris, se encontró congestión vascular durante los días 6, 8, 10, 12 y 14 y marginación leucocitaria en los días 10, 12 y 14. También se obtuvo infiltración linfocitaria perivascular ligera durante los días 10, 12 y 14 degeneración hialina en la pared de arteriolas y áreas de satelitosis en el cerebro. En los días 6, 8, 10 y 12 se encontró congestión vascular. La marginación leucocitaria se hizo presente los días 14 y 16,durante los días 12 y 14 se hallaron infiltraciones linfocitarias perivasculares ligeras. En éstos mismos días se presentaron hemorragias perivasculares e infiltración leucocitaria de las meninges del cerebro.

En la medula oblonga se halló congestión vascular durante los días 10, 12 y 14, hemorragias perivasculares y focales a los 8 y 10 días, marginación leucocitaria en los días 12, 14, 16 y 18, y degeneración hialina de arteriolas. El estudio del puente cerebral

nos demostró la presencia de congestión vascular y hemorragias perivasculares en los días 10, 12 y 14, marginación leucocitaria e infiltración linfocitaria perivascular durante los días 16, 18, 20, 22 y 24. En la medula espinal se hallaron hemorragias focales en los días 8 y 10 y marginación leucocitaria durante los días 12, 14, 16, 18, 20 y 22. En el ganglio semilunar no se observó ninguna lesión fuera de congestión vascular.

En la mucosa nasal al igual que en la tráquea sólo se observó congestión vascular en la submucosa. El pulmón presentó áreas de enfisema y atelectasia, congestión vascular y engrosamiento de la pared alveolar, hemorragias perivasculares y peribronquiales durante los días 6 hasta el 26, hemorragias y edema alveolares durante los mismos días. También presentó el pulmón marginación leucocitaria mononuclear a partir del día 8 hasta el final del experimento, en los animales sacrificados los días 12, 14 y 16 se encontró infiltración linfocitaria peribronquial con la formación de nódulos linfáticos. Degeneración hialina de la pared de arteriolas se presentó en el pulmón durante los días comprendidos entre el 14 y el 24. Se encontró macrófagos con hemosiderina en los pulmones de los animales sacrificados los días 26, 28 y 30, estenosis bronquial con hiperplasia del epitelio bronquial, especialmente de células caliciformes. Este cambio fue observado en la mayoría de los animales.

Al final del experimento la medula ósea fue muy celular. Se

encontraron focos de células rojas jóvenes, eritrofagocitosis por células reticulares, células reticulares con marginación de cromatina y vacuolización citoplasmática, neutrófilos, basófilos y eosinófilos inmaduros y abundantes.

En el riñón se observó congestión vascular y hemorragias perivasculares pequeñas tanto en la medula como en la corteza. Esto se encontró durante los días 6 hasta el 12. A partir del día 8 hasta el 29 se halló marginación leucocitaria. La hiperplasia endotelial fue observada desde el día 8 hasta el final del experimento. Durante los días 22, 24 y 26, se encontró adherencias entre las membranas de Bowman, parietal y visceral, líquido proteínico en la cavidad de Bowman y en la luz de los túbulos contorneados proximales. Estos últimos presentaron desde el día 10 hasta el 30, células vacuoladas e infiltración de hemoglobina en su citoplasma durante los días 24, 26 y 28. Ocurrió infiltración renal linfocitaria difusa, perivascular y periglomerular, a partir del día 8 hasta el final del experimento. En los túbulos colectores se observaron cilindros hialinos y degeneración vacuolar del epitelio en algunos casos. Se encontró congestión vascular en la submucosa de la vejiga urinaria, hiperplasia endotelial, marginación leucocitaria, desde el día 14 hasta el 30, nódulos linfáticos en la submucosa durante los días 20, 22, 24 y 26. En el tiroides en el pene no se encontraron lesiones.

Resumen

Se inocularon 15 terneros con B. bigemina para estudiar las alteraciones en hematología, medula ósea, química sanguínea, en orina y cambios macro y microscópicos.

Este experimento tuvo una duración de un mes, los terneros fueron sacrificados cada tercer día.

La temperatura determinada tanto en la mañana como en la tarde mostró un ascenso notable en la fase aguda, luego se encontró descenso en la etapa crónica de la enfermedad. Los hemoparásitos aparecieron en la corriente sanguínea al cuarto día de inoculación y persistieron aunque en bajo porcentaje durante todo el experimento. El hematocrito presentó un descenso marcado durante la fase aguda de la enfermedad, luego subió progresivamente hacia el final del estudio, alteraciones similares se presentaron en la hemoglobina.

En los días iniciales del experimento se observó un descenso marcado en el recuento leucocitario total, luego de la fase aguda se observó un aumento en éste tipo de células.

Inicialmente se presentó un descenso notorio en las célualo eritroides de la medula ósea especialmente en rubrocitos y metarrubrocitos, luego de la fase aguda se presentó un ascenso en todas las células de la línea eritroide. Las células de la líneas mieloide presentaron un ascenso ligero, en este ascenso también se incluyó linfocitos y plasmocitos, hacia el final se observo un

descenso paulatino de este tipo de células.

Las proteínas séricas totales presentaron descenso moderado hacia la parte intermedia del experimento, en los días finales del estudio estas experimentaron un ascenso moderado. Las variaciones presentadas por las globulinas séricas fueron escasas.

La glucosa sanguínea encontró aumentada tanto antes como después de la inoculación. La transaminasa oxaloacética glutámica presentó inicialmente un descenso en su nivel sérico y al final del estudio se observó un aumento que no sobrepasó los valores obtenidos antes de la inoculación. La dehidrogenasa de sorbitol, presentó un ascenso en la fase inicial, hacia el final del trabajo, esta presentó un descenso marcado. La bilirrubina total presentó un ascenso marcado en la fase aguda, luego decreció lentamente hacia el final del experimento. El calcio, el fósforo y la fosfatasa alcalina presentaron variaciones muy ligeras.

Los hallazgos macroscópicos consistieron en petequias y edemas en los nódulos linfáticos, aumento de volumen en los líquidos pericardíaco, pleural y peritoneal, el color de estos varió desde el ámbar hasta el rojo, se observaron petequias en varias serosas. El bazo se encontró aumentado de tamaño hasta 5 veces su volumen normal.

Microscopicamente se halló congestión vascular y marginación leucocitaria en todos los órganos. En los nódulos linfáticos se encontró hemosiderosis, eritrofagocitosis, degeneración hialina en

arteriolas, atrofia linfoide e hiperplasia reticular. Cambios similares se presentaron en el bazo, nódulos hemolinfáticos y tonsila. En el hígado se encontró infiltración linfocitaria periportal, marginación leucocitaria muy marcada en vena hepática terminal y en arteria hepática , hemosiderosis en área portal. Edema y nódulos linfoides fueron observados en la submucosa de la vesícula biliar. En el sistema nervioso central se encontró congestión vascular, hemorragias perivasculares, marginación leucocitaria y área de satelitosis.

En los pulmones se observó congestión vascular, marginación leucocitaria, edema y hemorragias alveolares, perivasculares y peribronquiales y degeneración hialina en la molecular de arteriolas.

La medula ósea se encontró muy celular especialmente en la línea eritroide hacia el final del estudio.

En el riñón se observó congestión vascular, marginación leucocitaria, hiperplasia endotelial de arteriolas renales, infiltración linfocitaria periglomerular, y perivascular. En los túbulos proximales del asa de Henle se encontraron glóbulos de hemoglobina dentro de las células epiteliales. Se hallaron nódulos linfáticos en la submucosa de la vejiga urinaria.

En este trabajo se demostraron las variaciones en la patología clínica y los cambios histopatológicos de terneros que padecían de babesiosis y se demuestra que la intensidad de los cambios está

relacionada con la virulencia de la cepa.

English Summary

Introduction

For many years, cattle production has been an important economic part of the gross national product of Latin American countries. In the last decades, the recognition of the importance of the production losses caused by animal diseases has become more evident. Within the realm of diseases, bovine babesiosis has been considered to be one of the more important diseases causing losses in tropical and subtropical climates. In Colombia, bovine babesiosis is caused by *Babesia argentina*, *Babesia bigemina* and *Babesia major* which invade and destroy red blood cells, hence causing anemia and death. *Boophilus microplus* serves as the major biological vector of this disease in Colombia.

Revision of the Literature

A thorough revision of the pertinent literature of babesiosis has been done in relation to the clinical, macroscopic, microscopic, biochemical and immunoserological alternations produced by the disease.

Materials and Methods

Fifteen Holstein-Friesian castrated male, intact calves, 4.6 months old, were demonstrated to be free of hemoparasites by anaplasmosis and babesiosis complement fixation, blood smear examination and by inoculation of a pooled blood sample from each of the calves into a splenectomized calf. Five ml of blood from a Babesia bigemina (Australian Isolate, Mahoney) carrier calf, with no detectable parasitemia, was injected subcutaneously into each of the 15 experimental calves. The following analyses were made on days -2, -1, 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30: PCV, parasitemia, total and differential leukocytes, hemoglobin, myeloid-erythroid ration, blood glucose, serum glutamic oxalo-acetic transaminase, sorbitol dehydrogenase, total serum proteins, serum protein electrophoresis, bilirubin, serum calcium and phosphorus, alkaline phosphatase, antibody complement fixation and urinalysis for color,specific gravity, pH, albumin, glucose, occult blood, bilirubin, ketone bodies, and uribilinogen. On each of the post-inoculation days 2 through 30, one calf was euthanatized with 110 v alternating current and subjected to a complete necropsy in which 45 tissues were collected and fixed in buffered 10% formalin. All tissues were later paraffin embedded, sectioned at 5 micron and stained with hematoxylin and eosin. The Gomori technique for iron, Mallory technique for hyaline and

the May-Grumwald-Giemsa stain were utilized for selected tissues.

Results

The clinical, clinical pathological and some pathological results are given in Tables 2, 3 and 5 and Figures 1, 2, 4,5, 6, 7, 8, 9, 10, 11, and 12. The photomicrographs were omited from the paper due to the difficulty of reproduction. The morning and afternoon temperatures spiked on day 8 PI during the acute phases of the babesiosis. Babesia bigemina parasites were observed from day 4 PI through day 30 PI, with the maximum parasitemia of 1.0% occurring on day 8 PI. The PCV and hemoglobin values began a rapid descent on day 2 PI to a low of 19% on day 13 PI from which day onward a gradual ascent to a maximum of 25% on day 30 PI was observed. The total leukocytes decreased on day 8 PI, to a low of 8,000/mm^3 (slight leucopenia) and rapidly increased to a plateau of approximate 13,000/mm3 (moderate leucocytosis) on day 12 PI. Lymphocytes and monocytes were moderately increased beginning on day 4 PI to day 24 PI of the experiment except day 12 PI on which the monocytes decreased to a low of 1%. The erythroid series of the bone marrow decreased continually beginning day 2 PI until day 18 PI following which a gradual ascent began. The myeloid series of the bone demonstrated a gradual ascent until 18 PI following which a descent occurred.

Few alternations of the total serum proteins or fractions of the total serum proteins occurred. Blood glucose spiked on day 18 PI at 95 mg%, while SGOT and SD continously showed a general trend of descent. Total bilirubin values reached a maximum concentration on day 12 PI following which a rapid descent occurred. Serum calcium, phosphorus and alkaline phosphatase showed no significant changes.

Macroscopic lesions included petechiation, hypertrophy and edema of lymph nodes, hydropericardium, hydrothorax, hydroperitoneum, splenomegaly and icterus, on days 8 through 12 PI.

Microscopic lesions included congestion and leucocytic margination and sedimentation in practically all organs. The lymph nodes, spleen, tonsil and hemal nodes had lesions of hemosiderosis, erythrophagocytosis, hyaline degeneration of arteriols, lymphoid atrophy and reticuloendothelial hyperplasia. The liver had intensive lymphocytic periportal infiltration, periportal hemosiderosis, Kupffer cell hyperplasia, and leucocytic engorgement or sedimentation of the terminal hepatic veins. The central nervous system had vascular congestion, perivascular hemorrhages and leucocytic sedimentation. The lungs had lesions similar to those of the central nervous system plus hyaline degeneration of arteriols. The kidneys had lymphocytic periglomerular and perivascular infiltrations, endothelial hyperplasia, vascular congestion,

leucocytic sedimentation, and deposition of hemosiderin in the proximal tubules.

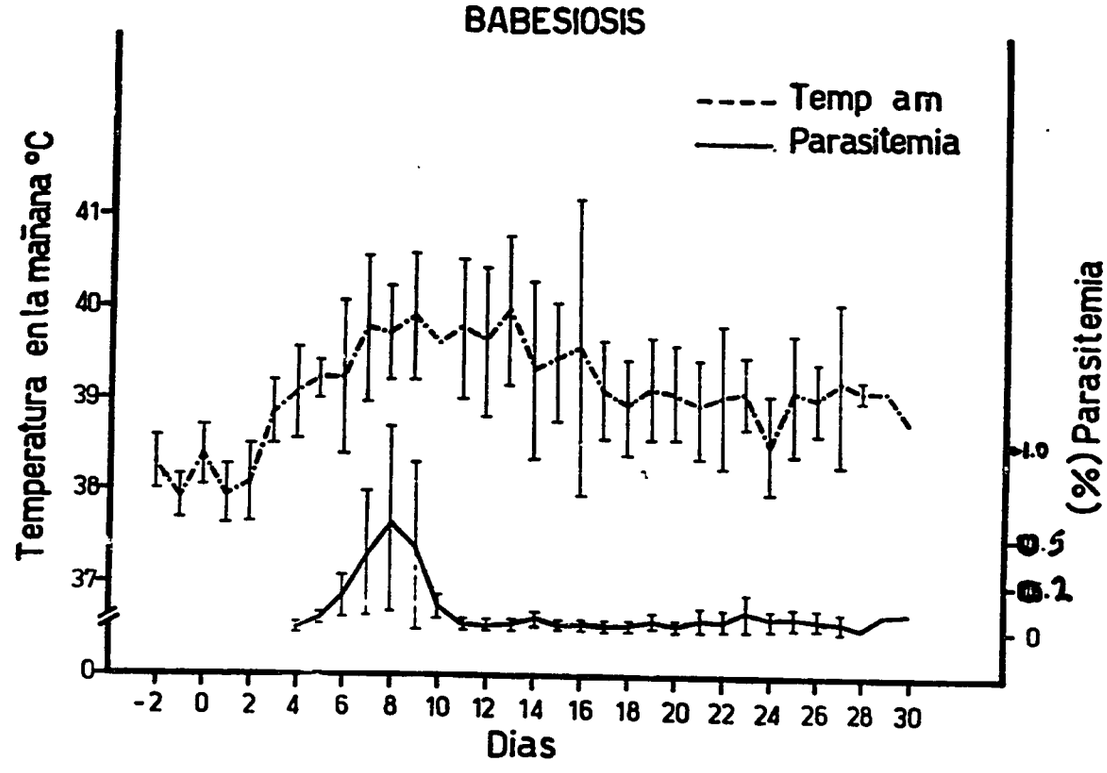


Figura 1. Promedio por grupo de la temperatura a.m. y de la parasitemia de los terneros inoculados con B. bigemina.- Las lineas verticales nos muestran las desviaciones estandar para cada determinación y para cada día.

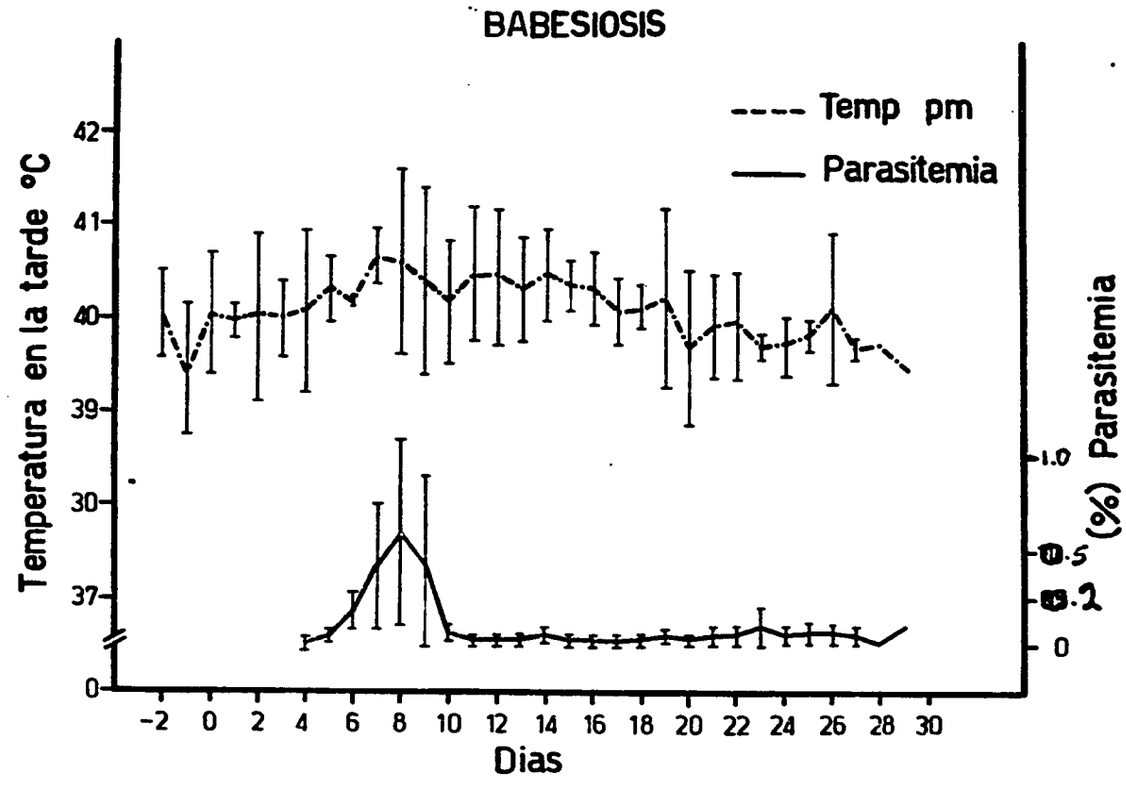


Figura 2. Promedio por grupo de la temperatura p.m. y de la parasitemia de los terneros inoculados con B. bigemina.- Las líneas verticales nos muestran las desviaciones estandar para cada determinación y para cada día.

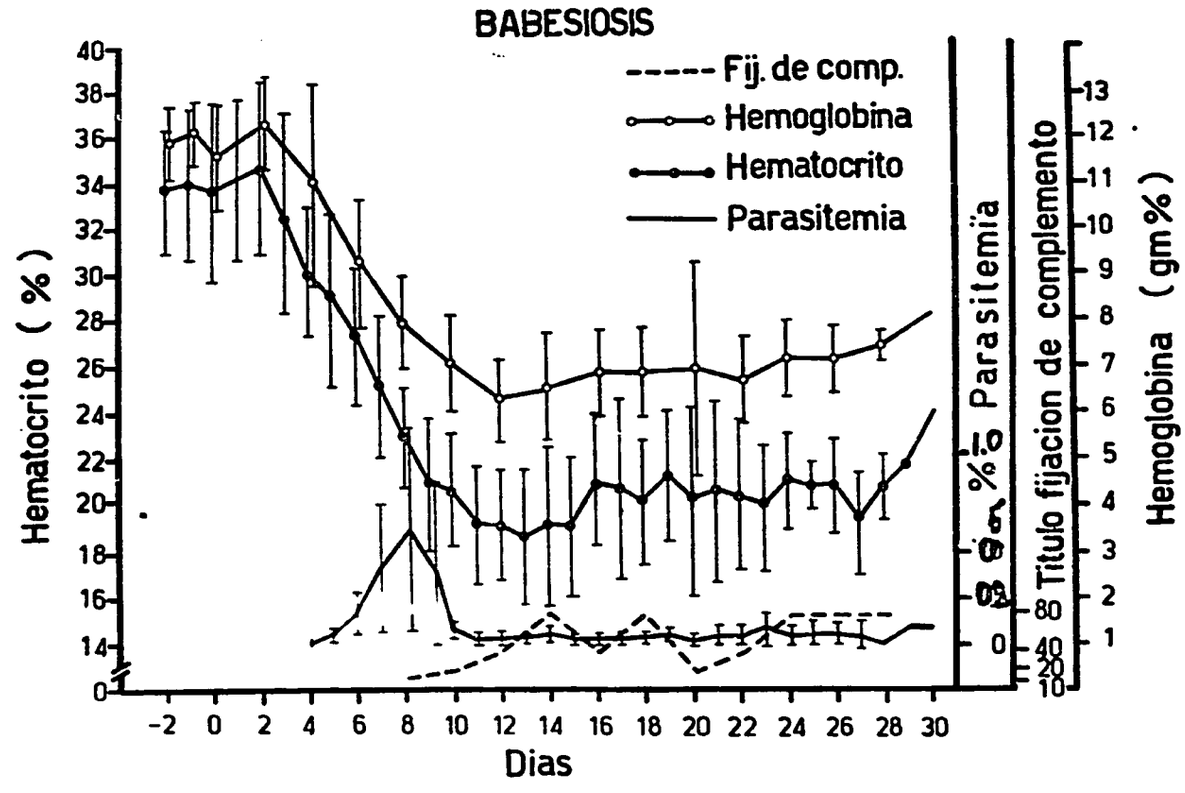


Figura 4. Promedio por grupo de los análisis de la fijación del complemento, de la hemoglobina, del hematocrito y de la parasitemia de los terneros inoculados con B. bigemina. Las líneas verticales nos muestran las desviaciones estandar para cada determinación y para cada día.

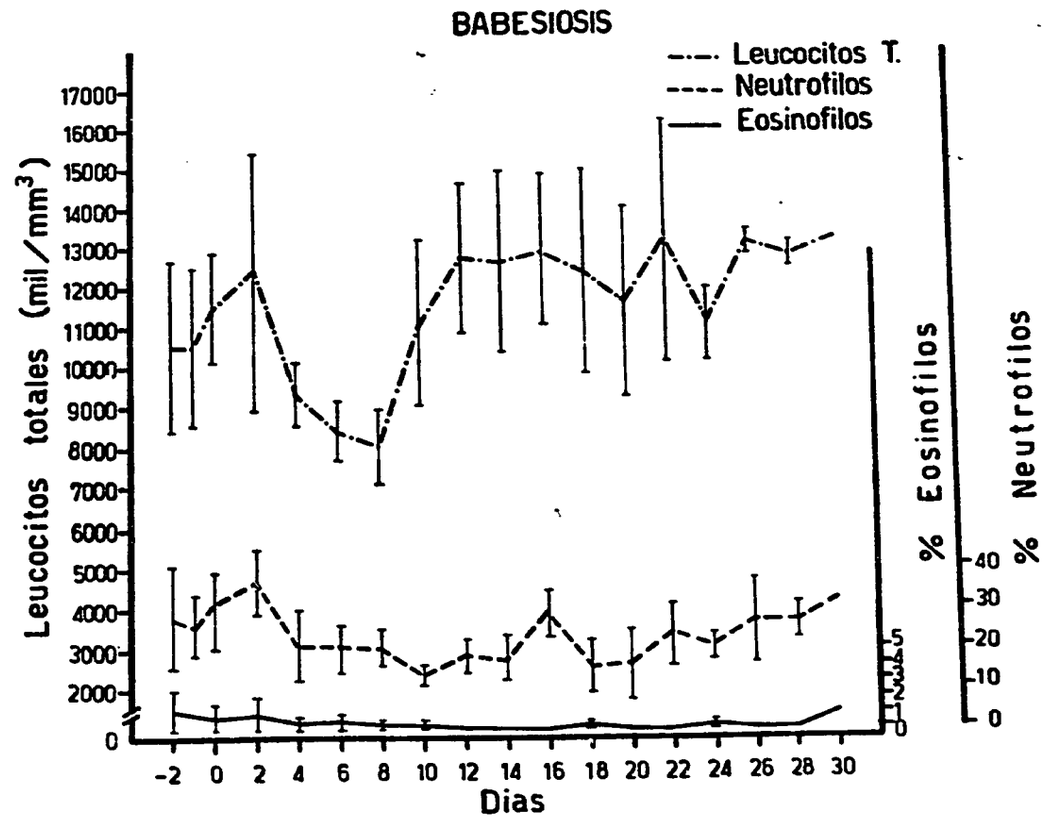


Figura 5. Promedio por grupo del recuento leucocitario total, porcentajes de neutrófilos y eosinfilos de terneros inoculados con B. bigemina.- Las líneas verticales nos muestran las desviaciones estandar para cada determinación y para cada día.

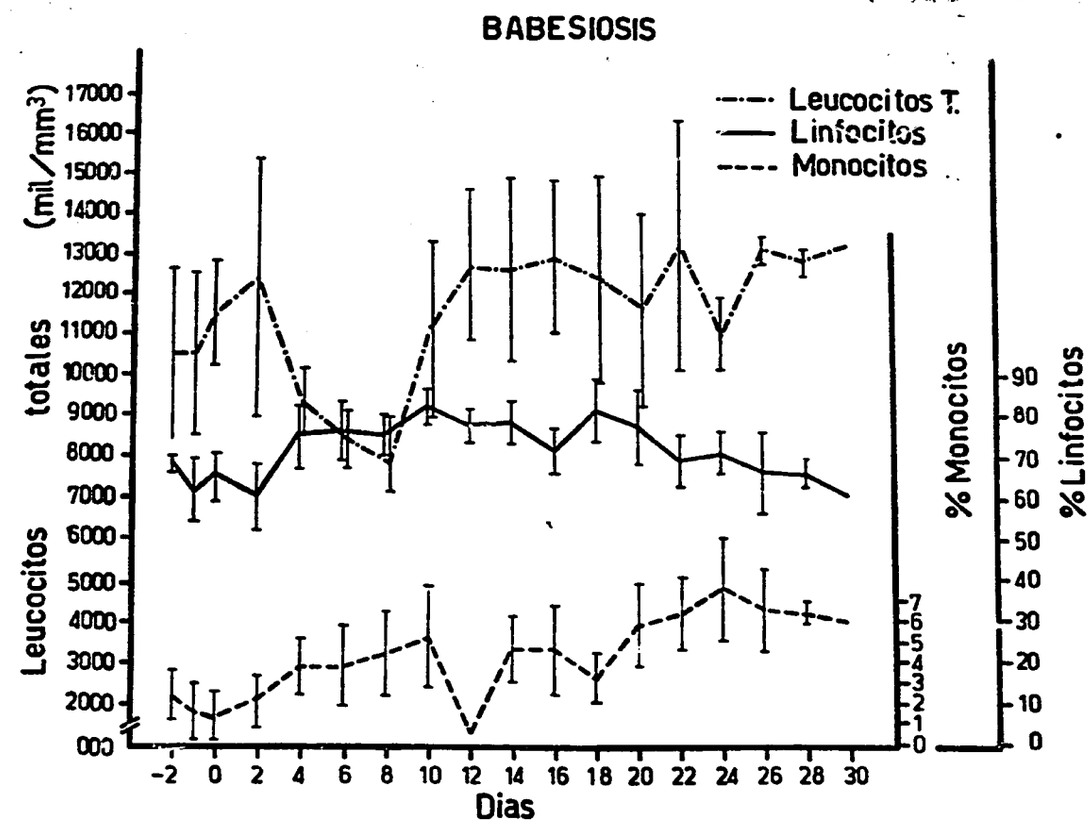


Figura 6. Promedio por grupo del recuento leucocitario total, porcentajes de linfocitos y monocitos de los terneros inoculados con B. bigemina.- Las líneas verticales nos muestran las desviaciones estandar para cada determinación y para cada día.

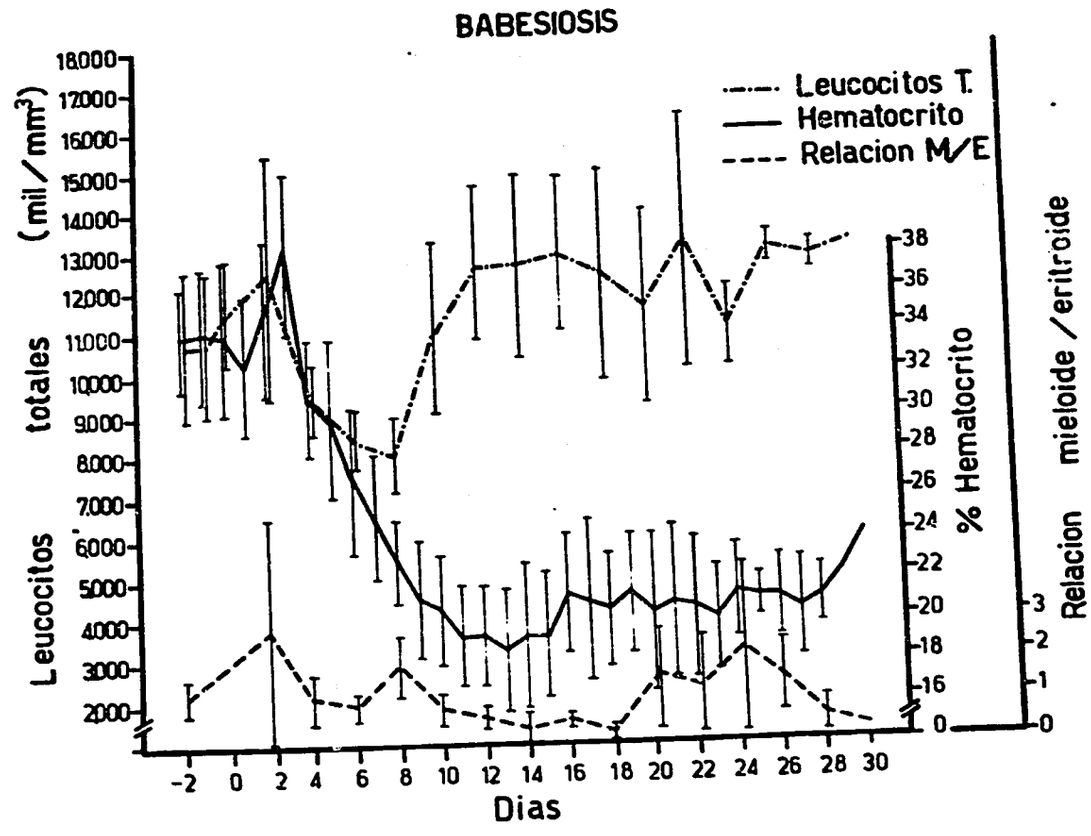


Figura 7. Promedio por grupo del recuento leucocitario total y de los cálculos del hematocrito y de la relación mieloide eritroide (M/E) de los terneros inoculados con B. bigemina.- Las líneas verticales nos muestran las desviaciones estandar para cada determinación y para cada día.

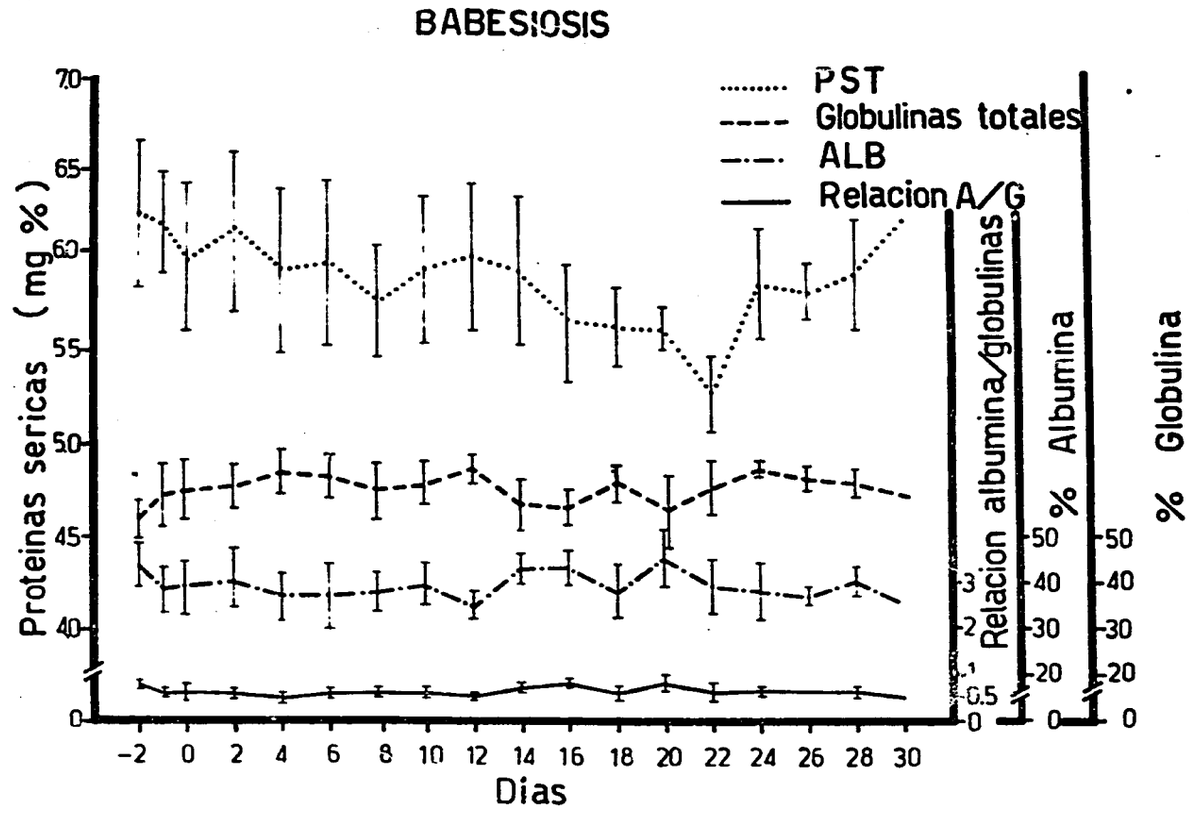


Figura 8. Promedio por grupo de los análisis de proteínas séricas totales, globulinas totales, albúmina y relación albúmina globulina (A/G) de los terneros inoculados con B. bigemina.- Las líneas verticales nos muestran las desviaciones estandar para cada una de las determinaciones y para cada día.

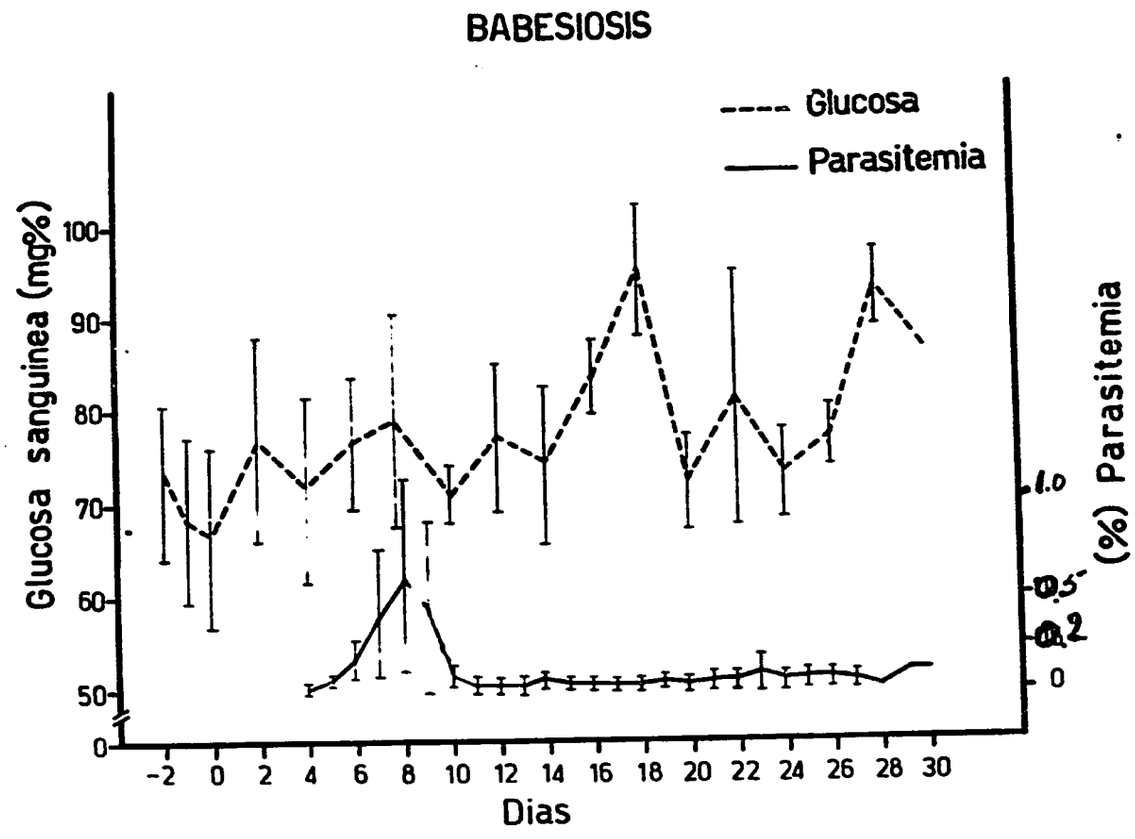


Figura 9. Promedio por grupo de los análisis de glucosa y parasitemia de los terneros inoculados con B. bigemina.- Las líneas verticales nos muestran las desviaciones estandar para cada una de las determinaciones y para cada día.

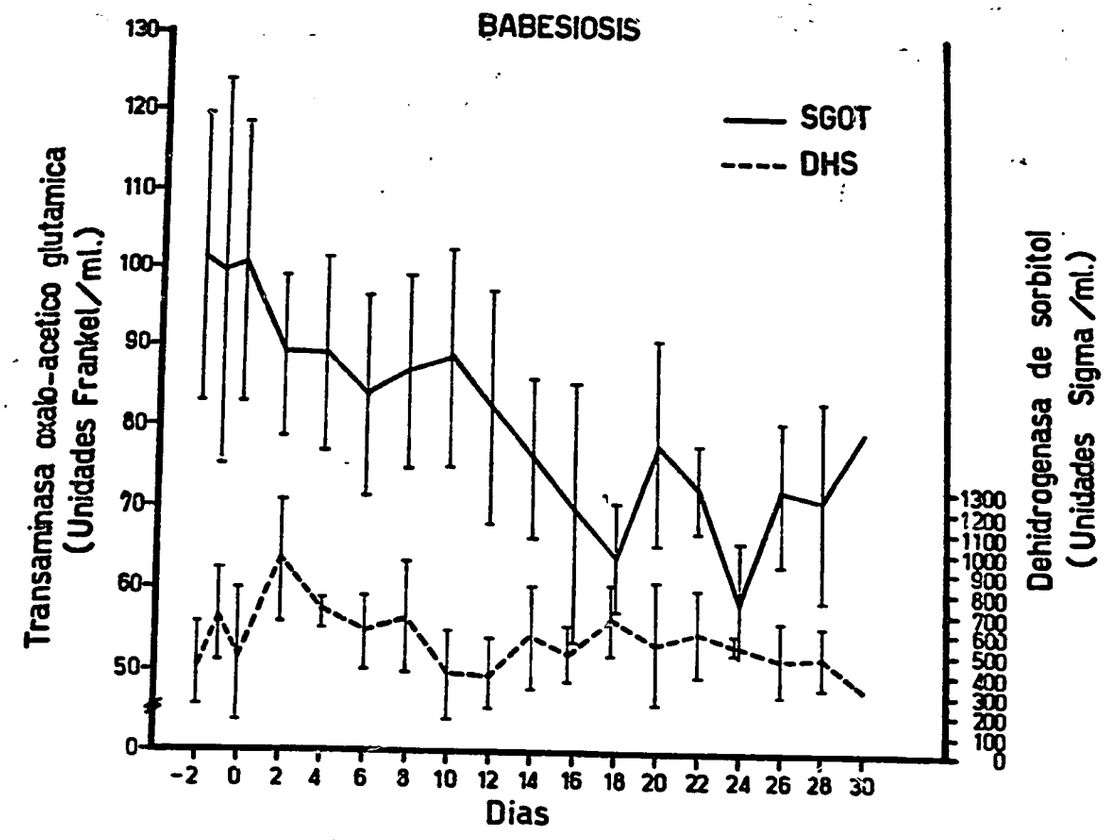


Figura 10. Promedio por grupo de los análisis de transaminasa oxaloacética glutámica y de la deshidrogenasa de sorbital de terneros inoculados con P. bigemina.- Las líneas verticales nos muestran las desviaciones estandar para cada una de las determinaciones y para cada día.

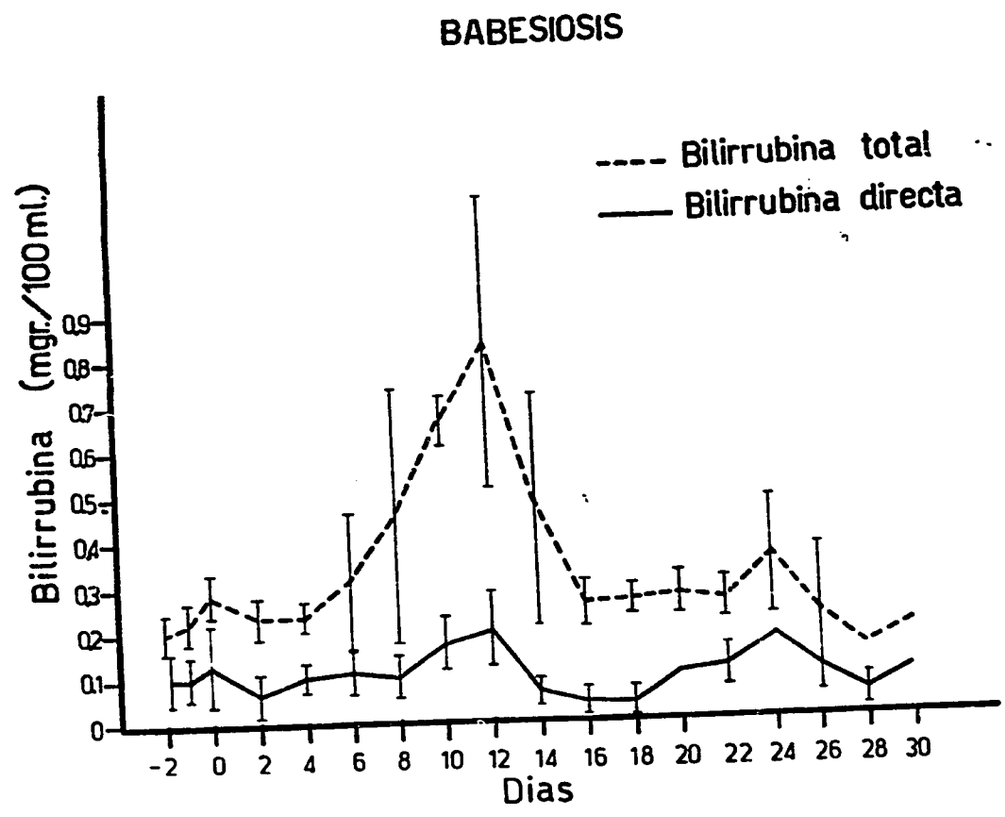


Figura 11. Promedio por grupo del análisis de la bilirrubina total y directa de los corderos inoculados con B. bigemina.- Las líneas verticales nos muestran las desviaciones estandar para cada una de las determinaciones y para cada día.

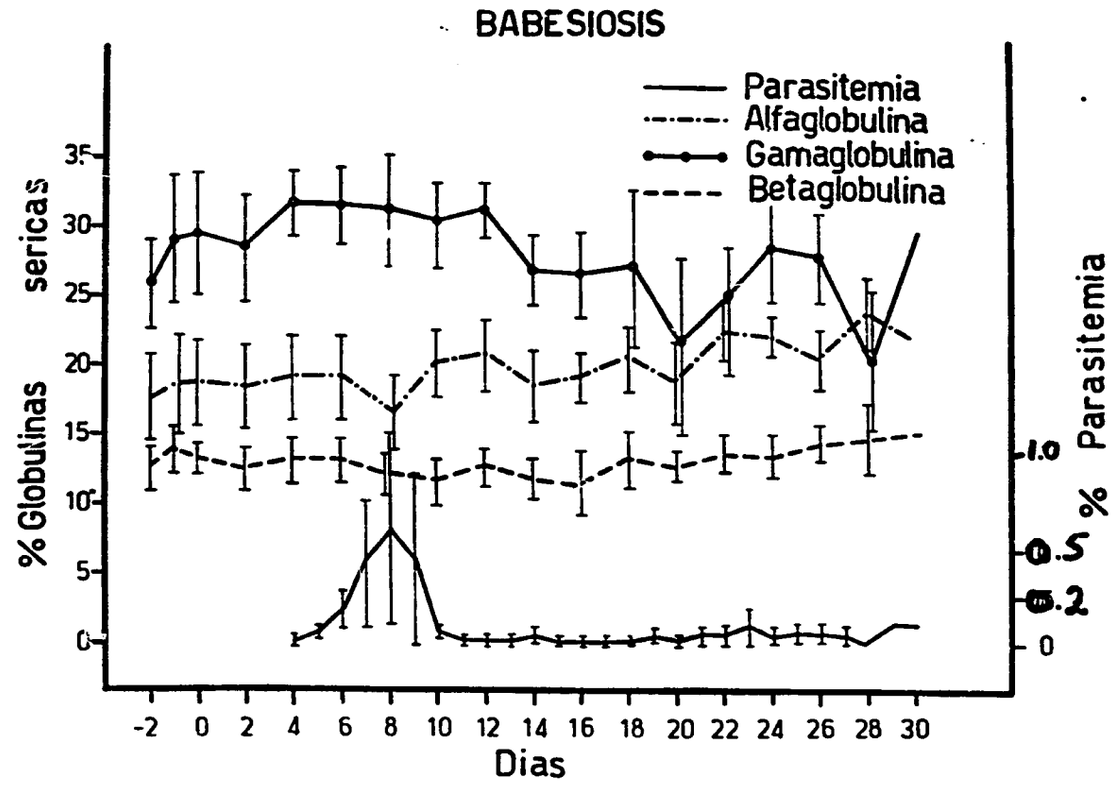


Figura 12. Promedio por grupo de los análisis de la parasitemia, alfa-globulina beta-globulina y gama-globulina de los terneros inoculados con B. bigemina. Las líneas verticales nos muestran las desviaciones standard para cada una de las de terminaciones y para cada día.

B A B E S I O S I S Tabla No. 2.

VARIACIONES EN EL RECUENTO DE LAS CELULAS ERITROIDES DE LA MEDULA OSEA *

DIAS / CELULAS	-2	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
RUB. BLASTOS	4.7	-	2.6	3.6	5.3	11.0	11.0	11.0	5.1	6.1	7.0	14.0	1.3	7.6	14.3	9.2	10.0
PROERITROCITOS	2.2	-	8.6	10.0	18.3	21.3	17.3	24.6	2.3	21.6	34.0	8.5	48.3	22.3	19.1	20.7	31.5
RUB. CITOS	91.9	-	66.0	101.0	129.0	76.6	121.6	114.5	163.0	129.8	123.7	48.5	104.8	91.5	94.6	135.3	141.0
MEGALOCITOS	118.8	-	83.0	133.0	122.0	70.3	126.3	141.3	179.5	169.0	230.2	6.8	89.5	77.6	85.6	174.2	175.1

* Estos resultados se obtuvieron al efectuar dos recuentos en cada uno de los cuales se contó un total de 500 células, luego se promediaron los resultados y así se obtuvo el recuento para cada muestra y para cada animal, finalmente se promediaron los resultados de los animáles examinados para así obtener cada uno de estos números.

B A B E S I O S I S Tabla No. 3

VARIACION EN EL RECUENTO DE LAS CELULAS MIELOIDES DE LA MEDULA OSEA*

Dias / Celulas	-2	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
Mieloblast.	6.3	-	6.6	6.6	8.0	13.3	12.3	10.0	8.1	3.3	3.5	9.5	17.8	14.0	10.0	5.2	7.5
Promieloc	3.8	-	2.6	4.6	4.0	6.0	4.6	3.3	1.3	1.3	2.2	2.5	7.5	5.1	3.0	9.5	2.5
Mie.Neutrof.	24.8	-	34.6	31.6	16.6	37.0	30.6	23.3	18.5	28.6	23.7	31.5	28.1	43.8	33.8	18.5	19.0
Metam.Neut.	42.0	-	66.0	30.3	32.3	54.0	36.0	32.8	14.0	21.5	14.7	24.5	32.3	32.8	26.3	27.0	12.5
Band.Neutrf.	57.0	-	66.0	46.0	38.6	60.6	38.0	22.6	33.6	31.8	18.5	80.8	48.1	81.1	74.3	37.7	40.0
Neutrofilos	56.4	-	86.3	75.0	44.0	72.0	4.7	30.5	21.3	42.6	18.2	38.5	47.5	63.1	78.1	39.2	44.0
Metam.Eosin.	14.7	-	17.0	3.6	14.3	12.0	6.6	8.0	1.1	3.0	9.7	--	10.0	2.3	--	--	0.5
Band.Eosinf.	14.6	-	15.3	3.3	16.3	12.0	6.3	8.6	8.0	2.3	1.2	--	5.8	2.1	--	--	1.0
Eosifofilos	4.9	-	2.6	3.6	4.0	4.6	0.6	2.6	4.5	1.6	--	4.0	7.8	3.5	0.5	0.2	--
Metam.Basóf.	0.6	-	0.6	0.3	1.0	1.0	0.6	0.8	0.6	0.6	0.2	---	2.0	0.5	0.5	0.7	--
Band.Basóf.	0.6	-	--	0.3	0.6	1.0	1.0	0.3	--	0.5	--	--	--	0.5	--	0.2	--
Basófilos	0.7	-	0.6	0.3	1.0	2.0	1.3	0.6	0.5	--	0.2	0.5	1.0	0.5	0.5	1.2	--
Linfocitos	22.1	-	20.0	42.0	23.0	18.0	23.3	22.3	16.1	35.2	13.7	55.0	13.3	37.8	14.6	15.5	8.0
Plasmocitos	1.4	-	2.0	1.6	3.0	1.3	1.3	0.6	2.0	2.0	0.5	8.5	4.8	4.6	4.0	1.5	0.5
Monocitos	7.0	-	8.3	4.6	10.3	16.3	11.0	7.1	5.6	6.0	3.2	3.0	15.0	10.3	7.1	2.0	4.0

* Estos resultados se obtuvieron al efectuar dos recuentos en cada uno de los cuales se contó 500 células, luego se promediaron los resultados y así se obtuvo el recuento para cada muestra, para cada animal, finalmente se promediaron los resultados de los 3 animáles examinados, para así obtener cada uno de estos números.

B A B E S I O S I S

VARIACIONES LINFOIDE Y RETICULAR EN NODULOS LINFATICOS.

NODULOS \ DIAS	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
PAROTIDEO	++ *	++ *	++ **	++ **	+ *	+++ **	++++ **	+++ *	++++ **	+++ *	++ **	+ ***	+ ***	++ ***	++ ***
SUPRAFARINGEO	++ *	++ *	++ **	++ **	++ **	++++ *	++++ **	+++ *	+++ **	+++ **	++++ **	+++ ***	++++ **	+ ***	++ ***
MANDIBULAR	+ *	+ *	++ **	++ **	++ **	+++ **	+++ **	++++ **	++++ *	++++ **	+++ ***	++ ***	++ ****	+++ **	+++ ***
PREESCAPULAR	+ *	+ *	+ **	++ ***	+++ ***	+++ ***	++++ **	++++ **	++++ **	++++ **	+++ ***	++ ***	+ ***	+ ****	++ **
MEDIASTINICO	- -	++ *	+++ *	+++ *	++ **	++ **	+++ **	++++ *	++++ **	+++ **	+++ **	++ ***	++ ***	+ ****	++ ***
HEPATICO	+ *	+ *	++ *	++ *	++ **	++ **	+++ **	+++ **	+++ **	++++ ***	++ ***	++ ***	++++ ***	++ ****	++ ***
ESPLENICO	++ *	++ *	++ *	++ **	+++ ***	+++ ***	++ **	+++ ***	++ **	++ **	++ ***	++ ***	++ *	++ **	++ **
PREFEMORAL	++ **	++ **	+++ *	++ **	+++ ***	+++ ***	++++ **	++++ **	+++ ***	++ ***	+++ ****	++ ***	++ ****	++ ****	+++ ****
MESENTERICO	+ *	++ *	++ *	+++ *	+++ **	+++ **	++++ **	++++ **	+++ ****	++++ ***	+++ ***	+ ***	+ ****	+ ****	+ ****

++ HIPERPLASIA LINFOIDE
- LIGERA..... +
- MODERADA..... ++
- MARCADA..... +++
- MUY MARCADA..... ++++

** HIPERPLASIA RETICULAR
- LIGERA..... *
- MODERADA..... **
- MARCADA..... ***
- MUY MARCADA..... ****

THE ISOLATION, SEPARATION, AND PRESERVATION OF *BABESIA BIGEMINA*

The history of the recognition of bovine babesiosis in Colombia and the identification of the 3 species responsible was recounted by Velásquez (1938). Bovine babesiosis exists in Colombia as a mixed infection of *Babesia bigemina*, *Babesia argentina* and *Babesia major*, and the incidence of infection appears to be related to the occurrence and activity of the tick-vectors at the various altitudes (Todorovic, *et al.*, 1969).

In an attempt to add to our knowledge of the mechanisms involved in acquired resistance to *B. bigemina*, a series of investigations were undertaken. As a starting point, it was necessary to have an isolate of *B. bigemina* free from contaminating organisms. However, *B. bigemina* carriers in Colombia are almost invariably infected with at least one other blood parasite. *Babesia argentina*, *B. major*, *Anaplasma marginale*, *Trypanosoma vivax*, and *Trypanosoma theileri* are also parasites of cattle in Colombia in areas where tick-vectors are present (Kuttler, *et al.*, 1969; Todorovic, *et al.*, 1969; Wells, *et al.*, 1970). Kuttler (1969) separated *Babesia spp.* and *A. marginale* from a mixed carrier from the Llanos Orientales (Eastern Plains) of Colombia. A pure *Babesia spp.* carrier was established by the simultaneous inoculation of mixed infected blood and an experimental drug

BW-356-C-61 (α-Ethoxyethylglyoxal Dithiosemicarbazone)* which prevented an A. marginale infection.

The organisms originally contaminating our isolate of B. bigemina were B. argentina, B. major, and A. marginale. This paper describes the isolation, separation, and preservation of a B. bigemina stabilate used at the Laboratorio de Investigaciones Médicas Veterinarias, Instituto Colombiano Agropecuario (ICA), Bogotá, Colombia.

Materials and Methods

Three-day-old male Holstein calves were obtained for the use as experimental animals from Facatativá, in an area free of Boophilus microplus. They were hand-reared on the Tibaitatá ICA experiment station, also in an area free of B. microplus.

The isolate of B. bigemina was made from a natural case of babesiosis. A four-month-old splenectomized calf was transported by airplane to the northern coastal area of Colombia and placed on a tick-infested pasture at the Turipaná ICA experiment station near Montería.

Thin smears were made with blood obtained from the jugular vein. The smears were fixed with methanol and stained with a

---

* Gloxazone, obtained from Burroughs-Wellcome and Co., Inc. Laboratories, Tuckahoe, New York.

1 to 20 solution of Giemsa stain for 20 minutes, using phosphate buffered water (pH 7.0-7.2) containing 0.01% alkyl phenoxy polyethoxy ethanol[a/] to prepare the stain solution. The smears were examined, using an oil immersion objective, for 30 minutes in cases when the first appearance of Babesia was thought to be imminent.

The method for separation of B. bigemina from contaminating organisms involved rapid passage through 5 splenectomized calves and was based on that used by Sergent et al. (1927) and Callow and Hoyte (1961a). The first calf was inoculated with blood carrying several different organisms, and subsequent subinoculations were done soon after blood smears from each calf were found to be positive for B. bigemina. Blood for inoculation was collected from the jugular vein without using an anticoagulant and each passage was carried out immediately by injecting the blood into the jugular vein of a susceptible calf.

The drugs used to treat the Babesia infections were trypan blue and diaceturato de 4,4'-diazoaminodibenzamidina[b/]. Trypan blue was used for suppressing a B. bigemina infection without interfering with a potential B. argentina infection.

---

a/ Triton X-100, Rohm and Hass Company, Independence Mall West, Philadelphia, Pa. 19105

b/ Ganaseg, Squibb, Cali, Colombia.

A frozen stabilate of the purified B. bigemina isolate was made using a modification of the method reported by Pipano and Senft (1966). Blood containing 2.5% B. bigemina parasitized erythrocytes was collected from the fifth splenectomized calf at the acute stage of the disease by venipuncture using ethylene diamine tetraacetic acid, disodium salt (Na_2H_2 EDTA, 2.0 mg/liter) as an anticoagulant. Glycerol was used as a cryoprotective agent, at a final concentration of 11.6%. Fifty ml of the blood-glycerol mixture were dispensed in 60 ml plastic narrow mouth bottles with screw caps,[c/] and incubated for 30 minutes at 4°C. After incubation the blood was stored in a dry ice cabinet at -79°C.

Results

The splenectomized calf that was placed on a tick-infested pasture at the Turipaná ICA station was found to be heavily infested with B. microplus ticks in the larva stage in 3 days, ticks in the nymphal stage in 8 days, and ticks in the adult stage in 14 days. After 16 days the body temperature of the calf rose to 40.4°C and B. argentina was found in thin blood smears stained with Giemsa stain. Two days later, B. bigemina was found in blood smears and 180 ml of blood were withdrawn from the jugular vein

---

c/ Kimble, Owens-Illinois, Toledo, Ohio 43601

using ethylene diamine tetraacetic acid dipotassium salt (1.3 gm/liter) as an anticoagulant. After airplane transportation to the ICA Laboratorio de Investigaciones Médicas Veterinarias in Bogotá, the blood was inoculated intravenously into a splenectomized calf, the first of a series of 5 splenectomized calves. The results of the passages through 5 splenectomized calves are summarized on Table I.

Babesia bigemina was found in blood smears from the first calf after 36 hours. Ninety minutes later, 12 ml of blood for the next passage were drawn from the first calf. A B. argentina infection, which developed in the first calf 4 days after the inoculation with blood, was treated with diaceturato de 4,4'-diazoaminodibenzamidina, 3 mg/kg for 5 days. Anaplasma marginale was present in smears from the first calf 44 days after inoculation.

The 12 ml of blood from the first calf were inoculated intravenously into the second splenectomized calf, immediately after the blood was drawn. Babesia bigemina was found in blood smears from the second calf after 30 hours. Within 1 hour, blood was taken A B. argentina infection, which developed in the second calf 4 days after the inoculation with blood, was treated with diaceturato de 4,4'-diazoaminodibenzamidina, 3 mg/kg for the following 2 days. The second calf died 1 day after the last treatment.

The third calf was found to be positive for B. bigemina 24

hours after inoculation with 12 ml of blood from the second calf, and the procedure of taking blood for passage into the fourth splenectomized calf was the same as for the previous calves in this series. The third calf was subsequently treated with an intravenous injection of 20 ml 1% trypan blue, which suppressed the B. bigemina infection. Babesia argentina and B. major infections, which developed in the third calf 15 days after inoculation with blood, were treated the following day with diaceturato de 4,4'-diazoaminodibenzamidina, 3 mg/kg. The third calf died 1 day after treatment.

The fourth calf was found to be positive for B. bigemina 37½ hours after inoculation with 12 ml of blood from the third calf. The procedure of taking blood and treating with trypan blue was repeated. The 12 ml of blood from the fourth calf were inoculated intravenously into the fifth splenectomized calf, immediately after the blood was drawn. Babesia bigemina was found in blood smears from the fifth calf after 57½ hours and within an hour 12 ml of blood were taken for intravenous passage into a non-splenectomized calf. A frozen stabilate of B. bigemina was made from the fifth 4 days after inoculation when the parasitemia had reached 2.5%. The day following freezing, 50 ml of the frozen stabilate was inoculated intravenously into a second non-splenectomized calf.

The non-splenectomized calf inoculated with 12 ml of blood

from the fifth calf was found to be positive for B. bigemina 2 days after inoculation. The second non-splenectomized calf inoculated with 50 ml of blood previously frozen from the fifth calf was found to be positive for B. bigemina 5 days after inoculation. The non-splenectomized calves did not require treatment with trypan blue. Babesia argentina, B. major and A. marginale were not found in any smear from either of the 2 non-splenectomized calves nor in any smear from the fourth and fifth splenectomized calves for 2 months after inoculation.

Discussion

Babesia bigemina was found in blood smears from a splenectomized calf 18 days after it was placed on a tick-infected pasture. Callow and Hoyte (1961a) observed the experimental transmission of B. bigemina by B. microplus and recorded patent infections with B. bigemina 11 to 17 days following the placement of larva on cattle. Callow and Hoyte (1961b) also reported that transmission of infection to the cattle did not take place until the ticks were nymphs. Adults were also shown to be capable of transmitting infection and B. bigemina was found to be present in maximum numbers in smears during the adult stage of the ticks. The observations of the experimental transmission of B. bigemina by B. microplus recorded by Callow and Hoyte (1961b) are in agreement

with the observations on a natural transmission of B. bigemina in our experiment.

The ability of B. bigemina to multiply so rapidly that it was found in thin blood smears from 24 to 57½ hours after calves had been inoculated with relatively small numbers of parasites, made it possible to separate B. bigemina from other blood parasites. Either the latter do not multiply as rapidly, or their predilection site is the visceral circulation. The prepatent periods for B. bigemina observed in our experiments were in agreement with those prepatent periods reported by Callow and Hoyte (1961a).

The apparent lability of newly isolated Babesia spp. emphasizes the need for the establishment of stabilates (Irvin and Brocklesby, 1969; Frerich, et al., 1969). Many factors, such as clonal selection, methods of transmission, type of host animal, and antigenic variation are probably involved in the lability of Babesia spp. (Phillips, 1969). A frozen stabilate of the B. bigemina isolated in this study was established.

Summary

This paper describes the origin of a Babesia bigemina stabilate used at the Laboratorio de Investigaciones Médicas Veterinarias, Instituto Colombiano Agropecuario, Bogotá, Colombia. Experiments were carried out to separate B. bigemina from contaminating

organisms. The method used was to passage the B. bigemina through 5 splenectomized calves. The first calf was inoculated with blood carrying several different organisms, and subsequent subinoculations were done soon after blood smears from a calf were found to be positive for B. bigemina.

Five blood passages were carried out in 6½ days. Babesia argentina, B. major and A. marginale were eliminated as contaminants of the B. bigemina isolate after four passages. A frozen stabilate of the isolated B. bigemina was established.

References

1. Callow, L. L. and Hoyte, H. M. 1961a. The separation of Babesia bigemina from Babesia argentina and Theileria mutans. Aust. Vet. J., 37: 66-70.

2. Callow, L. L. and Hoyte, H. M. D. 1961b. Transmission experiments using Babesia bigemina, Theileria mutans, Borrella sp. and the cattle tick, Boophilus microplus, Aust. Vet. J., 37: 381-390.

3. Frerichs, W. M., Holbrook, A. A., and Johnson, A. J. 1969. Equine Piroplasmosis: Production of antigens for the complement fixation test. Am. J. Vet. Res., 30: 1337-1341.

4. Irvin,A. D. and Brocklesby, D. W. 1969. Continuous high parasitemia in mice infected with Babesia (Nutallia) microti. J. Parasit., 55: 1190.

5. Kuttler, K. L. 1969. Isolation of a single hemoparasite from animals with multiple infections. Progress report of Hemotropic Disease Research, pp. 108-112. Texas A&M University.

6. Kuttler, K. L., Adams, L. G., and Zaraza, H. 1969. An epidemiological and geographic survey of Anaplasma marginale and Trypanosoma theileri in Colombia. J. Am. Vet. Med. Assoc., 154: 1398.

7. Phillips, R. S. 1969. Babesia rodhaini infections in the rat after acute primary parasitemia. Parasitology, 59: 349-356.

8. Pipano, E. and Senft, Z. 1966. Storage of Babesia bigemina, Babesiella berbera, Anaplasma marginale, and Anaplasma centrale in deep freeze. J. Protozool., 13 (Suplement): 34.

9. Sergent, E., Donatien, A., Parrot, L., Lestoquard, F., and Plantureux, E. 1927. Etudes experimentales sur les piroplasmoses bovines d'Algerie. Ann. Inst. Pasteur, 41: 721-785.

10. Todorovic, R. A., Adams, L. G., and Roberts, E. D. 1969. A study of bovine babesiosis in Colombia, South America. J. Am. Vet. Med. Assoc., 154: 1399.

11. Velásquez, J. 1938. Contribución al estudio de las piroplasmosis de los animales domésticos en Colombia. Revista de Medicina Veterinaria (Bogotá) Año VIII, No. 72, pp 67-108.

12. Wells, E. A., Betancourth, A., Page, W. A. 1970. The epidemiology of bovine trypanosomiasis in Colombia. Trop. Anim. Hlth. Prod., 2: 111-125.

IV. A. 3. Serodiagnosis of Babesiosis

Introduction

Past investigation was concerned with the development of a serologic technique which would aid in diagnosing babesiosis. A practical method of antibody detection would be most desirable for the early diagnosis of babesiosis during the various phases of infection when the parasites are not readily detectable in blood films (Malherbe, 1956). In spite of several attempts, no satisfactory practical procedure was devised for the serologic diagnosis of babesiosis, particularly of the latent form.

In this review an attempt was made to summarize and discuss the recent advances in sero-diagnosis of babesiosis with special attention to the serologic techniques used in Laboratorio de Investigaciones Médicas Veterinarias for the detection of babesial antibodies in infected cattle.

Literature Review

For more than 70 years, babesiosis has been recognized as specific infectious entity and a severe economic threat to the cattle industry in many areas of the world; however, the naute of the immune host-parasite interaction has remained little understood (Zuckerman and Ristic, L968).

A characteristic feature of Babesia infection is that animals which recover from an acute infection become carriers of the respective hemoparasite. These carrier animals cannot be diagnosed in blood films by any of the comtemporary staining methods. Thus, in order to identify carrier animals, it was necessary to develop serological methods to detect specific antibodies rather than the parasites themselves.

In the last two decades some fundamental knowledge concerning the immuno-serology of several Babesia species has led to the development of sero-diagnostic procedures which provided a means for studying the pathogenesis of babesiosis and the detection of animals with subclinical infection. The antigens used in these serological procedures originated from parasitized erythrocytes and serum or plasma of animals with acute babesiosis.

Parasitic-erythrocytic antigens may be used in a variety of tests, e.g., Complement fixation, gel-diffusion, fluorescent antibody, agglutination test, etc., for detection of babesial antibodies. The complement fixation (CF) reaction constituted one of the earliest tests for the diagnosis of babesiosis. A specific CF reaction for Babesia has been found useful in detecting CF antibodies in the serum of animals with chronic babesiosis. However, the practical application of CF test was not conclusively demonstrated.

Hirato et al. (1945) first reported the possibility of the application of CF test for diagnosing equine babesiosis. The antigen consisted of stromata of erythrocytes from acutely infected horses with Babesia caballi. By means of the CF test it was found that antibodies appeared in the blood 11 to 15 days after the onset of parasitemia and in one instance persisted for 100 days.

Mahoney (1962) prepared antigens from the blood of cattle with high parasitemic, pure infections of either Babesia bigemina or Babesia argentina. In the serums of calves with pure infections of these parasites, homologous complement-fixing antibodies developed 7 to 21 days after infection. Antibodies were detected at all stages from the advent of a parasitemia detectable in thick blood films to a time when the parasites were in numbers too low for detection by microscopic examination. The antigens appeared to possess a degree of species specificity showing only limited reaction with heterologous antibody. The use of the CF test in epizootiological studies of bovine babesiosis was reported by Mahoney (1964). Both antigens of B. bigemina and B. argentina were used in his study. During the initial infection, the complement-fixation reaction was positive in 94% to 100% of all cases studies. However, after 4 to 5 months the number of calves positive to the test dropped

below 50%. The same pattern followed calves born in enzootic areas except that the percentage of reaction dropped considerably. Homologous reactions were consistent and dependable (74%) during the first few months after infection, but positive reactions fell to 8% after several months infection with B. argentina. With B. bigemina antigen 95% reacted in the 3rd month of infection, but fell to 20% by the 7th month.

Schindler and Denning (1962) studied the CF reactions in animals infected with Babesia rodhaini and Babesia canis. An antigen for the CF test was prepared from erythrocytes of dogs acutely infected with B. canis by means of immunohemolysis or by exposing the erythrocytes to 0.3% saponin. Clear supernatant fluid of B. rodhaini lysed in distilled water and centrifuged to remove erythrocyte stromata served as the CF antigens. Positive CF reactions as the animals probably succumbed before the antidies could be demonstrated. Babesia canis antibodies were demonstrated in dogs 11 to 36 days after infection and were also detected in animals that had harbored parasites for years. No serologic CF cross-reaction was observed between B. rodhaini and B. canis using the antigens of the respective parasites. Lyophilization was found to be an efficient means for long-term preservation of the antigen.

Schindler et al (1966) studied the immunologic and serologic reactions to B. canis infection, applying both CF and the indirect

Coon's tests. Animals had been immunized against the infection with live Babesia canis or soluble antigens of B. canis. Immunity was acquired only after infection with live B. canis. This resistance remained even after B. canis disappeared from the blood and even after disappearance of CF antibodies. Dogs vaccinated with soluble antigen from B. canis produced CF antibodies but not protective immunity.

Mahoney (1967a) found that the most sensitive CF antigens for B. bigemina and B. argentina were a crude suspension of the parasites prepared by distilled water lysis of infected erythrocytes followed by centrifugation. In tests on serums obtained from non-exposed cattle, non-specific CF reactions were observed in low dilutions of serum. More non-specific reactions (4.5%) were found when B. argentina antigen was used in comparison with B. bigemina antigen (1.6%). The non-specific reaction to B. argentina were prevented by absorption of the serum with a suspension of normal bovine erythrocytes stroma. Cross-absorption tests indicated that the B. bigemina and B. argentina CF reaction each consisted of major species -specific and a minor group -specific component. These results suggested that substances associated with B. argentina rather than contaminated material of bovine origin in the crude suspensions were responsible for the differences in specificity.

Mahoney (1967b,c) reported the application of the CF test in the evaluation of resistance against bovine babesiosis produced by vaccination with killed-B. argentina vaccine or by passive immunization of calves against B. argentina. Animals immunized with killed-B. argentina reacted to the injection by the formation of CF antibodies. These CF-antibodies responses during the course of infection with killed-Babesia parasites were used as a guide that adequate antigenic stimulation was determined for production of protective immunity against babesiosis. However, in the experiments with passive immunization against B. argentina with special reference to the role of CF antibodies, the results were different. The failure of convalescent serum which contained high level of CF-antibodies to give protection suggests that these antibodies were not important in the immune reaction. Therefore, no positive correlation between protecting capacity and CF titer of serum could be demonstrated. The CF antibodies were unlikely to be concerned with immunological resistance to B. argentina.

Frerichs et al. (1969a,b) prepared an antigen from erythrocytes infected with B. caballi. Blood was collected at the height of parasitemia (3 to 7%) and infected erythrocytes were mixed with 10 volumes of cold distilled water, and lysed for 1 to 8 hours at $4^{o}C$. The lysate was passed through a refrigerated

centrifuge at the rate of 2-4 liters per hour at 2.8 kg of air pressure per square cm^2. The paste which collected on the wall of the centrifuge cylinder consisted of parasites and erythrocytic stroma which was mixed with veronal buffered saline, filtered and lyophilized and stored at -20°C. The serologic responses of fifteen horses were studied by CF test. The CF-titer persisted longer in 14 of the horses than did their capability to transmit the disease, and this was a reliable index of exposure to B. caballi. The authors concluded that the greatest difficulty in producing B. caballi antigen was due to the low yield of antigen that was caused by low rate of parasitemia. The use of splenectomized horses along with the administration of synthetic corticosteroids, greatly alleviated this difficulty. Loss of serologic specificity by Babesia equi after it was passaged in horses was the greatest problem in producing CF antigen. This problem could be overcome by (1) obtaining fresh isolates under field conditions or (2) storing the parasites in liquid nitrogen before specificity was lost.

Ristic and Sibinovic (1964) obtained an antigen from the lysate of erythrocytes of horses acutely infected with B. caballi by means of precipitation with protamine sulfate. The antigen was used to detect antibodies in horses with babesiosis in double-gel diffusion test. The reaction of equine sera in gel-precip-

itation test was correlated with the persistence of the latent infection as determined by transmitting infections from carriers into susceptible horses. The specificity of the test was shown by the abscence of reaction with sera of horses with various other infections, including viral infectious anemia.

Ristic et al (1964) used the fluorescent antibody technique to study the erythrocytic growth and developmental stages of B. caballi and B. equi isolated from horses in Florida. By means of FA the morphological structure of Babesia spp. from horses was identified.

Ristic and Sibinovic (1964) used the one-step fluorescent-labeled antibody inhibition test in which an antigen is reacted simultaneously with a mixture of fluorescein-labeled and unlabeled antibody to detect circulating anti-Babesia antibody. According to the authors the one-step fluorescein-labeled antibody inhibition test can be considered only as a research tool requiring further study and evaluation of its accuracy and specificity.

Madden and Holbrook (1968) used the indirect fluorescent antibody technique to detect antibody in carrier horses experimentally infected with B. caballi. The reactions were determined to be specific. There was no cross reaction with serums from carrier horses experimentally infected with B. equi when used in the indirect fluorescent antibody test for B. caballi and the

test was used to differentiate the two infections. According to the authors the procedure involved in preparing the reagents and conducting the test were rather straight forward, however, difficulty arises in reading and interpreting the results. Identification of a strongly positive reaction is relatively simple, but differentiation between a weakly positive reaction and a negative requires considerable experience.

Ross and Löhr (1968) developed an indirect fluorescent antibody test (IFA) for the detection of B. bigemina antibodies in bovine serum. The test was considered to be highly specific and accurate and sufficiently sensitive to detect reactors infected two years previously. Susceptible and immune clinical responses were obtained when groups of negative and positive reactors to the IFA were challenged with B. bigemina infected blood. By means of the test it was possible to demonstrate, test reliability, relationship between protective antibodies, and those detectable by immunofluorescence. Serologic responses to the challenge with B. bigemina infected blood were measured by the IFA test and it was found that titers reaching peaks at 21 days after infection, with maximum titers up 1:1280 waining gradually thereafter, but still above minimum levels at the end of six months periods. Some evidence was produced indicating a continued downward trend with minimum positive IFA responses still

detectable 18 to 24 months after a single experimental infection.

Curnow and Curnow (1967) reported an indirect hemagglutination test (IH) for diagnosing B. argentina infection in cattle. Formalinized sheep erythrocytes treated with tannic acid were sensitized with an extract of the parasite-erythrocyte stroma mixture used as the antigen in the CF test for B. argentina. This antigen was also used to inhibit the reaction in hemaglutination inhibition test. Bovine erythrocytic stroma antigen prepared from uninfected blood was used to inhibit the indirect (HI) hemagglutination test and to absorb serum before CF testing. By doing this it was possible to remove some of the non-specific reactions. The sensitivity and specificity of the IH and CF tests were found to be similar.

Todorovic et al (1967) reported a hemagglutination test for titrating antibodies in rats infected with Babesia rodhaini. Heparinized blood was collected from uninfected rats to provide erythrocytes for test. A 2% suspension of trypsinized erythrocytes were used. Serial dilutions of sera were prepared in physiologic saline. A positive test was indicated by a single clump which did not break up readily on manual agitation. Hemagglutinins were first detected on 6 day post-infection with the high titers occurring on 12 day which coincided with the peak of parasitemia.

Curnow (1968) reported an agglutination test for detection of specific B. argentina antibodies by using highly parasitized bovine erythrocytes as an antigen. The reaction appeared to be highly specific for each isolate of B. argentina.

Sibinovic et al (1968) used a heat stable polysaccharide antigen extracted from equine erythrocytes infected with B. caballi and B. equi. The antigen was employed for titration of antibodies in the serum of horses affected with babesiosis by means of a bentonite agglutination test and passive hemagglutination tests. In comparison with the gel precipitation reaction, bentonite agglutination and passive hemagglutination tests required less antigen and rendered results more rapidly.

Parasitic-serum antigens may be used in a variety of serologic tests for the detection of Babesia antibodies e.g. gel-precipitation, latex-agglutination. Sibinovic et al (1965) observed a precipitation reaction in double-gel diffusion test when serum from a horse acutely infected with B. caballi and B. equi was allowed to react with serum from a convalescent horse. Antibodies to the serum antigens were detectable at 3 months after infection and persisted at variable levels for an additional 7 months. The antigen disappeared from the serum of infected horses approximately one month after infection.

Mahoney (1966) detected a circulating antigen in acute-phase

of infection with B. bigemina and B. argentina by means of gel-diffusion technique.

The serum antigens isolated from dogs with acute Babesia canis infection were absorbed to latex particles (0.81 micron in diameter) in accordance with the method described by Todorovic et al (1968) for absorption of explasmodial antigens. These sensitized latex particles were used as agglutinogens in a tube-latex agglutination test (TLA) for titration of antibodies in sera of dogs infected with B. canis (Zuckerman and Ristic, 1968). The earliest that antibodies were detected in the TLA test was approximately 15 days following infections; about 2 months later they reached a maximal titer and persisted at low levels during the next four months after infection. The persistence of serum agglutinins was generally correlated with the persistence of a carrier stage of infection.

Mahoney and Gooder (1969) reported that two antigenic substances were present in serums collected from acute infection with B. argentina, however, they were absent from serum of non-infected cattle. This was revealed by gel-precipitation test. Antiserum to infected calf serum prepared in rabbits and then absorbed with normal calf serum, contained precipitating antibodies to components absent from the serum of noninfected calves. Such serum was used in attempts to characterize antigenic substances produced during acute B. argentina infection. The first of these

antigenic substances was immunologically identical with bovine fibrinogen, and the second appear to be a pigmented macromolecular complex found in the tissue of normal cattle. Soluble serum antigens of parasitic origin were not detectable, perhaps they either did not induce a strong precipitation response or were in such minute quantity, that detection was beyond the sensitivity of gel-diffusion test used in his study.

Ferris et al (1968) discussed the significance and application of Babesia serum antigens in serodiagnosis of babesiosis. A new terminology was suggested for these soluble antigens found in serum or plasma during acute Babesia infections. Hence, they are different of erythrocytic antigens they were termed ecto-antigens. The ectoantigens are complex in nature containing peptids, lipids, phosphatides and polysaccharides and can be used for stimulation of protective antibodies and also used in diagnosis of acute and chronic infection of Babesia species of animals and man.

Materials and Methods

Experiments were carried out to evaluate the various serologic techniques for detection of Babesia antibodies in cattle, and to develop a new serologic test for diagnosis of Babesia infection. Research was conducted in cooperation with the

Instituto Colombiano Agropecuario (ICA) in Bogotá, Colombia.

Complement Fixation Test: The complement fixation test used to detect babesial antibodies in the serum of infected animals was modified by Todorovic *et al* (1971) from the original test described by Mahoney (1962). The technique consisted of (1) the isolation, purification, and titration of the babesial antigens; (2) preparation of the hemolytic system; (3) complement titration; and (4) performance of the test in tubes and in the microtiter system.

I. Isolation and Purification of Babesial antigens.

A. Maintenance of Infection. Ten Holstein calves 2 to 3 months of age were splenectomized under general anesthesia (Equi-thesin, Jan-Sal, 25 to 40 ml I.V. per animal). After the operation the valves were examined for hemotropic infections at daily intervals for three weeks and thereafter weekly for two months. The calves were infected with pure strains of *Babesia bigemina* and *B. argentina* or both organisms, respectively. When the parasitemia developed to a high level, three passages were made through another group of splenectomized calves. After the third or fourth passage in splenectomized calves, approximately 50% parasitemia was observed for *B. bigemina* and approximately 25% parasitemia for *B. argentina*.

B. Preparation of Babesial Antigens. Two techniques were used for the isolation of *Babesia* antigens.

Technique 1. Blood from a highly parasitized, splenectomized calf was collected by canulation of the carotid artery. Three to 5 liters of blood were collected in flasks containing EDTA (1.2 gm per liter of blood) as an anticoagulant. After collection, the blood was stored for 3 hours at 3 to 5°C for further processing. The plasma was separated from the infected erythrocytes by centrifugation in IEC centrifuge at 2,500 rpm for 20 minutes. Thereafter, the infected erythrocytes were washed 2 times in a sterile physiologic saline solution (0.85%) by centrifugation as above, and finally, the erythrocytes were resuspended in 10 times of their volume with veronal buffer (pH 7.3-7.4). After washing the erythrocytes were disrupted by sonification. This process was performed on 150 ml of suspended erythrocytes in an ice water bath (3 to 5°C) on the sonifier for 6 minutes at a power of 50 watts (Sonifier Cell Disruptor, Model W 185, Heat Systems-Ultrasonics, Inc., Plainview, N. Y.). The pooled suspensions of sonified infected erythrocytes were centrifuged in a Sorvall Superspeed RC2B automatic refrigerated centrifuge for 30 minutes at 15,000 rpm (27,000xg).

After centrifugation, the supernatant was saved for further studies (Su-I) and the sediment (Se-I) was resuspended (10 times volume) veronal buffer, and homogenized in a tissue homogenizer (Pyrex 40 ml) for 15 strokes and recentrifuged in the Sorvall

centrifuge for 30 minutes at 27,000xg. The resulting supernatant (Su-2) was used in the complement fixation tests as Babesia antigen A. Sediment II was resuspended in veronal buffer, homogenized, and recentrifuged as in the process above. Supernatant III was collected and used in the complement fixation test as Babesia antigen B. This technique was repeated to obtain antigen C and D. The procedure described above can be repeated as long as antigenic activity can be found in the sediments. The above procedures should be performed on the same day that the blood is collected.

Technique 2. Blood from a highly infected splenectomized calf was collected as in Technique 1, and was centrifuged immediately at 2,500 rpm for 20 minutes. The plasma was decanted and saved for isolation of serum soluble antigens. The infected erythrocytes were washed twice in sterile 0.85% NaCl. Ten volumes of cold, sterile distilled water was added to sediment. This suspension was placed in the refrigerator at 3 to 5°C for an hour to allow for erythrocyte lysis. If lysis is not complete, the suspension was left in the refrigerator for one additional hour. This pooled lysate was centrifuged at 5,500 rpm (3,500xg) for 30 minutes in the Sorvall centrifuge. The sediment was suspended in an equal amount of veronal buffer pH 7.3 and 200 ml of the suspension sediments and veronal buffer were diluted to make

one liter. This suspension was homogenized in the Pyrex tissue homogenizer for a total of 15 strokes and stored in sealed 10 ml ampules at minus 60°C. The contents of the ampules were thawed and further processed for isolation of antigens A, B, C, and D, as described in technique 1, on the day when test was performed.

II. Titration of Babesial Antigens: The antigenic suspensions were diluted with veronal buffer pH 7.3 up to 1:128 or more. The titration was performed by using known positive serum, negative serum, antigenic fractions, veronal buffer pH 7.3, complement and hemolytic system according to the procedure described in Table 4, in a Manual for Conducting the Complement Fixation Test for Anaplasmosis, ARS, USDA (1958). When the titration end point was determined, the desired dilution of babesial antigen was calculated and used in complement fixation test.

III. Preparation of Hemolytic System: A normal adult female sheep was bled from the jugular vein under aseptic procedures into a 500 ml flask containing 150 ml of Alsever's solution until the level totaled 300 ml. When sufficient blood was collected to total 300 ml, the solution of blood in Alsever's was thoroughly and gently mixed and was stored at 4°C. This stock suspension was refrigerated one day prior to using in the complement fixation test and can be kept and used for two weeks if hemolysis does not occur.

Each day that sheep erythrocytes were needed for the CF test, the contents of the flask of stock supply were mixed by gentle rotation. Sufficient blood cells for test requirements were removed by a sterile pipette and placed in a graduated conical centrifuge tube. This suspension was centrifuged in a IEC centrifuge for 10 minutes at 1,700 rpm. The supernatant was decanted by suction through a capillary pipette taking off the total upper white layer of cells and supernatant. If the supernatant was not completely colorless on the third washing, the cells were considered too fragile and were not used in the complement fixation test. The tube was refilled with 5 volumes of veronal buffer and the cells were washed three times. Finally, the packed cells were suspended in veronal buffer to make a 2% suspension for test use.

Commercially produced hemolysin was used in the CF test. The anti-sheep hemolysin preserved with equal parts of glycerol was produced by Sycco-Sylvana, Millburn, New Jersey, U.S.A. Two units of hemolysin were diluted in veronal buffer pH 7.3 so that this quantity was contained in 0.5 ml. The hemolysin titration and dilution was performed in accordance to the procedure described in a Manual for Conducting the CF test for Anaplasmosis, ARS, USDA (1958). In the preparation of the hemolytic system equal amounts of the 2% suspension of sheep

erythrocytes and diluted titrated hemolysin were admixed and stored at 3 to 5°C for one day before using.

IV. Complement Titration: Lyophilized guinea pig complement, commercially produced by Texas Biological Laboratories, Inc., Fort Worth, Texas, consisting of pooled, fresh whole serum from adult normal animals were used for CF test. Five ml of restoring solution was added to the lyophilized complement. The complement was then titrated in accordance to the procedure described in a Manual for Conducting the CF test for Anaplasmosis, ARS, USDA (1958).

V. Performance of the Complement Fixation Test: When all of the components were standardized, the performance of the CF test was as follows: For each serum sample tested, 0.1 ml of serum was pipetted into each of two tubes containing 0.4 and 0.9 of veronal buffer pH 7.3, respectively. The second tube was used as a serum control. The tubes were placed in a water bath at 56°C for 30 minutes for inactivation. Tubes were removed from water bath and allowed to stand at room temperature for 90 minutes. Then, 0.5 ml containing 2 units of *Babesia* antigen was added to one of each tube being tested, no antigen was added to the second control tube. Two units, 0.5 ml of complement was added to both tubes, and the tubes were incubated for one hour in a water bath at 37°C. Then 1 ml of hemolytic system was

added to the tubes and again the tubes with contents were incubated for 45 minutes in a water bath at 37°C. The results were read immediately or after 12 hours. Immediate reading can be done after centrifugation at 1,000 rpm for 2 to 3 minutes in the IEC centrifuge. Delayed reading can be performed if the test tubes are refrigerated overnight at 4°C. All serum control should be completely hemolysed, because they contained no antige If any tube contains non-hemolysed erythrocytes, the sample was reported as "anticomplementary", false positive, and the status of the sample can be determined by special procedures. Those tubes which contained the test sample and Babesia antigen showed reactions from complete hemolysis (negative) to complete fixation (positive). The interpretation falling between these two reactions is as follows: 4 plus no hemolysis -strong positive; 3 plus, slight hemolysis, positive; 2 plus, medium hemolysis -suspicious positive; 1 plus, considerable hemolysis -suspicious; and complete hemolysis -negative.

VI. Serum Titration: When the screening test was performed to determine the number of positive samples, the next procedure was to establish the antibody titer. This was accomplished by serially diluting the serum in veronal buffer pH 7.3 until a final dilution of 1:1280 was attained according to technique described in the Manual for Conducting the CF Test for Anaplas-

mosis, ARS, USDA (1958) or by means of the microtiter technique described by Hidalgo and Dimopoullos (1967) for diagnosing bovine anaplasmosis. The microtiter technique as modified was performed as follows: In a sterile tube which contained 0.3 ml of veronal buffer, 0.2 ml of serum tested was delivered. The tube was placed in a water bath at 56°C for 30 minutes. By means of a plastic pipette (microtiter, long delivery tester, Cooke Eng. Co., Alexandria, Va.) one drop of veronal buffer (0.025 ml) was delivered into a plastic test plate, containing "U" shaped wells. Then a metal drop deliverer was immersed in a tube containing inactivated serum and delivered into first recess of plastic plate. The deliverer was rotated and transferred to next recess, so making serum dilutions from 1:5 to 1:640. By means of plastic pipette one drop (0.025 ml) of Babesia antigen was added to each dilution recess. Then, one drop (0.025 ml) of complement was added to each recess, and was incubated for one hour at 37°C. After incubation, two drops or 0.050 ml of hemolytic system was added to each dilution and the plastic plate was covered with pressure stick adhesive cover (Linbro Chemical Co, New Haven, Conn.) and was incubated at 37° C for 45 minutes. Reactions were read by means of concave mirror stand after storing the plate for 2 to 24 hours at 3 to 5°C (Figure 1).

Double-Gel Diffusion Test: The isolation of soluble *Babesia spp.* antigens which can be used in a gel precipitation test was accomplished by precipitating a supernatant lysate of erythrocytes from acutely infected cattle with sodium sulfate or column chromatography (Todorovic *et al*, 1968). By means of gel diffusion it was possible to study the serologic cross-reaction between *Babesia bigemina* and *Babesia argentina* (Figure 2). The use of immunoelectrophoresis (Todorovic *et al*, 1968) in the analysis of soluble antigens of *Babesia spp.* has again demonstrated the excellent resolving power of this technique.

Agglutination Tests: The hemagglutination of tanned erythrocytes (Curnow and Curnow, 1967) sensitized with soluble babesial antigens has been explored in our Laboratory for detection of babesial antibodies. Tube-latex agglutination test, (Todorovic *et al*, 1968) was also used, and the reactions observed can yield both quantitative and qualitative information.

Results and Discussion

The sera from the babesiosis experimentally and naturally infected cattle were subjected to the complement fixation test for a period of two years. A total of 5420 samples of cattle blood have been tested. These samples were obtained from animals in several experimental herds in Colombia located in

Valle del Cauca, Río Magdalena, Llanos and Montería, with known histories of babesiosis and also from animals artificially infected at the Laboratorio de Investigaciones Médicas Veterinarias (LIMV) in Bogotá. It was possible to obtain samples of serum from animals before infection and after artificial or tick-borne infections and to make tests of the daily, weekly bleedings from these animals. In the samples drawn before inoculation with the infected blood with B. bigemina and B. argentina the complement fixation reaction was negative and remained so until approximately the 8th day after inoculation. On the day 10 a 2 plus reaction was usually observed with titer 1:10. The following day the samples usually gave a 4 plus reaction with the titer 1:40. Babesia bigemina and B.argentina in the blood films stained with Giemsa were observed microscopically four days after infection, so the Babesia antibodies were detected after parasitemia. The peak of parasitemia on day 8 post inoculation coincided with the first day of complement fixing antibody response which persisted in a variable manner with the death of the animals. There has been exceptionally good agreement between the complement fixation reaction and the known histories of animals with blood-borne or tick-borne infections. Serum samples obtained from the private farms from Valle del Cauca, Río Magdalena, Llanos and Montería gave reaction in accordance

with the history of the animal. Approximately 95% were positive, whereas, approximately 5% gave discordant reactions. The latter were especially noted in serum from several of the carrier animals. Additional work is going forward to in field-checking the accuracy of the test through animal inoculation. The accuracy of the complement fixation test for bovine babesiosis will be the subject of our future investigation. The complement fixation test described by Mahoney (1962) has been used since 1964 for the control of B. argentina infection in cattle in Australia. Approximately 0.5% of cattle tested have shown a positive CF reaction, but have been negative to a transmission test. This is up to ten times the number of infected animals in some areas. False-positive reactions to the CF test were found also in our work. It has been questioned whether the infection with or vaccination against other diseases may result in cross-reaction with babesiosis. Future studies will be directed to assess the specificity of the test on sera collected from animals infected with other common bovine diseases in the tropics or premunized against bovine anaplasmosis. In addition, the specific nature of the CF test will be further studied in connection with the use of antigens from acute serum or plasma or more purified antigens of Babesia parasites, as well as the serologic relation between Babesia bigemina and Babesia argentina

of different isolates. The antigen used in our work could be produced in quantity, but had the disadvantage of containing too much hemoglobin and in some lots was anticomplementary. Future work is planned to apply column chromatography to produce a purer Babesia antigen. This process will remove most of the hemoglobin and will result in a more uniform antigen. Obviously, similar technical and mechanical errors which are inherant to other complement fixation procedures also apply to our technique. However, when the test is carefully conducted using an antigen isolated from high parasitemic blood, a minimum of error in accuracy and specificity should occur. The complement fixation test used in our laboratory for diagnosis of bovine babesiosis is proving to be specific. Antigens used in these procedures originated from erythrocytes and serum of animals with acute babesiosis and they were found to be useful for detecting specific Babesia antibodies. Because of the impurities of these antigens, they can be used only in the CF test, and under these circumstances, the variables and difficulties of the CF test become more pronounced and the dependability of the test is lowered.

In addition to the complement fixation test used in our laboratory attempts have been made to apply double-gel diffusion, immunoelectrophoretic techniques for characterization of the antigen-antibody reaction of B. bigemina and B. argentina. A cross reaction was observed in the agar diffusion system between

B. bigemina antigen and antibodies against *B. argentina*. The application of the latex agglutination and hemagglutination tests for detection of the *Babesia* antibodies, are still under investigation in our laboratory.

Methods devised to date for serologic diagnosis of bovine babesiosis are still obviously imperfect. Improvement must still be sought, and serologic procedures standardized especially for practical use. Nevertheless, the admittedly imperfect techniques now available are already capable of serving for diagnosis of bovine babesiosis.

Summary

The detection of the carrier state of bovine babesiosis has presented a particularly difficult problem, because the blood from a high percentage of carrier animals does not contain sufficient *Babesia* parasites on which to base the diagnosis. Therefore, a great deal of past interest was concerned with the development of serologic techniques which would aid in diagnosing babesiosis.

In this review an attempt has been made to summarize and discuss the recent advances on sero-diagnosis of babesiosis in infected cattle with special attention to the serologic procedures used in Laboratorio de Investigaciones Médicas Veterinarias

located in Bogotá, Colombia. In the last two decades fundamental knowledge concerning the immuno-serology of several Babesia spp. has led to the development of sero-diagnostic procedures for detection of Babesia antibodies. The antigens used in these techniques originated from parasitized erythrocytes and serum or plasma of animals with acute babesiosis, and they were applied in several serologic tests. The complement fixation reaction constituted one of the earliest tests for diagnosis of babesiosis. In recent years considerable progress was made to improve the complement fixation test for the diagnosis of babesiosis. In addition gel precipitation, fluorescent antibody, and agglutination techniques were applied for the detection of specific Babesia antibodies utilizing antigens from the parasitized erythrocytes as well as acute serum.

The investigations described in this report were conducted to develop the new techniques and to evaluate existing techniques for diagnosing bovine babesiosis. Research was executed in collaboration with the Instituto Colombiano Agropecuario in the Laboratorio de Investigaciones Médicas Veterinarias in Bogotá, Colombia. Antigens of Babesia spp. were isolated by means of two techniques and used in the complement fixation test for the detection of Babesia antibodies in cattle experimentally and naturally infected. By means of the complement fixation test

it was possible to detect specific antibodies in the serum of cattle 8 days after blood-borne infection. A total of 5420 serum samples of cattle infected with babesiosis were tested. The cattle were from several Colombian experimental herds with known histories of babesiosis located in Valle del Cauca, Río Magdalena, Llanos and Montería and from cattle artificially infected in the Laboratorio de Investigaciones Médicas Veterinarias in Bogotá. Approximately 95% of these samples were positive whereas about 5% gave discordant reactions. In addition to the complement fixation test used in our laboratory, attempts were made to apply the double-gel diffusion technique for characterization of *Babesia spp.* antigen-antibody reactions. A cross reaction was noted between *Babesia bigemina* and *Babesia argentina* in this system. The application of latex-agglutination and hemoagglutination tests for the detection of the *Babesia* antibodies are still under investigation in our laboratory. As a result of these investigations and observations, it is apparent that more investigation is needed for the development of a practical serologic technique for the diagnosis of babesiosis and to help solve this complex biological disease problem in tropical and sub-tropical areas of the world.

References

1. Anon. (1958) A Manual for Conducting the CF Test for Anaplasmosis. Laboratory Services, Animal Disease Eradication Division in Cooperation with Animal Disease and Parasite Research Division, Agricultural Research Service, USDA, Washington, D. C.

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

3. Curnow, J. A. (1968) In vitro Agglutination of Bovine Erythrocytes Infected with Babesia argentina. Nature, 267-268.

4. Ferris, D. H., Todorovic, R. and Ristic, M. (1968) Significance and Application of babesial and Plasmodial Ectoantigens To Comparative Medicine. J. A. V.M.A., 153: 1888-1896.

5. Frerichs, W., Holbrook, A. A., and Johnson, A. J. (1969a) Equine Piroplasmosis: Complement Fixation Titers of Horses Infected with Babesia caballi. Am. J. Vet. Res, 30: 627-702.

6. Frerichs, W., Holbrook, A.A., and Johnson, A. J. (1969b) Equine Piroplasmosis: Production of Antigens for the Complement Fixation Test. Am. J. Vet. Res., 30: 1337-1341.

7. Hidalgo, R. J. and Dimopoullos, G. T. (1967) Complement Fixation Microprocedures for Anaplasmosis. Am. J. Vet. Res., 28: 245-251.

8. Hirato, K., Ninomiya, M., Nwano, Y., Kutii, T. (1945) Studies of the Complement Fixation Reaction for Equine Piroplasmosis. Jap. Vet. Sci., 7: 197-205.

9. Madden, P. A. and Holbrook, A. A. (1968) Equine Piroplasmosis: Indirect Fluorescent Antibody Test for Babesia caballi. Am. J. Vet. Res., 29: 117-123.

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

11. Mahoney, D. F. (1964) Bovine Babesiosis. An assessment of The Significance of Complement Fixing Antibodies Bases upon Experimental Infection. Aust. Vet. J., 40: 369-375.

12. Mahoney, D. F. (1966) Circulating Antigens in Cattle Infected with Babesia bigemina or Babesia argentina. Nature, 24: 422.

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

14. Mahoney, D. F. (1967b) Bovine Babesiosis: The Passive Immunization of Calves Against Babesia argentina with Special Reference to the Role of Complement Fixing Antibodies. Exptl. Parasitol., 20: 119-124.

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

16. Mahoney, D. F. and Gooder, B. V. (1969) Babesia argentina: Serum Changes in Infected Calves. Exptl. Parasitol., 24: 375-382.

17. Malherbe, W. D. (1956) The Manifestations and Diagnosis of Babesia infections. Annals of the New York Academy of Sciences, 64: 128-146.

18. Ristic, M., Oppermann, J., Sibinovic, S., and Phillips, T. N. (1964) Equine Piroplasmosis: A Mixed Strain of Piroplasma caballi and Piroplasma equi Isolated in Florida and Studied by the Fluorescent Antibody Technique. Am. J. Vet. Res., 25: 15-23.

19. Ristic, M., and Sibinovic, S. (1964) Equine Babesiosis: Diagnosis by a Precipitin in Gel and a One-Step Fluorescent Antibody-Inhibition Test. Am. J. Vet. Res., 25: 1519-1526.

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

21. Schindler, R. and Denning, H. H. (1962) Demonstration of Babesia antibodies by the Complement Fixation Reaction. Z. Tropenmed. Parasitolog., 13: 480-488.

22. Schindler, R., Woratch, R. and Schroeder, G. (1966) Immunity and Serologic Reaction Against Babesia canis in Dogs after infection with Living Parasites and after Immunization with a Soluble Antigen. Z. Tropenmed. Parasitol., 17: 226-240.

23. Sibinovic, K. H., Ristic, M., Sibinovic, S., and Phillips, T. N. (1965) Equine Babesiosis: Isolation and Serologic Characterization of Blood Serum Antigens from Acutely Infected Horses. Am. J. Vet. Res., 110: 147-153.

24. Sibinovic, S., Sibinovic, K. H., and Ristic, M. (1969) Equine Babesiosis: Diagnosis by Bentonite Agglutination and Passive Hemagglutination Tests. Am. J. Vet. Res., 30: 691-695.

25. Todorovic, R., Ferris, D. H., and Ristic, M. (1967) Roles of the Spleen in Acute Plasmodial and Babesial infections in Rats. Exptl. Parasitol., 21: 354-372.

26. Todorovic, R., Ristic, M., and Ferris, D. H. (1968) Soluble Antigens of Plasmodium gallinaceum. Trans. Roy. Soc. Med. and Hyg., 62: 51-57.

27. Todorovic, R., Ferris, D. H. and Ristic, M. (1968) Antigens of Plasmodium gallinaceum. II. Immunoserologic Characterization of Explasmodial Antigens. Am. J. Trop. Med. and Hyg., 17: 695-701.

28. Todorovic, R., Ristic, M. and Ferris, D. H. (1968) A Tube Latex Agglutination Test for Diagnosis of Malaria. Trans. Roy. Soc. Trop. Med. and Hyg., 62: 58-68.

29. Todorovic, R. A., Vizcaíno, O. G. and Adams, L. G. (1971) Detection of Babesial Antibodies by the Complement Fixation Technique. Revista Instituto Colombiano Agropecuario (In Press)

30. Zuckerman, Avivah and Ristic, M. (1968) Blood Parasite Antigens and Antibodies. In "Infectious Blood Diseases of Man and Animals" eds. Weinman and Ristic, 79-122. Academic Press. N. Y. and London.

IV. A. 4. Caracterización Inmunoserológica de Antígenos de *Babesia argentina* y *Babesia bigemina* por Fijación del Complemento, Precipitación en Gel e Inmunoelectroforesis.

Introducción

La babesiosis bovina como entidad infecciosa reconocida (Babes, 1889) viene ocasionando graves problemas a las ganaderías del mundo. En Colombia dentro de los muchos problemas que afectan su ganadería se cuenta el de los parasitismos, siendo las especies de *Babesia* entre éstos parásitos quienes inciden sobre los animales disminuyendo su producción y deteniendo su progreso.

Las especies de Babesia, se reproducen dentro de los eritrocitos de los animales y son responsables de incalculables pérdidas, porque estos se atrasan en el crecimiento, disminuyen su producción y finalmente quedan en muy malas condiciones físicas generales cuando no sucumben. Su diagnóstico que es por tanto necesario se hace en la babesiosis aguda facilmente con frotis coloreados de la sangre. Pero en el estado crónico que es la forma más común en el campo, es difícil hacerlo por éste método porque los parásitos muy rara vez pueden observarse en la sangre circulante. En este caso hay que recurrir a algunas pruebas serológicas las cuales requieren la presencia de un antígeno.

Debido a que los antígenos de Babesia no son populares en el comercio, es de mucha importancia para nuestro país obtener uno específico, activo y con propiedades de estabilidad que permita hacer un diagnóstico seguro, caracterizar las distintas cepas de las especies de Babesia argentina y de Babesia bigemina y con estas bases poder iniciar un estudio de su prevalencia la cual tendría sin lugar a dudas, la importancia de orientar campañas futuras para su control.

Objetivos:

1. Obtener un antígeno específico para la B. argentina y para la B. bigemina.

2. Determinar de este antígeno su estabilidad serológica, algunas características físicas y químicas y con base en ellas, lograr un método eficiente y más adecuado para su preservación.

3. Evaluar algunas técnicas serológicas para la detección del título de anticuerpos (Fijación del complemento, precipitación en gel e inmunoelectroforesis).

4. Obtener la prueba más práctica para el diagnóstico de la babesiosis bovina crónica.

Revisión de Literatura

En la babesiosis como en muchas otras enfermedades, no siempre

es posible llegar a un diagnóstico definitivo porque la Babesia no se observa en alguna de sus fases, o hay interferencias de algunos factores inespecíficos y porque algunas pruebas serológicas se utilizan empiricamente que pecan por su inexactitud e inespecificidad. También muchas técnicas serológicas realizadas con antígenos que están compuestos por más de una fracción antigénica puede dar lugar según estudios hechos en 1967 por Sibinovic y colaboradores, a reacciones cruzadas que dificultarían observar bien la verdadera reacción. Otras veces la prueba no es bien clara por fallar en la potencia y especificidad del antígeno (Frerichs y colaboradores, 1969). Para obviar muchas de estas dificultades ha sido necesario desarrollar técnicas serológicas para descubrir anticuerpos específicos en animales que llegan al estado de portadores.

Un factor importante en el diagnóstico cuando se utilizan pruebas serológicas como la fijación del complemento, precipitación en gel e inmunoelectroforesis, es el antígeno, el cual debe ser bien específico y ojalá con un alto grado de pureza. Todorovic y colaboradores en 1968 obtuvieron un antígeno de Plasmodium gallinaceum libre de fracciones inespecíficas utilizando la ultracentrifugación diferencial y columnas de cromatografía con agar, sephadex, biogel.

Además de un antígeno puro y específico se pueden utilizar

técnicas adicionales para evitar al máximo reacciones inespecíficas. Así, por ejemplo Mahoney en 1967 las evitó absorbiendo el suero con una suspensión de eritrocitos de bovinos normales y luego utilizó este suero abosorbido en pruebas de fijación del complemento. Esta prueba, Hirato y colaboradores en 1945, la aplican para el diagnóstico de la babesiosis equina. El antígeno estaba constituído de estroma de eritrocitos de caballos agudamente infectados con Babesia caballi. Los anticuerpos aparecieron en la sangre 11 a 15 días después de comenzar la parasitemia y en un caso persistieron por 100 días.

Con B. bigemina y B. argentina Mahoney en 1962 detectó anticuerpos fijadores del complemento 7 a 21 días después de la infección. El mismo Mahoney en 1964 reportó el uso de la fijación del complemento en estudios epidemiológicos de la babesiosis bovina para el cual se usaron antígenos de B. bigemina y de B. argentina.

Estudios de Schindler y Denning en 1962 sobre la fijación del complemento en animales infectados con Babesia rodhaini y Babesia canis demostraron que los anticuerpos de B. canis aparecieron en perros 11 a 36 días después de la infección. Ellos prepararon para la fijación del complemento un antígeno de eritrocitos de perros agudamente infectados con B. canis por medio de inmunohemólisis o al exponer los eritrocitos a la acción de la saponina en una concentración del 0.3%. Los eritrocitos con B. rodhaini

se lisaron con agua destilada y el sobrenadante después que se centrifugó sirvió como antígeno para la fijación del complemento. Mahoney 1967a, igualmente encontró que los antígenos fijadores del complemento más sensibles para B. bigemina y para B. argentina fueron una suspensión de los parásitos preparados al lisar los eritrocitos infectados con agua destilada.

Frerichs y colaboradores 1969b, encontraron en el suero de 15 caballos, por medio de la prueba de la fijación del complemento, que el título permaneció por mayor tiempo que la capacidad de transmitir la enfermedad en 14 de los caballos. Observaron que la gran dificultad en producir el antígeno estuvo relacionada con el bajo porcentaje de la parasitemia. Pero con el uso de caballos esplenectomizados y la aplicación de corticosteroides sintéticos en dosis de 0.05 mg/kg mejoraron mucho esta dificultad.

Los antígenos solubles que se aislan del suero o plasma de animales agudamente infectados tienen aplicación en pruebas de precipitación en gel y de inmunoelectroforesis.

En 1964 Sibinovic y colaboradores aislaron un antígeno soluble en el suero sanguíneo de caballos infectados agudamente con B. caballi y demostraron los anticuerpos contra este antígeno por la técnica de difusión en gel. El suero de caballos convalescientes fue usado como anticuerpo y el antígeno lo constituyó el suero de caballos con infección aguda, el cual desapareció aproximadamente

un mes después de la infección experimental.

Trabajando con Trypanosoma lewisi, D'Alesandro en 1963 reportó un antígeno soluble y Seed y Weinman en 1963 encontraron un antígeno similar en el suero de ratones infectados con Trypanosoma rhodesiense.

Trabajando independientemente en 1960 Gray y Weitzen aislaron antígenos solubles en el suero de animales infectados con Trypanosoma vivax y Trypanosoma brucei.

Eaton en 1939 reportó que monos normales produjeron anticuerpos maláricos fijadores del complemento cuando se les inoculó suero libre del parásito de monos agudamente infectados con malaria.

Ristic y Sibinovic en 1964 demostraron un antígeno soluble de Babesia el cual precipitaron con sulfato de protamina de un lisado libre de parásitos de eritrocitos infectados. En el mismo año de 1964 Sibinovic y colaboradores obtienen antígeno soluble de caballos con babesiosis aguda precipitando el antigeno del suero con sulfato de amonio a diferentes concentraciones y utilizaron el antígeno soluble obtenido en pruebas de precipitación en gel e inmunoelectroforesis.

Antígeno soluble aislaron Todorovic y colaboradores en 1968 en el suero y plasma de pollos agudamente infectados con P. gallinaceum precipitando el suero o el plasma con sales o por ultracentrifugación, para extraer o separar materiales antígenicos

Los antígenos de eritrocitos parasitados los obtuvieron por congelación y descongelación de los glóbulos rojos o también por sonificación de los mismos. Sometieron el antígeno a pruebas técnicas dejando cantidades de 1 ml en pequeños tubos a diferentes temperaturas. Igualmente que a la acción de varias enzimas y diferentes pH.

En 1967 Curnow y Curnow reportaron el uso de la fijación del complemento para el diagnóstico de la B. argentina y usaron como antígeno estroma eritrocítico parasitado. Este antígeno también se usó para inhibir la reacción en la prueba de inhibición de la hemoaglutinación y para inhibir la prueba de la hemoaglutinación indirecta y para absorber los sueros antes de probarlos por fijación del complemento, prepararon un antígeno de estroma de eritrocitos bovinos no infectados.

Como lo demostró Mahoney y Gooder en 1969 por la prueba de precipitación en gel, hay dos sustancias antigénicas en los sueros recolectados en infección aguda con B. argentina. Gel antisuero del suero de ternero infectado que se preparó en conejos y que luego se absorbió con suero de ternero normal, contenía anticuerpos precipitantes que estaban ausentes en el suero de terneros no infectados.

Por muchas razones es necesario determinar la composición química del antígeno, su estabilidad serológica y algunas otras

propiedades físicas con el fin de obtener un medio eficaz para su preservación. Preservativos como liofilización, merthiolate y el fenol fueron utilizados por Todorovic y colaboradores en 1968. También Schindler y Denning encontraron en 1962 que la liofilización es un medio eficiente para preservar por mucho tiempo el antígeno. con la purificación y determinación del peso molecular por columnas de cromatografía como lo hizo Determann en 1968 utilizando geles o mediante ultracentrifugación con gradientes de densidad (Brakke, 1953), ha sido posible la obtención de antígenos puros y más específicos.

Los antígenos se obtendrán en lo posible inoculando en terneros esplenectomizados, cepas de B. argentina y de B. bigemina de la Costa Atlántica, del Valle del Cauca o de los Llanos Orientales por ser las zonas más representativas de la babesiosis bovina en Colombia. Se aislarán de eritrocitos altamente parasitados y del suero o del plasma de animales en fase aguda de la infección por Babesia utilizando para ello varias técnicas. Los antígenos se purificarán y concentrarán por columnas de cromatografía y las diferentes fracciones antigénicas que se aislen se utilizarán en pruebas de fijación del complemento, precipitación en gel e inmunoelectroforesis y se preservarán por varios métodos.

Materiales y Métodos

Catorce terneros Holstein de la Sabana de Bogotá de 6-8 meses de edad se esplenectomizaron. Un grupo de estos serviran para separar y purificar B. argentina y B. bigemina y el resto para la producción de los antígenos.

Para la separación se utilizarán drogas como el tripan azul o teniendo en cuenta el tiempo de incubación s i la sangre es inoculada artificialmente con jeringa o naturalmente con larvas de Boophilus microplus. El A. marginale se eliminará utilizando clorhidrato de oxitetraciclina (McKesson).

Antes y después de la esplenectomía, se hará controles de los animales examinando sus sangres para verificar su negatividad a Babesia y Anaplasma y registrar sus temperaturas y hematocritos normales. Con aproximadamente 200 ml de sangre (mezcla de todos los esplenectomizados) se inoculará uno de ellos para corroborar más esa negatividad. Comprobada la negatividad a hematozoarios se sangrarán 100 ml de cada uno de los animales en el grupo de esplenectomizados y de intactos para obtener suero negativo (control) para posteriores pruebas serológicas.

Otro grupo de 9 terneros Holstein intactos se inocularán con sangre infectada de las regiones de la Costa Atlántica, del Valle del Cauca, o de los Llanos para hacerlos portadores con los cuales se infectarán los animales esplenectomizados y además servirán

para producir suero positivo y para observar el comienzo y fluctuaciones durante el curso de la infección del nivel de los anticuerpos precipitantes y fijadores del complemento. Estos animales se sangrarán diariamente durante 6meses. Después de obtener la cepa de B. argentina o de B. bigemina complementamente puras, se congelará sangre con glicerol al 12% como medio crioprotectivo y estroma eritrocítico con Babesia pura, se inoculará a un ternero esplenectomizado el cual se controlará diariamente para verificar los parámetros de su parasitemia, hematocritos y temperatura.

Cuando la parasitemia alcance un alto porcentaje (25-30%) en B. argentina y (40-60%) en B. bigemina se inoculará 500 ml de esta sangre a otro ternero esplenectomizado igualmente que varios conejos y gallos con estroma eritrocítico o con el plasma o el suero de la misma sangre. El animal altamente parasitado se exanguinará canulando la arteria carótida recibiendo la sangre en un erlenmeyer con EDTA (ethylenediamine tetraacetic acid) 1.2 gm/litro de sangre.

Esta sangre colectada en EDTA se dejará antes de procesarla 1-2 horas en nevera (3-5°C) y luego en centrífuga automática refrigerada (Sorvall Superspeed RC2-B) se separarán glóbulos rojos del plasma utilizando rotor GSA a 2.500 rpm por 20 minutos.

El plasma o el suero se congelará en frascos plásticos a-20°C para aislar posteriormente antígenos solubles. El antígeno o

antígenos de los eritrocitos parasitados se aislará por dos técnicas: Técnica 1, sonificando los glóbulos rojos (Sonifier Cell Disruptor, model W185, Heat Systems-Ultrasonics, Inc., Plainview, N. Y.) y Técnica 2, lisando el estroma eritrocítico con agua destilada fría (Mahoney, 1967a).

Obtenido el antígeno se procederá a titularlo de acuerdo a Manual for Conducting the Complement Fixation Test for Anaplasmosis, ARS, USDA (1958) y luego se envasará en frascos de 5 ml los cuales una parte se dejará en refrigeración (3.5°C), otra en congelación (-65°C) y otra parte se liofilizará (Virtis Co., Inc. Research Equipment, Gradiner, N. Y. 12525). Con este antígeno a diferentes temperaturas y preservativos (glicerol, merthiolate, fenol) y sin preservativos, se harán pruebas de fijación del complemento y de precipitación en gel las cuales se repetirán mensualmente. El antígeno se someterá a pruebas técnicas y a la acción de diferentes pH.

Por columnas de cromatografía (Pharmacia-Uppsala, Sweden) se tratará de remover exceso de hemoglobina, averiguar peso molecular aproximado (Determann, 1968) y purificar fracciones antigénicas.

Antígeno del suero o del plasma. Se aislará precipitando el suero o el plasma con sulfato de amonio (Sibinovic y colaboradores, 1965). El antígeno se caracterizará como el que se obtenga del estroma eritrocítico y se preservará en igual forma. Como control para los antígenos eritrocíticos y para los del suero o del plasma, se pre-

parará un antígeno de sangre no infectada.

Preparación del Antisuero: El antisuero para las pruebas de precipitación en gel y de inmunoelectroforesis se obtendrá inoculando conejos y gallos con eritrocitos y suero normales y con los mismos con infección aguda (Sibinovic y colaboradores, 1965).

Teniendo en cuenta que en las especies de *Babesia* hay una especificidad de especie y también un componente específico de grupo (Mahoney, 1967a), se harán pruebas cruzadas con los antígenos de *B. argentina* y *B. bigemina* y sus sueros homólogos.

Trabajo Realizado

En 6 de 14 terneros esplenectomizados se purificó *B. bigemina* y en 8 de los mismos se obtuvo un antígeno por la Técnica 1. Con este antígeno se hizo screen y titulación por microtecnica con buenos resultados.

Recientemente se aisló un antígeno por Técnica 1 y Técnica 2 pero su título resultó un poco bajo.

Un ternero Holstein intacto se inoculó subcutaneamente con 5 ml de sangre de la región de Montería y luego se aplicó 5 dosis de trypan azul pero no fue posible separar *B. argentina* de *B. bigemina*. En este animal 3 dosis de oxitetraciclina (15 ml cada uno) mezcladas a partes iguales con agua destilada estéril fueron suficientes para eliminar el *A. marginale*. Hay en congelación sangre preser-

vada en glicerol al 12% con B. argentina pero posiblemente contaminada con B. major y sangre pura infectada con B. bigemina de la región de Montería y también en congelación sangre de los LLanos Orientales (B. bigemina, B. argentina, and A. marginale.).

English Summary

Immunoserologic Characterization of the Antigens of Babesia argentina and Babesia bigemina for Complement Fixation, Gel Precipitation and Immunoelectrophoresis.

Although bovine babesiosis has been studied for almost 80 years, little is known of the antigenic similarities and variation of B. argentina and B. bigemina. A study of the antigenic composition of the 2 above mentioned protozoa is necessary to understand cross-immunity between and among the species for future vaccination or prèmunition control programs as well as development of serologic diagnostic techniques.

The basic objectives of the experiment are to isolate and purify specific antigens from B. argentina and B. bigemina and to compare the antigens by complement fixation, gel precipitation and immuno-electrophoresis; also to characterize the nature of the antigens, physically and chemically as well as developing an optimum method of preservation of the antigens.

Pertinent and relevant literature on the subject of antigenic

characterization of Plasmodium spp., Babesia spp. and Trypanosoma spp. is reviewed and the relationship of the material is correlated with the purpose of the study.

Hemotropic disease-free intact and splenectomized calves inoculated with B. bigemina, and B. argentina will be utilized as a source to obtain both serum and erythrocytic derived antigens when the parasitemias are high. The crude serum and erythrocytic antigens will be purified by centrifugation and column chromatography, and subjected to complement fixation, gel precipitation and immunoelectrophoresis to study the possibility of cross reactions. Antigenic variations will also be studied by the above techniques in B. bigemina and B. argentina isolates from los Llanos, La Costa Atlántica and Valle del Cauca as well as by cross immunization procedures in cattle. The antigens will be preserved by liophilization, merthiolate, phenol and low temperature methods.

The serum and erythrocytic antigen of B. bigemina has been isolated by rapid serial passaged through 5 splenectomized calves and titrated by complement fixation. Experiments are in progress to isolate B. argentina by tick (Boophilus microplus) larval transmission in la Costa Atlántica.

IV. A. 5. Immune Response of Cattle Inoculated with Irradiated *Babesia bigemina*

Introduction

Attempts to induce immunity to bovine babesiosis have been undertaken by numerous investigators with varying degrees of success. The inconclusive results obtained with killed vaccines have been attributed to the loss or denaturation of functional antigens during the preparation of the vaccines or to the rapid elimination of parasites in the host. Cattle infected with *Babesia* by inoculation of blood containing living *Babesia* are relatively immune to natural infections. However, there is a narrow margin between the induction of fatal disease and mild infections resulting in protection.

Studies on the effects of irradiation on parasitic protozoa have repeatedly shown that while an extremely high radiation dose is necessary to cause immediate death of the parasite, much lower doses can interfere with its infectivity. Therefore, the use of irradiated *Babesia* parasites as vaccines might provide the special immunological properties of living *Babesia* parasites while suppressing the pathogenic effects of the vaccine.

In view of these considerations, a series of investigations were undertaken to study the effect of various radiation dosages on the infectivity and immunogenicity of the erythrocytic stages

of Babesia bigemina. The immunizing effect of irradiated B. bigemina which had been frozen and stored was also investigated. These studies were conducted toward the ultimate goal of producing irradiated vaccines against bovine babesiosis.

Materials and Methods

The isolation, separation, and preservation of the Babesia bigemina stabilate used throughout this study was previously described in this report. For the preparation of irradiated and challenge inocula, blood containing B. bigemina was collected from splenectomized calves at the acute stage of the disease after inoculation with 50 ml of the frozen stabilate of B. bigemina. Thus, although the calves were inoculated or challenged at different times, each inoculum was prepared from splenectomized calves previously inoculated with a standard dose of the same B. bigemina infected blood.

For irradiation treatment, 120 ml of inoculum were divided equally between two plastic Petri dishes. The inoculum in one Petri dish was exposed to the desired amount of irradiation using a cobalt-60 theletherapy unit* which delivered approximately

---

* Eldorado 8, Atomic Energy of Canada Limited: Commercial Products, P. O. Box 93, Ottawa, Canada.

200 rads/min at an exposure distance of 50 cm. The inoculum in the other Petri dish was placed in an adjacent room for the duration of irradiation and used as a non-irradiated control.

Five experiments were performed to study the effects of various gamma radiation dosages on the infectivity and immunogenicity of erythrocytic stages of B. bigemina in cattle. In experiment I, eight three-month-old calves were divided into four groups of two calves each. Three groups of calves were given one intravenous inoculation of blood containing 10^9 B. bigemina parasitized erythrocytes previously exposed to radiation doses of 22,000 rads, 26,000 rads, and 30,000 rads. One calf in the control group was given one intravenous inoculation of non-irradiated blood containing 10^9 B. bigemina parasitized erythrocytes and the other control calf received one intravenous inoculation of a similar volume of non-parasitized blood previously exposed to a radiation dose of 30,000 rads. All calves in experiment I were challenged intravenously with blood containing 10^9 B. bigemina parasitized erythrocytes 7 weeks after the last inoculation.

In experiment II, 6 three-month-old calves were divided into 3 groups of two calves each. Two groups of calves were given one intravenous inoculation of blood containing 10^9 B. bigemina parasitized erythrocytes previously exposed to radiation doses

of 36,000 rads and 42,000 rads. Those groups of calves that failed to show parasitic multiplication and reinvasion of erythrocytes were given a second inoculation in a similar manner 14 days after the first inoculation. This was done to enhance any immunizing effect that the irradiated parasites might have had. One calf in the control group was given one intravenous inoculation of non-irradiated blood containing 10^8 B. bigemina parasitized erythrocytes and the other control calf received two intravenous inoculations at a 14-day interval of a similar volume of non-parasitized blood previously exposed to a radiation dose of 42,000 rads.

In experiment III, 4 three-month-old calves were divided into 2 groups of 2 calves each. The 2 groups of calves were given one intravenous inoculation of blood containing 10^9 B. bigemina parasitized erythrocytes previously exposed to radiation doses of 36,000 rads and 42,000 rads. The inoculation schedules in experiments II and III were arranged so that all calves received their final inoculation on the same day. All calves in experiments II and III were challenged intravenously with blood containing 10^9 B. bigemina parasitized erythrocytes 3 weeks after the last inoculation.

In experiment IV, 31 four-month-old calves were divided into four experimental groups of four calves each and five control

groups of three calves each. The four experimental group of calves were given one intravenous inoculation of blood containing 10^{10} B.bigemina parasitized erythrocytes previously exposed to radiation doses of 24,000 rads, 36,000 rads, 42,000 rads and 60,000 rads.

The first control group of calves were given one intravenous inoculation of non-irradiated blood containing 10^{10} B. bigemina parasitized erythrocytes. To evaluate the possibility that some reduction in parasitemia might be the result of a reduction in the number of viable parasites injected rather than an attenuation of the parasites infected, the second and third control groups of calves received one intravenous inoculation of non-irradiated blood containing 10^7 and 10^4 B. bigemina parasitized erythrocytes, respectively, To test the hypothesis that living erythrocytic stages of B. bigemina exposed to a radiation dose sufficient enough to prevent progressive parasitemias are more immunogenic than non-living parasites, the fourth control group of calves were given one intravenous inoculation of blood containing 10^9 B. bigemina parasitized erythrocytes previously heat inactivated in a water bath at 56°C for 30 minutes. The fifth control group of calves received one intravenous inoculations of a similar volume of non-parasitized blood previously exposed to a radiation dose of 36,000 rads. All calves in Experiment IV were challenged intra-

venously with blood containing 10^9 B. bigemina parasitized erythrocytes 4 weeks after their last inoculation.

The fifth experiment was undertaken to study the effect of freezing on the immunogenicity of irradiated erythrocytic stages of B. bigemina. An aliquot of blood containing B. bigemina parasitized erythrocytes previously exposed to a radiation dose of 60,000 rads for the fourth experimental group in experiment IV was frozen using the method previously described for the preservation of B. bigemina. Four 4-month-old calves were given one intravenous inoculation of irradiated blood containing 10^{10} B. bigemina parasitized erythrocytes previously frozen for 48 hours. The inoculated calves in experiment V were challenged intravenously with blood containing 10^{10} B. bigemina parasitized erythrocytes 26 days after the last inoculation with an aliquot of the same challenge inoculum used in experiment IV and on the same day as in experiment IV.

Following all inoculations the calves were examined daily for the presence of parasitized erythrocytes by the use of combination thin and thick blood films previously described in this report. Subinoculations were performed the day before challenge from any group which received irradiated parasitized erythrocytes but failed to show detectable parasitemias to determine whether a subpatent parasitemia might have resulted.

Subinoculations from each group were accomplished by pooling a 100 ml blood sample from each calf in that group and injecting the pooled sample intravenously into one splenectomized calf. Daily packed cell volumes were determined by the micro-hematocrit method and bi-weekly complement fixation tests were performed on collected serum samples. Daily rectal temperatures and weekly body weight changes were also recorded. The duration and intensity (percentage of erythrocytes infected) of parasitemia were the major criteria of susceptibility in the challenged calves.

Results

The first series of experiments was undertaken to determine the effect of gamma radiation on the infectivity of Babesia bigemina in calves. Calves infected with B. bigemina parasitized erythrocytes exposed to doses up to and including 30,000 rads developed progressive parasitemias with obvious parasite multiplication and reinvasion of erythrocytes. Some calves receiving parasitized erythrocytes irradiated at levels of 36,000 and 42,000 rads did not develop progressive infections. Progressive infections were prevented by exposure to irradiation at 48,000 rads or higher.

The second series of experiments to determine the effect of gamma radiation on the immunogenicity of B. bigemina is now in progress. The complete results will be given in the next annual report.

IV. A. 6. Separation, Isolation and Purification of *Babesia argentina* From Cattle in the Sinú Valley

Introduction

The use of pure isolates of organisms for research purposes has been known to be necessary for years. In the Sinú Valley there are known to be at least 3 species of *Babesia*, *Babesia bigemina*, *Babesia argentina* and *Babesia major* of which the former 2 are transmitted by *Boophilus microplus*. It has been found in other countries that the smaller *Babesia* forms (*B. argentina*, *B. major*, etc) are more prevalent and have a higher incidence rate, hence it becomes evident that isolates of *B. argentina* must be acquired for further studies in pathogenesis, serologic diagnosis, antigenic comparisons and cross-immunity.

Materials and Methods

Method I.

Four splenectomized calves will be examined by CF testing, blood smears and pooled inoculation into another splenectomized calf, and followed for 2-3 months to be sure that they have no impending infection with blood parasites. The calves will be placed on a pasture known to be infested with *Boophilus microplus*, the accepted vector of *Babesia spp.* It has been found that

about 3 days are required for the larvae to find a site for attachment, and about seven days are necessary for the larvae to molt to nymphs in Turipaná.

It is reported that only the larvae of B. microplus transmit the smaller forms (Babesia argentina) of Babesia and that nymphs as well as adult transmit the larger forms (B. bigemina). Therefore the objective is to expose non-infected splenectomized calves to a pasture infested with B. microplus and before the larvae molt to nymphs, remove all ticks by use of insecticides, thus having exposed the calves to larvae only; they should only be infected with the smaller forms of Babesia, namely B. argentina.

Table I -Method for B. argentina Isolation

Day	Treatment
0	Exposure of 4 non-infected splenectomized calves to B. microplus larvae in the pasture.
1	Larval attachment
2	Larval attachment
3	Larval attachment
4	Observation of larvae
5	Observation of larvae
6	Observation of larvae
7	Observation of larvae

8 Remove 1st calf; spray daily with insecticides and place in isolation.

9 Remove 2nd calf with above procedure.

10 Remove 3rd calf with above procedure. Larval molt.

11 Remove 4th calf with above procedure. Larval molt.

The 4 calves will then be monitored by blood smears for the presence of B. argentina daily, and when observed, subinoculation into a splenectomized calf will be executed to ascertain the purity of the isolate.

Method II

A number of engorged adult female B. microplus will be obtained from a calf with acute babesiosis. The adults will be allowed to lay eggs, and the eggs will be permitted to molt to larvae. The larvae will be allowed to attach to laboratory animals or to a calf for development. When the larvae are about to molt to the nymphal instar, they will be removed and ground in a mortar with pestle in the presence of normal bovine serum and the supernatant will be injected subcutaneously below the base of the ear of a non-infected calf. The calf will be monitored as above.

Method III

Two non-infected intact calves will be premunized against

homologous B. bigemina only and inoculated with trypan blue for a 2 week period, following which the calves will be placed on a pasture infested with B. microplus and followed by the same procedure as in method I,

Upon finding B. argentina with a concommitant temperature rise, the infected blood will be passaged to a non-infected splenectomized calf. The calf will be monitored as above to insure purity.

Method IV

Four non-infected splenectomized calves will be infected with blood containing B. argentina and B. bigemina on day 0. When the calves exhibit detectable parasitemias of both organisms, they will be treated separately with one application of 4A65 at 1.2 mg/kg, S. Q., 1.0 mg/kg SQ, 0.8 mg/kg SQ, and 0.6 mg/kg SQ. According to Callow (1970), one application of 4A65 at 0.6 mg/kg or 1.0 mg/kg will sterilize B. bigemina infections, but not B. argentina. The calves will be monitored daily with PCV and blood smears and a subinoculation will be executed 10 days following treatment into normal splenectomized calves to verify the purity of the isolate.

Results

Methods I, II and IV have not been performed due to the lack of calves at present, but will be done in the near future.

Method II, infected B. microplus ticks are being reared at present in Turipaná. Larvae are being harvested to place on splenectomized calves to begin the isolation of B. argentina when the tick moats are completed in the new laboratory.

IV. A. 7. Establishment and Maintenance of a Non-Infected *Boophilus microplus* (tick) Colony

Introduction

Non-infected tick (*Boophilus microplus*) colonies are fundamental to the study of the transmission, life cycle and other facets of the vector-parasite-host relationships of bovine babesiosis. Normal ticks can be selectively infected with *Babesia* argentina and *Babesia bigemina* and other protozoan organisms to ascertain the role played by the tick in transmitting disease. Effects of acaricides plants (*Melinis minutiflora*) and other drugs can be ascertained on normal and infected ticks.

Materials and Methods

The procedure below is based on the fact that only 5-15% of the ticks from an enzootic area are infected with *Babesia* because the peripheral circulation of adult carrier cattle is very low since most of parasites are located in the internal and visceral circulation. Because the peripheral parasitemia of *Babesia* must be relatively high (approximately 10%) to infect *B. microplus* and adult carriers have low peripheral parasitemias, the probability of selecting non-infected ticks is high. Based on this assumption, female ticks collected at random are raised and the larval progeny are allowed to feed on normal splenectomized calves.

I. Establishment

Engorged adult female Boophilus microplus ticks, collected from adult cattle in Turipaná, are allowed to deposit eggs which later hatch to larvae. The engorged females, which deposit approximately 2000 eggs, are maintained separately so that the progeny of each individual adult is known. The 10,000 larvae from each of 5 adult females are placed on the ears of each of 5 normal splenectomized calves free of hemotropic diseases, hence 5 adult females will be screened for the abscence of Babesia infection. The splenectomized calves will be monitored for 3 months by PCv, blood smears, CF testing and subinoculation to verify the abscence of hemotropic diseases, at which time, the ticks will be said to be non-infected.

II. Maintenance

The non-infected B. microplus ticks will be maintained by feeding on disease free guinea pigs and cattle. Some of ticks will be kept in desicators, using different humidities produced by various solutions and temperatures in order to cause the ticks to undergo "diapause" so as to maintain them for long periods without feeding.

Results

The 5 engorged female B. microplus ticks have been collected and the eggs have been allowed to hatch into larvae which will be placed on the ears of 5 splenectomized calves.

IV. A. 8. Combination Thin and Thick Blood Films for the Detection of *Babesia* parasitemia

Introduction

During acute bovine babesiosis, parasitemia can be revealed by the routine examination of thin blood films. However, cattle which survive the acute stage of the disease become carriers, and the *Babesia* parasites are not readily demonstrable by the routine examination of thin blood films. Mahoney and Saal (1961) described a thick blood film method for detecting *Babesia* parasitemia which facilitated the examination of a greater quantity of blood.

For the diagnosis of malaria in man, a method for the preparation and staining of combination thin and thick blood films has been developed (Anon., 1970). It seemed that a similar method might prove effective for the detection of *Babesia* parasitemia in cattle with both acute and chronic infections. The method which evolved is described and some results of its application reported.

Materials and Methods

Preparation of combination thin and thick films. For the preparation of the thin film a small drop of blood was placed on

one end of a microscope slide and a spreader slide was gently drawn into the drop of blood. When the blood had spread along one-half of the width of the spreader slide by capillary action, the spreader slide was pushed forward rapidly. The size of the drop, the angle of the spreader slide, and the rapidity with which the spreader slide was pushed was standardized so that the extended thin film did not reach either side of the microscope slide nor cover more than one-half the length of the microscope slide. The thin blood film was then rapidly dried.

The method used for the preparation of the thick film was based on a technique described by Mahoney and Saal (1961). A loop of 2.5 mm external diameter was used to deliver a standard drop of blood on the end of the microscope slide opposite the thin film. The loop was made by twisting 30 gauge wire, conveniently taken from flyscreen gauze, tightly around a 14 gauge hypodermic needle. The loop delivered approximately 1.4 mm^3 of blood when touched on the microscope slide. After the remaining blood on the loop had been blotted on a clean paper towel, the loop was used to spread the drop evenly over an area of 28 mm^2 which was outlined by a circle 6 mm in diameter on a paper under the microscope slide. Before use on the next blood sample, the loop was rinsed first in water and then in alcohol which was allowed to evaporate. The thick blood film was then dried thoroughly in a horizontal

position. Initial drying was hastened by placing the slide near a light bulb.

The thin blood film was then fixed with absolute methyl alcohol. The slide was held in a slanted position with the thin film down and a few drops of absolute methyl alcohol placed on the thin film. To prevent alcohol or alcohol fumes from contacting the thick film, the slide was placed in a vertical position with the tick film up until the methyl alcohol had evaporated. The slides were then placed in a staining rack* and could be kept at this stage for several days before further processing.

Just before staining, the slides were placed in an incubator at 65°C for 45 minutes for final drying of the thick films. Final drying was required to ensure that the that the thick films did not wash off during the staining process. To prevent deterioration of the thick films, the combination thin and thick films were stained soon after final drying. Staining of the combination thin and thick films. The method used for the staining of the combination thin and thick films was based on a staining technique described for Plasmodium spp. in man (Anon., 1970). The thin and thick films previously prepared and placed

* Staining Rack Model S7680. Scientific Products, Division of American Hospital Supply Corporation, 1210 Leon Place, Evanston, Illinois 66201.

in staining racks were stained in a 1 to 50 solution of Giemsa stain* for 45 minutes. Phosphate buffered water (pH 7.0-7.1) containing 0.01% alkyl phenoxy polyethoxy ethanol** was used to prepare the stain solution. Stock solutions of alkaline buffer (Na_2HPO_4, M/15 solution), acid buffer ($Na_2H_2PO_4$, M/15 solution), and alkyl phenoxy polyethoxy ethanol (10% aqueous dilution) were prepared and kept in separate glass stoppered bottles for use in the preparation of the buffered water containing alkyl phenoxy polyethoxy ethanol. Fresh buffered water (pH 7.0-7.1) containing 0.01% alkyl phenoxy polyethoxy ethanol was prepared weekly by using 39 ml of stock acid buffer, 61 ml of stock alkaline buffer, 1 ml of stock alkyl phenoxy polyethoxy ethanol, and 899 ml of distilled water. An electric pH meter*** was used to determine the pH of the freshly prepared buffered water containing alkyl phenoxy polyethoxy ethanol. The stain solution with pH 7.0-7.1 was found to give the most satisfactory stain results.

Immediately following staining, the thin and thick films were rinsed briefly by dipping the slides in a staining dish

---

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

** Triton X-100. Rohm and Haas, Philadelphia, Pa. 19105

*** Beckman Expandomatic Model 76. Beckman Instruments, Inc., Scientific Instruments Division, Fullerton, California, 92634

filled with buffered water (pH 7.0-7.1) containing 0.01% alkyl phenoxy polyethoxy ethanol. A rubber band was then placed around the staining rack to hold the slides in place. The staining rack was then placed in a vertical position with the thick film immersed in the same buffered water containing alkyl phenoxy polyethoxy ethanol for an additional 3 to 5 minutes. Only the thick film was immersed in the water for the 3 to 5 minutes and the water was not allowed to cover any part of the thin film. The slides were then dried in the vertical position with the thick film down.

Results and Discussion

The use of a 1 to 50 solution of Giemsa stain provided osmotic pressures that caused lysis of the unfixed erythrocytes in thick films during the 45 minute staining period. Therefore, the usual morphological criteria for differential identification of parasites based on differences in size and in position in the erythrocyte are not applicable to thick films. The use of the surface-active agent, alkyl phenoxy polyethoxy ethanol, in the staining and washing solutions resulted in brighter and cleaner preparations with only parasites, leukocytes, and platelets remaining in the thick films.

The use of buffered (pH 7.0-7.1) Giemsa stain solution

provided dependable differential staining of the parasite cytoplasm deep blue and of the parasite nucleus brilliant red. For conformation of Babesia parasitemia on thick films, it was necessary to resolve the objects under examination into a red nucleus and blue cytoplasm and to find some parasites that were typically pyriform and arranged in pairs. The depth of the thick film allowed organisms to lie in any plane which provided some distortion from the typical appearance of the parasites seen in thin films. The typical pairs could, however, be easily recognized even when some distortion had occurred.

The thick film method described allows the examination of approximately 1.0 mm^2 of blood in 15 minutes. If the same amount of blood were spread out in a single layer as a thin film, an observer, examining at a rate of 20,000 cells per minute, would take approximately 5 hours to search the film. The parasite count on thick film can be estimated by counting the number of parasites per mm^2 of blood can then be converted to per cent parasitized erythrocytes from an estimate of the number of erythrocytes per mm^2 of blood. An estimate of the parasite count on thick films can also be made from the number of parasites seen on each field in proportion to the number of leukocytes present on each field. The number of parasites per mm^2 of blood can then be determined from an estimate of the number of leukocytes per mm^2 of blood.

The number of parasites per mm^2 of blood can then be converted to percent parasitized erythrocytes from an estimate of the number of erythrocytes per mm^2 of blood.

Summary

A method for the preparation and examination of combination thin and thick blood films for the detection of Babesia parasitemia was described.

A technique for the staining of the combination thin and thick films using a phosphate buffered Giemsa stain solution containing alkyl phenoxy polyethoxy ethanol was also described.

References

1. Anon. 1970. Manual for the Laboratory Diagnosis of Parasitic Diseases. II. Blood Parasites 8010-C. Parasitology Training Unit, National Communicable Disease Center, Atlanta, Georgia 30333.

2. Mahoney, D. F. and Saal, J. R. 1961. Bovine Babesiosis: Thick blood films for the detection of parasitemia. Aust. Vet. J., 37: 44-47.

V. A. 1. A Clinical, Pathological and Serological Study of Concurrent Anaplasmosis and Babesiosis in Experimentally Infected Calves

Introduction

Numerous investigators have commented on the common distribution of anaplasmosis and babesiosis, and on the high frequency of concurrent infection of cattle with both *Anaplasma* and *Babesia* organisms. The common distribution of these two diseases appears to be due to ecological factors which favor the survival and prevalence of tick vectors. Ticks have been shown to be the principal vectors responsible for the transmission of both anaplasmosis and babesiosis. Some 20 species of ticks have been reported to be capable of transmitting *Anaplasma*. At least 5 species of ticks are capable of transmitting *Babesia*. Several species of ticks have the ability to transmit both *Anaplasma* and *Babesia*.

Descriptions of the clinical nature of concurrent anaplasmosis and babesiosis have been few, and have consisted primarily of reports of observations made during premunition studies. Several investigators have observed an increased severity in clinical illness in cattle concurrently infected as compared to animals singly infected. Other investigators have reported the

results of experiments which indicate that either of these disease agents may initiate in the host animal a physiological state which is unfavorable for the multiplication of the second disease agent. The purpose of the investigation to be described and which is currently in progress, is to conduct an experiment especially designed to study the clinical course, pathological manifestations, and serological response of cattle concurrently infected with *Anaplasma marginale* and *Babesia bigemina*; and further, to study the biological behaviour of these two disease agents when present together during a concurrent infection.

Materials and Methods

Twenty-two, male, Holstein calves, 7 months of age are being used as experimental animals. Prior to experimental infection, all calves were screened for the presence of current or previous infection with *Anaplasma* or with *Babesia* hemoparasites by the complement fixation test, by the examination of stained blood smears, and by the subinoculation of a splenectomized calf with a pooled blood sample composed of 10 ml of blood collected from each experimental calf.

The calves were physically examined and blood and serum samples collected twice each week for 2 weeks prior to experimental infection to ascertain normal values for the following parameters:

hematocrit; hemoglobin level; total and differential leukocyte counts; reticulocyte count; total, direct, and indirect serum bilirubin concentration; serum glutamic-oxalacetic transaminase; serum sorbitol dehydrogenase; total serum proteins; serum protein electrophoretic patterns; body weight, and body temperature.

Following the collection of blood and serum samples necessary to ascertain preinfection values for the above mentioned parameters, the experimental calves were randomly divided into 4 groups. Group I and Group II contain 3 calves each. Group III and Group IV each contain 8 calves. The calves in Group I were inoculated with blood containing A. marginale. This group of calves is serving as controls to evaluate the response of calves singly infected with A. marginale. The calves in Group II were inoculated with blood containing B. bigemina. These calves are serving as controls to study the response of calves singly infected with babesiosis. The calves of Group III were inoculated with blood containing A. marginale. When the calves of this group had recovered from the clinical phase of anaplasmosis and had entered the latent or carrier stage of disease, they were inoculated with blood containing B. bigemina. This group of calves is being used to study what effect a secondary concurrent infection with B. bigemina has upon calves with chronic anaplasmosis. The calves of Group IV were inoculated with blood containing B. bigemina. Upon

recovery from the clinical phase of babesiosis and attainment of the carrier state of infection, these animals were inoculated with blood containing A. marginale. This group of calves is being utilized to study what effect a secondary concurrent infection with A. marginale has upon calves chronically infected with B. bigemina.

Inocula used to experimentally infect these calves was prepared from the blood of calves with pure patent infections. The infected blood was preserved by the addition of glycerol as a cryoprotectant, and stored at -79°C in a dry ice chest until used as inocula. Each experimental animal was inoculated with 5 ml of the appropriate inoculum containing Anaplasma or Babesia, subcutaneously utilizing sterile technique.

Whole blood and serum samples have been routinely collected twice each week for the duration of the experiment which is still in progress. This schedule has been altered during the acute clinical phases of anaplasmosis and babesiosis during which time samples have been collected 3 times each week to permit a more extensive analysis of experimental parameters during the acute phases of clinical disease. Body temperature has been recorded during each collection period and the general attitutde and condition of each calf noted. Percent parasitemia of erythrocytes is being determined by the examination of Giemsa stained blood smears, counting 10,000 to 12,000 cells located from ¼ to ½ inch from the feathered end of the blood smear.

Results

The calves of Group I, infected with A. marginale were observed to have an average incubation period of 21 days. The calves of this group have at this time recovered from the acute clinical phase of anaplasmosis and are now being observed as carrier animals. The calves in Group II, infected with B. bigemina were observed to have an average incubation period of 5 days. These calves have now recovered from acute babesiosis and are being observed as chronically infected carriers of B. bigemina. The calves of Group III infected with A. marginale had an average incubation period of 21.5 days. The calves of this group were infected on post inoculation day 70 with B. bigemina. By day 70 the calves of this group had passed through the acute phase of anaplasmosis and were far enough through the recuperative phase to be considered as carriers of A. marginale. Erythrocytes infected with B. bigemina have not as yet been seen in this group of calves following 8 days of incubation. Early indications are that the primarily anaplasmosis in these calves may be causing a prolonged incubation period for the secondary Babesia infection. However, sufficient data has not been collected at this time to determine if the secondary babesiosis is or is not being inhibited. The calves of Group IV, infected with B. bigemina had an average incubation period of 5 days. By day 37 post infection the calves

of this group had recovered from acute babesiosis, as indicated by the return of hematocrit levels to near preinoculation values. These calves were at this time inoculated with blood containing *A. marginale*. The secondary anaplasmosis in these calves was observed to have an incubation period of 24.5 days. At the present time the number of erythrocytes infected with *Anaplasma* parasites has reached 1.4% and appears to be increasing.

At this time insufficient data has been collected to evaluate the response of calves in Group III and IV concurrently infected with *A. marginale* and *Babesia bigemina*. The collection of samples and analysis of data is expected to be completed by June 1971. Data obtained from the analysis of samples collected from the concurrently infected calves of Groups III and IV will at that time be compared to data collected from the simply infected control calves of Group I and II.

V. A. 2. Control of Anaplasmosis and Babesiosis Simultaneously in Young Cattle

Introduction

A control system for anaplasmosis and babesiosis in young cattle could give an increase in beef production by preventing losses due to these diseases. The purpose of the experiment described below was to test 2 methods of control of these diseases; one using vaccination and the other using premunization with 2 new compounds as treatment.

Materials and Methods

In Bugalagrande (1000 m), Valle, Colombia, 30 Lucerna, two weeks old calves were selected and divided into 3 groups of 10 each which were maintained in concrete stalls for approximately 15 months after vaccination before field exposure. Group I was vaccinated subcutaneously with the Diamond Laboratories attenuated Anaplasmosis vaccine and with a *Babesia bigemina* and *Babesia argentina* killed adjuvant vaccine (described in V.A.3.) on day 0, and the killed-*Babesia* adjuvant vaccine was repeated on day 14 PI. Group II was inoculated subcutaneously on day 0 with 5 ml of blood from a non-patent bovine carrier of Llanos isolates of *A. marginale*, *B. bigemina* and *B. argentina* and was treated on day 5 PI with a

combination of 356-C-61 at 5 mg/kg I.V. and 4A65 at 1.0 mg/kg S. Q. Group III served as untreated controls. All calves were field challenged at approximately 15 weeks post-inoculation.

In Girardot (350 m), Cundinamarca, Colombia, 30 Zebu calves, approximately 8 weeks old were selected and divided into 3 groups of 10 each. The calves were under continuous challenge by Boophilus microplus which were dipped sporadically with insecticides. The Groups I, II and III were treated exactly as in Bugalagrande.

The thirty-3 month old calves used in the Montería experiment were secured in the Sabana de Bogotá and were free of hemotropic infection. Groups I, II and III were treated exactly as those of Group I. At 6 weeks postinoculation, the calves of all groups were flown to Montería for field exposure.

Parameters for PCV, parasitemia, anaplasmosis and babesiosis complement fixation were determined weekly. Body weights were measured monthly.

Results

The majority of the results are in the hands of the principal investigator, and are therefore, not fully available for publication here, however an interpretation of those results available will be attempted.

Discussion

In regard to vaccination with the Diamond attenuated *Anaplasma* vaccine, it was evident the organisms were not viable eventhough the vaccine was handled according to the company's recommendation. Neither a parasitemia nor a CF response due to the vaccine occurred and hence apparently no immunization against anaplasmosis occurred. The lack of anaplasmosis immunization from vaccination was also evident due to the high degree of susceptibility of the calves to field challenge, particularly in Montería.

Premunization against anaplasmosis, with the combined use of 4A65 and 356-C-61 on day 5 PI as treatment, appeared to cause either sterilization or such a severe reduction of the *Anaplasma* parasitemia that neither a significant CF response nor premunization against anaplasmosis apparently occurred. It was deduced that 4A65 might also be effective against anaplasmosis. Indeed, it was later discovered by a Wellcome Foundation investigator that 4A65 was in fact effective against *A. marginale*. It, therefore, appears that both control measures against anaplasmosis were unsuccessful.

The killed *Babesia* adjuvant vaccine caused slight elevations in complement fixation antibody levels, but apparently afforded little protection against babesiosis after field challenge. The

use of 4A65 (1.0 mg/kg) on day 5 PI as the treatment in babesiosis premunition apparently sterilized the infection as no parasitemias or significant CF responses were recorded. Apparently, both control systems against babesiosis were uneffective.

There were no appreciable differences between groups I, II and III at any of the 3 experimental sites, indicating that the control systems employed were unsuccessful.

V. A. 3. Simultaneous Control of Helminths, Anaplasmosis and Babesiosis in Cattle

Introducción

Las enfermedades causadas por Anaplasma y Babesia han sido reconocidas como causantes de grandes pérdidas económicas desde el año 1.900, sin embargo, aún no existen métodos eficaces para su control (6,7).

Las dos enfermedades mencionadas se hallan diseminadas en regiones tropicales y subtropicales y están asociadas con la presencia de garrapatas y otros ectopárásitos (10, 11).

Por otra parte, la incidencia de parásitos gastrointestinales es favorecida por la presencia de alta humedad y temperatura propias de los trópicos, resultando que la distribución de las enfermedades hemotrópicas coincide con la distribución de parásitos gastrointestinales (4). Esta asociación trae como consecuencia pérdidas económicas de incalculable valor que se hace manifiesta por alta morbilidad y mortalidad, baja rata de crecimiento, baja producción de leche y disminución de la capacidad de ganancia diaria de peso (5).

El objetivo del presente trabajo fue evaluar un sistema de control de las dos entidades (parasitismo hemático y gastrointestinal) que pueda usarse en condiciones ventajosas en fincas particulares.

Materiales y Métodos

El experimento se llevó a cabo en el Centro Nacional de Investigaciones Agropecuarias del ICA en Palmira.

Para el trabajo se utilizó un grupo de 50 machos Holstein de aproximadamente 3-4 meses de edad, nacidos y criados en el Centro Experimental de Palmira. Los animales fueron seleccionados, identificados individualmente y mantenidos en establo en grupos de 20, 20 y 10 durante los primeros 50 días del experimento; inicialmente se tomó sangre a cada uno para estudios hematológicos y materia fecal para estudio de parásitos gastrointestinales.

El Grupo I (20 animales) fue premunizado contra Anaplasma marginale, Babesia argentina y Babesia bigemina. Las cepas de estos parásitos se aislaron y purificaron así: se tomó sangre de varios animales utilizando EDTA como anticoagulante y se transportó en termos con hielo al LIMV en Bogotá, donde se inyectaron 100 cc a un ternero esplenectomizado. La parasitemia se presentó en este ternero unos pocos días después.

Los animales del Grupo I se premunizaron inyectando vía subcutánea 5 cc de sangre parasitada. Después de 7 días de inoculados los animales del Grupo I se encontraron infectados con Babesia y se trataron con el producto químico 4A65 a la dosis de 1 mg/kg I.M. (2). A los 21 días después de la inoculación con sangre

resultaron positivos a Anaplasma los animales del Grupo I y se trataron utilizando el compuesto químico 356-C-61 a la dosis de 5 mg/kg IV (11,8). Este tratamiento se repitió 2 veces consecutivas con 8 semanas de intervalo. Los animales fueron llevados a la pradera 50 días después de la premunición. Durante el período experimental se tomó sangre 2 veces por semana para medir el hematocrito y efectuar la prueba del complemento para Anaplasma y Babesia (13), se tomó temperatura y se hicieron frotis sanguíneos para evaluación de la parasitemia. Los animales se pesaron cada 2 semanas y se tomó muestra de materia fecal para examen mensualmente.

El Grupo II (20 animales) fue premunizado contra A. marginale y tratado en la misma forma descrita para el Grupo I al hablar de de Anaplasma. Este grupo no fue premunizado contra Babesia pero se le aplicó la vacuna tipo A-G-S a base de Babesia muerta, dos aplicaciones con intervalo de 14 días (3,9,12).

La vacuna de Babesia se preparó haciendo pases sucesivos en terneros esplenectomizados, hasta obtener el 40% de parasitemia y la cepa pura de Babesia separada del Anaplasma. Estas condiciones se obtuvieron en el sexto pase y en el ternero correspondiente se canuló la arteria carótida para extraer ocho litros de sangre usando EDTA como anticoagulante en la proporción de 1.2 gramos por litro de sangre.

La sangre así recolectada se centrifugó a 2,500 rpm, durante 20 minutos; se recolectó el sobrenadante, se liofilizó y se envasó en ampollas estériles de 20 cc. En el momento de la vacunación se mezcló el contenido de la ampolla con 20 cc de un adyuvante comercial oleoso H-37 Ra, para aplicar luego 10 cc vía subcutánea por animal. La vacunación se repitió 14 días más tarde.

Estos animales se llevaron luego a la pradera y se tomaron muestras para medir los mismos parámetros como quedó descrito para el Grupo I.

Los animales de los Grupos I y II fueron tratados contra parásitos gastrointestinales 6 días después del examen de materia fecal. Se utilizó Ripercol (Tetramisol) a la dosis de 1 cc por cada 5 libras de peso de una solución preparada utilizando 62.5 gramos en 1.000 cc de agua. El tratamiento con Ripercol se repitió 10 semanas después de que los animales salieron al campo.

El grupo III (control) no fue premunizado ni vacunado pero si se tomaron las muestras tomadas a los Grupos I y II. El muestreo se llevó a efecto durante 8 meses. A este grupo no se le administró Ripercol.

El Grupo control fue dividido en 2 sub-grupos, 5 enteros y 5 esplenectomizados. Esto se hizo con el objeto de detectar cualquier aparición de Anaplasma o Babesia en los animales experimentales.

El manejo de los animales siguió como el de rutina en la granja. La alimentación se hizo con ensilaje, maíz, caña de azúcar y un suplemento alimenticio comercial.

Cada semana se colectaron garrapatas para su clasificación.

Resultados

En los análisis de materia fecal se encontró los siguientes endoparásitos: Haemonchus, Trichostrongylus, Ostertagia, Cooperia, Oesophagostomum, Trichuris, Moniezia y Eimeria. Los recuentos iniciales de huevos de nemátodos fueron considerados como muy altos; la efectividad de la droga fue muy buena en el control de los parásitos antes mencionados; el tratamiento con Ripercol fue necesario repetirlo sólo cuando el número de huevos por gramo de materia fecal empezó a hacerse cada vez más alto.

Un total de 1.673 garrapatas fueron recolectadas de los animales de los 3 grupos. De estas garrapatas sólo 151 fueron clasificadas como Boophilus microplus (9%) y 1.514 fueron clasificadas como Dermacentor nitens (91%). Es de anotar que en la granja de Palmira el Dermacentor que es una garrapata de equinos sea la de más alta incidencia en bovinos.

Los resultados hematológicos (valor del hematocrito, parasitemia y fijación del complemento) se describen para cada uno de los 3 grupos. La figura 1 correspondiente al Grupo I indica

lo siguiente: Los animales premunizados del Grupo I desarrollaron anemia temporal que duró aproximadamente 7 semanas. En este caso el hematocrito bajó de 33 a 20.

Después de la exposición a las garrapatas en la pradera todos los animales mostraron aumento en el valor del hematocrito y la condición general de los animales mejoró notablemente.

Mas tarde (nueve semanas aproximadamente) se presentó anemia la cual fue debida a presencia de helmintos. En este caso fue necesario repetir el tratamiento con Ripercol.

Los resultados correspondientes al Grupo II (premunizados contra Anaplasma y vacunados contra Babesia) pueden ser facilmente interpretados en la Figura 2 correspondiente al Grupo II.

Los resultados de premunición para Anaplasma fueron semejantes a los descritos para el Grupo I (anemia temporal, baja en hematocritos) pero se recuperaron al ser tratados contra anaplasmosis con la droga 356-C-61.

Después de la exposición a garrapatas todos los animales se mostraron resistentes a Anaplasma y Babesia.

En comparación con el Grupo III (control) todos los 5 animales esplenectomizados murieron y todos los animales intactos sufrieron de anaplasmosis y babesiosis clínica y requirieron tratamianto. Siete semanas después de la exposición a garrapatas desarrollaron parasitemia con Babesia y 11 semanas después de la exposición a

garrapatas mostraron Anaplasma; los animales fueron tratados con Ganaseg (Babesia) y oxitetraciclina (Anaplasma). Ver figura 3 correspondiente al Grupo III.

Discusión

El control de parásitos es una necesidad apremiante en las ganaderías ya que las pérdidas económicas ocasionados por ellos ascienden a cifras incalculables (4).

En algunas partes del mundo el control y la erradicación de Babesia es posible haciendo control y erradicación del vector (garrapata) pero ésta parece que no es posible en Colombia (11). Se impone estudiar un sistema práctico que permita controlar las enfermedades por hematozoarios y este puede ser la premunización ó la vacunación. En un programa de premunización se debe tener en cuenta que la presencia de parásitos gastrointestinales puede interferir con el desarrollo de una inmunidad integral o puede debilitar tanto al animal que fisiologicamente no es capaz de desarrollar un sistema inmunogénico.

El control de parásitos gastrointestinales debe hacerse integrando diversos sistemas los cuales obrando en forma sinérgica pueden obrar en la supresión del parasitismo. Los antihelmínticos deben ser utilizados como una ayuda en el control de parasitismos pero no debe olvidarse la necesidad de buena sanidad animal, nutri-

ción apropiada y manejo animal apropiado (4).

Los resultados de este experimento indican que la premunización con cepas homólogas en combinación con quimioterapia es aún un buen sistema de control de hematozoarios (14). Es deber de los investigadores químicos continuar los trabajos tratando de hallar productos que sean de baja toxicidad, altamente efectivos, fáciles de usar y económicos (18).

En relación Babesia puede decirse que además de la inmunidad coinfecciosa la inmunidad estéril producida por vacunas de gérmenes muertos puede ser de igual valor, sin embargo, más investigación se necesita para ver que tiempo puede durar esta immunidad estéril, qué puede suceder cuando la población de garrapatas es alta y la exposición de los animales se efectúa en un corto tiempo (12) la experiencia previa de los bovinos a estas enfermedades (Babesia) también puede ser un determinante en la respuesta inmunogénica de la vacuna.

El control de parásitos gastrointestinales y el de hemoparásitos debe ser simultáneo, masivo y de carácter regional ya que los 2 problemas se presentan en un medio ecológico semejante. Este control debe iniciarse durante los primeros 3 a 6 meses de edad de los animales.

Resumen

Cincuenta terneros Holstein de 3 a 4 meses de edad fueron utilizados para evaluar un sistema de control simultáneo de parásitos hemáticos y gastrointestinales. El experimento se efectuó en la Granja del ICA a una altitud de 1.000 metros sobre el nivel del mar. Tres grupos fueron utilizados en el experimento. En el Grupo I (20 terneros) los animales fueron premunizados simultaneamente con Anaplasma y Babesia. A los 8 días se trataron contra Babesia usando 4A65, a los 21 y 56 días se trataron contra Anaplasma con 356-C-61. El grupo II (20 Animales) se premunizaron contra Anaplasma como en el Grupo I y se vacunaron con vacuna AGS más adyuvante contra Babesia (esta vacuna se repitió a las dos semanas). Los Grupos I y II se trataron 2 veces con Ripercol durante el experimento. El Grupo III sirvió como control (sin premunización ni vacuna). El experimento duró 8 meses y 2 veces por semana se tomó sangre para estudios sanguíneos (hematocrito, parasitemia, título de fijación del complemento) peso y exámen de materia fecal.

Los grupos I y II mostraron la efectividad de la premunición y la vacunación en contraste con el Grupo III en el cual los animales mostraron síntomas clínicos de la enfermedad y muerte.

Se considera de importancia el control simultáneo de estos

Figura 1. Curvas que indican el valor del hematocrito (H), el título de fijación de complemento (TFC) y la parasitemia en 20 terneros premunizados con Babesia y Anaplasma (Grupo I).

a. El valor promedio del hematocrito está indicado por la línea negra horizontal continua, las verticales indican la desviación standard.

b. El título de fijación del complemento (TFC) está indicado por la línea punteada y representa el valor promedio.

c. La parasitemia con Babesia (Pb) está indicada por la línea punteada y la parasitemia por Anaplasma (Pa) está representada por una línea continua delgada.

d. Las letras mayúsculas tienen el siguiente significado

$\frac{T}{-6}$ = Fecha del tratamiento con Ripercol

$\frac{P}{-5}$ = Fecha de premunización Babesia spp. y Anaplasma

T 4A65= Fecha de tratamiento contra Babesia.
T 356-C-61= Fecha de tratamiento contra Anaplasma
E = Fecha de exposición a garrapatas en el campo.
$\frac{T}{10}$ = Fecha de tratamiento con Ripercol

Nótese la anemia desarrollada después de la inoculación y la mejoría ocurrida después de tratados los animales.
Nótese que después de la exposición a garrapatas el hematocrito no descendió significativamente, pero luego ascendió rapidamente. Esto indica alto grado de inmunidad.

La anemia detectada en el segundo descenso de la curva se debió a endoparásitos y por eso se hizo el 2o. tratamiento con Ripercol.

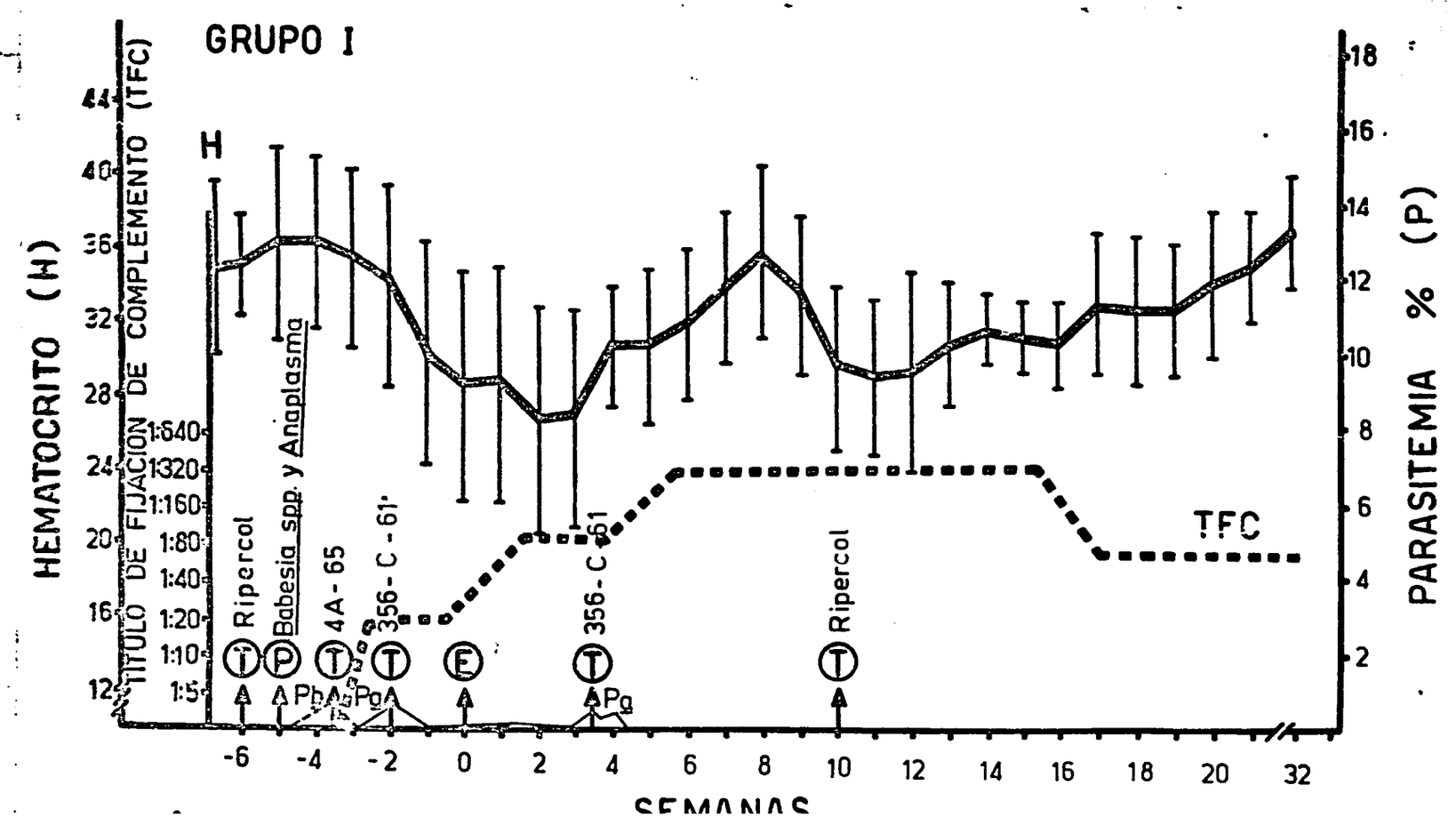

GRUPO I
HEMATOCRITO (H)
TITULO DE FIJACION DE COMPLEMENTO (TFC)
PARASITEMIA % (P)
H
TFC
Ripercol
Babesia spp. y Anaplasma
4A - 65
356 - C - 61
356 - C - 61
Ripercol
SEMANAS

Figura 2. Curvas que indicar el valor del hematocrito (H), el título de fijación de complemento (TFC) y parasitemia en 20 terneros (Grupo II) vacunados contra Babesia con vacuna AGS (vacuna muerta) y premunizados con Anaplasma marginale.

Todos los parámetros de la Figura 2 son iguales a los de la Figura 1, excepto que la vacuna (V) contra Babesia fue repetida a las dos semanas.

Nótese que como en el caso del Grupo I se presentó anemía después de la premunición pero los tratamientos contra Anaplasma (356-C-61) hicieron recuperar el valor del hematocrito.

El segundo descenso del hematocrito obedeció a presencia de parásitos gastrointestinales pero el tratamiento oportuno hizo ascender el hematocrito inmediatamente.

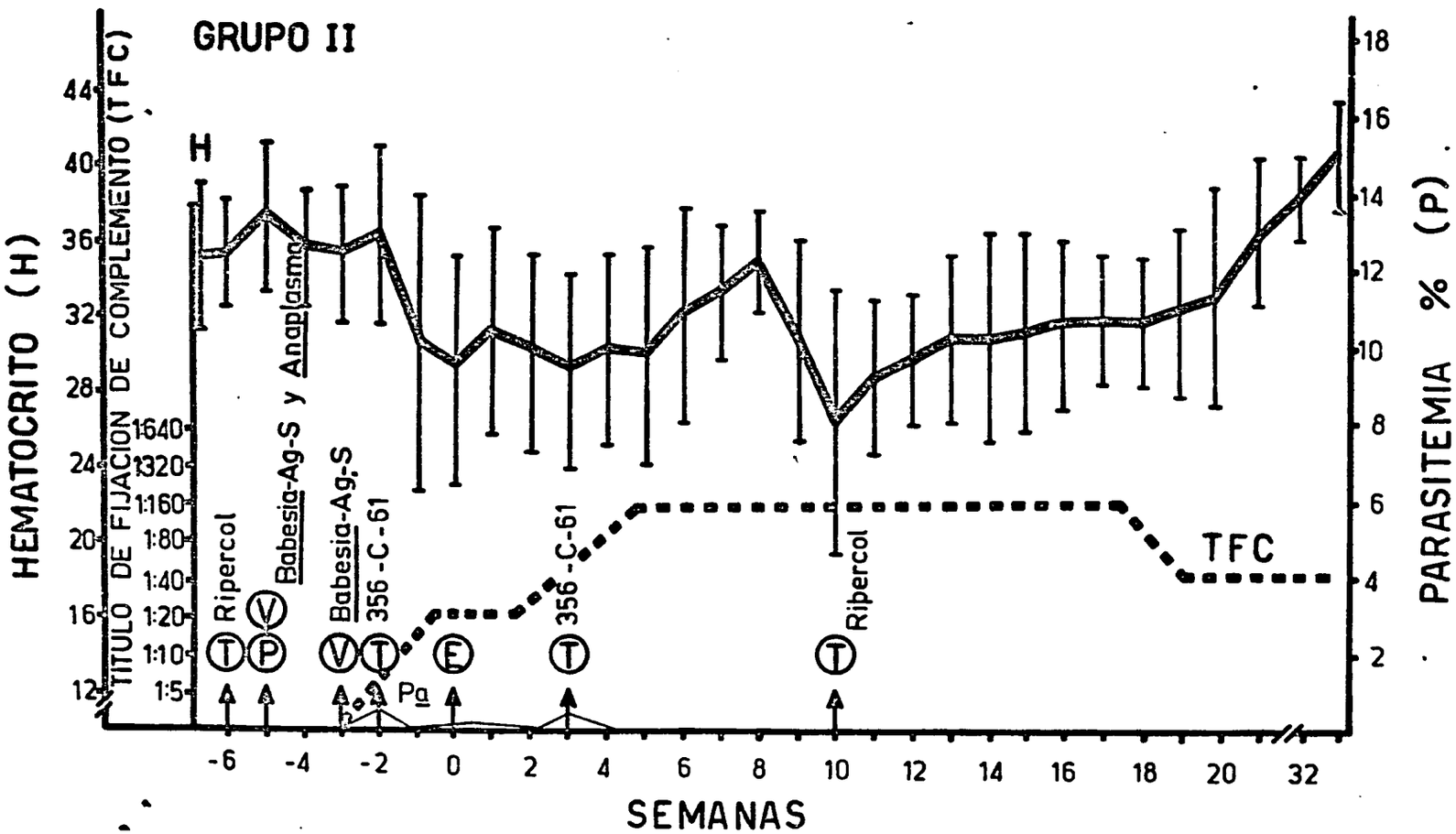

GRUPO II
HEMATOCRITO (H)
TITULO DE FIJACION DE COMPLEMENTO (T F C)
PARASITEMIA % (P)
SEMANAS
TFC
Ripercol
Babesia-Ag-S y Anaplasma
Babesia-Ag-S
356 -C - 61
356 -C-61
Ripercol
Pa
H
44
40
36
32
28
24
20
16
12
1:640
1:320
1:160
1:80
1:40
1:20
1:10
1:5
18
16
14
12
10
8
6
4
2
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
32

Figura 3. Curvas que indican el valor del hematocrito (H), el título de fijación del complemento (TFC) y parasitemia (Pb y Pa, para Babesia y Anaplasma) en 10 terneros (Grupo III) utilizados como control.

a. La letra E indica exposición a garrapatas en el campo.

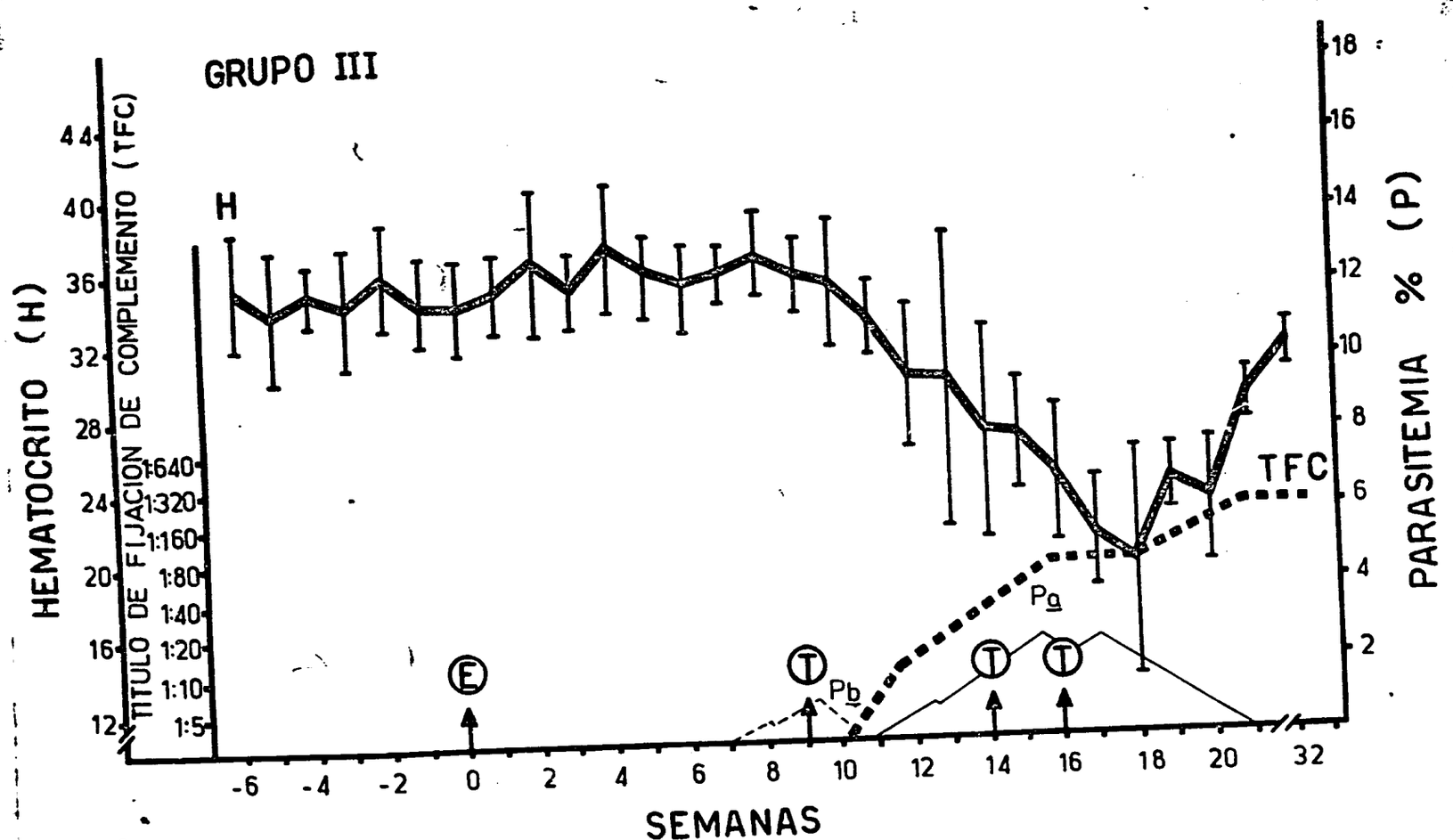
b. $\frac{T}{9}$ = Fecha tratamiento contra Babesia.

c. $\frac{T}{14}$, $\frac{T}{16}$ = Fechas tratamiento contra Anaplasma.

Nótese que los animales estuvieron en buenas condiciones antes de la exposición a garrapatas.

A las 7 semanas de la exposición se encontró Babesia, a las 11 semanas se encontró Anaplasma. En este caso la anemia fue grande y fue necesario hacer un tratamiento contra Babesia y dos contra Anaplasma.

Después de los tratamientos el hematocrito ascendió, como puede verse después de la semana 18.


GRUPO III
HEMATOCRITO (H)
TITULO DE FIJACION DE COMPLEMENTO (TFC)
PARASITEMIA % (P)
SEMANAS
H
TFC
Pa
Pb
E
T
T
T
44
40
36
32
28
24
20
16
12
1:640
1:320
1:160
1:80
1:40
1:20
1:10
1:5
18
16
14
12
10
8
6
4
2
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
32

parasitismos y se dan algunos conceptos sobre los posibles sistemas de control de ellos.

English Summary

A group of 50 male Holstein-Friesian calves, 3 to 4 months old were used to evaluate a control program for gastrointestinal and hemotropic parasites. The experiment was conducted at the ICA experimental station in Palmira, Cauca Valley at an elevation of 1000 m. The animals were divided into 3 groups.

Twenty animals were premunized simultaneously against babesiosis and anaplasmosis; 8 days later they were treated against babesiosis using the compound 4A65 at a dosage of 1 mg/kg of body weight, and 21 and 56 days after premunition they were treated intravenously with the compound 356-C-61 (5mg/kg IV) against anaplasmosis.

Twenty animals were premunized against anaplasmosis as it was done with the animals in Group I. Animals in this group were vaccinated with AGS plus adjuvant vaccine against babesiosis, The vaccine was repeated 14 days later. Animals in Group I and II were treated twice during the experiment treated twice during the experiment with Ripercol (Tetramisol) against gastrointestinal parasites.

Ten animals were not treated and were used as controls.

All three groups of calves were kept under the same environmental conditions and the same management. The experiment was carried out during a period of 8 months. Blood samples were collected to evaluate anemia and parasitemia. The antibody titer was determined by the complement fixation test. The body weights were measured and the fecal samples were examined for the presence of gastrointestinal parasites. Animals in Groups I and II had a high degree of resistance to babesiosis and anaplasmosis infections as a result of effective premunition and vaccination techniques. However, the animals in the control group had clinical babesiosis and anaplasmosis and high infestation with gastrointestinal parasites.

The importance of simultaneous control of gastrointestinal and hemotropic parasites is pointed out and methods to control these parasites are given.

References

1. Barret, P. A., Benveridge, E., Bradley, R. L., Brown, C. G. D., Bushby, S. R. M., Clark, M. L., Neal, R. A., Smith, R. and Wilde, J. K. H. (1965) Biological Activity of some a dithiosemicarbazone. Nature 206: 1340-1341.

2. Callow, L. L. and McGregor, W. (1970) The Effect of Imidocarb against Babesia argentina and Babesia bigemina Infections of Cattle. Aust. Vet. Journal 46: 195-200.

3. Ferris, D. H., Todorovic, R. and Ristic, M. (1968) Significance and Application of Babesial and Plasmodial Ecto-antigens to Comparative Medicine. J.A.V.M.A. 153: 1888-1896.

4. Mateus, G. 1968 Incidence and Control of Gastrointestinal Parasites in Cattle in Colombia. Ph.D. Thesis. The University of Wisconsin at Madison, Wisconsin.

5. Nieto, F. y Zaraza, H. 1965. Parasitismo gastrointestinal en los Bovinos Lecheros del Valle del Cauca. Agric. Trop. Vol. XXI, No. 2.

6. Riek, R. F. (1968) Babesiosis in Infectious Blood Diseases of Man and Animals. Ed. Weinman, D. and Ristic, M. pp. 219-268. Academic Press, N. Y. and London.

7. Ristic, M. (1968) Anaplasmosis. In Infectious Blood Diseases of Man and Animals. Ed. Weinman, D. and Ristic, M. pp. 473-542. Academic Press, N. Y. and London.

8. Roby, T. O., Amerault, T. E. and Spindler, L. A. (1968) The Inhibotory Effect of a Dithiosemicarbazone on Acute Infection of Anaplasma marginale. Research in Vet. Sci. 9: 494-499.

9. Todorovic, R., Ferris, D. and Ristic, M. (1967) Immunogenic Properties of Serum Antigens from Chickens Acutely Infected with Plasmodium gallinaceum. Annals of Trop. Med. and Parasitol. 61: 117-129.

10. Todorovic, R. A., Adams, L. G. and Roberts, E, D. (1969) A Study of Bovine Babesiosis in Colombia, South America. Proc. 106th Annual Meeting. Minneapolis, J.A.V.M.A. 154: 1399.

11. Todorovic, R. A.,Luque, F., G. y Adams, L. G. (1970) Contribución al Estudio de la Distribución de Garrapatas en Colombia, Sur América. Revista de la Facultad de Medicina Veterinaria y Zootecnia (In Press).

12. Todorovic, R. A., Adams, L. G., Vizcaíno, O. G. and González, E. F. (1970) Research and Control of Bovine Babesiosis in Colombia, South America. Proc. VI Congreso Panamericano de Medicina Veterinaria y Zootecnia. Santiago de Chile, Chile Sept. 28-30, 1970.

13. Todorovic, R. A., Vizcaíno, O. G. y Adams, L. G. Determinación de Anticuerpos de Babesia por la Técnia de Fijación del Complemento. Revista ICA (In Press).

14. Zaraza, H., Kuttler, K. L. y Roberts, E. D. (1969) Efectos de la Descarga Natural de Anaplasma marginale en Terneros Vacunados y no vacunados. Revista ICA 4: 139-146.

V. A. 4. Premunization Against Bovine Babesiosis and Anaplasmosis of Holstein Calves in Riopaila, Valle del Cauca.

Introducción

La incidencia de enfermedades ocasionadas por hematozoarios es elevada en la hacienda "Riopaila" y causa grandes pérdidas económicas anualmente. La leche producida por estos animales es utilizada en la elaboración de productos alimenticios. Por esta razón, las investigaciones se han dirigido a la búsqueda de un sistema eficaz que confiera al animal cierta inmunidad, para evitar la presentación de enfermedades causadas por hemoparásitos.

Materiales y Métodos

Para realizar este trabajo se escogió la hacienda "Riopaila" donde la incidencia de estas entidades es muy elevada ya que un 90 a 95% de los animales se hallan afectados. Trabajos anteriores hechos por el Centro Nacional de Investigaciones ICA en Palmira con gran éxito sirvieron de modelo para el control de éstas entidades en la Hacienda "Riopaila". La hacienda "Riopaila" cuenta con explotación de carne y leche con base en razas Charbray y Holstein respectivamente. Las garrapatas constituyen un problema permanente en la hacienda.

Esta ganadería cuenta con sus programas de cría de hembras de

reemplazo y venta, los machos son vendidos a los 8 días para elaboración de productos cárnicos.

El programa de cría y manejo es el recomendado por el Servicio de Administración Rural de la C.V.C. El confinamiento es total hasta los 56 días de edad con alimentación a base de leche y concentrado a voluntad; luego van a semi-estabulación con la misma alimentación, pastos de corte y algo de pastoreo (pangola) en donde el animal va a estar gradualmente en contacto con el medio ambiente y las garrapatas, esto dura hasta los 6 meses, aquí la incidencia de la enfermedad es muy baja 1 a 2%. Luego son llevados a pastoreo total en una hacienda cercana con alta infestación de garrapatas en las praderas, donde se enferman de 90% de los animales y en donde la mortalidad alcanza hasta en 10 ó 15% . El control de las garrapatas se efectúa con baños garrapaticidas a base de fósforo o arsenicales.

En EDTA, como anticoagulante, a la concentración de 1.2 gm x 1000 cc se colectaron 100 cc de sangre de un animal que no había recibido ningún tratamiento específico. Esto se hizo con el objeto de aprovechar las cepas existentes en la región para efectos de premunición ya que se presentan diferencias antigénicas en las distintas cepas del país.

Con esta sangre se inoculó un ternero esplenectomizado en el LIMV con el propósito de clasificar el organismo y reproducir la

enfermedad. Este animal fue controlado diariamente, se hicieron hematocritos y extensiones. Se evidención después de varios días que el animal se encontraba infectado con Babesia bigemina, Babesia argentina y Anaplasma marginale.

Sangre de este animal fue recolectada en anticoagulante y fue colocada en refrigeración transportada a la Hacienda "Riopaila". La Inoculación se hizo en un lote de 25 hembras de 1 mes de edad que se encontraban en confinamiento y por consiguiente libres de la infección. A cada animal fueron inoculados 2 ml por vía subcutánea.

Después de la inoculación los animales permanecieron en confinamiento hasta los 56 días de edad, luego fueron llevados a semi-estabulación durante dos meses y posteriormente fueron a pastoreo en la hacienda con alta infestación de garrapatas.

Dos animales fueron tratados 8 días después de la inoculación con el compuesto 4A65 a una dosis de 1 mg/kg y por vía intramuscular contra la babesiosis y 21 días post-inoculation se trataron con el compuesto 356-C-61 a la dosis de 5 mg/kg y esta misma dosis fue repetidad (30, 40, 45) cuando las extensiones demostraron un aumento en la parasitemia por Anaplasma en animales premunizados. Veinticinco animales sirvieron de testigos, pertenecían a la misma raza, la misma edad y las mismas condiciones de manejo. Los animales fueron sangrados 2 veces por semana para hacer evaluaciones

de hematocrito y parasitemia y pruebas serológicas. Cada dos semanas se recolectaban garrapatas para su clasificación.

Resultados

La clasificación de las garrapatas halladas en la finca correspondía al género Boophilus microplus. El animal esplenectomizado e inoculado con sangre proveniente de animales enfermos de la hacienda "Riopaila" reveló la presencia de B. bigemina, B. argentina y Anaplasma marginale. Una semana después de la inoculación de 2 ml de sangre por vía subcutánea al grupo experimental, desarrollaron fiebre, se evidenciaron además señales de inapetencia, pérdida de peso, anemia, con hematocritos de 25% en promedio. En todas las extensiones coloreadas fue posible evidenciar B. bigemina y B. argentina. Los animales se recuperaron 3 días después de la inoculación de la droga anteriormente citada contra babesiosis. Dos semanas después de la inoculación algunos animales mostraron porcentajes bajos de Anaplasma alrededor de los 21 días todos los animales mostraron parasitemia de 1 hasta 5% de Anaplasma. La droga citada previamente 356-C-61, se inyectó este mismo día en todos los animales y el tratamiento fue repetido 30 y 40 días después. Algunos animales necesitan un nuevo tratamiento en el curso de 7 semanas. Dos animales se recuperaron poco tiempo después y al término del experimento se hallaban en buen estado físico.

Después de 8 semanas los 25 animales en experimentación y los 25 animales testigo fueron a pastoreo permanentemente, en praderas altamente infestadas de B. microplus, todos los animales testigos sufrieron alternativamente babesiosis y anaplasmosis, con manifestaciones claras de pérdida de peso, anemia, inapetencia y parasitemia en (extensiones coloreadas) estos animales se trataron con drogas comerciales y específicas de uso ordinario en la hacienda. Los animales premunizados artificialmente fueron resistentes a la anaplasmosis y ala babesiosis, los valores de hematocrito fueron siempre normales.

English Summary

In view of the promising results obtained with premunition of cattle at the ICA-Palmira experimental station, similar studies were conducted at Holstein-Friesian dairy farm, "Riopaila", located in the northern part of the Cauca Valley at an elevation of 1,100 m. It was estimated that annual mortality due to babesiosis and anaplasmosis was approximately 10 to 15%, on this farm. The objectives were to identify the hemoparasites and develop a system of premunition to control these infections. Blood was collected in anticoagulant (EDTA, 1.2 gm/liter) from three infected animals on the farm and the pooled samples were injected into a splenectomized calf held at LIMV in Bogotá on the same day. Thereafter,

this calf was examined daily for hemotropic parasites. Anaplasmosis and babesiosis were reproduced and a strain of each of *A. marginale* and *B. bigemina* and *B. argentina* was obtained for premunition studies.

Twenty five, Holstein-Friesian, female calves one month old were infected with 2 ml of blood collected from the infected splenectomized calf. Twenty five animals of the same age and breed were not infected and were used as controls. Eight and 21 days after blood inoculation all animals were treated with compound No. 4A65 at a dosage of 1 mg/kg of body weight and No. 356-C-61 (5 mg/kg of body weight) respectively. The treatment against bovine anaplasmosis was repeated beginning 30, 40 and 45 days post premunition. Samples were collected two times per week for evaluation of parasitemia, anemia and CF antibody titers, Two months after premunition all animals were exposed to tick-borne (*B. microplus*) challenge. Animals premunized demonstrated a high degree of resistance to babesiosis and anaplasmosis. However, non-premunized animals suffered severe attacks of anaplasmosis and babesiosis and were treated to prevent death losses.

V. A. 5. Evaluation of the Compounds 4A65 and 356-C-61 as Chemoprophylactic Agents Against Bovine Babesiosis and Anaplasmosis under Field Conditions at San Juanito, Valle del Cauca

Introducción

Se ha encontrado que terneros mantenidos en condiciones experimentales e inoculados con los compuestos 4A65 y 356-C-61 fueron resistentes a babesiosis y anaplasmosis, manteniendo un hematocrito, no presentaron síntomas de estas enfermedades y no experimentaron pérdida de peso.

Se trata de demostrar los efectos de estas drogas en terneros que permanecen en condiciones naturales en donde estas enfermedades han sido endémicas y han ocasionado numerosos casos de mortalidad tanto en adultos como en jóvenes.

Materiales y Métodos

Para llevar a cabo este experimento se escogió la hacienda "San Juanito" la cual facilitó 30 terneros de raza Holstein cuyas edades están comprendidas entre 3 y 4 meses, 15 animales fueron tratados con droga y 15 terneros sirvieron de testigo.

El experimento fue iniciado a finales de 1969: todos los animales fueron pesados y sangrados para determinar el grado de para-

sitemia, hematocritos y exámenes serológicos para Anaplasma y Babesia, también se usaron.

Tanto el grupo en experimentación como el grupo testigo fueron mantenidos en la misma hacienda bajo el mismo régimen de manejo y condiciones nutricionales. Al iniciar el experimento todos los animales se pesaron con cinta métrica y se les inyectó el compuesto 4A65 contra Babesia utilizando una dosis de 2 mg/kg de peso IM. Esta misma dosis fue repetida a los 3 meses.

Cada semana fue tomada sangre para hematocrito y extensiones que se colorearon con Giemsa y se identificaron con el número respectivo del ternero y la fecha de la toma: 24 horas después de la recolección de la muestra de sangre se sacó el suero y se envió al LIMV de Bogotá para hacer estudios serológicos.

Dos semanas después de la primera inyección de 4A65 se inyectó a cada ternero el compuesto 356-C-61 contra Anaplasma, utilizando una dosis de 4 mg/kg IV, previa agitación para asegurar una buena homogenización de la droga. Esta dosis fue repetida a los 3 meses.

Los terneros permanecieron durante todo el tiempo del experimento en el campo. Cada dos semanas fueron recolectadas garrapatas en tubos con alcohol, los tubos estaban numerados y se tenía un tubo para cada animal, la recolección se hizo antes de que los animales fueran bañados con garrapaticida.

Cada 3 semanas se hizo control de peso de los animales.

Los datos obtenidos en este experimento fueron comparados con los datos obtenidos en experimentos realizados por el ICA, en animales en condiciones de laboratorio y con el grupo de controles que permaneció en el campo.

Resultados

Se encontró que los animales antes de la experimentación se hallaban infectados naturalmente de Babesia y Anaplasma, estos hallazgos se detectaron por extensiones coloreadas con Giemsa y pruebas serológicas. Los 15 animales que fueron tratados con drogas mostraron un aumento considerable en peso al término del experimento; en oposición al grupo testigo en el cual el aumento de peso no fue comparativamente muy grande. Al iniciarse el experimento los aniamles tenían un promedio de 70 kilos, al finalizar el experimento los animales alcanzaron 200 kilos, en promedio. El grupo testigo alcanzó al término del experimento 125 kilos de peso en promedio. El grupo testigo sufrió brotes esporádicos de Babesiosis y anaplasmosis y se trataron con drogas comerciales de uso frecuente en la hacienda para estos casos.

En todos los animales del experimento se detectó por fijación de complemento anticuerpos contra Babesia y Anaplasma, lo que puede indicarnos que los animales fueron infectados por garrapatas.

Los datos obtenidos en este experimento permiten concluír que

el compuesto 4A65 es muy efectivo en la prevención de babesiosis ya que los animales tratados con este compuesto no mostraron signos clínicos de babesiosis. Por el contrario el compuesto 356-C-61 protegió por un corto tiempo, cino o 6 meses, después de este tiempo 7 animales mostraron signos clínicos de anaplasmosis, es importante hacer énfasis aquí que la presentación de la enfermedad coincidió con el comienzo del verano, época muy buena para el desarrollo y multiplicación de la garrapata. Por esta razón se hace necesario aplicar el compuesto 356-C-61 con más frecuencia para hacer un control efectivo de la anaplasmosis.

English Summary

The objective of this experiment was to evaluate the chemoprophylactic action of the compound 4A65 and 356-C-61 (Burroughs-Wellcome Co., Tuckahoe, N. Y.) against bovine babesiosis and anaplasmosis under field conditions. The experiment was carried out at a Holstein-Friesian dairy farm "San Juanito" in Buga, Valle del Cauca, (altitude 1,000 m). A total of 30 female calves 3 to 4 months old were used to study the prophylactic effects of these compound. The calves were divided into two groups. Fifteen calves (Group A) were injected with drug No. 4A65 at a dosage of 2 mg/kg of body weight IM and two weeks later with drug No. 356-C-61 at a dosage of 4 ml/kg of bodyweight intravenously. This treatment was repeated beginning 90 days later. Control animals (Group B) received no treatment.

Observations for drug toxicity were made for 30-60 minutes following injection.

Four weeks following the first treatment, the calves (Groups A and B) were exposed to B. microplus ticks naturally infected with B. bigemina, B. argentina and A. marginale. The calves were examined twice weekly for 4 weeks before and weekly after field challenge during a period of 200 days. Packed cell volume (PCV), parasitemia (P), body weight and complement fixing antibody titers for bovine babesiosis and anaplasmosis were determined. Satisfactory chemoprophylactic protection against clinical babesiosis was obtained with drug No. 4A65 at doses of 2 mg/kg, but not with compound No. 356-C-61 against bovine anaplasmosis. Seven animals suffering clinical anaplasmosis were treated with oxytetracycline. It appeared that the chemoprophylaxis using drug No. 4A65 did not interfere with the normal development of acquired immunity to bovine babesiosis.

V. A. 6. Premuniton Against Bovine Babesiosis and Anaplasmosis in Brown Swiss Cattle in Aguasalada, Valle del Cauca

Introducción

Las enfermedades ocasionadas por hematozoarios causan graves pérdidas económicas en el Valle del Cauca y en especial en esta localidad donde el problema constituye una verdadera enzootia.

Se calcula que las pérdidas acumuladas en los 5 años anteriores llegan hasta un 25% especialmente en animales de un año de edad. Existe un grave problema con garrapatas, especialmente en periódos de transición de verano a invierno, y viceversa. Se trata de ensayar técnicas de premunización en combinación con terapia química como sistema de control de babesiosis y anaplasmosis en terneros, en condiciones naturales.

Materiales y Métodos

Se seleccionaron 15 terneros Pardo Suizo de 2 meses de edad para iniciar el experimento. Diez animales de la misma edad y raza sirvieron de controles. Se han encontrado diferencias antigénicas en las cepas de las diferentes regiones del país. De ahí la necesidad de efectuar programas de premunización en una región con cepas originarias de esa misma región con el fin de conferir al animal premunizado una inmunidad más específica. Por esta razón

10 ml de sangre fueron recolectados de cada animal enfermo. La sangre se tomó en EDTA como anticoagulante y a una concentración de ..2 gm por 1000 cc. Un ternero en el LIMV esplenectomizado fue inoculado con esta mezcla de sangres, por via subcutánea, para clasificar los hemoparásitos y reproducir la enfermedad. El ternero esplenectomizado demostró la presencia de A. marginale, B. argentina y B. bigemina.

Sangre de este ternero fue recolectada en EDTA como anti-coagulante fue mantenida en refrigeración y transportada a la hacienda "Aguasalada", un día después de llegada la sangre, se inoculó al grupo experimental con 2 ml de esta sangre por vía intramuscular, control de temperatura fueron hechos los primeros días de iniciado el experimento. Diez díaz después de la inoculación de la sangre parasitada se hizo la primera aplicación del compuesto 4A65 a la dosis de 1 mg/kg de peso por vía intramuscular contra Babesia y 20, 30, 40, 65 días después de la inoculación de sangre parasitada se hizo aplicación del compuesto 356-C-61 a la dosis de 5 mg/kg de peso por vía intravenosa contra Anaplasma.

Los animales fueron expuestos a las garrapatas a los 6 meses de edad ya que esta es la edad en la cual ellos van a pastoreo permanente. Todos los animales tanto el grupo experimental como el grupo control se hallan en las mismas condiciones de manejo, los animales fueron pesados cada 3 semanas con cinta métrica.

Se tomaron fotos al comenzar y al finalizar el experimento, de todos los animales en experimentación, controles y del resto de bovinos de la hacienda con el propósito de identificar los vectores. En el grupo no premunizado se tuvieron en cuenta los problemas ocasionados por hematozoarios y se consideraron las drogas empleadas en los tratamientos.

Resultados

Las garrapatas coleccionadas en la finca "Aguasalada" corresponden a: B.microplus, la sangre recolectada de animales enfermos e inoculada a un ternero esplenectomizado en el LIMV, permitió la reproducción de A. marginale, B. bigemina y B. argentina. De la inoculación de Babesia y Anaplasma los animales en experimentación sufrieron anemia ocasionada por los parásitos respectivos, pero después de la inoculación de droga estos animales se recuperaron notoriamente, apreciándose un aumento del hematocrito, y mejoramiento de las condiciones físicas del animal. Una sola inoculación del compuesto 4A65 a la dosis anteriormente citada es suficiente para impedir una reacción de Babesia después de la premunización. Sin embargo, una dosis única del compuesto 356-C-61, no puede evitar la aparición del Anaplasma, después de la premunización. En algunos terneros se hace necesario la aplicación de 4 dosis porque la parasitemia ocasionada por Anaplasma es

persistente y algunos animales sufren la sintomatología clínica, que se manifiesta con baja del hematocrito 20% en promedio, estos animales se recuperan después de 5 inyecciónes del compuesto 356-C-61. Una vez los animales fueron llevados a las praderas altamente infestadas con B. microplus, los animales previamente inmunizados demostraron una gran resistencia a los hematozoarios, en cambio animales no premunizados y que sirvieron de control altamente susceptibles a la babesiosis y anaplasmosis y fue necesario el cuidado permanente y tratamiento adecuado con drogas comerciales para evitar la mortalidad en este grupo.

Se observaron además en esta finca deficiencias nutricionales, parásitos gastrointestinales y otras enfermedades infecciosas.

English Summary

Control of bovine babesiosis involves control of the tick vectors by dipping or premuniton of animals by injecting infected blood followed by treatment to control but not eliminate the post-inoculation infection. The emergence of strains of Boophilus ticks resistant to commonly used acaricides has complicated the control situation considerably. In addition, varying breed susceptibility to ticks and infection with babesiosis and anaplasmosis are factors associated with host-parasite relationships and need more investigation. Before the control program against vectors or hemoparasites

can be applied on large scale in the tropics.

For these reasons, the experiments were conducted to identify hemoparasites and develop a system of premunition on a Brown-Swiss dairy farm "Aguasalada" in Buga, Cauca Valley (altitude 1,000 m). It was estimated that annual mortality due to babesiosis and anaplasmosis was approximately 25% on this farm. Furthermore, the resistance to acaricides was evident by the high tick population on animals treated with various acaricides. The purpose of this investigation was to identify the hemoparasites, vectors and to develop a system of premunition for the control of these infections. In order to keep the experiment on a practical basis, management practices, such as feeding and ration type were determined by the farmer.

A total of 25 female calves, two months old, were used in this study. Acaricides used for controling the tick population were Toxaphene and Asuntol (Bayer). Preinoculation blood samples were collected for the determination of pre-exposure infection with *Babesia spp.*, and *A. marginale*. Fifteen intact calves (Group A) were premunized with 2 ml of blood from a carrier calf infected with an isolate of *Babesia bigemina*, *B. argentina* and *A. marginale* from the "Aguasalada" farm. Ten intact calves (Group B) served as untreated controls. Calves which were premunized (Group A) were treated on day 10 after inoculation with Burroughs-Wellcome

compound No. 4A65 at a dosage of 1 mg/kg of body weight, and on days 20, 30, 40 and 65 after inoculation with Burroughs-Wellcome compound No. 356-C-61 (-dithiosemicarbazone) at a dosage of 5 mg/kg of body weight. Blood samples throughout the experiment were collected at 7 day intervals. Giemsa stained blood smears were prepared for the evaluation of parasitemia with Babesia spp. and A. marginale. The micro hematocrit method was used for determination of anemia. Serology (CF test forbabesiosis and anaplasmosis) and the other laboratory procedures were performed in the Veterinary Medical Investigations Laboratory (LIMV) in Bogotá. Total tick collections were performed monthly on all animals. Body weights were determined every 15 days on all animals.

On the basis of observations made with these experiments it can be concluded that Brown Swiss cattle may be more susceptible to tick infestation and hemoparasites than other breeds in the tropics. The species of ticks on animals located on this farm were identified as Boophilus microplus. Animals premunized with blood collected from the patent carrier of B. bigemina, B. argentina, and A. marginale (isolate Aguasalada) developed slight post inoculation reaction which was manifested by parasitemia, fever and anemia. The Babesia parasitemia was effectively controled by one application of compound 4A65, however 4 applications of 356-C-61 were necessary to control the Anaplasma parasitemia. Four weeks

post-premunition, these animals recovered from anemia. When these premunized animals were exposed to ticks (B. microplus) naturally infected with Babesia bigemina, B. argentina and A. marginale, they were highly resistant to clinical babesiosis and anaplasmosis, while control group became infected with babesiosis and anaplasmosis after 4 to 8 weeks of exposure and some of them died. It is concluded that the method of premuniton used in this experiment was effective against babesiosis, however the premunition technique against anaplasmosis requires more refinement.

V. A. 7. A Study of the Problem of Anaplasmosis and Babesiosis an d other Infections on the Farm San Felipe, Tolima

Introducción

Una mortalidad de animales mayores de un año, sin causa aparente, fue atribuída a hemoparásitos, ya que las praderas se encuentran altamente infestadas de garrapatas y porque también la hacienda se encuentra localizada en una zona endémica de babesiosis y anaplasmosis.

Los animales muertos son de raza Pardo Suizo de alta pureza, por lo que las pérdidas económicas son onerosas.

Materiales y Métodos.

La hacienda está situada en jurisdicción del municipio de Armero, Tolima y durante 20 años está dedicada a la cría y levante de ganado Pardo Suizo de alta pureza. Hay 200 animales aproximadamente.

Las hembras son llevadas a una sala de parición un mes antes del parto. El ternero es dejado con la madre durante 3 ó 4 días y su alimentación está constituída exclusivamente por calostro. Después de 3 ó 4 días de edad los terneros son llevados a "salacunas" donde permanecen recluídos por 6 meses, pero en este tiempo son llevados a pastoreo dos veces por semana, no se presenta mortali-

dad en este grupo de animales y problemas sanitarios nunca se presentan. Los animales son vacunados contra: carbón sintomático, pasteurellosis y edema maligno. Los mayores de 3 meses se vacunan contra fiebre aftosa y revacunaciones se hacen cada 4 meses. Los sistemas de manejo en general son buenos, los parásitos gastrointestinales están bien controlados. Baños garrapaticidas se hacen cada 15 días, los productos comerciales más utilizados son: Dursban, IE; Garafos, Asuntol, etc., a pesar de los baños aplicados, muchos animales presentan gran número de garrapatas al momento del examen. Los animales comienzan a presentar problemas cuando van a pastoreo, los animales en reclusión no presentan problemas.

El día de la visita 8 animales se encontraban enfermos, a todos se les tomó la temperatura y se les hizo sangría con el propósito de evidenciar la causa del problema.

Animales clinicamente sanos también fueron sangrados para determinar hematocritos y pruebas serológicas. La sangre fue coleccionada con anticoagulante para extensiones coloreadas y exámenes en fresco para Tripanosoma. Una mezcla de sangre de animales enfermos fue inoculada a un ternero en el LIMV. Se tomaron muestras de garrapatas para su clasificación.

Resultados

Las garrapatas recolectadas se clasificaron como B. microplus.

De un caballo se recolectó también una garrapata que se clasificó como Amblyomma cajennense"

El hematocrito de los 8 animales enfermos osciló entre 21 y 32%. En el grupo de los animales aparentemente sanos el hematocrito tuvo valores de 27 y 34%.

Los animales enfermos con hematocritos de 28 y 31 respectivamente presentaron baja parasitemia de Anaplasma marginale (0.05%). El animal inoculado con sangre de animales enfermos demostró evidencia de Anaplasma y Babesia y sin embargo, mostró fiebre fluctuante y ligera anemia.

Extensiones en fresco para Tripanosoma, no evidenciaron este parásito.

Prueba para brucellosis fueron negativas para todos los animales. Dos animales fueron positivos a Leptospira por el método de aglutinación-lisis (AL).

Dos meses después de la visita a la hacienda un animal con típicas manifestaciones de la enfermedad problema, fue traído para autopsia, las lesiones post-mortem parecen indicar que se trate de un caso de leptospirosis. Durante la visita a la finca se pudo observar que agua estancada en potreros adyacentes al establo podría estar ocasionando, el problema ya que constituye un medio favorable para la proliferación de Leptospira.

English Summary

The objective of this investigation was to determine the cause of high mortality of Brown Suiss cattle located on the privately owned dairy farm "San Felipe", Armero, Tolima (altitude 350 m) in the Upper Magdalena Valley. The mortality losses coincided with the rainy season when the animals were exposed to tick infected pastures. Blood samples were collected from eight infected animals for measurement of the following parameters: packed cell volume (PCV); parasitemia, complement fixing antibody titers, total white blood cell count, red blood cell count. Fifty ml of blood were collected from each infected animal in EDTA, 1.2 gm/l. The blood was pooled and injected into a single intact normal calf at the Veterinary Medical Investigation Laboratory (LIMV) in Bogotá, Colombia on the same day. Blood smears from this calf were examined daily for the presence of hemoprotozoan parasites using stained smears and wet mounts. The complement fixation test for babesiosis and anaplasmosis was performed weekly. Ticks collected from infected cattle were classified as B. microplus, and ticks collected from horses were classified as Amblyomma cajennense. The eight infected animals had PCV volumes between 21 and 32%. All animals had a low level of Anaplasma marginale infection (0.05%). Serologic tests for brucellosis (tube and plate)

were negative but were positive for babesiosis and anaplasmosis (CF) and leptospirosis by agglutination lysis (AL). Post mortem examination of one of the animals positive to hemoparasites, revealed typical lesions of leptospirosis, a disease whose prevalence is unknown in Colombia, but which may be a common concurrent infection in the tropics.

V. A. 8. Premunization of Adult Cattle Against Babesiosis and Anaplasmosis with 4A65 and Oxytetracycline in Tibaitatá

Introduction

Because the ICA National Dairy Program has plans for the dissemination and production of dairy cattle, it was proposed that these hemoparasite susceptible cattle which originate from the Sabana de Bogotá be premunized before sending them to enzootic tropical zones in Puerto Asís, Putumayo known to have a high prevalence of hemotropic diseases, particularly anaplasmosis and babesiosis.

Materials and Methods

Thirteen male calves 12 to 15 months of age of Holstein-Friesian, Brown Swiss, and Normand breeds were utilized in this experiment. Prior to premunization, one set of control data, consisting of packed cell volume, blood smears, complement fixation of anaplasmosis and babesiosis and body weights, were taken. On day 0, all cattle were inoculated subcutaneously with 5 ml of blood from a carrier of Montería strains of *A. marginale*, *B. bigemina* and *B. argentina* which had no demonstrable parasitemia. On day 8 and 9 PI, all cattle were inoculated with compound 4A65 at 1 mg/kg IM. On day 21 PI, all cattle were inoculated with

TABLE I: RESULTS

Day	Treatment	PCV	Anaplasma % Par.	Anaplasma CF	Babesia % Par.	Babesia CF	Body Wt.
0	5 cc blood S.Q.	33.2	Neg	Neg	Neg		249.8
8	4A65 1 mg/kg S.Q.	15.9	Neg	Neg	0.55		235.9
9	4A65 1 mg/kg S. Q.	16.7	Neg	Neg	Neg		-----
11	--------	18.9	Neg	Neg	Neg		-----
14	--------	21.4	Neg	Neg	Neg		244.5
21	Oxytetracycline 12 mg/kg IV	24.9	0.22	1:2	Neg		-----
24	--------	27.7	Neg	---	Neg		-----
28	--------	28.4	Neg	1:3	Neg		-----
31	--------	30.0	Neg	---	Neg		274.2
35	--------	30.9	Neg	---	Neg		276.9
38	--------	31.5	Neg	---	Neg		278.2
42	Challenge 5 cc S. Q.	31.7	Neg	1:120	Neg		285.3
45	Oxytetracycline 12 mg/kg IV	30.4	0.10	---	Neg		270.5
49	Oxytetracycline 12 mg/kg IV	22.6	4.53	1:208	Neg		-----
50	Oxytetracycline 12 mg/kg IV	----	Neg	---	Neg		-----
51	Oxytetracycline 12 mg/kg IV	----	Neg	---	Neg		-----
52	Oxytetracycline 12 mg/kg IV	19.6	2.04	1:268	Neg		-----
53	Oxytetracycline 12 mg/kg IV	20.7	0.41	---	Neg		-----
54	Oxytetracycline 12 mg/kg IV	----	Neg	---	Neg		-----
55	Oxytetracycline 12 mg/kg IV		Neg	---	Neg		-----
56	--------	20.8	0.05	---	Neg		-----
59	--------	21.4	0.03	1:247	Neg		282.8
63	--------	21.2	0.03	1:303	Neg		286.4
66	--------	20.5	0.01	1:129	Neg		281.3
70	--------	23.8	0.01	1:38	Neg		-----
78	--------	25.1	Neg	1:76	Neg		-----
85	--------	27.4	0.01	---	Neg		308.7
90	--------	30.5	Neg	---	Neg		314.0

oxytetracyclines (McKesson) at 12 mg/kg IV. Blood samples and serum were collected twice weekly and body weights were measured. All cattle were challenged subcutaneously on day 42 PI with 5 ml of blood from the same carrier as stated above. On days 45, 49, 50, 51, 52, 53, 54, and 55, all cattle were treated with oxytetracycline at 12 mg/kg intravenously. On day 90, all calves were sent to Puerto Asis, Putumayo after having been vaccinated against blackleg, malignant edema and hemorrhagic septicemia.

Discussion

Blood from the anaplasmosis and babesiosis carrier was infective as it did produce demonstrable parasitemias and a rapid decrease in PCV as evidenced from day 0 to day 8 PI and 21 PI respectively. 4A65 was proven to be very effective in the control of the babesia parasitemia and reduced it to zero within 24 hours. A second unnecessary application of 4A65 was administered as no parasites were seen on day 9, and following this day *Babesia* parasites were never seen again even after challenge. Both *B. bigemina* and *B. argentina* were observed on day 8 PI with an average low parasitemia of 0.55%. It is difficult to say whether the cattle were premunized against babesiosis or whether they were simply chemotherapeutically protected by the long residual action of 4A65, because it is know to protect against

B. bigemina 46 days (Todorovic et al., ITVM, Report, 1969) and 90 days (Neitz, personal communication) post inoculation. It was speculated that it was the latter case.

All cattle except 2 had an average A. marginale parasitemia of 0.22% on day 21 PI without a decrease in PCV. After one application of oxytetracycline, the low parasitemias became unevident. All cattle were challenged on day 42 PI with the same carrier blood with no demonstrable parasitemia, and all cattle had a relatively high A. marginale parasitemia of 4.53% on day 49 PI, but due to the lack of calves, the challenge inoculum could not be checked for infectivity. This surge of A. marginale parasitemia was probably due to a normal recrudescence stressed by the challenge inoculum and was accompanied by a rapid reduction in PCV. Treatment with oxytetracycline for 8 times was relatively uneffective in controlling the parasitemia due to A. marginale. From other investigations with 4A65 which will be and have been reported, it appears that 4A65 might possibly have some unknown effect to enhance the host susceptibility to anaplasmosis if applied at the correct time during the incubation period and at a moderately low dosage.

Body weights fluctuated directly proportional to the PCV which was manipulated by the hemotropic infections. All cattle were shipped to a hot tropical jungle zone in Puerto Asís,

Putumayo, Colombia where Anaplasma and Babesia infected Boophilus microplus ticks exist in large quantities. All cattle have done well for the last 6 months.

It is not considered that the method of premunition used in this experiment was effective until after the animals were challenged on day 42 PI and there is still doubt about the Babesia premunized state in this experiment. Emphasis was shifted from 4A65 to Ganaseg and oxytetracycline to a 356-C-61 and oxytetracycline combination.

V. A. 9. Premunization of Adult Cattle Against Anaplasmosis and Babesiosis with Ganaseg and a Combination of Oxytetracycline and 356-C-61 in Tibaitatá

Introduction

On the basis of the previous experimental premunization methods using 4A65 and oxytetracyclines, it was decided to try an improved method with better drugs and techniques on susceptible animals from the Tibaitatá dairy program which were to be sent to low tropical enzootic hemoparasite zones.

Materials and Methods.

Eleven cattle between 1 and 3 years of age of Holstein-Friesian Normand and criollo breeds were divided into 2 groups A and B. Both groups were bled twice weekly for the first month and thereafter weekly for a duration of 60 days for PCV, parasitemia and complement fixation for anaplasmosis and babesiosis. Weights were measured on days 0, 44 and 61 PI. Group A consisting of 5 males were treated with Ganaseg at 1.5 mg/kg IM on day 5 PI and with a combination of 356-C-61 at 5 mg/kg IV and oxytetracyclines at 12 mg/kg IV dissolved in 200 ml of PSS on days 18 and 22 PI. Group B consisting of 4 males and 2 females were treated exactly as group A with the exception that they were

treated on day 18 PI only with 356-C-61 and oxytetracycline. All cattle were inoculated with 5 ml of blood from a carrier of B. bigemina, B. argentina and A. marginale Montería strains on day 0. On day 60 PI, all cattle were sent to either Tolima or Putumayo, Colombia in enzootic hemoparasite zones.

Discussion

Cattle in groups A and B showed a very slight reaction to both anaplasmosis and babesiosis since the infections were completely controlled by the treatment regimen. No evidence of a recurring parasitemia was seen post-treatment. There were no apparent differences between the amount of reduction of the PCV in group A or B nor were differences between the magnitude of the control of the parasitemia between the groups. Group A developed a significantly higher anaplasmosis CF titer when compared to group B. Differences in weight gains were negligible when the large 712 kg bull was eliminated from group B. None of the cattle were blood-borne challenged, but were sent for field challenge. No report of their performance has been received except that none have died due to hemotropic diseases after having been in the different zones for 90 days, hence no definite conclusions of the immune status can be assessed at this time.

It is apparent that one application of Ganaseg at 1.5 mg/kg

I.M. was extremely effective for controlling the Babesia parasitemia and that one application of 356-C-61 plus oxytetracycline were equally effective for controlling the Anaplasma parasitemia whether applied once or twice in adult cattle, which are considered to be highly susceptible to these diseases. No clinical signs of hemotropic diseases were ever observed.

TABLE I : RESULTS GROUP A

Day	Treatment	PCV	Anaplasma % Par.	Anaplasma CF	Babesia % Par.	Babesia CF	Body Wt.
0	5 cc blood	34.2	Neg	Neg	Neg		237.4
3	--------	33.6	Neg	Neg	Neg		-----
5	Ganaseg 1.5 mg/kg I.M.	30.2	Neg	Neg	0.01		-----
	--------	31.8	Neg	Neg	Neg		-----
11	--------	31.0	Neg	1:2	Neg		-----
14	--------	38.3	Neg	----	Neg		-----
15	--------	28.6	0.09	1:18	Neg		-----
18	356C61 5 mg/kg IV Oxytetracycline 12 mg/kg IV	27.4	0.18	1:80	Neg		-----
22	356-C-61 5 mg/kg IV Oxytetracycline 12 mg/kg IV	23.6	Neg	1:184	Neg		-----
24	--------	28.0	Neg	1:139	Neg		-----
28	--------	29.8	Neg	1:35	Neg		------
35	--------	31.0	Neg	1:38	Neg		-----
44	--------	36.0	Neg	1:28	Neg		278.2
61	--------	33.2	Neg	----	Neg		295.0

TABLE II : RESULTS GROUP B

Day	Treatment	PCV	% Par.	CF	%Par.	CF	Body Wt.
0	5 cc blood	33.2	----	Neg	----		300.3
3	----------	33.3	----	Neg	----		-----
5	Ganaseg 1.5 mg/kg I.M.	30.8	----	Neg	0.07		-----
7	----------	32.5	----	Neg	----		-----
11	----------	32.5	----	1:20	----		-----
14	----------	32.2	----	----	----		-----
15	----------	32.8	0.01	1:38	----		-----
18	Oxytetracycline 12 mg/kg IV						
	356-C-61 5 mg/kg IV	27.7	0.01	1:39	----		-----
22	----------	25.8	----	1:42	----		-----
24	----------	29.0	----	1:43	----		-----
28	----------	28.5	----	1:7	----		-----
35	----------	29.7	----	1:4	----		-----
44	----------	34.5	----	1:4	----		329.8
61	----------	32.5	----	----	----		343.2

V. A. 10. Simultaneous Treatment of Bovine Anaplasmosis and Babesiosis with Compound 4A65

Introduction

Compound 4A65 (Burroughs-Wellcome Co.) is known to be highly effective for the treatment and chemoprophylaxis of bovine babesiosis and some communications with Burroughs-Wellcome Co. researchers have indicated effectivity against bovine anaplasmosis. Because bovine anaplasmosis and babesiosis frequently occur simultaneously in Colombia and 4A65 may have a dual efficacy the following experiment was designed to verify or disprove its role as a single drug effective against both diseases.

Materials and Methods

Drug	4A65 di HCl dissolved in distilled H_2O at 5%.
Animals	3 or 4 month old Holstein-Friesian calves from the Sabana of Bogotá, previously checked for the abscence of anaplasmosis and babesiosis by blood smears, complement fixation serology and pooled blood injection into a splenectomized calf. Four splenectomized calves.
Number	3 groups of 6 calves and one group of 2 control calves. 4 splenectomized calves.

Doseage	Group 1 -1.0 mg/kg I.M. Group 2 -2.0 mg/kg I.M. Group 3 -2.5 mg/kg I.M. Group 4 -Control
Challenge & Treatment	Day -16 -5 ml of blood SQ containing 12% parasitemia of *A. marginale*-Montería. Day -5 -5 ml of blood SQ containing 10% parasitemia of *B. bigemina* and *B. argentina*-Montería Day 0 -Treatment when *A. marginale* reached average 3.16% parasitemia and *B. bigemina* or *B. argentina* reached 0.095% parasitemia and the PCV decreased to an average of 14.2% with parasitemias of both organisms present. Day 81 -10 ml of blood SQ containing *A. marginale* (5% parasitemia) and Day 84 ten ml of blood containing *B. bigemina* and *B. argentina* (1.5% parasitemia) SQ for challenge. Although the challenge inoculum was not inoculated into a susceptible calf to check pathogenicity, both the splenectomized anaplasmosis and babesiosis carrier calves succumbed to acute infections indicating a degree of virulence.
Period	119 days.

Body weight	Twice weekly.
Hematology &	Samples were collected twice weekly through Day -11. Day -7 through Day 11, samples were examined daily; samples were collected 2 times weekly thereafter. Hematology consisted of PCV, blood smears for parasitemia and complement fixation testing for anaplasmosis and babesiosis.
Temperature	Coincided with blood sample collection.

An attempt was made to artifidally induce simultaneous anaplasmosis and babesiosis infections, with the parasitemias of both occurring simultaneously, for this reason. *Anaplasma marginale* was injected on Day -16 and *B. bigemina* and *B. argentina* were injected on Day -5 post-inoculation in order to allow the correct amount of time for the respective incubation periods. When the parasitemia of *A. marginale* reached 3.16% and *B. bigemina* or *B. argentina* reached 0.095% and the PCV reached 14.2% with both organisms present. Group I was be given 1.0 mg/kg I.M. Group II, 2.0 mg/kg I.M. and Group III was given 2.5 mg/kg I.M. These doseages were chosen in hopes that the drug would be effective against *A. marginale*, *B. argentina* and *B. bigemina* without sterilizing the calves for babesiosis. In other words, an attempt was made to successfully treat the 2 diseases without sterilizing the calves to retain the co-infectious immune state.

TABLE I: Results

	GROUP I				GROUP II				GROUP III				GROUP IV			
Day	PCV	Am%	Bab%	wt/kg	PCV	Am%	Bab%	wt/kg	PCV	Am%	Bab%	wt/kg	PCV	Am%	Bab%	wt/kg
-56	34.8	0.00	0.00	-----	35.8	0.00	0.00	-----	37.3	0.00	0.00	-----	34.5	0.00	0.00	-----
-42	34.5	0.00	0.00	-----	34.5	0.00	0.00	-----	37.3	0.00	0.00	-----	38.5	0.00	0.00	-----
-28	32.5	0.00	0.00	-----	33.3	0.00	0.00	-----	36.5	0.00	0.00	-----	37.0	0.00	0.00	-----
-16	All Groups inoculated SQ with 5cc blood containing 12% Anaplasma marginale.															
-11	26.5	0.00	0.00	93.7	29.6	0.00	0.00	82.5	28.8	0.00	0.00	92.0	31.5	0.00	0.00	109.5
-7	24.8	0.00	0.00	94.2	28.2	0.00	0.00	84.8	27.8	0.01	0.00	98.0	28.5	0.01	0.00	110.0
-6	22.2	0.41	0.00	-----	26.5	0.38	0.00	-----	24.0	1.03	0.00	-----	23.5	1.45	0.00	-----
-5	All Groups inoculated SQ with 5cc blood containing 10% B. bigemina and B. argentina.															
-4	20.0	0.48	0.00	85.8	24.5	0.60	0.00	78.8	22.8	1.40	0.00	88.7	21.0	2.20	0.00	99.5
-3	19.5	1.08	0.00	-----	23.5	1.18	0.00	-----	20.8	1.56	0.00	-----	19.0	2.40	0.00	-----
-2	17.5	1.60	0.00	-----	22.8	1.48	0.00	-----	18.1	2.00	0.00	-----	18.8	2.70	0.00	-----
-1	16.5	1.76	0.00	-----	18.3*	2.74	0.00	-----	16.5	3.47	0.01	-----	16.5	3.75	0.01	-----
0	14.2	2.13	0.06	97.3	14.8	3.24	0.12	94.8	12.7	3.45	0.07	100.3	15.0	3.80	0.13	111.5
	1.5 mg/kg SQ 4A65				2.0mg/kg SQ 4A65				2.5mg/kg SQ 4A65				Controls			
1	13.5	3.10	0.01	-----	14.8	1.98	0.00	-----	12.5	1.90	0.00	-----	14.0	2.00	0.28	-----
2	13.2	1.55	0.01	-----	13.0	1.12	0.00	-----	12.3	1.23	0.00	-----	13.0	2.60	0.85	-----
3	13.0	1.50	0.01	100.2	14.4	0.28	0.00	94.2	12.2	0.27	0.00	97.2	11.0	2.85	2.15	112.5
4	13.2	0.80	0.00	-----	16.2	0.08	0.00	-----	13.3	0.11	0.00	-----	10.0*	2.90	1.80	-----
5	14.2	0.66	0.00	-----	17.2	0.02	0.00	-----	14.0	0.04	0.00	-----	11.0	2.20	2.00	-----
6	14.5	0.63	0.00	-----	17.8	0.02	0.00	-----	14.3	0.03	0.00	-----	12.0	2.50	2.00	-----
7	14.2	0.98	0.00	101.0	17.8	0.03	0.00	95.4	15.0	0.03	0.00	96.0	17.0	1.50	0.10	108.0
8	14.5	0.15	0.00	-----	17.6	0.01	0.00	-----	15.0	0.01	0.00	-----	18.0	0.05	0.05	-----
9	16.2	0.30	0.00	-----	19.2	0.02	0.00	-----	17.2	0.02	0.00	-----	17.0	0.05	0.05	-----
10	17.4*	0.17	0.00	102.0	20.0	0.03	0.00	98.0	18.7	0.01	0.00	97.8	20.0	0.10	0.05	111.0
11	17.8	0.19	0.00	-----	18.8	0.05	0.00	-----	18.0	0.00	0.00	-----	19.0	0.15	0.20	-----
14	18.0	0.32	0.00	-----	21.0	0.01	0.00	-----	18.5	0.03	0.00	-----	17.0	0.05	0.00	-----
17	19.4	0.37	0.00	-----	21.2	0.08	0.00	-----	20.3	0.09	0.00	-----	15.0	0.05	0.00	-----
22	17.2	0.49	0.00	103.4	21.7*	0.23	0.00	98.3	18.8	0.22	0.00	101.5	17.0	0.00	0.00	120.0
24	17.6	0.34	0.00	104.6	21.5	0.35	0.00	92.3	18.5	0.08	0.00	104.0	17.0	0.20	0.00	111.0
28	17.6	0.57	0.00	109.2	18.3	1.08	0.00	93.0	18.8	0.15	0.00	105.8	17.0	0.00	0.00	117.0
31	16.6	0.56	0.00	-----	15.8	1.70	0.00	92.8	18.0	0.28	0.00	98.5	19.0	0.80	0.05	127.0
35	18.4	0.64	0.00	107.0	17.5	0.68	0.00	94.8	19.8	0.13	0.00	100.5	20.0	0.30	0.00	134.0
38	19.2	0.78	0.00	106.0	19.0	0.70	0.00	95.3	20.3	0.26	0.00	100.5	20.0	0.60	0.00	129.00
42	20.6	0.39	0.00	106.4	21.5	0.84	0.00	97.3	23.0*	0.08	0.00	106.2	22.0	0.40	0.05	129.0
45	22.0	0.26	0.00	108.8	21.3	1.20	0.00	100.8	22.0	0.21	0.00	109.4	24.0	0.70	0.00	128.0
49	22.8	0.21	0.00	112.2	22.8	1.19	0.00	106.3	23.6	0.21	0.00	112.4	23.0	1.00	0.00	135.0
52	23.0	0.23	0.00	117.2	21.8	0.91	0.00	107.0	24.2	0.21	0.00	116.4	20.0	1.20	0.00	138.0
56	19.8	0.21	0.00	117.0	22.7*	0.67	0.00	106.0	24.2	0.08	0.00	115.8	20.0	1.00	0.00	140.0
59	22.8	0.19	0.00	119.4	24.0	0.68	0.00	107.7	22.4	0.11	0.00	116.4	23.0	1.20	0.00	139.0
63	22.8	0.00	0.00	120.0	23.7	----	0.00	106.7	21.6	0.00	0.00	119.6	22.0	0.90	0.00	142.0
66	24.8	0.14	0.00	121.4	25.3	0.30	0.00	106.7	23.8	0.12	0.00	119.8	19.0	0.70	0.00	141.0
70	25.6	0.13	0.00	-----	26.7	0.30	0.00	107.0	23.4	0.08	0.00	123.4	25.0	0.60	0.00	137.0
73	25.6	0.13	0.00	125.6	25.7	0.18	0.00	108.3	22.2	0.08	0.00	125.0	18.0	0.50	0.00	140.00
77	25.4	0.00	0.00	123.2	26.7	----	0.00	109.3	23.4	----	0.00	127.0	18.0	----	0.00	142.0
80	25.4	----	0.00	125.6	26.3	----	0.00	112.3	23.2	----	0.00	129.6	20.0	----	0.00	143.0
81	All Groups challenged with 10 ml blood SQ containing 5% A. marginale.															
84	26.8	0.00	0.00	-----	26.7	0.00	0.00	-----	24.8	0.03	0.00	-----	23.0	0.40	0.00	-----
	All Groups challenged with 10 ml blood SQ containing 1.5% B. bigemina and B. argentina															
86	24.4	0.00	0.00	132.6	25.3	0.00	0.00	-----	22.6	0.00	----	125.2	22.0	0.00	0.00	130.0
88	24.8	0.00	0.12	-----	26.0	0.33	0.10	-----	25.4	0.00	0.08	-----	22.0	0.10	0.00	-----
91	16.0	0.00	0.56	127.4	17.7	0.20	1.52	116.7	20.4	0.20	1.10	121.8	24.0	0.60	0.00	142.0
94	15.6	0.00	0.40	-----	18.7	0.00	0.04	-----	15.2	0.00	0.04	-----	22.0	0.00	0.00	-----
98	18.2	0.01	0.00	-----	19.7	0.00	0.00	-----	17.0	0.00	0.00	-----	27.0	0.00	0.20	-----
101	18.0	0.01	0.00	-----	19.7	0.00	0.00	-----	17.5	0.00	0.00	-----	23.0	0.30	0.20	-----
105	18.4	0.00	0.01	-----	19.3	0.00	0.00	-----	19.8	0.00	0.00	-----	20.0	0.00	0.00	-----
108	20.6	0.00	0.04	-----	20.7	0.00	0.04	-----	18.3	0.02	0.07	-----	27.0	010	0.00	-----
112	21.8	0.00	0.00	-----	20.7	0.00	0.00	-----	19.8	0.00	0.00	-----	30.000.00		0.00	-----
115	21.4	0.02	0.00	-----	23.0	0.03	0.00	-----	19.8	0.06	0.00	-----	26.0	0.00	0.00	-----
119	23.0	0.00	0.00	130.6	22.0	0000	0.00	110.3	19.3	0.00	0.00	122.3	24.0	0.00	0.00	

* death of one calf

TABLE II

Day	Group I CF Ana	Group I CF Bab	Group II CF Ana	Group II CF Bab	Group III CF Ana	Group III CF Bab	Group IV CF Ana	Group IV CF Bab
-56	Neg		Neg		Neg		Neg	
-42	Neg		Neg		Neg		Neg	
-28	Neg		Neg		Neg		Neg	
-16	Neg		Neg		Neg		Neg	
-11	1:2		1:3		1:7		1:10	
-7	1:320		1:423		1:640		1:22	
-4	1:358		1:278		1:404		1:640	
-3	1:404		1:508		1:453		1:453	
-1	1:279		1:139*		1:320		1:320	
0	1:180		1:183		1:180		1:320	
1	1:254		1:320		1:359		1:453	
2	1:201		1:320		1:453		1:453	
3	1:485		1:485		1:404		1:453	
4	1:320		1:243		1:453		1:320*	
5	1:640		1:640		1:570		1:640	
6	1:640		1:485		1:640		1:640	
7	1:640		1:640		1:640		1:640	
8	1:211		1:243		1:320		1:320	
9	1:227		1:485		1:404		1:320	
10	1:640*		1:640		1:320		1:640	
11	1:557		1:279		1:404		1:640	
14	1:640		1:269		1:227		1:320	
17	1:557		1:243		1:180		1:160	
22	1:423		1:160*		1:127		1:160	
24	1:640		1:320		1:184		1:320	
28	1:580		1:320		1:160		1:80	
31	1:640		1:640		1:227		1:80	
35	1:580		1:380		1:190		1:80	
38	1:367		1:320		1:180		1:40	
42	1:184		1:227		1:121*		1:80	
45	1:121		1:227		1:106		1:80	
49	1:184		1:320		1:113		1:40	
52	1:168		1:269		1:135		1:40	
56	1:211		1:269*		1:81		1:80	
59	1:135		1:170		1:46		1:80	
63	1:168		1:160		1:67		1:80	
66	1:159		1:254		1:70		1:80	
70	1:139		1:254		1:92		1:160	
73	1:77		1:254		1:92		1:160	
77	1:75		1:404		1:160		1:320	
86	1:30		1:34		1:92		1:320	
88	1:90		1:234		1:135		1:320	
91	1:80		1:186		1:138		1:160	
94	1:160		1:254		1:105		1:160	
98	1:161		1:200		1:61		1:160	
101	1:211		1:160		1:113		1:320	
105	1:139		1:160		1:80*		1:320	
108	1:127		1:147		1:67		1:320	
115	1:80		1:127		1:80		1:160	
119	1:46		1:101		1:48		1:160	

*Death one calf

Discussion

All data represent the averages of the respective groups on a given day. Complement fixation data for anaplasmosis are completed, but babesiosis complement fixation data are not completed. Calculations of body temperatures are not completed at this time.

In Group I, calf 305 died on Day 10 PI with a PCV of 10% and a 1.3% parasitemia of *A. marginale* and 0.00% parasitemia of *Babesia spp.*

In Group II, calf 312 died on Day -1 PI with a PCV of 22% and a 1.0% parasitemia of *A. marginale* and 0.00% parasitemia of *Babesia spp.* Calf 309 of Group II died on Day 21 PI with a PCV of 12% and a 0.05% parasitemia of *A.marginale* and 0.00% parasitemia of *Babesia sp.* Calf 311, also of Group II, died on Day 56 PI with a PCV of 20% and a 2.3% parasitemia of *A. marginale* and 0.00% parasitemia of *Babesia sp.*

In Group III, calf 316 died on Day 42 PI with a PCV of 19% and 0.6% parasitemia of *A. marginale* and no detectable *Babesia* parasitemia. Calf 315 of Group III died on Day 105 PI and had a PCV of 12% and a 5% *Babesia bigemina* with no detectable *A. marginale* on Day 101 PI.

In Group IV, calf 323 died on Day 4 PI with a 10% PCV and 4% *A. marginale* and 3% *B. bigemina* and *B. argentina* parasitemias.

Compound 4A65 was very effective in the treatment of bovine babesiosis as dosages of 2.0 and 2.5 mg/kg completely eliminated the parasitemia of both B. bigemina and B. argentina within 24 hours, however, the Babesia parasitemia persisted 3 days PI at 1 mg/kg while in the controls, the Babesia parasitemia persisted for 11 days. In groups I, II and III, Babesia were never observed again until challenge, whereas in the control group IV, low Babesia parasitemias were observed on days 31 and 42 PI.

Compound 4A65 was effective in reducing the A. marginale parasitemia, average 2.3 days at 1.0, 2.0 and 2.5 mg/kg to reduce below 1.0% parasitemia versus the untreated controls with a persistent parasitemia of more than 1.0% for 7 days PI. The doseages of 2.0 and 2.5 mg/kg reduced the Anaplasma parasitemia to less than 0.1% within 3 and 4 days respectively while the 1.0 mg/kg doseage never reduced the parasitemia below 0.1%. It appeared that the recrudescence of the Anaplasma parasitemia was related to the 2.0 and 2.5 mg/kg doseages, not only was the recrudescence equal to or greater than the controls, but it occurred more rapidly (28 days PI vs. 49 days). All groups had a chronic persistent Anaplasma parasitemia and the 4 calves which died in groups I, II and III succumbed to acute exacerbations of chronic anaplasmosis.

Following challenge with 10 ml of 1.5% Babesia parasitemic

blood with a 32% PCV on day 84 PI, all groups exhibited B. bigemina and B. argentina within 17 days, however groups II and III at doseages 2.0 and 2.5 mg/kg had the highest parasitemias of 1.52% and 1.1% respectively on day 91 PI. The control group exhibited an extremely mild response of 0.02% parasitemia with a slight decrease in PCV. Groups I, II and III underwent a marked reduction in PCV to an average of 16.2%. It appeared that groups I, II and III were sterilized against babesiosis by the respective doseages 1.0, 2.0, and 2.5 mg/kg due to the reactions that they underwent, yet probably sterile immunity persisted since only one of the animals in group III died, and group I (1.0 mg/kg) underwent the least reaction and recovered the fastest, since in these calves the Babesia parasitemia was allowed to proliferate for 3 days PI to stimulate a greater degree of immunity. It also appeared that doseages up to 2.5 mg/kg of 4A65 were not chemoprophylactically effective up to day 84 PI.

All cattle were challenged with 10 ml of 5% Anaplasma parasitemic blood with a PCV of 24% following which the calves underwent a mild reaction as evidenced by the low parasitemias of 0.01% (group I), 0.33% (group II), 0.2% (group III) and 0.60% (group IV), all of the parasitemias became unevident within 2 days. Compound 4A65 did not sterilize against anaplasmosis and a co-infectious immune status developed in these calves.

Following the inoculation of anaplasmosis, the PCVs were reduced beginning day -11 and rapidly decreased until day -6, the following day -5, Babesia was inoculated and the PCVs were further rapidly decreased to a PCV of 15% or less in all groups on day 0. Following treatment the PCV of groups I, II, III, and IV (one calf died of anaplasmosis and babesiosis) gradually increased to approximately 20% on approximately day 14 PI,however the rate of recovery was not significantly different between treated and untreated groups. From day 22 PI through day 81 PI recrudescences occurred in groups II and IV on days 28 and 49 respectively which were accompanied by a decreased PCV. Following challenge the decrease in PCV was much more evident in the treated groups I, II and III than in the controls group IV due to babesiosis.

The calves of groups I, II, III and IV had an average weight gain per day from day -11 to day 80 PI of 350 gm, 327, 412 gm, and 368 gm respectively which are below expected weight gains. The weight gains and losses were directly related to the presence or abscence of parasitemias and PCVs produced by anaplasmosis and to a lesser extent to babesiosis since babesiosis was eliminated by treatment on day 0 in groups I, II and III. The calves in all groups were in a fair to good state of health at the beginning of the experiment. From day -16 to day 119 the calves were

in a poor state of health, failing to gain weight, stunted, no gloss to the hair coat etc. due to the chronic anaplasmosis.

Complement fixation data for anaplasmosis from day -56 to day 0 were very similar in all groups in that the first positive low titers were observed on day -11, following which, the titers rapidly increased to a maximum on days -3 and -4. The CF titers decreased slightly on days -1 and 0, only to rise again on days 2 through 7 PI. The relationship between the percentage of parasitemia and PCV was direct and somewhat delayed. From day 7 PI until day 77 PI, the anaplasmosis CF titers varied directly as did the recrudescent parasitemias which appeared to be related to the doseage of 4A65, particularly at 2.0 mg/kg. The titers of groups I and III were reduced by day 77, but group II (2.0 mg/kg) and group IV (Controls), had relatively high titers of 1:404 and 1:320 respectively which were probably due to the recrudescences. Following challenge on day 81 PI, the general trend of the CF titers was a moderate increase on approximately day 98 PI which then began a moderate decrease on day 105 PI in groups I, II and III, but group IV retained a higher increasing titer until day 108 PI.

Perhaps, two applications of 4A65 at 2.5 mg/kg SQ at a one day interval would be indicated to control anaplasmosis, but babesiosis sterilization would almost completely be certain to

occur and the cattle would be susceptible again. However, since only calf died in group I and 2 calves died in groups II and III, a lower level of treatment may be indicated, particularly because much less lacrimation and salivation occurred in group I as compared to groups II and III.

Summary

Compound 4A65 was used to treat a simultaneous infection of bovine babesiosis and anaplasmosis at 1.0, 2.0 and 2.5 mg/kg IM doseages. The compound was rapidly effective in the control of the Babesia parasitemia, but apparently sterilized the calves at the 3 above mentioned doseages, however, the calves probably retained a degree of sterile immunity in that all but one calf recovered following challenge.

Compound 4A65 at the 1.0, 2.0 and 2.5 mg/kg doseages, was moderately effective in controlling the Anaplasma parasitemias, but had no effect and even appeared to augment the appearance of recrudescent Anaplasma parasitemias in the chronic phases of the disease. The anaplasmosis complement fixation,weight gain and hematological data support these concepts.

V. A. 11. Simultaneous Sterilization of Anaplasmosis and Babesiosis Carrier Calves with 4A65.

Introduction

Because cattle originating from anaplasmosis and babesiosis enzootic zones are usually carriers of both diseases, and future control programs may require eradication in some countries, it becomes evident that a single drug effective against both diseases would have great value. The following experiment was executed to ascertain the property of 4A65 to sterilize anaplasmosis and babesiosis carrier cattle.

Materials and Methods

Drug	4A65 di HCl dissolved in distilled H_2O at 5%
Animals	7-month old Holstein-Friesian calves known to be anaplasmosis and babesiosis carrier. 1 Splenectomized calf. These animals were from Experiment V. A. 10.
Number	2 groups of 6 and 1 splenectomized calf.
Doseage	Group I -2.5 mg/kg I.M. Group II -2.5 mg/kg I. M.
Frequency	Group I -Every other day for 5 days. Group II -Every other day for 10 days.
Period	100 days.

Body weight	Days -3,0, 4, 36, 60, 74.
Hematology & Serology	Samples were taken 3 times weekly until day 18PI for PCV, parasitemia and complement fixation response, and thereafter twice weekly.
Calf Inoculation	- 63 days after the last treatment in Group II, pooled blood samples from each group will be inoculated into 2 splenectomized calves to check for infectivity or sterilization as the case may be.
Explanation	To determine the effectiveness of 4A65 to sterilize carriers of both diseases simultaneously.

Results

Fifty ml. of blood from each of 6 calves in group I was collected in EDTA di Na (1.2 mg/l) and inoculated subcutaneously into another splenectomized calf which is being bleed daily for 2 weeks and thereafter weekly for 60 days. At seven days PI in the splenectomized calf, no evidence had been found of anaplasmosis nor babesiosis.

Discussion

All data represent the averages of the respective groups on a given day. In group II, 5 calves died, one of which died due to foreign body pneumonia. The other 4 calves died with no

RESULTS: TABLE I

	Group I			CF		Group II			CF	
Day	PCV	Am%	Bab%	Ana	wt/kg	PCV	Am%	Bab%	Ana	wt/kg
-10	22.2	Neg	Neg	---	-----	24.3	0.18	0.01	---	-----
-7	23.3	0.08	Neg	1:19	-----	24.7	0.02	0.02	1:50	-----
-3	24.0	Neg	Neg	1:71	130.0	23.0	0.17	0.02	1:40	149.3
0	23.5	0.18	0.13	1:26	131.6	23.0	0.42	0.02	1:42	152.4
	2.5 mg/kg S.Q.					2.5 mg/kg S.Q.				
2	----	----	----	---	-----	----	----	----	---	-----
	2.5 mg/kg S. Q.					2.5 mg/kg S. Q.				
4	27.8	Neg	Neg	1:24	127.8	26.5	Neg	Neg	1:64	138.7
	2.5 mg/kg S. Q.					2.5 mg/kg S. Q.				
6	27.8	Neg	Neg		-----	25.7	Neg	Neg		-----
	2.5 mg/kg S. Q.					2.5 mg/kg S. Q.				
8	30.5	Neg	Neg		-----	28.5	Neg	Neg		-----
	2.5 mg/kg S. Q.					2.5 mg/kg S. Q.				
10	----	----	----		-----	----	----	----		-----
						2.5 mg/kg S. Q.				
12	32.5	Neg	Neg		-----	29.0	Neg	Neg		-----
						2.5 mg/kg S. Q.				
14	34.1	Neg	Neg		-----	29.7	Neg	Neg		-----
						2.5 mg/kg S. Q.				
16	----	----	----		-----	----	----	----		-----
						2.5 mg/kg S. Q.				
18	34.8	Neg	Neg		-----	33.0***	Neg	Neg		-----
						2.5 mg/kg S. Q.				
25	34.0	Neg	Neg		-----	34.0	Neg	Neg		-----
34	37.0	Neg	Neg		-----	36.3*	Neg	Neg		-----
36	36.8	Neg	Neg		143.3	36.5	Neg	Neg		160.0
39	34.5	Neg	Neg		-----	34.5	Neg	Neg		-----
46	34.3	Neg	Neg		-----	33.0	Neg	Neg		-----
50	33.2	Neg	Neg		-----	30.0	Neg	Neg		-----
53	31.0	Neg	Neg		-----	28.0	Neg	Neg		-----
60	33.8	Neg	Neg		153.2	29.0	Neg	Neg		179.5
67	32.7	Neg	Neg		-----	32.0*	Neg	Neg		-----
74	33.9	Neg	Neg		157.7	32.0	Neg	Neg		168.0
81	34.2	Neg	Neg		-----	31.0	Neg	Neg		-----

* Death one calf

detectable A. marginale nor Babesia spp. parasitemias and PCVs of 32% and 38% respectively. Necropsies of these 3 calves revealed no characteristic lesions, except hemorrhage, hydrothorax and hydroperitoneum, and it was postulated that they may have died due to intoxication.

Because the calves were selected from experiment number V. A. 10. and V. A. 13. and were challenged on day -41 with a 10 ml SQ inoculum of 5% A. marginale, parasitemia with a 24% PCV and on day -38 with a 10 ml SQ inoculum of 1.5% B. bigemina and B. argentina parasitemia with a 32% PCV it can be established that the calves were carriers of anaplasmosis and babesiosis. The carrier state of these calves was also evidenced by Anaplasma CF titers, parasitemias, low PCV due to both diseases on day 0. The calves were in a very poor state of health at the beginning of the experiment, having a poor hair coat, being stunted and "poor doers".

4A65 Had an immediate effect upon the parasitemias of both diseases, causing the low parasitemias to disappear within 2 days, never to be observed again.

The PCV of group I increased rapidly from a PCV of 23.5% on day 0 to 27.8% on day 4 PI and 30.5% on day 8 PI and from day 8 PI on, the PCV continued to increase up to a maximum PCV of 37% on day 34 PI, following which a slight unexplanable reduction

occurred. The PCV of group II followed a similar course, but with a much more gradual recovery than group I.

Weight losses occurred during treatment, but following treatment the weight gains increased rapidly in group I. The general health of the calves in group I improved drastically after treatment. No evaluation of weight gains could be made in group II, because 5 of 6 calves died due to an apparent intoxication. Upon inoculation of the 4A65 compound each time lacrimation and salivation occurred in all calves within 15 minutes and lasted 30 minutes.

Summary

Compound 4A65 eliminated Anaplasma and Babesia demonstrable parasitemias within 2 days, and indirectly caused a rapid elevation of PCV, however 2.5 mg/kg SQ every other day for a total of 10 injections was fatal in 5 of 6 calves, whereas 2.5 mg/kg SQ 5 times every other day was not fatal. Sterilization data is not complete at this time.

V. A. 12. A Study of the Use of An Integrated Control Program for Hemo, Endo and Ectoparasites of Cattle on the North Coast of Colombia

Introduction

Production losses in cattle on the Northern Atlantic Coast of Colombia due to infection or infestation, as the case may be, of the combination of hemoparasites, endoparasites and ectoparasites are apparently quite high and even restrictive in many zones of this region of Colombia. Previous studies have disclosed that anaplasmosis, babesiosis, trypanosomiasis, helminthiasis, acariasis and other insects are parasitic menaces causing, as a combination, high production losses. The control systems which the cattleman are now using are not optimally effective in reducing these losses, therefore, a better,modernized, economical control system should be developed.

The experimental control systems, herein described are designed to reduce these losses to a minimum with a reasonable investment of capital; for this reason, three control systems are described, each one is successively less intricate and costly, and are compared with an non-treated group. Should any of the 3 control systems prove economically acceptable, they would be, recommended for use on a national scale. Apart from the above mentioned objectives, a better insight as to age-incidence-prevalence of these diseases can

be accomplished for future application.

History of Project

The original proposition to execute a hemoparasite control program with existing information was strongly emphasized in the February, 1970 annual review meeting at Bogotá by the members of the reviewing committee. Beginning in April, 1970, a basic hemoparasite control program was designed, which indicated that addition experimentation was necessary, particularly as related to the dosages used in premunition against anaplasmosis and babesiosis. (VA8, VA9, VA13). Furthermore, the project was altered to include endo and ectoparasites at the request of ICA to make a complete parasite control "package". The project was then rewritten and submitted to the ICA and CIAT administrations for supportive consideration. The project was considered worthy and then underwent several revisions until it was ratified in December, 1970 by CIAT, ICA and TAMU.

During January, all of the necessary equipment, provisions, calves, etc. were accumulated and on Jan. 28, 1971, all of the paraphanelia was flown to Montería and the joint TAMU-ICA-CIAT project was begun on Feb. 5, 1971 with 23 cows and calves and now as of April 29, 1971, wer have 67 calves. The new laboratory does not have reliable electrical energy nor water, making the project

extremely difficult to execute.

Materials and Methods

Site — Turipaná ICA experimental Farm located at Montería, Córdoba, 20 m above sea level and the new Laboratorio de Investigaciones Médicas Veterinarias.

Group I

Optimal Control System of Hemo, Endo, and Ectoparasites

Animals	Newborn calves of Costeño con cuernos and Sanmartinero cows.
Number	20 calves
Drugs	Ganaseg (Squibb)
	Terramycin (Pfizer)
	356-C-61 (Burroughs-Wellcome)
	Dursban (Dow)
	Toxaphene (Cooper)
	Bovizole (Merck)
	Ripercol (Cynamid)
Dosage & Route	Ganaseg -1.0 mg/kg IM
	Terramycin -12 mg/kg IV
	356-C-61 -5mg/kg IV
	Dursban -1:600 topical
	Toxaphene -1:500 topical

	Bovizole -110 mg/kg oral
	Ripercol -80 mg/kg oral
Frequency	Ganaseg -Once 10 or 11 days PI
	Terramycin and 356-C-61 in saline- Once at 21 PI
	Dursban or Toxaphene -Each 10 days until 60 days are completed, thereafter each 17 days. Also the insecticides are alternated each 90 days.
	Bovizole or Ripercol -Each 34 days, alternating the anthelminthics, each 34 days.
Premunition	Day 0 -5 cc of blood containing Turipaná isolates of A. marginale, B. bigemina and B. argentina SQ
	Day 9 -Treatment against babesiosis with Ganaseg
	Day 21 -Treatment against anaplasmosis with oxytetracycline and 356-C-61 in saline.
Duration	-12 months.

Group II

Intermediate Control System of Hemo, Endo and Ectoparasites

Animals	Same as Group I
Number	20 calves
Drugs	4A65 (Burroughs-Wellcome)
	Dursban (Dow)
	Toxaphene (Cooper)
	Bovizole (Merck)
	Phenothiazine (Cooper)

Dosage & Route	4A65 -2.5 mg/kg SQ
	Dursban -1:600 topical
	Toxaphene -1:500 topical
	Bovizole -110 mg/kg Oral
	Phenothiazine -25 mg/kg Oral
Frequency	4A65 -Day and Day 45 PI
	Dursban or Toxaphene -Each 17 days, alternating the insecticides each 90 days
	Bovizole or Phenothiazine -Each 34 days, alternating the anthelminthic each 34 days
Duration	12 months

Group III

Minimal Control System of Endo and Ectoparasites

Animals	Same as group I
Number	20 calves
Drugs	Dursban (Dow)
	Toxaphene (Cooper)
	Bovizole (Merck)
	Phenothiazine (Cooper)
Dosis & Route	Dursban -1:600 Topical
	Toxaphene -1:500 Topical
	Bovizole -110 mg/kg Oral
	Phenothiazine -25 mg/kg Oral

Frequency	Dursban or Toxaphene -Each 17 days, alternating the insecticides every 90 days Bovizole or Phenothiazine -Each 34 days, alternating the anthelminthics every 28 days.
Duration	12 months

Group IV

Control

Animals	Same as Group I
Number	20 calves
Drugs	None
Dosis	None
Frequency	None
Duration	12 months

Parameters and Other Treatments

Packed cell volumes, blood smears, wet mounts, and complement fixation for anaplasmosis and babesiosis will be determined weekly until the calves are 4 months old, and thereafter every 17 days. Body weights will be determined every 34 days. Tick collection from 5 calves in each group will be performed every 17 days. Helminth egg and larvae counts will be done every 34 days. Temperatures will be taken during the premunition in Group I and when indicated in the other groups.

The mother cows of all the calves in the experiment were bled for parasitemias, PCV, wet mount and CF determinations. All calves were new-born, i.e., less than 10 days old when the experiment started. Day 0 is considered to be the day when the premunition procedure in Group I, the application of 4A65 in Group II and anthelminthics in Group III were started. An attempt was made to begin all treatments when the calves were 25 to 35 days of age. All cattle were routinely vaccinated as indicated against blackleg, hemorrhagic septicemia, malignant edema, and Foot and Mouth disease. All Groups were treated on day 73 and 87 against Dictyocaulus viviparus with Franozan (Cooper) and Luvorem (Squibb).

Discussion

It must be recognized that the experiment was begun 72 days hence and that all the results must be regarded as preliminary since 10 months of data is still lacking. All 80 calves have not yet been procured, but should be so done within 2 weeks, because the project must wait for the calves to be born.

In regard to the hemoparasitic control program, the results to date appear promising in Groups I and II. The premunition program in Group I was executed without a critical decrease in PCV (low of 21%) and both parasitemias were controlled by the drugs and dosages applied, however an Anaplasma parasitemia did

PRELIMINARY RESULTS

TABLE I: Percentage Packed Cell Volume.

DAY	GROUP I	GROUP II	GROUP III	GROUP IV
0	33.4	34.5	33.7	34.4
	5ml. S.Q. B. arg. .05%	4A65-2.5mg/Kg 5q		
	B. big. 0.65%			
	A. marg. 1.1%			
7	35.5	----	----	----
10	28.8	37.0	35.3	36.2
	Ganaseg 1.5mg/kg.			
12	25.6	----	31.2	33.1
18	29.0	----	----	----
20	30.3	36.3	33.0	34.8
21	Oxytetracycline 12mg/Kg.			
	356-C-61- 5mg/Kg.			
22	28.8	----	----	----
28	31.5	36.4	32.4	30.6
37	30.6	30.0	34.4	28.4
44	34.5	----	----	----
45	----	4A65-2.5mg/Kg.	----	----
46	31.8	41.0	----	----
54	33.0	35.8	33.7	30.2
72	32.9	36.0	33.8	30.0

TABLE II: Anaplasma and Babesia Parasitemias.

DAY	GROUP I		GROUP II		GROUP III		GROUP IV	
	An.	Bab.	An.	Bab.	An.	Bab.	An.	Bab.
0	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
7	neg.	+	---	---	---	---	---	---
10	neg.	+	+	neg.	neg.	neg.	neg.	neg.
12	neg.	+	---	---	+	neg.	+	neg.
18	neg.	neg.	---	---	---	---	---	---
20	+	neg.	neg.	neg.	neg.	neg.	neg.	neg.
22	+	neg.	---	---	---	---	---	---
28	+	neg.	+	neg.	neg.	neg.	neg.	+
37	neg.	neg.	---	---	neg.	neg.	+	neg.
44	neg.	neg.	+	neg.	---	---	---	---
46	+	neg.	+	neg.	---	---	---	---
54	+	neg.	+	neg.	neg.	neg.	+	neg.
72	+	neg.	+	neg.	neg.	neg.	+	neg.

TABLE III: Amaplasmosis CF Data

DAY	GROUPS I	GROUP II	GROUP III	GROUP IV
0	neg. 5ml. S.Q. B. arg. .05% B. big. 0.65% A. marg. 1.1%	1/190 4A65-2.5mg/Kg.	neg.	neg.
7	1/10	---	---	---
10	1/9 Ganaseg 1.5mg/kg	1/28	neg.	neg.
12	1/80	---	neg.	neg.
18	1/169	---	---	---
20	1/453	1/80	neg.	neg.
21	Ozytetracycline 12mg/Kg. 356-C-61- 5mg/Kg.	---	---	---
22	1/455	---	---	---
28	1/453	1/9	neg.	neg.
37	1/80	1/21	neg.	1/10
44	1/80	---	---	---
45	---	4A65-2.5mg/Kg.	---	---
46	1/80	---	---	---
54	1/453	1/80	neg.	1/453
72	---	---	---	---

TABLE IV: Body Weights in Kilograms

DAY	GROUP I	GROUP II	GROUP III	GROUP IV
0	48.8	41.4	45.4	43.2
20	73.5	73.0	72.0	64.6
54	96.6	108.4	102.4	95.8

become evident on day 46 PI and was still evident on day 72 PI. The parasitemia could be a recrudescence due to failure of 356-C-61 and oxytetracycline to control the parasitemia or due to field challenge as is seen in Control Group IV. Nevertheless, the *Anaplasma* parasitemia, which began on day 46, has not yet caused a significant decrease in the PCV. Group I has the highest and most persistent *Anaplasma* CF response as compared to the other Groups. Group II inoculated twice with 4A65 has the highest PCV, in spite of having the highest incidence of *Anaplasma* parasitemias. This observation suggests that 4A65 may in some manner augment the proliferation of *A. marginale*. Group II has the second highest titers for *Anaplasma* complement fixation antibodies and the highest PCV of all Groups. Group III has the lowest incidence of anaplasmosis, no CF reaction, and the second highest PCV, making it difficult to explain why this Group has done so well without hemoparasitic control. Group IV has the lowest PCV and appears to be undergoing anaplasmosis field challenge, as the incidence of anaplasmosis is rising as well as anaplasmosis CF titers. Group IV is the only group which has exhibited babesiosis due to field challenge, as all other Groups are completely negative from day 18 to day 72 PI. Obviously, no definite conclusions can be extracted, but it appears that the control Group IV is undergoing more stress than any of the other groups.

The endoparasites appear to be causing the most severe problems, particularly Dictyocaulus viviparus, followed by Eimeria spp.and strongyloides in order of decreasing severity. All Groups were treated against D. viviparus to prevent mortality. Following treatment against D. viviparus, calves usually develop solid immunity against the larvae.

Ectoparasites has been a relatively minor problem, with primarily Boophilus microplus being the major ectoparasite.

The crucial field challenge period has not yet begun since the rainy season will be beginning in June at which time the level of helminths, ticks, and therefore hemoparasites will increase drastically.

V. A. 13. Evaluation of Different Dosages of Ganaseg, Compound 4A65 and Oxytetracycline for the Premunition of Cattle Against Anaplasmosis and Babesiosis in Turipaná

Introduction

Although premunition has been practiced as an inexact method of protecting cattle against hemotropic diseases for many years, there still remains room for refinement of the technique, particularly as related to the dosage of the infecting organisms and the drug utilized for suppression of the clinical signs and the potential anemia. The drug dosage must be carefully regulated so as not to sterilize the cattle, thus making them susceptible to reinfection; for these reasons, the following experiment was designed to evaluate various dosages of various drugs to be used in the premunition experiment to be executed on the Turipaná ICA experiment station.

Materials and Methods

Animals Nineteen 5 month-old Holstein-Friesian calves known to be free of anaplasmosis and babesiosis as proven by negative complement fixation titers, blood smears and pooled inoculation into a splenectomized calf. 3 other splenectomized calves were used as a source

of inoculum for infection and challenge.

Number	Group I -4 calves
	Group II -4 calves
	Group III -4 calves
	Group IV -4 calves
	Group V -3 calves
Drugs	Ganaseg(R) Squibb-Mathieson
	4A65(R) Burroughs-Wellcome
	Oxytetracycline(R) McKesson
Dosage	Group I -1.5 mg/kg IM- Ganaseg; Oxytetracycline 12 mg/kg IV.
	Group II -2.0 mg/kg IM- Ganaseg; Oxytetracycline 12 mg/kg IV
	Group III -2.5 mg/kg IM- Ganaseg; Oxytetracycline 12 mg/kg IV.
	Group IV- 0.3 mg/kg SQ- 4A65; Oxytetracycline 12 mg/kg IV.
	Group V- No Treatment.
Frequency	Group I -Day 8 PI, Ganaseg; Day 21 PI, Oxytetracycline
	Group II -Day 8 PI, Ganaseg; Day 21 PI, Oxytetracycline.
	Group III -Day 8 PI, Ganaseg; Day 21 PI, Oxytetracycline.
	Group IV -Day 8 PI, 4A65; Day 21 PI, Oxytetracycline.

Group V - No treatment

Infection & Challenge	Day 0 -All cattle were given 5 ml subcutaneously of blood containing 10% Babesia bigemina and Babesia argentina and 1% Anaplasma marginale (Turipaná isolate).
	Day 141 -All cattle were given 10 ml subcutaneously of blood containing 5% Anaplasma marginale (Turipaná isolate).
	Day 144 -All cattle were given 10 ml subcutaneously of blood containing 1.5% Babesia bigemina and Babesia argentina (Turipaná isolate).
Duration	231 days
Parameters	Preinoculation blood samples were collected on days -51, -37, -22, -9 and after infection, twice weekly for PCV, parasitemia and complement fixation determination. Body weights were determined twice monthly.

Results

The data represented in Tables I, II, III and IV are given as the mean of the observations of the particular group on a given day.

TABLE I: Parasitemias

Day	GROUP I		GROUP II		GROUP III		GROUP IV		GROUP V	
	% Am	%Bab	% Am	%Bab	% Am	%Bab	% Am	%Bab	% Am	%Bab
-51	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
-37	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
-22	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
-9	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
0	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	All Groups received 5 ml SQ of 10% B. bigemina and B. argentina and 1% A. marginale.									
5	Neg	0.03	Neg	0.18	Neg	0.30	Neg	0.05	Neg	0.47
8	Neg	0.09	Neg	0.29	Neg	0.63	Neg	0.25	Neg	0.62
	Ganaseg 1.5 mg/kg I.M.		Ganaseg 2.0 mg/kg I.M.		Ganaseg 2.5 mg/kg I.M.		4A65- 0.3 mg/kg S.Q.		NT	
11	0.11	0.01	0.14	Neg	Neg	0.05	0.05	Neg	0.37	0.18
14	0.55	Neg	0.15	Neg	0.08	Neg	0.06*	Neg*	0.17	0.53
18	0.40	Neg	0.03	Neg	0.80	Neg	0.06	Neg	0.45*	0.25*
21	0.97	Neg	0.10	Neg	1.08*	Neg*	0.13	Neg	1.00	Neg
	Oxytetracycline 12 mg/kg I. V.								NT	
25	0.23	Neg	Neg	Neg	1.35	Neg	Neg*	Neg*	0.90	Neg
28	0.20	Neg	0.01	Neg	2.02	Neg	0.05	Neg	0.80*	Neg*
32	Neg	Neg	0.01	Neg	Neg*	Neg*	0.10	Neg	0.90	0.10
35	Neg	Neg	0.08	Neg	0.05	Neg	0.55	Neg	0.40	Neg
39	0.14	Neg	0.05	Neg	0.03	Neg	0.05	Neg	---*	---*
42	0.01	Neg	0.03	Neg	0.03	Neg	0.03	Neg	----	----
46	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	----	----
49	Neg	Neg	0.25	Neg	Neg	Neg	0.03	Neg	----	----
53	Neg	Neg	0.50	Neg	Neg	Neg	Neg	Neg	----	----
56	0.42	Neg	0.38	Neg	Neg	Neg	0.08	Neg	----	----
60	0.78	Neg	0.48	Neg	Neg	Neg	0.60	Neg	----	----
63	0.89	Neg	0.29	Neg	Neg	Neg	0.80	Neg	----	----
67	0.43	Neg	0.10*	Neg*	0.45	Neg	0.25	Neg	----	----
74	0.20	Neg	0.05	Neg	0.45	Neg	0.20	Neg	----	----
77	0.06	Neg	0.08	Neg	0.23	Neg	0.15	Neg	----	----
82	0.08	Neg	0.05	Neg	0.40	Neg	0.10	Neg	----	----
84	0.08	Neg	0.03	Neg	0.28	Neg	0.45	Neg	----	----
88	0.11	Neg	Neg*	Neg*	Neg	Neg	0.60	Neg	----	----
91	0.49	Neg	0.05	Neg	0.78	Neg	0.90	Neg	----	----
95	0.53	Neg	0.27	Neg	1.60	Neg	0.80*	Neg*	----	----
98	0.16	Neg	0.25	Neg	1.10	Neg	0.40	Neg	----	----

(Continued)

TABLE I: Parasitemias (Continued)

Day	GROUP I		GROUP II		GROUP III		GROUP IV		GROUP V	
	% Am	%Bab	% Am	%Bab	% Am	%Bab	% Am	%Bab	% Am	%Bab
102	0.02	Neg	0.08	Neg	0.80	Neg	0.30	Neg	----	----
105	0.01	Neg	Neg	Neg	0.50	Neg	0.30	Neg	----	----
109	0.01	Neg	Neg	Neg	0.55	Neg	0.30	Neg	----	----
112	0.02	Neg	Neg	Neg	0.80	Neg	0.05	Neg	----	----
116	0.01	Neg	0.03	Neg	0.80	Neg	0.80	Neg	-----	----
119	0.02	Neg	0.03	Neg	0.95	Neg	0.60	Neg	----	----
123	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	----	----
126	0.01	Neg	Neg	Neg	Neg	Neg	Neg	Neg	----	----
130	Neg	Neg	Neg	Neg	0.03	Neg	Neg	Neg	----	----
133	0.01	Neg	0.06	Neg	0.20*	Neg*	Neg	Neg	----	----
137	Neg	Neg	0.10	Neg	0.20	Neg	Neg	Neg	-----	-----
140	Neg	Neg	0.03	Neg	Neg	Neg	Neg	Neg	----	----
141	10 ml S.Q. 5% *A. marginale* with 24% PCV									
144	Neg	0.03	Neg	Neg	Neg	Neg	Neg	Neg	----	----
	10 ml S. Q. 1.5% *B. bigemina* and *B. argentina* with 32% PCV									
146	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	----	----
148	Neg	0.03	Neg	0.50	Neg	0.50	0.01	0.10	----	----
151	0.20	0.60	Neg	0.35	Neg	0.20	0.30	0.01	----	----
154	0.13	0.01	0.05	Neg	Neg	Neg	Neg	Neg	----	----
158	0.08	Neg	Neg	Neg	Neg	Neg	Neg	Neg	----	----
161	0.05	Neg	0.10	Neg	Neg	Neg	Neg	Neg	----	----
165	0.18	Neg	0.05	Neg	0.05	Neg	Neg	Neg	----	----
169	0.23	Neg	Neg	Neg	Neg	Neg	Neg	Neg	----	-----
173	0.53	0.01	0.30	Neg	0.10	Neg	Neg	Neg	----	----
176	0.06	Neg	Neg	Neg	Neg	0.05	Neg	Neg	----	----
180	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	----	----

* Death one calf

TABLE II: Packed Cell Volume

Day	Group I PCV	Group II PCV	Group III PCV	Group IV PCV	Group V PCV
-51	34.5	34.5	30.8	31.8	34.7
-37	35.3	32.3	31.5	35.3	34.0
-22	33.5	33.2	32.8	33.0	33.7
-9	34.8	35.0	33.0	36.3	35.0
0	All Groups received 5 ml SQ of 10% B. bigemina and B. argentina and 1% A. marginale with 5% PCV				
5	35.5	34.3	28.8	35.8	32.0
8	25.3	23.3	22.8	26.5	23.8
	Ganaseg 1.5 mg/kg I.M.	Ganaseg 2.0 mg/kg I.M.	Ganaseg 2.5 mg/kg I.M.	4A65-0.3 mg/kg I.M.	NT
11	29.3	27.8	22.8	25.0	21.7
14	27.5	23.8	22.8	26.3*	18.3
18	26.0	22.3	20.3*	23.0	19.5*
21	24.8	21.0	20.7	19.7	16.0
		Oxytetracycline 12 mg/kg I.V.			NT
25	24.0	24.0	16.3	22.5*	13.0
28	23.0	22.0	14.7	20.0	15.0*
32	23.5	21.5	22.0*	20.0	15.0
35	23.5	22.0	21.5	20.5	13.0
39	24.0	25.8	23.5	23.5	----*
42	24.0	25.2	22.5	.9.5	----
46	24.0	21.5	21.5	20.0	----
49	23.7	22.5	23.0	21.5	----
53	23.3	25.5	22.0	22.5	----
56	22.0	19.3	20.5	20.5	----
60	20.5	17.5	22.0	21.0	----
63	17.8	16.8	21.5	20.0	----
67	19.0	19.7*	21.5	19.5	----
74	19.8	19.3	16.5	16.5	----
77	20.0	19.3	16.5	18.0	----
82	20.3	17.0	15.0	18.5	----
84	18.5	17.3	14.5	18.0	----
88	20.3	20.0*	14.5	19.0	----
91	19.0	21.0	15.5	13.0	----
95	19.8	21.5	16.5	17.0*	----
98	20.3	21.5	17.0	19.0	----

(Continued)

TABLE II: Packed Cell Volume (Continued)

Day	Group I PCV	Group II PCV	Group III PCV	Group IV PCV	Group V PCV
102	22.0	23.0	19.0	21.0	----
105	22.0	22.0	19.5	23.0	----
109	23.8	23.5	20.5	24.0	----
112	24.3	23.5	20.5	25.0	----
116	24.3	22.5	23.0	25.0	----
119	24.3	24.5	20.0	20.0	----
123	23.3	24.0	19.5	23.0	----
126	25.5	25.5	20.5	24.0	----
130	27.0	23.5	15.0	25.0	----
133	26.0	27.0	22.0*	25.0	----
137	27.3	24.0	25.0	23.0	----
140	25.8	21.5	27.0	25.0	----
141	10 ml S. Q. 5% *Anaplasma marginale* with 24% PCV				
144	25.8	24.0	26.0	25.0	----
	10 ml S.Q. 1.5% *B. bigemina* and *B. argentina* with 32% PCV				
146	23.3	28.0	26.0	24.0	----
148	26.3	21.0	24.0	23.0	----
151	19.5	14.0	20.0	22.0	----
154	19.8	14.0	21.0	18.0	----
158	21.5	16.5	26.0	22.0	----
161	22.3	16.5	25.0	19.0	----
165	19.5	16.0	27.0	19.0	----
169	20.3	16.0	24.0	22.0	----
173	20.3	16.5	26.0	22.0	----
176	21.0	18.0	27.0	22.0	----
180	19.5	22.5	28.0	22.0	----

0 Death one calf

TABLE III: Anaplasmosis CF Titers

Day	Group I	Group II	Group III	Group IV	Group V
-51	Neg	Neg	Neg	Neg	Neg
-37	Neg	Neg	Neg	Neg	Neg
-22	Neg	Neg	Neg	Neg	Neg
-9	Neg	Neg	Neg	Neg	Neg
0	Neg	Neg	Neg	Neg	Neg
	All Groups received 5 ml S. Q. of 10% *B. bigemina* and *B. argentina* and 1% *A. marginale* with 5% PCV				
5	Neg	Neg	Neg	Neg	Neg
8	Neg	Neg	Neg	Neg	Neg
	Ganaseg 1.5 mg/kg I.M.	Ganaseg 2.0 mg/kg I.M.	Ganaseg 2.5 mg/kg I.M.	4A65 -0.3 mg/kg S.Q.	NT
14	1:2	1:8	1:10	1:2*	1:2
18	1:5	1:24	1:34*	1:5	1:3*
21	Oxytetracycline 12 mg/kg I.V.				NT
32	Neg	1:8	Neg	1:4*	1:160*
35	Neg	1:3	1:20	1:6	1:320*
39	Neg	Neg	1:4	1:20	----
42	Neg	1:2	Neg	1:13	----
46	1:2	1:3	Neg	1:6	----
49	1:20	1:9	1:3	1:9	----
53	1:76	1:9	1:10	1:9	----
56	1:11	1:32	1:14	1:23	----
60	1:11	1:21	1:20	1:178	----
63	1:25	1:27	1:14	1:113	----
74	1:15	1:15*	1:320	1:227	----
77	1:15	1:15	1:320	1:156	----
82	1:15	1:19	1:443	1:113	----
84	1:11	1:30	1:443	1:113	----
88	1:6	1:6*	1:443	1:320	----
91	1:11	1:4	1:441	1:18	----
95	1:10	Neg	1:320	Neg*	----
98	1:5	1:6	1:227	1:20	----

(Continued)

TABLE III: Anaplasmosis CF Titers (Continued)

Day	Group I	Group II	Group III	Group IV	Group V
102	1:5	1:6	1:160	1:20	----
105	1:4	Neg	1:227	1:40	----
109	1:5	1:4	1:113	----	----
112	1:2	1:4	1:160	1:20	----
116	1:7	1:3	1:28	1:10	----
119	1:2	1:4	1:13	Neg	----
123	Neg	1:3	1:160	Neg	-----
126	1:4	1:3	1:160	1:10	----
130	1:5	1:6	1:357	Neg	----
133	1:2	1:4	1:320*	Neg	----
137	Neg	1:2	1:160	Neg	----
141	10 ml S. Q. 5% *Anaplasma marginale* with 24% PCV				
144	1:2	Neg	Neg	Neg	----
	10 ml S. Q. 1.5% *B. bigemina* and *B. argentina* with 32% PCV				
146	1:4	Neg	1:80	Neg	----
148	1:13	1:20	1:80	1:10	----
151	1:4	1:40	1:80	1:20	----
154	1:4	1:57	1:80	1:20	----
158	1:6	1:20	1:80	1:20	----
161	1:38	1:13	1:40	1:40	----
165	1:67	1:40	1:20	1:20	----
169	1:57	1:20	1:40	1:10	----
176	1:23	1:57	1:80	1:10	----
180	1:19	1:28	1:160	1:40	----

* Death one calf

TABLE IV: Body Weights in Kilograms

Day	Group I	Group II	Group III	Group IV	Group V
0	All Groups received 5 ml blood S. Q. with a 10% B. bigemina and B. argentina and 1% A. marginale with 5% PCV.				
8	Ganaseg 1.5 mg/kg I.M. 95.3	Ganaseg 2.0 mg/kg I.M. 98.0	Ganaseg 2.5 mg/kg I.M. 88.8	4A65 -0.3 mg/kg S.Q. 82.8	NT 90.7
21	Oxytetracycline 12 mg/kg I.V. 97.3	99.5	91.0*	90.3*	NT 97.0*
42	99.8	97.8	87.0*	93.0*	----**
56	100.0	89.3	78.0	88.0	----
67	105.5	104.3	84.0	97.0	----
82	109.5	114.7*	105.0	98.5	----
98	104.5	104.5*	104.5	80.0*	----
112	110.0	114.5	106.0	82.0	----
126	116.5	116.0	110.0	80.0	----
140	126.8	123.5	120.0*	84.0	----
141	10 ml S.Q. 5% Anaplasma marginale with 24% PCV				
144	10 ml S. Q. 1.5% B. bigemina and B. argentina with 32% PCV				
146	128.0	130.5	118.0	85.0	----
180	133.5	138.5	124.0	93.0	----

* Death one calf

Discussion

All calves were infected and developed a detectable parasitemia for anaplasmosis, but 3 failed to demonstrate a detectable Babesia parasitemia. Both parasitemias were rapidly reduced to either negative or low levels in Groups I, II, III and IV, however oxytetracycline at 12 mg/kg IV on day 21 PI was minimally effective.

The oxytetracyclines did cause a momentary decrease in the Anaplasma parasitemias, but the parasitemias increased gradually thereafter and underwent recrudescences and persisted in Groups I, II, III, IV whereas all calves in the control Group V died by day 35 PI due to acute babesiosis followed by acute anaplasmosis with relatively low parasitemias. Groups I, II, III and IV had recrudescences of anaplasmosis beginning approximately 1.5 months following treatment on day 21 PI. Five calves died of anaplasmosis in Groups II, III, IV after treatment against anaplasmosis on day 21 PI indicating that one application of oxytetracyclines was not effective. Group I almost invariably had the lowest percentage of Anaplasma parasitemia and the highest PCV and there were no mortalities. Groups II, III and IV suffered from more severe Anaplasma recrudescences, lower PCV and had 5 mortalities, however the reasons for these results are not evident. Perhaps, 4A65 and higher dosages of Ganaseg, have some adverse effect on the host

to favor the proliferation of anaplasmosis.

Following the Anaplasma challenge, all Groups had a definite decrease in PCV and a slight elevation in Anaplasma parasitemia which could have been due to the challenge or a natural recrudescence since all of the calves had had prolonged Anaplasma parasitemias. The decrease in PCV was probably not associated with anaplasmosis. All calves survived the challenge, and hence were apparently immune to anaplasmosis.

Complement fixation results for babesiosis are not yet completed. Complement fixation results for anaplasmosis are completed and indicate that Groups I and II developed relatively low maximum average titers of 1:76 and 1:57 respectively eventhoug the calves had demonstrable Anaplasma parasitemias. Groups II and III developed low titers until recrudescence occurred and then higher titers were observed. Groups I, II, III and IV, which were treated with oxytetracycline developed low titers in comparison with the control Group V eventhough demonstrable Anaplasma parasitemias were evident. Following challenge on day 141 PI, the Anaplasma CF titers increased slightly. The CF results are not conclusive and addition testing will be done to confirm the CF response.

Compound 4A65 at 0.3 mg/kg SQ and Ganaseg at 1.5, 2.0 and 2.5 mg/kg IM on day 8 PI were very effective in controlling the

B. bigemina and B. argentina parasitemia, however low Babesia parasitemias were observed on day 11 PI in Groups I and III. After day 11 PI, no Babesia parasites were detected until day 148 PI which was 4 days following challenge. If the duration of the Babesia parasitemias of Groups I, II, III and IV are compared with Group V, it can be seen that the parasitemias of Group V persisted up to 21 days longer. No mortalities occurred in Groups I, II, III and IV due to babesiosis, however babesiosis was a significant factor in the cause of death of the 3 calves in the control Group V. The inoculation on day 0 produced a significant decrease in the PCV by day 8 PI, and after the inoculation of Ganaseg, the PCV of Groups I and II increased, indicating that the lower dosages were effective. Following challenge on day 144 PI, the PCV of Groups I, II, III and IV were definitely reduced on day 151 PI with Babesia parasitemias becoming evident on day 148 PI and persisted as long as day 176 PI. Because the calves in Groups I, II, III and IV underwent clinical babesiosis apparently Ganaseg and 4A65 caused sterilization at the given dosages, however a degree of sterile immunity persisted since none of animals succumbed to the challenge.

The daily weight loss for Groups I, II, III and IV over a 132 day period before challenge averaged 9 grams which indicates

that the calves were severely stressed by the persistent anaplasmosis.

Summary

Simultaneous premunition against bovine anaplasmosis and babesiosis was attempted by the inoculation of A. marginale, B. bigemina and B. argentina with one subsequent treatment on day 8 PI with Ganaseg (1.5, 2.0 and 2.5 mg/kg IM) or 4A65 (0.3 mg/kg SQ) against babesiosis and one treatment on day 21 PI with oxytetracycline (12 mg/kg IV) against anaplasmosis. The cattle were challenged on days 141 and 144 with anaplasmosis and babesiosis respectively. On the basis of the data, one application of oxytetracycline is not sufficient to prevent post-premunization Anaplamosis reaction.

Ganaseg and 4A65 were effective in the control of the premunization Babesia parasitemia, however at the above mentioned dosages they apparently eliminated the Babesia infection to induce a degree of sterile immunity, since none of the calves succumbed to the Babesia challenge. It became apparent that the doseage of Ganaseg must be decreased to approximately 0.5 to 1.0 mg/kg SQ in order not to eliminate the Babesia infection. Also anaplasmosis must be treated with either a higher dosage more frequently or with other drugs on combination of drugs in order to prevent the chronic recrudescence.

Addendum

Subsequent to the above experiment, 2 additional experiments were executed. In the first experiment, 5 five month old, Holstein-Friesian calves were divided into 2 groups, which were inoculated subcutaneously with 5 ml of blood from a B. bigemina and B. argentina carrier calf on day 0. Group A consisting of 2 calves with an average PCV of 15% and an average 0.2% Babesia parasitemia, were treated once on day 12 PI with 0.5 mg/kg I.M' of Ganaseg (Squibb) which suppressed the parasitemia to 0.0% by day 15. Group B, consisting of 3 calves with an average PCV of 11.7% and an average 0.10% Babesia parasitemia were treated once on day 12 PI with 1.0 mg/kg IM of Ganaseg, which suppressed the parasitemia to 0.0% by day 15, never to be observed again.

In the second experiment 2 five month old Holstein-Friesian calves were inoculated subcutaneously on day 0 with 5 ml of blood containing a 0.06% A. marginale parasitemia. On day 19, the calves had an average PCV of 20% with an average 0.06% Anaplasma parasitemia. They were treated once on day 19 PI, with a combination of 356-C-61 (Burroughs-Wellcome) (5mg/kg IV) and oxytetracycline (McKesson) (12 mg/kg IV) diluted in 200ml of sterile PSS. On day 22, and thereafter no detectable Anaplasma parasitemia was observed and the PCV demonstrated a slight increase.

It is evident that Ganaseg is an extremely effective drug against bovine babesiosis and that at 0.5 mg/kg and 1.0 mg/kg it is effective for the control of the parasitemia. The combination of 356-C61 and oxytetracycline is effective in controlling the *Anaplasma* parasitemia. From these data, a good anaplasmosis and babesiosis premunition program, with frozen titrated and standardized inocula of *A. marginale*, *B. argentina* and/or *B. bigemina* and subsequent treatment with low dosages of Ganaseg and a combination of 356-C-61 and oxytetracycline, is feasible and practical.

VI. A. 1. A Sequential Study of the Pathogenesis of Disease Caused by *Trypanosoma vivax* in Experimentally Infected Calves, Utilizing Clinical, Pathological, Histopathological and Immunofluorescent Techniques

Introduction

Bovine trypanosomiasis is caused by a flagelated protozoan belonging to the genus *Trypanosoma*. Kubes, in 1944, presented evidence that *Trypanosoma vivax* had spread through certain countries and adjacent islands in Central and South America during this century. His findings were based on the morphologic similarities of organisms found there with *T. vivax* isolated during the original African studies.

Wells, *et al.* in 1969 found evidence that clinically normal cattle of various ages harbored *T. vivax* in Colombia, South America, although the vector was not discovered. The tsetse fly which is the known vector of bovine trypanosomes has never been found in the New World.

The importance of trypanosomiasis in South American cattle has not been established. The blood of cattle frequently contains *T. vivax* as well as *Anaplasma marginale* and/or *Babesia spp.* A need therefore existed to characterize the clinical, hematologic, biochemical and pathological aspects of trypanosomiasis alone in

cattle, and to obtain a basic understanding of the pathogenetic process.

Literature Review

The pathogenicity of the disease caused by T. vivax in African cattle is generally regarded as of major importance. Fiennes stated that T. vivax often caused either a chronic or benign disease with a high recovery rate, and only exceptionally caused peracute or acute disease. Hudson reported that T. vivax caused fever initially, and subsequently hypoglycemia and hemorrhages in the tongue, adrenals and kidney. Death occurred in the subacute stage from severe anemia rather than hypoglicemia. In another study, Fiennes found multiple thromboses of the smaller blood vessels in the spleen, liver, lymph nodes, lung and adrenal glands of cattle with Trypanosoma congolense. The tunica adventitia of many of the organs studied became hyperplastic with loss of elasticity, and the endothelial cells reportedly underwent "metaplasia" and passed into the general circulation.

Foci of chronic infection by both T. congolense and T. vivax were found in bovine cardiac tissue. The cattle had been exposed to infected tsetse flies and then treated with trypanosomicidal drugs. Most of the organisms were degenerated or lysed, but some were normal, and were always found in capillaries. The occurrence

of myocardial necrosis varied, but often was severe. Lodged parasitic emboli were suspected to have caused local ischemia.

Most clinical pathological data from cattle infected with T. congolense or T. vivax included decreased hemoglobin values and erythrocyte counts, eosinopenia, neutrophilia or neutropenia, lymphocytosis, periodic monocytosis associated with a reduction in numbers of circulating trypanosomes, elevated serum glutamic-oxalacetic transaminase (SGOT) levels and no change in bilirubin levels. Serum albumin levels decreased in sheep with T.vivax, in rats with Trypanosoma brucei and in rabbits with Trypanosoma evansi. Gamma globulins increased. Blood glucose levels remained normal in cattle with T. congolense, but decreased in rats with T. brucei.

Fiennes summarized certain theories as follows: Direct intervention by the parasite can not be substantiated because parasites can not be demonstrated by detailed examinations. Intracellular developmental forms have been proven to exist only with Trypanosoma cruzi. Histologic lesions caused by intoxication induced by dead trypanosomes has not been proven. An antigen-anti body reaction causing injury to the somatic cells has been thought to occur.

Because of the lack of information on the pathogenesis of disease caused by T. vivax, data on other trypanosomal diseases has been reviewed. Results of experimental disease caused by

T. brucei in mice indicated that infectivity and pathogenicity appeared to be related. Both varied according to the antigenic type involved. Gordon et al. exposed rabbits to Trypanosoma rodhesiense from carrier insects and observed local multiplication of the parasites, "chancre" formation and invasion of the blood stream.

Nigerian swine infected with Trypanosoma simiae developed fever and died acutely without anemia. Thrombi were present in hepatic arterioles, pulmonary arterioles, interlobular veins of the renal cortex, arterioles at the hilus and trabeculae of lymph nodes, small vessels of the brain, medium sized vessels of the adrenals and submucosal vessels of the intestine. The thrombi contained fibrin, mononuclear cells and in some cases whole or fragmented trypanosomes. Endothelial cells of several thrombosed capillaries were hypertrophic.

That trypanosomes are influenced by body temperature was suggested by a report from India in which the parasites were found chiefly in the skin capillaries of cattle infected with T. evansi. Fiennes found T. congolense infections in the skin of cattle and mentioned the rapid removal of the parasites once they entered the main circulation. He stated that serum antibody titers and body temperatures above 38 C were probably prime factors in the defense mechanism. Markinelle studied highly pathogenic T. cruzi infections

in mice and found that a high (36 C) environmental temperature may protect mice for months.

Rabbits infected with Trypanosoma gambiensi developed skin lesions containing high concentrations of trypanosomes before death. A vascular permeability-increasing factor was demonstrated in aqueous extracts of the parasite when inoculated intradermally following systemic introduction of Evans' blue dye.

T. brucei was studied in rabbits using ear chambers, contrast media with X-ray films and India ink. The endothelium of venules and capillaries became irregular and blood flow was impeded. Many large macrophages which lined the vessels contained carbon particles. Trypanosomes appeared cyclically in spaces between vessels, and were often seen when the parasitemia was low. Just before death the vessels became "sticky" and were lined with leucocytes. When vascular circulation ceased, the vessels disintegrated within a few hours. Fiennes described vascular lesions in the bovine species that resembled the vascular lesions in rabbits.

It has been established that mammalian host develop antibody titers against trypanosomes, and that in some unknown way, the antigenic character of the trypanosomes is altered. The ability of trypanosomes in mammalian hosts to change in antigenic character has been offered as an explanation for the fluctuations in parasitemias which are commonly observed. Recently it has been repor-

ted that although chick embryos infected with T. rodhesiense or T. brucei developed neither agglutinating antibodies nor antibodies detectable with fluorescent antibody techniques, fluctuations in trypanosome numbers occurred.

Antibody levels in cattle with T. brucei have been related to increased kinin activity following each peak of parasitemia, and to a decrease of the precursor of kinin in the blood. The kinins are 10 times as active as hisamine, causing increased vasodilatation and capillary permeability with resulting edema and pain.

Schroeder and Ristic demonstrated a heat-stable opsonin in calves infected with Anaplasma marginale. Titers appeared to be correlated with intensity and persistence of anemia and with the amount of erythrophagocytosis in the bone marrow. An autoimmunization mechanism was proposed as the cause of the anemia.

Zuckerman proposed 4 models to account for the various evidence of autoimmunization in protozoa: 1. The infectious agent may share an antigen with the host cell, with antiparasitic antibody then combining with the heterogenetic antigen in the host cell. 2. The target host cell might be coated with parasitic antigen, which attracts antiparasitic antibody, followed by cellular damage. 3. The target host cell may be directly coated with antiparasitic antibody and be sensitized. 4. The target host cell may be so modified by an infectious agent, or its products, or by a drug used

in treating the infectious agent, as to become autoantigenic. Zuckerman stated that protozoan diseases, in which it was suspected that autoimmunity had occurred, were all diseases of the blood and tissues where the organisms had intimate contact with cells, and often were intracellular in nature. Intracellular forms of T. vivax have not been demonstrated as they have with T. cruzi, in which development occurs in a series of mesenchymal cells, preferentially in cardiac tissue.

Materials and Methods

The isolate of T. vivax which was used in the experiment was obtained from a clinically ill cow near Neiva, Colombia, and was identified by its non-infectivity for laboratory mice, by its behavior in culture and by its morphology. The isolate was passed once in sheep to eliminate Babesia spp. and then in a calf which was treated with Oxytetracycline HCl* intravenously at the rate of 12 mg/kg for 12 days and with B-W Drug No. 356-C-61** intravenously at the rate of 5 mg/kg for 3 days to eliminate A. marginale. Serum from this calf remained free of complement fixing antibody titers for Anaplasma and Babesia antigens.

---

*Chas. Pfizer Co., Inc., New York, N. Y.

**Burroughs-Wellcome Co., Research Park Triangle, N. C.

Eleven intact male and 4 female Holstein-Friesian calves 4 to 6 months were used. All of the calves were raised on pastures located in the Sabana of Bogotá, an area considered to be trypanosome free. The calves were examined hematologically for trypanosomiasis and serologically for anaplasmosis and babesiosis and found to be free of these diseases. The calves were maintained as a group in one section of a barn, and were not allowed to mix with other cattle. Prior to their inoculation with trypanosomes, 5 cc of blood from each of the 15 experimental calves was inoculated intravenously into a susceptible splenectomized calf, cultured on enriched blood agar, inoculated intraperitoneally into 2 laboratory mice at the rate of 1.0 cc each, and examined in wet mounts.

One calf served as an experimental control in which the continued absence of blood parasites throughout the experiment indicated that contamination from outside sources was not likely. The control calf was subjected to necropsy last, and the tissues served as negative controls for the immunofluorescence study.

Oxylated blood from the carrier calf was examined daily, and a relative scale for quantitating the degree of parasitemia was established. In each test, 10 microscopic fields of fresh blood in a wet mount slide were examined at 400 magnification. If no trypanosomes were found, the test was considered negative, and if 1 trypanosome was found in a total of 10 fields it was designated

a "1 plus". If an average of 1 trypanosome was found in each of 10 fields it was designated a "2 plus". When there was more than 1 trypanosome in each of 10 fields it was designated a "3 plus", and if a high number of trypanosomes were present in each of 10 fields it was designated a "4 plus".

Each of the 14 experimental calves was given intravenously 5.0 cc of citrated blood containing approximately 100,000 organisms per cc. A sample of infective blood was frozen in glycerol using the technique of Cunningham, et al. The sample was stored at -79 C, insuring a stabilate of trypanosomes (as defined by Lumsden) for future work.

Beginning 5 days prior to the inoculation of the calves and continuing throughout the experiment, rectal temperatures were measured each morning and afternoon. Samples of blood from each calf were collected on each of 4 days before inoculation and subsequently on each day of scheduled necropsy. Aliquots of the blood were treated with sodium fluoride or sodium oxalate and other aliquots were allowed to clot. Blood glucose concentrations, packed cell volumes, hemoglobin concentrations, sorbitol dehydrogenase levels, serum glutamic oxalacetic transaminase concentrations total serum proteins, bilirubin concentrations, total and differential leucocyte counts and serum protein electrophoretic patterns were determined. These data obtained from the 14 infected calves

are summarized in Figures 1, 3 and 4. The group mean and standard deviation are shown on each day of observation.

The packed cell volume was determined by standard laboratory methods. The hemoglobin concentration was determined by the Hycel* cyanmethemoglobin technique. Serum bilirubin content was assayed by the technique described by Gibson and Goodrich. Total serum protein was determined by a hand held temperature compensated Goldberg refractometer (A-O TS meter).** Serum protein electrophoresis on cellulose polyacetate membranes was done using a Gelman deluxed electrophoresis chamber, Model 51210.*** Aliquots of 5.0 lambdas of serum were applied to cellulose acetate membranes soaked in fresh cold barbital buffer having pH 8.6 with an ionic strength of 0.05. Electrophoresis was continued for 45 minutes at 1.5 to 1.8 milliamperes at a constant direct current voltage of 350 volts maintained by a Gelman voltage supply, Model 38201.*** The cellulose polyacetate membrane was stained for 5 minutes in a solution of 0.1% Ponceau S with 5% trichloracetic acid, differentiated in 5% acetic acid (1 minute in each of 3 baths) and cleared for 30 seconds in a mixture of 10% glacial acetic acid and 90% methanol. It was

---

*Hycel Co., Houston 2, Texas

**American Optical TS Meter, Buffalo, N. Y.

***Gelman Instrument Co., Ann Arbor, Mich.

dried on glass slides at 60 C for 20 minutes and quantitated by scanning on a Gelman automatic recording and integrating scanner, Model 39372.*

Serum glutamic oxalacetic transaminase levels were measured by a modification of the original Reitman and Frankel method. Blood glucose concentrations were determined using a photometric adaptation of the Somogyi method.

One calf, selected by random sampling, was euthanatized by electrocution at each of the post inoculation times of 12 hours, 1, 2, 3, 5, 8, 12, 17, 23, 30, 38, 45 and 88 days. One calf was necropsied on day 37 following death due to trypanosomiasis. The control calf was the last calf to be killed.

As each calf was dissected at necropsy, triplicate sets of tissues were collected. One set was preserved in buffered 10% formalin and tissue sections were stained with hematoxylin and eosin, using standard techniques. The other 2 sets were frozen onto wooden blocks using Cryoform** and stored in separate freezers at -20 C for immunofluorescence studies.

Emphasis was placed on the histologic study of sections of tissues from the major organs and lymph nodes, although tissues

---

*Gelman Instrument Co., Ann Arbor, Mich.

**International Equipment Co., Needham Heights, Mass.

from each of the following anatomical sites were examined: lip, tongue, dental pad, ear, eye, nasal mucosa, mandibular lymph node, mesenteric lymph node, prefemoral lymph node, hemal lymph node, lung, heart, aorta, bone marrow, adrenal, kidney, urinary bladder, spleen, pancreas, rumen, reticulum, abomasum, duodenum, ileum, colon, liver, gall bladder, ovary, uterus, penis, testicle, cerebrum, cerebellum, medulla oblongata, choroid plexus, pituitary, spinal cord, bone, semilunar ganglion, semitendinous muscle, diaphragm, and mediastinal lymph node. All samples of a given organ except those samples representing gross lesions were taken from similar locations within the organ.

Bone marrow biopsies were taken from the ribs of 3 calves on each scheduled day of necropsy. The 3 calves included the calf to be dissected, plus 2 other calves selected at random. After all the calves had been subjected to 1 biopsy, the selection process was repeated, using ribs from the opposite sides of the calves.

On day 23 a quantity of blood was withdrawn from an infected calf selected at random and allowed to clot in order to obtain serum containing antibodies against T. vivax. The 112 cc of serum was put in dialysis tubing with an air pocket at each end and then placed in a 1000 cc. beaker containing half saturated $(NH4)_2SO_4$ solution. The solution was agitated overnight with a magnetic stirrer in a refrigerator set at 4C. The solution was changed twice. After

24 hours the serum volume had been reduced to 46 cc. An equal volume of physiological saline containing 1 drop of 1.0 N NaOH per liter was added. To this was added, drop by drop, 56 cc of saturated $(NH_4)_2SO_4$, using a magnetic stirrer. After 1 hour of stirring, it was centrifuged at 10,000 rpm for 15 minutes using a refrigerated Sorvall* Model RC2-B centrifuge. The supernatant was discarded, and the precipitate was washed with half saturated $(NH_4)_2SO_4$. The centrifugation-wash cycle was repeated 2 more times. The precipitate was then redissolved in a minimum quantity of 0.5 molar Na_2CO_3-$NaHCO_3$ solution at pH 9.0 (1 part 0.5 molar Na_2CO_3 and 4 parts 0.5 molar $NaHCO_3$). The resuspended material was placed in new dialysis tubing and dialyzed against 0.05 molar $NaHCO_3$ solution at pH 7.5 in a 1000 cc beaker. The solution in the beaker was changed twice overnight. The material in the dialysis tubing, now having a volume of 51 cc, contained 4.9% protein. A sample of it was examined by cellulose acetate zone electrophoresis and contained gamma globulin with no albumin. The 51 cc of solution was expanded to 100 cc by mixing with 38 cc of cold physiological saline and then slowly adding 11 cc of buffer solution pH 9.0 (1 part 0.5 molar Na_2CO_3 and 4 parts 0.5 molar $NaHCO_3$). One milligram of fluorescein

---

*Ivan Sorvall, Inc., Newton, Conn.

isothiocyanate (FITC)* was used for each 20 milligrams of protein in the solution. The FITC was dissolved in 2 cc of acetone and was added slowly to the swirling solution which was then stirred overnight in the refrigerator. The conjugated preparation, which had a PH of 8.9, was passed through 2 columns containing Sephadex** G-25 (Course) and the gammaglobulin containing portion was collected in fractions and tested for protein concentration. It was filtered, using a Swinny filter holder containing a Milipore*** filter with a 0.45 micron pore size, and stored in 1.0 cc aliquots at -20 C.

Positive control slides for the fluorescent antibody study were prepared as follows: Blood from an infected calf having a 4-plus parasitemia was collected in tubes containing the sodium salt of ethylenediaminetetraacidic acid (EDTA). The blood was centrifuged at approximately 500 rpm until the supernatant was clear. The supernatant was collected in a separate tube containing a very small quantity of the erythrocytes from the original tube. This material was mixed by swirling, and 1 drop was placed on each glass slide, spread evenly across the surface and allowed to air

---

*Nutritional Biochemicals Corp., Cleveland,Ohio

**Pharmacia Fine Chemicals, Inc., Piscataway, N. J.

***Milipore Filter Corp., Beford, Mass.

dry. The slides were fixed for 10 minutes with acetone at -20 C and then allowed to dry. They were stored in slide boxes surrounded by plastic bags containing a small quantity of $CaCl_2$ in a freezer at -20 C.

Sections of uninfected tissues were cut from frozen blocks of mediastinal lymph node and kidney taken from the control calf. Sections for FA studies were cut at 4 microns. Those tissues which were examined by FA techniques included those of the ear, cerebrum, thymus, heart, lung, mediatinal lymph node, prescapular lymph node, liver, spleen, kidney and bone marrow. The sections were mounted on new glass slides that had been previously cleaned in acetone and air dried. The tissues were fixed in acetone at -20 C.

Tissues from each infected calf were stained and examined as a group along with at least 1 positive control slide and 1 each of the negative control slides. The slides were allowed to warm to room temperature. The conjugate was thawed and diluted 1:50 with 0.01 molar NaH_2PO_4 buffered saline with pH 7.5, and carefully added to the tissue so that its entire area was covered. Each preparation was placed on supports in a separate covered Petri dish containing moistened filter paper and incubated for 30 minutes at 37 C. Then the sections were removed from the incubator and washed for 15 minutes in each of 3 successive changes of phosphate-buffered saline that had been chilled previously. The sections were air dried and

cover slips were applied, using a non-fluorescing glycerin solution at pH 9.0. They were examined within 1 hour, using a Leitz Ortholux binocular microscope equipped with 10X oculars and 10X, 40X, 54X oil immersion and 100X oil immersion objectives and a dark field condenser. Non-fluorescing immersion oil was employed. The light source was an Osram Mercury super pressure HBO-200W lamp with a diffusion filter, a BG 12 excitor filter and a K 470 barrier filter.

Results

Clinical Manifestations. The first clinical sign was an increase in the morning rectal temperature which averaged 2.5 C above normal (Fig. 1). The fever was at a maximum 24 hours after inoculation and had decreased by day 3. The average afternoon rectal temperatures are shown in Fig. 1.

Changes in the appearance of the calves were detectable approximately 2 weeks post inoculation. The calves gradually became emaciated and their hair coats became dry. At approximately 4 weeks post inoculation the calves appeared more docile, their heads dropped, and general weakness gradually became apparent. At 5 weeks post inoculation submandibular edema could be detected in some calves. The edema receded in all animals as death became imminent and in 2 calves the edema had regressed completely before

death. Dehydration and lacrimation were apparent and the abdomens became gaunt after 5 weeks on experiment. Appetite remained near normal, but there was a general lack of vigor in this phase. The calves moved more sedately toward their feed and appeared to eat in a more languid manner.

Clinical Pathology. The parasitemia became evident on day 5 and reached a maximum (4-plus level) on day 7 (Fig. 1). Thereafter, the degree of parasitemia in a given calf varied from zero to a maximum relative value of 4-plus.

The infected calves became anemic as the experiment progressed. Hemoglobin levels and packed cell volumes decreased steadily (Fig. 1). There was an overall decrease in the myeloid: erythroid (M:E) ratio, (Fig. 1). Total and direct bilirubin levels increased slightly, and then fluctuated thereafter (Fig. 2). The SGOT levels increased in the last half of the experiment and the standard deviation increased (Fig. 2). Serum sorbitol dehydrogenase levels increased and then regressed (Fig. 2).

The level of total serum protein had decreased by day 8 but remained constant thereafter (Fig. 2). A slight decrease in the absolute amount of serum albumin occurred. Although there was a relative increase in the percent of globulins, there was actually a slight decrease in the absolute amount of globulins present due to the decrease in total serum proteins. The albumin:globulin

(A;G) ratio decreased slightly (Fig. 2). The absolute amount of gamma globulins increased, while the absolute amount of alpha and beta globulins decreased (Fig. 2).

Blood glucose levels remained normal until after day 30, when the mean values decreased to one half those of the pre-inoculation values (Fig. 3).

Total leucocyte counts decreased by day 8 and remained fairly constant until day 30 when there was an abrupt increase to a level above normal (Fig. 3). The leucopen9a was influenced by the development of neutropenia in the acute phase (Fig. 4). This condition persisted until near the end of the experiment, when a neutrophilia developed.

There was a slight decrease in the number of lymphocytes by day 8 (Fig 3). However, there was a relative lymphocytosis due to the considerable decrease of total leucocytes. The absolute number of lymphocytes was approximately normal at the end of the experiment. A very slight eosinopenia and monocytopenia developed gradually throughout the course of the experiment (Fig. 3).

Gross Pathology. The tissues and organs of the calves killed at 12 hours, 1, 2, 3 and 5 days were not observed to have lesions except for an occasional small area of consolidation in the apical or cardiac lobes of the lungs.

In the calf killed on day 8 the lymph nodes were slightly

edematous and the hemal lymph nodes were slightly enlarged.

The abdominal cavity of the calf killed on day 12 contained approximately 30 cc of amber fluid. One prescapular lymph node contained multiple petechiae. The thymus appeared edematous and the spleen was slightly enlarged.

The lymph nodes were enlarged and edematous in the calf killed on day 17. The hemal lymph nodes were slightly enlarged. The thymus was reduced in size.

The lymph nodes of the calf killed on day 23 were moderately enlarged and slightly edematous. The hemal lymph nodes were normal in size. The thymus was small, but of normal consistency. One small abcess containing thick greenish exudate was present in 1 tonsil. Red bone marrow extended half way down the femoral shaft, contrasting with red marrow that occupied approximately the proximal 20% of the femur in calves killed previously.

The lymph nodes of the calf killed on day 30 were slightly enlarged and edematous. One prescapular lymph node contained multiple ecchymoses. The hemal lymph nodes were greatly enlarged. The thymus was not evident. The abdominal cavity contained approximately 40 cc of amber fluid.

An experimental calf in a weakened condition died on day 37 after routine blood samples were taken. Although not detected on external examination of this calf, the connective tissue of the

intermandibular space was found to be edematous. All of the lymph nodes were moderately enlarged and edematous and hemorrhages were present in the prescapular and renal lymph nodes. The thymus was small and edematous. The epicardial surface contained multiple petechiae, the right ventricle was rounded and there was serous atrophy of pericardial fat. The pericardial sac contained approximately 30 cc of amber fluid. The liver capsule and parenchyma were slightly more difficult to cut than normal. The kidneys had multiple petechiae on their surfaces and there was serous atrophy of perirenal fat. The cortices of the adrenal glands were congested.

The calf killed on day 38 was weak, and its eyes were sunken. Submandibular edema was conspicuous. All of the lymph nodes were moderately enlarged and edematous, and 1 prefemoral lymph node had several ecchymotic hemorrhages. The hemal lymph nodes were greatly enlarged and prominent, and the thymus was small and edematous. Depot fat in the animal was minimal. The cut surface of the ventricular myocardium was pale, and the pericardial sac contained approximately 10 cc of amber fluid. The liver was pale, and the capsule and parenchyma were more resistant to cutting than normal. The abdominal cavity contained approximately 25 cc of amber fluid. The capsular surfaces of both kidneys contained multiple petechiae. The thymus was gelatinous in consistency.

The lymph nodes of the calf killed on day 45 were moderately enlarged and edematous, and the hemal lymph nodes were greatly

enlarged. Although no free fluid was observed in the body cavities, considerable fibrin was present in the ventral abdomen. The mesentery of the spiral colon contained a large quantity of edema. Serous atrophy of fat was evident. The thymus was small and edematous. The right ventricle of the heart was moderately rounded. The apical lobes, the ventral portions of the cardiac lobes and small ventral portions of the diaphragmatic lobes of the lung were red and firm. The trachea contained white foam. The hepatic capsule and parenchyma were moderately resistant to cutting. Red bone marrow extended more than half way down the length of the femur.

The last calf was killed on day 88. This calf had chronic cystitis. The gross, microscopic and clinical pathological data did not reflect uncomplicated chronic trypanosomiasis, therefore data from this calf are not included.

Microscopic Pathology.

Lungs: Within 12 hours after inoculation, hypertrophy was evident in the endothelium of the pulmonary arterioles. There appeared to be an excess number of mitotic figures in the endothelium of arterioles. Within 24 hours post inoculation some alveolar spaces contained serofribrinous exudates and erythrocytes. Intraalveolar aggregations of macrophages were seen by day 8, and in sections of lung tissue collected from calves killed subsequently, there were similar aggregations of macrophages in blood vessels. Some small

blood vessels observed in longitudinal section contained multiple small dark bodies. By day 23 some alveolar walls were slightly thickened and contained eosinophilic material. The interalveolar lesion became more prominent with time. This lesion was randomly distributed throughout the parenchyma within pulmonary lobules. Typically, an affected lobule was located adjacent to a normal lobule, and therefore the affected tissue was dispersed in a random pattern. By the end of the experiment the interalveolar tissue was seen to contain numerous macrophages, some of which apparently contained engulfed material. Lung sections collected on days 30, 37 and 38 processed with Periodic Acid Schiff or Masson's Trichrome Stain* indicated the basement membranes of the vessels were normal, and there was no evidence of deposition of excess material along the vessels.

Heart: Evidence of progressive changes were found in the myocardium as early as day 2. At that time small lymphocytes had infiltrated the interstitial tissue around blood vessels and lymphatics. Larger lymphocytes were recognizable by day 5 and day 12. The endothelial cells of arterioles were hyperplastic. After a month there were many plasma cells in the interstitial tissue along the blood vessels, lymphatics, nerves and between muscle bundles. Some myocardial

---

*PAS & Masson's Trichrome Stains: AFIP Protocol. 1957

nuceli were altered, with the chromatin being arranged in a long, thin, wavy line along the central axis of the cell, such as is described for the "caterpillar cell" of certain human cardiac myopathies. Sarcosporidia were common in heart muscle as well as in skeletal muscle.

Liver: Initial changes in the liver were observed by day 3. There were areas of acute focal necrosis which were infiltrated with neutrophils. Some of the hepatocytes located near terminal hepatic veins had undergone fatty metamorphosis. A few lymphocytes had infiltrated into portal tracts by day 5, and they were more numerous by day 8. By day 23 there were large aggregations of macrophages in terminal hepatic veins, and a few neutrophils had infiltrated into the parenchyma. Kupffer cells scattered throughout the tissue were filled with globules of hemosiderin. Periacinar congestion was marked by the fifth week, and in most, but not all calves there were many degenerated hepatocytes aroun terminal hepatic veins, with fatty metamorphosis being present in the midzonal regions. Periacinar fibrosis could not be demonstrated by special staining techniques.

Kidneys: In the kidneys of the calf killed on day 3 there was excessive proteinaceous material in the proximal convoluted tubules, and the glomerular tufts were slightly hypercellular. The degree of proteinuria had decreased to a low but persistent level by the

end of the second week. Endothelial hypertrophy was evident in arterioles oriented near glomeruli. The lumens of some of these arterioles were very small during the second week. Perivascular infiltration of lymphocytes, plasma cells and other mononuclear cells became more prominent as time elapsed. At the end of 3 weeks, large accumulations of cells were present around the arcuate arteries.

The degree of hyper cellularity of the glomerular tufts increased and at the end of 5 weeks the tufts completely filled some of the Bowman's spaces. Macrophages filled with hemosiderin were located predominently in the medulla. Accumulations of neutrophils in the proximal convoluted tubuels and elsewhere in the kidney were visible on day 38.

Lymph nodes: In general, by 12 hours post inoculation mitotic activity was at a high level in the germinal centers of most lymph nodes. During the experiment most lymph nodes had evidence of lymphoid or reticuloendothelial hyperplasia, however, toward the end of the experiment some germinal centers appeared exhausted. Occasionally small hemorrhages and edema were seen. Macrophages containing hemosiderin were scattered throughout the lymphatic tissue during the latter portion of the experiment.

The mediastinal lymph nodes were typical of those nodes in which lymphoid hyperplasia occurred. During the first week

there was a moderate degree of hyperplasia and many necrotic cells were present in germinal centers. In the second week there apparently was an increase in the number and size of the germinal centers. Marked lymphoid hyperplasia was evident during the last 4 weeks of the experiment.

In the prescapular lymph nodes a mild reticuloendothelial cell hyperplasia was evident by the end of the first wek, and the condition developed to a moderate or marked level during the second week. The condition regressed considerably near the end of the experiment. Concurrently, the concentration of lymphocytes appeared slightly decreased on day 2 and on day 12 there was moderate depletion, although the concentration of lymphocytes was approximately normal at the end of the experiment.

Bone Marrow (Femur): Specimens of bone marrow from the first 2 calves killed consisted mostly of depot fat with some evenly distributed hemopoeitic cells. After day 3 the blood forming tissue increased at the expense of the fatty tissue and by day 12 there were confluent areas of hemopoietic tissue. Cells of the erythroid series were generally more numerous than cells of the myeloid series. It was estimated that there were about twice as many erythroid cells as myeloid cells on day 23. After this time reticuloendothelial (RE) cells were numerous, and in some areas they had aggregated in discrete foci. Congestion was apparent in tissue collected after 3 weeks, and tissue collected on day 38 contained many cells of

the neutrophilic series.

Ear: Endothelial hyperplasia was evident in a few subcutaneous arterioles by day 3, but this was not evident in calves killed toward the end of the end of the experiment. A slight to moderate infiltration of eosinophils, lymphocytes and macrophages had occurred in the interstitial tissue along blood vessels and lymphatics. The concentration of infiltrating cells in the subcutaneous tissue remained at a fairly constant level during the course of the disease.

Thymus: During the first week there was a slight increase in numbers of eosinophils and neutrophils in the interstitial tissue between lobules. The cortices of the lobules appeared thin by day 12, and by day 23 there was definite depletion of thymocytes. No parenchyma remained by day 30, and on days 37 and 38 all that remained was supporting connective tissue and vessels, with no distinctly discernable boundary between cortex and medulla. There was a moderate degree of atrophy present in some of the thymus tissue collected on day 45 but in other areas the tissue was normal.

Spleen: There was moderate congestion and moderate to marked hemosiderosis in sections of spleen collected after the first week, and this condition persisted throughout the experiment. Neutrophils collected in the red pulp, mostly near Malphigian corpuscles, in the late stages of the disease.

Hemal Lymph Nodes: There was a variable degree of congestion.

Fluorescent antibody stained tissue: Trypanosomes fluoresced with a typical apple green color in smears from infected blood. Smears were improved by removing most, but not all, of the erythrocytes as less time was required per slide. The optimum dilution of the conjugate was 1:50.

Trypanosomes with somewhat less fluorescence than that of the controls were detected in the mediastinal lymph node and spleen of the experimental calf killed on day 23. The parasite seen in the mediastinal lymph node was intact, and the nucleus and kinetoplast both had a stron fluorescence. The rest of the parasite was easily visible, but fluorescence was weak. The second trypanosome, seen in splenic tissue, possessed similar fluorescing qualities as the one described in the lymph node. It was intact except for a small portion of the anterior extremety. In neither case was it possible to say if the parasites were located in vessels. Because the organisms were essentially of the same shape as the trypanosomes observed in blood films, they probably were lying free in the interstitial fluid. Almost certainly they were not intracellular. An examination of tissue cut from the same frozen blocks and examined by light microscopy failed to locate any portions of parasites.

Microscopic structures having an evenly distributed apple

green fluorescence were observed in tissue from certain organs of the experimentally infected calves but not from tissue of the control calf. In cardiac tissue collected at 12 hours a blood vessel was seen to be surrounded by an area of evenly distributed fluorescence. In renal tissue taken later in the experiment there was a similar type of fluorescence that was evenly distributed through the glomerular tufts of some Bowman's spaces and in the interstitial tissue aroun vessels associated with the convoluted tubules and to a lesser extent with descending tubules.

In tissue from the control calf and most infected calves nonspecific fluorescence was seen in the form of multiple, evensized circular granules which were not trypanosomes. The granules had an apple green fluorescence and were sufficiently small that as many as 200 were estimated inside some cells. The cells were suspected of being macrophages. Usually, however, the number in a cell was much less, although the size of the granules remained fairly constant. Organs most often affected included lymph nodes, spleen and thymus, while the cerebrum was rarely involved.

Granules similar to those seen intracellularly were observed intercellularly in bone marrow of at least 1 infected calf. In some areas the granules were packed tightly together in the intercellular spaces and often were sufficiently numerous as to form a syncytium which tended to outline the normal cellular structures.

Summary

T. vivax obtained from a clinically sick cow near Neiva, Colombia, South America was passed in a sheep and in a calf, and then inoculated into the jugular vein of 14 Holstein-Friesian calves. Fever occurred by 24 hours and recurring parasitemia commenced after 72 hours post inoculation. It was estimated that practically all of the 14 calves would have died spontaneously of trypanosomiasis within 3 months if none of the calves had been euthanatized.

Associated with the first and subsequent parasitemias were decreases in hemoglobin, PCV, M:E ration, serum albumin, A:G ratio and neutropenia, and slight increases in total and direct bilirubin and SGOT levels. Later changes included slightly elevated, fluctuating total bilirubin levels, slightly increased SGOT and gamma globulin levels and slightly decreased alpha and beta globulin levels. Blood glucose levels decreased in only 1 calf after day 30.

After 2 weeks all calves exhibited a gradual loss of body condition and later submandibular edema usually became evident. The latter condition sometimes regressed and this sign along with a PCV below 20% indicated that death was near. After 4 weeks the post mortem lesions considered sufficiently consistent to be of diagnostic value included conspicuously hypertrophied, edematous lymph nodes and hypertrophied hemal lymph nodes, emaciation, rounded right heart,

palpably firm liver, atrophied thymus and hypertrophied femoral bone marrow.

Associated with the T. vivax of the infecting inoculum and of succeeding parasitemias was a generalized endothelial hypertrophy and mononuclear cell infiltration along blood and lymph vessels, with proteinuria and bone marrow hyperplasia. Generalized lymphatic and RE hyperplasia occurred, but was not proven to be due to the trypanosomiasis. At 3 weeks post inoculation there was an aggregation of macrophages containing engulfed material distributed along capillaries in pulmonary interalveolar tissue, and this lesion in combination with the anemia and apparent cardiac insufficiency were thought to be important in the development of anoxia and probably contributed to the single fatality observed.

Periacinar congestion and fatty metamorphosis of midzonal hepatocytes were probably related to the failing heart and indirectly to the pulmonary lesion. Cystitis and pyelonephritis in the last 2 calves to be killed probably was a reflection of generalized debility due to chronicity of the trypanosome infection.

The detection of intact trypanosomes or their residue in histologic sections, utilizing a direct fluorescent antibody technique, was of limited success. Generalized fluorescence of interstitial tissue associated, at least in part, with vessels was observed in tissue from infected calves but not from the control

calf. It was suspected that the fluorescence was related to the presence of soluble antigens to the trypanosome.

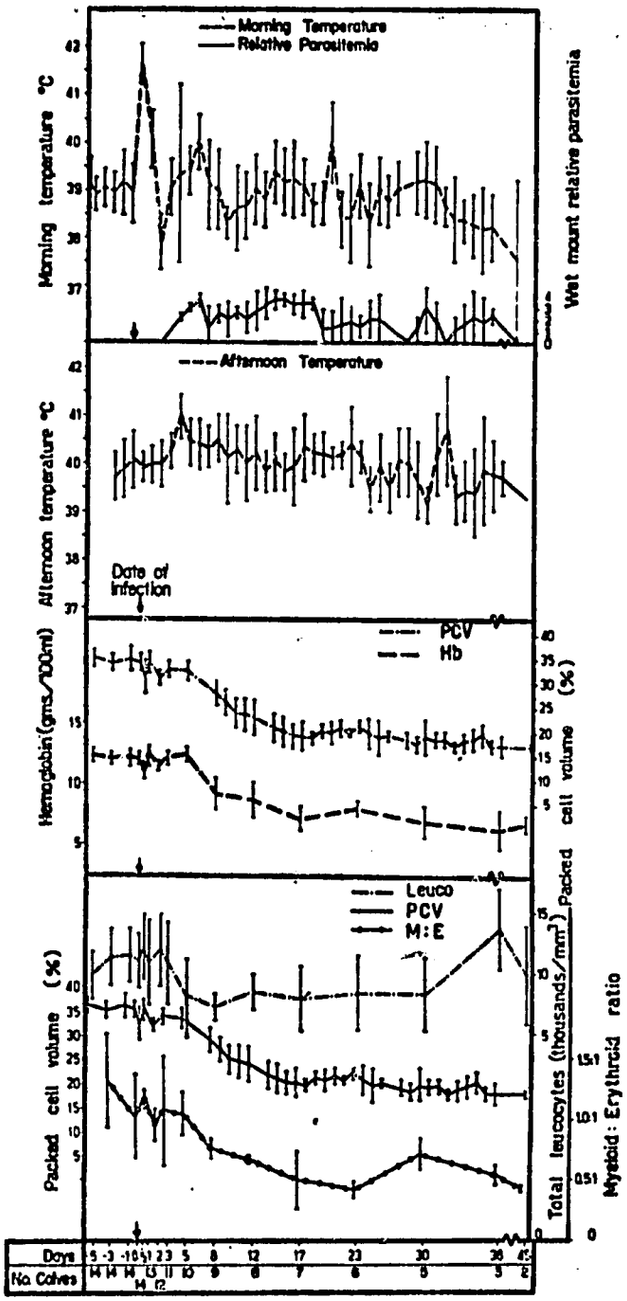


Fig. 1. Morning and afternoon rectal temperatures and Hematological studies. Group means are presented and vertical lines represent ± standard deviation. Number of calves decreased from 14 to 2 due to aerial necropsy. Arrow indicates time of intravenous inoculation with Trypanosoma vivax.

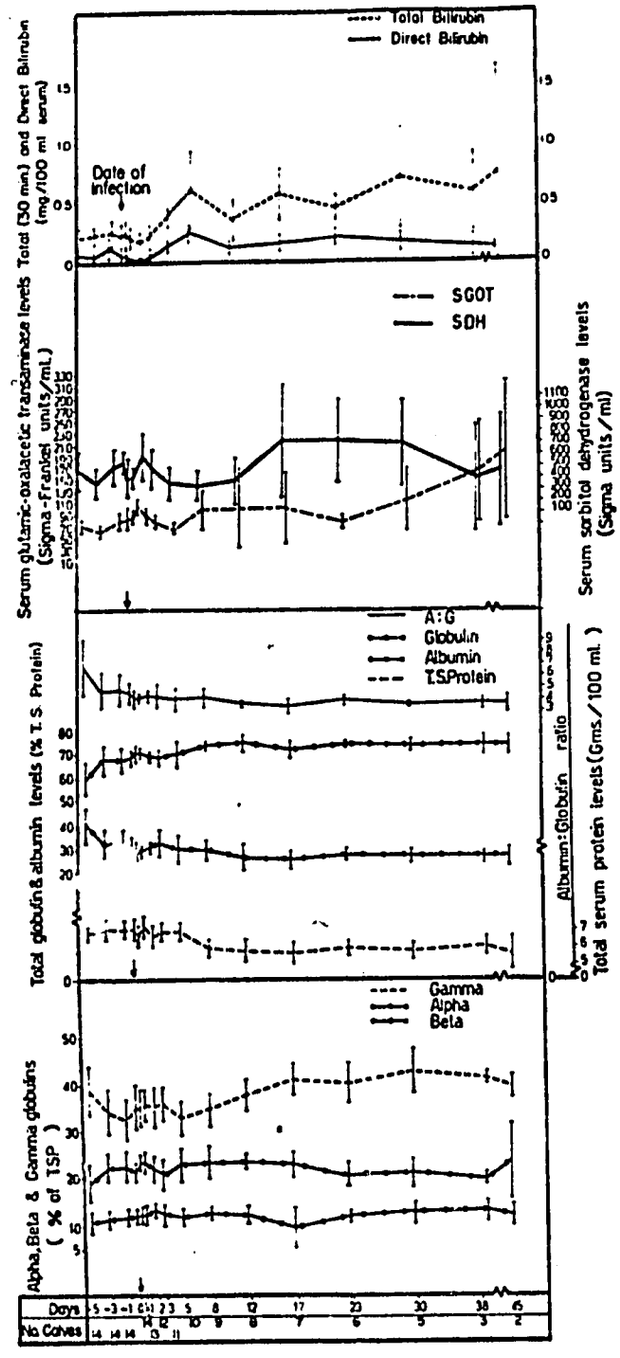


Fig. 3. Hematological data. Group means are presented and vertical lines represent ± standard deviation. Number of calves decreased because of selective serial necropsy. Arrow indicates time of intravenous inoculation with *Trypanosoma vivax*.

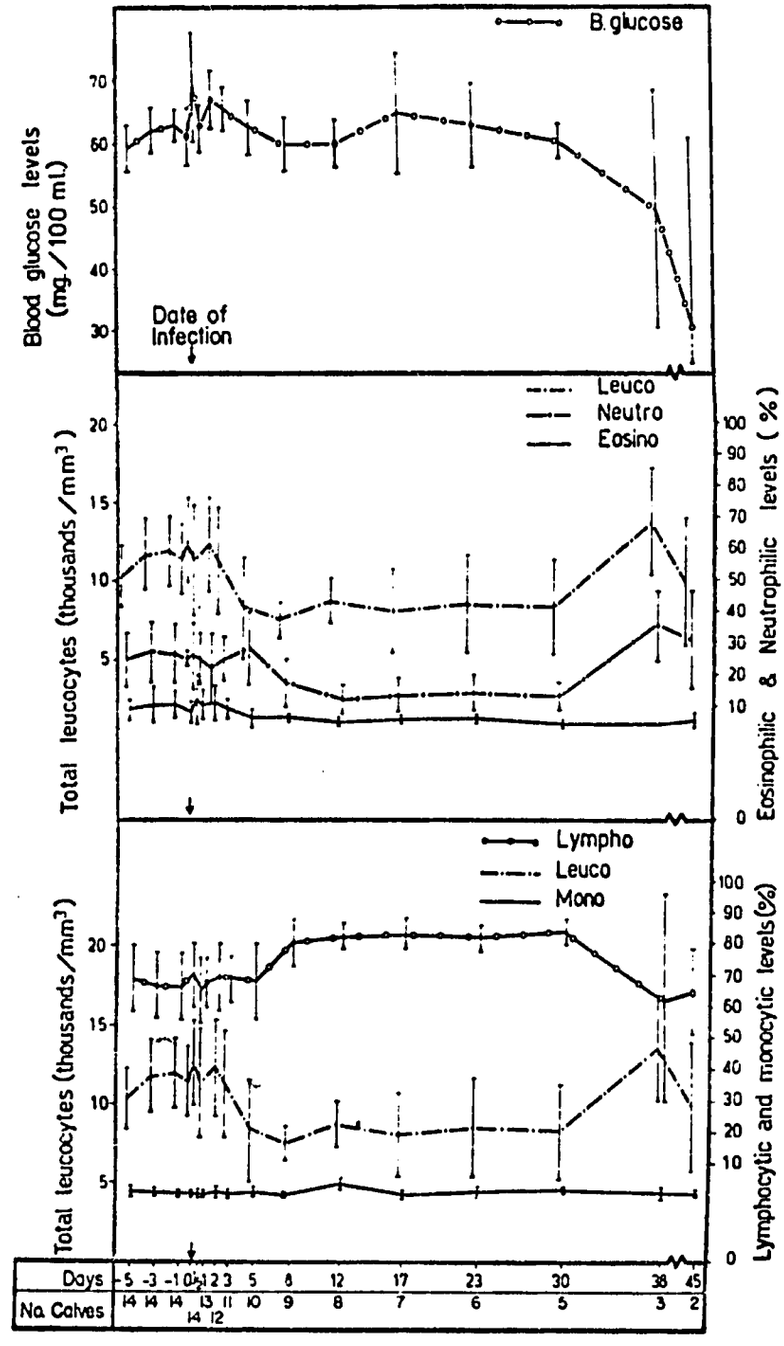


Fig. 4. Hematological data. Group means are presented and vertical lines represent ± standard deviation. Number of calves decreased because of selective serial necropsy. Arrow indicates time of intravenous inoculation with Trypanosoma vivax.

VII. A. Participation in and Direction of Post-Graduate Degree Theses

Participation in the training of veterinarians has consisted primarily in the direction and counseling of 13 students working toward either a degree of Master of Science or Doctor of Philosophy in Veterinary Pathology or Veterinary Microbiology. The names of the students alphabetically are as follows: Drs. Adolfo Arboleda, John Bishop, Fernando Botero, Donald Corrier, Charles Daley, Wilson Ferreira, José Hernández, Floyd Jones, Fernando Lozano, Héctor Muñoz, Jaime Payán, Kenneth Thompson and Otoniel Vizcaíno. Four of the students have completed their respective degrees.

In addition to the above participation, the Texas A&M University Group has been responsible for the "in laboratory" training of 5 veterinarians and several technicians in the field of hemotropic diseases.

Seminars on the subject of hemotropic diseases were presented sporadically throughout the year, to various groups of veterinarians.

VII. B. Attendance and Participation in Scientific Meetings.

Drs. Adams and Todorovic attended and participated in the Third National Congress of Veterinary Medicine and Animal Science on May 17-23, 1970 in Lima,Perú. A paper entitled "Bovine Babesiosis, Its Problem and Control in Colombia, South America" by Todorovic, R. A., Adams, L. G. and González, E. F. was presented. Furthermore, a one week seminar on the etiology, ultrastructure, pathogenesis, diagnosis, epizootiology, therapy, and control of anaplasmosis, babesiosis and trypanosomiasis was presented to a large audience of Peruvian veterinarians.

Dr. Adams participated in the Caribbean Veterinary Medical Association on June 9-12, 1970 in Georgetown, Guyana. Dr. Adams presented a paper entitled "The Treatment and Control of Bovine Babesiosis" to the Veterinarians attending the congress.

Dr. Todorovic participated in the 107th Annual Meeting of the American Veterinary Medical Association on June 22-26, 1970 in Las Vegas, Nevada. He presented a paper entitled "Immune Response of Cattle Vaccinated Against Babesiosis in Colombia, South America" to the American Association of Veterinary Parasitologist section.

Drs. Adams and Todorovic attended and participated in the 6th Panamerican Congress of Veterinary Medicine and Animal Science on September 28-October 3, 1970 in Santiago, Chile. Five papers were presented which were entitled as follows: "A Study of the

Pathogenesis of Anaplasmosis in Intact Calves; Including Clinical Clinical Pathological, Histopathological, Serological and Immuno fluorescent Techniques" by Adams, and Todorovic, "Research and Control of Bovine Babesiosis in Colombia, South America", by Todorovic, Adams, Vizcaíno, and González, " A Study of the Pathogenesis of Babesiosis in Intact Calves; Including Clinical, Clinical Pathological, Histopathological and Immunofluorescent Techniques" by Lozano, Adams, and Todorovic, "A Study of the Pathogenesis of Hepatic Granulomas of Turkeys Produced by Streptococcus fecalis var. liquefaciens by Hernández, Roberts, Adams and Vera, and "The Ultrastructure of Babesia bigemina" by González, Todorovic and Adams.

Dr. Adams attended the Annual Meeting of the American College of Veterinary Pathologist on November 16-18, 1970 in New Orleans, Louisiana where he took his Board Examinations and passed them to become a Diplomate.

Drs. Todorovic and Adams were nominated and invited to submit a paper entitled "Serologic Diagnosis of Babesiosis" to the XIXth World Congress of Veterinary Medicine, August 15-21, 1971 in Mexico City, Mexico. The paper was approved and will be presented to the Symposium: Protozoal Diseases; Babesiosis and Anaplasmosis.

Scientific Visit of the East African Veterinary Research Organization with Special Emphasis on East Coast Fever Investigation.

Introduction

A great deal of scientific progress has been made toward practical solutions of East Coast Fever (ECF) of cattle caused by Theileria parva. Several of the techniques utilized by the investigators working on ECF have applications for the solutions of problems related to other hemotropic diseases of cattle such as anaplasmosis, babesiosis and trypanosomiasis. Due to the general applications of the techniques used in ECF investigation, it was recognized and recommended that a scientific visit would be profitable and useful for the Texas A&M University Group who are doing research on anaplasmosis, babesiosis and trypanosomiasis of cattle in Colombia, South America. Furthermore, the need for the establishment of a scientifid working relationship between the African and South American continents was recognized, and a scientific visit was recommended to ascertain the liaison. Dr. L. G. Adams, Project Leader of the Texas A&M University Group on Bovine Hemotropic Diseases of the Centro Internacional de Agricultura Tropical located in Colombia, was asked to make the scientific visit, which was paid with US-AID funds.

Report

Dr. Adams attended the "East Coast Fever and Related Diseases: A Technical Conference" in Rome, Italy on March 8, 1971, where he was introduced to several of the investigators, and the financial supportive agency's advisors. He was able to be reviewed on the latest ECF information achieved by the investigators. Valuable concepts and techniques were acquired for future use toward solutions of other bovine hemotropic diseases as well as professional contacts for future collaboration. Following the Rome meeting, Dr. Adams went to Kenya to visit the East African Veterinary Research Organization (EAVRO), Muguga, Kenya.

While in Africa, Dr. Adams visited the following persons:

Dr. George Corry, Acting Director of EAVRO

Dr. I. E. Muriithi, Director of Kenya Veterinary Services, Kabete, Kenya

Dr. Mathew Cunningham, Project Manager of the FAO Tick-Borne Disease Project, EAVRO.

Dr. C. G. D. Brown, FAO Project, EAVRO

Dr. Mike Burridge, FAO Project, EAVRO

Dr. Tony Ervin, FAO Project, EAVRO

Dr. Alan Young, Serengeti Research Institute, EAVRO

Mr. Clem Kimber, FAO Project, EAVRO

Dr. Ordway Starnes, Director of the East African Agricultural and Forestry Research Organization, Muguga, Kenya.

Dr. Winston Malmquist, USDA, EAVRO

Dr. Jack Moulton, Faculty of Veterinary Science, University of Nairobi, Kabete, Kenya.

Dr. James Howe, Director USAID, Office of Regional Activities, Nairobi.

Mr. Eugene Reed, USAID, Regional Economic Advisor, Nairobi.

Dr. Bill Davis, Agricultural Attaché, United States Embassy, Nairobi.

Dr. Jan Le Roux, Kenya Veterinary Services Laboratories, Kabete Kenya.

Dr. John Tremlett, Chief, Kenya Veterinary Services Laboratories Kabete, Kenya.

Dr. Allan Wilson, East African Trypanosomiasis Research Organization, Tororo, Uganda.

Dr. C. K. Dar, East African Trypanosomiasis Research Organization, Tororo, Uganda.

Dr. T. Capstick, Wellcome Research Foundation, Kabete, Kenya

Dr. J. Burger, Wellcome Research Foundation, Kabete, Kenya.

Dr. Ruth Simpson, Wellcome Research Foundation, Kabete, Kenya.

Dr. W. O. Neitz, Onderstepoort Veterinary Research Institute, Union of South Africa.

Dr. J. vander Walt., Onderstepoort Faculty of Veterinary Science, Union of South Africa

To give the details of the conversations with each of the above persons would be contraindicated due to the length of the discussions, hence an attempt is made to summarize the general conversations in regard to organization, research on ECF, trypanosomiasis, babesiosis and anaplasmosis, and future possibilities of Texas A&M University participation in Africa.

The EAVRO, EAFFRO

Kenya Veterinary Services, University of Nairobi Faculty of Veterinary Science, Onderstepoort Veterinary Research Institute and Onderstepoort Faculty of Veterinary Science all appeared to be very well organized and productive institutions in training and services as well as research functions. The personnel in each case was of superior scientific quality. The working interrelationship between the various institutions was, however, fair. At EAVRO the possibility of Texas A&M University participation was discussed and was received quite well in that they were very receptive to the idea.

In regard to the research activities on ECF, a good understanding of the disease was ascertained. At length discussions on ECF in regard to, the new bovine splenic and lymph node lymphoblast tissue culture techniques, indirect fluorescent antibody diagnostic

techniques for piroplasms and macroschizonts, lymph node biopsies, low temperature preservation of ground up tick supernatant inoculum, *in vitro* tick feeding techniques, tick dissection techniques, use of chemotherapy in premunition techniques, *in vitro* arthropod tissue culture techniques, tissue culture vaccination methods, blood spot indirect fluorescent antibody diagnostic procedures, irradiation attenuated vaccine techniques, histocompatibility problems of tissue culture vaccine, histopathology and the epizootiology of ECF of cattle and game animals (cape buffalo, especially), were carried out with all of the research personnel. From these discussion, it was apparent that a considerable number of the ECF research techniques will have direct application to research on anaplasmosis, babesiosis and trypanosomiasis of cattle in Latin America, and many of the techniques will be utilized in the near future by the Texas A&M University Group in Colombia. It was interesting to observe the direct correlation between the approach used by the ECF workers and the approach used by the Texas A&M University Group in Colombia toward the solution of somewhat similar problems. The workers in East Africa have been extremely efficient and productive toward solving the problems of ECF control in cattle.

In reference to trypanosomiasis, detailed research techniques were discussed in relation to the indirect fluorescent antibody diagnostic test on serum and blood spots, antigenic variation tests,

pathogenesis, low temperature preservation procedures, chemotherapy, chemoprophylaxis, epizootiology and the use of insecticides to control tsetse populations.

The anaplasmosis and babesiosis research were discussed primarily in the realm of chemotherapy, premunition, vectors and serologic diagnostic techniques.

In all instances it was evident that most investigators thought that studies related to the pathogenesis of the hemoprotozoal infections of the arthropod vectors and the bovine host were absolutely essential to a final solution of the control of this group of diseases. From pathogenesis studies the investigators maintained that several by-products could be used to define better practical control programs because the most vulnerable part of the life cycle would be determined. Quantitative inoculla of determined clinical response mortality, incubation period and drug responsiveness were also indicated by most investigators to be of primary importance.

The scientific visit made by Dr. Adams was successful and highly productive for the Texas A&M University Group in Colombia, and it appears that participation by the Group in Africa is agreeable and indicated for the near future. A working liaison between hemotropic disease investigators in Africa and South America was apparently established for future collaboration.