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## NARRATIVE SUMMARY OF ACCOMPLISHMENTS AND UTILIZATION

The Institute of Tropical Veterinary Medicine (ITVM) at Texas A&M University was created in 1966 for the purpose of generating and disseminating new information related to the control of animal diseases occurring in the tropics. The hemoprotozoal, arthropod-transmitted diseases of cattle were selected for initial emphasis in recognition of their widespread incidence and the significant deleterious influence they have on meat and milk productivity.

The complexity of these problems in cattle and domestic livestock is best illustrated by comparisons with human malaria, which like these cattle pathogens is also a hemoprotozoal, arthropod-transmitted infection. Worldwide efforts by W.H.O. and other agencies to eradicate malaria have generally been unsuccessful, notwithstanding generally effective insecticides for the control of the arthropod vectors and drugs to control the disease. Eighty-eight years after the discovery of the causative organism, an effective vaccine for the prevention of malaria has yet to be developed.

The final solution for Anaplasma and Babesia infections of cattle, as with malaria in humans, is yet to be found. Even so, significant progress has been made in developing methods which will reduce cattle losses and increase productivity in susceptible cattle imported into highly endemic areas.

Controlled field studies using premunition to prevent losses have yielded highly significant results. Susceptible cattle premunized or vaccinated for Anaplasma and Babesia before being moved into an area where these diseases were endemic (Monterria) showed greater weight gains. Vaccinated cattle as a group weighed over three times that seen in the non-vaccinated control group when measured ten months after arrival in the endemic area.

As the result of this and other corroborative studies, more extensive field trials are currently being undertaken to evaluate premunition as a method of control. Serologic and cross immunity tests have failed to identify major strain differences in either Anaplasma and/or Babesia which would endanger the success of the proposed programs.

Chemotherapeutic studies have continued with variable success. Anaplasma infections can be eliminated with Imidocarb, when given two times at a 14-day interval using 5 mg/kg. This level of medication, however, has been found dangerously near the toxic level. Detailed studies in sheep, cattle, goats, and horses of the toxic properties, pathologic changes associated with drug use, and tissue residue following treatment have been made. Significant Imidocarb residues have been detected nine months after treatment.

Efforts at disease eradication based solely on chemotherapy in a herd showing 80% Anaplasma infection were unsuccessful. This level of infection is comparable to those seen in highly endemic zones of Colombia.

Treatment of the entire herd with Imidocarb effectively terminated death losses due to anaplasmosis, and based on the serologic evidence possibly eliminated infection in 75% of the cattle after three to four months. Transmission occurred later, however, to the extent that one year after treatment the incidence of infection was essentially the same as seen originally.

Imidocarb has been found effective in the treatment of babesiosis at levels about one-fifth or one-tenth the amounts needed to treat anaplasmosis. Babesia bigemina has been consistently eliminated from infected cattle with as little as 1 mg/kg given one time. Treatment one time with 5 mg/kg Imidocarb in cattle infected with Babesia and infested with Boophilus ticks eliminated Babesia infection in both the cattle and the ticks. Treatment with this level of Imidocarb prevented babesiosis for up to six weeks, and blocked the infection in ticks for up to four weeks after treatment.

Successful and useful serologic diagnostic techniques for babesiosis have been developed using complement-fixation, a rapid card agglutination test, and an indirect fluorescent antibody test. These tests have provided the basis of identifying Babesia infections in Haiti, the Mariana Islands, Nicaragua, Mexico, and Texas during the past year.

During the year, personnel in Colombia were transferred to the new research facilities in CIAT. Presently we have 3 senior staff, Drs. Galvin, Corrier, and Thompson, and 1 junior staff, Dr. Hopps, in Colombia, for a total of 4 professional man year equivalents. At Texas A&M, we have 3, staff located at Texas A&M, each being financed one-half time by the project for a total of 1.5 professional man years. As of September 1974, it is anticipated that this ratio will be 4 man years in Colombia and 1.25 man years in Texas.

A total of 10 U.S. graduate students have participated in this program since its beginning. Of this number, 5 have completed work for the Master of Science degree, 3 have completed the requirements for the Ph.D. degree, and 3 are currently involved in Ph.D. programs, with plans to be finished within the next year.

The close association of staff from Texas A&M with Colombian research workers has been very advantageous. This is best illustrated by the large number of papers that are co-authored with Colombian staff (a total of 20 papers--see Appendix I). This close association of both Colombian and U.S. veterinarians has established a rapport which has strengthened the programs in both countries.

## GENERAL BACKGROUND

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

Many tropical and subtropical 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. A major group of these diseases comprises the blood parasitic diseases of cattle including anaplasmosis, theileriasis (East Coast Fever), babesiosis (piroplasmiasis), and trypanosomiasis. 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 premunization 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

Many of the objectives listed regarding anaplasmosis and babesiosis have been accomplished, with techniques and information evolving which offer hope for significant improvement in present control practices. Even so, losses continue and the disease problem persists. There are many vital questions that have gone unanswered. Continued research is the best way to solve these problems and to reach the ultimate objective of disease control.

As research on babesiosis and anaplasmosis proceeds to demonstrate

methods which provide effective control, more attention can be given to trypanosomiasis, theileriasis, or other parasitic diseases for which control techniques are relatively inadequate.

#### 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 21 titles have been added to the list of publications and abstracts in Appendix I.

##### Anaplasmosis:

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

Comparisons of available vaccines, including the attenuated organism and the killed adjuvant vaccine, have shown them to be unreliable when used in uninfected cattle moved into the highly endemic area of the north coast (Monteria) of Colombia. Admittedly, the conditions of challenge and secondary stress factors were extreme, but under these conditions no advantage was seen in using adjuvant vaccine, and only slight value was associated with the attenuated organism.

- b. To develop a more effective killed vaccine.

One study was made this year in which a vaccine, employing Freund's complete adjuvant (FCA), was compared with the commercially available adjuvant vaccine. The FCA vaccine was clearly responsible

for a more marked immunologic response based on the response of vaccinated calves to challenge.

Trials to wash out or remove red blood cell stroma from antigenic material have been partially successful on the basis of complement-fixation activity. The mass of antigen was significantly reduced by washing without affecting the total antigenic units.

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

An experiment (Monteria #3) conducted on the north coast (Monteria-Turipana) has clearly demonstrated the value of premunition or calf vaccination in susceptible calves being introduced into an infected herd. A total of 24 Holstein calves free of Anaplasma and Babesia were divided into 3 groups of 8 calves each. The average weights of calves in each of these 3 groups were not significantly different at the onset of the experiment. Group 1 calves were premunized with both Anaplasma and Babesia. Group 2 calves were treated with Imidocarb at the level of 5 mg/kg to serve as a chemoprophylactic agent. Group 3 animals served as non-vaccinated and non-treated controls.

The comparison of Groups 1, 2, and 3 dramatically showed the advantage of premunition. All calves in Group 1 had gained a total of 816 pounds at 6 months, and 2,040 pounds at 10 months. In Group 2 the 6 month gain was 688 pounds and the 10 month gain 1,640 pounds. In Group 3 the 6 month gain was 48 pounds and the 10 month gain 602 pounds. Based on a \$0.25 per pound value at 10 months, Group 1 had made \$510.00, Group 2 \$410.00, and Group 3 \$150.00. None of the calves in Group 1 died; 1 died in Group 2, but 4 or 50% of the calves in the control group

died as the result of hemotropic infection.

A similar study was made on calves native to the endemic area (Monteria). The premunized or vaccinated group failed to show any marked advantage when compared to the non-vaccinated controls. This finding was not unexpected since previous survey work has shown calves born in this area become exposed very early in life, probably while maternal antibodies are still persistent. Such infections are usually mild and often go undetected. Under these conditions, vaccination or premunition would obviously be redundant. A field study of several ranches in the Monteria area verifies this assumption. On two such ranches the average age at which infection occurred ranged from 36 to 90 days.

Much of the area in Colombia lies in zones outside this endemic area where Anaplasma and Babesia are sporadic in occurrence. In this situation as high as 60% to 70% of the calves may fail to obtain a naturally occurring exposure, and then be exposed later in life when they become highly susceptible. Under these circumstances, calfhood vaccination appears to be indicated. Based on this reasoning, relatively large-scale premunition efforts are being planned for the coming year for the control of both anaplasmosis and babesiosis in the intermediate areas.

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

A study was completed in Colombia in which three Anaplasma isolates were tested for possible antigenic variations. One isolate was obtained from Texas and represented the organism that has been

maintained for years in splenectomized calves and used routinely for anaplasmosis research. The second isolate was obtained from an infected sheep. This strain supposedly was an attenuated organism, but the pattern of infection in splenectomized calves failed to support this assumption. Isolate number three was obtained from naturally infected cattle in the Monteria area. Splenectomized calves were infected with one of the three isolates. The pattern of virulence was monitored. After the acute episode and when the infections had entered a relatively quiescent phase, cross infections were induced to determine immunologic similarities of these isolates (two from the U.S. and one from Colombia). The results showed little or no significant difference in the initial response to infection between the three isolates. No immunologic differences were detected following cross challenge. Calves premunized with the Texas isolate were immune to challenge from the Colombian isolate and vice versa. Based on this experiment, we may reasonably assume that if antigenic differences do exist they are minor in so far as immunogenicity is concerned.

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

Three tick species have been investigated for possible involvement in the transmission of anaplasmosis: Amblyoma americanum, A. cajennense, and Boophilus microplus. No evidence of transstadial transmission occurred with A. americanum and A. cajennense. Transovarial trials were not made. Boophilus microplus larvae were fed

through the nymphal stage on a calf with anaplasmosis. Engorged nymphs were removed, allowed to molt, and placed on a susceptible splenectomized calf. Anaplasmosis occurred 16 days after ticks were released. A calf not infested with ticks did not develop anaplasmosis. Only one transovarial transmission effort was made; this proved to be negative.

- g. To evaluate therapeutic compounds for treatment.

Imidocarb (4A65) is still the most encouraging single drug for use against Anaplasma and Babesia. It has produced consistent results in eliminating Babesia when used in low levels. In the treatment of anaplasmosis, it is less consistent, and must be used in higher dose ranges. As reported last year, some evidence of toxicity was detected in cattle treated with therapeutic levels.

With this in mind, rather comprehensive studies of drug toxicity and tissue residue were undertaken.

A total of 20, 10-12 month old, calves were divided into 4 groups of equal size. Groups 1, 2, and 3 were treated with 5, 10, and 20 mg/kg I.M., respectively, twice at a 14-day interval. Group 4 served as a non-treated control. The LD<sub>50</sub> in calves was calculated 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. No significant alterations occurred in weight gains, leucocyte counts, and serum proteins. Increasing quantities of Imidocarb were associated with increases in blood urea nitrogen, increases in serum glutamic oxalacetic transaminase, and a left shift in

neutrophilic leucocytes.

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 accentuated hepatic lobules. The most prominent histopathological lesions were acute tubular necrosis of the proximal convoluted tubules of the kidney and acute periacinar hepatic necrosis.

A similar study in Angora goats showed toxic levels to be about 6.75 mg/kg.

A third study, financed by Burroughs Wellcome Laboratories, was made of the toxic effects of Imidocarb in horses. A complete pathologic study was made on 20 horses receiving 2 I.M. injections of Imidocarb at a 24-hour interval of varying amounts, ranging from 2 to 32 mg/kg in twofold increments. The LD<sub>50</sub> was determined to be 16 mg/kg. Deaths were attributed to renal and hepatic failure.

Rather extensive tissue residue studies have been made in cattle and sheep treated with Imidocarb. Following tissue extraction techniques, the level of Imidocarb is determined by spectrophotometric methods. Blood Imidocarb levels were found to persist for days and possibly weeks, but do disappear in a few weeks. Tissue levels, however, were very persistent. The Anaplasma carrier status was removed from an aged cow after treatment with 5 mg/kg Imidocarb given 2 times at 14-day intervals. This cow was slaughtered 9.3 months after the last treatment, and tissues tested for residues.

No detectable levels were found in plasma, whole blood or kidneys. The heart showed 4.5 ppm, skeletal muscle 3.3 ppm, liver 9.4 ppm, and spleen 2.3 ppm.

Results to date with Imidocarb suggest that it may have a very useful place in the treatment and prophylaxis of babesiosis, but toxicity and tissue residues following its use in levels required for anaplasmosis therapy presents serious problems for which a ready answer is not yet available. For this reason recent studies have been initiated combining Imidocarb and Gloxazone for the treatment of anaplasmosis, in the hope that Anaplasma sterilization can be achieved at reduced levels of both drugs. Results to date are inconclusive.

Babesiosis:

- a. To develop and evaluate immunologic techniques.

The technique of premunization appears to offer the greatest probabilities for success. The results of trials on the north coast of Colombia with respect to premunition against anaplasmosis and babesiosis have been previously discussed. Babesia premunition will form a part of the calf vaccination field trial previously mentioned.

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

On two recent occasions, the presence of babesiosis in Texas has first been detected by serologic tests and then confirmed by calf inoculation. Both of these outbreaks were associated with Boophilus tick infestations. The threat of this disease to the U.S. is

stimulating added support for further investigations in this area.

We have been called upon on several occasions to perform service testing on serum from cattle suspected of having babesiosis. Samples from Haiti, Mexico, Nicaragua, and the Mariana Islands have been tested. Serologic evidence of Babesia and Anaplasma have been found in each instance.

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

We have not been actively engaged in this area, but contact with USDA and Texas Animal Health Commission personnel have shown their program of tick eradication to be progressing satisfactorily in Texas. Large areas previously quarantined, because of the presence of Boophilus microplus, have been released from quarantine as the result of a consistent and thorough tick eradication program. This has been accomplished in the presence of large deer herds which are known to be capable of harboring the tick. This success suggests that a similar approach might be feasible on selected farms in Colombia.

Tick colonies, including Boophilus microplus, Boophilus annulatus, Anocentor nitens, Amblyoma americanum, and Amblyoma cajennense, are being maintained for vector work.

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

Two field trials, one in Haiti and the other in the Mariana Islands, have shown Imidocarb to be a useful compound in temporarily preventing or moderating the onset of Babesia infection. A group of 44, 10-18 month old, cattle negative for both Anaplasma and Babesia

were shipped from Texas to Haiti. Upon arrival all cattle were inoculated with Imidocarb S.C. at the rate of 3 mg/kg. Four months later, all cattle were alive even though carrying a heavy Boophilus tick load. Most were showing evidence of having had Anaplasma and Babesia infection. Treatment was probably responsible for reducing death losses.

A group of approximately 750 dairy type heifers were moved from New Zealand to Tinian Island, one of the Mariana chain. Prior to shipment, they were vaccinated with a killed Anaplasma vaccine, and upon arrival at Tinian were injected with 3 mg/kg Imidocarb. This treatment apparently afforded good protection for about five weeks in the face of severe tick challenge. Death losses began to occur after five weeks and a second injection of Imidocarb was made. Deaths did not exceed 1%. After the second treatment a marked relief from the effects of babesiosis was noted. This experiment is still underway and additional data will be forthcoming.

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

Babesia complement-fixation tests have been conducted on five intact, Babesia-infected cattle over an 8-month period. To date all but one have become negative to the CF test even though they have been shown to be injected by sub-inoculation. These cattle became positive an average of  $10.6 \pm 2.9$  days after exposure and retained a positive titer  $130 \pm 71$  days after exposure.

Animal Number	First CF Pos. (in days)	Last CF Pos. (in days)
361	11	130
496	11	74
497	11	116
498-500	14	83
543	<u>6</u>	<u>&gt;251</u>
	10.6 ±2.9	130 ±71

In addition to the Babesia CF and rapid card agglutination tests, an indirect fluorescent antibody test system has been developed. It is hoped that this system will overcome some of the inherent difficulties of the CF test and be sufficiently sensitive to detect infection beyond the limits of the CF test.

- 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 or other suitable in vitro systems are continuing. Experiments have been carried out using bovine lymph node, hemalymph node, spleen and kidney cells in culture. The results were negative. Since the B. bigemina parasite normally grows in the erythrocytes of the bovine, it was decided to explore the possibility of erythrocyte suspensions as a suitable media in which to grow Babesia. Normal erythrocytes have been successfully maintained for 7 to 10 days, a time which should be long enough to support growth. To date there has been no evidence of

Babesia penetration or multiplication in the erythrocytes in vitro.

Experiments conducted on the micro-environment of in vitro erythrocytes does point to three specific areas where better control would be useful. These areas are the acid base balance, blood gas concentrations of oxygen and carbon dioxide, and specific nutrients, mainly glucose. Efforts to better control these factors are in progress.

Theileriasis:

A Theileria resembling T. mutans has been isolated from cattle native to Texas on two occasions. These two isolates are morphologically indistinguishable from each other, and closely resemble T. parva and T. annulata, both highly pathogenic for cattle.

Initially the isolates were contaminated with A. marginale. Treatment of dually infected calves with Imidocarb and Imidocarb-Gloxazone combinations successfully eliminated Anaplasma leaving a pure infection of Theileria. Passage of Theileria in intact calves fails to produce evidence of pathogenicity, and the organism is observed in blood films only sporadically. In splenectomized calves, parasitemias as high as 13% have been observed, associated with a moderate drop in PCV. Cattle with Theileria infection do not react with the CF test.

Means of natural transmission are not known. Efforts at transstadial transmission using A. americanum and A. cajennense have been unsuccessful.

## RESEARCH DESIGN

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

It is intended that there be four staff positions in Colombia, with about one-third this number in man/hour equivalents at Texas A&M.

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 of plans and detailed procedures for field use of preimmunization as a calfhood vaccination program to protect cattle in the intermediate zone are 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 as 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, 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.

As of August 1, 1974, Dr. Galvin will return to Veterinary Parasitology at Texas A&M and will be replaced by Dr. R. A. Todorovic. Dr. Adams will remain half-time in Pathology and half-time ITVM. Dr. Kuttler is half-time Microbiology and half-time ITVM. Dr. Maurer will be one-fourth time under this project and three-fourths time on the 211-D project (effective September 1, 1974).

In the coming year it is planned that Drs. Todorovic, Corrier, Thompson, and Hopps will be stationed in Cali. Drs. Wyss and Craig may be assigned to Colombia late in the funding period. Drs. Adams and Kuttler will remain at Texas A&M on a half-time status.

State and industry support of activities at the Institute of Tropical Veterinary Medicine has been significant during the past year, amounting to about \$34,000.00. We have received funds totaling about \$25,000.00 from Burroughs Wellcome Laboratories for continued research on Imidocarb. Project proposals have been submitted to the Texas Agricultural Experiment Station for support of both anaplasmosis and babesiosis research.

These proposals are in response to the recognized needs of the state of Texas for additional information on both anaplasmosis and babesiosis. The USDA has acquired property on the Mexican border for expanded Boophilus research and work is currently underway to construct facilities for this research. Use of this facility would greatly increase the scope of our research in Texas and thereby contribute directly to studies in Colombia.

A budget statement for 1973-74 outlining expenditures and obligations

for each of the major inputs and the major targets is given (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.

Work will continue on tissue culture growth of Babesia, and while as

yet unsuccessful it is hoped that future work will be more encouraging. Work will proceed using new therapeutic agents and combinations for treatment of anaplasmosis and babesiosis. Increased emphasis will be placed on the production of a more purified killed Anaplasma vaccine.

A budget statement for 1974-75 showing planned expenditures for each of the major inputs and the major work targets is given (Appendix III).

**APPENDIX I**

**Institute Staff Publications  
1968 - 1974**

## 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, 39-49.

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

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

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

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, (December 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 (submitted).

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 (submitted).

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.

KUTTNER, 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.

KUTTNER, 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.

KUTTLER, 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 (Estreptotricosis cutanea) en Colombia. Revista ICA, (March 1970), 5, (1): 3-16.

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

KUTTNER, 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.

KUTTNER, K. L., ADAMS, L. G. and ZARAZA, H.: Estudio Epizootiologico de Anaplasma marginale y 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. G. and GONZALEZ, E. F.: 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, (abstract).

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

Experiments were carried out at the Palmira Instituto Colombiano Agropecuario (ICA) experimental station in Valle del Cauca (altitude 1,000 meters) to produce co-infectious and sterile immunity against bovine babesiosis. Calves randomly selected were divided into 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 Bacti-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 cultural 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 radiation up to 60 kRad was inoculated into calves. Calves infected with  $1 \times 10^{10}$  B. bigemina parasitized erythrocytes exposed to doses up to and including 30 kRad developed progressive parasitemias. Some calves receiving  $1 \times 10^{10}$  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  $1 \times 10^{10}$  B. bigemina irradiated at 48 and 60 kRad was similar to the acquired resistance developed in calves inoculated with  $1 \times 10^{10}$  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 in non-fatal infections. The acquired resistance to infection with B. bigemina developed in calves inoculated with  $1 \times 10^{10}$  B. bigemina irradiated at 48 and 60 kRad was much greater than the acquired resistance to infection developed in calves inoculated with  $1 \times 10^{10}$  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.

KUTTLER, 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.

KUTTLER, K. L.: Promising Therapeutic Agents for the Elimination of Anaplasma marginale in the Carrier Animal. Proceedings of the 75th Annual Meeting of the USAHA, 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.

KUTTLER, K. L., GRAHAM, O. H. and JOHNSON, S. R.: Apparent Failure of Boophilus annulatus to Transmit Anaplasmosis to White-Tailed Deer (Odocoileus virginianus). Journal of Parasitology, (June 1971), 57: 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: Descripción 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 hemo-agglutination 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 Porcinas Atribuida a Aflatoxicosis. Revista ICA (accepted for publication).

Nine of the 56, 4- to 6-month-old Duroc male and female pigs died 2 months after consuming a ration consisting of 87.5% moldy peanut meal. The pigs exhibited weight loss, roughened hair coats, anorexia, lethargy, icterus, melena, increased followed by decreased rectal temperature and death. The livers of the remaining 45 pigs were condemned due to cirrhosis. Serum sorbitol dehydrogenase activities, glutamic-oxaloacetic transaminase activities, bilirubin concentrations, serum beta globulin levels, serum gamma globulin levels, and total serum protein concentrations were increased as serum albumin/globulin ratios, albumin levels, packed cell volume and hemoglobin contents were decreased. No changes were observed in total leukocyte counts or serum alpha globulin levels.

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

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 lachrymation. The increase in rectal temperature coincided with the onset of parasitemia while the packed cell volume decreased after the onset of parasitemia. The average incubation period and standard deviation was  $14.9 \pm 3.5$  days post-splenectomy. Treatment with 2-di-(Beta, gamma-dioxipropil)-(aminofenol)-(4 arseno 5)-Beta-(benzaxozalil)-(2)-mercaptol-propionato de sodio at 29 mg/kg intramuscularly caused the parasitemia to become undemonstrable within 24 hours with further recrudescence occurring within 6 weeks. This is the first report of bovine eperythrozoonosis due to 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  $3 \times 10$  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-ethoxyethyl-glyoxal dithiosemicarbazone) and 4A65 (3,3-Bis-(2-imidazolin-2-yl) carbanilide dihydrochloride). The third group of calves was used as a control. Calves selected for use at Monteria were not native to that area. All calves were subjected to natural exposure. Ticks were collected and identified at each site.

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

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

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

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  $2 \times 10^{10}$  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 hemoparasite, 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, (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 acaracides 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 premunizing 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. Proceedings of the 7th Panamerican Veterinary Congress, July 1973, Bogota, Colombia, 39-40, (abstract).

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  $\mu$ 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  $-79^{\circ}$  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 LD<sub>50</sub> 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 LD<sub>50</sub> at 14 days in calves receiving only one injection of imidocarb dipropionate was determined to be 15 mg/kg, and the LD<sub>50</sub> 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 periacinar 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 (accepted for publication).

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 (accepted for publication).

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., CAMP, B. J., DAVIS, R. H. and KUTTLER, K. L.: Studies on Imidocarb: I. Spectrophotometric and Thin-Layer Chromatographic Detection of Imidocarb in Biologic Specimens. Journal of Pharmacology and Experimental Therapy (submitted).

Imidocarb is a diamidine currently under evaluation for the treatment of babesiosis and anaplasmosis. Sensitive and highly specific spectrophotometric and thin-layer chromatographic methods for quantitative and qualitative determination of imidocarb in biologic specimens are described. Imidocarb is extracted under basic conditions from plasma, urine, milk, bile and homogenized tissue samples into organic solvents. Following extraction and concentration into acid, the drug strongly absorbs ultraviolet light in 3% HCl and can be qualitatively identified by thin-layer chromatography. Results can be obtained within 3-4 hours of receipt of a specimen and the detection limit for estimation of pure imidocarb in aqueous solution is 0.01 µg/ml, which is equivalent to a concentration of 0.05 µg/ml in plasma and 0.20 µg/g in tissue.

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  $1 \times 10^{10}$  B. bigemina parasitized erythrocytes exposed to 24 krad developed progressive parasitemias. Some calves receiving  $1 \times 10^{10}$  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.: Treatment of an Anaplasmosis Infected Herd Using Imidocarb (Dipropionate). (manuscript prepared for publication 1974).

A range herd of 469 cattle located in south Texas, near Laredo, was found to have an 82-88% Anaplasma infection rate. Treatment with 5 mg/kg imidocarb dipropionate (salt weight) was given each animal twice at a 2-week interval.

Treatment was followed by a marked drop in average complement-fixation (CF) titers, increase in average packed cell volume, and a complete disappearance of Anaplasma parasitemias. It is possible that infection may have been eliminated in as many as 75% of the animals treated, but a reinfection or recrudescence rate was such that a year later there was very little difference between pre-treatment incidence.

Treatment, while ineffective in eradicating infection, did show considerable therapeutic promise.

\* KUTTLER, K. L. and CRAIG, T. M.: Isolation of a Bovine Theileria. American Journal of Veterinary Research (submitted).

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 Research Capabilities. A 93 page report compiled as a member of a National Academy of Science Committee to render such a report for the use of USAID. (To be published June 1974)

\*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 Imido-carb 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.

**APPENDIX II**

**Protocol for the Premunition of Calves  
for the Control of Anaplasmosis and Babesiosis**

## APPENDIX II

### PROTOCOL FOR THE PREMUNITION OF CALVES FOR THE CONTROL OF ANAPLASMOSIS

A Colombian isolate which has been used in previous studies at Palmira will be inoculated into a splenectomized calf and at an optimum time, i.e., when the parasitemia is in excess of 20% and the PCV is in excess of 20, the calf will be exsanguinated. The blood for stabilate preservation will be collected in 12% sodium citrate at the proportion of 0.5 cc Na citrate to 10 cc blood, and prepared according to the following procedure:

1. Centrifuge at 2000 RPM for 10 minutes and remove plasma and buffy coat.
2. Wash cells in sterile phosphate buffered (pH 7.2) saline solution (PBS).
3. Centrifuge at 2000 RPM for 10 minutes and aspirate and discard PBS and remaining buffy coat.
4. Add equal volumes of 4 molar dimethyl sulfoxide (DMSO) to packed, washed erythrocytes.
5. Place 2 ml volume in rubber/metal stoppered serum vials (+5 cc capacity).
6. Place immediately in liquid nitrogen for minimum of 2 hours.
7. If space requirements dictate, vials can be stored later at approximately  $-70^{\circ}\text{C}$  (dry ice chest or Revco ultra-low temperature freezer).
8. Stabilates should be satisfactory for use for a minimum of one year.
9. Storage at higher temperatures may cause decrease in viability.

#### Directions for Use:

1. Thaw stabilate by placing vials in  $37^{\circ}\text{C}$  water bath. This should require not more than 5 minutes. For field use, care should be taken to

insure that temperatures in excess of 37°C are not used for thawing.

2. Dilute 1 cc of thawed stabilate with sterile PBS (pH 7.2, temperature less than 4°C), the exact volume of which is determined by previous titration (preferably 9 cc or 99 cc).
3. Maintain diluted stabilate at less than 4°C until used. Do not freeze.
4. Do not use diluted stabilate more than 1 hour after preparation.
5. Administer 2 cc of the diluted stabilate intravenously.
6. Premunized calves should demonstrate infection by CF titers and/or parasitemia.
7. Recovery is to be indicated when the PCV returns to 75% of normal, approximately 2 weeks after peak of infection. Babesia premunition should not proceed until the PCV indicates that recovery from Anaplasma premunition has occurred.

Titration of Stabilate for Field Use:

The frozen stabilate is to be characterized by titration in susceptible calves. Dilutions of  $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$  will be inoculated intravenously using 1 splenectomized and 2 intact calves for each dilution to determine incubation time and virulence.

It is preferable to use the greatest dilution which will consistently produce infection in both splenectomized and intact calves with an incubation time of less than 50 days. Infection is to be evidenced by parasitemia and/or serological response.

Criteria for Initial Herd Selection for Premunition to Control Anaplasmosis:

Premunition will be used only in calves 4 to 10 months old. Preferably the calves will be on a dairy where standard management practices include maintenance of calves in concrete-floored stalls until they are approximately 6 months old. Ideally, over 80% of the calves should be susceptible to anaplasmosis as determined by serological tests.

Approximately 20% of the susceptible calves must be maintained as nonpremunized controls. Adult cattle in the same herd should have a high percentage (greater than 40%) of positive carriers. There should be a tick population of sufficient numbers to expose the test calves to hemoparasites after they are changed from stable to pasture conditions. There should, however, be some form of tick control by spraying and/or management so that ticks alone do not constitute a serious health burden.

Following premunition, blood samples will be collected from all pre-munized and control calves at weekly intervals for 2 months, and at monthly intervals thereafter. Packed cell volumes, serologic titers, and parasitemias will be determined. The calves will be weighed 2 to 3 times upon termination of the study. The study has a scheduled duration of 3 years.

If natural infection of a significant number of control calves has not occurred within one year, approximately 20% of the premunized and control calves will be artificially challenged to determine effect of premunition.

At approximately 6-month intervals, as additional animals are born and attain a suitable age for premunition, the calves will be allocated to pre-munition or control groups and handled similarly to the first group.

Therapy:

If therapy is indicated, one injection of oxytetracycline will be given at the rate of 11 mg/kg body weight. Further injections will be given if needed. Treatment will be given if the PCV drops below 15.

Premunition of Adult Cattle:

If a decision is made to preimmunize adult cattle, it is suggested to vaccinate first with Anaplaz killed vaccine. After evidence of immunity is established, the adult cattle would be preimmunized with diluted stabilate as for calves.

NOTE: There are 3 major situations in which cattle are encountered in the tropics, each of which presents different problems in their protection against hemoprotozoal diseases:

1. indigenous cattle in enzootic areas;
2. the importation of hemoprotozoan-free cattle into enzootic areas;
3. cattle in the tropics which may not be exposed to infected ticks until they are so mature as to be seriously infected or killed by these diseases.

This initial trial is set up to prevent illness in the third category, so we are selecting herds that fit that situation, i.e., dairy cattle that are now so managed that they frequently are lost when first exposed to the disease. Later trials will be conducted with cattle in other typical situations. Each major situation will require some procedural modifications but, once arrived at, methods will be established for each of the 3 types of

situations so that it will then be possible to preimmunize large numbers of cattle with relatively little surveillance to assure a satisfactory protection at low cost.

PROTOCOL FOR THE PREMUNITION OF CALVES  
FOR THE CONTROL OF BABESIOSIS

Colombian isolates of Babesia bigemina and Babesia argentina which have been used in previous studies at Palmira will be inoculated into separate splenectomized calves. At an optimum time, i.e., when the parasitemia is in excess of 5% for B. bigemina and 2% for B. argentina and the packed cell volumes are above 10, the calves will be exsanguinated. The blood for stabilate preservation will be collected in 12% sodium citrate at the proportion of 0.5 cc Na citrate to 10 cc blood, and prepared according to the following procedure:

1. Centrifuge at 2000 RPM for 10 minutes and remove plasma and buffy coat.
2. Wash cells in sterile phosphate buffered (pH 7.2) saline solution (PBS).
3. Centrifuge at 2000 RPM for 10 minutes and aspirate and discard PBS and remaining buffy coat.
4. Add equal volumes of 4 molar dimethyl sulfoxide (DMSO) to packed, washed erythrocytes.
5. Place 2 ml volumes in rubber/metal stoppered serum vials (+5 cc capacity).
6. Place immediately in liquid nitrogen for minimum of 2 hours.
7. If space requirements dictate, vials can be stored later at approximately  $-70^{\circ}\text{C}$  (dry ice chest or Revco ultra-low temperature

freezer).

8. Stabilates should be satisfactory for use for a minimum of one year.
9. Storage at higher temperatures may cause decrease in viability.

Directions for Use:

1. Thaw stabilate by placing vials in 37°C water bath. This should require not more than 5 minutes. For field use, care should be taken to insure that temperatures in excess of 37°C are not used for thawing.
2. According to previous titration, the stabilates should be injected undiluted or diluted with sterile PBS (pH 7.2, temperature less than 4°C).
3. Maintain diluted stabilate at less than 4°C until used. Do not freeze.
4. Do not use diluted stabilate more than 1 hour after preparation.
5. Administer intravenously suitable volumes of each stabilate as indicated by titration.
6. Premunized calves should demonstrate infection by CF titers and/or parasitemia.

Titration of Stabilate for Field Use:

The frozen stabilates are to be characterized by titration in susceptible calves. Suitable dilutions of each stabilate will be injected intravenously using 1 splenectomized and 2 intact calves for each dilution of each stabilate. Dilutions will be made using an assumption that a minimum infective dose is approximately  $10^6$  viable organisms. For subsequent use in premunition, a solution which will produce infection (and subsequently immune carriers) but

not clinical disease will be selected.

The incubation period should be 5 to 8 days for B. bigemina and 6 to 12 days for B. argentina. Infection is to be evidenced by parasitemia and/or serological response.

Criteria for Herd Selection for Premunition to Control Babesiosis:

Premunition will be used only in calves 4 to 10 months old. Preferably the calves will be on a dairy where standard management practices include maintenance of calves in concrete-floored stalls until they are approximately 6 months old. Ideally, over 80% of the calves should be susceptible to babesiosis as determined by serological tests.

Approximately 20% of the susceptible calves must be maintained as nonpremunized controls. Adult cattle in the same herd should have a high percentage (greater than 40%) of positive carriers. There should be a tick population of sufficient numbers to expose the test calves to hemoparasites after they are changed from stable to pasture condition. There should, however, be some form of tick control by spraying and/or management so that ticks alone do not constitute a serious health burden.

Following premunition, blood samples will be collected from all pre-munized and control calves at weekly intervals for 2 months, and at monthly intervals thereafter. Packed cell volumes, serologic titers, and parasitemias will be determined. The calves will be weighed 2 to 3 times when premunition is being initiated, at monthly intervals during the study, and 2 to 3 times upon termination of the study. The study has a scheduled duration of 3 years.

If natural infection of a significant number of control calves has not

occurred within one year, approximately 20% of the premunized and control calves will be artificially challenged to determine effect of premunition.

At approximately 6-month intervals, as additional animals are born and attain a suitable age for premunition, the calves will be allocated to premunition or control groups and handled similarly to the first group.

Therapy:

If therapy is indicated, one injection of Ganaseg will be given intramuscularly at the rate of 0.5 mg/kg body weight.

**APPENDIX III**

BUDGET STATEMENT FOR PAST YEAR  
(1973-1974)

Statement of Expenditures According to Major Work Goals

	Anaplasmosis 34%	Babesiosis 45%	Trypanosomiasis 20%	Theileriasis 1%	Total (100%)
Personnel Salary & Allowances	78,420.00	103,790.00	46,129.00	2,306.00	230,645.00
Travel and Transportation	9,188.00	12,161.00	5,405.00	270.00	27,024.00
Equipment	4,341.00	5,746.00	2,553.00	128.00	12,768.00
Supplies and Operation	15,307.00	20,259.00	9,004.00	451.00	45,021.00
Overhead	19,529.00	25,848.00	11,488.00	574.00	57,439.00
<b>Total</b>	<u>\$126,785.00</u>	<u>\$167,804.00</u>	<u>\$74,579.00</u>	<u>\$3,729.00</u>	<u>\$372,897.00</u>

BUDGET STATEMENT FOR COMING YEAR  
(1974-1975)

Statement of Expenditures According to Major Work Goals

	Anaplasmosis 40%	Babesiosis 40%	Trypanosomiasis 15%	Theileriasis 5%	Total (100%)
Personnel Salary & Allowances	78,839.00	78,839.00	29,565.00	9,855.00	197,098.00
Travel and Transportation	10,672.00	10,672.00	4,002.00	1,333.00	26,679.00
Equipment	1,292.00	1,293.00	485.00	162.00	3,232.00
Supplies and Operation	27,864.00	27,863.00	10,449.00	3,483.00	69,659.00
Overhead	27,293.00	27,293.00	10,235.00	3,412.00	68,233.00
<b>Total</b>	<u>\$145,960.00</u>	<u>\$145,960.00</u>	<u>\$54,736.00</u>	<u>\$18,245.00</u>	<u>\$364,901.00</u>