

AGENCY FOR INTERNATIONAL DEVELOPMENT WASHINGTON, D. C. 20523 BIBLIOGRAPHIC INPUT SHEET	FOR AID USE ONLY <i>Batch 40</i>
---	--

1. SUBJECT CLASSIFICATION	A. PRIMARY Serials	Y-AL72-0000-0000
	B. SECONDARY Food production and nutrition--Pests of animals	

2. TITLE AND SUBTITLE
 Annual research report: April 1,1974 to March 31,1975

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

4. DOCUMENT DATE 1975	5. NUMBER OF PAGES 83p.	6. ARC NUMBER ARC
--------------------------	----------------------------	----------------------

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

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

9. ABSTRACT

10. CONTROL NUMBER PN-AAC-727	11. PRICE OF DOCUMENT
12. DESCRIPTORS Livestock Protozoan infections	13. PROJECT NUMBER
	14. CONTRACT NUMBER CSD-1947 Res.
	15. TYPE OF DOCUMENT

CSD-1947 Reso
PN-AAC-727

Annual Research Report
A Research and Training Program in Tropical Veterinary Medicine
(AID-csd-1947, in Colombia)

Program Director: F. D. Maurer, D. V. M., Ph.D.
Director, Institute of Tropical Veterinary Medicine
College of Veterinary Medicine
Texas A&M University
College Station, Texas 77843

Period Covered by Report: April 1, 1974 to March 31, 1975

Including Appendix I, II and III

Annual Research Report

A Research and Training Program in Tropical Veterinary Medicine
(AID-csd-1947, in Colombia)

Program Director: F. D. Maurer, D.V.M., Ph.D., Assoc. Dean
Director, Institute of Tropical Veterinary Medicine
College of Veterinary Medicine
Texas A&M University
College Station, Texas 77843

Contract Period: July 1968 to March 31, 1975

Period Covered by Report: April 1, 1974 to March 31, 1975

Total AID Funding of Contract to Date: \$1,775,000.00

Total Expenditures and Obligations Through Previous Contract Year: \$1,397,731.00

Total Expenditures and Obligations for Current Year: *\$377,269.00

Estimated Expenditures for Next Contract Year: \$305,055.00

*\$18,349 of these funds (\$377,269) represent the amount obligated but not actually spent during the year 1974-75. A total of \$358,920 was actually spent during the year 1 April 1974 to 31 March 1975 (See Appendix III).

Professional Staff:

L. G. Adams, D.V.M., Ph.D. - Texas A&M University (TAMU) - College Station

D. E. Corrier, D.V.M., Ph.D. - Centro Internacional de Agricultura
Tropical (CIAT) - Cali, Colombia

T. J. Galvin, D.V.M., Ph.D. - CIAT, Cali, Colombia - terminated July, 1974
Now at Department of Parasitology, TAMU

E. F. Gonzales, D.V.M., M.S. - CIAT - Cali, Colombia

D. C. Hopps, D.V.M., CIAT - Cali, Colombia

K. L. Kuttler, D.V.M., Ph.D. - TAMU, College Station

K. C. Thompson, D.V.M., Ph.D. - CIAT - Cali, Colombia - terminated Nov. 1974

R. A. Todorovic, D.V.M., Ph.D. - TAMU until July, 1974 - transferred to
CIAT - Colombia

J. H. Wyss, D.V.M. - TAMU (started 1 December 1974)

ACKNOWLEDGMENTS

The Institute of Tropical Veterinary Medicine at Texas A&M is indebted to several national and international organizations for their help and cooperation in carrying out its research.

The work in Colombia would not have been possible without the cooperation and assistance of the Instituto Colombiano Agropecuario (ICA) and the support by their staff members. Our research staff in Colombia have been considered an integral part of the CIAT Animal Health Program, thus enjoying the same support and assistance as resident staff. This association has been an essential factor in the success we have had in Colombia.

The financial and moral support received from USAID, Washington, is gratefully acknowledged. The understanding, encouragement, and helpful suggestions by the program reviewers have been and continue to be most helpful.

TABLE OF CONTENTS

Narrative Summary of Accomplishments and Utilization

General Background	1
Project Objectives	2
Continued Relevance of Objectives	4
Accomplishments to Date	
Anaplasmosis	5
Babesiosis	11
Research Design	16
Dissemination and Utilization of Research Results	17
Statement of Expenditures and Obligations and Contractor Resources	17
Work Plan and Budget Forecast for the Coming Year	19
Appendix I Titles and Abstracts of Institute Staff Publications (1968-1975)	
Appendix II Systems of Anaplasmosis Immunization	
Appendix III Budget Statement for Past Year (1973-1974) Budget Statement for Coming Year (1974-1975)	

NARRATIVE SUMMARY OF ACCOMPLISHMENTS AND UTILIZATION

The year extending from April 1, 1974, to March 31, 1975, has seen the development and limited utilization of a practical system of premunition or immunization against both Anaplasma and Babesia infections. This technique has been cumbersome, expensive and not always safe in the past but, when used as directed, it can now safely induce a replicating infection of moderate intensity which will subside and leave the animal basically immune to future attacks.

This system for anaplasmosis employs dilute stabilates and produces infection within a predictable time range. Therefore, after exposure to the infecting inocula or vaccine, the time of parasitemia can be predicted. In calves less than 8 months old, no further treatment is needed after 1 vaccination. In older animals treatment is administered at a predetermined time to moderate the infection.

Similar techniques using Babesia argentina and B. bigemina are possible. These organisms are highly susceptible to a number of drugs, which actually facilitates premunition. Stabilates of these organisms have been prepared, characterized, and are ready for use in intermediate or sporadic zones of Colombia to premunize or vaccinate cattle in these areas. It is in these areas where exposure may or may not occur that animals acquire sufficient age before exposure, and then, when infection does occur, extreme clinical signs may follow.

This system of immunization, while practical, does require judgment and a degree of expertise to use it safely. Even then there is an element of danger involved. With this in mind, efforts toward the development of other methods have been pursued. Some success with a killed adjuvant Anaplasma vaccine has occurred. Intradermal inoculation of 1/10 and less of the antigenic material has produced CF responses equal to or greater than 10 times this amount given subcutaneously. On challenge, the level of immunity is still below standards needed to prevent clinical anaplasmosis.

Aspects of cell mediated immunity are under study. Results show that this aspect of the immune response is important in both anaplasmosis and babesiosis. The role of humoral antibodies is also important, and studies currently under way are designed to establish the relationship of these systems in naturally and artificially acquired immunity. Hopefully these basic studies will lead to safer and more effective means of immunization.

Chemoprophylaxis, in the case of babesiosis, has once again proven feasible under field conditions. The injection of 3 mg/kg of Imidocarb into susceptible cattle going into endemic zones has apparently prevented outbreaks of babesiosis. This field trial conducted in the Dominican Republic on 145 Santa Gertrudis cattle was apparently effective, based on previous similar trials without the drug.

Anaplasmosis chemotherapy, due to FDA restrictions, has slowed considerably. Only one new compound was tested this year (Hoffman-LaRoche). This drug was therapeutically effective but, in levels tried for sterilization, was apparently toxic. Administration of the drug was followed in practically every instance by relapsing infections.

Advances have been made in the serology associated with Babesia. In addition to the complement-fixation tests (CF), techniques involving indirect fluorescent antibody (IFA), indirect hemagglutination (IHA) and a rapid card agglutination test (CT) have been developed. Comparisons between the CF and IFA show similar declines in titer response following infection whereas the IFA continues positive for a much longer period of time, suggesting the presence of a non-complement fixing antibody detectable by IFA.

These serologic tests have greatest usefulness in epizootiological studies which are being made in Colombia. These studies have confirmed the widespread incidence of infection.

There have been a number of significant personnel changes during this past year. Dr. Galvin returned to TAMU, and Dr. Todorovic replaced him in Colombia. Dr. Corrier remained in Colombia. Mr. Ray Long and Dr. E. Gonzales were employed for expansion of the Colombian project to include greater utilization studies. Dr. K. Thompson resigned in November, 1974, to assume duties in CIAT on tick studies. Dr. J. Wyss was employed at TAMU to replace the position vacated by Thompson. Drs. Maurer, Adams, and Kuttler remain at TAMU. As of April, 1975, we have only three graduate students active in the program. Two of these (Kyzar and Reynolds) are supported entirely by the U.S. Army. The third graduate student, Dr. D. Hopps, remains in Colombia and plans to complete his work by July or August, 1975. Drs. Wyss and Craig completed their research for the Ph.D. degree this year.

After the first of the year (January, 1975) the apparent reduction in grant support dictated a reduction in force of those people associated with the project. This policy is currently being pursued. It is anticipated that Dr. E. Gonzales and Mr. Ray Long will be shifted to other funds. Dr. Adams and his technician have been shifted to other support. Dr. J. Wyss plans to take a position elsewhere.

GENERAL BACKGROUND

Generally man has enjoyed his greatest development in latitudes above 30° where the climate was invigorating and disease problems less severe.

Disease control is a basic prerequisite for animal health and survival in any locale, but becomes a major consideration and a decisive factor in the tropics where environmental factors favor the development and transmission of disease. Historically, the validity of this statement has been established in nearly every instance where man or animals from temperate zones have been introduced into the tropics.

In relatively recent history diseases such as yellow fever, malaria, and sleeping sickness, all arthropod-borne diseases, stymied efforts to settle and colonize the tropical zones where these infections occurred. Only with scientific progress in controlling these diseases has it become possible for these areas of the world to develop.

Counterparts to these diseases in man occur among food producing animals, where trypanosomiasis, theileriasis, babesiosis, anaplasmosis and a host of encephalidities occur among animals in the tropics. As with those diseases of man, all of the above are arthropod-borne. Tropical and subtropical climate favor the development of arthropods. Many of these areas of the world are noted for profuse forage and roughage production, unsuited for human food but capable of utilization by ruminant ungulates which can convert this resource into a high quality protein for human consumption. This production could contribute to the health and economic well-being of millions of people now living on marginal diets under poverty conditions. A major limiting factor preventing livestock production in these areas is animal disease.

Wilson et al. (Bulletin World Health Organization, 28, 595-613) estimates that the area in Africa virtually devoid of cattle, as the 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. Such a step could rival, if not surpass, the so-called "Green Revolution" that has so significantly contributed to cereal grain production in recent years.

Past research efforts have contributed greatly to our knowledge of these diseases, but more information is needed to provide workable, practical control programs.

It was with this background that our original project was submitted to AID for funding. The 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.

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 (specific objectives)
 - a. To evaluate, under field conditions, vaccines presently available.

- b. To develop a more effective killed vaccine.
- c. To evaluate preimmunization methods as a means of prophylaxis.
- d. To measure possible antigenic variations among Anaplasma organisms.
- e. To investigate the prevalence of disease and natural transmission by arthropod vectors.
- f. To investigate vector control measures as preventive measures.
- g. To evaluate therapeutic compounds for treatment.
- h. To conduct pathogenesis studies.
- i. To evolve and test practical control procedures based on available information.

B. Babesiosis (specific objectives)

- a. To develop and evaluate immunologic techniques.
- b. To determine the prevalence of disease and the potential number of vectors in various geographic areas.
- c. To evaluate a vector control program as a means of Babesia control.
- d. To investigate various ixodicides in achieving tick control.
- e. To evaluate therapeutic compounds, their efficacy and toxicity, in relation to treating acute and chronic infections.
- f. To develop serologic tests for the detection of latent as well as acute infection.
- g. To determine the antigenic variations of Babesia organisms, by serologic and other means.
- h. To determine the Babesia incidence in cattle and wild fauna, and the role of wildlife in maintaining infection.
- i. To develop non-bovine sources of Babesia organisms from ticks and tissue culture for use in vaccines.
- j. To conduct pathogenesis studies, with emphasis on pathologic changes that influence and affect animal productivity.

C. To develop information on other related blood diseases, including Trypanosoma and Theileria infections.

- 2. To train graduate students in research methods applicable to tropical diseases and to collect information, specimens, and illustrations for use in this training program.
- 3. To provide trained faculty and staff to operate veterinary programs in developing countries, and to serve as consultants to related tropical disease problems.

CONTINUED RELEVANCE OF OBJECTIVES

Until such time as animal disease is eradicated as a threat to food production, the basic research objectives directed at this goal will continue to be relevant.

Progress in accomplishing many of these objectives has been marked. Emphasis has shifted and will continue to do so as results occur. It is impossible to predict how and when animal disease will be controlled. Methods adequate in Texas may be entirely unacceptable in the tropics and vice versa.

For this reason, control procedures and our research have been directed on a broad front including: 1.) Vaccination with live antigens, 2.) Vaccination with dead antigens, 3.) Chemotherapy, 4.) Chemoprophylaxis, and 5.) Epizootiology.

Even though many of our original objectives have been completed and accomplished disease problems persist. There are many vital questions that have gone unanswered and continued research is the best and possibly the only way to solve these problems and to reach the ultimate objective which is to control disease.

ACCOMPLISHMENTS TO DATE

Several review papers on anaplasmosis and babesiosis have been prepared and published in addition to technical papers describing the results of research. A total of 14 titles have been added to the list of publications and abstracts in Appendix I.

Anaplasmosis:

- a. To evaluate, under field conditions, vaccines presently available.

The apparent superiority of premunition as a method of vaccination has led us to emphasize this technique in both field and control trials. This work will be discussed more completely under heading c.

- b. To develop a more effective killed vaccine.

Efforts to concentrate antigens have continued this year. The rationale for antigen concentration is twofold. First, if the antigen can be concentrated, an intradermal route of application would become feasible. Second, in the process of purification, the red cell contaminants would be removed, thus reducing the likelihood of the neo-natal isoerythrolysis now associated with vaccinations.

By sonication and centrifugation cycles the total mass of antigen was reduced to 1.7% of the original. During this procedure the antigenic activity, as measured by CF activity, was reduced to 75% of the original. While removing 98.3% of the mass, only 25% of the activity was lost. Antigen concentration (CF units per 1 gram) increased from 368 units per gram to 15,759 units per gram, resulting in over a 40-fold increase in concentration.

Only a few trials of vaccine activity have been conducted on splenectomized calves. The concentrated antigens have been combined with Freund's complete adjuvant (FCA), and injected I.D. CF response has been good, but later challenge reactions have not shown a satisfactory degree of immunity. These vaccines are comparable to the present commercial vaccine, but below an acceptable efficacy level. Studies

in intact animals are indicated.

A study comparing the moderating effect of the commercial vaccine on intact heifers inoculated with Anaplasma marginale will be discussed with premunition.

- c. To evaluate premunization methods as a means of prophylaxis.

Two Anaplasma stabilates have been prepared for use in premunition work, one in Colombia and the other in the U.S. Both stabilates employed DMSO as a cryoprotectant; freezing and storage in Colombia was in a CO₂ chest, whereas liquid nitrogen was used for freezing and a -70°C Revco box was used for storage in the U.S. The Colombian stabilate has been characterized for use at 10⁻³, whereas the Texas stabilate is being used at 10⁻².

A trial in which 21 yearling heifers were exposed to 2 ml I/V of the 10⁻² Texas stabilate was made under varying conditions. Seven heifers were vaccinated with the Ft. Dodge vaccine prior to exposure (Group I), nine received only the infected inoculum (Group II), and five received treatment 32 days after exposure (Group III). A fourth group, of four heifers, was inoculated with 5 ml whole blood from a calf having an 8% parasitemia and a 20% PCV. The results are given in Table 1. All cattle were safely premunized. A prior vaccination with "Anaplaz" appeared to slightly lengthen the incubation time. A significantly higher CF response was observed in the vaccinated group, but otherwise few differences were detected. Infection produced by acute blood significantly reduced the incubation time, and generally produced a more severe clinical response, and a significantly higher parasitemia. There were few, if any, significant differences between Groups II and III,

TABLE I

	Avg. Incub. Time-- Days	Avg. Low PCV %	Avg. High Paras. %	High CF Response	No. of Animals
Gr. I	33 ± 4	19.7 ± 3.5	3.8 ± 2.3	1:780	7
Gr. II	32 ± 4	18.5 ± 6.4	8.7 ± 4.8	1:254	9
Gr. III	26 ± 2	21.4 ± 2.9	5.8 ± 3.4	1:320	5
Gr. IV	8 ± 2	15.8 ± 5.7	14.1 ± 11.2	1:269	4
Significance	P<0.01	NS	P<0.05	P<0.05	
DRS	5.5		8.7		

- Gr. I Vaccinated before premunition with Ft. Dodge Anaplaz vaccine
- Gr. II No vaccination, no treatment: 2 ml 10⁻² stabilate only
- Gr. III 2 ml 10⁻² stabilate followed by 1 treatment with 5 mg/lbs oxytetracycline on day 32
- Gr. IV 5 ml undiluted blood showing an 8% parasitemia

although animals treated with oxytetracycline appeared to recover slightly faster.

The most striking feature of these trials was the very large animal variation. This factor suggests the need for extreme caution in premunizing cattle with virulent organisms. The stabilates have the marked advantage (See Groups I and II) of producing infection within a predictable incubation time.

All cattle were solidly immune to challenge, and showed no demonstrable response to challenge. The value of premunition is well established; the problem is in accomplishing this in a safe and efficient manner. Stabilates offer an approach which contributes to this goal.

- d. To measure possible antigenic variation among Anaplasma organisms.

The only work in this area was to challenge cattle referred to in "c" with a highly infectious inoculum of different history and origin. Cattle previously premunized showed no relapse or reaction that could be attributed to the challenge inoculum. Once again, no demonstrable antigenic drift could be observed under the conditions of these trials.

- e. To investigate the prevalence of disease and natural transmission by arthropod vectors.

A survey of two ranches in Nicaragua revealed a 46% and 31% incidence of anaplasmosis. Studies in Colombia on 37 ranches in the Eastern Plains, 4 ranches on the Atlantic Coast and 6 ranches in the Cauca Valley revealed 75%, 91% and 71% positive serologic results in each of the 3 areas tested in the order of their listing. Anaplasmosis is probably endemic in all 3 areas.

Transmission studies have been minimal this year. A new facility for tick research is being jointly constructed at Falcon Heights, Texas, on the Rio Grande River, by the USDA and Texas A&M University. The USDA has completed one building, which we now share with them. It is anticipated that TAMU will have its first building completed by the end of 1975. Preparatory work is now being carried out in anticipation of full scale activities within the next 6-8 months.

g. To evaluate therapeutic compounds for treatment.

A new compound designated 20-1263, obtained from Hoffman-LaRoche, was tested for therapeutic efficacy, and for its potential as a sterilizing compound. The company provided funds for this trial. Comparisons made with oxytetracycline did not show this compound to have any advantage. It was therapeutically active, but failed to eliminate infection even though it was very effective in suppressing infection. About 45-60 days after treatment all 8 cattle treated with this new drug developed submandibular edema, lacrimation, and variable incoordination and weakness. Six of the treated cattle died of apparent toxic signs, while two recovered. The long delay after treatment before toxic signs occurred is not understood, but similar delays are seen with Imidocarb, which grossly are similar. Histopathology is different from those seen with Imidocarb toxicity.

h. Pathogenesis studies.

An association between vaccination with "Anaplaz" (an anaplasmosis vaccine) and neonatal Isoerythrolysis (N.I.) in calves born to vaccinated dams has been well established. These calves often die, but most

will survive. The pathologic changes during the course of the disease in calves affects the spleen. There have been instances of calves severely affected by anaplasmosis after having recovered from N.I. To help test the hypothesis that N.I. calves might be more susceptible, three calves of this category that had recovered from N.I. were therefore challenged with Anaplasma. In every instance the disease produced resembled the severe form seen in splenectomized calves. Following recovery, the spleens were removed from each of the 3 calves. Their weights were about 1/10 normal, which probably accounts for the severe reaction.

This evidence suggests a second adverse reaction associated with the "Anaplaz" vaccine.

The influence of Dexamethazone (Azium), an anti-inflammatory synthetic corticosteroid, was tested for its influence on Anaplasma parasitemias in splenectomized calves. The injection of 8-17 ml Azium 3 times a week for 3 weeks appeared to be associated with an increase in parasitemia, drop in PCV, but without an increase in CF titer. These relapses were apparently exacerbated by the treatment, which theoretically inhibited the humoral immune response allowing a sudden upsurge in parasitemia.

Babesiosis:

- a. To develop and evaluate immunologic techniques.

Premunition is the approach which offers the greatest possibilities for success. B. bigemina and B. argentina stabilates have been prepared both at TAMU and CIAT. Both stations are using DMSO as cryoprotectants, but a CO₂ chest is being used in Colombia in contrast with liquid nitrogen and a Revco deep-freeze at TAMU.

Serial needle passage of B. bigemina at CIAT has reportedly resulted in an attenuated strain, which may be useful for vaccine purposes. A total of 10 serial passages of B. argentina have been made in a similar attempt. A 10th passage is currently under test to evaluate the severity of infection related to this inoculum.

Three older, mature bulls were premunized against A. marginale, B. argentina, and B. bigemina. This was successfully accomplished using available stabilates and chemotherapy. The lowest PCV during the course of B. argentina premunition was 21% in 1 animal; the lowest for Anaplasma premunition was 25%. All 3 animals recovered uneventfully, and have been shipped to Mexico.

A leucocyte migration-inhibition test (LMIT) has been developed for measuring cell mediated immune response in cattle with babesiosis. It is highly probable that this type of immune response is important in the animal's defense mechanism. The relationship of cell-mediated immunity and humoral antibody response is being studied to further elucidate a practical system of disease prevention.

- b. To determine the prevalence of disease and the potential number of vectors in various geographic areas.

The prevalence of 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 all age groups were included. A total of 3,698 serum samples were collected and tested using a B. bigemina antigen.

The prevalence of B. 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 is not evenly distributed. The percentage of reactors among 37 ranches varied from 5 to 98%, suggesting the potential of future babesiosis outbreaks. Babesia may be considered endemic in all 3 areas.

Boophilus microplus ticks were identified on each of the 37 ranches in the Eastern Plains, and were nearly equally distributed based on tick counts. In addition, ticks identified as Amblyoma cajennense, A. triste, and Anocentor nitens were collected on 3 of the ranches. The significance of these ticks as vectors is not known.

- e. To evaluate a vector control program as a means of Babesia control.

For years the hypothesis has persisted that molasses grass (Melinis minutiflora) has an adverse effect on Boophilus ticks. This assumption was partially checked using small study plots comparing tick activity on Melinis minutiflora and Paspalum plicatulum (pasto negro). Immature and mature strands of molasses grass apparently had no adverse effect, such as repelling or inhibiting larval stages of Boophilus microplus from crawling up the plant to the leaf tips. If molasses grass has an adverse effect, the mode of action is yet to be described. Studies are continuing

to determine if this grass may influence egg laying, hatchability, or viability of the larvae.

Strains of Boophilus microplus, Anocentor nitens, Ixodes spp. and Amblyoma spp. are being maintained or introduced into the CIAT laboratories. Ticks are being collected, identified, and classified from the epidemiological studies under way in the Llanos. These ticks will be used in hemoparasite transmission studies, in vector ecology studies and in training programs.

The complete eradication of Boophilus microplus in South Texas has been reported. This was done in the presence of a large deer population. The deer numbers were equal to or greater than cattle. It was feared that the deer, an acceptable host for Boophilus ticks, would perpetuate the infestation. It now appears that eradication is possible when the effort and motivation are sufficient to persist with a constant and consistent program.

Expansion of tick research in Texas is anticipated with the completion of building construction at Falcon Heights, Texas. It is our understanding that funds have been appropriated by the Texas Legislature for construction of such a facility, which should be completed within the next year.

- d. To investigate various ixodocides in achieving tick control.

Some work is anticipated in this area this year at the Falcon Dam facility, in cooperation with the USDA.

- e. To evaluate therapeutic compounds, their efficacy and toxicity, in relation to treating acute and chronic infections.

A field trial involving 145 head of registered Santa Gertrudis cattle

on the La Romana Ranch, Dominican Republic, was conducted combining a system of Anaplasma premunition and Imidocarb treatment for Babesia. Past experience has shown that these cattle, when moved from the quarantine station onto the ranch proper, suffer heavy losses due to tick-borne disease. Imidocarb therapy given 2 weeks after release from quarantine was apparently effective. No losses have been observed during the first 5 months when exposed to ticks. This method is simple and apparently effective. It has the drawback that FDA's refusal to clear the drug for use in the U.S. has led others to follow this policy, even though the drug is registered and is being used in Australia and in some countries of Europe.

- f. To develop serologic tests for the detection of latent as well as acute infection.

Numerous Babesia bigemina antigenic fractions have been prepared and tested in the rapid card agglutination (BCT) procedure. A fraction preserved with penicillin and streptomycin, sealed in glass vials and stored at 4°C gave the most consistent reaction for the longest period of time, approximately 6 months.

To date the BCT has been performed on 300 serum samples. Complement-fixation (CF) and indirect fluorescent antibody (IFA) are being used for comparative purposes. To date these comparisons are not complete, but preliminary data shows 91% positive with IFA, 76% positive with the BCT, and 57% positive reactions with the CF test.

In addition to the IFA, CF, and CT (card test) an indirect hemagglutination procedure is being studied to further evaluate different serologic techniques for babesiosis. These different tests are being

developed for both B. bigemina and B. argentina. There are some cross reactions, but it is believed that when these studies are completed the basis for serologic differentiation of the Babesia species will exist.

A comparison of CF and IFA reactions on a group of 5 adult cattle artificially injected with B. bigemina was made to evaluate the nature of each test. The maximum CF titers occurred a few days in advance of the IFA, but the latter were much higher. CF and IFA titers showed a significant regression which was almost identical until day 100, when the CF titers dropped below the diagnostic level and the IFA titers leveled off at a level above the diagnostic level. It would appear that possibly the CF active antibody may have been reacting in both tests but, being transient in nature, it disappeared in a detectable level at about day 100. A second more persistent antibody appears to be inactive in the CF system, but capable of attaching to antigen and hence detectable on the IFA test. This antibody was shown to persist beyond 180 days.

Even though both the CF and IFA are laboratory tests, the IFA technique has advantages over the CF test in simplicity, economy and speed of performance.

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

Efforts to grow Babesia in tissue culture have been discontinued. Experiments have been carried out using bovine lymph nodes, hemolymph nodes, spleen, kidney, and mature erythrocytes. The results were negative. Normal erythrocytes maintained in cell culture media provided some encouragement. Babesia inoculated into such media survived 3 days

at 37°C, whereas the control Babesia held the same time at 37°C failed to survive. A similar effort to maintain infectivity 8 days was unsuccessful.

RESEARCH DESIGN

The major emphasis in Colombia has been on babesiosis and in Texas on anaplasmosis.

At the current level of funding, it is anticipated that there will be 1 full-time professional equivalent in Colombia, and equal professional staff in Texas. Technical, clerical, and labor support for each staff member will be provided.

Staff activities will remain largely unchanged, but support from other sources will be required. Some shift in emphasis may result in keeping with the policies and philosophy of the granting agency.

The work in Colombia and Texas is complementary and contributes a greater understanding and appreciation of the problem by providing an up-to-date exchange of information between all professional staff, which facilitates implementation of research findings and allows rapid confirmation, or second party examination, of research findings. Our present approach has worked well; however, there is room for improvement. An opportunity for the research staff to assemble more frequently than the present annual review would facilitate the exchange of information. Greater participation by research staff in field trials both in Colombia and the U.S. might increase the yield of information and accelerate progress.

We are basically following the procedures and plans as outlined in our proposals.

DISSEMINATION AND UTILIZATION OF RESEARCH RESULTS

A list of titles and abstracts of presentations made to scientific meetings and publications appears in Appendix I. An outline entitled "Systems of Anaplasmosis Immunization" is presented in Appendix II.

The research capabilities established first at ICA, and later CIAT, have been used not only by our staff but also by Colombian veterinarians and students working on similar problems, and often in cooperation with our team. This project has markedly stimulated local interest in these problems and has contributed to a better appreciation for what can and should be done. The Colombian government (ICA) has constructed a modern laboratory at Turipana for the study of tropical hemotropic diseases.

There have been numerous Colombian veterinarians and research workers who have worked with Texas A&M staff and students and associates, both in Colombia and Texas, who continue to cooperate and assist in our program. This association has proven to be mutually advantageous.

On several occasions ITVM staff have contributed as consultants to various government and private livestock enterprises, in areas such as Mexico, Nicaragua, Peru, Ecuador, West Pakistan, Haiti, Panama, Dominican Republic, Guyana, and the Mariana Islands (western Pacific). These opportunities have afforded the chance to further test procedures which we believe will become integral parts of any future control programs.

STATEMENT OF EXPENDITURES AND OBLIGATIONS AND CONTRACTOR RESOURCES

Funds were spent in the U.S. and in Colombia for budgeted items including manpower, equipment, travel, etc. In Colombia local expenditures for

wages, travel, etc., are made by CIAT on our behalf, and CIAT is periodically reimbursed from the AID account maintained at Texas A&M by the Office of International Programs. All other purchases and staff salaries (in Texas and Colombia) are handled through this office.

Dr. T. J. Galvin terminated his assignment with ITVM in July, 1974, and is now full time in Veterinary Parasitology. Dr. K. C. Thompson terminated his appointment with ITVM in November, 1974, and is now a full time CIAT employee in charge of tick research. During the past year, Dr. L. G. Adams has been one-half time Veterinary Pathology and one-half time ITVM. As of May, 1975, Dr. Adams is full time Veterinary Pathology. Dr. Todorovic was transferred to CIAT, replacing Dr. Galvin, in July, 1974, and is full time ITVM. Drs. Wyss and Craig maintained graduate student classification until December, 1974, and January, 1975, when Wyss was assigned full time with ITVM, and Craig became full time Veterinary Parasitology. Dr. Wyss will terminate his appointment with ITVM no later than August 31, 1975.

State and industry support of our projects have continued with hopes of marked increases in the coming year. A line budget item of over \$30,000.00 annually has been approved by TAES and requested from the legislature. The Texas State Department of Agriculture has requested legislative support in the amount of \$35,000.00 for a tick research building at Falcon Heights. Hoffman-LaRoche Laboratories provided \$7,000.00 for a drug testing trial, which is now complete.

A budget statement for 1974, outlining expenditures and obligations for each of the major inputs and the major targets is given in Appendix III.

WORK PLAN AND BUDGET FORECAST FOR THE COMING YEAR

In general the programs at Texas A&M and Colombia will be oriented toward the completion of present projects and utilization of past research results to evolve systems and techniques for the more efficient and economic control of hemotropic diseases of food-producing ungulates.

Work in Colombia will include studies on all three major hemotropic diseases: anaplasmosis, babesiosis, and trypanosomiasis, but with an emphasis on the first two. A major effort has begun to test and evaluate premunition as a technique for Anaplasma and Babesia control in the intermediate zone (see Appendix II). It is expected that this will last at least three years and will involve vaccinating all 6 to 10 month old calves on a continuing basis to establish immune herds. An appropriate number of unvaccinated controls will also be observed.

The establishment of a USDA lab on U.S. territory for the study of Boophilus ticks will greatly facilitate vector studies, both in relation to Anaplasma and Babesia. The availability of Boophilus ticks will permit us to more closely simulate natural field challenge of both Anaplasma and Babesia under controlled conditions. This factor will facilitate vaccine studies for both anaplasmosis and babesiosis. In addition, the pathogenesis of both Anaplasma and Babesia induced by tick infestation can be compared to infections induced by needle inoculation of infected blood. Increased emphasis will be placed on the production of a more purified killed Anaplasma vaccine.

A budget statement for 1975-76 showing planned expenditures for each of the major inputs and the major work targets is given (Appendix III).

A P P E N D I X I

Institute Staff Publications
1968 - 1975

APPENDIX I

KUTTLER, K. 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 5th National Anaplasmosis Conference, February 28-29, 1968, Stillwater, Oklahoma.

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 test (CF), 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.

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

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 non-irradiated 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.

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.

CORRY, G. L. and KUTTLER, K. L.: Serological Activity of a Soluble Antigen of Theileria cervi. Experimental Parasitology (Prepared - not published)

Two basic cellular erythrocytic antigens were prepared from erythrocytes obtained from a white-tailed deer (Odocoileus virginianus) infected with Theileria cervi. The first antigen was prepared from erythrocytes lysed by freezing, the second from erythrocytes lysed with distilled water. The serologic activity as determined by the complement-fixation (CF) test was greater in the antigen lysed by freezing. Both antigens when solubilized at pH 7.2 using ultrasonic disintegration increased markedly in titer.

The two antigens were pooled and disrupted by ultrasonic disintegration in buffered mediums, ranging from pH 5 to pH 11. Optimal solubilization and serologic activity as measured with the CF test was obtained at pH 11.

The antigen solubilized at pH 11 was used to determine antibody in sera from infected deer by (CF) and by passive hemagglutination (PHA) tests. Both tests resulted in similar but not identical antibody titers.

A gel diffusion test and a ring (interfacial) test gave no valid results.

INDEX DESCRIPTORS: Theileria cervi effect of soluble antigen on complement-fixation and passive hemagglutination.

Theileria cervi is a hemoparasite of the white-tailed deer (Odocoileus virginianus), first described by Schaeffler (1961). It was thought by Marburger and Thomas (1965) and Robinson et al. (1967) to be a contributing factor of death losses among deer in Texas.

Other organisms of the same genus occur in different parts of the world where they cause diseases of varying severity in domestic and wild animals.

Laboratory diagnosis of these diseases depends primarily on microscopic detection of the parasites in stained smears. Serologic tests have been described by Schaeffler (1963), Kuttler and Robinson (1967), Kuttler et al. (1967), and Gadir et al. (1970). The antigens used in these tests were, for the most part, particulate. In this state, the cell membrane is most responsible for antigenic and serologic activity with the internal structure of the parasite cell not so greatly involved. It is not unreasonable to assume that the cytoplasm of the parasite cells contains a mosaic of antigens which when dispersed in molecular phase, i.e., when in solution, are capable of more specific or sensitive reactions. Such antigens could find wide use in serologic tests, and possibly prove of greater value in elucidating the antigenic relationship of similar intraerythrocytic parasites.

The purpose of this work, therefore, was to investigate the possibility of establishing a method of obtaining a soluble antigen from the erythrocytic stage of T. cervi that would react with the homologous antibody in an in vitro system.

KUTTLER, K. L.: Serial Passage of an Attenuated Anaplasma marginale in Splenectomized Calves. Proceedings of the 73rd Annual Meeting of the USAHA, October 12-17, 1969, Milwaukee, Wisconsin, 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.

KUTTLER, K. L., ADAMS, L. G. and ZARAZA, H.: An Epidemiologic and Geographic Survey of Anaplasma marginale and Trypanosoma theileri in Colombia. 106th Annual AVMA Convention, July 1969, Minneapolis, Minnesota. 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 T. 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.

KUTTNER, K. L. and ZARAZA, H.: Premunization With An Attenuated Anaplasma marginale. Proceedings of the 73rd Annual Meeting of the USAHA, October 12-17, 1969, Milwaukee, Wisconsin, 104-112.

An attenuated Anaplasma marginale infection has 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 using a Texas isolate of A. marginale. Experimental and natural challenge with a Colombian isolate resulted in evidence of acute anaplasmosis in both vaccinated and non-vaccinated animals.

TODOROVIC, R. A., ADAMS, L. G. and ROBERTS, D. E.: A Study of Bovine Babesiosis in Colombia, South America. Proceedings of the 106th Annual AVMA Convention, July 1969, Minneapolis, Minnesota. 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.

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). (Spanish translation of: Kuttler, K. 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.)

ADAMS, L. G., HIPOLITO, O., MORALES, H., GONGORA, S. and JONES, L. P.: Dermatofilosis Bovina (Streptotricosis 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 Dermatophilus congolensis.

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.

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, September 28 - October 3, 1970, Santiago de Chile, 37.

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 one calf was euthanized 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.

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.

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: 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.

KUTTLER, K. L., ADAMS, L. G. and ZARAZA, H.: Estudio Epizootiologico del Anaplasma marginale y et Trypanosoma theileri en Colombia. Revista ICA, (June 1970), 5, (2). (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.)

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: 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.

TODOROVIC, R. A.: Babesiellosis 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.

TODOROVIC, R. A., ADAMS, L. G., VIZCAINO, O. and GONZALEZ, E.: Research and Control of Bovine Babesiosis in Colombia. Proceedings of the VI Congreso Panamericano de Medicina Veterinaria y Zootecnia, September 18 - October 3, 1970, Santiago de Chile, 36.

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 four 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 infection, 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.

TODOROVIC, R. A., GONZALEZ, E. F. and ADAMS, L. G.: Immune Response of Cattle Vaccinated Against Babesiosis in Colombia, South America. Proceedings of the American Veterinary Medical Association and the American Association of Veterinary Parasitologists, June 22, 1970, Las Vegas, Nevada.

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 were exposed to tick-borne (Boophilus microplus) challenge. The second group consisted of 20 male, 95 kg, Holstein, 4-month-old calves subdivided into 4 groups and vaccinated with a killed Babesia vaccine derived from the erythrocytes and plasma, respectively, of animals acutely infected with Babesia bigemina and Babesia argentina. The animals were inoculated with vaccine with or without Bacto-Adjuvant Complete H 37 Ra. The third group of 40 male, 80 kg, Holstein, 3-month-old calves was divided into sub-groups. The first sub-group consisted of 20 animals which were preimmunized with Babesia bigemina and Babesia argentina and 8 days later were treated with a new experimental babesiacidal drug. The second sub-group which consisted of 20 animals was simultaneously 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 Boophilus microplus naturally infected with Babesia bigemina and Babesia argentina. The fifth group consisted of 10 animals used as controls. Responses to vaccination and tick-borne challenge were evaluated by packed cell volumes, percentage of parasitemia, body temperatures, body weight, complement fixing antibody titers, general physical conditions, and percent recoveries after tick-borne challenge. Results in general indicate that resistance to babesiosis can be produced by co-infectious or sterile immunity. Experiments in prophylaxis, based on residual action of the babesiacidal drug, have given consistent and satisfactory results. In the future, it may be possible to develop control programs against bovine babesiosis based on these observations. The present status of these studies was described.

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

Five cases of ovine neo-natal necrobacillosis, in the Sabana 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.

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 an including 30 kRad developed progressive parasitemias. Some calves receiving 1×10^{10} parasitized erythrocytes irradiated 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} non-irradiated 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.

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 submandibular 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.

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 have been studied by means of electron microscopy.

Although there are reports in the literature of the fine structure of Babesia canis which affects dogs, Babesia caballi which affects horses, and Babesia rodhaini which affects rodents, there is no report on the ultrastructure of B. bigemina which infects cattle.

B. bigemina was isolated from naturally infected cattle in the Valle del Cauca, Colombia, and maintained in splenectomized calves in the Laboratorio de Investigaciones 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 by 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 is the largest internal structure of the parasite and occupies one-fourth to one-third 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 two 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.

KUTTLEK, K. L.: Efficacy of Oxytetracycline and a Dithiosemicarbazone in the Treatment of Anaplasmosis. American Journal of Veterinary Research, (September 1971), 32: 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.

KUTTLEK, K. L.: Promising Therapeutic Agents for the Elimination of Anaplasma marginale in the Carrier Animal. Proceedings of the 75th Annual Meeting of the USAHA, October 27, 1971, Oklahoma City, Oklahoma, 92-98 (published 1972).

Two new drugs, a dithiosemicarbazone (356C61) and 3,3'-bis(2-imidazolin-2-yl)-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 A. 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.

KUTTLEK, 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: 657-659.

Transovarial transmission of anaplasmosis occurred when two 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.

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 tissues. 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.

TODOROVIC, R. A. and ADAMS, L. G.: Serologic Diagnosis of Babesiosis.
Proceedings of the XIX World Veterinary Congress, August 15-22, 1971,
Mexico City, 1114-1116.

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

In this review an attempt has been made to summarize and discuss the recent advances on sero-diagnosis of babesiosis in infected cattle with special attention to the serologic procedures used in the Laboratorio de Investigaciones Medicas Veterinarias located in Bogota, Colombia. In the last two decades fundamental knowledge concerning the immuno-serology of several Babesia spp. has led to the development of sero-diagnostic procedures for detection of Babesia antibodies. The antigens used in these techniques originated from parasitized erythrocytes and serum or plasma of animals with acute babesiosis, and they were applied in several serologic tests. The complement-fixation reaction constituted one of the earliest tests for the diagnosis of babesiosis. In recent years considerable progress was made to improve the complement-fixation test for the diagnosis of babesiosis. In addition, gel precipitation, fluorescent antibody, and agglutination techniques were applied for the detection of specific Babesia antibodies utilizing antigens from the parasitized erythrocytes as well as acute serum.

The investigations described in this report were conducted to develop the new techniques and to evaluate existing techniques for diagnosing bovine babesiosis. Research was executed in collaboration with the Instituto Colombiano Agropecuario in the Laboratorio de Investigaciones Medicas Veterinarias in Bogota, Colombia. Antigens of Babesia spp. were isolated by means of two techniques and used in the complement-fixation test for the detection of Babesia antibodies in cattle experimentally and naturally infected. By means of the complement-fixation test it was possible to detect specific antibodies in the serum of cattle 8 days after blood-borne infection. A total of 5,420 serum samples of cattle infected with babesiosis were tested. The cattle were from several Colombian experimental herds with known histories of babesiosis located in Valle del Cauca, Rio Magdalena, Llanos and Monteria and from cattle artificially infected in the Laboratorio de Investigaciones Medicas Veterinarias in Bogota. Approximately 95% of these samples were positive; whereas, about 5% gave discordant reactions. In addition to the complement-fixation test used in our laboratory, attempts were made to apply the double-gel diffusion for characterization of Babesia spp. antigen-antibody reactions. A cross reaction was noted between Babesia bigemina and Babesia argentina in this system. The application of latex-agglutination and hemagglutination tests for the detection of the Babesia antibodies are still under investigation in our laboratory. As a result of these investigations and observations, it is apparent that more investigation is needed for the development of a practical serologic technique for the diagnosis of babesiosis and to help solve this complex biological disease problem in tropical and sub-tropical areas of the world.

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

A group of 50 male, Holstein-Friesian calves, 3 to 4 months old, were used to evaluate a control program for gastrointestinal and hemotropic parasites. The experiment was conducted at the ICA experimental station in Palmira, Valle del Cauca, at an elevation of 1,000 meters. The animals were divided into 3 groups.

Twenty animals were premunized against anaplasmosis and babesiosis simultaneously; 8 days later they were treated against babesiosis using the compound 4A65 at a dosage of 1 mg/kg of body weight, and 21 and 56 days after premunition they were treated intravenously with the compound 356C61 (5 mg/kg IV) against anaplasmosis.

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

Ten animals were not treated and were used as controls.

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

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

TODOROVIC, R. A. , LUQUE, G. F. and ADAMS, L. G.: Contribution to the Study of the Tick Distribution in Colombia, South America. Revista de la Facultad de Medicina Veterinaria y Zootecnia (accepted for publication).

The purpose of this work was to collect and identify tick species involved in the epizootiology of bovine babesiosis in Colombia. Bovine babesiosis was reported in Colombia in 1888 but there is not any published evidence about tick species involved in the transmission of the disease. Although Boophilus microplus is the predominant tick in medium and hot climates, the exact distribution of the tick in the different regions of Colombia is not known. To develop an effective control program, the distribution of tick species needs to be determined. This is the first attempt to obtain this information about tick distribution in Colombia.

Animals naturally infected with Babesia bigemina and Babesia argentina were used as a tick collection source. The infectivity of these animals was determined by blood smears and complement-fixation techniques. The animals were located on farms in Palmira (Valle del Cauca) and Turipana (north coast), Magdalena River and Sumapaz River. Ticks were collected from different breeds: Holstein-Freisian, Zebu, Blanco Orejinegro, and Costeno con Cuernos, in animals of different ages. The ticks were collected from different parts of the animal bodies and preserved in Ethanol 95%. Adults, nymphs, and larvae were collected from both sexes for identification purposes during a 12-month period (January - December).

Dermacentor nitens was found in the animals infected with babesiosis in the Valle del Cauca, north coast, Sumapaz River; Amblyomma cayennense was found in animals infected with babesiosis and anaplasmosis in the Magdalena River and the north coast; Boophilus microplus was found in the same animals infected with babesiosis and anaplasmosis used in this experiment. Until the present time the significance of the findings of Amblyomma and Dermacentor ticks in epidemiology of babesiosis is not clear.

Experiments are in progress to determine the population and distribution of the tick species in other parts of Colombia for the purpose of investigating the exact role of Dermacentor nitens and Amblyomma cayennense in the transmission of bovine babesiosis

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.

ADAMS, L. G.: Epizootia Espontanea de Hepatitis Toxica en Porcinos Atribuida a Aflatoxicosis. *Revista ICA*, (March, 1974), 9: 31-48.

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 transamination 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.

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

Eperythrozoon wenyoni, E. tejanodes and E. 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 ±3.5 days post-splenectomy. Treatment with 2-di-(Beta, gamma-dioxipropil)-(aminofenol)-(4 arseno 5)-Beta-(benzaxozalil)-(2)-mercaptol-proprionato 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 Eperythrozoon wenyoni, E. tejanodes and E. tuomii in Colombia.

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.

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 Streptococcus faecalis var. liquefaciens at a dilution of 3×10 on the MacFarland 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.

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-ethoxyethylglyoxal dithiosemicarbazone) and 4A65 (3,3-Bis-(2-imidazolin-2-yl) carbanilide dihydrochloride). The third group of calves was used as a control. Calves selected for use at Monteria were not native to that area. All calves were subjected to natural exposure. Ticks were collected and identified at each site.

There was no apparent significant difference in weight gains and resistance to anaplasmosis and babesiosis between groups at any site. At Bugalagrande and Girardot the absence or 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 is apparent that the vaccinated and preimmunized calves did not develop resistance to anaplasmosis and babesiosis due to the use of antigenically different organisms; the simultaneous injection of the preimmunization drugs at 5 days post inoculation; the lack of sufficient sterile immunity to suppress tick-borne infection; or the inability of the very young calves to develop sufficient resistance.

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

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

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.

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 A. 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.

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 (Odocoileus virginianus) deer 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.

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, (abstract).

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.

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.

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, B. major and A. marginale were eliminated as contaminants of the B. bigemina isolated after four passages. A frozen stabulate of the isolated B. bigemina was established.

CORRIER, D. E. and ADAMS, L. G.: Observations During Concurrent Anaplasmosis and Babesiosis in Experimentally Infected Calves. Proceedings of the 6th National Anaplasmosis Conference, March 1973, Las Vegas, Nevada, 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.

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° C. 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 stablitate 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 10^9 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.

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 - April 5, 1973, Barnett, Texas, (abstract).

The increasing presence of both Boophilus annulatus and Boophilus microplus 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.

KUTTLER, K. L.: East Coast Fever (Theileriasis, Theileriosis, Rhodesian Tick Fever, Rhodesian Red Water). Submitted for publication in the 1973 revision of the "Foreign Animal Diseases" manual, U. S. Animal Health Association, Committee on Foreign Animal Diseases.

A review of the literature with a comprehensive discussion of East Coast Fever is given. This hemoprotozoal agent (Theileria parva) is considered by many to be the single most serious tick-borne disease occurring in East Africa. The 3 host tick, Rhipicephalus appendiculatus, is difficult to control and most ranches where it occurs will dip their cattle every week. The disease organism is readily transmitted by this and other tick vectors and produces a disease which may produce mortality of over 90%. This disease is limited to the African continent; however, similar infections caused by T. annulata and T. mutans have a much broader distribution. Theileria mutans is generally considered non-pathogenic, but T. annulata is a major disease producing hemo-parasite, but generally of less virulence than T. parva.

KUTTLER, K. L.: Review: Current Status of Control and Treatment With Drugs. Proceedings of the 6th National Anaplasmosis Conference, March 1973, Las Vegas, Nevada, 93-97 (abstract).

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 an oral tetracycline for 60 days at the rate of 5 mg/pound of body weight.

KUTTLER, K. L. and TODOROVIC, R. A.: Arthropod Borne Protozoan Infections (Affecting Domesticated, Food-Producing Animals). Submitted for publication in the 1973 revision of the "Foreign Animal Diseases" manual, U. S. Animal Health Association, Committee on Foreign Animal Diseases.

A review of the literature with a comprehensive discussion of anaplasmosis and babesiosis is given. In addition, tables of different Babesia spp., Theileria spp., Trypanosoma spp., Anaplasma spp., and Leucocytozoon spp. are given with reference to animals affected, morphology of the organism, and potential vectors. Brief descriptions of besnoitiosis and leucocytozoonosis are also given.

Despite progress in developing more effective acaricides and more efficient therapeutic agents, the arthropod borne hemoparasites remain a major disease problem in the tropics and the subject of intense research.

KUTTLER, K. L. and TODOROVIC, R. A.: Techniques of Premunization for the Control of Anaplasmosis. Proceedings of the 6th National Anaplasmosis Conference, March 1973, Las Vegas, Nevada, 106-112, (abstract).

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

Premunization is a practical approach for the 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 the animals being premunized, (2) virulence, potency, and size of the pre-munizing inoculum, and (3) the strain, or size, of the expected challenge exposure. In some instances, the 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.

MAURER, F. D.: The Need for Knowledge of Foreign Animal Diseases.
Introductory chapter for the 1973 revision of the "Foreign Animal Diseases"
manual, U. S. Animal Health Association, Committee on Foreign Animal
Diseases.

As the introduction to a text on the diagnosis, prevention and control of diseases foreign to the United States, it is a chapter of justification for a national interest in exotic animal diseases. Justification is based upon the need to protect U. S. livestock industries, as animal diseases are major handicaps to efficient livestock production, hence, the food supply in the U.S.A. and abroad. Further, with the world's population rapidly exceeding its food supply and many people suffering from protein deficiencies, there is an urgent need to develop all sources of animal protein. Only ruminant animals are capable of converting range forages and many crop by-products into food for man.

While all animal diseases are a handicap to efficient production, the most highly fatal, infectious diseases which impose the greatest burdens are among those exotic to the United States. This results in a marked contrast in livestock productivity between developed and developing countries. With only 40% of the world's livestock in developed countries, they produce 80% of the world's meat, milk and eggs.

If we are to protect U. S. livestock against the threat of exotic disease and help developing nations feed themselves, we must have knowledge of the major diseases and know how to control them. Should we neglect opportunities to improve world food production, we increase the chances of regional starvation with the associated social, economic and political instability which lead to aggression.

MAURER, F. D.: Rinderpest. Submitted for publication in the 1973 revision of the "Foreign Animal Diseases" manual, U. S. Animal Health Association, Committee on Foreign Animal Diseases.

Rinderpest is an acute, febrile, virus disease which spreads by direct and indirect contact between ruminants, primarily cattle and buffalo. It is characterized pathologically by inflammation, hemorrhage, necrosis and erosion of the digestive tract accompanied by a wasting, frequently bloody diarrhea. Less acute forms may occur in resistant cattle. Since ancient times, Rinderpest has been the world's most devastating disease of cattle and, as such, has had a major influence on man's food supply. Prior to 1949, Rinderpest killed over 2 million cattle and buffalo per year. Losses continued until effective vaccines were developed and immunity maintained. It is only through such immunity that cattle raising is now profitable in Africa, the Middle East, and Asia. The presence of Rinderpest anywhere serves as a constant threat to the rest of the world. The U. S., like other free nations, must be alert to its recognition, and be prepared to accomplish prompt confirmatory diagnosis and control.

MAURER, F. D. and McCULLY, R. M.: African Horsesickness. Submitted for publication in the 1973 revision of the "Foreign Animal Diseases" manual, U. S. Animal Health Association, Committee on Foreign Animal Diseases.

The chapter describes the history, geographic distribution, etiology, clinical character, pathology, diagnosis, preventive immunity, epidemiology, and control of this most destructive disease of equine animals. African Horsesickness is a highly fatal, insect-borne, febrile, virus disease clinically dominated by an acute pulmonary edema or a hemorrhagic myocarditis associated with localized areas of inflammatory edema and hemorrhage. Long confined to south and equatorial Africa, in the 1960's it spread via the ubiquitous *Culicoides* vectors throughout 11 countries of the middle east, North Africa, and Spain, killing many hundreds of thousands of equines. In view of the essentially worldwide distribution of *Culicoides* vectors, African Horsesickness is a prevailing threat to the western hemisphere and elsewhere. With 9 known antigenically different strains of virus, polyvalent vaccines oriented to the strains involved are required to protect threatened equines. Each country, including the United States, needs to be prepared to promptly diagnose and, if need be, vaccinate against African Horsesickness.

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.

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 the diagnosis and control of bovine babesiosis in Colombia, South America. Antigens of *Babesia bigemina* and *Babesia argentina* were isolated and used in the complement fixation and rapid agglutination tests for the diagnosis of babesiosis in cattle. Three systems were evaluated for the control of bovine babesiosis: (1) vaccination of susceptible cattle with killed *Babesia* spp. vaccine to produce resistance based on sterile immunity; (2) premunition of cattle with virulent *Babesia* spp., followed by chemotherapy to produce resistance based on co-infectious immunity; and (3) chemoprophylaxis based on the activity of babesiacidal compounds with prolonged residual action. All these systems were found effective in controlling bovine babesiosis under the conditions of these experiments. The epizootiological conditions of babesiosis enzootic areas will indicate which system is applicable. In zones with a high incidence of *Boophilus microplus*, the premunition is indicated; in areas where the tick population is controlled, or in areas at constant risk of tick exposure, the system of inducing resistance with killed *Babesia* spp. or chemoprophylaxis is indicated.

TODOROVIC, R. A., GONZALEZ, E. F. and ADAMS, L. G.: Bovine Babesiosis: Sterile Immunity to Babesia bigemina and Babesia argentina Infections. Tropical Animal Health and Production, (1973), 5: 234-245.

Killed Babesia bigemina and Babesia argentina vaccine was prepared from the infected erythrocytes (AG-E) and from the 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 two and one-half 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 the data obtained in these experiments, 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.

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 babesiasis were evaluated in 29 calves. The compound had prophylactic and therapeutic properties in calves artificially or 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 the development of acute babesiasis 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 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 of imidocarb per kilogram 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 babesiasis. 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 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.

VIZCAINO, O. G. and TODOROVIC, R. A.: Produccion de Antigenos Solubles de Babesia argentina y Babesia bigemina para Pruebas de Immunodiffusion. (Soluble Antigens of Babesia bigemina and Babesia argentina and Their Application in Serologic Tests.) Proceedings of the 7th Panamerican Veterinary Congress, July 23-28, 1973, Bogota, Colombia, 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 test 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.

ADAMS, L. G.: A Study of the Toxicity of Imidocarb Dipropionate in Horses.
(manuscript in preparation 1974)

An experiment was designed to study the potential systemic toxicity of imidocarb dipropionate in which 12 male and 12 female horses from 2 to 8 years of age were divided into 6 groups of 2 males and 2 females each. Five groups of 4 horses each were intramuscularly injected twice at a 24-hour interval with 2, 4, 8, 16, and 32 mg/kg, respectively. One group of 4 horses was injected intramuscularly with physiological saline solution and served as controls. Two of 4 horses injected with 16 mg/kg and 4 of 4 horses injected with 32 mg/kg died between days 2 and 6 following the first injection; therefore, the LD₅₀ was determined to be 16 mg/kg at 21 days following the first injection. Increasing quantities of injected dipropionate correlated with increasing mortality rates, rapidity of mortality, systemic reaction, local injection site reactions, and increasing levels of serum urea nitrogen, increasing enzymatic activities of serum glutamic oxaloacetic transaminase, serum sorbitol dehydrogenase, serum creatinine phosphokinase, serum lactic dehydrogenase, a left shift in neutrophilic leukocytes, higher respiratory and pulse rate, and an increasing severity of hepatic renal and pulmonary lesions. The most prominent pathological lesions were an acute tubular necrosis of the proximal convoluted tubules of the renal cortex and an acute periportal hepatic lipidosis and necrosis. Death was attributed to renal and hepatic failure.

ADAMS, L. G. and CORRIER, D. E.: A Study of the Toxicity of Imidocarb Dipropionate in Cattle. (manuscript in preparation 1974)

An experiment was designed to determine the potential systemic toxicity of imidocarb dipropionate in which 20, 10 to 12 month old, calves were divided into 4 groups of 5 each. Three groups were intramuscularly treated twice at a 14-day interval with 5, 10, and 20 mg/kg, respectively, while the other group was treated only with physiological saline solution and served as a control. The LD₅₀ at 14 days in calves receiving only one injection of imidocarb dipropionate was determined to be 15 mg/kg, and the LD₅₀ at 67 days in calves receiving two injections was determined to be 15 mg/kg. None of the calves treated either once or twice at 5 or 10 mg/kg died, while 5 of 5 calves treated either once or twice at 20 mg/kg died within 18 days following the first injection.

No significant alterations occurred in the average daily gain, total serum proteins, total leukocytes, absolute lymphocytes, absolute monocytes, and absolute eosinophils. Increasing quantities of injected imidocarb dipropionate correlated with increasing levels of blood urea nitrogen, increasing enzymatic activity of serum glutamic oxaloacetic transaminase, and a left shift in neutrophilic leukocytes. In cattle treated with 20 mg/kg, the most prominent gross pathological lesions were hydrothorax, hydroperitoneum, pulmonary edema, perirenal edema, enlarged pale kidneys with prominent alternating red and white bands in the renal cortex, and enlarged, friable, pale livers with accentuating hepatic lobules. The most prominent histopathological lesions were acute tubular necrosis of the proximal convoluted tubules of the renal cortex and acute periportal hepatic necrosis.

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 and Production*, (1974), 6: 71-78.

The chemotherapeutic efficacy of imidocarb dihydrochloride [3,3'-bis(2-imidazolin-2-yl)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.

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 and 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 parasitaemias 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.

ALIU, Y. O.: Absorption, Distribution, and Excretion of Imidocarb Dipropionate [3,3'-bis-(2-imidazolin-2-yl)carbanilide] in Sheep. Ph.D. Dissertation, Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, August 1974, (106 pages).

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 suggests 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.

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.

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.

GUZMAN, V. H., ADAMS, L. G. and GALVIN, T. J.: Verminosis Laringea Bovina Producida por Mammomonogamus laryngeus en Colombia. Revista ICA (accepted for publication).

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.

KUTTLER, K. L.: Use of Imidocarb to Control Anaplasmosis. The Southwestern Veterinarian, (1975), 28, (1).

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.

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 T. mutans were reproduced in splenectomized calves inoculated with pooled blood samples from east Texas cattle. Theileria can be obtained in pure form by treating cattle, with dual infections, with Gloxazone and imidocarb which eliminated Anaplasma but not the Theileria. These Theileria infections were responsible for mild, transient reductions in packed red cell volume.

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 (accepted for publication).

Treatment of calves with 5 mg/kg imidocarb as dipropionate given intramuscularly 14 days before and 14 days after exposure to Babesia infected Boophilus microplus larvae rendered the next generation larvae incapable of transmitting Babesia infection. The drug, when administered to calves 14 and 28 days before tick exposure, prevented the development of clinical babesiosis, but the larval progeny of ticks reared on the calf treated 28 days before infestation were infective for Babesia. 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.

MAURER, F. D.: Support for Research in Animal Health. Journal of the American Veterinary Medical Association (accepted for publication).

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.

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

The work of an international committee, of which F. D. Maurer was the veterinary member. The report 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.

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 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 indirect fluorescent antibody 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. Indirect fluorescent antibody test serum titers of 1:100 or greater were considered to be positive. No cross-reactivity of the indirect fluorescent antibody test was observed between Trypanosoma 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 indirect fluorescent antibody test for Trypanosoma vivax. The indirect fluorescent antibody 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 indirect fluorescent antibody test by eluting serum from dried impregnated filter paper discs produced results nearly equal to those obtained by using conventional serum samples. The indirect fluorescent antibody test was up to 20 times more effective in detecting Trypanosoma vivax positive cattle than the thick blood smear technique. The indirect fluorescent antibody test demonstrated the presence of Trypanosoma 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.

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.

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

TODOROVIC, R. A.: Serologic Diagnosis of Babesiosis: A Review. Experimental Parasitology (submitted).

In the last three decades some fundamental knowledge concerning the immunoserology of Babesia spp. infections has led to the development of serologic techniques which provide a means for studying the pathogenesis of babesiosis and the detection of animals with subclinical infections. The antigens used in the serologic 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 serologic 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 summarize and discuss the recent advances in the serodiagnosis of babesiosis, together with conditions where the use of serologic methods may be valuable.

TODOROVIC, R. A., GONZALEZ, E. F. and ADAMS, L. G.: Babesia bigemina, Babesia argentina and Anaplasma marginale: Co-infectious Immunity in Bovines. Experimental Parasitology (submitted).

Forty-eight intact and 8 splenectomized calves were used to evaluate different systems of co-infectious immunization against Babesia bigemina, Babesia argentina and Anaplasma marginale. Co-infectious immunity was induced by two methods: (1) blood of calves acutely infected with B. bigemina, B. argentina and A. marginale was used as the source of inoculum and the post injection reactions were chemotherapeutically controlled with Imidocarb, Ganaseg, Gloxazone or Liquamycin; and (2) by artificially inducing babesiosis with the blood of carrier calves 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 co-infectious immunity were treated chemotherapeutically to prevent death losses.

TODOROVIC, R. A. and KUTTLER, K. L.: A Babesiasis Card Agglutination Test. American Journal of Veterinary Research (accepted for publication).

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.

TODOROVIC, R. A., LOPEZ, L. A., LOPEZ, A. G. and GONZALEZ, E. F.: Control of Bovine Babesiosis and Anaplasmosis in Cauca River Valley. Experimental Parasitology (submitted).

Experiments were carried out to evaluate two systems: (1) premunition and (2) chemoprophylaxis for the control of bovine babesiosis and anaplasmosis in the Cauca River Valley. 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 post premunition 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 post premunition reaction, but failed to prevent clinical anaplasmosis under the conditions of this investigation.

TODOROVIC, R. A. and TELLEZ, C. H.: The Premunition of Adult Cattle Against Babesiosis and Anaplasmosis in Colombia, South America. Tropical Animal Health and Production (accepted for publication).

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 by Gloxazone (dithiosemicarbazone) combined with oxytetracycline. Systems of premunition for Babesia spp. were found effective and practical; however, systems of premunition for A. marginale were found less effective and not practical under the conditions of these experiments.

CORRIER, D. E.: The Epidemiology of Bovine Anaplasmosis and Babesiosis in the Lowland Tropics of Colombia. Presented at Hemoparasite Workshop - CIAT, 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 per cent 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 per cent in the Eastern plains, 91 per cent on the Atlantic coast and 71 per cent 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 per cent in the Eastern plains, 77 per cent on the Atlantic coast and 75 per cent 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 per cent, which indicated the disease is not evenly distributed throughout 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 identified 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 Amblyoma cajennense, Amblyoma 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.

DAY, W. C. and KUTTLER, K. L.: Animal Health Considerations Involved in the Movement of U.S. Cattle to Haiti. (Accepted for publication in The South-western Veterinarian).

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 in 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.

MAURER, F. D.: Livestock, a World Food Resource Threatened by Disease. Journal of the American Veterinary Medical Association, (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.

MILLER, R.M.: Investigations On Transstadial Transmission of Bovine Anaplasmosis and Benign Bovine Theileriosis in Cattle by Two Species of Amblyomma (Acarina: Ixodidae). 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 the engorgement of the 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 resembling T. mutans 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 that they were susceptible to the blood parasites at the time of tick infestation.

PLATT, K. B. and ADAMS, L. G.: Evaluation of the Indirect Fluorescent Antibody Test for Detecting Trypanosoma vivax in South American Cattle. Submitted for publication to Research in Veterinary Science.

The indirect fluorescent antibody test (IFAT) as used in Africa for detecting bovine trypanosomiasis was adapted for use in South America and evaluated. Antigen consisted of Trypanosoma vivax laden bovine blood fixed in a 60:40:: acetone:methanol solution. The test detected initial titers of 1:50 and 1:100 at an average of 13.1 and 15.9 days post parasitemia (PP). Maximum titers as high as 1:400 developed in 8 calves at an average of 23.4 days PP. In another calf, 109 days PP were required. Efficacy in detecting sero-positive calves throughout the course of infection was 81.1 and 96.4% at serum dilutions 1:100 and 1:50 respectively. No false positive reactions occurred when serums from 36 hemoparasite-free-calves were tested. Cross reactivity did not occur when serums from calves singularly infected with Trypanosoma theileri, Trypanosoma evansi, Anaplasma marginale, Babesia argentina, Babesia bigemina and Eperythrozoon spp. were similarly tested in the IFAT. No significant differences were found in IFAT results of surveys in which both conventional serum samples and serums eluted from dried filter paper blood samples from the same calf were used.

REYNOLDS, S. D.: Evaluation of Methods of Premunition to Anaplasma marginale. 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.

A frozen stablitate prepared from a pooled sample of blood containing red blood cells highly parasitized with A. marginale was used as the infective organism in this trial.

Twenty-five yearling crossbred heifers were divided into 4 experimental groups. Group I consisted of 7 animals which were vaccinated with 2 injections of Anaplaz vaccine at 4-week intervals prior to the first infection with live organisms. Group II, consisting of 9 animals, was designated as the untreated group. The 5 animals in Group III received oxytetracycline 11 mg/kg when their parasitemias reached 4.6%. Group IV, consisting of 4 animals, was designated the control group.

Clinical manifestations of the infection were mild. During the infection the animals had a rough hair coat and poor body condition. There was some decrease in weight gains but nothing remarkable clinically.

A complement fixation titer preceded the appearance of a parasitemia by up to a week. Associated with the appearance of a parasitemia were decreases in PCV, RBC, and Hb and a rise in MCV.

The recovery rates for the animals in each of Groups I, II, and III showed no significant differences. The animals in Group II (untreated) displayed a faster rate of recovery than either Group I or Group III for PCV, RBC, and Hb, but had a slower rate of weight gain.

THOMPSON, K. C., TODOROVIC, R. A. and HIDALGO, R. J.: Antigenic Variation of an Isolate of Babesia bigemina. Tropical Animal Health and Production (1975).

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

Antigens were characterized by means of the complement fixation (CF), gel diffusion (GD), agar gel immunoelectrophoresis (AGI) and the indirect haemagglutination tests (IHA).

Differences were detected between the four sources of antigen.

THOMPSON, K. C., TODOROVIC, R. A. and HIDALGO, R. J.: Immune Response of Cattle to Babesia bigemina Isolated from Acute and Chronic Blood and Tick Borne Infections. Tropical Animal Health and Production (1975).

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

Thirty-six intact calves were divided into 16 groups of 2 (or 3) and each group inoculated with infective Babesia bigemina erythrocytic stabilates. Twenty-eight days later they were homologously and heterologously challenged with the original stabilates, and monitored for an additional 20 days. The host's apparent reduced response to homologous challenge and the marked immune response observed with heterologous systems indicated antigenic differences of the B. bigemina and confirmed the serological data under the experimental conditions used in the investigation.

THOMPSON, K. C.: The Maintenance, Life Cycle and Experimental Infection of the South American Boophilus microplus Tick. Tropical Animal Health & Production (1975)

Techniques were 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.

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. (Prepared for publication).

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.

TODOROVIC, R. A. LONG, R. F. and McCALLON, B. R.: Comparison of Rapid Card Agglutination Test with the Complement Fixation Test for Diagnosis of Anaplasma marginale Infection in Colombian Cattle. (Prepared for publication).

A total of 342 serum samples were collected from Colombian cattle before and during natural infection with Anaplasma marginale on the North Coast of Colombia. The serum samples were used to compare the complement fixation (CF) and rapid card agglutination (CT) tests for the diagnosis of anaplasmosis. On the basis of the results with CF and CT tests, both were found to be in agreement in detection of anaplasmosis infected cattle. It appeared that the CF and CT can detect Anaplasma marginale specific antibodies at approximately the same time after exposure, however, the positive agglutination reaction of the CT occasionally developed several days after the first CF reaction. The positive CT reaction persisted once it had become positive in contrast to the CF reaction which fluctuated between trace and 1:80 titers. The advantages of the CT test for the diagnosis of Anaplasma marginale infection under field conditions are simplicity, economy and speed of performance.

WYSS, J. H. and GALVIN, T. J.: A Standardized Simultaneous Immunization Procedure for Anaplasma marginale, Babesia argentina, and Babesia bigemina. (Submitted to Tropical Animal Health and Production).

A 42 day standardized simultaneous immunization procedure for Anaplasma marginale, Babesia argentina and Babesia bigemina using stabilates and drug moderation is described. The reactions of eight calves to the procedure are presented and the weights, packed cell volumes and temperatures of the immunized calves are compared to control calves during the 42 day period. All eight calves responded to the injection of stabilates, as evidenced by parasitaemia and sero-conversion from negative to positive, for each of the three organisms. Although expected significant differences in packed cell volumes and temperatures did occur during the immunization period all calves were successfully immunized and in good condition for transportation to the field by the end of the period.

WYSS, J. H., MATEUS, G., GALVIN, T.J. and ZARAZA, H.: Evaluation of Immunization Versus Imidocarb Chemoprophylaxis of Susceptible Calves Introduced Into an enzootic Zone of Anaplasmosis and Babesiosis. (Submitted to Tropical Animal Health and Production).

Results of a 392 day field trial comparing two different haemoparasite control procedures on susceptible calves introduced into an enzootic area of anaplasmosis and babesiosis are presented. One group of calves was previously immunized by a standardized simultaneous procedure for Anaplasma marginale, Babesia argentina and Babesia bigemina; a second group received Imidocarb chemoprophylaxis, and a third group was maintained as untreated controls. During the field challenge there were no deaths in the immunized group in contrast to 12.5 per cent mortality in the chemoprophylaxis group and 50 per cent mortality in the control group due to haemoparasites. In addition, the two groups which received some type of treatment for haemoparasites did significantly better in respect to weight gains and showed much less evidence of anemia than the control group. An economic comparison of the three groups is presented.

MILLER, R. M., PRICE, M. A. and KUTTLER, K. L.: Investigations on Transstadial Transmission of Bovine Anaplasmosis and Benign Bovine Theileriosis in Cattle by Two Species of Amblyomma (Acarina: Ixodidae). (Manuscript prepared for publication).

Amblyomma americanum and A. cajennense nymphal stages were fed on Anaplasma and Theileria infected cattle. Following molting, the adult ticks were fed on non-infected fully 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 ticks tested.

A P P E N D I X I I

Systems of Anaplasmosis Immunization

SYSTEMS OF ANAPLASMOSIS IMMUNIZATION

Introduction

The most successful methods developed for immunization against anaplasmosis in the tropics have involved the principle of premunition. Killed vaccines involving 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.

Efforts to achieve this goal have employed a number of means, some of which work well and others of which are totally inadequate.

Virulent Field A. marginale

It has not been uncommon 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 are refractory to this type of treatment.

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 occurs with these artificially maintained strains. The use of diluted stabilates to induce infection is also a factor in producing 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 10^2 to 10^3 dilution of the frozen stabilate. These frozen stabilates can be stored in liquid nitrogen for periods in excess of one year; 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 eliminate 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 daily. A single intravenous inoculation of 5 mg/lbs is usually sufficient when given early in the course of the 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 its drawbacks. 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 (<6 months) calves the invasiveness of the organism is below the level needed to produce replicating infections; 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 and 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 final consideration of this attenuated organism must be its availability. From 1967 to 1971, the organism was available for use and testing from Diamond Laboratories. Since that time, the organism has had only limited access by research workers. There is no indication of price or volume that might be produced when and if it becomes available commercially.

There is a need for independent testing of this product, with

statistical comparisons between it, A. centrale, and the "dilute stabilate" products.

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 methods of frozen stabilates are in use for this vaccine and, with the proper screening and standardization as used for the "dilute stabilates," this hazard can be minimized.

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 stabulates for use in preimmunizing campaigns. This can be accomplished following procedures published in the literature by almost any properly equipped laboratory with technical competency.

Killed Vaccine

The only such 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. In addition to this the duration of immunity is short, requiring frequent re-vaccination. Vaccination of producing cows has been associated with a neo-natal hemolytic disease of calves which can produce serious losses.

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 for use

++++ 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.

APPENDIX III

BUDGET STATEMENT FOR PAST YEAR

1 April 1974 - 31 March 1975

Statement of Expenditures According to Major Work Goals

	Anaplasmosis	Babesiosis	Trypanosomiasis & Theileriasis	Total
Personnel Salary & Allowances	89,322.00	93,292.00	15,880.00	198,494.00
Travel and Transportation	16,804.00	17,550.00	2,987.00	37,341.00
Equipment	7,789.00	8,136.00	1,385.00	17,310.00
Supplies and Operation	22,543.00	23,545.00	4,008.00	50,096.00
Overhead	25,056.00	26,169.00	4,454.00	55,679.00
Total	161,514.00	168,692.00	28,714.00	358,920.00

BUDGET STATEMENT FOR COMING YEAR

1975 - 1976

Statement of Expenditures According to Major Work Goals

	Anaplasmosis	Babesiosis	Trypanosomiasis & Theileriasis	Total
Personnel Salary & Allowances	76,051.00	76,052.00	16,900.00	169,003.00
Travel and Transportation	13,075.00	13,074.00	2,906.00	29,055.00
Equipment	1,200.00	1,200.00	267.00	2,667.00
Supplies and Operation	22,837.00	22,837.00	5,075.00	50,749.00
Overhead	24,111.00	24,112.00	5,358.00	53,581.00
Total	<u>137,274.00</u>	<u>137,275.00</u>	<u>30,506.00</u>	<u>305,055.00</u>