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ITVM

PROJECT REPORT

1968 - 1978

~~U.S. Agency for International Development
Washington, D.C. 20523~~

A Research and Training Program in Tropical Veterinary Medicine

(USAID - csd - 1947, Colombia)
(USAID - ta - c - 1335)

Hemoparasitic Diseases of Ruminant Livestock

Colombia

Program Leader: F.D. Maurer 1968-1976

Program Leader: G.S. Trevino 1976-1978
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* These men spent a number of years, not included in the above statement, in Colombia as graduate students where they rendered invaluable service to the project.

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ACKNOWLEDGEMENTS

Personnel and staff of the Institute of Tropical Veterinary Medicine (ITVM) have over the years been the recipients of support, encouragement, advice, and assistance, from a large number of individuals and agencies. We greatly appreciate this assistance and wish to acknowledge all who so generously shared with us their resources. It is impossible to list individually all those who so helped, but we would like to mention some of those agencies who contributed to our program.

In 1966, The Rockefeller Foundation, New York, N.Y., (RF), gave ITVM a generous 3 year grant for hemoparasite research and training in Colombia, which was augmented in 1968 and replaced in 1969 by grants from the United States Agency for International Development (USAID) for continuation of this work. Support by USAID of this work has been continuous since 1968, and will officially terminate in 1978.

In 1967 we moved into the Instituto Colombiano Agropecuario (ICA), Laboratorio de Investigaciones Médicas Veterinarias (LIMV), Bogotá, Colombia where we remained until 1972. The cooperation and assistance of our Colombian colleagues was exemplary. In addition ICA allowed us to use their field stations at Tibaitatá, El Nus, La Libertad, Palmira, Turipaná, Carimagua, and others in pursuit of our studies.

In 1972 the ITVM team moved to Cali and occupied laboratories at the Centro Internacional de Agricultura Tropical (CIAT), where we were integrated into the Animal Health Program of that establishment. Our research staff in Colombia were considered an integral part of the CIAT Animal Health Program, and so enjoyed the same prerogatives as other CIAT resident staff. This association was an essential factor in the success we have had in Colombia.

Besides our indebtedness to RF, USAID, ICA, LIMV, AND CIAT we wish to acknowledge the support, cooperation, and assistance of the following institutions and agencies:

- 1.) Burroughs-Wellcome Laboratories - Research Triangle, N.C.
- 2.) Diamond Laboratories, Des Moines, Iowa
- 3.) School of Veterinary Medicine, University of Caldas, Manizales, Col.
- 4.) USDA-ARS Livestock Insects Laboratory, Kerrville, Texas
- 5.) Royal (Dick) School of Veterinary Studies, Centre for Tropical Veterinary Medicine, University of Edinburg, Scotland.
- 6.) Delta Regional Primate Research Centre, Tulane University
- 7.) U.S. Naval Medical Research Unit No. 3, Medical Zoology Department, Cairo, Egypt.
- 8.) Pfizer Inc., Groton, Conn. and Terre Haute, Ind.
- 9.) Ministry of Agriculture and National Development, Guyana.
- 10.) Texas State Veterinary Diagnostic Laboratory, College Station, Texas.
- 11.) College of Veterinary Medicine (Departments of Microbiology, Pathology, and Parasitology), College Station, Texas.
- 12.) USDA Haemoprotozoan Diseases Laboratory, Beltsville, Maryland.
- 13.) Department of Entomology, Texas A&M University, College Station, Texas.
- 14.) International Programs Office, Texas A&M University, College Station, Texas.
- 15.) Texas State Department of Agriculture, Austin, Texas.
- 16.) University of Illinois, College of Veterinary Medicine, Urbana, Ill.
- 17.) Hoffman La Roche, Nutley, N.J.
- 18.) School of Veterinary Medicine, National University, Bogota, Colombia.
- 19.) Animal Production and Health Division - FAO - Rome
- 20.) Vakgroep Tropische Diergeneeskunde en Protozoologie; Rijksuniversiteit te Utrecht, Utrecht, Netherlands.
- 21.) Emergency Programs, Veterinary Services, APHIS, USDA, Hyattsville, Md.
- 22.) Texas and Southwest Cattle Raisers Assn.
- 23.) Callaghan Ranch, Encinal, Texas
- 24.) Texas Animal Health Commission
- 25.) Texas Agricultural Experiment Station, Texas A&M University, College Station, Texas

NARRATIVE SUMMARY:

In 1968, at the inception of the program aimed at research and training in Tropical Veterinary Medicine several basic guide lines were established which for the past 10 years have significantly influenced our activities.

Obviously a major research effort on all livestock diseases of the tropics was not within our capability, nor was sufficient funding envisioned to support such an effort. It was decided that the major effort would be directed toward the arthropod-borne hemoparasitic diseases affecting ruminant livestock, principally cattle. Included within this group of diseases were anaplasmosis (Anaplasma marginale), babesiosis (Babesia bigemina, B. bovis), trypanosomiasis (Trypanosoma vivax), and theileriosis (Theileria cervi, T. mutans). With the exception of theileriosis all were diseases of major economic concern to the livestock producer in South and Central America and were serious constraints to animal production. Theileriosis is a major disease of East Africa, and the work done here with local Theileria organisms served as models of the pathogenic organisms.

At the recommendation of the Rockefeller Foundation, and with concurrence of the local authorities and the U.S. Agency for International Development, Colombia was selected as the principal overseas headquarters for our research and teaching program. We were first housed in the Laboratorio de Investigaciones Médicas Veterinarias (LIMV) facilities at Bogota and then in 1972, at the Centro Internacional de Agricultura Tropical (CIAT), Cali, where we remained until the termination of this program in 1978.

The urgent need for workable solutions to these problems led to an emphasis on applied rather than basic research. Research stressed the development of practical diagnostic serologic tests, to determine the incidence and immunologic and therapeutic studies to moderate losses. Anaplasmosis, a serious disease problem in the U.S. as well as Latin America, was studied mainly in the U.S. Babesiosis, while occurring sporadically in the U.S. is very rare, so this disease with its vectors was emphasized in Colombia. Trypanosomiasis (*T. vivax*) was studied in Colombia, and the Theileria sp. models (*T. mutans* and *T. cervi*) were studied in the U.S. where they normally occur.

Anaplasmosis:

Both the complement-fixation (CF) and the Card Agglutination (CA) tests are recognized by the USDA as standards in identifying Anaplasma carrier cattle. Comparisons between the CF and CA tests show a high degree of agreement. A new test, developed by ITVM staff in Colombia, based on an indirect fluorescent antibody (IFA), reaction has been found reliable, with some apparent advantages. Surveys using these tests showed a clear pattern of Anaplasma incidence in Colombia. Very little infection occurs above 6,000 feet elevation. Below 3,000 feet elevation, anaplasmosis is endemic.

The bulk of the work on anaplasmosis has been in the areas of vaccination and chemotherapy. We probably conducted the first laboratory and field trials in South America of the attenuated Anaplasma organism developed at Illinois by serial sheep passage. This vaccine produced a mild preimmunizing infection in animals, which were then completely resistant to needle challenge. Field trials on the North coast were less encouraging, although a level of resistance was clearly observed. Early work showed that preimmunization with virulent Anaplasma produced a

stronger level of immunity than the attenuated organism under North coast conditions. With this in mind, virulent stabilates were prepared by freezing in liquid nitrogen, titrated, characterized and used as preimmunizing inocula. Hundreds of cattle have been successfully vaccinated by these methods.

Trials were conducted, using the killed vaccine currently being marketed (Ft. Dodge, Anaplaz) in the U.S. The level of immunity produced by this preparation was generally inadequate.

Successful chemotherapeutic trials have been conducted on:

- 1.) Oral tetracyclines (American Cyanamid)
- 2.) Parenteral tetracyclines (Pfizer, Inc)
- 3.) Alpha-dithiosemicarbazone (Gloxazone, Burroughs Wellcome)
- 4.) Imidocarb dipropionate and dihydrochloride (Burroughs Wellcome)
- 5.) A long acting Terramycin (Pfizer Inc.)

A number of other experimental compounds have been tested, but found to be ineffective. Only the tetracyclines have been approved for use in the U.S. and South America. Even though Gloxazone and Imidocarb are probably more effective than the tetracyclines in use, a new long acting Terramycin offers considerable promise, is safe, and can be marketed. A practical, consistent and safe chemotherapeutic regime for removing the carrier state of anaplasmosis has yet to be developed, but all of the above drugs are therapeutically active.

The transmission of anaplasmosis is still poorly understood, and is an area where significant control measures might be possible. The concept that Boophilus ticks act as biological, transovarial vectors has recently been questioned even though there is an abundance of circumstantial evidence that these ticks are involved in the transmission of anaplasmosis.

There is a great deal of basic information needed which would contribute to our better understanding of anaplasmosis. Optimum control of anaplasmosis will probably require this type of information, derived from future research. Proper application, however, of the knowledge now available could prevent major losses from anaplasmosis. Vaccines can be used to protect clean cattle introduced into endemic areas, and calfhood vaccination procedures in zones of sporadic infection have proven adequate.

Babesiosis:

Serologic diagnostic procedures were not available to us when this project began, so techniques and antigens were developed based on literature, mainly from Australia, with modifications as seemed appropriate. The following serologic antigens and procedures have been developed for Babesia diagnosis:

- 1.) Complement fixation
- 2.) Indirect fluorescent antibody
- 3.) Indirect hemagglutination
- 4.) Latex Particle agglutination
- 5.) Rapid card agglutination
- 6.) Gel diffusion
- 7.) Enzyme labeled antibody

The first two are in routine use and have been used for incidence survey work throughout Colombia. In general, Babesia incidence parallels Anaplasma infection and Boophilus tick infestation. Little or no infection is encountered above 6,000 feet elevation, where as at lower elevations the disease is endemic.

Following procedures developed in Australia, with modifications, premunizing vaccines have been developed. A stabilate procedure is used, in which the vaccine is stored in liquid nitrogen, titrated and characterized before administration in much the same way as with Anaplasma vaccines. A marked attenuated effect has been induced in the

Babesia bovis organism, by serial passage through splenectomized calves. Animals receiving these preimmunizing vaccines occasionally require treatment, especially older animals. Ganaseg. (Squibb, 4,4' - Diaminodiazobenzene-di-acetamido-acetate) administered intramuscularly after the first sign of preimmunizing infection, using 0.5 mg/kg body weight, successfully moderates the clinical effects of infection without eliminating the infection, thus establishing a carrier infection and subsequent immunity. This treatment level is 1/6 the recommended therapeutic level.

Preliminary research at ITVM suggests that a killed, non-infective vaccine can produce a sterile immunity, sufficient to withstand a moderate Babesia challenge.

Two babesias, B. bigemina and B. bovis (argentina) have been recognized in the Western Hemisphere for years. It has generally been considered that B. bigemina is the organism primarily responsible for disease losses. Our evidence in the Cauca Valley indicates that B. bovis may be the more pathogenic. A method was developed by our team in Colombia to separate these two infections in situations where mixed infections occur.

Chemotherapy experiments have been very successful. Ganaseg is highly effective, and experiments have shown that as little as 0.25 mg/kg of this drug given early in the acute phase of B. bigemina infection will successfully block a fatal infection. This represents 1/12 the recommended dosage. A new drug, Imidocarb dipropionate (Burroughs Wellcome), has also been tested and found to have unusually high therapeutic efficacy. Administered at the rate of 5 mg/kg it effectively eliminates B. bigemina infections in the treated cattle and also eliminates the infection in the ticks feeding on treated cattle. This drug acts

as a chemoprophylactic for up to 6 weeks, and will rid ticks of their infections if they feed on cattle treated as long as 2 weeks previously.

Trypanosomiasis:

Trypanosoma vivax, a salivarian trypanosome, is usually thought of in association with tsetse fly transmission in Africa. However, this organism occurs in South America in the absence of tsetse flies and produces a debilitating, often fatal infection in sheep and cattle.

An indirect fluorescent antibody test was developed in Colombia which proved to be highly specific for the detection of serum antibodies. A survey of 2000 cattle revealed trypanosome infections in 5 of 11 departments.

Even though this infection is known to be widespread, little is known concerning the method of transmission, or the severity of the problem relative to actual production losses.

Incidence of Trypanosoma theileria in La Libertad was 62% (25 of 40 animals).

Theileriosis:

Both Theileria cervi and a benign bovine Theileria morphologically similar to T. mutans have been isolated and studied. The T. cervi occurring exclusively in white tailed deer is pathogenic and under some conditions is responsible for heavy death losses. Attempts to transmit this organism to cattle were unsuccessful. The tick Amblyoma americanum was found to be a transtadial vector of the infection.

The bovine Theileria is readily transmitted by blood inoculation, produces a low level of parasitemia in splenectomized calves, with a slight drop in PCV. The infection in intact calves is non-apparent. Efforts to transmit this organism with A. americanum and A. cajenense were unsuccessful. No serologic antigens were produced. This Theileria

was refractory to treatment with tetracyclines, Ganaseg and Imidocarb.

Ticks (*Boophilus*):

Boophilus microplus (Tropical Cattle Fever Tick) is prevalent in most areas of Colombia below 5000 feet elevation and is not only the principal vector of *Babesia*, but also the cause of ixodiasis, or tick worry, which produces a significant adverse impact on livestock production. Tick colonies have been established, methods of infecting ticks with *Babesia* have been described and extensive research conducted on the influence of certain types of vegetation on tick survival. It was found that ticks become infected during the last 24 hours of feeding on an infected host. Of 6 grass species analyzed, *Melinis minutiflora* (Molasses grass) showed the highest tick deterrent properties. This method of tick control has not been field tested but merits further investigation.

Training Programs:

These programs were emphasized early in the course of our work, largely because of the shortage of U.S. professional staff trained and experienced in the field of hemoparasites. The initial concept was to enroll U.S. veterinarians in the Graduate College at Texas A&M University where they could complete their course work, then spend a year or more in Colombia doing their research in the tropics, on tropical animal disease problems. It was felt that individuals trained both in the U.S. and in the tropics would then be better prepared to assume research and training responsibilities in developing tropical countries. It is gratifying to note that 9 of the 14 U.S. graduate students have or have had positions in which they were involved in veterinary programs in developing countries of Latin America. Four of these were affiliated with the Texas program in Colombia as staff members.

All but 1 have now found adequate placement elsewhere. Three of the 14 were associated with the military and their assignments have prevented continued pursuit of careers in tropical veterinary medicine. One student left Texas after receiving a Master of Science degree and went to Iowa State to pursue a Ph.D. program, and another joined a diagnostic laboratory in the U.S. The impact of these students in the area of animal health in the tropics is sure to be significant in years to come.

A list of U.S. graduate students, and the terminal degrees pursued in our program are presented in the Personnel Section; titles and abstracts of theses and dissertations are listed in Appendix 1.

It soon became obvious that our obligation and opportunities for training would not be limited to U.S. graduate students. There were a large number of academically qualified, and eager Colombian veterinarians seeking the opportunity to broaden their professional education through graduate studies. Through R.F. and USAID scholarships a number of Colombian students have come to TAMU for further studies. In addition, our staff in Colombia has actively participated in graduate programs in pathology and microbiology, offered by the veterinary faculty of the National University, Bogota, Colombia. Our staff not only participated in lectures, but also served on committees where they supervised research. Titles and abstracts of these studies are presented in Appendix 1.

In addition to formal courses and graduate degree programs offered to U.S. and foreign students there have been a large number of professional colleagues who have spent time at both the TAMU and Colombian laboratories to become acquainted with our program, and the techniques

developed at these laboratories. We have had students and trainees from Colombia, Peru, Brazil, Dominican Republic, Costa Rica, Bolivia, Nigeria, Kenya, Sudan, Guyana, Mexico, Japan, and Australia. Our staff have taken numerous trips to the countries of Central and South America, and the Carribean where seminars and training sessions were conducted. In addition we have traveled to Australia, Guam, and Malaysia for similar activities.

While it is sometimes hard to assess accomplishments associated with teaching and training programs, it is probable that in the future these efforts may well surpass in overall significance the research achievements of this program.

Introduction and Background

The tropics, historically, have not been associated with efficient, high-producing cattle, sheep, and goat industries; yet these areas are potentially highly productive. The abundant solar energy and adequate rainfall are responsible for rapid and luxuriant growth of forage plants. These forages are for the most part not utilized for human food, and unless converted by livestock into meat and milk are often wasted. It is doubtful if we can long continue to disregard this resource and potential, in view of the expanding world population and the ever-increasing demands for food.

There are several recognized deterrents to cattle production in the tropics, not the least of which are the arthropod transmitted blood diseases. For years such diseases of man such as malaria, trypanosomiasis, and yellow fever have stymied progress in the tropics. To a large extent the first two are still serious problems to human health. In tropical veterinary medicine, such diseases as theileriasis, anaplasmosis, babesiosis, and trypanosomiasis occur among food-producing animals.

Wilson et al. (Bulletin World Health Organization, 28: 595-613) estimates that the area in Africa virtually devoid of cattle, as a result of trypanosomiasis alone, exceeds the size of the United States. He estimates that this area could support 125,000,000 head of cattle were it not for this devastating cattle disease. Control of this disease, plus the other blood parasitic diseases mentioned above, would produce an economic-social impact on affected areas that could hardly be measured.

Experience in the tropics has emphasized the many facets of animal

production, perhaps more drastically than seen in temperate zones where diseases are not such a dominant factor. Disciplines such as genetics, nutrition, soils, forages, entomology, etc., as well as veterinary medicine, are essential to a maximum utilization of tropical resources for animal production. If we could consolidate expertise in all disciplines concerned with animal production into an integrated, cooperative effort, the results could well rival, if not surpass, the accomplishments of the so-called "Green Revolution" that has so significantly contributed to cereal grain production.

It was with this background, that our original project was submitted to AID for funding. This research proposal was aimed primarily at the applied, production-oriented aspects, but in so doing it was recognized that basic studies would be required to provide the groundwork for applied techniques. General objectives of the program were the following:

1. To develop diagnostic techniques for the purposes of identifying the problem, and studying the epidemiologic aspects for possible control procedures.
2. To develop immunization systems to curb losses, and reduce the severity of infection.
3. To investigate therapeutic agents for their use in:
 - a. Treating sick animals and preventing death.
 - b. Eliminating the infection on a herd basis from both the vertebrate and invertebrate hosts.
4. To develop an integrated control program based on scientific facts uncovered by the above studies.
5. To enroll veterinarians in graduate programs in tropical veterinary medicine, for research and application of disease control techniques.

PROJECT OBJECTIVES

1. To conduct research on tropical diseases aimed at developing information and methods for the control of these diseases, thus promoting productivity of beef and milk in developing countries. Initial emphasis will be placed on arthropod-borne blood diseases (anaplasmosis, babesiosis, theileriasis, and trypanosomiasis).
 - A. Anaplasmosis:
 - 1.) To evaluate and develop practical, and reliable serologic techniques for the diagnosis of anaplasmosis.
 - 2.) To survey anaplasmosis incidence in Colombia and to determine the possible economic impact of this disease.
 - 3.) To develop immunogens to prevent anaplasmosis.
 - a. Premunition
 1. To study and evaluate an attenuated Anaplasma as a vaccine.
 2. To develop Anaplasma stabilates from local strains which could be given safely.
 3. To compare these strains with the existing A. centrale organisms.
 - b. Sterile Immunity
 1. To assess quality of resistance imparted by killed vaccines.
 - 4.) To determine the source of transmission, with the goal of finding a method of control by eliminating transmission.
 - 5.) To evaluate chemotherapy.
 - a. To treat acute infection thus moderating clinical effects.
 - b. To eliminate infection and the carrier state of chronically infected animals.
 - 6.) To conduct pathogenic studies at high and low elevations, in

tropical and temperate zones.

7.) To study and evaluate control systems.

B. Babesiosis:

1.) To evaluate and develop practical and reliable serologic techniques for the diagnosis of babesiosis.

2.) To survey babesiosis incidence in Colombia and to determine the possible economic impact of this disease.

3.) To develop immunogens to prevent babesiosis.

a. Premunition- using both B. bigemina and B. bovis.

1. Prepare stabilates of each organism.

2. To study the attenuating effect of passage in splenectomized calves.

3. To evaluate possible strain differences which might influence the degree of immunity.

b. Sterile Immunity

1. Soluble plasma antigens.

2. Particulate blood based antigens.

3. To determine optimum adjuvant.

4.) To evaluate a vector control program to control disease in Colombia.

5.) To develop non-bovine sources of Babesia organisms from ticks and tissue culture for possible use in vaccines.

6.) To evaluate chemotherapy as a means of treating acute infections as well as eliminating chronic infections.

7.) To conduct pathogenesis studies to better understand the course of infection, and factors which might influence the course of disease.

8.) To study and evaluate control systems.

- C. To develop information on other related blood diseases, including Trypanosoma and Theileria infections.
- D. To train graduate students in research methods applicable to tropical diseases and to collect information, specimens, and illustrations for use in this training program.
- E. To provide trained faculty and staff to solve veterinary problems, design comprehensive animal health programs in developing countries, and to serve as consultants to related tropical disease problems.

Anaplasmosis: Serologic Diagnosis

Serologic diagnostic procedures have been described by others, and have been adopted for use both in Colombia and the U.S. The basic complement-fixation (CF) is an accurate test, but does require a trained technician, and a moderately well equipped laboratory. In recent years a rapid card test (CT) has been described by the USDA which can be performed on plasma or serum either at the laboratory or in the field within minutes of blood collection. A CT was performed on 469 head of cattle previously determined by CF tests to have over 80% positive reactors. These CT's were conducted at the ranch using plasma. The equipment, a small centrifuge and a rotator plate was powered by a car battery. There was an overall agreement of 93% between the CF and CT. These 2 tests were compared on a second herd of 430 cattle with a low incidence of infection (<10%). In this instance, the CT was done on serum, and the agreement was 95.8%. For the 899 samples the overall agreement between CT and CF tests was 94.3%.

In recent years a new test, the indirect fluorescent antibody test (IFA) has been adopted for use by our staff in Colombia. This test shows promise and on limited trials is even more specific and sensitive than either the CF or CT, particularly in long standing infections.

Anaplasmosis: Incidence and Distribution in Colombia

The incidence of anaplasmosis in a given area is dependent on the presence of suitable vectors, a susceptible host and a reservoir of infection.

Colombia, located on the northern tip of South America, borders Panama on the north, Venezuela and Brazil on the east, and Peru and Ecuador on the south, is the only South American country with extensive coastlines on both the Pacific and Caribbean. The equator passes through the southern part of Colombia, which for the most part lies between 0° and 10° north. The northern Andes, forming 3 mountain chains or cordilleras, effectively divide the country into mountain highlands, valleys, and lowland plains located on the north coast and the Llanos. The "Llanos" comprise almost half of Colombia and lie east of the mountains in the Orinoco and Amazon basins.

Even though Colombia is in the latitude normally considered tropical, a wide variety of climatic conditions do occur which reflect the different altitude zones.

It is generally recognized that more severe problems with hemotropic infections occur in the more tropical zones, but the incidence or severity of infection has not been determined by serologic methods. The complement fixation test using USDA antigen was used to determine the prevalence of infection at 5 stations located in 5 different climatic zones. Attention was given to variations in age and breed in the hope of securing a better understanding of the epidemiologic characteristics of anaplasmosis. Serums from a total of 603 cattle of varying ages, and breeds, were tested. A summary description of each station is tabulated in Table 1.

TABLE 1

Climate Description* of ICA Stations

Name	Location in Colombia	Size Acres	Annual Precip- itation	Annual Mean Temperature	Elevation	Climate
Tibaitatá	Cundinamarca	1,325	740 mm	14°C	2600 M.	Cold
El Nus	Antioquia	4,164	1390 mm	23°C	1200 M.	Medium
Palmira	Valle	1,046	1005 mm	24°C	1000 M.	Medium- Hot
La Libertad	Meta	3,254	4261 mm	26°C	450 M.	Hot
Turipaná	Cordoba	4,292	1112 mm	28°C	13 M.	Hot

*Obtained from Instituto Colombiano Agropecuario Report dated July 5, 1966, and signed by Fernando Penaranda Canal, General Director of ICA.

Tibaitatá, located only a few miles from Bogotá, is situated on the Bogotá Savannah at an altitude of 2600 meters. It is cool and there is very little seasonal variation in temperature.

El Nus is located in the foot hills of the Andes a short distance from the Magdalena River. This station has an altitude of 1200 meters. The lush vegetation gives a false impression of rolling hills, where as in reality the slopes are very steep.

Palmira at 1000 meters is located in the Cauca Valley near Cali between the western and central Cordilleras. This land is very fertile, making possible high stocking rates. The cattle tested in this area were exclusively Holstein-Friesian. The temperature here is noticeably warm.

La Libertad is located on the western edge of "Los Llanos" and at the base of the eastern Cordillera having an altitude of 450 meters. This area is characterized by extremely heavy rainfall and high mean temperatures.

Turipaná, located on the north coast at an altitude of 13 meters, is very hot and humid, and characteristically tropical. The soil in this area is fertile, but because of drainage problems is not being used to its capacity. Improved pastures have high stocking rates, but the climate is generally considered undesirable for some livestock.

Complement-fixation tests for anaplasmosis were conducted using essentially the technique described by the USDA. Serum titrations were conducted using a microtiter technique. At the 1/5 dilution, serums were considered positive if the reactions were classed as 3+ or 4+ (0 to 25% hemolysis); suspicious if the reactions were classed 1+ or 2+ (50 to 75% hemolysis); negative if the reactions were absent or virtually so (only a few cells remaining).

Age and breed were recorded on all animals for comparisons at each station.

Cattle were selected at random, but an effort was made to obtain sizeable groups less than 1 year of age, 1-2 years of age, 2-3 years of age and animals over 3 years of age. This was done at all stations except at El Nus where it was necessary to group 1-3 year old animals together to give a sufficiently large number for statistical analysis.

A variety of cattle breeds were encountered. The following cattle breeds and crosses were used:

- 1.) Holstein-Friesian (Palmira, Tibaitatá, Turipaná)
- 2.) Blanco Orejinegro, (BON). (El Nus)
- 3.) BON 3/4 x Jersey 1/4 cross (El Nus)
- 4.) San Martinero (La Libertad)
- 5.) Zebu (La Libertad, Turipaná)
- 6.) Angus (La Libertad)
- 7.) Jersey (La Libertad)
- 8.) Costeño con Cuernos (CCC) (Turipana)
- 9.) Romosinuano (R.S.) (Turipaná)

At Tibaitatá and La Libertad blood cultures were made on 31 and 40 animals respectively for the detection of Trypanosoma theileri. Each of 4 blood agar slants were inoculated with 0.5 ml oxalated blood from each animal tested.

Tibaitatá:

Complement-fixation tests for anaplasmosis on 92 cattle at Tibaitatá resulted in 3 suspicious reactions with the remainder negative (Table 2). Cattle over 3 years of age showed an average PCV of 31.9%. The remaining age groups, 2-3, 1-2, and less than 1 year of age showed PCVs of 31.8%, 34.7%, and 33.9% respectively. The two latter groups, 1-2 and less than 1 year of age, showed significantly higher PCVs than the two older groups.

Blood cultures for T. theileri were all negative.

TABLE 2
 Anaplasmosis Survey at
 Tibaitata*
 (Holstein Friesian)
 CF and PCV Results in Varying Age Groups

	Avg. Age months	No. of Animals	Avg. PCV	Complement-Fixation		
				Pos.	Susp.	Neg.
>3 years of age	88.6 ±33.4	20	31.9%	0	1 (5%)	19 (95%)
2-3 years	31.1 ± 1.1	11	31.8%	0	0	11 (100%)
1-2 years	15.2 ± 2.6	24	34.7%	0	2 (8%)	22 (92%)
<1 year	7.0 ±1.2	37	33.9%	0	0	37 (100%)
Significance			P<0.01 DRS= 1.6			
Totals		92	33.4%	0	3 (3%)	89 (97%)

* Trypanosoma theileri cultures made on 31 samples were 100% negative.

DRS = Difference required for significance.

TABLE 3

Anaplasmosis Survey at
El Nus(BON and $\frac{1}{4}$ Jersey-BON Crosses)

CF and PCV Results in Varying Age Groups

	Avg. age Months	No. of Animals	PCV	CF Tests			Blood Smears			Avg. CF Titer of Pos. Samples
				Pos.	Susp.	Neg.	Pos.	Susp.	Neg.	
>3 yrs. of age	99 ±30	40	34.6	21 (52%)	10 (25%)	9 (23%)	1 (2%)	1 (2%)	38 (95%)	1/8
1-3 yrs. of age	22.4 ±4.4	20	35.6	8 (40%)	3 (15%)	9 (45%)	1 (5%)	0	19 (95%)	1/8
<1 yr. of age	4.8 ±2.3	30	35.0	2 (7%)	2 (7%)	26 (86%)	0	1 (3%)	29 (97%)	1/10
Significance			N.S.							
TOTALS		90	35.0	31 (34%)	15 (17%)	44 (49%)	2 (2%)	2 (2%)	86 (96%)	

N.S. - Not Significant

BON - Blanco Orejinegro

TABLE 4

E1 Nus

Breed Comparisons of Anaplasmosis Incidence and PCV

	No. of Animals	Avg. Age in Months	PCV	CF Results			CF Titers of Pos. Samples
				Pos.	Susp.	Neg.	
BON	23	114 ±8	36.1	12 (52%)	8 (35%)	3 (13%)	1/7
¼ Jersey-BON	17	79 ±36	32.6	9 (53%)	2 (12%)	6 (35%)	1/9
Significance			P<.01				
BON	18	4.7 ±2	34.6	2 (11%)	0	16 (89%)	1/10
¼ Jersey-BON	12	4.8 ±3	35.4	0	2 (17%)	10 (83%)	0
Significance			N.S.				

BON - Blanco Orejinegro

N.S. - Not significant

TABLE 5
 Anaplasmosis Survey at
 Palmira
 CF and PCV Results in Varying Age Groups

	Age in Months	No. of Animals	Avg. PCV	CF Tests			Blood Smears		
				Pos.	Susp.	Neg.	Pos.	Susp.	Neg.
>3 years	62.3 ±2.7	58	29.5	32 (55%)	14 (24%)	12 (21%)	2 (3%)	5 (9%)	51 (88%)
2-3 years	28.7 ±3.6	30	29.6	18 (60%)	7 (23%)	5 (17%)	3 (10%)	7 (23%)	20 (67%)
1-2 years	15.8 ±2.0	17	29.4	2 (12%)	7 (41%)	8 (47%)	0	1 (6%)	16 (94%)
<1 year	5.8 ±3.7	44	30.9	10 (23%)	4 (9%)	30 (68%)	1 (2%)	0	43 (98%)
Significance			N.S.						
TOTALS		149	29.9	62 (42%)	32 (21%)	55 (37%)	6 (4%)	13 (9%)	130 (87%)

N.S. - Not significant

E1 Nus:

Complement-fixation tests of serums from 90 cattle at E1 Nus resulted in 31 positive, 15 suspicious and 44 negative (Table 3). The incidence was higher in the older animals, with 77% showing either a positive or suspicious reaction in cattle over 3 years of age, 55% reacting in the 1-3 year old group and 14% reacting in those animals less than 1 year of age. No significant differences were detected in PCVs observed in the 3 groups. Average serum CF titers of positive samples from the 3 age groups failed to show any significant difference.

The 23 Blanco Orejinegro (BON) cattle over 3 years of age showed 52% positive and 35% suspicious reactions. Of 17 Jersey-BON crosses, 53% positive and 12% suspicious reactions were noted (Table 4). Among 30 approximately 5 month old calves no significant differences in response to the CF test were noted. The average PCV of the 17 Jersey-Bon crosses over 3 years of age was 32.6%, which proved to be significantly lower than the average 36.1% observed in the 23 BON cattle, with a probability of error of less than 0.01. No significant differences were detected between average PCVs in calves of similar breeding.

Palmira:

Complement-fixation tests on 149 Holstein-Friesian cattle at Palmira resulted in 62 (42%) positive, 32 (21%) suspicious and 55 (37%) negative. (Table 5) Generally, the incidence was greatest in the older animals. Either a positive or suspicious CF response was observed in 79% of the cattle over 3 years of age, 83% of the cattle 2-3 years of age, 53% of the cattle 1-2 years of age and 32% of the calves less than 1 year of age. No significant differences in PCVs occurred in the 4 age groups tested, even though the herd average of 29.9% was lower than seen in other areas.

La Libertad:

The survey results at La Libertad are recorded in Tables 6 and 7.

TABLE 6

La Libertad

(San Martineros, Zebu, Angus, Jerseys)

	Age in Months	No. of Animals	Avg. PCV	CF Tests			Blood smears		T. Theileri		Avg. CF Titer of Positive Samples
				Pos.	Susp.	Neg.	Pos.	Susp.	Neg.	Cultures	
>3 years of age	78 ±29	29	34.3	12 (41%)	16 (55%)	1 (3%)	0	0	29 (100%)	9/9Pos.	1/6
2-3 yrs. of age	28 ± 3	10	34.9	4 (40%)	5 (50%)	1 (10%)	0	0	10 (100%)	2/2Pos.	1/5
1-2 yrs. of age	18 ± 2	47	32.3	19 (40%)	16 (34%)	12 (26%)	1 (2%)	4 (9%)	42 (89%)	10/14Pos.	1/8
>1 year of age	7 ± 2	35	33.8	4 (11%)	6 (17%)	25 (71%)	1 (3%)	0	34 (97%)	4/15Pos.	1/14
Significance			N.S.								
TOTALS		121	33.4	39 (32%)	43 (36%)	39 (32%)	2 (2%)	4 (3%)	115 (95%)	25/40Pos.	

N.S. - Not significant

TABLE 7

La Libertad

Breed Influence on the Incidence of Anaplasmosis and PCVs in Yearling Cattle

	No. of Animals	Avg. Age Months	Avg. PCV	CF Results			Avg. CF Titer of Positive Samples	<u>Tryp. theileri</u> Cultures
				Pos.	Susp.	Neg.		
San Martinero	14	17.9 ± 9	30.4	6 (43%)	5 (36%)	3 (21%)	1/8	3/3 Pos.
Zebu	17	18.5 ± 9	35.2	7 (42%)	5 (29%)	5 (29%)	1/7	3/5 Pos.
Jersey	9	17.8 ± 8	31.0	3 (33%)	4 (44%)	2 (22%)	1/8	2/2 Pos.
Angus	7	18.0 ± 7	31.0	2 (29%)	3 (43%)	2 (29%)	1/7	2/3 Pos.
Significance			P<0.01					
		DRS:	4.2					

DRS: Difference Required for Significance.

Of 121 cattle tested, 29 were over 3 years of age, 10 were 2-3 years of age, 47 were 1-2 years of age and 35 were less than a year of age. Among the 4 age groups no significant difference in PCV occurred. The older animals showed a greater incidence of CF response. The number of positive or suspicious reactions in cattle over 3 was 28 (96%), 2-3 years of age 9 (90%), 1-2 years of age 35 (74%), and among calves less than 1 year of age 10 (28%). The overall herd response was 39 (32%) positive, 43 (36%) suspicious and 39 (32%) negative.

Differences between 14 San Martineros, 17 Zebu, 9 Jersey, and 7 Angus, averaging approximately 18 months of age, were considered in Table 7. Zebu animals showed a significantly higher PCV when compared to the other 3 breeds. Breed response to the CF test did not appear different. There was no difference in CF titers, or trypanosome incidence that could be attributable to breed.

A total of 25 of 40 (62%) of all animals tested were positive for I. theileri. Higher numbers of positive cultures occurred in the older animals. The incidence decreased in younger animals.

Turipaná:

Complement-fixation tests performed on serums from 151 cattle at Turipaná resulted in 116 (77%) positive, 21 (14%) suspicious, 13 (9%) negative, with 1 sample anti-complementary (Table 8). Of 27 calves less than 1 year of age, 74% were either positive or suspicious to the CF test. All other groups showed over 90% reacting, with 100% of the cattle 1-2 years of age showing either a positive or suspicious response. A significantly lower average PCV was observed in the 1-2 year old group. No other differences in PCVs were observed.

Complement-fixation titers of positive serums were significantly higher in young animals ranging from an average of 1/39 in those less than 1 year of age to 1/10 in cattle over 3 years of age.

TABLE 8

Turipaná

(Costeño con Cuerno, Romosinuano, Zebu, and Holstein)

>3 years of age	91 ±35	55	31.3	34 (62%)	16 (29%)	5 (5%)	0	1 (2%)	54 (98%)	1/10
2-3 yrs. of age	29 ± 3	33 ^a	30.9	29 (88%)	2 (6%)	1 (3%)	1 (3%)	6 (18%)	26 (79%)	1/15
1-2 yrs. of age	17 ± 3	36	25.8	34 (94%)	2 (6%)	0	11 (31%)	7 (19%)	18 (50%)	1/23
<1 year of age	4 ± 3	27	31.1	19 (70%)	1 (4%)	7 (26%)	12 (44%)	4 (15%)	11 (41%)	1/39
Significance			F=9.31 P<0.01							F=6.77 P<0.01*
		DRS	3.2							
TOTALS		151	29.9	116 (77%)	21 (14%)	13 (9%)				

- *1.) Significance is reached between adult cattle (>3 yrs. of age) and the 1-2 year old and calves (<1 year old).
 2.) Significance is reached between 2-3 yrs. old and calves <1 year of age.

DRS: Difference required for significance
 a.- one was anticomplementary

At Turipaná, blood smears for anaplasmosis were positive in 44% of the calves less than 1 year of age and 31% of the 1-2 year old animals, with only 1 or 3% positive in the 2-3 year old group and none in adult cattle over 3 years of age.

Breed differences are recorded in Table 9. Among a total of 55 adult cattle representing 4 breeds, there were 5 negative to the CF test. Of these 5, 4 were Costeño con Cuernos (CCC). The average PCV of 25 CCC while lower than Romosinuano (RS) and Zebu, failed to reach significance. A group of 10 Holstein cows had an average PCV of 24.3% which was significantly lower than averages for the other 3 groups.

Among the 2-3 and 1-2 year of age groups, significantly lower PCVs occurred with the CCC, when compared to RS. Nearly all animals of these age groups reacted to the CF test.

Comparisons of 11 CCC and 15 RS calves under 1 year of age failed to show any significant difference in PCV, even though the CCC followed the previous trend of being lower than RS.

Among 94 animals less than 3 years of age, only 7 were negative to the CF test. Of these 7, 6 were CCC and 1 a RS calf.

A summary of station response to the CF test as correlated to altitude and mean temperature is presented in Table 10. The incidence of CF response (positive and suspicious reactions combined) appears to be related to mean temperature and elevation. As the altitude decreased, the mean temperature and incidence of CF response increased. At Tibaitatá, with a mean temperature of 14, the CF response was 3%, El Nus at 23°C had 51% reactors, Palmira at 24°C had 60% reactors, La Libertad at 26°C had 67% reactors and Turipaná, at 28°C had 91% reactors. This correlation between temperature and anaplasmosis incidence is highly significant ($r= 0.99$, $P<0.01$).

In view of both breed and age influence on PCV, a comparison between stations is possible only on animals of comparable age and breed. Such

TABLE 9

Breed Influence on PCVs and Incidence of Anaplasmosis

	No. of Animals	Avg. Age in Months	Avg. PCV	CF Results			Avg. CF Titer of Positive Samples
				Pos.	Susp.	Neg.	
>3 years of age: CCC	25	110 ±32	31.6	13 (52%)	8 (32%)	4 (16%)	1/7
R.S.	15	76 ±31	34.3	9 (60%)	6 (40%)	0	1/7
Holstein	10	61 ± 9	24.3	7 (70%)	2 (20%)	1 (10%)	1/18
Zebu	5	102 ±33	34.8	4 (75%)	1 (25%)	0	1/10
Significance		DRS = 4.2	(P<0.01)				N.S.
2-3 yrs. of age CCC	15	27 ± 2	26.5	13 (93%)	0	1 (7%)	(1 AC) 1/16
R.S.	18	31 ± 2	34.6	16 (89%)	2 (11%)	0	1/15
Significance			P<0.01				N.S.
1-2year of age CCC	19	16 ±3	22.5	17 (89%)	2 (11%)	0	1/30
R.S.	16	19 ±2	30.2	16 (100%)	0	0	1/15
Significance			P<0.01				N.S.
<1 year old CCC	11	2.5 ±3	30.5	6 (55%)	0	5 (45%)	1/30
R.S.	15	5.2 ±3	32.1	13 (87%)	1 (7%)	1 (7%)	1/67
Significance			N.S.				N.S.

CCC - Costeño con Cuernos

R.S. - Romo sinuano

DRS - Difference Required for Significance

N.S. - Not Significant

TABLE 10

Summary of CF Response at Each of the 5 Stations Tested

Location	Altitude	Mean Temperature	CF Results	
			Pos. and Susp.	Negative
Tibaitatá	2600M	14°C	3%	97%
El Nus	1200M	23°C	51%	49%
Palmira	1000M	24°C	60%	40%
La Libertad	450M	26°C	67%	33%
Turipaná	13M	28°C	91%	9%

TABLE 11

Summary of Packed Cell Volume and Anaplasmosis Incidence in
Holstein Adult Cows at Tibaitatá, Palmira, and Turipaná

	No. of	Avg. Age	PCV	CF		
				Pos.	Susp.	Neg.
Tibaitatá	20	88.6 ±33.4	31.9	0	0	20
Palmira	58	62.3 ±14.5	29.5	32 (55%)	14 (24%)	12 (21%)
Turipaná	10	61.1 ± 9.1	24.3	7 (70%)	2 (20%)	1 (10%)
Significance			P<0.01 DRS = 2.4			

DRS - Difference Required for Significance

a comparison (Table 11) is possible with adult Holstein cows at Tibaitatá, Palmira, and Turipaná. At Tibaitatá an average PCV of 31.9% was recorded for 20 animals. At Palmira an average PCV of 29.5% was recorded for 58 animals and at Turipaná observations on 10 cows showed an average of 24.3%. These differences proved to be highly significant. The incidence of CF response also increased at Palmira and Turipaná.

An attempt to show a relationship between the prevailing PCVs and CF response at El Nus, Palmira, and La Libertad was unsuccessful. At Turipaná (Table 12) a significant correlation between CF titer and PCV was noted. In 58 animals 2 years of age and under, a correlation coefficient of -0.404 was noted, which is significant at the 0.01 level. Lower PCV values were observed in association with higher CF serum titers. In 61 animals over 2 years of age a trend of lower average PCVs at the higher serum titers occurred but failed to reach significance.

Discussion and Conclusions:

Cattle native to the Bogotá Savannah were found to be free of anaplasmosis. The constant year round cool temperature apparently provides an unfavorable environment for the usual arthropod vectors.

Since no restrictions exist to prevent the importation of infected cattle, it is highly probable that infected cattle are on the Savannah, but significant transmission does not occur.

Even though the exact vector or vectors for Trypanosoma theileri are unknown, the consistent absence of infection on the Bogotá Savannah is probably the result of an absence of transmission.

El Nus, Palmira and La Libertad show a gradually increasing incidence of anaplasmosis in this order. The somewhat higher CF titers in the younger animals probably reflects more recent acute disease.

Age did not influence PCVs, but breed differences occurred at El Nus and La Libertad. Mature milking cows at El Nus with ¼ Jersey

TABLE 12

Turipana'

Average PCV in Animals Reacting to the CF Test

CF Response and Titer	No. of Animals <2 yrs of age	Average PCV	No. of Animals >2 yrs of age	Average PCV
Negative, Suspicious And 1/5 titers	12	28.6%	40	32.5%
1/10 and 1/20	21	28.0%	7	30.5%
1/40	14	27.8%	9	29.7%
1/80 and over	11	22.2%	5	28.8%
Correlation Coefficient		-0.404 P<0.01		-0.221 NS

NS - Not significant

breeding had lower PCVs than a comparable group of pure BON cows. The reason for this is not fully understood, but cannot positively be attributed to a greater incidence of anaplasmosis, or even to a more severe reaction to anaplasmosis.

The trend of greater anaplasmosis incidence in older animals, decreasing progressively to the calves, was a consistent observation at all stations. At El Nus, Palmira, and La Libertad a majority of animals become infected before reaching 3 years of age, and probably are infected within the first 2 years of life.

At La Libertad, Zebu yearlings, while showing essentially the same rate of anaplasmosis infection, showed significantly higher PCVs. This apparent genetic difference may well play a role in the relative efficiency of this breed in the tropics.

In contrast to El Nus, Palmira, and La Libertad, tests at Turipaná suggest active infections with a current rapid rate of transmission. The 70% positive CF reactions in calves less than a year of age reflect a much more rapid rate of infection than seen elsewhere. The effects of anaplasmosis and probably, babesiosis, are seen most markedly in the 1-2 year old group as a significantly lower PCV. The increased Anaplasma activity in these young animals is also reflected by a high parasitemia on blood smears, and the increased CF titers in this group.

This depression of PCV is even more impressive when it is remembered that calves of comparable age at Tibaitatá, free of hemotropic disease, showed significantly higher PCVs than older animals.

It seems probable that the massive exposure and re-exposure to Anaplasma of even young calves is sufficient to result in a depressed PCV during the first 1 to 2 years of life. If this is the case, it could well be a partial explanation for "poor doing" calves, and animals that are slow maturing and slow to gain weight.

It is probable that the active nature of anaplasmosis infections at Turipaná accentuated breed differences in PCVs which were evident in this survey. Among adult cattle, the most significant finding was the low average PCV seen among Holsteins. In addition to low PCV, the average CF titer was somewhat higher in this group, suggesting a greater level of anaplasmosis activity among these animals. In cattle over 3 years of age the average PCVs of 2 beef breeds, Zebu and Romo Sinuano (RS), were higher than the Costeño con Cuernos (CCC) dairy animals. Among 2-3 and 1-2 year old animals this PCV difference reached significance, comparing RS and CCC. It is not understood why dairy animals, even non-lactating animals, should show this depressed PCV. It is probable that factors other than anaplasmosis are involved. The development of reliable serologic tests for babesiosis and trypanosomiasis will contribute to better understanding concerning the interaction of these hemoparasites. Until the development and use of such tests, the interpretation of low PCVs will have to remain speculative.

These studies in general show not only the geographic distribution of anaplasmosis, but also indicate the urgent need for a control program. The early age of infection suggests the need for a program of calf immunization or premunization.

The absence of anaplasmosis above 2600M, and the endemic nature of the disease below 1200M suggests the need for extreme caution in moving cattle from the high altitude zones to the tropical areas, which as seen in Colombia, may be only a few miles apart.

Anaplasmosis: Transmission

The transmission of anaplasmosis in the tropics despite well publicized theories and concepts remains an enigma.

In tropical areas of Colombia, and throughout the tropical world, Boophilus ticks have long been recognized as a major vector. In Australia, and South Africa the occurrence of Boophilus ticks is directly related to the incidence of anaplasmosis. Anaplasmosis does not occur outside of the Boophilus areas, and yet in recent years work by our team in Colombia, in Texas, and reports from Australia have thrown considerable doubt on probability of transovarial transmission of anaplasmosis by this tick. Since Boophilus ticks are 1-host ticks, theoretically they could only transmit disease by transovarial means. Efforts to accomplish transovarial transmission have uniformly failed in Colombia and Australia. Trials in Texas have been erratic, but for the most part also have failed. In 3 instances anaplasmosis occurred in splenectomized calves after Boophilus larvae were released. In all instances the incubation times were prolonged. In two instances the release of B. annulatus larvae was followed by acute anaplasmosis with death occurring on day 65. A third successful trial involved B. microplus ticks which were intentionally exposed to Anaplasma marginale while feeding. The larval progeny of these ticks were then placed on a susceptible splenectomized calf, which developed acute anaplasmosis 62 days later, a parasitemia of 20% with eventual recovery. There have been many other similar attempts at Texas which were unsuccessful in transmitting Anaplasma.

A recent trial to evaluate 3 premunizing vaccines was conducted on the north coast of Colombia (Turipaná) which produced further circumstantial evidence that Boophilus may not be the principal vector of anaplasmosis. It is well documented and accepted that the incubation

time for anaplasmosis is directly proportional to the level of exposure. Anaplasmosis can be produced in 2 days or in 80 days depending on the size of the exposing inocula. In this experiment, as yet unpublished, a total of 54 calves were moved to the Turipaná station where 26 were inadvertently exposed to an extreme tick stress with half body counts of over 300 in 18 days. The remaining 28 animals showed an average body count of < 10. Despite over a thirty fold difference in the number of ticks present on the heavily infested calves, the Anaplasma incubation time was 51.3 days for the light tick infestation and 54.1 days in those animals with heavy tick infestation, which was not significantly different.

Workers in South Africa tend to believe that biting flies are not a major factor in the transmission of anaplasmosis. In Australia the same feeling exists, where as in the U.S., and other parts of Africa, flies are considered an important vector. There is experimental evidence to show that transmission occurs with at least 20 species of ticks including Boophilus, Hyalomma, Rhipicephalus, Dermacentor, Ixodes, and Haemaphysalis ticks and at least 10 species of biting flies, but under conditions of natural field transmission there are many unexplained paradoxes which create problems in unequivocally establishing the means of disease spread.

Anaplasmosis: Systems of Immunization

The only successful methods developed for immunization against anaplasmosis in the tropics have involved the principle of premunition. Killed vaccines using adjuvants and concentrated antigens have, to date, been unsatisfactory.

The ideal approach is to produce a replicating infection of Anaplasma without producing serious clinical effects. The retention of chronic, non-apparent infections will provide adequate protection from the supposedly constant exposure to Anaplasma occurring in the tropics.

Virulent Field A. marginale:

It has been common in the past to artificially premunize calves born in endemic areas of the tropics with blood from local cattle known to be carriers of the infections. Young animals (<4 months of age) can safely receive these inocula, which will result in premunition and immunity to future Anaplasma exposure, but such a practice is not recommended. Perhaps the greatest hazard is the potential of transmitting other diseases such as leptospirosis, babesiosis, trypanosomiasis, ephemeral fever, theileriosis, viral mucosal disease complex, lymphosarcoma and a host of other infectious diseases of cattle. The possibility of disease transmission by the indiscriminate transfer of blood creates strong doubts about the value of this procedure. In effect this approach might well result in greater health problems, with subsequent reduced productivity, than it would prevent.

The use of broad spectrum antibiotics to moderate the effects of Anaplasma in older animals does not solve the basic objection to this method of premunition, since most of the viral agents potentially capable of being transmitted are refractory to tetracyclines.

Dilute Stabilate:

The Anaplasma used in these stabilates, while traceable to virulent strains, has the advantage of numerous needle passages, during which time the purity of infection has been well established. A slight reduction in virulence is thought to occur with these artificially maintained strains. The use of diluted stabilates is also a factor in producing extended incubation times and milder initial response.

Perhaps the greatest advantage of dilute stabilates, however, is the ease, safety, and economy of production. Approximately 24,000 doses of vaccine could be prepared from 1000 ml. of blood removed from a single 400 pound Anaplasma-infected calf with a packed cell volume of 24% and a parasitemia of about 20%. The calf need not be sacrificed by this procedure. The size and amount of the infecting inocula is dependent on results of standardized titrations. We have found an optimum incubation time to be between 30-34 days. This can be achieved using 1-5 ml. of a 10^{-2} or 10^{-3} dilution of the frozen stabilate. These frozen stabilates can be stored in liquid nitrogen for periods in excess of 2 years; however, annual standardization and safety checks are recommended for each stabilate. Safety checks on the infecting inocula, and the prolonged storage and use of the same stabilates essentially eliminates the hazard of accidental transmission of other infectious agents.

These inocula can be used safely in young animals up to one year of age; however, in older animals treatment with a tetracycline is recommended. Since the incubation time is known, a single treatment can easily be synchronized to correspond with the parasitemia without laboratory confirmation. The tetracyclines can be given either orally or parenterally. If oral treatment is preferred, an oral preparation is fed for a four-day period at a level of 3 to 5 mg/lbs (chlortetracycline)

daily. A single intravenous inoculation of 5 mg/lbs (oxytetracycline) is usually sufficient when given early in the course of developing parasitemia.

The degree of protection thus induced has been found superior to that produced by attenuated Anaplasma when challenged by field exposure.

Attenuated A. marginale:

This Anaplasma is thought to have been attenuated by a large number of passages through sheep over a period of years. It is assumed that during this process the organism became attenuated for cattle, but retained the same basic antigenic make-up.

Experimental evidence by workers, other than those who developed it or the company producing the vaccine, have confirmed the attenuated nature of the organism and the antigenic similarities to A. marginale. Basically, premunition with this organism can be safely done without treatment and, if replicating infections occur, the animals will be immune, or at least resistant, to field challenge.

This approach to vaccination is not without some hazards. First, severe reactions have been observed in lactating dairy cattle and in older beef cattle. These reactions resulted in packed cell volumes so low that death losses might on occasion be expected. Second, in very young calves (< 6 months) the virulence of the organism is below the level needed to produce replicating infections in all animals; hence, some of these cattle might remain susceptible following vaccination. This factor might be compensated for by adjusting the infecting inocula, and by a serum check after a suitable interval (40 to 50 days). The infecting titer of frozen sheep blood is generally low, and represents a foreign protein when injected into cattle. Injection of large volumes of sheep blood intravenously into cattle will nearly always be associated with severe pulmonary distress, possibly collapse and

death. The inocula of 1 to 5 ml is not hazardous, however. A third drawback which has been detected is that, under severe field challenge, animals premunized with attenuated *Anaplasma* were not as solidly protected as were those that had been premunized with a virulent organism. The reasons for this are not known since, on needle challenge, animals carrying the attenuated strain were solidly protected. A fourth disadvantage which needs documentation is that reversion of the organism to virulence has been demonstrated both by Texas and Florida investigators. A significant increase in virulence was attained by serial back passage of the attenuated organism in splenectomized calves. After 12 such passages, a decided increase in virulence was noted. It would seem advisable to compare this strain with an antigen derived from *A. centrale*. In all probability *A. centrale* would not revert to virulence, but this is not well established. Finally, it should be borne in mind that injection of the sheep-derived antigen into cattle might inadvertently transmit bluetongue or malignant catarrhal fever to cattle.

This vaccine is now commercially available in many Latin American countries, particularly Mexico, and Central America.

Anaplasma centrale:

This is a naturally attenuated *Anaplasma* first discovered over sixty years ago by Theiler, and used since that time as a vaccine for anaplasmosis in Africa, Asia, Australia and South America. This organism is widespread and readily available. It has probably had greater use than almost any other veterinary biological. Even so, it is not without drawbacks. Generally, it can be safely used in all age groups; caution is indicated in lactating dairy cattle and older beef animals.

A. centrale is not as closely related antigenically to A. marginale as is the attenuated organism previously discussed. It does, however, protect against virulent challenge and reduces the severity of infection and prevents death losses.

It has a further handicap in that cattle are the source of the premunizing inocula, hence posing the threat of transmitting other cattle diseases as previously discussed. The use of frozen stabilates for this vaccine with the proper screening and standardization as used for the "dilute stabilates," could minimize this hazard.

Comparisons of infections observed in cattle of varying ages showed that those produced by the attenuated Anaplasma and A. centrale to be about the same in relative virulence.

In addition to the long history of successful use, A. centrale has the added advantage of being readily available. The organism can be maintained for years in splenectomized calves, or can be produced as frozen stabilates for use in premunizing campaigns. This can be accomplished in accordance with procedures documented in the literature by almost any properly equipped laboratory with technical competency. It is now being successfully used in a number of South American countries. A notable exception in South America is Colombia, which has consistently refused to allow its use, even though encouraging premunition with a virulent organism.

Killed vaccine:

The only such commercially available vaccine currently on the market is not recommended for use in the tropics. Experimental evidence suggests that the degree of immunity produced is inadequate to withstand field challenge, and the duration of immunity is short, requiring frequent re-vaccination. In the tropics where a year round

TABLE 13

SYSTEMS OF ANAPLASMOSIS IMMUNIZATION

Method	Calves < 8 mo.	8 mo. to 2 years	Cattle > 2 yrs.	Relative efficacy
Virulent field <u>A. marginale</u>	Safe N.R.	Not safe N.R.	Not safe N.R.	++++
Virulent field <u>A. marginale</u> with therapy	Safe N.R.	Safe N.R.	Not safe N.R.	++++
Dilute stabilate	Safe R.	Safe N.R.	Not Safe N.R.	++++
Dilute stabilate with therapy	Safe R.	Safe R.	Safe R.	++++
Attenuated <u>A. marginale</u>	Safe N.R.**	Safe R.	Safe R.*	+++
<u>Anaplasma</u> <u>centrale</u>	Safe R.	Safe R.	Safe R.*	++
Killed vaccine	Safe N.R.	Safe N.R.	Safe N.R.	+

* Not recommended for lactating dairy cattle

** The mildness of this organism is such that a satisfactory replicating infection is not always produced in calves of this age.

R. Recommended for use in the tropics

N.R. Not recommended.

++++ Maximum protection against needle and field challenge

+++ Solid protection against needle challenge - variable response against some field challenges

++ Partial protection against both needle and field challenge. Prevents death losses by either challenge.

+ Partial protection against needle challenge, and little or no protection against field challenge as tested in Colombia. Has a short acting immune response which limits its effectiveness in areas of year-round vector problems.

vector problem exists and transmission may occur throughout the year, this vaccine is not thought to produce adequate protection. Vaccination and re-vaccination of cows has been associated with a neonatal hemolytic disease of calves which can produce serious losses.

Anaplasmosis: Treatment

There are two distinct phases of treatment for anaplasmosis. The one involves treatment of the clinically ill animal to prevent death and alleviate symptoms. A second is aimed at eliminating infection in the chronic carrier, which appears perfectly normal, but acts as a reservoir of infection for other animals and is hence a disease threat. In the endemic areas of South America treatment is usually limited to the first type, but in areas of low incidence such as the high elevations in Colombia, or many areas of the U.S., the second phase assumes importance. We have worked on both areas of treatment with considerable success, but with some disappointment in that two of the most promising drugs cannot be cleared for use in food producing animals because of residue and toxicity problems.

Treatment of Acute Infection:

A common pitfall in the evaluation of therapeutic agents used for anaplasmosis is the fairly large number of spontaneous recoveries which might mislead the observer to believe that a specific drug or therapeutic procedure was effective, when in reality recovery would have occurred without treatment. A second problem is that often the animal with anaplasmosis may go unrecognized until the terminal phases of infection, at which time even specific therapy is unsuccessful. Early treatment is recognized as being important to ensure success.

Prior to the introduction of the tetracyclines and other chemotherapeutic compounds, treatment of acute anaplasmosis was limited largely to supportive therapy including a variety of hematinics, blood transfusions, good nursing, and care. These procedures were aimed largely at the elimination of stress and alleviation of the anemia until such time as the immune mechanism and hemopoietic system could adequately respond. It would appear that blood transfusions could and

should play an important role in the treatment of acute anaplasmosis but there are conflicting reports and some suggestion of caution in this procedure. There is always a danger in treating weakened animals, particularly range cattle when the excitement and exertion associated with handling and restraining may do more harm than the treatment will do good. Extensive treatment of the semi-wild range animal may be difficult and impractical; whereas, treatment of a docile dairy animal may be useful and successful.

Until the last few years, the only specific chemotherapeutic compounds have been the tetracyclines, principally oxytetracycline, chlor-tetracycline, and tetracycline hydrochloride. These compounds all appear similar in suppressing the reproduction of the Anaplasma organism, and are generally effective when given intramuscularly at the rate of 3-5 mg/lbs. of body weight early in the course of infection. The tetracyclines have a marked chemoprophylactic effect when given as low as 0.5 mg/lbs. orally over an extended period. We have successfully used 5 mg/lbs. orally to moderate clinical signs in adult bulls being preimmunized prior to shipment to Mexico.

Three new compounds have been tested and show promise: 356C61 (Gloxazone, Alpha-Ethoxyethyl-glyoxal Dithiosemicarbazone, Burroughs-Wellcome), 4A65 (Imidocarb, 3,3'-Bis-(2-imidazolin-2-yl)-carbanilide dihydrochloride or dipropionate, Burroughs-Wellcome), and a long acting terramycin formulated to contain 200 mg. oxytetracycline/ml (T-200), (Pfizer).

In preimmunization experiments, we have shown 356C61 (5mg/kg) and 4A65 (4mg/kg) superior to oxytetracycline (12 mg/kg) in moderating the course of infection in adult cattle intentionally exposed to virulent A. marginale. Roby has reported that the development of acute anaplasmosis in splenectomized calves has been inhibited with a single injection of 2.5 mg/kg 4A65. These drugs, 356C61 and 4A65, are not available

commercially and, hence, have not been used extensively in cases of acute anaplasmosis but on limited experimental work show promise.

The long acting Terramycin (T-200) has a reasonable chance of clearance and use in the U.S. and hence South America so considerable effort has gone into testing this compound.

This formulation has been compounded to provide sustained oxytetracycline plasma levels over a 3-5 day period. Two recently conducted experiments clearly indicate the value of T-200 in treating acute anaplasmosis when compared with oxytetracycline (liquamycin formulated to contain 50 mg/ml) (T-50).

The first experiment tested the efficacy of 3 compounds in treatment of artificially induced anaplasmosis in the early stages of an ascending parasitemia (1-4%), in 23 splenectomized calves. Group I, consisting of 5 calves served as non-treated controls. Four calves (Group II) were treated 1 time with 10 mg/kg oxytetracycline (T-50) intramuscularly (IM); 5 calves (Group III) were treated 3 times at daily intervals with 10 mg/kg oxytetracycline (T-50) IM; 5 calves (Group IV) were treated 1 time with 20 mg/kg T-200, and 4 calves (Group V) were treated 1 time with 10 mg/kg Vibramycin.

All control calves died in Group I, 1 calf died in Group II. No other deaths occurred. All treatments were effective in moderating the infectious process, but T-50 given 3 times and T-200 given 1 time were significantly more effective than T-50 given 1 time and Vibramycin given 1 time.

There appeared little or no difference between T-50 given 1 time and Vibramycin and between T-50 given 3 times and a single injection of T-200.

These results are tabulated in Tables 14, 15 and 16 with the pre-injection data presented in Table 14 and the influence of infection in each of the 5 groups tabulated in Tables 15 and 16.

TABLE 14

Comparison of Four Therapeutic Methods in Anaplasmosis
Pre- Treatment Baseline Data for each Treatment Group

	I	II	III	IV	V	Significance
Number of animals	5	4	5	5	4	
Average Pre-exposure PCV	29.6%	30.5%	31.8%	31.6%	31.2%	NS
Average Pre-exposure weight	236 ±56	217±36	215 ±42	206 ±47	237 ±48	NS
Average Incubation time*	23.4	22.2	22.6	22.6	21.8	NS
Days after exposure treatment was administered	24.6	23.5	24.0	24.2	23.2	NS

Group I treated with PSS - 0.1 ml/kg., IM, 1 time

Group II treated with oxytetracycline - 10 mg/kg, IM, 1 time.

Group III treated with oxytetracycline (T-50) 10- mg/kg, IM, 3 times at daily intervals

Group IV treated with T-200 - 20 mg/kg, IM, 1 time.

Group V treated with Vibramycin - 10 mg/kg, IM, 1 time.

*Number of days after exposure that a 0.5% parasitemia was observed.

NS: Not significant

TABLE 15

Comparison of Four Therapeutic Methods in Anaplasmosis
Influence of Treatment on Parasitemia

	GROUPS					Significance
	I	II	III	IV	V	
Average parasitemia on day of treatment (%)	2.7	2.2	2.6	3.0	2.6	NS
Average maximum parasitemia (%)	59	20	25	8	21	P<0.01 DRS: 34
Days after treatment that high parasitemia occurred	6	12.2	19	19	8.5	P<0.01 DRS: 5.6
Parasitemia regression coefficient *	11.06	1.02	-0.293	-0.170	2.322	P<0.01 DRS: 1.54

NS Not significant

DRS Difference required for significance.

NT Not tested

* Calculations based on daily parasitemia values during the first 10 days after treatment or until animal died if this came first.

TABLE 16

Comparison of Four Therapeutic Methods in Anaplasmosis
Influence of Treatment on PCV

	GROUPS					Significance
	I	II	III	IV	V	
Average PCV on day of treatment	27.4 ±4.0	28.2 ±1.5	28.8 ±5.9	29.6 ±4.8	28.5 ±4.2	NS
Average low PCV (%)	6.0	9.0	9.1	12.6	9.0	P < 0.01 DRS: 3.9
Days after treatment Low PCV Occurred	7.8	13.5	21.6	18.6	11.2	P < 0.01 DRS: 3.6
Regression Coefficient *	-3.59	-1.19	-0.625	-0.901	-2.05	P < 0.01 DRS: 1.11
Deaths	5/5	1/4	0/5	0/5	0/4	

NS: Not Significant

DRS: Difference required for significance

* Calculated on daily PCV values during the first 10 days after treatment or until time of death if this came first.

The second experiment involved 43 adult Angus cows 6-9 years of age in which anaplasmosis was induced by the inoculation of infected blood.

When the subsequent Anaplasma marginale parasitemia reached a level of 4-10%, 15 cows were treated intramuscularly (I.M.) with 20 mg/kg of T-200. Another 15 infected cows were treated I.M. on two successive days with 10 mg/kg/day of Liqueamycin (T-50) and 13 cows remained as infected, nontreated controls.

Both oxytetracycline formulations were highly effective in moderating the course of infection and resulted in rapid recovery, whereas 2 of 13 nontreated controls died and the survivors showed higher parasitemias, lower packed cell volumes and greater weight loss than the treated animals. There were no significant differences between the two treatment groups. One injection of the T-200 was comparable in efficacy to two injections of the T-50 and because of the concentration, the volume of T-200 required was only one-fourth the volume of T-50.

Pre-treatment base line data is presented in Table 17 and the effects of treatment in Table 18.

TABLE 17

Comparison of Two Different Compounds in Treatment of Anaplasmosis

Pre-Treatment Baseline Date

	Controls	T-50	T-200	Significance
No. of Cattle	13	15	15	
Average Age (Pre-infection) Yrs.	6.5 ± 2.1	6.8 ± 1.4	7.4 ± 1.6	NS
Avg. Weight (Pre-infection) Kg.	432 ± 45	431 ± 32	427 ± 45	NS
Avg. PCV (Pre-infection)%	37.4 ± 3.3	38.1 ± 2.4	37.5 ± 3.0	NS
Average Incubation Time (Days)	24.9 ± 2	25.3 ± 2.8	25.1 ± 2.4	NS
Avg. Parasitemia at time of Treatment (%)	NA	5.6 ± 1.3	5.3 ± 1.5	NS
Avg. PCV at Time of Treatment (%)	30.8 ± 3.7	30.7 ± 2.9	32.3 ± 2.4	NS
Avg. Time after Exposure Treatment Given (Days)	NA	28.1 ± 2.7	27.9 ± 2.5	NS

±: Standard Deviation

NS: Not Significant

NA: Not Applicable

TABLE 18

RESULTS OF TREATMENT ON ACUTE ANAPLASMOSIS WITH TWO TETRACYCLINE COMPOUNDS

	Controls	T-50	T-200	Significance	DRS
No. of Animals	13	15	15		
Average High Parasitemia (%)	21.3 ± 12.4	5.9 ± 1.4	5.6 ± 1.6	P < 0.05	6.2
Avg. Low PCV (%)	13.1 ± 3.5	24.1 ± 3.3	26.0 ± 2.6	P < 0.01	2.8
Average Weight Loss (Kg.)	70 ± 44	61 ± 30	47 ± 33	NS	-
PCV - 10 Day Regression Coefficient-After Treatment	-2.50	-0.236	-0.390	P < 0.01	-0.73
Parasitemia - 10 Day Regression Coefficient-After Treatment	3.50	-0.879	-0.862	P < 0.01	1.40
Deaths	2/13	0/15	0/15		

DRS: Difference Required for Significance

±: Standard Deviation

NS: Not Significant

Treatment of Carrier Infections to Eliminate Anaplasma

Soon after the tetracyclines were observed to inhibit growth of Anaplasma, experiments were conducted to evaluate these drugs in relation to the removal of carrier infections. These experiments indicated that such therapy would eliminate carrier infections, but only after the prolonged administration of fairly heavy doses. Nevertheless, this significant breakthrough stimulated a great deal of research on this subject with the evolution of numerous successful treatment regimens which will effectively eliminate carrier infections. A list of these procedures is given in Tables 19 and 20 which under varying conditions have proven successful.

Carrier status has been eliminated by the IV or IM injection of 5 mg/lb tetracycline daily, repeated 10 times. The injection of oxytetracycline and chlortetracycline at the same dosage is also effective when repeated daily for 12 to 16 days. These drugs are relatively non-toxic; however, they do produce considerable irritation at the site of I/M injections, and if given too rapidly IV can produce a transient mild to moderate respiratory distress.

The smallest amount of chlortetracycline that has proven effective in eliminating infection was 0.5 mg/lb orally for a 120-day period. This treatment was administered during the winter season when vector activity, thus re-exposure, was absent. The negative status of treated animals was established by serologic means. A similar experiment, using 0.5 mg/lb orally for 90 days, conducted during the vector season failed to eliminate the Anaplasma; however, suppression and reduced infectivity were detected.

It is probable that oral treatment with low-level tetracyclines in feed, while effective, may be influenced by factors such as continued exposure, lack of uniform consumption by animals, or differences in individual tolerance, so that

TABLE 19
COMPARISON OF DIFFERENT DRUG LEVELS IN ELIMINATION OF ANAPLASMA INFECTION

DRUG	RATE OF ADMIN.	ROUTE	NO. OF TREATMENTS	INTERVAL
Tetracycline	5 mg/lbs	I/V or I/M	10	Daily
Oxytetracycline	5 mg/lbs	I/V or I/M	12-14	Daily
Chlortetracycline	15 mg/lbs	I/V	16	Daily
Chlortetracycline	1.0 mg/lbs	Orally	41	Daily
Chlortetracycline	2.5 mg/lbs	Orally	45	Daily
Chlortetracycline	0.5 mg/lbs	Orally	120	Daily
Chlortetracycline	5 mg/lbs	Orally	30-60	Daily
Chlortetracycline	5 mg/lbs	Orally	60	Daily
Chlortetracycline	2.5 mg/lbs	Orally	60	Daily
Chlortetracycline	1.5 mg/lbs	Orally	60	Daily
Chlortetracycline	5 mg/lbs	Orally	45-60	Daily
Chlortetracycline	5 mg/lbs	Orally	30	Daily

TABLE 20
RESULTS OF DRUG TRIALS IN SUCCESSFUL ELIMINATION OF ANAPLASMA INFECTION

DRUGS	RATE OF ADMIN	ROUTE	NO. OF TREATMENTS	INTERVAL
Oxytetracycline*	11 mg/kg	I/V	3	24 or 48 hours
356C61	5 mg/kg			
4A65*	5 mg/kg	I/M or S/C	3	Daily
4A65*	2 mg/kg	I/M or S/C	3	Daily
356C61	5 mg/kg			
4A65 (1)	5 mg/kg	I/M or S/C	2	14 Days

*Splenuctomized calves used
(1) Adult cattle used

completely reliable results may not always occur. For these reasons chlor-tetracycline levels higher than 0.5 mg/lb are generally used. A treatment consisting of 5 mg/lb daily for 30 to 60 days has generally been successful. This treatment regimen gives room for animal variation and has provided more consistent removal of carrier infections.

The prolonged periods of treatment discourage their use, as they disrupt normal management practices. This, plus the expense of the drug and labor to implement such a program, has led many workers to search for a better system. In 1965, Barrett et al. described an alpha dithiosemicarbazone (356C61) which had specific activity against Anaplasma. This dithiosemicarbazone is an insoluble powder which is prepared as an aqueous suspension. If injected IV, it may produce a respiratory distress. When diluted in PSS this can be minimized. Comparisons between 5 mg/kg 356C61 and 11 mg/kg oxytetracycline when given IV to splenectomized calves showed 356C61 to be superior based on a significantly faster return of packed cell volumes to normal following treatment. The injection of 356C61 at a level of 5 mg/kg on each day of treatment for a total of ten times produced a fatal toxicosis in six of seven animals. Deaths occurred as early as one day and as late as 41 days after the last of 10 injections. Clinically, the animals showed chronic bloating, rumen atony, and depression before dying. Animals were treated symptomatically with tuminal stimulants and laxatives but failed to respond. Relief of bloat by trocharization or the passage of a stomach tube was only transitory.

Other attempts to eliminate the Anaplasma infection in carriers were made using 356C61. A total of 5 injections, using 5 mg/kg once every 24-hour interval, was unsuccessful. The injection of 356C61 at the rate of 5 mg/kg once every two-days for 3 times, four times over a two-week period at three and four day intervals, three times at weekly intervals, and three times at two week intervals were all unsuccessful.

The addition of oxytetracycline to 356C61, giving both drugs simultaneously, was consistently successful in abolishing the carrier state in Anaplasma-infected splenectomized calves not previously treated. Oxytetracycline (11 mg/kg) was combined with 356C61 (5 mg/kg), both drugs being diluted in 150 ml sterile saline and then injected IV. Three treatments at either 24 or 48 hour intervals were successful. At 72-hour intervals, only two or three responded favorable.

Imidocarb (4A65), a white, readily soluble powder, originally recognized for its babesiacidal activities, has also shown promise for use in anaplasmosis. Imidocarb given once daily at the rate of 4,5, or 6 mg/kg for three successive days has successfully eliminated the Anaplasma carrier status in splenectomized calves. The addition of 356C61 to 4A65 appears compatible and was effective in eliminating Anaplasma with reduced dose rates of 4A65. The use of 2 mg/kg 4A65 when given together with 5 mg/kg 356C61 once daily for a total of 3 times was effective. In this instance, 4A65 was administered IM and the 356C61 IV. Another regimen consisting of 5 mg/kg 4A65 and 2 mg/kg 356C61 was also effective when administered as described. Unpublished evidence is available that the carrier status of splenectomized calves may be terminated with as little as 2 mg/kg of each 4A65 and 356C61 given once every 24 hours for 3 days.

A total of 15 mg/kg 4A65 when given over a three-day period was effective, but 15 mg/kg 4A65 given in one injection was ineffective in eliminating the carrier state. A single injection of 4 and 6 mg/kg 4A65 combined with 15 mg/kg 356C61 was also ineffective.

The apparent synergistic or at least additive effect of oxytetracycline and 356C61 suggested the possible desirability of combining oxytetracycline and 4A65, but every such trial was unsuccessful.

Most recently Roby and Mazzola have described the use of 4A65 at the level of 5 mg/kg in adult carrier cattle. A single injection was ineffective in eliminating the carrier status, but a total of two injections at this dosage, each given two weeks apart, was successful in eliminating infection in all five cattle so treated. They used both the dihydrochloride and the dipropionate salts successfully. Both salts had identical effects on the parasites; however, the dipropionate was less irritating at the site of inoculation.

Imidocarb at 5 mg/kg can be given safely either S/C or I/M. As little as 3 mg/kg given IV may produce immediate respiratory collapse and death. The more likely responses are labored breathing, excessive salivation, and lacrimation. These same signs often follow the SC or IM injection of 5 mg/kg. There appears to be considerable individual variation in tolerance to 4A65. On one occasion the SC injection of calves with 15 mg/kg produced nothing more than transitory discomfort; whereas, on another occasion the SC injection of 10 mg/kg was followed by death two days following the injection.

The toxicity of 4A65, while known to exist, is still poorly understood. There have been unconfirmed reports of delayed signs of toxicity characterized by edema, lacrimation, depression, and death sometimes associated with elevated blood urea nitrogen and serum glutamic oxalacetic transaminase values. These signs have been seen three to five weeks after drug inoculation.

A field trial on 469 cattle was conducted to test the efficacy of 2 injections of 4A65 at the 5 mg/kg level in the elimination of carrier infections. A preliminary screen test revealed 88% to be positive or suspicious to the complement-fixation (CF) test. For this reason, all cattle were treated in an effort to blanket the herd and eliminate infection. The results are presented in Table 21.

TABLE 21

Anaplasma Survey of an Infected Herd Treated with Imidocarb Dipropionate (5 mg/kg I/M)

Date Day	No. Cattle Tested	Percent of Total Herd	CF Negative (%)	CF Positive and Suspicious (%)	Avg. CF Titer of Those Pos.	% Showing >0.5%AM* Parasit.	Avg. PCV
December 13 Day 0	469	100%	12	88	1:24	15.5	29.7 ±6.1
January 30 Day 48	170	36%	67	33	1:5	0	33.2 ±3.7
March 8 Day 85	149	32%	44	56	1:15	6.0	29.8 ±4.1
April 12 Day 120	152	32%	41	59	1:12	3.9	29.7 ±4.3
July 17 Day 216	331	71%	38	62	1:10	1.5	32.5 ±3.9
December 12 Day 365	199	42%	20	80	1:8	0	32.8 ±3.35

* AM = Anaplasma marginale

Treatment was therapeutically effective, but failed to produce the desired prophylactic control. An initial drop in positive serum response as measured by the CF was noted after treatment. This was followed by a gradual increase, thought to be due to reinfection. One year after treatment the rate of positive serum tests was essentially the same as before treatment.

Evaluation of imidocarb's usefulness in removing Anaplasma infection is difficult in this trial because of the apparent reinfection or relapsing infections which occurred. The drug probably did not eliminate Anaplasma infections in all cattle, so that in the absence of vector control rapid reinfection appeared to occur. If Anaplasma infection had been eliminated in all treated cattle then a secondary reservoir would have to be present. This could have been either the vectors or possibly wildlife. Even though white-tailed deer are very numerous in this area, there is no direct evidence to suggest that they are acting as reservoirs of infection.

Failure in this trial emphasizes the need for strict isolation and vector control for success to be achieved in Anaplasma control by chemotherapy.

Babesiosis: Diagnosis

A definitive diagnosis of babesiosis is made by two methods: first by identifying the Babesia on stained blood smears, the second by the detection of antibodies specific for Babesia antigens. Demonstrable parasitemias are usually transient, often occurring for only a few days during the acute phase of infection. Thick blood smears, or brain biopsies improve the chances of identifying the organism, and when the organisms are seen there is no doubt of the positive diagnosis. The absence of a demonstrable parasitemia does not in all cases signify a negative animal. Observable parasitemias are very uncommon in latent infections, which may persist for extended periods of time. The epidemiology, and identification of carrier animals is therefore dependent on identifying specific antibodies.

Probably the first successful serum test employed was the complement fixation (CF) procedure. A wide variety of tests have been applied to the diagnosis of babesiosis over the past few years, and work has encompassed the following: Gel precipitation, (gel-diffusion, G.P.), fluorescent antibody (F.A.), indirect fluorescent antibody (IFA), hemagglutination (HA), indirect hemagglutination (IHA), capillary-tube agglutination (CA), card agglutination test (BCT), latex particles agglutination, and slide agglutination test (SA). A list of tests, the principal developer of the tests, and the Babesia species used are listed in Table 22.

The detection of Babesia antibodies is not always an infallible indication of current babesiosis, since a positive test may indicate a previous exposure to Babesia antigens not necessarily a current infection, may indicate chronic or active infection, may indicate a previous acute infection that has been eliminated by chemotherapy. Both false positive and false negative reactions are possible and do on occasion occur, but nevertheless,

TABLE 22

Serological tests for diagnosis of Babesiosis

Author(s) & date	Serological Test	Babesia spp.	Source of:	
			Antigen	Antibody
Hirato et al (1945)	CF	<i>B. caballi</i>	RBC	Equine
Mahoney (1962)	CF	<i>B. bigemina</i> , <i>B. argen.</i>	RBC	Bovine
Schindler and Dennig (1962)	CF	<i>B. canis</i> , <i>B. rodhaini</i>	RBC	Canine, Murine
Mahoney (1967a)	CF	<i>B. argentina</i> , <i>B. bigem.</i>	RBC	Bovine
Frerichs et al (1969a)	CF	<i>B. caballi</i>	RBC	Equine
Todorovic et al (1971)	CF	<i>B. bigemina</i> , <i>B. argen.</i>	RBC	Bovine
Ristic and Sibinovic (1964)	GP	<i>B. caballi</i>	RBC	Equine
Sibinovic et al (1965)	GP	<i>B. equi</i> , <i>B. caballi</i>	Serum	Equine
Mahoney (1966)	GP	<i>B. bigemina</i> , <i>B. argen.</i>	Serum	Bovine
Mahoney and Goodger (1969)	GP	<i>B. argentina</i>	Serum	Bovine
Todorovic et al (1971)	GP	<i>B. bigemina</i> , <i>B. argen.</i>	Serum	Bovine
Vizcaino and Todorovic (1973)	GP	<i>B. bigemina</i> , <i>B. argen.</i>	RBC	Bovine
Ristic et al (1964)	FA	<i>B. caballi</i> , <i>B. equi</i>	RBC	Equine
Ristic and Sibinovic (1964)	FA	<i>B. caballi</i>	RBC	Equine
Garnham and Voller (1965)	FA	<i>B. divergens</i>	RBC	Simian
Ludford (1969)	FA	<i>B. bigemina</i> , <i>B. argen.</i> , <i>B. canis</i> , <i>B. rodhaini</i>	RBC	Bovine, Canine Murine
Madden and Holbrook (1968)	IFA	<i>B. caballi</i>	RBC	Equine
Ross and Lohr (1968)	IFA	<i>B. bigemina</i>	RBC	Bovine
Zwart et al (1968)	IFA	<i>B. major</i> , <i>B. bigemina</i>	RBC	Bovine
Cox and Turner (1970)	IFA	<i>B. microti</i>	RBC	Murine
Ross and Lohr (1970)	IFA	<i>B. bigemina</i>	RBC	Bovine
Brocklesby et al (1971)	IFA	<i>B. major</i> , <i>B. bigemina</i>	RBC	Bovine
Donnelly et al (1972)	IFA	<i>B. divergens</i>	RBC	Bovine
Goldman et al (1972)	IFA	<i>B. bigemina</i> , <i>B. berbera</i>	RBC	Bovine
Joyner et al (1972)	IFA	<i>B. major</i> , <i>B. divergens</i>	RBC	Bovine
Leeflang and Perie (1972)	IFA	<i>B. bigemina</i> , <i>B. argen.</i> <i>B. major</i> , <i>B. divergens</i>	RBC	Bovine
Ludford et al (1972)	IFA	<i>B. argentina</i>	RBC	Bovine
Burridge et al (1973)	IFA	<i>B. bigemina</i>	RBC	Bovine
Curnow (1968)	HA	<i>B. argentina</i>	RBC	Bovine
Todorovic et al (1967)	HA	<i>B. rodhaini</i>	RBC	Murine
Curnow and Curnow (1967)	IHA	<i>B. argentina</i>	RBC	Bovine
Sibinovic et al (1969)	IHA	<i>B. equi</i> , <i>B. caballi</i>	RBC	Equine
Goodger (1971)	IHA	<i>B. bigemina</i> , <i>B. argen.</i>	RBC	Bovine
Lohr and Ross (1969)	CA	<i>B. bigemina</i>	RBC	Bovine
Ristic et al (1971)	CA	<i>B. canis</i>	RBC	Canine, Simian, Human
Todorovic and Kuttler (1974)	BCT	<i>B. bigemina</i>	RBC	Bovine
Curnow (1973)	SA	<i>B. bigemina</i>	RBC	Bovine

these tests have proven very helpful in identifying infection and establishing the prevalence of infection. A practical test for use in the serological diagnosis of babesiosis needs to meet the following criteria: (1) the test has to be simple to perform; (2) the interpretation of results must be free of subjectivity; (3) the test must be rapid; (4) the cost must be minimal; (5) the results have to be sufficiently sensitive and specific; and (6) the test must be capable of performing uniformly reliable results in various laboratories and under different field conditions.

The CF reaction constituted one of the earliest tests for diagnosis of babesiosis. Although complement fixing antibodies are unquestionably produced in the course of Babesia spp. infection, results of the CF test vary according to the antigens used. The CF test described by Mahoney has been used for identification of Babesia spp. infections in Australian cattle. Approximately 0.5 percent of cattle tested have shown a positive CF reaction but have been negative to a transmission test. On the other hand, infected cattle, after 8-12 months show a declining CF antibody level and the majority of animals showed no CF antibody in the diagnostic range, but still carried infection as revealed by subinoculation. The CF test, which has the lowest sensitivity in comparison with other serological tests, may reliably detect antibodies to B. argentina for 7 months and to B. bigemina for 4 months after a single infection. There are limited CF reactions between heterologous infections of B. bigemina and B. argentina.

The presence of colostral antibodies that react in the CF test is a source of error. Complement fixing antibodies of colostral origin remain at levels detectable by the CF tests for an average of 2 to 3 weeks after birth.

Immunofluorescence techniques which were introduced to study Babesia spp. infections a decade ago have, for the first time, made a test

available in which the whole intraerythrocytic Babesia spp. parasites, instead of extracts, constitute antigen. The FA tests are more sensitive than the CF test, and periods of reactivity from a single infection in excess of 2 years have been reported.

The commercial availability of fluorescein-labelled antiglobulin and the observation that tests can be made from eluent obtained from dried blood samples on filter paper as a source of Babesia spp. antibodies make it possible to carry out tests in any laboratory where a fluorescent microscope and blood parasitised with Babesia spp. are available. Serious limitations in the IFA test for immunodiagnosis of babesiosis result from the subjective nature of the interpretation and from the lack of specificity due to cross reacting antigens when a whole parasite is used. Colostral antibodies may react in the IFA test for an average of 4 to 5 months after birth which precludes the use of the IFA test to record the infection rate in calves of this age. Even with these drawbacks the IFA is gradually evolving into the test of choice. This is particularly true with B. bovis (argentina).

The gel precipitation test appears to be a very useful serological technique for antigenic characterization of Babesia spp.; however, the practical application of this technique in diagnosing babesiosis was not demonstrated.

Agglutination reaction provides a sensitive means for detecting Babesia spp. antibodies in various systems, such as haemagglutination, latex particle agglutination, capillary, slide and card agglutination techniques. The IHA technique using tanned or formalin treated erythrocytes coated with Babesia spp. soluble antigens have been used and found to be highly sensitive with relatively reproducible results; however, some non-specific reactions do occur.

Agglutination reaction reported in the capillary agglutination (CA) test, slide agglutination (SA) and card agglutination (BCT) test

appears to be of significant importance and the practical application of these reactions needs to be further investigated.

Although several serological tests have been reported (Table 22) and noteworthy advances have been made in attempts to purify Babesia spp. antigens, some serological cross reactions have been shown among the various Babesia and Plasmodium spp. The differences in titre of homologous and heterologous sera are significant.

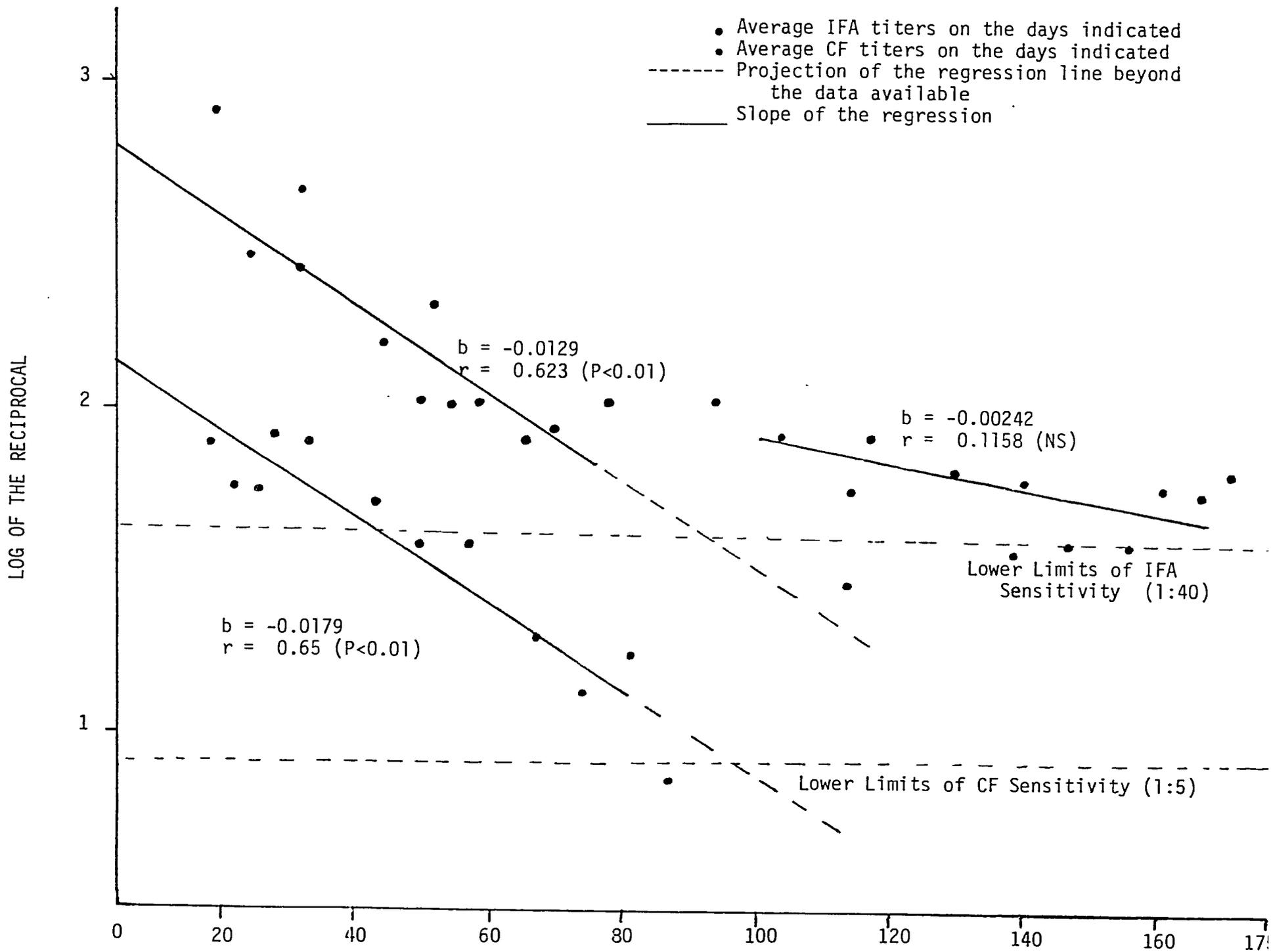
In selecting the test of choice it is essential to have a precise understanding of the purpose for which serology is to be utilised. Methods devised to date for serological diagnosis of babesiosis are imperfect and improvements must be sought. Nevertheless, until better techniques can be developed, the serological techniques now available are capable at diagnosing infections caused by Babesia spp.

Detailed comparisons between the complement-fixation (CF) and the indirect fluorescent antibody (IFA) reactions were made on 5 cattle infected with B. bigemina by blood inoculation (in Texas) and on 4 calves naturally infected at Monteria on the north coast of Colombia. Both B. bigemina and B. bovis infections occurred in the calves, and tests were made using both B. bigemina and B. bovis antigens.

In the absence re-infection, 5 cattle infected and sequentially sampled showed an initial high rise in both CF and IFA titers. This serum response immediately started to decline based on serum titrations. (Figure 1)

Both tests were effective in detecting specific antibodies for the first 84 days of infection, with 57 of 60 (95%) serums tested being positive on the CF test and 57 of 57 (100%) tests being positive to the IFA test. During the interval from 98 to 175 days, 24 of 60 (40%) of the serums tested were positive with the CF test, and 53 of 56 (95%) were positive with the IFA test.

Figure 1
Comparative Decrease in IFA and CF Serum Titers on Post-infection
Days 18 to 84 and on 98 to 175 Days (IFA only)



During the first 84 days, a similar linear regression occurred in both CF and IFA serum titers, but after 98 days the IFA regression flattened out, whereas the CF titers decreased below the sensitivity threshold in 60% of the serums tested.

Among 4 calves naturally infected on the north coast of Colombia, and sequentially sampled at weekly intervals a somewhat different pattern of serologic response emerged. The IFA test on an average became positive, 7.8 weeks after arrival, to B. bovis antigen and 5.8 weeks after arrival with B. bigemina antigen. The CF test became positive on an average at 11.8 weeks with B. bovis antigen and 9.5 weeks with B. bigemina antigen. Instead of the rapid decline in titers seen in the previous experiment the reactions persisted with both CF and IFA during the 210 day period of observation. (Figures 2 and 3)

After the first evidence of serological response to B. bigemina infections, CF positive reactions were measured in 88 of 92 test or 96% of those conducted, and in the case of B. bovis infections, 91 of 107 tests or 85% were positive. After the first evidence of serological response to B. bigemina infection, IFA positive reactions were measured in all of 120 tests conducted, and in the case of B. bovis infections all of 118 tests were positive. The persisting serum reactions are supposedly the result of continuing re-infection or re-exposure of calves under conditions seen on the north coast of Colombia.

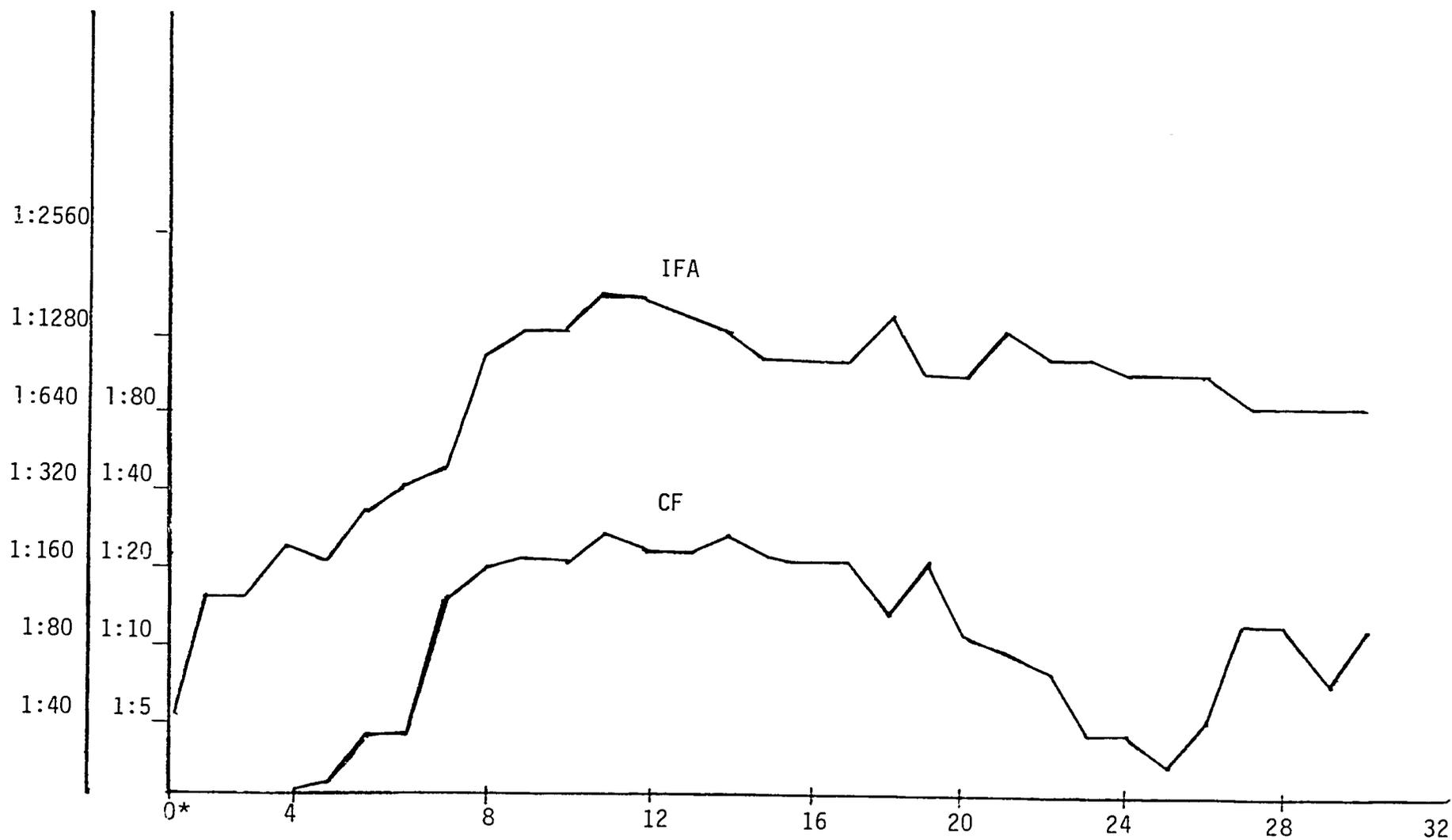
Under naturally occurring conditions of infection, the CF test is probably more useful than the data at Texas would suggest. Even so the IFA appears to drop in titer less, and is more rapid to occur initially, so has some distinct advantages as well as the disadvantages previously mentioned.

Figure 2

B. bigemina Antigen
(Colombia)

IFA
Titers

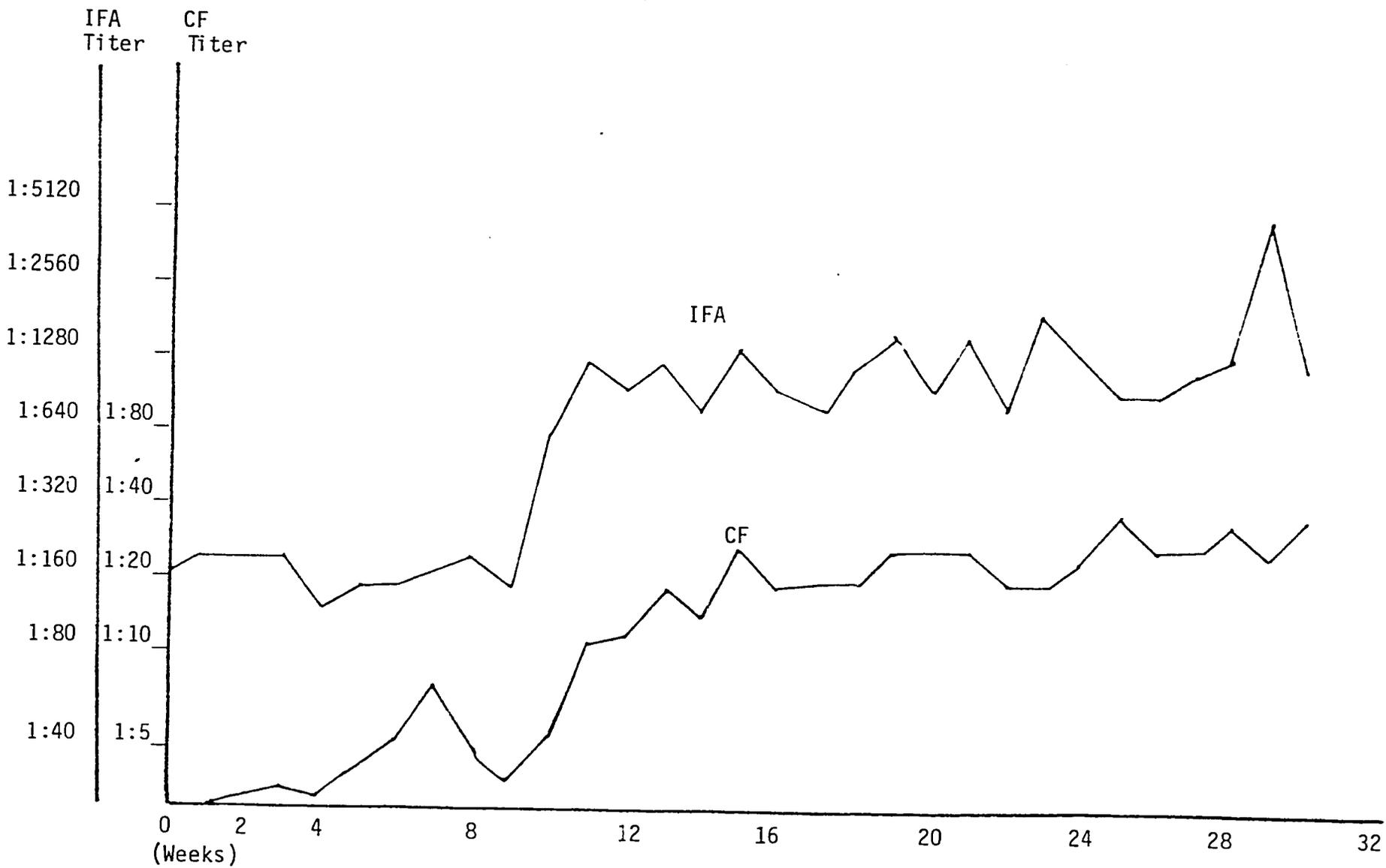
CF
Titer



* Onset of IFA approximately 7.8 weeks after arrival at Turipana

Figure 3

B. bovis Antigen
(Colombia)



Babesiosis: Epidemiology (Prevalence Studies)

The epidemiology of anaplasmosis and babesiosis in Colombia is influenced by several geographical factors. Colombia is divided longitudinally by 3 Cordilleras of the Andean mountain range. The western Cordillera separates the narrow Pacific coast lowlands from the interior of the country. The central Cordillera divides Colombia's two major valleys formed by the Cauca and the Magdalena rivers; while the eastern Cordillera separates the western part of the country from the low eastern plains, Los Llanos Orientales, which comprise nearly three fifths of Colombia's surface area and extend nearly 640 kilometers eastward to the Venezuelan border.

Three major climatic zones exist, reflecting differences in elevation associated with the 3 Cordilleras of the Andes. A hot, lowland zone is found below 800 meters with an average temperature of 25°C. An intermediate zone occurs at 800 to 2100 meters with a temperature range of 17 to 23°C, while a high zone occurs above 2100 meters with an average temperature of 13°C or lower.

Colombia's estimated 3 million dairy cattle are found in the high mountainous zone and in the intermediate climatic zone. Beef cattle number approximately 19 million head and are found in the hot lowlands and in the intermediate climatic zone.

Three areas were selected for Babesia prevalence studies:

1. The eastern plains (Los Llanos Orientales);
2. the North coast; and
3. the Cauca Valley.

The area in Los Llanos and the North coast are located in the lowland tropical zone, while the Cauca Valley area is in the lower intermediate climatic zone. The 3 areas differ topographically, ecologically and in the systems of cattle management employed.

I. The Eastern Plains

The study area extends from the Piedmont region of the eastern Andean Cordillera eastward approximately 450 kilometers through the Department of Meta and into the Comisaria of Vichada. The area consists of savannah grasslands and varies in elevation from 500 meters in the Piedmont region to 200 meters in the savannahs of Vichada. The average annual temperature is approximately 26°C with rainfall decreasing from 3000 mm. in the Piedmont to 1200 mm. in Vichada. Rainfall is equally distributed during the wet season from April to November with little or no rainfall during the dry season from December through March. Cattle operations on the savannahs are extensive in nature with open-range type management or minimum management being common. Ranches of 5,000 to 10,000 hectares are not considered unusually large. Average stocking rates of 0.4 animal units per hectare were reported for the Department of Meta, while stocking rates of 0.1 to 0.05 head per hectare were reported for the Llanos region in general. Cattle production on the savannahs is oriented toward the production of 3 to 4 year old feeders which are moved into the Piedmont region and grass fattened for 8 to 10 months prior to slaughter. Predominant cattle breeds are Zebu, native Criollo cattle of Spanish descent, and Zebu-Criollo crosses.

The study area was subdivided into 5 study zones based on distance from the Piedmont region, and on differences in cattle operations and the level of ranch management.

Thirty-seven ranches were visited during the study and a total of 3,034 cattle examined.

Each of the ranches visited during the dry season from June to November and a random group of cattle representing a minimum of

10 per cent of the total herd were sampled ensuring that animals less than 1 year, 1 to 2 years, and more than 2 years of age were included in the sample group. The age, sex and breed of each animal were noted, and serum samples collected. Ticks were collected from infested cattle for later classification and the level of tick infestation assessed.

The serum samples were tested for babesiosis using the Complement-Fixation (CF) screen test and a B. bigemina antigen. Test results were read as negative, trace, or 1+, 2+, 3+ and 4+ reactions. All 1+, 2+, 3+ and 4+ reactions were considered as reactors.

Forty-two per cent, 1270 of the 3,034 cattle tested, were reactors to B. bigemina infection (Table 23). Babesia reactors were present in each of the 37 herds, ranging from 5 to 94 per cent incidence.

Forty-six per cent of the calves between 1 and 3 months of age were Babesia reactors, while 65 per cent of the calves between 4 and 6 months were reactors (Table 24). Sixty-five per cent of the 7 to 12 month old group were reactors; 48 per cent of the 13 to 24 month group; and 30 per cent of all cattle tested over 24 months of age were Babesia reactors.

Ticks collected from cattle during the study included: Boophilus microplus; Amblyomma cajennense; Amblyomma triste and Anocentor nitens. Boophilus microplus was the only species found on all the 37 ranches, while the other species were each found on 3 ranches. The differences in the number of ticks between the 5 zones within the study area was not significant. Differences in the level of tick infestation between individual ranches was related to the type of tick control program employed, and the period of time elapsed since the cattle were last sprayed or dipped.

Clinical cases of babesiosis were not reported. A disease complex,

TABLE 23

Babesia bigemina Prevalence in the Eastern Plains of Colombia

June - November 1974

Herd No.	No. of Cattle in Herd	No. of Cattle Tested	No. of Reactors*	Percent Reactors
1	445	45	21	47%
2	232	113	36	32%
3	484	99	51	52%
4	168	100	28	28%
5	455	108	20	19%
6	380	52	30	58%
7	386	60	14	23%
8	866	74	12	16%
9	254	53	26	49%
10	432	64	25	39%
11	326	85	41	48%
12	1060	99	56	57%
13	227	94	44	47%
14	1022	110	85	77%
15	1309	100	46	46%
16	1170	98	46	47%
17	1480	99	38	38%
18	1006	94	36	38%
19	867	100	23	23%
20	532	60	48	80%
21	523	83	33	40%
22	125	49	46	94%
23	247	70	54	77%
24	1430	100	35	35%
25	1340	100	31	31%
26	153	79	54	68%
27	246	87	36	41%
28	244	70	47	67%
29	968	103	60	58%
30	980	100	59	59%
31	620	55	19	35%
32	654	60	13	22%
33	400	100	24	24%
34	293	43	2	5%
35	357	30	4	13%
36	400	100	8	8%
37	545	99	19	19%
Total	22,626	3,034	1,270	42%**

* 1+, 2+, 3+ and 4+ reactions CF screen test.

** Percentage of reactors in the 3034 animals tested.

TABLE 24

Babesia bigemina Prevalence in the Eastern Plains of Colombia

June - November 1974

Age (Months)	No. of Cattle Tested	No. of Reactors*	Percent Reactors
1-3	144	66	46%
4-6	254	165	65%
7-12	416	269	65%
13-24	412	197	48%
> 24	660	194	30%
Total	1886	891	47%**

* 1+, 2+, 3+ and 4+ reactions CF screen test

** Percent reactors in the 1886 cattle tested.

commonly referred to as "Secadera" was reported most often as the most important disease problem on the ranch. "Secadera" occurs most commonly during the dry season and is probably a complex disease of multiple etiologies, of which nutritional stress, gastrointestinal helminthiasis, anaplasmosis and babesiosis are suspected principal causes.

II. The North Coast

The epidemiological study on the North coast was carried out on 4 ranches. Three of the ranches are located in the Department of Cordoba in the municipalities of Las Cordobas and Pueblo Nuevo, while the fourth ranch is located in the Department of Sucre in the municipality of Sampues. The ranches are located on the broad undulating Atlantic coastal plain which varies in elevation from sea level to a few hundred meters. The climate is tropical with an average annual temperature of 28°C.

Rainfall is equally distributed during the wet season from April to November with the dry season lasting from December through March. Cattle production is more intensive than that in the Llanos of Colombia with an average stocking rate of 1.9 animal units per hectare. Ranches of 300 to 600 hectares are average in size. Predominant cattle breeds in the area are Zebu, Native Criollo, and various Zebu crosses with Criollo, Holstein and other breeds.

Thirty pregnant cows, in their sixth to ninth month of gestation, were selected on each of the 4 ranches and identified by ear tag, brand or tattoo number. A serum sample was collected from each cow prior to parturition and again within 2 weeks following parturition.

Abortions, death of several calves and infection with other diseases resulted in only 107 calves being included in determining age at first infection with babesiosis.

Blood and serum samples were collected from each calf as soon after birth as possible and at biweekly intervals thereafter until the calves had reached 6 months of age. Giemsa stained thin blood films were prepared and examined microscopically to determine the earliest age at which parasitized erythrocytes could be detected and to determine the level of parasitemia in subsequent weeks. Mean parasitemias of Babesia parasites were calculated such that week "0" corresponded to the week when infection was first diagnosed in each calf and subsequent levels of parasitemia corresponded to the same stage of infection in each calf, thus allowing maximum mean parasitemias to be observed.

Packed cell volumes (PCV) were determined using the microhematocrit technique. Changes in the mean PCV were calculated such that week "0" and subsequent weeks corresponded to the same stage of infection in each calf thus allowing maximum decreases in the PCV to be observed.

Serum samples collected from the cows and calves were tested for babesiosis using the complement-fixation (CF) test as previously described.

The age at which the calves were first infected by Babesia bigemina was determined using the serological results and the results of blood smear examinations. Positive CF reactions which occurred in calves at 2 weeks of age were attributed to the presence of maternal antibodies and were not considered to be due to Babesia infection.

The effect of first infection with Babesia parasites was evaluated from the number of infected erythrocytes which were observed, and from the decreases which occurred in the PCV's.

Sixty-eight of the 120 pregnant cows tested for babesiosis were found to be B. bigemina reactors (Table 25). The percent reactors on

TABLE 25

Babesia bigemina Prevalence on the North Coast of Colombia

November 1973 - July 1974

Name of Farm	No. of Cows examined	No. of CF Reactors*	Percent CF Reactors
Las Delicias	30	5	17%
Sabana Acosta	30	18	60%
La Rebeca	30	21	70%
Nueva Colombia	30	24	80%
Total	120	68	57%**

* 1+, 2+, 3+ and 4+ reactions CF Screen Test

** Percentage reactors in the 120 cows tested.

the 4 ranches ranged from 17 to 80 per cent, with an overall prevalence of infection of 57 per cent.

The mean age of infection with B. bigemina for the 107 calves included in the study was 11 weeks (Table 26). The earliest age of infection with B. bigemina was 2 weeks with the latest age of infection being 34 weeks.

Apparent clinical infection attributable to infection with anaplasmosis and/or babesiosis was observed in 2 calves. The calves appeared weak and listless and had PCV's of 11 and 14 per cent respectively. The calves were not treated for anaplasmosis or babesiosis and made an uneventful recovery with the PCV's returning to 30 per cent or more, 4 weeks following the signs of clinical illness.

III. The Cauca Valley

The Cauca Valley follows the Cauca river for approximately 250 kilometers extending north and south through the Department of Valle. The Valley is located in the lower intermediate climatic zone and varies in elevation from approximately 900 meters on the valley floor to 1500 meters in the foothill regions. Rainfall is nearly equally distributed throughout the year, with the months of December and January receiving less rainfall than the remaining months of the year. Dairy cattle number slightly more than beef cattle in the Department of Valle with approximately 850,000 head of dairy cattle and 600,000 head of beef cattle reported. Dairy herds are found both in the intermediate climatic zone of the Valley and foothills and also in the cool climatic zone in the mountainous regions of the Cordilleras on either side of the Valley. The movement of dairy cattle from the high mountainous zone down to the valley and foothills is considered to be dangerous by dairymen with losses of up to 50% thought to be due to anaplasmosis and/or babesiosis.

TABLE 26

Babesia bigemina Prevalence on the North Coast of Colombia

November 1973 - July 1974

Age at First Infection with Babesia bigemina

Name of Farm	Number of Calves Examined	Age at First Infection (weeks)	
		Mean	(Range)
Las Delicias	27	15	(4-26)
Sabana Acosta	25	13	(4-34)
La Rebeca	27	8	(2-22)
Nueva Colombia	28	7	(2-12)
Total	107*	11**	

* All of the calves (107) were infected by 34 weeks of age.

** Mean age of infection for all 107 calves.

Six dairy herds ranging in elevation from 930 to 1100 meters were visited during the study. Two of the herds were located in the southern end of the valley, 3 in the middle one-third, and one herd at the northern end of the valley. Serum samples were collected from a minimum of 10 per cent of the animals in the herd when possible.

The serum samples were tested for babesiosis using B. bigemina antigen and the complement fixation test as described for the preceding studies.

The results of the CF tests for B. bigemina indicated that there were 319 reactors which represented 75 per cent of the 428 cattle tested (Table 27). The per cent B. bigemina reactors on the 6 ranches ranged from 59 to 100 per cent.

Twenty-seven per cent of the calves tested between 1 and 6 months of age were B. bigemina reactors, while 58 per cent of the calves between 7 and 12 months were reactors (Table 28). Seventy per cent of the cattle tested in the 13 to 24 month old age group were reactors and 94 per cent of all cattle tested greater than 24 months of age were B. bigemina reactors.

Discussion- Llanos Study

The 42 per cent prevalence of B. bigemina reactors in the Llanos indicates that although a large percentage of the cattle are infected, 58 per cent remain susceptible to clinical babesiosis. The data would suggest an unstable situation in which clinical cases of babesiosis could be expected to occur in indigenous cattle. However, clinical cases of babesiosis during the previous year were not reported by any of the ranch managers on the 37 ranches. The large decrease in Babesia reactors from 65 per cent in the 7 to 12 month age group to 30 per cent in the age group over 24 months suggest that either a large proportion of the cattle are not reinfected following the initial infection,

TABLE 27

Babesia bigemina Prevalence in the Cauca Valley of Colombia

March 1974 - February 1975

Results of Complement-Fixation Test for Babesia bigemina

Herd No.	No. of Cattle in herd	No. of Cattle Tested	No. of Reactors*	Percent Reactors
1	383	88	81	92%
2	300	23	23	100%
3	186	28	25	89%
4	196	31	29	94%
5	300	30	27	90%
6	243	228	134	59%
Total	1608	428	319	75%**

* 1+, 2+, 3+ and 4+ reactions CF screen test.

** Percentage reactors in the 428 cattle tested

TABLE 28

Babesia bigemina Prevalence in the Cauca Valley of Colombia

March 1973 - February 1974

Ages (Months)	No. of Cattle Tested	No. of Reactors*	Percent Reactors
1-6	60	16	27%
7-12	50	29	58%
13-24	77	54	70%
> 24	139	131	94%
Total	326	230	71%**

* 1+, 2+, 3+ and 4+ Complement Fixation screen test.

** Percent reactors in the 326 cattle tested.

or that a large percentage of older cattle are subclinical carriers which were not detected by the CF test.

The 5 to 94 per cent variation in the number of Babesia reactors among the 37 herds in the Llanos study indicated the potential occurrence of clinical babesiosis associated with the movement of cattle from a herd with low prevalence to a herd with high prevalence. Another probable factor is herd management.

The role of anaplasmosis and babesiosis in the "Secadera" complex needs to be clarified. Secadera was most frequently reported as the major single disease problem on the 37 ranches and is reported to occur most frequently during the dry season in association with nutrition stress. The syndrome is characterized by a continual loss in body weight accompanied by anemia. Anaplasmosis and/or babesiosis are considered to be important causal factors in association with nutritional stress.

B. microplus ticks were identified on each of the 37 ranches visited in the Llanos and were nearly equally distributed within the study area as indicated by the nonsignificant difference in tick counts among the 5 study zones. The infrequent occurrence of the other 3 species of ticks identified indicated that their importance as vectors or potential vectors of anaplasmosis and/or babesiosis is limited. The effect of annual climatic variation, such as occurs during the wet and dry seasons, on tick infestation of cattle in the Llanos needs to be determined to accurately assess the correlation of outbreaks of babesiosis with instability in the vector population. Unstable epidemiological situations may be expected when tick populations are either naturally or artificially reduced to such low levels that the frequency of transmission is insufficient to maintain infection in young animals when passive immunity and natural resistance is highest.

North Coast Study (Table 29)

The 100 per cent infection of the 107 calves with B. bigemina suggested that babesiosis due to infection with B. bigemina was endemic on the 4 ranches studied and that the 57 per cent reactors observed in the 120 pregnant cows tested was probably lower than the true prevalence of infection. The low number of reactors in the pregnant cows was attributed to the failure of the CF test to identify subclinical carriers as reactors. Positive transmission tests with CF negative cattle and the relatively short period of time following single infection during which the CF test will detect B. bigemina infection indicated that some CF negative cattle may actually be subclinically infected.

Cauca Valley Study (Table 29)

The 75 per cent prevalence of B. bigemina observed on the 6 ranches included in the study indicate that babesiosis is endemic in the Cauca Valley. The distance of the 6 ranches from each other and the location of the ranches at the north, south and middle portion of the Cauca Valley suggest equal distribution of disease throughout the Valley.

The high prevalence of babesiosis in cattle greater than 24 months of age, (94%), indicated the importance of early infection of calves when resistance is maximum, and the probability of clinical infections occurring in calves has decreased.

The danger of severe clinical infection occurring when susceptible cattle from non-endemic areas are introduced into the valley was substantiated.

TABLE 29
 EPIDEMIOLOGICAL STUDY OF BOVINE ANAPLASMOSIS AND BABESIOSIS IN THE LOWLAND TROPICS
 OF COLOMBIA

November 1973 - February 1975

Study Area	No. of Cattle Tested	Percent Reactors* <u>Anaplasma marginale</u>	Percent Reactors* <u>Babesia bigemina</u>
Eastern Plains	3034	75%	42%
North Coast	232	91%	77%
Cauca Valley	432	71%	75%
Total	3698	75%**	48%***

* 1+, 2+, 3+, 4+ reactopms Complement-Fixation screen test.

** Percentage anaplasmosis reactors in the 3698 cattle tested.

*** Percentage Babesia bigemina reactors in the 3698 cattle tested.

Babesia - Vector and Transmission

The literature and our experience indicates that the 1 host ticks belonging to the genus Boophilus are the principal vectors of babesiosis caused by B. bigemina and B. microplus. Since the latter are blood parasites that are capable of mechanical transmission as in anaplasmosis, the role of alternate vectors comes into focus as a problem that merits further study. Transmission of babesiosis can be experimentally induced by mechanical means such as contaminated instruments or biting flies, needles, etc., but the history of this infection is such that these methods are of little importance in maintaining infection. In the 1920's, 30's, and 40's when Boophilus ticks were eradicated throughout the southeastern U.S., including Texas, cases of babesiosis declined, and subsequently ceased to occur. This did not happen with anaplasmosis which has persisted in this area, obviously being transmitted also by other means. The elimination of the tick vector of babesiosis has always been associated with elimination of babesiosis. The prevailing attitude in South America that ticks cannot be eradicated is so strong that research in this area has been stymied and has never received even nominal support. Our efforts in this area have therefore been minimal in comparison to areas such as vaccination, diagnosis and treatment.

The life cycle of Babesia in Boophilus ticks is not yet entirely understood, but efforts to establish pure Babesia infections, either bigemina or bovis in clean ticks, have been successful. Female ticks apparently become infected during the last 24 hours of attachment on the host, and the resulting progeny larvae and nymphs transmit B. bovis and B. bigemina respectively.

Efforts have been made to characterize and identify grass species which in themselves are unfavorable to tick survival. For years

stockmen have held the concept that certain pastures were not as ticky as others, and that this was significantly influenced by the type of vegetation. The development of a tick control program based on pasture improvement, using selected species might prove a real step forward to tick control in endemic areas.

Of six grass species analyzed, Melinis minutiflora (Molasses grass) showed the highest tick deterrent properties while Andropogon gayanus (Gamba grass) exhibited the ability to maintain a defined and constantly low tick infestation. Based on this finding it was concluded that molasses grass could best be used in a tick control package in a marginal tick zone while gamba grass has the advantage within an endemic zone. The use of these grasses plus strategic acaricide application, should achieve tick control at a minimum cost, being particularly well adapted for the small livestock producer.

Babesia - Immunology

Immunization of cattle against babesiosis via a premunition technique has been practiced almost as long as the disease entity has existed. Early workers near the turn of the century observed that cattle having had red water, or coming from red water endemic zones were healthy but contact of these cattle, and more specifically, the ticks from these cattle with local cattle in the north was followed by devastating cattle losses. It was further observed that calves born to carrier dams often became naturally infected with a sub-clinical form of the disease with subsequent recovery and a continuing immunity. It is common to confirm today the observations made over 80 years ago, that if one takes ticks from known infected cattle and places them on susceptible cattle to induce carrier infection, a state of premunition or immunity is imparted.

In recent years some attention has been given to the development of sterile immunity through the use of killed vaccines, or by treating the infected animal in such a way as to eliminate carrier infections. Even though this approach was first reported as long ago as 1967, the basic techniques of premunition continue to be preferred, and with variation, are primarily the approaches still used.

Premunition:

There are two situations where premunition can fill an important function. The need is most urgent when clean cattle from Europe or the U.S. are being shipped to the tropics, either South-Central America, Africa or Asia, where ticks and babesiosis occur. These cattle if not premunized, rapidly develop babesiosis soon after exposure to Boophilus ticks, and unless promptly treated will die in large numbers. Another instance where premunition is desirable is in the so called intermediate, or unstable zones where ticks occur, but

with insufficient consistency to produce a 100% infection in the calves, and where re-exposure is insufficient to maintain immunity, thus a susceptible population builds periodically, with subsequent acute infections occurring among these cattle. In this situation calf vaccination or premunition is desirable and practical, and economically advisable.

Experimentally we have used both whole blood from carrier animals, and red cell frozen stabilates. Both techniques work well for both Babesia bovis and B. bigemina. The stabilate preparations are preferred for the same reasons mentioned under the heading of anaplasmosis. These Babesias freeze well, and can be stored for years in liquid nitrogen. The stabilates can be titrated annually, and their purity verified in splenectomized calves, thereby establishing a bank of known infectious material of known potency. This eliminates the need to maintain carrier animals which may develop other infections transmissible to cattle, may lose the infection through a spontaneous recovery, or who may cycle into acute infections through some unrecognized stress and when used as a source of premunizing blood be more infectious than anticipated.

Stabilate-vaccine production is quite simple and easily accomplished. In the case of B. bovis (argentina), the Australians have reported marked attenuation of this strain following 10-12 serial passages in splenectomized calves. This technique has been followed and apparent attenuation has occurred. Similar serial passage of B. bigemina has clearly established pure infections, and to a lesser extent some slight attenuation. The desired infection (either B. bovis or B. bigemina) is induced in splenectomized calves, and when the parasitemia reaches 2% or greater 3-400 ml of blood is collected for freezing. The volume is related to the size of the donor calf, the need for stabi-

late, and storage capacity. This blood is then washed 2 times in veronal buffer pH 7.2 - 7.4, and the packed cells mixed with equal parts 4 molar dimethylsulfoxide. Immediately after thorough mixing, the blood is poured into small vials in sufficient quantity to insure the recovery of 2 ml, stoppered with rubber caps, sealed with metal caps, and immersed in liquid nitrogen. The mixing with DMSO is done in small amounts (15 ml DMSO + 15 ml packed cells) to reduce the time during which the red cells are exposed to the action of DMSO prior to freezing. Prior to use the stabilates are thawed rapidly and immediately diluted 1:10 in 10% fetal calf serum in isotonic phosphate buffer solution, pH 7.2. The diluted stabilate is kept cold, on ice, and used within 20 minutes. That which has not been used in this length of time is discarded. Premunizing inocula are given intravenously. The final dilution, and amount of inocula is pre-determined by titration. The usual dilution for Babesia is 1:10 to 1:100, but dilutions of up to 1:1000 have been used. Usually 2 ml are given per animal in the jugular vein.

Many experiments and trials in Colombia and the U.S. have demonstrated the safety, reproducibility, and effectiveness of this approach. The incubation time is reasonably consistent so that if treatment is required all animals in a given group could be treated simultaneously without the need for individual examination.

Two drugs have proven highly effective in moderating clinical disease in cases where this is needed. Ganaseg (also called berenil) (4,4' -Diamidino-diazoaminobenzene-di-acetamidoacetate) is highly effective against Babesia. In those cases where zero parasitemias are to be avoided the recommended dose of 3-3.5 mg/kg b.w. is reduced to 0.5 mg/kg b.w. This low level (1/7 the recommended dose) has in nearly all instances been entirely satisfactory in moderating clinical effects without sterilization. Imidocarb (3,3' -bis (2-imidazolin-2yl)

carbanilide as either the dipropionate or dihydrochloride) has also been highly effective in treating Babesia. This drug is not accepted for use in most areas, but has been used experimentally at as low as 0.5 mg/kg in the same way as Ganaseg with highly successful results. It is anticipated that when this drug is released for commercial distribution, it will add a second highly effective agent against Babesia.

A trial was recently conducted in 19, 2 year old bred Holstein heifers destined for shipment to Nicaragua. Ten heifers were retained as non-premunitized controls. Each of the 19 heifers was inoculated IV simultaneously with 2 ml B. bigemina stabilate at a 1:10 dilution, and 2 ml B. bovis stabilate at a 1:10 dilution. The controls were not injected. Two heifers had sufficiently severe reactions as to require treatment with one IM injection of 0.5 mg/kg ganaseg. The average animal response to premunitization is given in Table 30.

One of the 10 heifers failed to develop a demonstrable replicating infection of B. bigemina based on parasitemia and on CF serologic response, and 2 of 19 failed to show a B. bovis response based on these criteria. The parasitemias referred to in Table 30 were B. bigemina. Only 3 of 19 cattle showed a B. bovis parasitemia and these were very low. No deaths due to Babesia premunitization were seen.

These cattle when taken to Nicaragua were apparently protected against local Babesia challenge, based on reports after their arrival. This approach combined with Anaplasma premunitization is highly recommended for use in cattle being shipped from the U.S. to endemic areas of South America and Africa.

Premunitization can also be used to advantage in areas of intermediate infection. A calf vaccination or premunitization approach has been extensively tested in the Cauca Valley, utilizing a grant from USAID for this purpose. Results indicate that these techniques are an unqualified success. The

details of these results will be presented in the USAID termination report for this project. (Project number - AID/ta-C-1220)

Sterile Immunity

These vaccines have been prepared on various occasions, generally with good results, but they have not found acceptance for routine use. A recently conducted trial at Texas A&M is representative of the value of these vaccines, which clearly suggest that greater emphasis should be placed in this area.

A B. bigemina antigen was prepared from the blood of a splenectomized calf showing a 65% B. bigemina parasitemia. The erythrocytes were lysed in hypotonic saline and distilled water with centrifugation to collect stroma and parasites which were concentrated into antigen with a CF titer activity o. 1:29. This antigen was mixed with equal parts complete Freund's adjuvant and inoculated in 2 ml amounts subcutaneously 2 times at a 4-6 week interval. Three adult cows and 5 splenectomized calves were vaccinated. Two non-vaccinated adult controls plus the 3 vaccinated adult cows were all challenged with 5 ml whole blood from a calf showing an ascending B. bigemina parasitemia of 0.5%. The challenge inocula was given IV, 67 days after the preimmunized cows had received the last vaccination. Thirty-three days after vaccinating the 5 splenectomized calves, they and four non-protected splenectomized control calves were all challenged with 2 ml of a 10^{-1} dilution of a B. bigemina frozen stabilate. The results are presented in Table 31. All of the non-vaccinated controls died of acute babesiosis. The vaccinated animals all survived. Infection was detected in 2 of 3 adult vaccinated cattle and 3 of 5 vaccinated splenectomized calves following virulent challenge, but the challenge response was moderate, with no severe clinical signs being seen.

It is not known how long this type immunity may persist, but even if it were no longer than 3 months it would be very useful in clean cattle transported to endemic zones. The vaccine is entirely safe and if natural exposure occurred as expected within the first 10 weeks after arrival, sufficient immunity would be present to allow a change from clean to infected status without the attendant hazards of acute babesiosis.

A few limited field trials early in the course of our project by Drs. Todorovic and Admas confirmed the value of killed vaccines and the potential of sterile immunity.

TABLE 30

Response of 2 Year Old Holstein Heifers to Babesia Premunition

	No. of Animals	Avg. PCV at time of Premunition	Avg. Low PCV due to Premunition	Avg. % Drop in PCV due to Premunition	Avg. High Parasitemia (Bb)	Avg. Incubation Time (Days)	Animals showing signs of replicating Infection	
							Bb	Ba
Babesia Premunized Holstein Heifers	19	29.6% ±1.9	19.4% ±4.5	34.3% ±14.8	0.27%	7.0	18	17
Non-Premunized Holstein Heifers	10	27.8% ±3.3	27.3% ±2.7	(1) --	--	--	0	0
Significance:		NS	P<0.01					

(1) Values taken at the same time the low PCV occurred in the premunized group.

TABLE 31

Response to *Babesia bigemina* (Bb) Needle Challenge
on Cattle Previously Exposed to a Killed-Adjuvant Bb Vaccine

	No. of Cattle	CF Response to Vaccination	Day of Challenge	No. Showing Challenge Response	Avg. Incub. Time (days)	Avg. High Parasit. (%)	High CF Titer	Low PCV	Deaths
Adult Cattle Vaccinated	3	1:25	67	2/3	6	<0.1	1:452	30	0/3
Adult Cattle Controls	2	--	--	2/2	2	1.1	---	8	2/2
Splenectomized Calves Vaccinated	5	1:13	33	3/5	7	0.7	1:254	17	0/5
Splenectomized Calves Controls	4	--	--	4/4	5	27	---	6	4/4

Vaccination interval was 3 weeks in the adult cattle and 3 and 4 weeks in the splenectomized calves. Adult cattle were challenged with 5 ml whole blood I/V from a calf showing an ascending Bb parasitemia of 0.5%. Splenectomized calves were challenged with 2 ml. IV, 10^{-1} dil., of Bb frozen stabilate.

Babesia - Treatment

The management of babesiosis can best be accomplished by preventive measures such as tick control, vaccination, and good management, but even when these measures are properly applied, there will be an occasional animal developing acute babesiosis and then the only recourse is to treat with effective babesiacidal agents. The drug most commonly used at the present time for this purpose is Berenil (or Ganaseg). Berenil (4,4' -Diamidino-diazoaminobenzene-di-acetamido-acetate) is highly effective, and has low toxicity at the levels used.

Berenil was recently titrated in splenectomized calves infected with B. bigemina, to establish effective drug levels. The recommended dosage is 3.5 mg/kg b.w. Using 3.5 mg/kg as a base, levels of 1.0 mg/kg, 0.5 mg/kg, 0.25 mg/kg, and 0.125 mg/kg were used to treat splenectomized calves when the B. bigemina parasitemias reached 1.8 to 3.2% but prior to any drop in PCV. The results are tabulated in Table 32, and clearly show that the drug was quite effective well below the recommended levels. As little as 0.25 mg/kg was sufficient to prevent death losses, and 1.0 mg/kg was apparently equal to the 3.5 mg/kg in limiting infection. B. bovis is known to require higher levels of therapy in many instances, so these low levels (0.25 mg/kg) while effective against B. bigemina may not be against B. bovis. Even so the drug is highly effective. We have routinely had success at 0.5 mg/kg, in animals being premunized.

A new drug, Imidocarb, (3,3' - bis- (2-imidazolin - 2-yl) carbanyl dipropionate or dihydrochloride) has been tested and found highly efficient in the treatment of babesiosis. Titrations have not been made, as was done with Berenil, but the use of this drug in low levels (0.5 mg/kg) has led to the conclusion that the level of activity

TABLE 32

Titration of Ganasey on
Babesia bigemina Infection
in Splenectomized Calves

Drug Amount	3.5 mg	1 mg	0.5 mg	0.25 mg	0.125 mg	-0- Control
No. of Calves	2	2	2	2	2	3
Avg. Weight	109 kg	100 kg	114 kg	112 kg	109 kg	105 kg
Avg. Parasitemia at time of Treatment (%)	2.4	3.2	3.0	2.4	3.2	1.8
Avg. High Parasitemia after Treatment (%)	1.1	1.5	2.2	2.8	6.4	5.8
Avg. time (hours) required for Parasitemia to disappear	24	36	32	64	96	---
PCV at time of treatment (%)	29.5	29.5	30	30	31.5	28.7
Avg. low PCV after treatment (%)	21.5	23.5	18	18	11.5	5.8
Avg. % Reduction in PCV (Normal PCV - Low PCV/Normal PCV)	28	20	40	40	64	80
Avg. PCV Regression Slopes*	-0.096	-0.114	-0.142	-0.195	-0.448	-0.252
Deaths	0/2	0/2	0/2	0/2	1/2	3/3

* Calculated from day 1 to the time (expressed as hours post treatment) that the low PCV occurred.

is about comparable. An assay technique was developed at Texas A&M to measure tissue residues of this compound and it was found that following 2 injections of 5 mg/kg each, administered 2 weeks apart, significant levels of drug were detected for as long as 9 months after infection in skeletal muscle, heart muscle, and liver. The persistence of this drug will probably prove a major drawback in its final clearance by FDA for marketing in the U.S. but also suggests it may have chemoprophylactic activities of value.

To test this hypothesis eight dairy type, bull calves approximately four to six months old were used. Calves 1,2,3, and 4 were treated with 5 mg/kg Imidocarb dipropionate injected IM. Calf 1 was treated 14 days after the release of B. argentina-infected Boophilus larvae, at a time when a Babesia parasitemia was present. Calves 2,3, and 4 were treated 14, 28 and 42 days before similar larval release. Calves 5, 6, 7 and 8 remained as untreated controls, paired with calves 1,2, 3 and 4, respectively. A total of 20 engorged female ticks were collected from each calf. Each of the engorged females, at the time of collection, and the resulting egg mass 18 days later, were weighed. The egg/female weight ratio was determined to evaluate further possible influence of treatment on the female tick and her fecundity. After weighing each total egg mass originating from a single calf, they were pooled and divided into 500 mg aliquots for future use.

When ready to feed, the larval progeny of engorged female ticks collected from calves 1 and 5, 2 and 6, and 3 and 7 were released on each of six susceptible splenectomized calves to determine larval infectivity. The diagnosis of Babesia infection and parasitemia in calf 4 was confirmed by inoculating a splenectomized calf with 10 ml. of its blood intravenously, collected 42 days after the fever response.

The influence of Imidocarb treatment on Babesia infection in calves and on Boophilus, ticks is tabulated in Tables 33 and 34, respectively.

Calf 1 developed signs of Babesia infection before treatment on day 14. Calf 1 recovered following treatment. The larval progeny of ticks recovered from calf 1 were not infective for Babesia when placed on a susceptible splenectomized calf.

Calf 2, treated 14 days before tick infestation, failed to develop evidence of Babesia infection. Larval progeny of ticks recovered from calf 2 were not infective for Babesia when placed on a susceptible splenectomized calf.

Calf 3, treated 28 days before tick infestation, failed to develop evidence of Babesia infection. The larval progeny of ticks recovered from calf 3 transmitted Babesia infection to a splenectomized calf.

Calf 4, treated 42 days prior to tick release, developed mild clinical signs of babesiosis. The occurrence of infection in calf 4 was confirmed by inoculating 10 ml. of whole blood into a susceptible splenectomized calf, which died of acute babesiosis (B. argentina) 14 days after injection.

Control calves 5,6,7 and 8, receiving no treatment, developed evidence of babesiosis after infestation with B. microplus larvae. The larval progeny from calves 5,6, and 7, when released on splenectomized calves, consistently produced babesiosis.

The average weights of 20 engorged female ticks collected from all calves are recorded in Table 34. The average egg yield from each female plus the ratio egg weight/female weight are also recorded. No clear pattern of influence attributable to either treatment of infection on female weights or fecundity was evident.

The apparent success of Imidocarb as a therapeutic and chemopro-

TABLE 33

Prophylactic effect of Imidocarb Against Babesia Infection in Calves and Ticks

Calf Number	Day of Treatment*	First Day of Fever	Response of Tick		Infestation Babesia parasitemia	Infectivity of larval progeny
			Min PCV	Max temp. °C		
1	+14	13	10	40.3	Pos.	Neg.
2	-14	None	25	39.1	Neg.	Neg.
3	-28	None	26	38.9	Neg.	Pos.
4	-42	14	30	40.4	Pos.**	NT
5	None	13	12	40.7	Pos.	Pos.
6	None	13	13	41.1	Neg.***	Pos.
7	None	13	16	40.2	Pos.	Pos.
8	None	15	25	40.4	NT	NT

* Calf 1 was treated 14 days after tick exposure; calves 2,3 and 4 were treated 14, 28 and 42 days before tick exposure.

** Confirmed by sub-inoculation

*** Negative on thin smear, but clinical signs of babesiosis were observed.

NT No Test

TABLE 34

Influence of Imidocarb Treatment on Size and Fecundity of B. microplus Ticks

Calf Number	Avg. Wts. of 20 engorged females (mg)	Avg. egg wts. from 20 engorged females (mg)	Ratio of egg/engorged female weights
1	404±	228±28	0.56±0.06
5	409±	235±29	0.57±0.05
2	348±34	203±21	0.58±0.04
6	438±58	248±54	0.56±0.12
3	442±41	263±26	0.59±0.04
7	385±42	128±30	0.34±0.09
4	509±43	304±60	0.60±0.12
8	461±40	253±40	0.55±0.09

± Standard deviation

phylactic agent against bovine babesiosis in both the vertebrate and invertebrate hosts suggests the possible use of this compound in future control programs. In situations where tick eradication is not feasible, or is impractical, Babesia control and possible eradication might be considered using this compound. Additional field studies would be required and the practical dose range and treatment interval determined, but if Babesia can be eliminated in both hosts then theoretically with repeated treatments the reservoir of infection could be eliminated even in the presence of Boophilus ticks.

In these trials it is not clear whether the drug killed the Babesia spp. in the tick or if the drug merely prevented the development of the organism in the vertebrate host and hence interfered with reinfection of the tick. It was, regrettably, not determined if calf 3 actually developed a sub-clinical infection, hence reinfesting ticks, or if the ticks retained infection to the next generation, in the absence of infection in calf 3.

This study prompted the field use of Imidocarb as a chemoprophylactic agent, in lieu of premunition, in 44 young charolais cattle which were moved from Texas to Haiti.

To determine what infections were present in Haiti, blood samples were taken from 31 head of adult, indigenous cattle randomly selected. Complement-fixation (CF) tests for anaplasmosis and babesiosis were conducted. These tests showed that 24 (71%) were positive or suspicious for Anaplasma and 17 (55%) were positive or suspicious for Babesia.

Of 44 Charolais cattle to be shipped, 10 were 6 month old heifers, 24 were 6 month old bulls, and 10 were 18 month old bulls. Prior to shipment, serum samples were collected from each and tested for evidence of previous exposure to Anaplasma and Babesia, all test were negative.

All cattle were vaccinated for anaplasmosis, one week prior to shipment to Haiti. Only 1 injection of Anaplaz vaccine was given.

The cattle were moved by truck to Miami, by plane to Cape Haitian, Haiti, and then trucked to the plantation. The day after arrival, all cattle were injected subcutaneously with Imidocarb at the rate of 2.8 mg/kg of body weight.

Following this treatment, all 44 cattle were put on pasture in contact with native cattle. No efforts were made to control ticks until 130 days after arrival, at which time all cattle were sprayed with Co-Ral to remove ticks. Blood samples were taken from all cattle 130 days after arrival; serum was collected and tested to evaluate the prevalence of Anaplasma and Babesia infection that had occurred.

The cattle became severely infested with Boophilus ticks. The incidence of Anaplasma and Babesia infections during the first 130 days is recorded in Table 35. There were 41 (93%) positive or suspicious serologic reactions for anaplasmosis. Of the 32 positives, the average CF serum titer was 1:186, indicative of recent acute infections. There were 34 (77%) positive or suspicious reactions to the Babesia CF test.

During the first 130 days no deaths occurred.

In view of the obvious massive natural infection occurring in these cattle, and the history of 20-50% death loss in similar cattle introduced into this situation, it is most impressive that there were no death losses. It would strongly appear that Imidocarb could well have chemoprophylactic activity against both Babesia and Anaplasma. Studies since this trial strongly suggest that a treatment with Imidocarb 2 weeks after arrival might be even more effective, and prolong the effect of treatment. This treatment technique for clean cattle being introduced is probably the least hazardous, and most economical of techniques currently being tested. It is dependent however, on prompt exposure of the introduced cattle at a time when the Imidocarb blood level is sufficient to prevent a fulminating infection.

Table 35

Babesiosis and Anaplasmosis Incidence 130 Days After Arrival in Haiti

	Anaplasmosis			Babesia		
	Pos.	Susp.	Neg.	Pos.	Susp.	Neg.
Pre Shipment	0	0	44 (100%)	0	0	44 (100%)
130 days after arrival in Haiti	32 (73%)	9 (20%)	3 (7%)	16 (36%)	18 (41%)	10 (23%)

Theileriosis

Pathogenic infections have not been described in Colombia, although it is probable that non-pathogenic strains of Theileria occur. In the U.S. white-tailed deer are naturally infected with Theileria cervi, which can under stress conditions become a highly pathogenic infection. This organism appears host specific and will not infect cattle.

It was with considerable interest that we discovered an apparently non-pathogenic Theileria in cattle located in East Texas. This organism morphologically closely resembles T. mutans, and T. parva, but is not pathogenic. No schizonts have been seen, only the blood piroplasms, which are readily transmitted by blood inoculation.

Initially the organism was seen along with anaplasmosis. Anaplasma was completely removed from the calf having a mixed infection, using tetracyclines, leaving only the Theileria which was apparently completely refractory to treatment.

Efforts to transmit these Theileria with Amblyomma americanum, and A. cajennense by transtadial means were unsuccessful.

We have not been successful in preparing frozen stabilates of this organism, but have maintained a chronic infection in splenectomized calves for over 2 years. It is interesting that parasitemias ranging from 0.1 to 2.0 % are almost always present and have maintained this level for over 2 years.

Trypanosomiasis

In our early survey work on anaplasmosis blood cultures were made to detect the presence of Trypanosoma theileri. This organism was found on the North coast and in the Llanos at La Libertad. It was readily diagnosed by placing 0.5 ml of sterile whole blood on blood agar slants containing penicillin and streptomycin. After incubation for 4-7 days the trypanosomes were readily identified in wet mounts, and on giemsa stains. These trypanosomes were entirely non-pathogenic insofar as we could determine, and closely resembled the same organism observed in the U.S.

Even though the tsetse fly is not present in South America a pathogenic, salivarian trypanosome does occur and has been identified as T. vivax. This organism was isolated from both sheep and cattle where it produces a long debilitating disease often terminating in death. The vector in Colombia is not well established, but is presumed to be a biting fly which transmits infection mechanically. There have been instances where significant cattle losses have been ascribed to this organism, but its distribution, prevalence, and economic impact in Colombia was basically unknown.

To partially answer some of the questions concerning this problem an indirect fluorescent antibody test (IFAT) for Trypanosoma vivax was developed for a survey involving over 2000 cattle distributed throughout 11 departments and territories in Colombia.

Antigen for the IFAT was derived from a strain of T. vivax isolated from a cow in the Department of Cordoba, Colombia. The strain was maintained via serial passage in hemoparasite-free calves. Inoculation of approximately 2×10^9 trypanosomes into splenectomized, triamcinolone acetonide-treated calves resulted in parasitemias suitable for

IFAT antigen production at 66 to 69 hours post inoculation.

Thin blood smears were prepared from citrated T. vivax laden blood, air dried and fixed in acetone: methanol::60:40, -20°C for 30 minutes. Fixed antigen slides were stored dry at -35 and -70°C and used successfully in the IFAT for 144 and 116 days respectively.

Indirect fluorescent antibody test serum titers of 1:100 or greater were observed at a mean time of 15.9 days post parasitemia (PP) with a range of 6 to 32 days. Maximum titers of 1:400 developed in 14 to 33 days PP in 8 calves. In one calf, 109 days PP were required before the maximum titer was attained. Serum titers persisted in calves up to 278 days PP. Maternal antibodies capable of causing a positive fluorescent reaction at 1:100 serum dilution persisted for 29 days after birth in 1 calf.

At serum dilutions of 1:100, the IFAT detected positive reactions in 80.4% of 133 serum samples obtained from 9 calves at various times during the course of T. vivax infections. At serum dilutions of 1:50, the percentage of positive reactions increased to 97%.

The IFAT was specific for T. vivax. No cross reactivity was observed between T. vivax and Anaplasma marginale, Babesia argentina, Babesia bigemina, Eperythrozoon spp. or Trypanosoma theileri at 1:50 serum dilutions. Suspicious reactions were occasionally observed when Trypanosoma evansi-positive serum diluted 1:50 and 1:100 was used in the IFAT for T. vivax. No false positive reactions were observed when serums from 36 hemoparasite-free calves were tested. Results from the IFAT were repeated within plus or minus 1 dilution approximately 80% of the time using different antigen lots on the same and different days.

Samples obtained for the IFAT by eluting serum from dried blood-impregnated filter paper that had been stored at -20°C for 10 days or less, produced results nearly equal to those obtained by using conventional serum samples.

In field cases of trypanosomiasis, the IFAT was up to 21 times more effective in detecting T. vivax-positive cattle on the basis of antibody presence than the thick blood smear technique.

Results of the survey revealed the presence of T. vivax antibodies in cattle from the departments of Boyaca, Cordoba, Meta, Sucre and Valle. No T. vivax IFAT antibodies were detected in serums obtained from cattle in the departments of Antioquia, Cauca, Caqueta, Cundinamarca, Tolima and Vichada.

APPENDIX I

1. KUTTLER, L. L., ZARAZA, H. and ROBERTS, E.D.: Hematologic and Clinical Response to Anaplasmosis Vaccines and the Comparative Efficacy of These Vaccines, As Measured by Field and Experimental Challenge. Proceedings of the Fifth National Anaplasmosis Conference, Stillwater, Oklahoma, (February 28-29, 1968): 39-49.

Twenty, 3-month-old calves were divided in 4 equal groups. Group 1 was inoculated with an attenuated Anaplasma marginale; group 2 received an A. marginale adjuvant vaccine; group 3 was infected with virulent A. marginale followed by treatment; and group 4 remained as unvaccinated controls. All animals were moved into an Anaplasma endemic zone 3 months later and allowed to undergo natural field challenge. Evidence of acute anaplasmosis was observed in all calves, except those preimmunized by virulent A. marginale. No significant evidence of protection was produced by either the attenuated A. marginale or the adjuvant vaccine when compared to the unvaccinated controls. The group preimmunized with virulent A. marginale failed to respond to natural exposure.

Hematologic response to virulent, attenuated, and killed A. marginale vaccines was measured in 18 mature cattle divided into 3 groups. The group receiving virulent A. marginale was treated 25 days after infection (Burroughs Wellcome Compound 356C61). No death losses occurred in this group, but moderate infections were observed to result in a significant reduction of PCV. The attenuated A. marginale vaccine produced a low level parasitemia, a marked serological response as measured by the complement-fixation (CF) test, and a very slight drop in PCV, which was not significantly different from values observed in an unvaccinated, non-infected, control group. The group receiving adjuvant vaccine showed only a low level, transient, CF serological response.

An experimental challenge was administered 8 weeks after vaccination to cattle receiving the attenuated and adjuvant vaccines, along with a group of 5 unvaccinated controls. All controls reacted to challenge with severe acute signs of anaplasmosis. One animal was allowed to die, a second would probably have died had it not been treated. Cattle receiving the attenuated vaccine showed no signs of active infection resulting from challenge. Cattle receiving the adjuvant vaccine reacted to challenge, but less severely than did the controls.

2. ZARAZA, H. KUTTLER, L. L. and ROBERTS, E. D.: Respuesta Hematologica y Clinica a Diferentes Vacunas de Anaplasmosis y la Eficacia Comparativa de Estas, Evaluadas por la Inoculacion Experimental. Revista ICA, (December 1968), 3, (4): 323-331. (Spanish translation of above.)

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3. CARSON, C. A.: An Antigenic and Serologic Comparison of Two Virulent Strains and an Attenuated Strain of Anaplasma marginale. A Thesis submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Master of Science, August 1969.

An antigenic and serologic study was conducted using virulent strains of Anaplasma marginale from Texas and Colombia and an attenuated strain of Anaplasma marginale. Soluble antigens of the three A. marginale strains were compared by agar gel diffusion and immunoelectrophoresis. Serum proteins from calves infected with each of the three A. marginale strains were separated electrophoretically and reacted with rabbit anti-bovine serum in immunoelectrophoresis systems.

No differences between the soluble antigens of the three A. marginale isolates were detectable by agar gel diffusion. All three antigens moved to the same mobility zone in agar gel electrophoresis systems and each antigen formed an arc of precipitation when reacted with serum from calves infected with homologous or heterologous strains of A. marginale.

A beta and a gamma serum protein component, not exhibited in normal bovine serum, were present in the serums of animals infected with either of the virulent A. marginale strains or the attenuated strain.

4. GONZALEZ, E. F. and TODOROVIC, R. A.: Ultraestructura de la Babesia bigemina Revelada por el Microscopio Electronico. Proceedings of the VII Congreso Nacional de Medicina Veterinaria y Zootecnia, Ibague, Colombia, (November 8-12, 1969).
5. KUTTLER, K. L.: Serial Passage of an Attenuated Anaplasma marginale in Splenectomized Calves. Proceedings of the 73rd Annual Meeting of the USAHA, Milwaukee, Wisconsin, (October 12-17, 1969): 131-135.

Twelve serial passages of an attenuated Anaplasma marginale were made in splenectomized calves by blood inoculation. The severity of infection produced at the twelfth passage level in 4 splenectomized calves was compared to the infection occurring in 4 similar calves at a second passage level. Significantly higher parasitemias and lower packed cell volumes occurred in the twelfth passage group, suggesting an increased virulence. No deaths occurred among animals of the second passage group; whereas, 1 of 4 died in the twelfth passage group.

6. KUTTLER, K.L., ADAMS, L. G. and ZARAZA, H.: An Epidemiologic and Geographic Survey of Anaplasma marginale and Trypanosoma theileri in Colombia. Proceedings of the 106th Annual AVMA Convention, Minneapolis, Minnesota, (July 1969). Journal of the American Veterinary Medical Association, (June 1, 1969), 154: 1398, (abstract).

Anaplasmosis complement-fixation tests, packed cell volumes, and stained blood smears were made on 603 cattle located at 5 experiment station farms in Colombia. These farms were situated in differing climatic zones varying from 2,600 meters to 13 meters in altitude and from 13°C to 28°C in mean temperature. Specific reference was made to breed susceptibility, the influence of age, and climatic condition on the incidence and severity of infection.

A direct correlation was noted between mean temperature and incidence of anaplasmosis. At 13°C the incidence was nil; whereas, at 28°C over 90% infection was noted. The mean temperature is directly associated with altitude.

Incidence of infection in enzootic areas was generally greater in older animals, but the effect of infection as characterized by anemia was more noticeable in young animals. The incidence of anaplasmosis in European breeds did not appear greatly different when compared to native and Zebu cattle, but in some instances PCVs were significantly lower in European breeds. This was most marked at the lower elevations.

Blood cultures for Trypanosoma theileri from 71 cattle at 2 experiment stations resulted in a pattern of infection similar to anaplasmosis. A high incidence of infection was noted at the lower elevation with a high mean temperature and no evidence of infection at 2,600 meters with a low mean temperature.

7. KUTTLER, K. L. and ZARAZA, H.: Premunization with an Attenuated Anaplasma marginale. Proceedings of the 73rd Annual Meeting of the USAHA, Milwaukee, Wisconsin, (October 12-17, 1969): 104-112.

An attenuated Anaplasma marginale infection had been established in 21 calves and 12 mature cattle. The resulting infections were found to be significantly less severe than virulent A. marginale in 12 calves and 5 mature cattle. A slightly milder response to the attenuated A. marginale occurred in calves at Bogota with a mean temperature of 14°C when compared to calves similarly infected at Palmira with a mean temperature of 24°C.

Calves and mature cattle previously premunized with the attenuated organism appeared to be immune to virulent challenge with a Colombian isolate resulted in evidence of acute anaplasmosis in both vaccinated and non-vaccinated animals.

8. TODOROVIC, R. A., ADAMS, L. G. and ROBERTS, E. D.: A Study of Bovine Babesiosis in Colombia, South America. Proceedings of the 106th Annual AVMA Convention, Minneapolis, Minnesota, (July 1969). Journal of the American Veterinary Medical Association, (June 1, 1969), 154: 1399, (abstract).

Our research program on bovine babesiosis is a part of the Institute of Tropical Veterinary Medicine, College of Veterinary Medicine, Texas A&M University, with the research program being sponsored by the Rockefeller Foundation and conducted at the Laboratorio de Investigaciones Medicas Veterinarias laboratories, Bogota, Colombia, in cooperation with the Instituto Colombiano Agropecuario. This research effort is directed mainly toward the study and control of bovine babesiosis and the training of Colombian veterinarians and graduate students involved in these research projects.

Although bovine babesiosis is eradicated in the United States, the disease still occurs in most of the world and is of great importance as a threat to livestock industry, especially in the tropical areas of Latin American countries. In Colombia, babesiosis was first described by Lleras (1908) and later recognized as a widely distributed disease, causing great losses in purebred dairy cattle imported into enzootic areas. At the present time the incidence of babesiosis in Colombia is difficult to estimate. The disease exists 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.

The experiments were carried out to identify the existing Babesia species occurring in Colombia by morphologic, immunoserologic, pathologic, and chemotherapeutic methods. The immunoserologic relationship of Babesia spp. and strains were studied by gel-double diffusion precipitation, immunoelectrophoresis, and fluorescent antibody techniques. Attempts were made to develop a sensitive and practical serologic test for the diagnosis of the latent Babesia infection. Several groups of intact and splenectomized calves were inoculated with various antigens isolated from the blood of cattle with acute babesiosis and the blood from patent carriers, respectively. Response to vaccination, premunition, and challenge by tick-borne Babesia was recorded. The results of these experiments were discussed.

9. VIZCAINO, O. G., TODOROVIC, R.A. and ADAMS, L. G.: Diagnostico de Babesiosis Bovina en Frotis Delgados de la Sangre. Proceedings of the VII Congreso Nacional de Medicina Veterinaria y Zootecnia, Ibague, Colombia, (November 8-12, 1969).
10. ZARAZA, H., KUTTLER, K. L. and ROBERTS, E. D.: Efectos de la Descarga Natural de Anaplasma marginale en Terneros Vacunados y no Vacunados. Revista ICA, (September 1969), 4, (3).

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11. ADAMS, L. G., HIPOLITO, O., MORALES, H., GONGORA, S. and JONES, L. P.:
Dermatophilosis Bovina (Estreptotricosis cutanea) en Colombia.
Revista ICA, (March 1970), 5, (1): 3-16.

Four cases of bovine dermatophilosis were diagnosed in Cordoba, Colombia, and confirmed by bacteriological culture methods. Macroscopic and microscopic descriptions were made of the lesions caused by Derma-
tophilus congolensis.

12. ADAMS, L. G. and KUTTLER, K. L.: Toxicity of Alpha-Ethoxyethylglyoxal
Dithiosemicarbazone in Cattle. American Journal of Veterinary
Research, (August 1970), 31: 1493-1495.

Alpha-ethoxyethylglyoxal dithiosemicarbazone, administered 10 consecutive days at the dose rate of 5 mg/kg/day, caused axonal and myelin degeneration of the vagus nerve in 2 of 7 calves. Of the 7 experimental calves, 6 died of tympanites.

13. ADAMS, L. G. and TODOROVIC, R. A.: A Study of the Pathogenesis of Anaplasmosis in Intact Calves: Including Clinical, Clinical Pathological Serological, and Immunofluorescent Techniques. Proceedings of the VI Congreso Panamericano de Medicina Veterinaria y Zootecnia, Santiago, Chile, (September 28- October 3, 1970): 37, (abstract).

Twelve, 4-month-old, male, hemotropic disease-free, Holstein calves were inoculated subcutaneously with blood containing a Colombian isolate of Anaplasma marginale. Previous to inoculation 3 control samples were taken for bone marrow and blood determination.

Thereafter, samples were collected every 2 days and 1 calf was euthanatized every 2 days to collect a complete set of tissues for gross and microscopic pathological lesions, as well as for the immunofluorescent study using the indirect technique. Results obtained are discussed, except those related to immunofluorescent study.

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14. CARSON, C. A., ADAMS, L. G. and TODOROVIC, R. A.: An Antigenic and Serologic Comparison of Two Virulent Strains and an Attenuated Strain of Anaplasma marginale. American Journal of Veterinary Research, (June 1970), 31, (6): 1071-1078.

Soluble antigens of 3 Anaplasma marginale strains were compared by agar gel diffusion and immunoelectrophoretic techniques. Serum proteins from calves infected with each of the 3 A. marginale strains were separated electrophoretically and tested with rabbit anti-bovine serum in immunoelectrophoretic systems. There was no detectable difference between the soluble antigens or the 3 A. marginale strains. A beta globulin arc, which was not detectable in normal bovine serum, was present in serum of acutely affected calves, and a gamma globulin arc was lengthened in the latter serum as compared with that in serum of normal calves.

15. GADIR, F. A., HIDALGO, R. J. and KUTTLER, K. L.: Complement-Fixation Antigen Production for Theileria in White-Tailed Deer (Odocoileus virginianus). American Journal of Veterinary Research, (May 1970), 31: 879-885.
16. KUTTLER, K. L. and ADAMS, L. G.: Comparative Efficacy of Oxytetracycline and a Dithiosemicarbazone in Eliminating Anaplasma marginale Infection in Splenectomized Calves. Research in Veterinary Science, (July 4, 1970), 2, (4): 339-342.

Comparisons between oxytetracycline and a dithiosemicarbazone (356C61) were made in 11 splenectomized, Anaplasma marginale infected calves. Oxytetracycline was administered at the rate of 11 mg/kg intravenously (i.v.) for 5 and 10 consecutive days. Compound 356C61 was administered at the rate of 5 mg/kg i.v. for 5 and 10 consecutive days.

Compound 356C61 appeared to be relatively more effective in the treatment of anaplasmosis, as indicated by the relative increase in packed cell volume (PCV) following treatment, and by the apparent elimination of the carrier status in animals receiving the 10 daily treatments. Compound 356C61 administered daily for 10 consecutive days resulted in rumen atony, tympanites, and death.

17. KUTTLER, K. L., ADAMS, L. G. and ZARAZA, H.: Estudio Epizootiologico del Anaplasma marginale y el Trypanosoma theileri en Colombia. Revista ICA, (June 1970), 5, (2): 127-148. (Spanish translation of: Kuttler, K. L., Adams, L. G. and Zaraza, H.: An Epidemiologic and Geographic Survey of Anaplasma marginale and Trypanosoma theileri in Colombia.)

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18. KUTTLER, K. L. and ZARAZA, H.: A Preliminary Evaluation of a Dithiosemicarbazone for the Treatment of Anaplasmosis. Research in Veterinary Science, (July 4, 1970), 2, (4): 334-338.

Trials were conducted on 3 splenectomized calves treated with a single intravenous (i.v.) inoculation of a dithiosemicarbazone (356C61) using 5 mg/kg, at different stages of induced anaplasmosis infection. When compared to an untreated control, this compound was effective in reducing the severity of the infection. Hematological response was least severe in the animal receiving treatment before signs of parasitemia or a decrease in packed cell volume had occurred.

Treatment with compound 356C61 (5 mg/kg i.v.) of 5 splenectomized calves and 6 intact adult cattle early in the course of an artificially induced Anaplasma marginale infection prevented death loss and reduced the severity of the subsequent reaction when compared with non-treated controls.

19. TODOROVIC, R. A.: Babesiosis Bovina en Australia. Revista de la Facultad de Medicina Veterinaria y de Zootecnia, (1970), 32, (1 & 2): 45-59.

Bovine babesiosis is still of great importance as a threat to the livestock industry in Australia. Due to the complexity of the epidemiology of this disease and other factors, the eradication of this hemoprotozoan malady is not possible at the present time.

The Commonwealth Scientific and Industrial Research Organization (CSIRO) is actively engaged in control and research on Babesia. Other research and teaching institutions involved in the same problem include: the University of Queensland; New South Wales, Department of Agriculture, Cattle Tick Research Station; Queensland State Department and Animal Health Station. All of these research projects on Babesia are sponsored mainly from the Government of Australia.

The Australian research workers have contributed more than a hundred scientific publications on the various areas of Babesia research; they are foremost in this field and the best trained in the world. The research laboratories are equipped with modern scientific tools, and staffed with well-trained technicians who successfully operate these instruments. The facilities are excellent and designed particularly for Babesia research. (Slides of these facilities are available for those who are interested.)

The experience from this visit and knowledge obtained through discussion with Australian scientists working on different research projects will be invaluable for organizing a similar research program on Babesia in Colombia, South America. Furthermore, the Australian scientists with whom I visited all realized the importance of our mission in South America and expressed their willingness to cooperate with us in any manner in the future. They will be able to come to Colombia and spend time on short- or long-term assignments if funds are available.

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20. TODOROVIC, R. A., ADAMS, L.G. and GONZALEZ, E. F.: Bovine Babesiosis: Its Problem and Control in Colombia, South America. Proceedings of the III Congreso Nacional de Medicina Veterinaria y Zootecnia, Lima, Peru, (May 17-23, 1970): 105-107, (abstract).

21. TODOROVIC, R. A., ADAMS, L. G., VIZCAINO, O. G. and GONZALEZ, E. F.: Research and Control of Bovine Babesiosis in Colombia, South America. Proceedings of the VI Congreso Panamericano de Medicina Veterinaria y Zootecnia, Santiago, Chile, (September 28 - October 3, 1970): 36, (abstract).

Research was carried out to develop an effective program for the control of bovine babesiosis in Colombia.

Experiments were carried out at the Palmira Instituto Colombiano Agropecuario (ICA) experimental station in Valle del Cauca (altitude 1,000 meters) to produce co-infectious and sterile immunity against bovine babesiosis. Calves randomly selected were divided into 4 groups according to the experimental design used to evaluate the immunoserological responses to vaccination against babesiosis and tick-borne challenge. The degree of this immunity was determined by tick- and blood-borne challenge. The percentage of parasitemia (P), body temperature (T), and percentage of mortality (M) were used as the basis for comparing the reaction produced after vaccination and challenge. Experiments were conducted to evaluate the prophylaxis, therapy, effects, dosage, route of injection, toxicity, and response of the animals injected with a new Burroughs Wellcome babesiacidal drug, No. 4A65.

On the basis of the observations made from these experiments, conclusions can be drawn that some degree of sterile immunity exists, besides the well-known co-infectious (premunition) immunity in Babesia infections. To understand the exact mechanism of this type of immunity, more work needs to be done. The degree of resistance and the duration of immunity in relationship to different environmental conditions, strain differences, and the pathogenicity of the Babesia spp., and the quality of tick-borne challenge need to be determined.

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22. TODOROVIC, R. A., GONZALEZ, E. F. and ADAMS, L. G.: Immune Response of Cattle Vaccinated Against Babesiosis in Colombia, South America. Proceedings of the 107th Annual AVMA Meeting, Las Vegas, Nevada, (June 22-26, 1970).

Attempts to produce co-infectious and sterile immunity in cattle against Babesia infections have been carried out by vaccinating animals with live or killed Babesia vaccines at Palmira, Valle del Cauca, Colombia (altitude 1,000 meters). Immune responses of the vaccinated animals were evaluated by several immunoserologic methods. The degree of resistance to tick-borne challenge (Boophilus microplus naturally infected with Babesia spp.) was determined by the percentage of recovery to normal parameters used in this study.

According to the experimental design used, a total of 110 animals were divided in 5 experimental groups to ascertain the immunologic responses. The first group consisted of 20 male, 85 kg., Holstein, 3-month-old calves which were preimmunized with Babesia bigemina, Babesia argentina, and 4 weeks later exposed to tick-borne (Boophilus microplus) challenge. The second group consisted of 20 male, 95 kg, Holstein, 4-month-old calves subdivided into 4 groups and vaccinated with a killed Babesia vaccine derived from the erythrocytes and plasma, respectively, of animals acutely infected with B. bigemina and B. argentina. The animals were inoculated with vaccine with or without Bacto-Adjuvant Complete H 37 Ra. The third group of 40 male, 80 kg., Holstein, 3-month-old calves was divided into sub-groups. The first sub-group consisted of 20 animals which were preimmunized with B. bigemina and B. argentina and 8 days later were treated with a new experimental babesiacidal drug. The second sub-group which consisted of 20 animals was simultaneously preimmunized with Babesia spp. and Anaplasma marginale and later treated with their respective specific drugs. The fourth group consisted of 20 female, 75 kg, Holstein, 3-month-old calves prophylactically treated with drug No. 4A65 and 3 weeks later exposed to B. microplus naturally infected with B. bigemina and B. argentina. The fifth group consisted of 10 animals used as controls.

Responses to vaccination and tick-borne challenge were evaluated by packed cell volumes, percentage of parasitemia, body temperatures, body weight, complement fixing antibody titers, general physical conditions, and percent recoveries after tick-borne challenge.

Results in general indicate that resistance to babesiosis can be produced by co-infectious or sterile immunity. Experiments in prophylaxis, based on residual action of the babesiacidal drug, have given consistent and satisfactory results. In the future, it may be possible to develop control programs against bovine babesiosis based on these observations. The present status of these studies was described.

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23. ADAMS, L. G. and FERREIRA, W. L.: Necrobacilosis Neo-Natal en Ovinos. Revista ICA, (September 1971), 6, (3).

Five cases of ovine neo-natal necrobacilosis, in the Savannah of Bogota, were diagnosed in lambs less than 2 weeks of age. Macroscopic and microscopic lesions were described and the diagnosis was confirmed by bacteriological culture techniques. This report constitutes the first known notice of the disease in neo-natal lambs in Colombia.

24. BISHOP, J. P., THOMPSON, K. C., CORRIER, D.: Aislamiento, Separacion y Preservacion de Babesia bigemina del Valle del Sinu. Resumen de los Trabajos Presentados al VII Congress Nacional de Medicina Veterinaria y de Zootecnia, Circuta, Colombia, November, 1971.

Se describe en este trabajo el origen de una cepa estable y pura la B. bigemina usada para efectos investigativos en el Laboratorio de Investigaciones Medicas Veterinarias de Bogota y en el Laboratorio de Investigaciones Veterinarias Tropicales de Monteria, pertenecientes al Instituto Colombiano Agropecuario (ICA).

Los experimentos se efectuaron con el proposito de separar la B. bigemina de otros organismos contaminantes. El metodo consistio en hacer pasajes de sangre contaminada a traves de cinco terneros esplenectomizados. El primer ternero fue inoculado con sangre que portaba diversos hemoparasitos y las subsiguientes inoculaciones se hicieron cuando las extensiones sanguineas del ternero inoculado, mostraban formas de Babesia bigemina.

Se hicieron cinco pasajes de sangre en seis y medio dias. Despues del cuarto pasaje fue aislada Babesia bigemina pura, las formas de Babesia argentina, Babesia major y Anaplasma marginale fueron eliminadas en el curso del estos pasajes sucesivos. Se congelo una cepa para estable de B. bigemina.

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25. VIZCAINO, O. G., THOMPSON, K. C., MATEUS, G.: Aislamiento de Babesia Argentina Utilizando Larvas de Boophilus microplus, Resumen de Los Trabajos Presentados al VII Congreso Nacional de Medicina Veterinaria y Zootecnia, Cucuta, Colombia, November 1971.

Las garrapatas, especialmente las de la familia Ixodae, transmiten en sus fases de desarrollo, numerosos agentes infecciosos a los animales domesticos. El ejemplo mas evidente de este fenomeno es el de la transmision de protozoarios del tipo de las babesias.

Al inocular animales susceptibles, con sangre de animales portadores de hematozoarios se desarrollan, segun el periodo prepatente del agente especifico, los cuadros clinicos de babesiosis o de anaplasmosis.

En Colombia la babesiosis bovina es ocasionada por tres generos de babesias: Babesia bigemina, B. argentina y B. major transmitidas principalmente por la garrapata Boophilus microplus, al igual que sucede en otras areas tropicales y subtropicales.

El objetivo de este trabajo fue aislar una cepa de B. argentina en estado puro, libre de otros protozoarios, utilizando Larvas de Boophilus microplus. El aislamiento de la cepa pura de B. argentina se hace necesario para producir antigenos especificos utilizables en pruebas serologicas. El trabajo se realizo en el LIMV de Bogota y la Granja de Turipana en Monteria.

26. BISHOP, J. P.: Immune Response of Cattle Inoculated With Irradiated Babesia bigemina. A Dissertation submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Doctor of Philosophy, December, 1971.

Babesia bigemina parasitized blood exposed to varied doses of gamma radiated up to 60 kRad was inoculated into calves. Calves infected with 1×10^{10} B. bigemina parasitized erythrocytes exposed to doses up to and including 30 kRad developed progressive parasitemias. Some calves receiving 1×10^{10} parasitized erythrocytes irradiation at levels of 36 and 42 kRad did not develop progressive infections. Progressive infections were prevented by exposure to irradiation at 48 kRad or higher. Subinoculations into susceptible splenectomized calves from parasites thus treated failed to produce active infections.

A degree of acquired resistance to infection with B. bigemina developed in calves after 1 inoculation with B. bigemina parasitized blood irradiated at 48 and 60 kRad. The resistance was sufficient to suppress multiplication of the Babesia and to permit calves to survive otherwise severe clinical infections with nonirradiated parasites. There was also less erythrocytic destruction and a smaller increase in rectal temperatures following challenge. Presumably, the irradiated parasites were responsible for the development of resistance since irradiated nonparasitized blood did not produce a discernable acquired resistance.

The acquired resistance to infection with B. bigemina developed in calves inoculated with 1×10^{10} B. bigemina irradiated at 48 and 60 kRad was similar to the acquired resistance developed in calves inoculated with 1×10^{10} nonirradiated B. bigemina. It seems likely that the protective immunity produced with irradiated B. bigemina may be similar to that produced with living pathogenic B. bigemina developed in calves inoculated with 1×10^{10} B. bigemina irradiated at 48 and 60 kRad was much greater than the acquired resistance to infection developed in calves inoculated with 1×10^{10} heat-killed B. bigemina. Thus, it seems likely that immunization with irradiated Babesia may provide the special immunological properties of living parasites important for producing a strong immunity while suppressing the pathogenic effects of the parasite. The Babesia parasites could be irradiated and frozen without apparent loss of immunizing properties.

27. DALEY, C.A.: A Sequential Study of the Pathogenesis of Disease Caused by Trypanosoma vivax in Experimentally Infected Calves, Utilizing Clinical, Pathological, Histopathological and Immunofluorescent Techniques. A Thesis submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Master of Science, May 1971.

Trypanosoma vivax obtained from a clinically sick cow near Neiva, Colombia, was passed in a sheep and a calf and inoculated into the jugular vein of 14 Holstein-Friesian calves. Fever occurred by 24 hours, and recurring parasitemia commenced after 72 hours. Associated with the first and subsequent parasitemias were decreases in hemoglobin, PCV, M:E ratio, serum albumin, A:G ratio and neutropenia.

All calves exhibited gradual weight loss by 2 weeks and later sub-mandibular edema usually became evident. Consistent post mortem lesions seen after 4 weeks were conspicuously hypertrophied, edematous lymph nodes, hypertrophied hemal lymph nodes, emaciation, rounded right heart, palpably firm liver, atrophied thymus and hypertrophied femoral bone marrow.

Associated with T. vivax of the infecting inoculum and succeeding parasitemias were generalized endothelial hypertrophy and mononuclear cell infiltration along blood and lymph vessels with proteinuria and bone marrow hyperplasia. At 3 weeks there were aggregations 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 important in the development of anoxia, and probably contributed to the single fatality observed.

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28. GONZALEZ, E. F., TODOROVIC, R. A. and ADAMS, L. G.: Ultraestructura de la Babesia bigemina. Revista ICA, (March 1971), 6, (1): 89-112.

The morphology and some aspects related to the reproductive and feeding mechanism of Babesia bigemina were studied by means of electron microscopy.

Babesia bigemina was isolated from naturally infected cattle in the Valle del Cauca, Colombia, and maintained in splenectomized calves in the Laboratorio de Investigaciones de Medicas Veterinarias in Bogota. Blood samples were collected from the splenectomized animals at a time when the percentage of parasitized erythrocytes was 25%, and these samples were used for electron microscopic studies.

By means of the electron microscope, different stages of B. bigemina were revealed, such as oval, conoid, and most commonly pear shaped. The sizes of these forms were 2.5 to 6.5 microns in length by 2.3 microns in width. The young forms of the parasite were 1.5 to 2.5 microns. All these forms of parasites are surrounded by a dense cytoplasmic membrane which contained endoplasmic reticulum in the form of vesicles; these vesicles are composed of granules of different density. The endoplasmic reticulum appears as a homogenous mass with transparent vacuolar structures which are oval and spherical in shape. In addition to the endoplasmic reticulum, well defined dense polar bodies were found which appeared as oval shaped organelles, which communicated with the conoid part of the parasite by canals. The nucleus was the largest internal structure of the parasite and occupies 1/4 to 1/3 of its body. The nucleus is surrounded by a single membrane. Nucleoli were not revealed by electron microscopy.

Reproduction of B. bigemina appears to be carried out in 2 ways: by budding and binary fission. On the basis of these observations it is not clear which means of reproduction is more predominant. It is possible that both forms take place at the same time.

The feeding mechanism is not apparent. It appears that polar bodies play some role in this mechanism. These polar bodies could assume the function of food reservoirs of the parasite. It was also revealed that food vacuoles are similar to those in malarial parasites. The formation of food vacuoles probably results from an end process of pinocytosis as was described for Plasmodium species. We believe that both processes are involved in the feeding mechanism of Babesia parasites. Results of this study confirm the previously reported observation that there is no formation of pigment granules in Babesia; this implies that digestion of the host hemoglobin is complete; in contrast, malarial parasites form hemozoin, a blood pigment, as an end product of metabolism.

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29. KUTTLER, L. L.: Efficacy of Oxytetracycline and a Dithiosemicarbazone in the Treatment of Anaplasmosis. *American Journal of Veterinary Research*, (September 1971), 32, (9): 1349-1352.

The combination of a dithiosemicarbazone (356C61) and oxytetracycline proved more efficacious in the treatment of anaplasmosis than did either drug administered alone. The Anaplasma marginale carrier state in splenectomized calves was suppressed for as long as 120 days and was possibly eliminated by 3 injections of 356C61 (5 mg/kg) and oxytetracycline (11 mg/kg) given simultaneously at 48-hour intervals.

30. KUTTLER, K. L.: Promising Therapeutic Agents for the Elimination of Anaplasma marginale in the Carrier Animal. Proceedings of the 75th Annual Meeting of the USAHA, Oklahoma City, Oklahoma, (October 27, 1971): 92-98.

Two new drugs, a dithiosemicarbazone (356C61) and 3,3'-bis(2-imidazolyl)-carbanilide dihydrochloride (4A65) have been successfully used to treat splenectomized calves with anaplasmosis. Carrier infections were eliminated with 5 or 10 mg/kg 356C61 and 11 mg/kg oxytetracycline when given 3 times at either a 24- or 48-hour interval. In addition, 5 mg/kg 356C61 plus 2 mg/kg 4A65 given 3 times at 24-hour intervals was effective in eliminating Anaplasma marginale infections. Levels of 4 and 6 mg/kg of 4A65 given 3 times at 24-hour intervals has proven successful in eliminating A. marginale infection.

31. KUTTLER, K. L., GRAHAM, O.H. and JOHNSON, S. R.: Apparent Failure of Boophilus annulatus to Transmit Anaplasmosis to White-Tailed Deer (Odocoileus virginianus). *Journal of Parasitology*, (June 1971), 57, (3): 657-659.

Transovarial transmission of anaplasmosis occurred when 2 splenectomized calves were infested with unfed larvae of Boophilus annulatus, but no evidence of infection was detected in 2 intact white-tailed deer after they were infested with other larvae of common origin. All attempts to isolate Anaplasma marginale from the 2 deer by transfer of blood into splenectomized calves were unsuccessful.

32. MULLENAUX, C. H. and ADAMS, L. G.: La Oncocercosis Equina Asociada con el Mal de la Cruz en Colombia: Descripcion de dos casos. Revista ICA, (September 1971), 6, (3).

Two cases of equine fistulous withers were diagnosed in which Onchocerca spp. was found to be present in the affected tissue. One of the horses had a brucellosis antibody titer of 1:50 using the rapid plate agglutination method and, in the same animal, Brucella spp. was cultured from the suppurative materials of the nuchal bursitis of the withers. Macroscopic and microscopic pathological lesions caused by the nematode Onchocerca spp. were described.

33. TODOROVIC, R. A. and ADAMS, L. G.: Serodiagnosis of Babesiosis. Proceedings of the XIX World Veterinary Congress, Mexico City, Mexico, (August 15-22, 1971): 1114-1116, (abstract).

Detection of the carrier state of bovine babesiosis has presented a particularly difficult problem because blood from a high percentage of carrier animals does not contain sufficient Babesia parasites on which to base the diagnosis. In the last two decades fundamental knowledge concerning the immuno-serology of several Babesia spp. has led to development of serodiagnostic procedures for detection of Babesia antibodies. This review summarizes recent advances in the serodiagnosis of babesiosis in infected cattle. Special attention is given to the serologic procedures used in the Laboratorio de Investigaciones Medicas Veterinarias (Bogota, Colombia), in collaboration with the Instituto Colombiano Agropecuario, specifically the complement-fixation test, the double-gel diffusion test, the latex-agglutination and hemo-agglutination tests.

34. TODOROVIC, R. A., GONZALEZ, E., MATEUS, G. and ADAMS, L. G.: Simultaneous Control of Helminths and Parasites in Cattle. Revista de la Facultad de Medicina Veterinaria y Zootecnia, Bogota, (1971), 33: 47-58.

Experiments were conducted at the ICA experimental station in Palmira, Colombia, to evaluate a control program for gastrointestinal and hemotropic parasites. As a result of effective premunition, vaccination, and treatment techniques, animals had a high degree of resistance to babesiosis and anaplasmosis infections. However, the animals in the control group had clinical infections of Babesia and Anaplasma 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.

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35. TODOROVIC, R. A., VIZCAINO, O. G. and ADAMS, L. G.: Determinacion de Anticuerpos de Babesia por la Tecnica de la Fijacion del Complemento. Revista ICA, (1971), 6: 213-233.
36. VIZCAINO, O., TODOROVIC, R.A. and ADAMS, L. G.: Caracterizacion Inmunoserologica de Antigenos de Babesia bigemina y Babesia argentina por Fijacion del Complemento, Precipitacion en Gel e Inmunoelectroforesis. Proceedings VII Congreso Nacional de Medicina Veterinaria y de Zootecnia, Cucuta, Colombia, (November 10-15, 1971): 6-7, (abstract).
37. ZARAZA, H. and KUTTLER, K. L.: Comparative Efficacy of Different Immunization Systems Against Anaplasmosis. Tropical Animal Health & Production, (1971), 3: 77-82.

Animal response to anaplasmosis vaccination was measured using an attenuated organism, a killed adjuvant vaccine, and a virulent Anaplasma marginale. A total of 7 calves (2-4 months of age) and 5 heifers (18 months of age) received the attenuated organism; 8 calves were given the adjuvant vaccine; 7 calves were preimmunized with virulent A. marginale; and 7 calves remained as non-vaccinated controls. The animals were vaccinated at Tibaitata on the Bogota Savannah and later moved to the north coast of Colombia, an anaplasmosis enzootic area.

All vaccination methods produced positive CF results. The live agents resulted in low parasitemias in most instances, although the attenuated organism was particularly mild in the younger animals.

Protection from field challenge was observed in all calves preimmunized with virulent organism, and in 2 of 5 heifers preimmunized with the attenuated organism. All other vaccinated animals developed anaplasmosis which was equally as severe as seen in the non-vaccinated controls.

38. CORRIER, D. E.: A Clinical, Serologic and Pathological Study of Concurrent Anaplasmosis and Babesiosis in Experimentally Infected Calves. A Thesis submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Master of Science, December 1972.

Concurrent and single infections of Anaplasma marginale and Babesia bigemina were studied in 22, 7 month old, male, non-splenectomized Holstein-Friesian calves. Clinical manifestations of disease were mild, consisting primarily of slight fever, poor body condition, and reduced weight gains. Anaplasma marginale appeared to be the more pathogenic of the 2 organisms.

Associated with the appearance of parasitized erythrocytes were decreases in packed cell volume, hemoglobin, albumin:globulin ratio, and serum albumin, and slight increases in the levels of serum bilirubin, serum glutamic oxalacetic transaminase, and alpha and beta serum globulins. Decreases in PCV and hemoglobin concentration were more prolonged and severe in the concurrently infected calves. Complement fixing antibodies for Anaplasma occurred on days 17 to 26 in association with increases in alpha and beta globulins. Complement fixing antibodies for Babesia were first observed on day 12 post inoculation.

Gross lesions observed in the concurrently infected calves included a moderately excessive quantity of yellow fluid in the peritoneal and pleural cavities, moderate lymph node enlargement, splenomegaly and hepatomegaly, moderate renal congestion, and occasional serous atrophy of depot fat.

Hepatocellular degeneration and necrosis were observed in the centrilobular areas of the liver. Lymphoid hyperplasia was observed in the malpighian corpuscles of the spleen and in the lymphoid follicles of the lymph nodes. Hemosiderosis of the spleen, liver, kidney and lymph nodes was attributed to the increased removal of damaged erythrocytes from the circulation with the subsequent release of breakdown products of hemoglobin.

The biological relationship of A. marginale and B. bigemina during the concurrent infection appeared to be one of independency. Neither an inhibitory nor a synergistic relationship was apparent during the investigation. The clinical and pathological manifestations of concurrent infection were more severe than those observed during infection with either of the hemotropic parasites alone, and were attributed to the concurrent infection being additive in nature.

39. HERNANDEZ, J. D., ROBERTS, E. D., ADAMS, L. G. and VERA, T.: Pathogenesis of Hepatic Granulomas in Turkeys Infected with Streptococcus faecalis var. liquefaciens. Avian Diseases, (January-March 1972), 16, (2): 201-216.

The pathogenesis of hepatic granulomas in turkeys has been studied by reproducing the lesions experimentally with Streptococcus faecalis var. liquefaciens isolated during a field outbreak of turkey hepatic granulomas in Colombia. The 170 turkey poults (Bronze) used were 4 weeks old. Groups of poults were inoculated intravenously or orally with 0.1 ml. of a 24-hour culture of S. faecalis var. liquefaciens at a dilution of 3 X 10 on the McFarland Nephelometer Standard 10. The oral route of inoculation reproduced a disease most similar to the naturally occurring disease.

Clinically, the acute phase of infection was characterized by a high mortality rate in the first to seventh days but only sporadically thereafter. The septicemic phase produced the formation of septic thrombi which localized in various organs, producing infarction with heterophilic infiltration. Once the septicemic phase of the problem passed, the disease was manifested primarily by a focal hepatitis initiated primarily as a focal necrotic cholangial lesion. The biliary epithelium had hyperplastic to degenerative processes which participated in the formation of biliary thrombi. Granulomas were characterized by focal areas of necrosis surrounded by Langhans-type giant cells and macrophages.

40. JONES, F. M.: Control of Anaplasmosis and Babesiosis in Young Cattle. A Thesis submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Master of Science, December 1972.

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

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

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

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

41. KUTTLER, K. L.: Combined Treatment With a Dithiosemicarbazone and Oxytetracycline to Eliminate Anaplasma marginale Infections in Splenectomized Calves. Research in Veterinary Science, (November 1972), 13, (6): 536-539.

A total of 12 treatment schedules combining oxytetracycline and an alpha-dithiosemicarbazone (356C61) were tested on 36 splenectomized calves carrying Anaplasma marginale infections. Anaplasma infection was eliminated following the administration of 5 or 10 mg/kg 356C61 combined with 11 mg/kg oxytetracycline, and given 3 times at 24 or 48 hour intervals. Treatments employing lower levels, fewer injections, or at greater time intervals failed to eliminate infection.

Treated, splenectomized calves failing to show evidence of an A. marginale relapsing infection within 62 days were found to be free of infection on the basis of infectivity trials conducted an average of 87 days after treatment, and by re-inoculation with A. marginale an average of 164 days after treatment.

42. KUTTLER, K. L.: Comparative Response to Premunization Using Attenuated Anaplasma marginale, Virulent A. marginale and A. centrale in Different Age Groups. Tropical Animal Health & Production, (1972), 4: 197-203.

Premunizing infections using virulent Anaplasma marginale (VAM), attenuated A. marginale (AAM) and Anaplasma centrale (AC) have been induced in 46 mature cattle, 33 intact calves, and 38 splenectomized calves, for the purpose of comparing the relative response to these infections.

The VAM produced significantly more severe reactions in adult cattle and splenectomized calves, and a slightly more severe response in intact calves; however, these animals were relatively more resistant to all three infections. There was no detectable difference between the reactions caused by AAM and AC when measured in adult cattle and intact calves. Among splenectomized calves, however, the AAM infections resulted in a milder response as measured by the relative drop in packed cell volume and percent parasitemia. The CF response was significantly lower in the AC infection.

43. KUTTLER, K. L., GRAHAM, O. H., JOHNSON, S. R. and TREVINO, J. L.: Unsuccessful Attempts to Establish Cattle Babesia Infections in White-Tailed Deer (Odocoileus virginianus). Journal of Wildlife Diseases, (January 1972), 8: 63-66.

Attempts to induce a demonstrable cattle Babesia infection by feeding known infected ticks on two white-tailed deer (Odocoileus virginianus) were unsuccessful. The injection of known Babesia carrier blood into an intact and a splenectomized deer failed to result in evidence of infection.

All deer were checked for possible sub-patent infections by inoculating their blood into splenectomized calves at weekly intervals for 5 weeks following exposure, but no infections were produced in the calves.

Babesia infected ticks having undergone one generation on deer were unable to transmit infection to splenectomized calves on the succeeding generation.

44. ARMSTRONG, J. M. and TODOROVIC, R. A.: Anaplasmosis of Cattle. Texas Agricultural Extension Service, A Fact Sheet, No. 10 M-6-73, (1973): 1-4.

A brief description of anaplasmosis, with special emphasis on recent achievements in the field of diagnosis and control was discussed and summarized for Texas A&M University Extension Service publication. This fact sheet was written principally for livestockmen to make them aware of recent developments in the field of anaplasmosis control and action that can be taken for prevention and treatment of this hemotropic disease.

45. BISHOP, J. P. and ADAMS, L. G.: Combination Thin and Thick Blood Films for the Detection of Babesia Parasitemia. American Journal of Veterinary Research, (September 1973), 34, (9): 1213-1214.

A method for preparing and examining combination thin and thick blood films for the detection of Babesia spp. parasitemias was developed. A technique for staining the combination thin and thick films, using a phosphate-buffered Giemsa stain solution containing alkyl phenoxy polyethoxy ethanol (APPE), was also described.

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46. BISHOP, J. P., ADAMS, L. G., THOMPSON, K. C. and CORRIER, D. E.: The Isolation, Separation and Preservation of Babesia bigemina. Tropical Animal Health & Production, (May 1973), 5: 141-145.

Experiments were performed in Colombia to separate Babesia bigemina from contaminating organisms. Babesia bigemina was passaged serially through five splenectomized calves. 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. Five blood passages were carried out in 6.5 days. Babesia argentina, Babesia major and Anaplasma marginale were eliminated as contaminants of the B. bigemina isolated after four passages. A frozen stabulate of the isolated B. bigemina was established.

47. CORRIER, D. E. and ADAMS, L. G.: Observations During Concurrent Anaplasmosis and Babesiosis in Experimentally Infected Calves. Proceedings of the Sixth National Anaplasmosis Conference, Las Vegas, Nevada, (March 1973): 60-65.

The clinical, serological and pathological manifestations of disease in intact calves concurrently infected with Anaplasma marginale and Babesia bigemina were investigated. Clinical signs were more severe in the concurrently infected calves than in singularly infected controls. Decreases in packed cell volume, albumin:globulin ratio, myeloid:erythroid ratio and increases in the number of reticulocytes, total serum proteins and serum gamma globulins were more pronounced in the concurrently infected calves. The concurrent infections had no apparent effect on the production of complement fixing antibodies. Gross lesions observed in the concurrently infected calves included: pleural and peritoneal transudates; splenomegaly; hepatomegaly; and moderate lymph node enlargement. Histological lesions included: moderate hepatocellular degeneration; lymphoid hyperplasia in the spleen and lymph nodes; and hemosiderosis of the spleen, lymph nodes, liver and kidneys. The relationship of A. marginale and B. bigemina during the concurrent infections appeared to be one of independency. The increased severity of the clinical and pathological signs of disease in the concurrently infected calves was attributed to the concurrent infections being additive.

48. CRAIG, T. M.: Infectivity and Cross Immunity Studies of Colombian Bovine Babesia Species. A Thesis submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Master of Science, December 1973.

Colonies of bovine hemotropic disease free Boophilus microplus ticks were established. Adult B. microplus females and eggs were incubated at 28 to 30° at a relative humidity of from 70 to 80%. Larvae were maintained at 24 to 28° C. and a relative humidity of 60 to 80% for maximal survival.

A colony of B. microplus infected with Babesia argentina was established by allowing non-infected ticks to feed on normal cattle for 10 to 11 days, at which time a stabilate of B. argentina was inoculated into the cattle subcutaneously. This resulted in a parasitemia at the time of final tick engorgement. The organism was maintained in ticks by allowing non-infected ticks to feed on a calf which was later infected by the release of infected larvae 11 to 13 days after the non-infected larvae commenced feeding. Diagnosis of Babesia spp. in ticks was done by examination of hemolymph.

Diagnosis and estimation of the effects of Babesia spp. infections in cattle were made on the basis of thick and thin blood films, packed cell volumes, rectal temperatures, body weights, cerebral biopsies, complement fixation titers and clinical signs.

Boophilus microplus eggs, larvae and nymphs infected with B. argentina were disrupted by several methods and the resulting material inoculated subcutaneously into splenectomized calves. None of the calves showed signs of infection and proved to be fully susceptible when challenged with B. argentina.

Babesia bigemina was isolated from other bovine hemotropic agents by rapid serial passage through splenectomized calves. This isolate was compared with a laboratory strain previously isolated from a different geographic region of Colombia. Two groups of 8 calves each were inoculated subcutaneously with 10^7 B. bigemina organisms of each isolate. A third group of 8 calves remained as untreated controls. Twenty-eight days later, 4 calves in each of the 3 groups were challenged with 2×10^{10} B. bigemina organisms of each isolate. The challenge groups were homologous, heterologous and control. Both homologous and heterologous groups demonstrated immunity to challenge. No differences in the virulence of the two isolates were demonstrated.

49. KUTTLER, K. L.: Current Status of the Tropical Cattle Fever Tick Boophilus microplus and Texas Wildlife. Presented to the Wildlife Society, Texas Chapter, Annual Meeting, Barnett, Texas (April 5, 1973), abstract.

The increasing presence of both Boophilus microplus and Boophilus annulatus have created considerable concern among both the Texas livestock industry and those interested in the preservation and maintenance of wildlife. Even though both ticks can complete their life cycles on deer, B. microplus appears better adapted to deer. This tick is very versatile and is capable of maintaining itself on several wildlife species. Neither B. annulatus nor B. microplus are capable of transmitting any known diseases from deer to cattle or from cattle to deer.

Eradication plans have been successful in the past, particularly where B. annulatus was present. The feasibility of B. microplus eradication by similar means was discussed.

50. KUTTLER, K. L.: Review: Current Status of Control and Treatment With Drugs. Proceedings of the Sixth National Anaplasmosis Conference, Las Vegas, Nevada, (March 1973): 93-97.

A review of the literature was given, emphasizing those treatment techniques and reports since the initial use of the tetracyclines for anaplasmosis in 1951. Two new drugs, Imidocarb and Gloxazone, were discussed. These drugs, while more effective than the tetracyclines, have not been cleared by the FDA and remain experimental. At the present time, the tetracyclines are the only effective therapeutic compounds available commercially for the treatment of anaplasmosis. Current recommendations for the elimination of carrier infections are to feed on oral tetracycline for 60 days at the rate of 5 mg/pound of body weight.

51. KUTTLER, K. L. and TODOROVIC, R. A.: Techniques of Premunization for the Control of Anaplasmosis. Proceedings of the Sixth National Anaplasmosis Conference, Las Vegas, Nevada, (March 1973): 106-112.

Attempts at Anaplasma premunization in varying age groups were reported using virulent A. marginale of Texas origin, virulent A. marginale of Colombian origin, attenuated A. marginale and A. centrale. Results of premunization response and response to field and artificial challenge were reported.

Premunization is a practical approach for prevention of clinical losses associated with anaplasmosis, but a series of variables must be considered if success is to be achieved. Some of these factors are: (1) age of animals being premunized, (2) virulence, potency and size of premunizing inoculum, and (3) strain, or size, of expected challenge exposure. In some instances, use of a highly virulent A. marginale in adult cattle resulted in overly severe reactions even with treatment. Gloxazone (356C61) and Imidocarb 4A65 were superior to oxytetracycline in moderating the premunizing infection. Attenuated strains of A. marginale when used in young intact calves failed to produce the desired premunizing effect; however, attenuated strains were very effective in adult cattle. Premunization is dependent on establishing an active infection, and in the absence of such infection, susceptibility to field or experimental challenge occurs. Successful premunization resulted in almost complete protection following challenge with antigenically similar A. marginale isolates. Protection was only partial, however, in instances where heterologous challenge was encountered.

52. MAURER, F. D. et al.: Livestock Production and Marketing in Pakistan. A 107 page report to AID based upon a month's survey in August 1973.
53. TODOROVIC, R. A., GONZALEZ, E. F. and ADAMS, L. G.: Bovine Babesiosis: Sterile Immunity to Babesia bigemina and Babesia argentina Infections. Tropical Animal Health & Production, (1973), 5: 234-245.

Killed Babesia bigemina and Babesia argentina vaccine was prepared from infected erythrocytes (AG-E) and infected plasma (AG-S) collected from acutely infected calves with B. bigemina and B. argentina. The vaccine was tested in Colombian cattle under field conditions in the Cauca Valley. A total of 40 calves 2-1/2 months of age received killed-Babesia vaccine. Five calves were not vaccinated; they served as controls. Vaccinated and non-vaccinated control calves were exposed to field-borne challenge with Boophilus microplus infected ticks. On the basis of data obtained, it was found that a high degree of sterile immunity to B. bigemina and B. argentina can be produced in calves injected with killed-Babesia vaccine. It appears that sterile immunity plays an important role in the mechanism of acquired immunity to babesiosis, other than well-known co-infectious immunity known as premunition.

54. TODOROVIC, R. A., VIZCAINO, O. G., GONZALEZ, E. F. and ADAMS, L. G.: Chemoprophylaxis (Imidocarb) Against Babesia bigemina and Babesia argentina Infections. American Journal of Veterinary Research, (September 1973), 34, (9): 1153-1161.

The chemoprophylactic effects of imidocarb (3,3'-bis(2-imidazolin-2-yl)-carbanilide dihydrochloride) against bovine babesiosis were evaluated in 29 calves. The compound had prophylactic and therapeutic properties in calves artificially and naturally infected with Babesia bigemina and Babesia argentina of Colombian (South American) origin. Administered intramuscularly at the dose level of 2 mg/kg, imidocarb suppressed development of acute babesiosis in calves treated 46 days previously and later exposed to a lethal dose of Babesia spp.-infected blood. Imidocarb failed to protect against Anaplasma marginale infection. Calves treated intravenously with imidocarb at the dose level 2 mg/kg and challenge inoculated 20 days later with a lethal dose of Babesia spp.-infected blood were protected. For 90 days after challenge, none of the calves had Babesia spp. parasitemia, as determined by examination of stained blood films and by subinoculation of blood into susceptible splenectomized calves. Calves intravenously treated 21 days previously with 3 mg/kg imidocarb resisted tick-borne challenge of Boophilus microplus. This resistance was evidenced for 15 weeks of field exposure by negative results of examinations of stained blood films and death of nontreated calves from acute babesiosis. All calves treated with imidocarb and subsequently exposed to blood or tick-borne Babesia spp. responded with an increase of complement-fixing antibodies.

Imidocarb readily controlled very severe acute infections with B. bigemina and B. argentina when the compound was given at dose rates of 1 mg/kg by both intramuscular or subcutaneous routes. Signs of acute toxicosis were observed in calves given intravenous injections of 3 mg/kg. Three calves died, having signs of embarrassed respiration, oral respiration, excessive salivation, muscular fasciculations, urination, defecation, incoordination, and prostration. Signs of toxicosis were milder with intramuscular or subcutaneous injections of imidocarb.

55. VIZCAINO, O. G. and TODOROVIC, R. A.: Produccion de Antigenos Solubles de Babesia argentina y Babesia bigemina para Pruebas de Immunologia. Proceedings of the VII Panamerican Congresss of Veterinary Medicine and Zootecnic, Bogota, Colombia, (July 23-28, 1973): 38-39, (abstract).

Diagnosis of bovine babesiosis during the acute phase of infection is made by examination of Giemsa-stained blood films; however, during the chronic phase of disease, several serologic tests are used for detection of specific Babesia spp. antibodies. The purpose of the present investigation was to isolate soluble antigens of Babesia bigemina and Babesia argentina from blood acutely infected with these hemotropic parasites and use them in immunodiffusion tests for detection of specific antibodies.

Soluble antigens of B. bigemina and B. argentina were isolated from plasma collected from animals acutely infected with these parasites. By means of column chromatography (DEAE-cellulose and Sephadex-G2000), soluble antigens of B. bigemina and B. argentina were purified from host material and found antigenically specific in gel diffusion tests. Antigenic fractions obtained by above procedures were found to contain protein at 280 μ w of optical density.

By means of DEAE-cellulose column chromatography, it was possible to separate host hemoglobin from soluble antigens of B. bigemina and B. argentina. Three protein peaks were recorded during fractionation, but only the second peak contained soluble antigens contaminated with host serum proteins. By means of Sephadex-G2000 column chromatography, it was possible to separate normal serum proteins from soluble B. bigemina and B. argentina antigens. When serum samples collected from cattle infected with B. bigemina and B. argentina were subjected to react with soluble antigens in the gel diffusion tests a line of precipitation reaction was observed. Twenty-four or more hours of incubation was necessary for visible reaction.

Specific antibodies to B. bigemina and B. argentina were detected in sera of cattle infected with these parasites for 73 and 83 days of infection in the homologous system tested. An attempt was made to characterize these soluble antigens by means of immunoelectrophoresis. It was found that both antigens migrate a short distance to the positive pole. Antigenic reactivity of B. bigemina and B. argentina soluble antigens was preserved for 6 months at -79° C.

56. ADAMS, L. G.: Epizootia Espontanea de Hepatitis Toxica en Procinos Atribuida a Aflatoxicosis. Revista ICA, (March 1974), 9: 3]-48.

Nine of the 56, 4- to 6-month-old Duroc male and female pigs died 2 months after consuming a ration consisting of 8.75% moldy peanut meal. The pigs exhibited weight loss, roughened hair coats, anorexia, lethargy, icterus, melena, increased followed by decreased rectal temperature and death. The livers of the remaining 45 pigs were condemned due to cirrhosis. Serum sorbitol dehydrogenase activities, glutamic-oxaloacetic transaminase activities, bilirubin concentrations, serum beta globulin levels, serum gamma globulin levels, and total serum protein concentrations were increased as serum albumin/globulin ratios, albumin levels, packed cell volume and hemoglobin contents were decreased. No changes were observed in total leukocyte counts or serum alpha globulin levels.

The principal macroscopic lesions consisted of generalized icterus, petechial and ecchymotic hemorrhages with yellow transudates occurring in the body cavities. Subendocardial as well as subserosal ecchymotic hemorrhage were commonly observed. Ulceration of the gastric fundus occurred which filled the stomach, duodenum, jejunum, ileum, and colon with free digested and undigested blood. The liver was pale yellowish-brown, firm (increased cutting resistance), and cirrhotic with very accentuated hepatic lobules outlined by translucent bands. Hundreds of irregular round yellow to brown foci of hepatic nodular regeneration were interspersed throughout the hepatic parenchyma. The gall bladder was moderately edematous and contained a small amount of light green bile. The principal microscopic lesions of the liver were disorganization of the hepatic architecture, acinus formation, severe sinusoidal fibrosis, mild biliary hyperplasia, advanced hepatic nodular regeneration, extensive hepatocellular megalocytosis, hepatocellular anisocytosis, mild hepatocellular necrosis, fatty metamorphosis, and moderate cholangiolar bile plug formation. The diagnosis and etiology of these 4 cases of porcine chronic toxic hepatitis was attributed to aflatoxicosis apparently produced by Aspergillus flavus growing on peanut meal. The present article is the first report of aflatoxicosis in Colombia.

57. ADAMS, L. G. and TODOROVIC, R. A.: The Chemotherapeutic Efficacy of Imidocarb Dihydrochloride on Concurrent Bovine Anaplasmosis and Babesiosis. I. The Effects of a Single Treatment. Tropical Animal Health & Production, (1974), 6: 7]-78.

The chemotherapeutic efficacy of imidocarb dihydrochloride (3,3'-bis(2-imidazolyl)carbanilide dihydrochloride) administered as single intramuscular doses of 1.0, 2.0, and 2.5 mg/kg, against concurrent bovine anaplasmosis and babesiosis, is reported. Dosages of 2.0 and 2.5 mg/kg of imidocarb dihydrochloride rapidly inhibited acute ascending concurrent parasitaemias of Anaplasma marginale, Babesia bigemina and Babesia argentina; however, 1.0 mg/kg had a minimal effect on A. marginale, but was very effective against B. bigemina and B. argentina. Imidocarb dihydrochloride at 1.0, 2.0 and 2.5 mg/kg inhibited the development of immunity of the acute Babesia spp. infections, making the calves more susceptible to babesiosis upon challenge.

58. ADAMS, L. G. and TODOROVIC, R. A.: The Chemotherapeutic Efficacy of Imidocarb Dihydrochloride on Concurrent Bovine Anaplasmosis and Babesiosis. II. The Effects of Multiple Treatments. *Tropical Animal Health & Production*, (1974), 6: 79-84.

Intact *Anaplasma marginale*, *Babesia bigemina* and *Babesia argentina* carrier calves treated intramuscularly 5 or 10 times with 2.5 mg/kg of imidocarb dihydrochloride at 48-hour intervals eliminated the *Babesia* infections, but not *Anaplasma* infections. The parasitemias became microscopically undemonstrable within 4 days following the first treatment, and the packed cell volumes increased significantly within 18 days. Intoxications resulting in fatalities occurred in 5 of 6 calves given 10 intramuscular treatments of 2.5 mg/kg of imidocarb dihydrochloride at 48-hour intervals.

59. ALIU, Y. O.: Absorption, Distribution, and Excretion of Imidocarb Dipropionate [3,3'-bis-(2-imidazolyl)carbanilide] in Sheep. A Dissertation submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Doctor of Philosophy, August, 1974.

Spectrophotometric and thin-layer chromatographic methods for quantitative and qualitative determination of Imidocarb in biologic specimens are described. Imidocarb was extracted under basic conditions from plasma, urine, milk, bile, and homogenized tissue samples in organic solvents. Following extraction and concentration in 0.82 N HCL, the drug was quantitatively identified by spectrophotometry. The limits of accuracy are estimated to be 1.0 µg/ml in plasma and other body fluids and 5.0 µg/gm in tissues.

High plasma levels were reached in 4 hours after the intramuscular injection of 4.5 mg/kg Imidocarb. This was followed by rapid decline initially but later the rate of decline was reduced so that trace amounts were still present weeks after the injection. High and persistent tissue residues were characteristic of this drug. Approximately 11-17% of the administered drug was excreted in the urine within 24 hours, but thereafter the excretion rate was low. The relatively high concentrations of the drug found in the bile suggest that biliary excretion is an important route of drug elimination. High concentrations were found in the milk of lactating ewes. When the milk was fed to nursing lambs, no drug could be detected in their plasma.

60. BISHOP, J. P. and ADAMS, L. G.: Babesia bigemina: Immune Response of Cattle Inoculated with Irradiated Parasites. Experimental Parasitology, (1974), 35: 35-43.

Effects of various radiation dosages on the infectivity and immunogenicity of Babesia bigemina were studied. Calves infected with 1×10^{10} B. bigemina parasitized erythrocytes exposed to 24 krad developed progressive parasitemias. Some calves receiving 1×10^{10} parasitized erythrocytes irradiated at 36 krad did not develop progressive infections. Progressive infections were prevented by exposure to irradiation at 48 and 60 krad. A degree of acquired resistance to infection with B. bigemina developed in calves after inoculation with parasites irradiated at 48 and 60 krad. The resistance developed was sufficient to suppress multiplication of the Babesia and to permit calves to survive otherwise severe clinical infections due to challenge with nonirradiated parasites. Irradiated parasites were frozen without apparent loss of immunizing properties.

61. BISHOP, J. P. and KUTTLER, K. L.: Infectivity and Immunogenicity of Irradiated Babesia rodhaini. Journal of Protozoology, (1974), 21, (5): 758-760.

Babesia rodhaini parasitized mouse blood exposed to varied doses of gamma radiation up to 30,000 r was inoculated into mice. Mice inoculated with nonirradiated B. rodhaini developed progressive infections and died 7 to 11 days after inoculation. Mice infected with B. rodhaini parasitized blood exposed to doses up to and including 22,000 r developed progressive parasitemias which were delayed in comparison to mice inoculated with nonirradiated B. rodhaini. Some mice receiving parasitized blood irradiated at 26,000 r did not develop progressive parasitemias. Progressive infections were prevented by exposure to irradiation at 30,000 r.

The results of two separate experiments revealed that one inoculation of parasitized blood exposed to 30,000 r or higher apparently stimulated a resistance to a challenge infection with nonirradiated parasitized blood. While 20 out of 20 control mice died as a result of challenging infections, 9 out of 28 mice previously exposed to irradiated parasitized blood survived.

The injection of irradiated nonparasitized blood did not produce a discernable acquired resistance to B. rodhaini. Presumably the irradiated parasitized blood was responsible for the development of acquired resistance to B. rodhaini.

62. CORRIER, D. E.: A Clinical, Histological and Ultrastructural Study of the Toxic Effects of Imidocarb Dipropionate in Goats. A Dissertation submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Doctor of Philosophy, August 1974.

The toxic effects of imidocarb dipropionate were studied in adult goats following the intramuscular injection of a lethal dosage of the drug. The acute clinical signs of toxicosis were transient and included excessive salivation, diarrhea, dyspnea, anorexia and inactivity. Significant increases in the mean serum urea nitrogen concentrations, serum glutamic oxaloacetic transaminase activities, and absolute neutrophilic leukocytes occurred.

The most prominent gross pathological lesions were enlarged, pale kidneys with the presence of alternating red and white streaks in the renal cortex, hydrothorax, hydropericardium, ascites, and pulmonary edema. The histological alterations included severe acute tubular necrosis of the proximal convoluted tubules of the renal cortex beginning as early as 6 to 12 hours post-injection and massive pulmonary edema.

Ultrastructural lesions were observed at 3 hours and progressed rapidly in the next 24 hours to include disruption of plasma membranes, dilation and proliferation of the endoplasmic reticulum, swollen electron dense mitochondria, and rarefaction of the cytoplasmic ground substance. Finally, complete disruption of the plasma membrane with fragmentation of the microvilli, loss of junctional complexes and cellular disjunction became evident from 12 to 24 hours post-injection.

Progressive decreases were observed in succinic dehydrogenase and adenosine triphosphatase activities beginning at 12 hours and 24 hours post-injection, respectively. The loss of ability of the epithelial cells of the proximal convoluted tubules to regulate cell volume was considered to have been the initial event responsible for the subsequent ultrastructural, histological and histochemical changes observed following the injection of imidocarb dipropionate.

63. KUTTLER, K. L.: Chemotherapy and Eradication of Anaplasmosis. Proceedings of the Third International Congress of Parasitology, Munich, Germany, (August 25-31, 1974), 3: 1308-1309, (abstract).

Methods of anaplasmosis eradication have been described, based on the principle of identifying carrier or infected cattle and the removal of this infection. Infection may be eliminated by segregation, slaughter, or segregation and treatment. The latter method is still handicapped by the relatively expensive procedures involved with long-term feeding of tetracyclines. Tests of new and experimental drugs show that improved techniques are more practical in terms of the number of treatments necessary for the elimination of Anaplasma infection.

Both Imidocarb [3,3'-bis-(2-imidazolin-2-yl)carbanilide dihydrochloride/dipropionate] and Gloxazone [α -ethoxyethylglyoxal dithiosemicarbazone] (Burroughs Wellcome Company, Inc. - Research Triangle Park, North Carolina) have been shown to be therapeutically active against Anaplasma. Imidocarb has effectively eliminated Anaplasma infection when administered 3 times at 24 to 48 hour intervals, using 4, 5, 6 mg/kg, or giving 5 mg/kg twice at a 2-week interval. Gloxazone, while therapeutically effective against Anaplasma, will not eliminate the infection in sub-toxic levels. Anaplasma infection can be eliminated with reduced amounts of Gloxazone when it is combined with oxytetracycline. The combination of Gloxazone and Imidocarb has been shown successful in eliminating infection with as little as 1 mg/kg Gloxazone and 3 mg/kg Imidocarb, when each compound is given twice at a 2-week interval.

Anaplasmosis is endemic in much of the intermountain west and Pacific west coast. The principle vectors there are ticks, and deer are known to act as a reservoir of infection. It is doubtful if eradication of Anaplasma under these circumstances is practical or even possible with present-day techniques.

There are large areas in Africa and South America where ticks are the principle Anaplasma vectors, where non-bovine reservoirs probably exist which would pose a similar problem. Chemotherapy under these conditions can best be used for control rather than eradication. All 3 compounds used in reduced amounts are effective in reducing the severity of infection, allowing the animals to become carriers of infection, hence immune to further reinfection.

64. MAURER, F. D.: Obtaining Support for Research on Animal Health.
Journal of the American Veterinary Medical Association, (July 15, 1974), 165, (2): 196-197.

The principle source of funds for research in the colleges of veterinary medicine has long been from agencies of the Federal government. In general, Federal agencies have placed emphasis upon human health related problems even though experimental animals and veterinarians were involved. As a result, there has been a relative neglect of those diseases of livestock which reduce U.S. production by 11 to 15% per year.

Rather than for the livestock industry to wait for government assistance, it is urged that livestock associations support research toward the solution of their own problems. Other industries find it economically profitable to plow back some 15% of annual profits into research and development; this could apply to livestock as well.

65. MAURER, F. D.: African Agricultural Research Capabilities.
National Academy of Sciences, Washington, DC, 1974. A publication of 221 pages.

The work of an international committee, of which F. D. Maurer was the veterinary member, compiled this report which constitutes a review of the needs, opportunities, facilities and personnel for research on the major agricultural crops and livestock. Emphasis is upon research required to solve major problems which now handicap crops and livestock production. Our primary area of concern was for research on animal disease problems. The committee's work was financed by USAID.

66. MILLER, R. M.: Investigations on Transstadial Transmission of Bovine Anaplasmosis and Benign Bovine Theileriosis in Cattle by Two Species of Amblyomma (Acarina : Ixodidae). A Thesis submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Master of Science, August 1974.

Nymphal stages of both Amblyomma americanum (Linnaeus) and A. cajennense (Fabricius) engorged either on a holstein bull calf chronically infected with Anaplasma marginale (Theiler), or on a holstein bull calf chronically infected with a Theileria organism resembling Theileria mutans (Theiler). After natural detachment and molting, the exposed adult ticks subsequently engorged on non-infected splenectomized holstein bull calves.

During engorgement of exposed adult ticks and for 75 days after their natural detachment, the splenectomized calves were monitored for the presence of blood parasites using both complement-fixation tests and Giemsa-stained thin blood smears. No evidence of infection was observed. After 90 days, the splenectomized calves were challenged to see if they were actually susceptible to either of the two blood parasites. Inoculations of blood demonstrating a parasitemia of either A. marginale or the Theileria were administered to the splenectomized calves which had been previously exposed to the test group of adult ticks. The splenectomized calves developed evidence of both anaplasmosis and theileriosis, suggesting they were susceptible to the blood parasites at the time of tick infestation.

67. PLATT, K. B.: The Development of an Indirect Fluorescent Antibody Test for Trypanosoma vivax in Colombia. A Thesis submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of master of Science, May 1974.

An indirect fluorescent antibody (IFA) test for Trypanosoma vivax infections was developed for a survey involving over 2,000 cattle distributed throughout 11 departments and territories in Colombia. The antigen for the IFA test was prepared from the blood of infected calves by making thin blood smears that were air-dried and fixed in acetone: methanol: 60:40 at -20° C for 30 minutes. The antigen prepared in this manner was useful up to and including 144 days when stored at -70° C. IFA test serum titers of 1:100 or greater were considered to be positive. No cross-reactivity of the IFA test was observed between T. vivax and Anaplasma marginale, Babesia argentina, Babesia bigemina, Eperythrozoon sp. or Trypanosoma theileri at 1:50 serum dilutions. Suspicious reactions occasionally were observed when Trypanosoma evansi positive serum was diluted 1:50 and 1:100 and used in the IFA test for T. vivax. The IFA test could be repeated within plus or minus one serum dilution approximately 80% of the time using different antigen lots on the same and different days. Samples obtained for the IFA test by eluting serum from dried impregnated filter paper discs produced results nearly equal to those obtained by using conventional serum samples. The IFA test was up to 20 times more effective in detecting T. vivax positive cattle than the thick blood smear technique. The IFA test demonstrated the presence of T. vivax antibodies in cattle from 5 departments in Colombia, while antibodies were not detected in the serum of cattle from 6 other departments of Colombia.

68. THOMPSON, K. C.: A Comparison of the Antigenic Properties of Erythrocytic Babesia bigemina in Acute and Chronic Blood Borne and Tick Borne Infections in Cattle. A Dissertation submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Doctor of Philosophy, May 1974.

This study was made to determine possible antigenic differences in a Babesia bigemina isolate in acute and chronic blood borne and tick borne infections of cattle.

On the basis of the serological results, antigenic variation within an isolate of B. bigemina occurred. Antigenic variation appeared to be influenced by the mode and duration of infection. The hosts' apparent reduced response to homologous challenge and the marked response observed with heterologous systems indicated antigenic differences of B. bigemina.

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69. TODOROVIC, R. A.: Bovine Babesiosis: Its Diagnosis and Control. American Journal of Veterinary Research, (August 1974), 35, (8): 1045-1052.

The investigation was conducted to develop new systems and to evaluate existing ones for diagnosis and control of bovine babesiosis in Colombia, South America. Antigens of Babesia bigemina and Babesia argentina were isolated and were used in complement-fixation (CF) and rapid agglutination (RA) tests for the diagnosis of babesiosis in calves.

Three systems were evaluated for control of bovine babesiosis: (1) vaccination of calves with killed Babesia spp. vaccine to produce resistance (based on sterile immunity), (2) premunition of calves with virulent Babesia spp. and then administration of a chemotherapeutic drug to produce resistance (based on coinfectious immunity), and (3) chemoprophylaxis, using a babesiacide having long residual activity. The 3 systems controlled bovine babesiosis under the conditions of the present experiments.

Epizootiologic conditions in enzootic areas, however, will indicate which system is preferable. In zones having a high population of the tick Boophilus microplus, premunition (system 2) is indicated; in areas where the tick population is controlled or in areas where cattle are at constant risk of tick exposure, vaccination with killed Babesia spp. (system 1) or chemoprophylaxis (system 3) are indicated.

70. TODOROVIC, R. A.: Prevention and Control of Bovine Anaplasmosis and Babesiosis (Piroplasmosis). Proceedings of the Texas Animal Production Conference for Latin American Cattle Producers, College Station, Texas, (January 21, 1974), abstract.

The purpose of this report was to discuss the epizootiological similarities between anaplasmosis and babesiosis, and to emphasize recent developments concerned with prevention and control. In addition, the mechanism of immunity of these hemotropic diseases was discussed.

71. TODOROVIC, R. A. and KUTTLER, K. L.: A Babesiasis Card Agglutination Test. American Journal of Veterinary Research, (October 1974), 35, (10): 1347-1350.

A babesiasis card agglutination test (BCT) has been developed for detecting specific antibodies in cattle infected with Babesia bigemina. The agglutinating antigen was isolated from the blood of a splenectomized calf having 22% B. bigemina parasitemia. The antigen was preserved with 0.02% formalin and stained with fast green dye. The BCT was performed by adding 1 drop of antigen and 2 drops of plasma or serum on a card and mixing for 5 minutes by rotation. Agglutination was visible in instances of positive reactions immediately after rotation.

In cattle intentionally exposed to B. bigemina, the BCT detected agglutinating antibodies simultaneously with the onset of first parasitemia. This reaction was observed to persist as long as 3 months, or long after the disappearance of parasitemia. Because of its simplicity and apparent specificity, the BCT may have use as a field test to aid in the diagnosis of B. bigemina infections. The BCT results showed 100% agreement with the complement-fixation (CF) test on those serums prepared from blood collected within 3 months of infection.

72. VIZCAINO, O. G. and TODOROVIC, R. A.: Inmunidad Cruzada en Bovinos Portadores de Babesia argentina y Babesia bigemina. Proceedings of the IX Congreso Nacional de Medicina Veterinaria y Zootecnia, Barranquilla, Colombia, (July 24-28, 1974).

73. ADAMS, L. G., CRAIG, T. M., PLATT, K. B. and WYSS, J. H.: Bovine Eperythrozoonosis in Colombia. Revista ICA, (December 1975).

Eperythrozoon wenyoni, Eperythrozoon tejanodes and Eperythrozoon tuomii were diagnosed in 14 of 37 splenectomized, Holstein-Friesian, 4-to-11-month-old calves that originated from the Sabana de Bogota. Eleven calves had pure infections of E. wenyoni, 2 calves had dual infections of E. wenyoni and E. tejanodes, and 1 calf had a pure infection of E. tuomii. The diagnosis was determined on Giemsa-stained blood smears, and morphological descriptions of the Eperythrozoon spp. were given. Six splenectomized calves exhibited depression and anorexia, but all 14 calves had elevated rectal temperatures. Two calves had serous conjunctivitis with excessive lacrimation. The increase in rectal temperature coincided with the onset of parasitemia while the packed cell volume decreased after the onset of parasitemia. The average incubation period and standard deviation was 14.9 \pm 3.5 days post-splenectomy. Treatment with 2-di-(Beta, gamma-dioxipropyl)-(aminofenol)-(4 arseno 5)-Beta-(benzaxozalil)-(2)-mercaptol-propionato de sodio at 29 mg/kg intramuscularly caused the parasitemia to become undemonstrable within 24 hours with further recrudescence occurring within 6 weeks.

This is the first report of bovine eperythrozoonosis due to E. wenyoni, E. tejanodes and E. tuomii in Colombia.

74. CORRIER, D. E.: The Epidemiology of Bovine Anaplasmosis and Babesiosis in the Lowland Tropics of Colombia. Proceedings of the Seminario Sobre Hemoparasitos (Anaplasmosis y Babesiosis), CIAT, Cali, Colombia, (March 17-22, 1975).

The prevalence of anaplasmosis and babesiosis was determined on 37 ranches in the Eastern plains, 4 ranches on the Atlantic coast, and on 6 ranches in the Cauca Valley of Colombia. A random group of cattle representing a minimum of 10% of the total herd were sampled on each ranch ensuring that animals less than 1 year, 1 to 2 years and more than 2 years of age were included in the sample group. A total of 3,698 serum samples were collected and tested using the complement fixation test. Tick counts were made and ticks were collected for classification on each of the 37 ranches visited in the Eastern plains.

The prevalence of Anaplasma reactors was determined to be 75% in the Eastern plains, 91% on the Atlantic coast and 71% in the Cauca Valley. The prevalence and even distribution of Anaplasma reactors among the 37 ranches in the Eastern plains indicated anaplasmosis is endemic within the entire study area. The prevalence of Anaplasma reactors on the 4 ranches on the Atlantic coast, and the 6 ranches in the Cauca Valley, though based on inadequate sample sizes for the areas in general, suggests that anaplasmosis is probably endemic in both areas.

The prevalence of Babesia bigemina reactors was determined to be 42% in the Eastern plains, 77% on the Atlantic coast and 75% in the Cauca Valley. The prevalence of infection with B. bigemina in the Eastern plains indicated the area is endemic. However, the percentage of reactors among the 37 ranches varied from 5 to 98%, which indicated the disease is not evenly distributed through out the area. The prevalence of B. bigemina reactors on the Atlantic coast and in the Cauca Valley suggests that babesiosis is probably endemic in both areas.

The high prevalence of anaplasmosis and babesiosis within the 3 areas in which the study was conducted indicates the importance of exposing calves to infection at an early age when maternal antibodies and natural resistance provide maximum protection against clinical disease.

The necessity of providing protection through immunization or other procedures to susceptible cattle which may be transferred into the areas was strongly indicated.

Boophilus microplus ticks were indentified on each of the 37 ranches in the Eastern plains and were nearly equally distributed as indicated by nonsignificant differences in the tick counts. Ticks identified as Amblyomma cajennense, Amblyomma triste and Anocentor nitens were collected on 3 of the ranches indicating that their role as vectors or potential vectors of anaplasmosis and/or babesiosis is limited.

75. CRAIG, T. M.: The Prevalence of Bovine Parasites in Various Environments Within the Lowland Tropical Country of Guyana. A Dissertation submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Doctor of Philosophy, December 1975.

The variety and relative abundance of bovine hemo, external and helminth parasites were compared between four ecologically distinct areas of Guyana. The coastal area, the richest agricultural area within the country, contains the highest livestock populations. The cattle for the most part are secondary to crop production on the alluvial soils reclaimed from mangrove swamps. The forested areas of Guyana are a true rain-forest with a four layer tree canopy. Livestock production is being developed in areas cleared of forests. The mid-savannahs area natural brown sand savannahs dominated by Trachypogon plumosus. This grass is unpalatable to cattle for much of the year. The Rupununi is a large savannah with an 8 month dry period. The dominant grass in this savannah is also T. plumosus.

The following hemoparasites were identified: Trypanosoma vivax in 0.7% of the coastal cattle; Anaplasma marginale in 85% of the cattle with the highest prevalence in the mid-savannahs and the lowest on the coast; the serological prevalence of Babesia bigemina was 31% and Babesia argentina 11% with a lower prevalence in the forested areas.

The tropical cattle tick, Boophilus microplus, was found in all areas of the country except at the village of Aishalton in the Rupununi. Other species of ticks found parasitizing cattle or in the vicinity of cattle populations were Amblyomma cajennense, Amblyomma triste and Anocentor nitens.

Insects identified parasitizing cattle were Haematopinus quadripertusus on the coast, Dermatobia hominis, Cochliomyia hominivorax and Haematobia irritans in the mid-savannahs; and Simulium haematopotum in the Rupununi.

The following helminth parasites were identified in Guyana: an apparently undescribed species of Ostertagia from the coast; Haemonchus placei and Haemonchus similis on the coast; H. similis in the Rupununi; Trichostrongylus axei, Cooperia punctata, Bunostomum phlebotomum, Strongyloides papillosus, Toxocara vitulorum, Capillaria bovis, Oesophagostomum radiatum, Trichuris discolor, Dictyocaulus viviparus, Mammomonogamus laryngeus, Onchocerca lienalis, Setaria labiatopapillosa, Stephanofilaria stilesi, Moniezia sp. and Cotylophoron cotylophorum.

Parasite profiles compared the coast and Rupununi helminth fauna. Bioclimatographs for the various regions of the country were prepared considering some of the more important genera of helminths and B. microplus. Fasciola and Cysticercus, two helminths expected to be encountered in a country like Guyana, were not found.

This study evaluates the factors concerned with the variety and abundance of various parasites considering edaphic, climatic, botanic, zoologic and social conditions which may be involved in their distribution. It was essential to determine what parasites are likely to be of economic importance, and what practices may be used to control these parasites.

76. DAY, W. C. and KUTTLER, K. L.: Animal Health Considerations Involved in the Movement of U.S. Cattle to Haiti. *Southwestern Veterinarian*, (1975), 28, (3): 229-232.

A total of 44 young Charolais cattle were moved from Texas to Haiti. They were vaccinated against anaplasmosis (1 injection only), anthrax and shipping fever. They were treated with 2,8 mg/kg body weight of Imidocarb before being exposed to infected Boophilus ticks.

Based on serologic evidence, infections with Anaplasma occurred over 90% of the calves within the first 130 days. Babesia infections apparently occurred in over 70% of the calves within this same period of time. No deaths, however, occurred during the first 130 days.

77. GONZALES, E. F. and TODOROVIC, R. A.: Evaluacion de la Inmunidad Coinfecciosa v Esteril en el Control de la Babesiosis Bovina. Proceedings of the II Congreso Latinoamericano de Buiatria, Sesion III. Enfermedades Transmisibles y por Hematozoarios, Maracaibo, Venezuela, (February 23-28, 1975).
78. GUZMAN, V. H., ADAMS, L. G. and GALVIN, T. J.: Verminosis Laringea Bovina Produciada por Mammomonogamus laryngeus en Colombia. *Revista ICA*, (June 1975).

Eight cases of bovine laryngeal verminosis were diagnosed in Valle del Cauca, Colombia, and confirmed by parasitological studies. Macroscopic and microscopic descriptions were made of the lesions caused by Mammomonogamus laryngeus.

79. KUTTLER, K. L.: Diagnosis of Anaplasmosis: A Review. Proceedings of the Seminario Sobre Hemoparasitos (Anaplasmosis y Babesiosis), CIAT, Cali, Colombia, (March 17-22, 1975). CIAT Series CE-12, 93-103.

Reliable tests are available to diagnose both acute and chronic anaplasmosis. A high degree of correlation and agreement occurs between the complement-fixation (CF) and the capillary tube agglutination test, and between the CF and the rapid card test (CT). Both the CF and CT are recognized as official tests for interstate movement of cattle where regulations require a preshipment negative test.

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80. KUTTLER, K. L.: Hemoparasites as They Influence Native-Born and Introduced Cattle in the Tropics. Proceedings of the Continuing Education Programme in Animal Production and Health - Beef Cattle, Malaysia, (August 18-22, 1975).

The arthropod-borne, hemoparasitic diseases (anaplasmosis, babesiosis, theileriosis, and trypanosomiasis) occur throughout the world, but more intensely in tropical and subtropical zones where adequate vectors are present to maintain and transmit the disease agent. Control programs are discussed. The selection of the best system for use in Malaysia will have to be determined by research and by evaluating each system under local conditions. Malaysia is fortunate in not having areas infected with virulent Theileria or Trypanosoma, since these conditions would probably survive and produce cattle losses if introduced. Extreme caution is recommended in the importation of cattle.

81. KUTTLER, K. L.: Use of Imidocarb to Control Anaplasmosis. Southwestern Veterinarian, (1975), 28, (1): 47-52.

A field trial was conducted on 469 cattle to determine the effectiveness of imidocarb [3,3'-Bis-(2-imidazolin-2-yl)-carbanilide dipropionate] which was injected intramuscularly 2 times 14 days apart at a level of 5 mg/kg body weight. Treatment was therapeutically effective, but these methods failed to produce the desired control. An initial drop in positive serum response as measured by the complement-fixation test was noted after treatment. This was followed by a gradual increase, thought to be due to reinfection. One year after treatment the rate of positive serum tests was essentially the same as before treatment.

Even though effective drugs are available to treat anaplasmosis, caution is indicated in those herds in which the infection rate is high and transmission is active.

82. KUTTLER, K. L. and CRAIG, T. M.: Isolation of a Bovine Theileria. American Journal of Veterinary Research, (March 1975), 36, (3): 323-325.

Dual infections of Anaplasma marginale and a Theileria, resembling Theileria mutans, occurred in splenectomized calves inoculated with pooled blood samples from eastern Texas cattle. Theileria was obtained in pure form by treating dually infected cattle with glöxazone and imidocarb which selectively eliminated Anaplasma. These Theileria infections were responsible for mild, transient reductions in packed red blood cell volume.

83. KUTTLER, K. L., GRAHAM, O.H. and TREVINO, J. L.: The Effect of Imidocarb Treatment on Babesia in the Bovine and the Tick (Boophilus microplus). Research in Veterinary Science, (1975), 18: 198-200.

Treatment of calves with 5 mg/kg Imidocarb [3,3'-bis-(2-imidazolin-2-yl)carbanilide dipropionate] given intramuscularly 14 days before and 14 days after exposure to Babesia infected Boophilus microplus larvae rendered the next generation of larvae incapable of transmitting Babesia infection. When administered to calves 14 or 28 days before tick exposure, the drug prevented the development of clinical babesiosis; the larval progeny of ticks reared on the calf which was treated 28 days before infestation were infective. Treatment of a calf 42 days before exposure to infective larvae did not prevent the development of a Babesia parasitemia but appeared to reduce the severity of infection.

84. KUTTLER, K. L. and ZARAZA, H.: Evaluacion Preliminar del Dithiosemicarbazono para el Tratamiento de la Anaplasmosis. Revista ICA, (June 1975).

85. MAURER, F. D.: African Swine Fever. A 16 page chapter in the 4th edition of Diseases of Swine, a textbook by H. W. Dunne and A. D. Lemay, 1975.

This is a comprehensive detailed review of African Swine Fever as the most serious single disease threat to the world's swine industries. Sections cover history and distribution of ASF, the etiology with details about the virus, and its characteristics as an antigen, the host range, transmission, clinical character of the disease, the gross and microscopic pathology with considerable illustrated detail of the histopathology, with emphasis on features most important to the diagnosis. The serologic diagnosis has become increasingly important in view of the increased pathologic similarity of chronic ASF to hog cholera, as ASF now appears in Spain.

86. MAURER, F. D.: Livestock, a World Food Resource Threatened by Disease. Journal of the American Veterinary Medical Association, (May 1, 1975), 166. (9): 920-923.

This is an editorial-type article which stresses the essential role of livestock as a world food resource and the importance of disease control for efficient livestock production, hence, man's food supply.

87. MAURER, F. D.: The Work of the Institute of Tropical Veterinary Medicine, Texas A&M University. Proceedings of the Seminario Sobre Hemoparasitos (Anaplasmosis y Babesiosis), CIAT, Cali, Colombia, (March 17-22, 1975).

This report outlines the Institute's origin, purpose, objectives, and research.

88. REYNOLDS, S. D.: Evaluation of Methods of Premunition to Anaplasma marginale. A Thesis submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Master of Science, May 1975.

Twenty-one yearling, crossbred, beef heifers were divided into 3 experimental groups and premunized by inoculating intravenously 2 ml of a 10^{-2} dilution of an Anaplasma stabulate of Texas origin. A similar group of 4 heifers was maintained as non-infected controls.

Seven cattle in group I were vaccinated with "Anaplaz" 2 times, at a 4-week interval, prior to the premunizing infections. Nine cattle in group II were premunized and allowed to go untreated during the course of infection. Five cattle in group III were each treated with 11 mg/kg oxytetracycline intravenously when the Anaplasma parasitemia was about 4.6%.

Complement-fixation (CF) titers preceded the appearance of an Anaplasma parasitemia by up to a week. Clinical manifestations associated with Anaplasma parasitemia were mild in all groups and limited to rough haircoat, an unthrifty appearance and a slight loss of weight. The infection in all groups was characterized by lower packed cell volume (PCV), reduced red cell counts, low hemoglobin and increases in the mean corpuscular volume when compared to the controls; however, no significant group differences in these parameters were detected. Cattle of group I showed slightly longer incubation times and higher CF titers than cattle in groups II and III. The recovery rates for cattle in groups I, II and III showed no significant differences.

A challenge consisting of 5 ml whole, fresh blood from a splenectomized calf showing an 8% Anaplasma parasitemia and a 20% PCV was administered intravenously to all 25 experimental cattle after the premunizing infection had subsided. This challenge was calculated to represent over 100,000 times more infectious particles than the premunizing infection. Premunized animals were solidly immune to challenge, whereas the controls were severely affected.

89. TODOROVIC, R. A.: Babesiosis. Proceedings of the XX World Veterinary Congress, Symposium on Immunology of Animal Diseases Caused by Blood Protista, Thessaloniki, Greece, (July 6-12, 1975): 269-270.
90. TODOROVIC, R. A.: Non-Chemical Control of Blood Parasites. Proceedings of the XX World Veterinary Congress, Section on Parasitology, Thessaloniki, Greece, (July 6-12, 1975): 222-223.
91. TODOROVIC, R. A.: Serological Diagnosis of Babesiosis: A Review. Tropical Animal Health & Production, (1975), 7: 1-14.

In the last three decades some fundamental knowledge concerning the immunoserology of Babesia spp. infections has led to the development of serological techniques which provide a means for studying the pathogenesis of babesiosis and the detection of animals with subclinical infections.

The antigens used in the serological procedures originated from the parasitized erythrocytes, plasma, and tissues of animals with acute babesiosis. Parasitic and serum soluble antigens were applied in a variety of serological tests, e.g., complement fixation, gel precipitation, agglutination, and fluorescent antibody, for detection of Babesia spp. antibodies.

In this review an attempt was made to summarise and discuss the recent advances in the serodiagnosis of babesiosis, together with conditions where the use of serological methods may be valuable.

92. TODOROVIC, R. A. and GONZALEZ, E. F.: Avances Recientes en el Sero-diagnostico de Babesiosis Bovina. Proceedings of the II Congreso Latinoamericano de Buiatria, Sesión III. Enfermedades Transmisibles y por Hematozoarios, Maracaibo, Venezuela, (February 23-28, 1975).
93. TODOROVIC, R. A. and GONZALEZ, E. F.: Evaluación de la "Premunición" Para el Control de Anaplasmosis y Babesiosis en Fincas Comerciales del Valle del Cauca (Colombia). Proceedings of the Seminario Sobre Hemoparasitos (Anaplasmosis y Babesiosis), CIAT, Cali, Colombia, (March 17-22, 1975).

94. TODOROVIC, R. A. and GONZALEZ, E. F.: Inmunizacion contra Babesiosis Bovina con Vacuna a Base de Parasitos Muertos. Revista ICA, (March 1975), 10: 87-99.

Non-living Babesia bigemina and Babesia argentina antigens were prepared from infected erythrocytes and from plasma collected from acutely infected calves. The antigens were lyophilized and stored at -20° C. The preparations were tested in Colombian cattle under field conditions in the Cauca Valley.

A total of 16 calves distributed in 4 groups of 4 calves each were injected and 4 calves not injected were used as controls. Calves (Group A) were injected with lyophilized plasma only; calves (Group B) were injected with lyophilized plasma plus adjuvant; calves (Group C) were injected with lyophilized parasitized erythrocytes only; and calves (Group D) were injected with lyophilized parasitized erythrocytes plus adjuvant. The inoculations were given twice at 2-week intervals. Vaccinated and non-vaccinated calves were exposed to field challenge with Boophilus microplus infected ticks. Immunological response was measured by packed cell volume (PCV), parasitemia (P), complement fixation test (CF), body weights and mortality.

It was found that a high degree of sterile immunity to B. bigemina and B. argentina can be produced in susceptible calves by injecting them with non-living Babesia spp. antigens.

95. TODOROVIC, R. A., GONZALEZ, E. F. and ADAMS, L. G.: Babesia bigemina, Babesia argentina, and Anaplasma marginale: Coinfectious Immunity in Bovines. Experimental Parasitology, (1975), 37: 179-192.

Forty-eight intact and 8 splenectomized cattle were used to evaluate different systems of coinfectious immunization against Babesia bigemina, Babesia argentina, and Anaplasma marginale. Coinfectious immunity was induced by 2 methods: (1) blood of cattle acutely infected with B. bigemina, B. argentina and A. marginale was used as the source of inoculum and the post vaccination reactions were chemotherapeutically controlled with Imido-carb, Ganaseg, Gloxazone, and Liquamycin, and (2) by artificially inducing babesiosis with the blood of carrier cattle with chronic infections of B. bigemina and B. argentina without chemotherapy. The degree of resistance was determined by blood-borne and tick-borne challenges. Ticks were collected from cattle and identified as Boophilus microplus and Dermacentor nitens. Vaccinated cattle demonstrated a high degree of resistance to babesiosis and anaplasmosis; however, cattle without coinfectious immunity were treated chemotherapeutically to prevent death losses.

96. TODOROVIC, R. A., LOPEZ, L. A., LOPEZ, A. G. and GONZALEZ, E. F.: Bovine Babesiosis and Anaplasmosis: Control by Premunition and Chemoprophylaxis. *Experimental Parasitology*, (1975), 37: 92-104.

Experiments were carried out to evaluate 2 systems: (1) premunition and (2) chemoprophylaxis for the control of bovine babesiosis and anaplasmosis in the Cauca River Valley, Colombia. Control of these diseases was achieved by inoculating cattle with virulent Babesia bigemina, Babesia argentina, and Anaplasma marginale and subsequent treatment with Imidocarb and Gloxazone to moderate the postpremunition reactions. Chemoprophylactic treatment with Imidocarb and Gloxazone was administered to cattle before and during field exposure. Premunized cattle were highly resistant to tick-borne (Boophilus microplus) challenge. Imidocarb had therapeutic and chemoprophylactic properties against babesiosis, but appeared toxic. Gloxazone moderated the A. marginale postpremunition reaction, but failed to prevent clinical anaplasmosis under the conditions of this investigation.

97. TODOROVIC, R. A. and TELLEZ, C. H.: The Premunition of Adult Cattle Against Babesiosis and Anaplasmosis in Colombia, South America. *Tropical Animal Health & Production*, (1975), 7: 125-131.

Twenty-five cattle (Bos taurus) between 2 and 3 years of age were premunized with virulent Babesia bigemina, Babesia argentina and Anaplasma marginale. The Babesia spp. premunition reaction was controlled by Imidocarb or by Ganaseg therapy. The A. marginale post premunition reaction was controlled by oxytetracycline alone, or combined with Gloxazone (dithiosemicarbazone). Systems of premunition for Babesia spp. were found effective and practical; but systems of premunition for A. marginale were found less effective and not practical under the conditions of these experiments.

98. CALDERON, G. A., and TODOROVIC, R. A.: Obtencion de Antigenos para la Prueba de Fijacion del Complemento de Plasma y Eritrocitos Parasitados con Babesia bigemina y Babesia argentina. Revista del Instituto Veterinario de Investigaciones Tropicales y de Altura, Lima, Peru. (1975).

Babesia bigemina and Babesia argentina complement fixation (CF) antigens were isolated from the plasma (P) of acutely infected splenectomized calves, respectively. These antigens were used in CF tests to compare their serologic reactivity with the CF antigens isolated from infected erythrocytes (E). It was found that CF plasma antigens can be used successfully in the CF test for detection of specific Babesia bigemina and Babesia argentina antibodies. This finding is of significant economical and practical importance because the plasma was discarded in procedure for isolation of Babesia species antigens previously reported.

99. VIZCAINO, O. G. and TODOROVIC, R. A.: Caracterizacion de los Antigenos de Babesia argentina y Babesia bigemina por los Metodos de Fijacion del Complemento, Immunodifusion, Inmunoelectroforesis e Inmunidad Cruzada. Revista ICA, (March 1975), 10: 77-85.

Babesia argentina and Babesia bigemina antigens were isolated from blood of acutely infected splenectomized calves and used in complement fixation tests. Soluble antigens for both B. argentina and B. bigemina were isolated by chromatography with DEAE cellulose and Sephadex G-200 from plasma collected from acutely infected calves and were characterized by means of immunodiffusion and immunoelectrophoresis techniques. The antigens of B. argentina and B. bigemina used in the complement fixation test reacted specifically with the homologous sera and at a lower percentage with the heterologous sera. Soluble antigens of B. argentina and B. bigemina had reactions of identity and non-identity in the double gel diffusion test and detected precipitating antibodies in the serum collected from animals with chronic babesiosis. The soluble antigens of B. argentina and B. bigemina had slow electrophoretic mobility toward the anode with clear precipitation arcs. In the cross immunity studies, a slight cross protection against B. argentina and B. bigemina infections was demonstrated.

100. MAURER, F. D.: African Swine Fever and Hog Cholera - a Comparison. Proceedings of the United States Department of Agriculture Seminar, Athens, Georgia, (January 1976).

This paper designed as a review of these diseases for U.S. veterinarians covered the history of ASF, then compared the etiology, host range, transmission, clinical character, gross and microscopic pathology with hog cholera. Emphasis was placed on those features of each most significant in the differential diagnosis of these very important diseases of swine.

101. WYSS, J. H.: The In Vitro Cultivation of Babesia bigemina Utilizing Bovine Cells in Culture. A Dissertation submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Doctor of Philosophy, May 1976.

Babesia bigemina in vitro cultivation experiments utilizing primary and continuous monolayer cultures were conducted. Experiments to infect normal non-infected cells by in vitro inoculation using fresh or stabilate B. bigemina-infected blood as inoculum were conducted with primary monolayer cultures of bovine spleen, lymph node, hemal node, and fetal kidney and continuous monolayer cultures of African Green Monkey kidney cells. When fresh infected blood was used as inoculum, the B. bigemina organisms dissociated from their host erythrocytes by day 2 and extracellular parasites were identifiable for up to 5 days on the surface of the cultured cells. When stabilate preparations were used as inoculums, the majority of the parasites remained intraerythrocytic with few extracellular parasites being observed. Babesia bigemina-infected erythrocytes present in the inoculum were observed up to 14 days on the surface of the cultured cells; however, the parasites were degenerative and pyknotic in appearance. No differences were observed between the various types of cultured cells and there was no evidence that parasitic infection of culture cells or multiplication of organisms took place in the original cultures or subsequent subcultures.

Experiments with primary monolayer cultures derived from B. bigemina-infected calves were conducted with spleen, lymph node, hemal node and leucocyte cultures. Five days after culture seeding, B. bigemina organisms could be found only in splenic monolayer cultures and could be identified in such cultures for 11 days post culture. The number of B. bigemina organisms decreased with time and there was no evidence that infection of cultured cells occurred or multiplication of the parasite took place. The subsequent 7 subcultures of the monolayer cultures did not demonstrate any evidence of being infected with B. bigemina and no subcultures of detached cells suspended in media could be established.

Babesia bigemina in vitro cultivation experiments utilizing erythrocyte maintenance suspension cultures were conducted. Experiments to infect normal non-infected erythrocytes maintained in suspension culture were conducted using fresh and stabilate infected blood preparations. In addition, B. bigemina-infected blood was also placed in maintenance cultures. Before the erythrocyte maintenance procedures were improved, a similar situation existed as with monolayer cultures. When fresh infected blood was used as inoculum or placed in maintenance culture, the B. bigemina became extra-erythrocytic within 24 hours and failed to infect other non-infected erythrocytes. When stabilate preparations were used, infected ghost erythrocytes were observed up to 2 days. Morphologically the B. bigemina were degenerative. As the erythrocyte maintenance procedures improved, infected erythrocytes were observed up to 4 days and infected erythrocytes held in maintenance culture 3 days were proven infective for a susceptible splenectomized calf.

102. KYZAR, C. T.: Evaluation of a Babesiosis Card Agglutination Test.
A Thesis submitted to the Graduate College of Texas A&M University
in partial fulfillment of the requirement for the degree of
Master of Science, 1976.

A babesiosis card agglutination test (BCT) was evaluated as a means of detecting specific antibodies in cattle infected with Babesia bigemina. The agglutinating antigen was prepared from the blood of a splenectomized calf having a 58% B. bigemina parasitemia.

Two methods of antigen preparation were evaluated, one using a pure B. bigemina parasite suspension and the other a crude suspension of B. bigemina parasites and parasite particles with erythrocytic stroma. The following methods of antigen preservation were evaluated: (1) dilution with phosphate buffered physiologic saline solution (PBS), (2) addition of 0.05% phenol, (3) addition of 0.01% thimersal, (4) addition of penicillin and streptomycin, (5) dilution with Walpole's acetate buffer containing 0.1% methyl-P-hydroxybenzoate, (6) lyophilization, and (7) freezing.

In order to determine suitability for testing, fresh and frozen serum and serum that had remained with the clot for 48 hours and plasma containing sodium citrate, ammonium heparin, and dipotassium ethylenediaminetetraacetic acid (EDTA) as anticoagulants were evaluated.

The BCT was performed on serums and plasma collected from animals experimentally infected with B. bigemina, Babesia argentina, and Anaplasma marginale. Using serum and plasma samples collected from 299 cattle from 4 different ecological areas of Colombia, the BCT was compared to 2 other serological tests, the complement-fixation (CF) test and the indirect fluorescent antibody (IFA) test.

The BCT, using the crude antigen suspension preserved with either the addition of penicillin and streptomycin or dilution with PBS, compared favorably with the IFA test under both laboratory conditions using serums and field conditions using plasma collected with sodium citrate as the anticoagulant. Significant differences were detected when the BCT was compared to the CF test.

103. HOPPS, D. C.: An Evaluation of Colostral Immunity and the Acquired Immune Response to Bovine Babesiosis using the Complement Fixation and the Indirect Fluorescent Antibody Tests. A Thesis submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Master of Science, (1976).

The complement fixation (CF) and indirect fluorescent antibody (IFA) tests for Babesia bigemina and Babesia argentina were applied to serums collected from 3 experiments. Experiment 1 involved 6 calves infected once with Babesia argentina and 6 calves infected once with Babesia bigemina. In the Babesia argentina infected calves, the CF and IFA titers were positive at the end of one year, and there was only a low level heterologous titer to Babesia bigemina which persisted for 2 to 4 months. In the Babesia bigemina infected calves, homologous CF titers were low and did not persist over 4 months, but heterologous titers to Babesia argentina were higher and persisted for over 7 months. Homologous IFA reactions were of high titer to Babesia bigemina and persisted the entire year; heterologous reactions were of low titer and persisted for only 3 months.

In experiment 2, six calves born in an endemic zone of Colombia were continually exposed to natural tick-transmitted infections of Babesia argentina and Babesia bigemina for one year, but they did not develop clinical babesiosis. Serologic titers were positive for both organisms during the first week of life, and protection from the clinical effects of Babesia infections was considered due to protective antibodies in the colostrum. Repeated natural exposure caused fluctuating positive titers during the first year of life.

In experiment 3, serums were collected from 5 noninfected calves before ingestion of colostrum from cows with antibody titers to babesiosis, and weekly for 6 months thereafter. Highest CF and IFA titers were measured in the serum of each calf at 1 week of age, intermediate titers were measured in the colostrum itself, and the lowest titers were detected in the serum from each cow. Antibodies persisted for as long as 20 weeks, depending on the original titer. Both the CF and IFA tests were approximately equal in their ability to detect colostrum antibodies.

The IFA test is recommended as the test of choice for a reliable and sensitive means of obtaining serologic evidence of babesial infection. The CF test, especially for Babesia bigemina, often lacked sensitivity and reliability

104. TODOROVIC, R. A.: Bovine Babesiosis in Colombia. *Veterinary Parasitology*, (1976), 2: 97-109.

Babesiosis is a tick-borne disease of cattle which occurs in many tropical and subtropical areas of the world. Despite the extensive investigations which have been carried out since the discovery of the organism (Babes, 1888) many problems of major importance remain to be solved in Babesia spp. -host complex. In Colombia (South America) the experiments were carried out to identify the existing Babesia spp. by morphologic and immunoserologic methods. The immunoserologic relationship of Babesia spp. were studied by several serologic techniques. Attempts were made to develop a sensitive and practical serologic test for diagnosis of latent Babesia spp. infections. Several groups of intact and splenectomized calves were inoculated with various antigens isolated from Babesia spp. infections and the response to vaccination, premunition and tick-borne challenge were studied. The second part of this investigation was mainly concerned with evaluating the system of chemoprophylaxis against Babesia spp. infections under actual field conditions.

105. TODOROVIC, R. A. and LONG, R. F.: Comparison of Indirect Fluorescent Antibody (IFA) with Complement Fixation (CF) Tests for Diagnosis of Babesia spp. Infections in Colombian Cattle. *Zeitschift fur Tropenmedizin und Parasitologie*, (1976), 27: 169-181.

A total of 372 serum samples were collected from Colombian cattle before and during the course of natural Babesia spp. infection on the North Coast of Colombia. The serum samples were used to compare indirect fluorescent antibody (IFA) with complement fixation (CF) tests for diagnosis of babesiosis. The IFA technique detected Babesia argentina antibodies an average of 4.0 weeks earlier than the CF test and Babesia bigemina an average of 2.5 weeks earlier. Both IFA and CF were capable of differentiating B. argentina and B. bigemina infections, however in some cases cross reactions were observed. In general IFA titers were at relatively high levels of 1:640 to 1:5120 in comparison with CF titers of trace to 1:80. In cases of mortality due to babesiosis, both IFA and CF serologic techniques were very useful in indicating the cause of death. Although both IFA and CF are laboratory tests, the IFA technique had advantages over the CF in simplicity, economy and speed of performance.

106. THOMPSON, K. C. and ROA, J. C.: The Transmission of Anaplasma marginale by the Tropical Cattle Tick Boophilus microplus. International Conference of Tick-borne Diseases and their Vectors, University of Edinburgh, Centre of Tropical Veterinary Medicine, Edinburgh, Scotland, (September, 1976).

Laboratory Boophilus microplus tick strains were used to infest splenectomized Holstein-Friesian calves infected with a purified Anaplasma marginale stabilate. The engorged B. microplus females were held in tick incubators (70 + % relative humidity at 28 to 30° C) during oviposition. The resulting larvae were used for the subsequent transmission trails when they were 14 to 21 days of age.

After eight repeated tick transmission trials, the only successful modes of Anaplasma transmission were by trans-stadial and intrastadial methods. Transovarial (biological) transmission did not occur.

It is suggested that for a tick species to be an efficient mechanical vector of Anaplasma, it would most likely be a two or three host tick and not a one host tick. This would hold true only if there were no great amount of intrastadial movement (especially of males) between cows.

107. THOMPSON, K. C.: The Problem of Bos Taurus Cattle Introduced into the Tropical Areas of Colombia. Second International Conference of Institutions of Tropical Veterinary Medicine, West Berlin, Germany, (October 1976).

Invariably the greatest problem imposed on any introduced breed of cattle into the tropics is one of acclimatization. Not only does the lack of photoperiod seasonality, temperature and humidity play havoc (often sterility; drop in milk production), but inefficient utilization of tropical forages and protein supplements may also cause intermittent to profuse diarrhea with subsequent drastic loss of body weight

After this period (which differs between breeds), one usually finds ticks with their diseases (anaplasmosis and/or babesiosis) taking their toll.

Surviving this, one has the age old problems of different cultural practices of cattle management to deal with along with other infectious (brucellosis, foot and mouth, clostridial diseases, anthrax, leptospirosis, IBR) and non-infectious (mastitis, foot rot, screw worm) disease syndromes.

Hence, any procedure designed to lessen or alleviate any or all of these effects, results in greater economical gain for the cattleman.

108. THOMPSON, K. C.: A Technique to Establish a Laboratory Colony of Boophilus microplus Infected with Babesia bigemina. A Dissertation submitted to the Graduate College of Texas A&M University in partial fulfillment of the requirement for the degree of Doctor of Philosophy, (February, 1976). (abstract)

A technique was evolved for the establishment and maintenance of a colony of Boophilus microplus free of infection with Anaplasma marginale and Babesia spp., and for their subsequent infection with a pure isolate of Babesia bigemina. Confirmation was obtained that the ticks are infected normally during the last 24 hours of attachment on the host. The life cycle of Boophilus microplus was described for a single situation on the Atlantic Coast of Colombia.

109. GONZALEZ, E. F., TODOROVIC, R. A., and THOMPSON, K. C.: Immunization against Anaplasmosis and Babesiosis: Part I. Evaluation of Immunization Using Minimum Infective Doses under Laboratory Conditions. Tropenmed. Parasit., (1976), 27: 427-437.

A method of immunization against anaplasmosis and babesiosis using minimum infective doses was developed under laboratory conditions. Stabilates of Anaplasma marginale stored at -60°C were found infective when diluted 10-fold to 10^{-5} . Stabilates of Babesia argentina and Babesia bigemina stored under the same conditions were infective when diluted 10-fold to 10^{-1} . Intact calves inoculated with the above dilutions of stabilates developed moderate parasitemias and recovered from infection without treatment. There was an immune response to vaccination with the formation of specific antibodies to A. marginale, B. bigemina and B. argentina as measured by the complement fixation (CF) test. All calves were found resistant to artificial challenge with lethal doses of the respective parasites.

110. TODOROVIC, R. A., LONG, R. F., MCCALLON, B. R.: Comparison of Card Agglutination Test with the Complement Fixation Test for Diagnosis of Anaplasma marginale Infection in Colombian Cattle. Veterinary Microbiology, (1977), 2: 167-177.

342 serum samples were collected from 9 Holstein-Friesian calves before and during natural infection with Anaplasma marginale on the North Coast of Colombia. The samples were used to compare the complement fixation (CF) and rapid card agglutination tests (CT) for the diagnosis of anaplasmosis. The positive agglutination of the CT always developed several days after the first CF reaction but then persisted. In contrast, the CF test showed an initial sharp rise in titer but then showed fluctuations between trace and 1:80 titers. The results indicated that under field conditions the CT was the simpler and more reliable test for the diagnosis of anaplasmosis in a single animal. The CF test remains useful in experimental situations where the earliest knowledge is required of the magnitude of immunological response to challenge.

111. THOMPSON, K. C., TODOROVIC, R. A., and HIDALGO, R. J.: Antigenic Variation of Babesia bigemina, Research in Veterinary Science (1977), 23: 51-54.

The purpose of the study was to determine whether antigenic differences occurred in four stabilates of Babesia bigemina derived from a single purified isolate and propagated as acute and chronic, blood-borne and tick-borne infections in Colombian cattle.

Antigens were characterised by means of the complement fixation (CF), gel diffusion (GD), agar gel electrophoresis (AGE) and the indirect haemagglutination tests (IHA).

Differences were detected. Acute blood and chronic blood antigens were similar, as were acute tick and chronic tick antigens, when compared by IHA and GD. Similarities were observed between acute blood and acute tick and between chronic blood and chronic tick when these antigens were compared by AGE and CF.

112. LONG, R. F., GONZALEZ, E. F., GUZMAN, M. I. and TODOROVIC, R. A.: An Indirect Fluorescent Antibody (IFA) Procedure for the Diagnosis of Bovine Anaplasmosis and Babesiosis. VIII Congreso Panamericano de Medicina Veterinaria y Zootecnia, Santa Domingo, Republica Dominicana. (1977).

A rapid accurate, reproducible serological test in which the easy preparation of antigens, simple storage requirements, minimum use of expensive materials and the capability of simultaneous testing of sera for multiple hemoparasite antibodies are combined to provide clinical or research laboratories and veterinarians with valuable diagnostic tool.

Detailed instructions for antigen preparation, test performance and interpretation of results are given and potential trouble areas are discussed and practical remedies outlined.

113. KUTTLER, K. L., ADAMS, L. G., and TODOROVIC, R. A.: Comparison of the Complement Fixation and Indirect Fluorescent Antibodies. American Journal of Veterinary Research. (1977), 38: 153-156.

Complement-fixation (CF) and indirect fluorescent antibody (IFA) antigens were prepared from Babesia bigemina isolates obtained in Texas. These serologic procedures were evaluated on 130 serum samples sequentially collected from 5 B. bigemina infected mature cattle, beginning on the day of exposure and continuing for 175 days thereafter.

Both tests were effective in detecting specific antibodies for the first 84 days of infection, with 57 of 60 (95%) serums tested being positive on the CF test and 57 of 57 (100%) tests being positive to the IFA test. During the interval from 98 to 175 days, 24 of 60 (40%) of the serums tested were positive with the CF test, and 53 of 56 (95%) were positive with the IFA test.

During the first 84 days, a similar linear regression occurred in both CF and IFA serum titers, but after 98 days the IFA regression flattened out, whereas the CF titers decreased below the sensitivity threshold in 60% of the serums tested.

110. TODOROVIC, R. A., LONG, R. F., MCCALLON, B. R.: Comparison of Card Agglutination Test with the Complement Fixation Test for Diagnosis of Anaplasma marginale Infection in Colombian Cattle. Veterinary Microbiology, (1977), 2: 167-177.

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114. THOMPSON, K. C.: El Problema de la Introduccion de Ganado Bos taurus a las Zonas Tropicales de Colombia. Revista El Cebie, Volume XVI Ano XXII, Bogata, Colombia, (Oct./Nov., 1977), 184: 18-19.

Existe la creencia entre los ganaderos de America Tropical, de la necesidad de introducir ganado Bos taurus como elemento mejorante de las razas nativas. Esta creencia se basa en la mayoria de los casos en estudios economicos realizados en paises de ecologia diferente a la nuestra, con una tradicion ganadera respaldada por muchos anos de experimentacion y desarrollo tecnologico.

115. THOMPSON, K. C., TODOROVIC, R. A. and HIDALGO, R. J.: The Immune Responses to Antigenic Variants of Babesia bigemina in the bovine. Research in Veterinary Science, (1977), 24: 1-4.

Four Babesia bigemina stabilates were used to determine the immune response of cattle to acute and chronic-blood and tick-borne infections.

Thirty-two intact calves were divided into 16 groups of 2 and each calf was inoculated with infective B. bigemina erythrocytic stabilates. Twenty-eight days later they were challenged with homologous and heterologous stabilates, and monitored for an additional 20 days. The hosts apparently reduced response to homologous challenge indicated antigenic differences between the isolates and confirmed the conclusions reached by examination of the serological data.

116. LOPEZ, G. V., TODOROVIC, R. A.: Rapid Latex Agglutination Test (RA) for the Diagnosis of Babesia argentina Infection. Veterinary Parasitology, (1978), 4: 197.

A rapid test, utilizing latex particles (0.81-um diameter), sensitized with Babesia argentina antigens, proved to be effective in the diagnosis of B. argentina in natural and experimental infections. Two drops of plasma or serum and one drop of B. argentina antigen placed on a glass plate were used in the test. Reaction was observed after 3-10 minutes rotation. The positive agglutination reaction was characterized by the formation of fine latex particle clumps. In experimental infections with B. argentina, the first detectable positive agglutination reactions coincided with the appearance of parasitemia in thin blood films. Plasma from animals with natural infections of B. argentina, proven by blood smears and indirect fluorescent antibody and complement fixation tests, also showed a reaction to the latex agglutination test.

117. TODOROVIC, R. A., and GARCIA, R.: Comparison of the Dried Blood on Filter Paper and Serum Techniques for the Diagnosis of Bovine Babesiosis Utilizing the Indirect Fluorescent Antibody (IFA) Test. *Tropenmedizin u Parasitologie*, (1978), 29.

A comparison between the techniques of dried blood on filter paper and serum for the diagnosis of babesiosis utilizing the indirect fluorescent antibody (IFA) test was evaluated. Dried blood on filter paper was used as a method to detect B. bigemina and B. argentina antibodies of Colombian cattle under laboratory and field conditions and the technique was compared with the serum of the same animals. A high relationship was found between the results of the dried blood and the serum from calves experimentally infected with Babesia spp. and calves from enzootic hemoparasites-free zones in Colombia. There were no significant differences in the sensitivity and specificity of both techniques. The samples on filter paper could be practical for field use due to their easy management and storage at different temperatures. This indicates that the use of dried blood may be a valuable aid for the epizootiologic studies of Babesia spp. infections in bovines.

118. THOMPSON, K. C., TODOROVIC, R. A., MATEUS, G. and ADAMS, L. G.: Methods to Improve the Health of Cattle in the Tropics. Immunisation and Chemoprophylaxis Against Haemoparasitic Infections. *Tropical Animal Health Production*, (1978), 10.

A study of methods to improve the health of native cattle in tropical areas of Colombia showed an advantage using immunisation techniques against haemoparasitic infections in comparison with other control methods. The control of anaplasmosis and babesiosis by immunisation of cattle with full virulent Anaplasma marginale, Babesia argentina and B. bigemina is feasible in tropical cattle when the post-immunisation reaction is controlled by appropriate drug therapy. Chemoprophylaxis was found less effective in controlling haemoparasitic diseases; however, treated cattle surviving the acute stage of infection showed weight gains not significantly different from those of the immunized calves. Both methods were found to be advantageous with calves born and raised in an endemic area of anaplasmosis and babesiosis. Tick and gastro-intestinal parasitic control without haemoparasitic control in calves had an advantage over no control system at all. These methods though were inferior to the immunization and chemoprophylactic techniques.

119. KYZAR, C. T., THOMPSON, K. C., and GONZALEZ, E. F.: Visualization of Babesia bigemina and Associated Bodies by Acridine Orange Staining, Libro Azul, Hoechst, Germany. (1977)

Using the described acridine orange technique the erythrocytes appear as olive green discs on a dark background, and the Babesia bigemina organisms stain as orange pyriform bodies. Associated with the infection are often found small round green bodies which may be seen in both infected and non-infected erythrocytes or free in the plasma. The theory is given that these small bodies are the infective units of B. bigemina.

120. CORRIER, D. E., CORTES, J. M., THOMPSON, K. C., RIANO, H., BECERRA, E. and RODRIGUEZ, R.: A Field Survey of Bovine Anaplasmosis, Babesiosis and Tick Vector Prevalence in the Eastern Plains of Colombia. Tropical Animal Health Production, (1977).

The prevalence of Anaplasma marginale, Babesia bigemina and Babesia argentina serological reactors in 37 cattle herds in the eastern plains of Colombia was found to be 74, 62 and 13 percent, respectively. Boophilus microplus was the only cattle tick equally distributed across the region.

121. KUTTLER, K. L.: East Coast Fever (Theileriasis, Theileriosis, Rhodesian Tick Fever, Rhodesian Red Water). "Foreign Animal Diseases" manual, U.S. Animal Health Assoc., (1976), 85-95.

A general discussion of East Coast Fever was presented with emphasis on diagnosis, epizootiology, and control. This disease is a highly pathogenic, tick transmitted, febrile infection of cattle, caused by the protozoan parasite Theileria parva, which primarily involves the lymphopoietic and hemopoietic systems. It is a serious economic constraint to profitable cattle production primarily in East Africa where the principle vector, Rhipicephalus appendiculatus, occurs.

122. KUTTLER, K. L., and TODOROVIC, R. A.: Arthropod Borne Protozoan Infections (Affecting Domesticated, Food-Producing Animals.) "Foreign Animal Diseases" manual, (1973), 291-322.

The arthropod borne protozoan infections include a wide range of infectious agents which are generally hemotropic in nature and are transmitted by blood sucking insects such as ticks, flies, mosquitoes, etc. Transmission may occur mechanically by the transfer of infected blood from one animal to another by insects or improperly cleaned surgical instruments. More often, biological vectors are involved, in which the causative agent undergoes cyclical development in the invertebrate host, prior to producing infection in the susceptible vertebrate host.

These protozoan agents may be extra- or intra-erythrocytic in nature, but are generally characterized by febrile reactions in the acute phase, varying degrees of anemia, and carrier infections following recovery.

Included under this heading are diseases caused by Theileria, Trypanosoma, Anaplasma, Babesia, Leucocytozoon, and Besnoitia. A list of agents with the disease they produce, animal species affected, and the principal vectors is given in Table 1. East Coast Fever (Theileria) and trypanosomiasis (Trypanosoma) have been discussed in detail in separate chapters. Babesiosis and anaplasmosis, major animal disease problems throughout the world, are discussed in some detail in this chapter.

123. MILLER, R. M., PRICE, M. A., KUTTLER, K. L.: Investigations on Transstadial Transmission of Bovine Anaplasmosis and Benign Bovine Theileriosis in Cattle by Two Species of Amblyomma (Acarina: Ixodidae). The Southwestern Entomologist, 1, (3), (September 1976): 107-110.

Amblyomma americanum and Amblyomma cajennense nymphal stages were fed on Anaplasma and Theileria infected cattle. Following molting, adult ticks were fed on susceptible splenectomized calves in an effort to demonstrate transmission. In no instance was an infection of either Anaplasma or Theileria produced by either of the tick species tested.

124. YOUNG, M. F., KUTTLER, K. L., ADAMS, L. G.: Experimentally Induced Anaplasmosis in Neonatal Isohemolytic Anemia Recovered Calves. *American Journal of Veterinary Research*, (1977), 38 No. 11: 1745-1747.

Anaplasma marginale infections were induced in 3 calves previously affected with neonatal immunohemolytic anemia (NIA). Similar infections were induced in 6 splenectomized and 7 intact calves. The response to infection by 3 calves (NIA recovered) closely resembled infections seen in splenectomized calves, being markedly more severe than similar infections in intact calves.

Spleens from 3 (NIA recovered) calves after splenectomy were about one-tenth normal size. Marked recrudescing anaplasma infections were not detected after splenectomy of the calves (NIA recovered), whereas marked recrudescing infections were observed after splenectomy of 2 intact calves having recovered from the primary infections.

125. KUTTLER, K. L. and JOHNSON, L. W.: Anaplasma and Babesia Pre-munition of Two-Year-Old Holstein Heifers Destined for Shipment to Nicaragua. *VM/SAC*, (1977), 1354-1358.

Nineteen Holstein-Friesian heifers were pre-munized by injection of diluted bovine blood stabilates containing Babesia bigemina, Babesia argentina, and Anaplasma marginale. An additional 20 heifers were pre-munized with a bovine blood stabilate of A. marginale only. Twenty of the 39 animals were given an attenuated strain (DAM) of A. marginale, and 19 were given a non-attenuated strain (TAM). Treatment consisting of one injection of 6.6 mg/kg oxytetracycline was administered to 19 of the heifers that received DAM or TAM stabilates. Twenty heifers received no treatment. There were 10 non-infected controls.

All 39 heifers receiving A. marginale, either DAM or TAM, showed evidence of replicating infections, resulting in pre-munition. No deaths occurred in the treated or untreated groups. Treatment had only a minimal, non-significant effect on packed red cell volumes and weight gains. Cattle receiving DAM showed significantly milder response compared to the response of those receiving TAM. Cattle that were also infected with Babesia had significantly prolonged incubation times for both DAM and TAM infections, but no other effects were observed.

Evidence of replicating infections was seen in 18 of 19 heifers pre-munized with B. bigemina. Seventeen of 19 cattle given B. argentina were assumed to be pre-munized. This assumption was based on either a positive parasitemia or positive complement-fixation response to B. argentina antigen.

All cattle were shipped to Nicaragua at the completion of the trials, where they were apparently resistant to field challenge.

126. KUTTLER, K. L. and ADAMS, L. G.: Influence of Dexamethasone in the Recrudescence of Anaplasma marginale in Splenectomized Calves. American Journal of Veterinary Research, (1977), 38: 1327-1330.

Dexamethasone was administered at the dose rate of 0.2 mg/kg of body weight to 11 splenectomized Anaplasma-carrier calves (groups 1 and 3) on Monday, Wednesday, and Friday for 3 weeks. Observations were made on these calves and on 7 nontreated, comparable calves (group 2) to determine the influence of treatment on carrier infections.

Dexamethasone treatment was associated in every instance with an exacerbation of the Anaplasma parasitemia and a decrease in packed red cell volume. The episode of acute anaplasmosis was of short duration, resembling the primary response, except that complement-fixation response did not increase accordingly. Serum protein electrophoresis of serums from 4 calves (Group 3) undergoing the drug-induced response failed to show any significant change during the 3-week treatment period, but did show a significant increase in γ -globulin immediately after treatment.

127. KUTTLER, K. L., YOUNG, M. F. and SIMPSON, J. E.: Use of an experimental long-acting Oxytetracycline (Terramycin L/A) in the Treatment of Acute Anaplasmosis. VM/SAC, (February, 1978), 187-192.

Anaplasmosis was induced in 43 adult cows ranging from 6 to 9 years of age. When the subsequent Anaplasma marginale parasitemia reached a level of 4 to 10%, 15 cows were treated intramuscularly with 20 mg/kg of Terramycin /LA (T-200). This formulation has been compounded to provide sustained oxytetracycline plasma levels for three to five days. An additional 15 infected cows were treated on two successive days with 10 mg/kg/day of Liquamycin (T-50), also given intramuscularly. The 13 remaining cows served as infected, nontreated controls.

Both oxytetracycline formulations were highly effective in moderating the course of infection and resulted in rapid recovery. In comparison, 2 Of 13 nontreated controls died and the survivors showed higher parasitemias, lower packed cell volumes, and greater weight loss than did the treated animals. There were no significant differences between the two treatment groups. One injection of the T-200 (200 mg oxytetracycline/ml) was comparable in efficacy to two injections of the T-50 (50 mg oxytetracycline/ml.). Because of the concentration, the required volume of T-200 was only one-fourth that of T-50.

128. KUTTLER, K. L., MCWHORTER, G. M.: Review of Cattle Fever Ticks and Disease They Transmit in New South Wales and Queensland, Australia, TAES DIR, No. 77-1, (1977).

Because of persistent Boophilus infestations and the threat of disease transmission in Texas, the Texas Agricultural Experiment Station (TAES) has assembled ad team of agricultural scientists representing wildlife sciences, animal science, range science, agricultural economics, entomology and veterinary medicine to study this problem.

To assist in the establishment of research priorities in Texas, representatives from Texas A&M University (TAES) visited Australia during the month of February 1977 to review Boophilus tick and tick-borne disease research. Australia had been faced with a tick problem similar to that of the United States prior to tick eradication in the early 1940's. Boophilus microplus ticks were introduced into the northern territory in 1872 from whence they rapidly spread down the east coast of Australia into New South Wales (NSW), then westward from the coast until climatic factors limited tick survival. Because of the importance of the livestock industry to the Australian economy a large scale dynamic research program has evolved and is one of the most advanced in the world. Task Force representatives Dr. Kuttler (Veterinary Medicine) and Dr. McWhorter (Entomology) spent 4 weeks in Australia visiting research facilities, field programs involving tick control and regulatory officials to review Boophilus and Babesia research from the Australian perspective. The following installations, facilities, and people were visited.

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129. KUTTLER, K. L., SIMPSON, J. E.: Comparative Efficacy of Two Oxytetracycline Formulations and a Synthetic Antibacterial in the Treatment of Anaplasmosis. 8th Pan American Veterinary Congress, Santo Domingo, D.R., (August, 1977), (abstract).

An experimental oxytetracycline injectable (Terramycin/LA), formulated to contain 200 mg/ml (T-200), and a synthetically derived tetracycline (doxycycline), formulated to contain 100 mg/ml (D-100), were compared with the commercially available oxytetracycline (Liquamycin) containing 50 mg/ml (T-50) in the treatment of induced anaplasmosis in splenectomized calves.

All experimental animals were exposed to Anaplasma marginale by the inoculation of known infected stabilates. A total of 23 splenectomized calves were infected, divided into 5 groups and treated as indicated when the ascending parasitemia reached 1-4%. Five calves served as untreated controls; 4 calves were treated 1 time with 10 mg/kg T-50 intramuscularly (I.M.); 5 calves were treated 3 times at 24 hour intervals with 10 mg/kg T-50 I.M.; 5 calves were treated 1 time with 20 mg/kg, T-200, I.M., and 4 calves were treated 1 time with 10 mg/kg, D-100, I.M.

All control calves died from acute anaplasmosis, and one calf treated once with T-50 died. No other deaths occurred. All treatments were effective in moderating the infectious process, but the T-50 given 3 times and the T-200 given 1 time were significantly more effective than T-50 given 1 time and the D-100 given 1 time.

In a second experiment, A. marginale infections were induced in 43 Aberdeen Angus cows averaging 6.9 years of age. These cattle were divided into 3 groups and 2 groups were treated when the ascending parasitemia reached 4-10%. The remaining group served as untreated controls. Fifteen cows were treated 2 times, 24 hours apart, with 10 mg/kg T-50 I.M. Fifteen cows were treated 1 time with 20 mg/kg T-200.

Among the 13 non-treated control cows 2 died, and all showed evidence of acute anaplasmosis. Treatments with T-50 twice, and T-200 once were both effective in moderating the course of infection. Highly significant values in favor of both treated groups were observed when compared with the non-treated controls, but no significant differences in the course of infection were apparent between the 2 treatment groups. One injection of the T-200 was therapeutically comparable to 2 injections of T-50. A 454 kg cow treated with T-50 at the rate of 10 mg/kg twice received 182 ml of the drug I.M., whereas only 45 ml of the T-200 was required to produce comparable results.

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130. KUTTLER, K. L., SIMPSON, J. E.: Relative Efficacy of Two Oxytetracycline Formulations and Doxycycline in the Treatment of Acute Anaplasmosis in Splenectomized Calves. American Journal of Veterinary Research, (February, 1978), 39: 347-349.

The efficacy of 3 antibiotic formulations was measured in the treatment of artificially induced anaplasmosis in the early stages of an ascending parasitemia (1% to 4%) in 23 splenectomized calves. Group 1, consisting of 5 calves, served as nontreated controls. Four calves (group 2) were treated 1 time with 10 mg of oxytetracycline (T-50)/kg of body weight I.M.; 5 calves (group 3) were treated 3 times with 10 mg of T-50/kg I.M.; 5 calves (group 4) were treated 1 time with 20 mg of an experimental oxytetracycline (T-200)/kg I.M.; and 4 calves (group 5) were treated 1 time with 10 mg of a synthetically derived antibacterial agent, doxycycline (D-100)/kg I.M.

All control calves died and 1 of 4 calves died that was treated 1 time with T-50. Other deaths did not occur. All treatments were effective in moderating the infective process, but T-50 given 3 times and T-200 given 1 time were markedly more effective than T-50 and D-100 given 1 time.

There appeared to be little or no difference in therapeutic efficacy between T-50 and D-100 given 1 time and between T-50 given 3 times and T-200 given 1 time.

131. KUTTLER, K. L.: Anaplasmosis in White-Tailed Deer-Submitted for Publication as a Chapter in a volume entitled Diseases of White-Tailed Deer.

Anaplasmosis in white-tailed deer (WTD) (Odocoileus virgianus) is an infectious, non-contagious disease capable of producing a mild anemia with spontaneous recovery characterized by a persisting chronic non-apparent infection. It is caused by the microorganism, Anaplasma marginale which invades the erythrocytes where it is thought to multiply by binary fission, eventually escaping to infect other erythrocytes, being transmitted from one animal to another by arthropod vectors or the inoculation of infected blood. Anaplasmosis is primarily a disease of cattle where it produces acute or sub-acute infections, characterized by severe anemia, high fever, and icterus. Death is not uncommon in cattle, and when recovery does occur, severe weight and production losses usually accompany and follow infections.

Anaplasmosis at one time was thought to be confined to the tropics and sub-tropics, affecting only cattle, but it is now recognized throughout the world where suitable vectors occur. The Anaplasma organism cannot be propagated on chick embryo, or in small laboratory animals, but will infect a wide range of wild ruminants including deer (Odocoileus virgianus, O. hemionus hemionus, O. hemionus columbianus), elk (Cervus canadensis canadensis), bighorn sheep (Ovis canadensis canadensis), pronghorn antelope (Antilocapra americana), and many African antelope.

Infections produced in species other than cattle are usually mild and often non-apparent. The greatest concern of anaplasmosis in wild animals involves the epizootiological aspects in which these secondary hosts act as reservoirs of infection for cattle.

132. THOMPSON, K. C., ROA, J. E., and ROMERO, T.: Anti-tick Grasses as the Bases for Developing Practical Tropical Tick Control Packages. Tropical Animal Health and Production, (1978).

Of six grass species analysed, Melinis minutiflora (Molasses grass) showed the highest anti-tick deterrent properties while Andropogon gayanus (Gamba grass) exhibited the ability to maintain a defined, constantly low, initial host tick infestation property and lengthy but low to moderate field tick population.

The conclusion is that Molasses grass is a species which would best be used in a tick control package within a marginal tick zone while Andropogon has the advantage within an endemic tick zone.

The goal being to produce an economical, practical tick control package by using anti-tick pastures plus limited, strategic acaricide application which will yield low cost, efficient tick control and an increased beef production for the small livestock producer who lacks the resources for conventional tick control methods.

133. TODOROVIC, R. A., and GONZALEZ, E. F.: Immunization Against Bovine Babesiosis Using a Live Vaccine. *Revista ICA*, 10: 243-254.

Forty male calves, Holstein-Friesian, were used to evaluate various systems of immunization against bovine babesiosis using a vaccine containing live Babesia parasites. Two methods were utilized to produce immunity: the first was using blood from animals carriers of B. bigemina and B. argentina, and the other one was using blood from splenectomized calves inoculated with B. bigemina and B. argentina and collected during the acute time of infection. The degree of resistance was measured by natural challenge to ticks (B. microplus) infected with Babesia in an endemic area of Cauca Valley. The premunized cattle showed a high degree of resistance against babesiosis while the control group, non premunized, was treated to prevent mortality. The response to vaccination was much better in cattle premunized with blood from a carrier animal. Weight gains were compared in vaccinated and nonvaccinated animals. There was a slight decrease in weight two months after vaccination but once the calves were exposed to the field challenge, the control group lost 53 kg in average during the six months following the exposure to ticks, compared with the vaccinated group.

134. GONZALEZ, E. F., TODOROVIC, R. A., LOPEZ, G., and GARCIA, O.: Attenuation of a Babesia Argentina isolate of Colombian Origin. *Scientific Proceedings of X World Congress of Buiatrics, Mexico*, (1978).

Babesia argentina (B. bovis), isolated in Monteria, Colombia, in 1971 was attenuated by continuous passages in splenectomized calves. The attenuated behavior was observed beginning with the 23 passage. A comparative statistical study made in a group of 60 cattle inoculated with 10^{10} B. argentina parasites showed significant comparative results between groups of animals inoculated with the virulent (passage No. 6) and attenuated (passage No. 27) parasites and a non-inoculated group. Of the 20 animals inoculated with the virulent parasite, 19 showed clinical symptoms of acute babesiosis and 9 animals died between days 11 and 14 post-inoculation (PI). None of the 30 animals inoculated with the attenuated parasite showed clinical symptoms of the disease. Only a slight decrease in average hematocrit on day 14 PI (-6.5%) and slight rise in the average temperature on day 11 PI (+.32°C) were observed. The implications of the utilization of this attenuated B. argentina parasite for the control and prevention of bovine babesiosis are obvious.

135. TODOROVIC, R. A.: Hemoparasite Control in Bovine., Proceedings of the 20th World Veterinary Congress, 6-12 July, 1975, Thessaloniki, Greece, Vol. 1, 569-581.

Bovine babesiosis is a widely spread disease in the tropical and subtropical areas of the world where ticks exist. A program of vector control using chemical methods decreases the incidence of the disease but it still is a problem which causes great economic losses in these areas of the world. Other control methods such as artificial immunization with blood from infected animals (premunition) and chemo-prophylaxis have been used.

Two systems of immunization against babesiosis have been evaluated under experimental and field conditions. The first system is based on obtaining a co-infectious immunity (premunition) using two methods: (a) with blood from calves with acute infections of B. bigemina and B. argentina used as inocula and controlling the post-innoculation reaction with specific drugs and (b) inducing an artificial infection with blood of calves that are infected carriers of B. bigemina and B. argentina without chemotherapy. For this trial, 48 intact and 8 splenectomized calves were used. The degree of resistance was determined by artificial inoculation of infected blood and exposition to ticks under field conditions. The premunized cattle showed a high degree of resistance against babesiosis, however, the non-premunized cattle had to be treated since they suffered a severe attack of babesiosis.

The second system is based on obtaining a sterile immunity using dead Babesia bigemina and Babesia argentina parasites and their antigens as inocula. For this, vaccines were prepared from infected erythrocytes (AG-E) and infected plasma (AG-S). The vaccines were tried in cattle under field conditions, using 20 calves which were distributed into 4 groups of 4 calves each and one control group. The immunological responses were measured by hematocrit value, parasitemia, complement fixation, weight and mortality. It was found that a good degree of sterile immunity against babesiosis could be produced using a dead vaccine based on these parasites, which indicates that it plays an important role in the mechanism of immunity against bovine babesiosis.

136. TODOROVIC, R. A., GONZALEZ, E. and LOPEZ, G.: Immunization Against Anaplasmosis and Babesiosis. Part II. Evaluation of Cryo-Preserved Vaccines Using Different Doses and Routes of Inoculation. VIII Panamerican Congress of Veterinary Medicine and Zootechnics, Santo Domingo, Dominican Republic, (August, 1977).

Anaplasma marginale, Babesia argentina and Babesia bigemina infected blood used as vaccines for immunization trials in Valle del Cauca, were preserved with 4 Molar Dimethyl-Sulfoxide (4M DMSO) and stored in liquid nitrogen (-196°C). The effectivity of the vaccines was determined in 87 healthy calves utilizing serial 10-fold dilutions. The effects of dose, inoculation routes, time and temperature were determined. The minimum infective dose for A. marginale was 10^{-3} (2×10^6) when 2 ml of vaccine were given intravenously (i.v.). The same dose when given subcutaneously (s.c.) was not infective. The 10^{-2} dilution (2×10^7) was infective when given through both routes, however, the incubation periods were statistically different. The average incubation period using 2 ml s.c. was 30 days, but when the dose was increased to a 5 ml and given s.c. decreased the average incubation period to 22 days. The minimum infective doses for B. bigemina and B. argentina were 10^{-1} dilutions (4×10^{-7}) and 10^{-2} (4×10^6) respectively, when 2 ml of vaccines were injected i.v. Infectivity was also recorded when Babesia spp. vaccines were injected s.c. at dosages of 5 ml of dilution 10^{-1} (1×10^{-8}).

137. GONZALEZ, E. F. and TODOROVIC, R. A.: Economical Impact of Anaplasmosis and Babesiosis in a Dairy Herd From an Endemic Area of Colombia. VIII Congreso Panamericano de Medicina Veterinaria y Zootecnia, Santo Domingo, Republica Dominicana, (1977). Abstract.

138. ALIU, Y.O., DAVIS, R.H., JR., CAMP, B.J., and KUTTLER, K.L.: Absorption, Distribution, and Excretion of Imidocarb Dipropionate in Sheep. American Journal of Veterinary Research, (December, 1977), 12, (12): 2001-2006.

Spectrophotometric and thin-layer chromatographic methods for determination of imidocarb in biological specimens are described.

Following intravenous injection of imidocarb (2.0 mg/kg) into 3 sheep, plasma concentrations, initially averaging 10.8 $\mu\text{g/ml}$, decreased to an average of 1.9 $\mu\text{g/ml}$ within 1 hour and then to less than 1 $\mu\text{g/ml}$ within the next 4 hours. When imidocarb (4.5 mg/kg) was injected intramuscularly (IM) into 7 sheep, peak plasma concentrations averaging 7.9 $\mu\text{g/ml}$ were achieved within 4 hours and then rapidly decreased to 4.6 $\mu\text{g/ml}$ within the next 2 hours. Plasma values then decayed very slowly by first-order kinetics and trace amounts were still present 4 weeks after treatment. Imidocarb was bound to plasma proteins and the apparent volume of distribution was estimated to be slightly higher than the total body water. The concentrations of the drug in the plasma and in the erythrocytes were approximately equal. Detectable amounts were present in all examined tissues 4 weeks after IM administration. Twenty-four hours after IM administration, the highest concentrations were in kidney, liver, and brain. The C-labeled imidocarb could be detected in all regions of the central nervous system examined, in the hypophysis, and in the pineal body.

Metabolic or biotransformation products were not detected by the methods used. Of the administered IM dose, 11 to 17% was excreted in the urine within 24 hours; thereafter, the excretion rate was low, and detectable amounts were still present in the urine for 4 weeks. Renal clearance of imidocarb was less than glomerular filtration rate, indicating net tubular reabsorption.

The relatively high concentration of imidocarb in the bile suggests that the bile is an important route of excretion. High concentrations were also found in the milk of lactating ewes, but the drug could not be detected in the plasma of lambs fed milk from these ewes.

MANUSCRIPTS IN PREPARATION
SUBMITTED TO SCIENTIFIC JOURNALS
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1. FURNISS, Sean B. and THOMPSON, K. C.: Birds in Colombia as Host for Ticks.
2. GONZALEZ, E. F., LONG, R. F., TODOROVIC, R. A.: Comparisons of the Complement-Fixation, Indirect Fluorescent Antibody and Card Agglutination Tests for the Diagnosis of Bovine Anaplasmosis.
3. LOPEZ, G. V., THOMPSON, K. C. BAZALAR, H.: Transmission Experimental de Trypanosoma Vivax por la Garrapata Boophilus Microplus.
4. THOMPSON, K. C. and ROA, J. C.: Transmision de Anaplasma marginale por la Garrapata Boophilus microplus.
5. THOMPSON, K. C., TODOROVIC, R. A., MATEUS, G. and ADAMS, L. G.: Methods to Improve the Health of Cattle in the Tropics: Conclusion and Economical Appraisal.
6. THOMPSON, K. C., TODOROVIC, R. A., MATEUS G., ADAMS, L. G.: Methods to Improve the Health of Cattle in the Tropics: Ecto-and Endoparasite Control.
7. GONZALEZ, E. F., CORRIER, D. E., TODOROVIC, R. A. and LOPEZ, G.: Epidemiology of Bovine Anaplasmosis and Babesiosis in the Cauca River Valley.
8. TODOROVIC, R. A., GONZALEZ, E. F. and GARCIA, O.: Evaluation of Different Immunization systems to Control Bovine Anaplasmosis and Babesiosis.
9. GARCIA, O., TODOROVIC, R. A., AND GONZALEZ, E. F.: Evaluation of Mono and Bivalent Vaccine Systems against Bovine Babesiosis.
10. TODOROVIC, R. A., GONZALEZ, E. F. and GARCIA, O.: Testing of a New Drug Against Anaplasmosis (Pfizer T-200)