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TECHNICAL ASSESSMENT REPORT

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AGRICULTURAL RESEARCH UMBRELLA
SUBPROJECT No. 4

INTRACELLULAR BLOOD PROTISTA WITH PARTICULAR REFERENCE TO IMMUNOPROPHYLAXIS AND CONTROL

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EXECUTIVE SUMMARY

The purpose of the subproject was to strengthen the capability of Indian Veterinary Scientists to conduct research on arthropod-borne blood diseases by utilizing modern immuno chemical, cultural and biotechnological technics. The main objective was the development and implemention under laboratory and field conditions of immunoprophylactic and diagnostic measures designed to prevent and control three major tick transmissible protistan blood diseases of food producing animals such as cattle, sheep and goats and working animals such as horse. In order of their importance to the livestock industry of India these diseases are theileriosis, caused by theileria annulata, babesiosis caused by Babesia bigemina and B. Bovis, and anaplasmosis caused by Anaplasma marginale.

For the achievement of its objectives the subproject utilized modern laboratory methodologies including recent advances in cellular engineering and conducted studies in an atmosphere of mutual collaboration between Indian and U.S. scientists. An integral component of the research phase of this program was an advance training for selected Indian scientists in the fields of immunology, sero-epidemiology, in vitro cultivation techniques and novel vaccine formulation procedures for blood protistan agents offered at appropriate institutions in the U.S.A. and elsewhere.

The project was designed to utilize in an unified manner all available Indian scientific talent and experience in the field of blood protistan diseases. Based on the information gained from the review of scientific literature and that gathered by the officials of the Indian Council of Agricultural Research and supplemented by an U.S. consulting service the work under this subproject was assigned to six principal Indian institutions. These institutions are : the Department of Medicine, Haryana Agricultural University, Hissar; the Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, U.P.; the Animal Disease Diagnostic Laboratory, National Dairy Development Board, Anand, Gujarat; the Department of Immunology, Punjab Agricultural University, Ludhiana, Punjab; Department of Parasitology, Tamil

Nadu Agricultural University, Madras Veterinary College, Madras and the Department of Parasitology, Bihar Veterinary College, Patna.

Detailed project objectives were developed for each participating station.

The U.S. input into the program was in three areas. These are : provision of scientific advisory service, sponsoring advance training for Indian scientists selected from the six participating stations and provision of suitable equipment and specialized supplies. The contribution of the Government of India into subproject covers several essential areas of research programs of the respective network stations. These inputs include salaries and allowances for the participating scientists to work on the subproject, funds for participation in the annual workshop, some equipment and supplies, and in-country travel and communication.

Although this subproject was sanctioned in 1985 it did not become operational until 1987. Hence, the progress made during the 3-year period has been considerable. Vaccines for theileriosis have been developed and successfully tested under laboratory and field conditions at four participating stations. The vaccine of one of these stations is being commercially marketed. The University of Illinois technology for production of babesiosis vaccine was successfully implemented at one station. A controled vaccination and challenge experiment produced positive results. Similarly, introduced technology for the production of anaplasmosis vaccine is under development. Field applicable serodiagnostic procedures for each of the three diseases have been developed and used for epidemiologic studies and follow-up of immune responses to vaccines.

The U.S. contribution has been very important particularly in the provision for advance training of Indian scientists and consulting services. Some of the requested equipment is in place and the remaining should be delivered before the termination of the program in early 1992.

The ultimate goal of this subproject has been the development of a triple vaccine in order to provide for a simultaneous protection against all three diseases. The work thus far done clearly points to the fact that such vaccines must be administered very early in the life of recipient animals, namely before their natural exposure to the three pathogens. It is therefore recommended that an arrangement be made to test the application of a triple vaccine during the remaining period of the subproject. These trials should be conducted on at least 4 geographic locations using calves 2 to 6 months of age. Theileria and Babesia vaccines should be produced in India and this consult should make arrangements for provision of a modified-live Anaplasma vaccine from the U.S.A.

I. SUBPROJECT DESCRIPTION

A. Purpose

1. Statement of Purpose

The purpose of this subproject is to strengthen the capability of the Indian Veterinary Science system to conduct research by utilizing modern immunochemical and cultural techniques toward the development of control measures for arthropod-borne blood protistan diseases.

These diseases exist throughout India because the widely prevalent transmitting agents, i.e., ticks, horse flies and other arthropod vectors. It is estimated that more than 200 million ruminants are at risk due to blood protistan diseases. In order of their importance to the livestock industry of India, these diseases are theileriosis, babesiosis, and anaplasmosis. Aside from heavy mortality, these diseases retard the growth and impair milk production in animals which recover from acute infection or those with the subclinical form of infection. The three

diseases also affect the small food producing ruminants such as goats and sheep. Those animals which provide sizeable amount of milk, meat, wool and hide, are indispensable for the livestock production programs of India. Consequently, these diseases constitute an important hinderance to the animal food production programs of India.

2. Linkage to Higher Goal

The Government of India had launched several projects designed to introduce the germ plasm of high yielding *Bos taurus* into indigenous breds in order to augment their milk production potential. Consequently, the value of cross-breed animals has increased and it has become an asset to the economy of a farmer and livestock owners in general, and is preceded only by the investment in crop production. The landless and marginal farmers are being encouraged under a 20-point program of development, to purchase highly producing cross-bred animals in order to enhance their economic condition.

Accordingly, the development of control measures for blood protistan diseases of food producing animals by use of novel biotechnological methods, such as *in vitro* collection of protistan agents and generation of protective antigens coupled with the application of modern animal husbandry methods should signal the arrival of the "White Revolution" as a follow up of a successful achievement in the "Green Revolution", the latter being considered among the most important products of agricultural research in this century.

B. Specific Objectives

1. Statement of Problems/Constraints

The objective of this subproject is directed

toward the development of immunoprophylactic and diagnostic methods for the prevention and control of leucocytic and erythrocytic protistan diseases transmitted by arthropods, primarily ticks. The program is divided into two closely integrated aspects. The first aspect covers the research of the above agents and the disease they cause in their respective hosts. The second aspect is concerned with the training of selected Indian scientists on the use and application of modern biotechnology and cellular engineering toward the development of control measures for blood protistan diseases.

The three blood protistan diseases and their causative agents are among the oldest known. Babesia was first recognized by the end of the 19th century, while such evidence for Anaplasma and Theileria goes back to the beginning of this century.

The great economic impact on the bovine industry exerted by diseases caused by these agents had been clearly recognized for many decades, yet the progress toward the development of control measures has been relatively slow. One of the principal reasons for this was the lack of available means for the study of these agents outside their natural hosts. The progress in the development of procedures for continuous in vitro cultivation of T. annulata and B. bovis took place during the last decade, but it is still lacking for A. marginale. However, selection of A. marginale mutant strain by adaptation of the virulent organism to growth in a non-bovine host resulted in the development of a modified-live A. marginale vaccine. The availability of these in vitro cultivation procedures and modified live A. marginale, coupled with the application of modern biotechnology and cellular engineering, has led to practical

advances in the control of theileriosis, babesiosis and anaplasmosis.

Aside from this strictly scientific constraint toward the rapid implementation of objectives, there are few other problem-contributing factors that should be considered. Modern research requires some limited provision of specialized equipment and supplies, particularly of chemical and immunochemical reagents. Most of these are readily available in the U.S.A., but their procurement and delivery to participating Indian institutions may not have been as efficient as one would anticipate. The communication and exchange of ideas among participating Indian stations is also a problem. Reasons given point toward the lack of funds for travel and communication; however, personality conflicts, research jealousies and other human relationship factors would also appear to have been a part of this shortfall.

2. Descriptive Statement of Objectives

The overall objective of this subproject is directed toward the development of immunoprophylactic and serodiagnostic methods for prevention and control of the three blood protistan diseases. The following research and training approaches have been used toward achievement of this objective.

a. Theileriosis vaccine

The schizont of *Theileria annulata*, the causative agent of theileriosis has been subjected to continuous *in vitro* propagation using bovine lymphocyte cell cultures. This system provided for attenuation of the virulence of the organism. Hence, high *in vitro* culture passages of the organism rendered avirulent organism useful for vaccination of susceptible cattle.

b. Babesiosis vaccine

The vaccine against babesiosis uses the technology developed by this consultant and his co-workers (see Section C). In short, it is an exoantigen product, a soluble glycoprotein derived from the surface of *in vitro* propagated *Babesia bovis* and *Babesia bigemina* parasites, the most important causative agents of bovine babesiosis. This parasite-free soluble antigen is highly effective immunogen-vaccine useful for prevention of bovine babesiosis.

c. Anaplasmosis vaccine

The consultant and his associates have developed a modified-live *Anaplasma marginale* vaccine by subjecting the virulent organism through continuous passages in an un-natural animal host, the sheep. This modified live *A. marginale* was found safe and effective as a vaccine for prevention of anaplasmosis in susceptible cattle.

d. Serodiagnostic methods

The application of the above vaccines is intimately associated with the availability of serodiagnostic tests useful for epidemiologic studies and a follow-up of immune responses to each of the above vaccines. To this effect tests, i.e. DOT-ELISA and Indirect fluorescent antibody (IFA) tests useful for detection and titration of antibodies to the above three agents are being used. Another test, the capillary tube agglutination (CA) test is being used as a field screening test for anaplasmosis and babesiosis.

The research under this subproject is being conducted at six Indian institutions. These are : Department of Medicine, Haryana Agricultural University, Hissar; the Division of Parasitology,

Indian Veterinary Research Institute, Izatnagar, U.P.; The Animal Disease Diagnostic Laboratory, National Dairy Development Board, Anand, Gujarat; The Department of Immunology, Punjab Agricultural University, Ludhiana, Punjab; The Department of Parasitology, Madras Veterinary College, Madras; and the most recent member of the subproject, The Department of Parasitology, Bihar Veterinary College, Patna, Bihar.

Training of Indian Scientists

The second aspect of the program is concerned with the training of Indian scientists, selected from the above six institutions, on the use and application of modern biotechnological methods toward the development of serodiagnostic and immunoprophylactic methods for control of blood protistan diseases.

C. Input/Output Matrices by Objective

1. Specification of Inputs

a. U.S. Inputs

The major U.S. scientific input into the program has been in the area of transfer to the project of a novel vaccinal technology for babesiosis and anaplasmosis.

Babesiosis vaccine

A major breakthrough in a century long struggle against bovine babesiosis has been achieved at the University of Illinois at Urbana. Researchers led by this consultant developed the first method of *in vitro* propagation of the causative agents of bovine babesiosis. They then found that babesial merozoites are surrounded by a surface coat antigen.

Furthermore, it was established that the surface coat was a glycoprotein essential for the attachment and entry of the parasites into erythrocytes but that following the entry, this surface antigen is left free in the supernatant of the culture medium. Consequently, glycoprotein material, termed exoantigen was shown to be highly immunogenic for induction of protective immunity to babesiosis. This novel technology has been extensively tested in many regions of the world, put into commercial production for Babesia canis vaccine in France, and introduced to India for the development of a bovine babesiosis vaccine.

Anaplasmosis vaccine

Unlike the above inactivated vaccine, the consultant and his associates have developed a modified-live vaccine for prevention of bovine anaplasmosis. The vaccine was developed by selection of an attenuated Anaplasma marginale mutant through continuous blood passages in sheep of the virulent anaplasma organism. This adaptation by a continuous passage of the organism in the atypical host, the sheep, over a period of 8 years, resulted in the development of a stable attenuated organism useful for prevention of anaplasmosis. This vaccinal organism has been made available to the subproject.

Advisory Services

To this effect U.S. scientists with advanced knowledge and experience in immunological, pathological and molecular aspects of arthropod-borne diseases are visiting the subproject and are providing consulting services to scientists and administrators of the respective participating stations. Some of these visitors are also providing short courses on

specific subjects of relevance to research on blood protista. Other visitors are taking part in the annual workshops of the subproject and are providing their criticism on the work completed and are making recommendations for future research.

Training of Indian Scientists

Selected scientists from the six participating stations have been trained in the U.S.A. The focus of this training was on the application of novel immunochemical techniques toward *in vitro* propagation of the causative agents of the three blood protistan diseases. The antigens generated by these means are then subjected to purification and quantitation and finally formulated in combination with adjuvants into vaccines. Upon termination of their training in the U.S.A., these Indian trainees are continuing similar studies in their own country using blood protistan agents of Indian origin. Aside from this formal laboratory training of approximately five months, other Indian scientists are making short-term visits to various institutions in the U.S.A. and elsewhere for briefings on new scientific developments and methodologies applicable to the field of hemotropic diseases.

Provision of Equipment and Supplies

Research equipment and supplies requested by the six stations is being delivered. It is anticipated that all equipment will be delivered before the end of the program in 1992.

b. Government of India

The contribution of the Government of India into subproject covers several essential areas of research programs of the respective network stations. These

inputs include salaries for participating scientists, funds for participation in annual workshops, in-country travel and some equipment and supplies. Principal investigators of each contributing station are requested to submit a research grant proposal to ICAR covering expenditures linked to the specific activity of their station. The proposal contains research objectives, methods of procedure for implementation of these objectives, and a supporting budget. The latter covers the provision for scientific staff, physical infrastructure and operating expenses. Each research grant proposal is reviewed by internal and, frequently external, scientific reviewers with established competence in specific research areas. Project leaders of each approved project are responsible for submitting annual progress reports to ICAR. These reports are also presented at the annual meetings of the subproject . This consultant is responsible for attending annual meetings and filing a report on accomplishments of the subproject and making recommendations for future research. This report is submitted for comments and approval to Winrock International, ICAR and AID.

2. Specification of Linked Outputs

A joint monetary, organizational and administrative effort between the U.S. Government and the Government of India, through their respective agencies, (AID and ICAR) provides for positive outputs that are of benefit to both countries. Strictly from the research point of view, the findings to be reported below should be of great practical and economic benefit to the livestock industry of India. Successful development and field application of a vaccine against theileriosis and preliminary evidence of a success with babesiosis vaccine would mean that

mortalities and morbidities of many thousands of cattle will be avoided which in turn will mean that many additional hundreds of thousands litres of milk will be made available to the consumers of India.

In addition, by training Indian scientists in the U.S.A. in the field of modern biotechnology, cellular engineering and immunochemistry and by their interaction and exchange of ideas with visitors from abroad, a new cadre of scientific prominence is being introduced into the veterinary and animal health prevention programs of India.

The benefits derived to the U.S.A. alone is best exemplified by giving an opportunity to the American scientists to observe and study a transmissible disease, i.e. theileriosis of food-producing animals that is not known to exist in the U.S.A. Infectious and vector-borne diseases do not always limit distribution and incidence to permanently established boundaries. The availability of U.S. scientists with prior knowledge and understanding of diseases that are currently strange to the U.S. may eventually be very important.

The second even more important benefit to the U.S.A. is the evidence that vaccines against babesiosis and anaplasmosis developed in the U.S.A. can effectively be implemented in a country with greatly different ecological, epidemiological and livestock management settings.

II. Input/Output Progress by Objectives

1. Assessment of Inputs Provided

a. United States

Material and academic input by the U.S.A. toward the achievement of the objectives of this program in

the areas of advisory services to the scientists of the six stations and training of Indian scientists in the U.S.A. has been generally appropriate and timely. Between 1987 and 1990 five U.S. scientists visited the subproject for periods of one to three and a half months. Their activities were concerned with offering lectures and seminars, reviewing research progress making recommendations for the future research and conducting workshops, i.e. one on monoclonal antibodies at IVRI during November 1988 and February 1989.

In the area of travel for Indian scientists to the U.S.A. for specialized training and briefings on recent progress on arthropod-borne blood diseases, eight junior and senior scientists visited the U.S.A. for periods of two weeks to five months.

The quality of input in both advisory services and specialized training has been good. However, the procurement and delivery of the equipment to the project has been of target. Many reasons have been given for these delays. Some of these are : need for updating specifications, checking of bids required much more time than anticipated, re-biding frequently needed, and suppliers not being prompt in providing proformas as projected.

b. Government of India

The input of the Government of India into individual research projects of the six participating stations has been generous and appropriate. The selection and the organization of individual research projects has been well planned and structured so that those fully complement the overall objectives of the subproject.

Aside from the formal research reports, visits of principal investigators to the ICAR have been

frequent, hence, productive by fully resolving certain administrative and research aspects of the project These visits also served to identify problem areas or constraints which may be inhibitory to the attainment of set objectives. Other issues concerned with physical infrastructure and a need for the accomplishment of research objectives. On other occasions representatives of the ICAR have visited respective stations for the purpose of discussing the above matters including the utilization of the operating budget of a given research project.

Under the circumstances and within the frame of available resources the GOI input into the subproject has been most appropriate, timely, and of good quality.

2. Assessment of Outputs Achieved

Based on research achievements by the subproject and the acquisition of academic and practical research knowledge by Indian scientists through advanced training in biotechnology (see below) the output by this joint venture must be considered productive. Blood protistan diseases being investigated by the subproject are not limited to India alone. These diseases are common to all countries in the tropical and semi-tropical regions of the world with some 700 million cattle being affected and threatened by these diseases.

Current status with reference to each of the three diseases is as follows :

Theileriosis

Four of the participating stations have produced and successfully tested T. annulata vaccines under laboratory and field conditions. This achievement of Indian scientists on bovine theileriosis has been

remarkable, hence, projecting these scientists as world leaders in the development of control measures for this disease.

At least one station has successfully utilized the vaccine technology developed at the University of Illinois. An important outcome at this study was the fact that the vaccine derived from cultures of Mexican Babesia bigemina strain effectively protected vaccinated cattle that were challenged with Indian virulent B. bigemina strain.

Anaplasmosis

Using Indian strain of A. marginale, one station is implementing U.S. technology toward the development of a vaccine for anaplasmosis.

Diagnostic Serology

Field applicable diagnostic tests for identification of antibodies to each of the three pathogens has been accomplished.

Other Outputs Achieved are :

Construction of Central Laboratory for Blood protista study is under an advanced stage in Hissar; A mini van was purchased at the Central Laboratory to be used for field investigations, collection of data and transportation of experimental animals and biological materials; An annual workshop was organized every year to review the progress of work done and to discuss the plan of work for the ensuing year. So far five annual workshops were held; A special workshop of the project was held to demonstrate a DOT-ELISA test for theileriosis developed by one collaborating station.

USAID provided for the following :

- Two workshops on "Hybricloma technology" and "Genetic engineering of Protozoan diseases" were held at the Veterinary Research Institute at Izatnagar.
- A team of three Indian scientists visited U.S.A. to identify institutions suitable for advance training of Indian scientists.
- Five Indian scientists visited U.S.A., each for three weeks duration to study recent advances in vaccine technology.
- Four Indian scientists were each trained for twenty weeks at the University of Illinois on the use of modern immunological and cultural techniques for study of blood protista and the development of vaccines against these agents.
- This consultant visited the program four times for the purpose of attending annual workshops of the project, to review the progress of work done and to discuss the research plan of work for the next year.
- Drs. Mike Buridge and Richard Dicrks also visited the programme for the similar purpose.

In the area of intellectual and academic advancement, a nucleus of well trained Indian scientists is being built capable of fully utilizing the most modern technology toward the elucidation and research management, not only of blood protistan diseases but also other vector-borne and tropical diseases that are of great economical and social importance not only to India, but Asia as a whole.

B. Progress Toward Objectives

1. Appropriateness of Objective Priorities

a. ICAR/SAU System

The two main objectives of the subproject originally agreed upon by the ICAR/SAU system are :

(1) the development of immunoprophylactic and diagnostic methods for prevention and control of the three blood protistan diseases (bovine theileriosis, babesiosis and anaplasmosis) and (2) provision for advance training of Indian scientists in the U.S.A. on use and application of modern biotechnology are highly complementary and appropriate for the solution of economic and social burden exerted by these diseases upon the Indian livestock industry. Consequently, the favourable research accomplishment by the subproject clearly evidence functional inter-relationship between these two objectives.

Specific accomplishments

Theileriosis vaccine : Major research accomplishments have been realized in the development of control measures for bovine theileriosis. Firstly, the government of India has issued a provisional license to the National Dairy Development Board (NDDB) for production and commercial marketing of a *Theileria annulata* vaccine. The vaccine, represents the 150th passage of *T. annulata* schizonts propagated *in vitro*. More than hundred of thousands of doses of the vaccine have been used thus far and the input from the field has been generally good. The recommendation regarding the use of the above vaccine is to be implemented on animals four months of age and older. However, the consensus of opinion of scientists from other collaborating institutions is that the morbidity and mortality caused by *T. annulata* are greatest among very young animals. Accordingly, these scientists feel that there is a need for a vaccinal procedure that can be effectively implemented on animals as old as two months of age. Accordingly, scientists at the HAU, IVARI and PAU are focusing, with good preliminary results, toward the development of a *T. annulata*

vaccine that would be particularly effective when administered to calves less than four months of age. This consultant and Dr. M.N. Malhotra of the I.C.A.R. have prepared a special experimental outline for a large triple vaccine field trials designed to elucidate this research approach during the remaining period of the project.

Babesiosis vaccine : The research with the B. bigemina exoantigen system for immunization purposes is underway at IVRI, and the results of some preliminary laboratory vaccination and challenge studies are very favourable. In a controlled experiment three susceptible crossbred cattle were vaccinated with exoantigens derived from cultures of Mexican B. bigemina and then challenged with a native (Indian) strain of this organism along with three non-vaccinated susceptible cattle. From the clinical point of view all three vaccinated animals fully resisted the challenge while three control animals developed typical clinical signs of acute babesiosis and died. This is a very meaningful result from two points of view. Firstly, it documents that the vaccinal technology developed in the U.S.A. is applicable to Indian livestock and secondly, it substantiates general scientific concept that B. bigemina antigens are identical in all parts of the world.

Similar laboratory vaccination studies are being initiated with B. bovis at HAU. At both of these locations scientists have been well trained at the University of Illinois on the use of culture-derived Babesia exoantigens as a means of immunoprophylaxis against bovine babesiosis.

Anaplasma vaccine : Work was initiated at HAU on attenuated, modified-live anaplasmosis vaccine

developed at the University of Illinois. The effort is aimed at the development of a similar sheep-adapted strain using Indian virulent A. marginale. For this purpose, 500 ml. of citrated blood from a calf having an Anaplasma infection was collected and inoculated in two calves with spleen in situ. One of these calves was later spleenectomised and the infection flared up in this calf. Forty ml. of blood from this calf was collected and exposed to gamma irradiation at 70 krads and then injected into two spleenectomised sheep at 20 ml. each. However, none of the two sheep revealed any Anaplasma in their periferal blood circulation. Further research efforts are underway.

Immunodiagnosis : A simple and highly specific immunodiagnostic test DOT.ELISA has been developed for epidemiological studies and monitoring of vaccine regimens against theileriosis. About 2000 serum samples have so far been collected from various livestock farms for screening by this test. Similar tests for use in babesiosis and anaplasmosis are under development. Also an Indirect fluorescent antibody (IFA) test has been effectively used for diagnosis of bovine theileriosis and babesiosis.

Particular antigen of Babesia equi has been prepared in accordance with a method developed by this consultant for diagnosis of anaplamosis. By use of this test called Capillary Tube Agglutination (CA) test 695 serum samples collected from Hissar and adjoining areas of Rajasthan and from the National Research Center on Equine research have been screened for prevalence of equine babesiosis. Amongst these 252 serum samples were found serologically positive.

Hybridoma cell culture system has been developed by fusion of mouse myeloma cells with mouse spleen B-lymphocytes primed to T. annulata piroplasm antigen.

Antibodies produced by these hybrid cells were detected by DOT.ELISA test.

Epidemiology : A prerequisite for any disease control programs is the knowledge of the epidemiology of such a disease. Approximately 13,000 blood films of cattle, buffaloes, sheep, goats, horses and dogs were examined for the presence of protozoan parasites. The study revealed the presence of the following pathogens in these animals.

Cattle	:	T. annulata, B. bigemina, B. bovis, A. marginale and Trypanosoma evansi.
Buffaloes	:	T. annulata, B. bigemina, A. marginale, T. evansi.
Sheep	:	T. hirci, B. ovis.
Goats	:	T. hirci, A. marginale
Horses	:	B. cabali, T. evansi
Dogs	:	B. canis, Hepatozoan canis, Ehrlichia canis.

Ticks collected from the above animals were also identified.

Chemotherapy : More than 600 clinical cases of theileriosis were successfully treated with a single dose of Buparvaquone (2.5 ml/kg body weight). At this dose rate the drug was found ineffective for treatment of equine babesiosis.

Long lasting oxytetracycline was found effective in the treatment of anaplasmosis. Supportive therapy was found to hasten the recovery process.

b. Indian System

Research accomplishments by the program initiated by ICAR/SAU and contributed to by AID are expected to

positively reflect on the Indian system as a whole and in particular on the progress and improvement in the area of food animal production. Foremost among animal food products is the milk which is considered a vital source of protein for the Indian population. The availability of methods for control of mortalities and morbidities among milk producing cattle would also serve as an impetus to the small and relatively poor farmers to engage in maintaining a few cows as a source of milk primarily for home consumption. Also, since bovine species are frequently used as working animals, the availability and maintenance of healthy cattle would mean an insurance when planning various seasonal farming activities that are essential for the well being of small farming communities. As the health risk for the purchase and maintenance of more costly crossbred cattle decreases due to this new prophylactic management, the livestock owners are expected to engage in more vigorous cattle purchases and in this way upgrade the quality level of Indian livestock industry. Finally, the private industry sector may become interested in commercializing T annulata vaccine if a more practical and economical vaccine delivery system can be worked out.

2. Importance of U.S. Interests

a. Current

One of the areas where AID on behalf of the U.S.A. can make an important contribution to the Government of India is agricultural production strategy, and agricultural research and education. During the 1950's and 1960's, U.S.A. Agricultural Universities, with support from AID and the Ford and Rockefeller Foundations, have already made a major contribution in this area when they were engaged in the development of India's agricultural universities

and research institutions. The current U.S. interest and investment is in a group of heterogenous subprojects with two common denominators, research and education in the area of agricultural commodities. Under this structural arrangement, the high pay-offs from the subprojects that are successful, as is the case with this subproject, is likely to be sufficient to make up for those that are not. Consequently, this collective positive output will serve to protect the importance of U.S. interests.

b. Future

This project has made considerable progress toward the achievement of its objectives. There is, however, much more to be done before the results of the current and future research can be put to practice for the benefit of Indian livestock programs. With the exception of minor difficulties and some delays the project has been proceeding smoothly and effectively. Accordingly, the project should be allowed to continue to its full completion. At the end of this project period, the Indian Agricultural Research institutions participating in this subproject would be expected to have acquired the capability to generate the scientific and technical advances required for a sustained growth in the area of vector-borne blood protistan diseases.

3. U.S. Role and Advantage in Cooperation

The Indian agricultural system (IAS) is receiving useful assistance from the world-wide donor community. The traditional assistance of AID to the IAS has been well recognized by way of its reputation and accomplishments record. India is the largest developing country with traditionally a well

established true democratic system. Agricultural production and commodities are among the major assets of India. In order to reap the full potential of new agricultural technologies, India's agricultural research efforts must be strengthened. For the U.S.A. as a leading industrial power, with highly organized and advanced agricultural system, the opportunity to assist technologically and otherwise Indian agriculture development is obviously an advantage and always a new scientific experience for participating U.S. scientists.

C. Progress Toward Purpose

1. Relevance

a. ICAR/SAU System

The key to the transition from traditional to science based agriculture is the establishment of indigenous capabilities for research and innovation. When ICAR/SAU system proposed the two objectives, the development of methods for the control of the three diseases and providing for advance training of Indian scientists they understood well that these two objectives are highly interdependent. Solving the disease problem is a practical matter of great socio-economic importance. However, building a cadre of young scientists capable of understanding and using modern biotechological tools does exceed the above as it provides an element of national security for dealing with future similar problems. Consequently, the progress toward the purpose with full relevance to the needs and anticipation of ICAR/SAU is being gradually fulfilled by the subproject.

b. Indian System

Direct reflection of achievements by the

subproject on Indian system has been discussed above under IIBb. The progress of the project toward overall purpose will obviously have more desirable and further reaching impact to the Indian system. New findings toward disease control generated by this subproject are bound to have a stimulating effect on the Indian Pharmaceutical Industry. It is conceivable that basic concepts on disease control developed by this project could be put into practical use by those skilful in the field of biological industrial science. Such a development would in turn produce a new and unique industrial activity resulting in bio-products which will be in demand not only by the Indian Livestock Industry but also in other countries which are faced with similar disease problems.

Education is considered a primary goal for every progressive nation. Through specialized advanced training and scientific interaction, selected Indian scientists have reached the level of great scientific competence and vision. These individuals and those yet to receive such an advanced training are expected to further disseminate their knowledge to a new generation of Indian scholars and in this way continue to strengthen the capabilities of the Indian agricultural research system. This occurrence is destined to have a long-lasting, positive impact on the Indian system.

2. <u>Mutuality of Interests</u>

a. <u>Current</u>

This and remaining subprojects under ARP deal with scientific areas identified by the Indo-U.S. Agricultural Subcommission as high priority for U.S. collaborative assistance. The actual aim of all subprojects is to enable India to benefit from

available technology in the U.S.A. This goal is being reached by collaborative research between the U.S. and Indian research scientists and their respective institutions.

Regardless of the exact reasons that prompted this collaborative effort, the fact is that it is mutually beneficial. Current benefits to each party are primarily academic as these provide an opportunity for scientists of one country to become familiarized with animal disease problems and research philosophies toward their solution of the other country. The remarkable follow-up of advance training of Indian scientists in the U.S. is that soon upon return to their home institutions, they started with practical implementation of the knowledge acquired in the U.S.A. However, this time they are using pathogens of Indian origin, crossbred animals typical of India, and laboratory facilities and epidemiological settings characteristic of their special region.

By virtue of a continuous scientist-to-scientist correspondence and collaboration, the U.S. counterparts are now benefitting by finding whether biological systems they developed are also applicable in a country with a different ecological and epidemiological settings.

b. Future

Based on the current pace of research, progress toward the development of preventive and control measures for the three blood protistan diseases, it is reasonable to hope that these measures will be developed. The implementation of these disease preventive measures will provide for greater production of milk and other food commodities and for more vigorous trading and purchases of crossbred

cattle. In turn, these developments should exert favourable economic effects on the livestock industry of India.

Since the U.S.A. has played an important part in the intellectual aspects of the project, it would be reasonable to anticipate that U.S. scientists will continue to be intimely associated with the program at the time of its field application. Also, the U.S. industry is expected to be associated with programs as a source of modern equipment and special reagents.

3. Follow-on Cooperation

a. Scientist-to-Scientist

Cooperative interaction of scientist-to-scientist between U.S.A. and India dates back to the 1950's and 1960's when AID was involved in the development of India's agricultural universities and research institutions. The modern and more sophisticated research subjects coqperatively introduced by this subproject will require more vigorous and more profound scientist-to-scientist interaction, planning and consultation. This scientific interchange will include provision to Indian colleagues of up-to-date scientific literature on specific research aspects and special immunochemical reagents, i.e., monclonal antibodies and conjugated specific immunoglobulins. In turn, Indian colleagues are expected to respond by providing their U.S. colleagues with the results and observastions made at laboratory and field studies in which the reagents and methodologies provided by the U.S. were used.

b. Inter-Institutional

Scientist-to-scientist interactions are frequently expanded to include inter-institutional cooperation and interaction. This is particularly

very likely to occur in this subproject where the need for graduate research and training is very strong. Indian veterinarians are expected to enter graduate programs in U.S. universities and interested scientists from these universities will be invited to visit the program in India. Also, it is likely that some institutional arrangements may be made whereby Indian graduate students would complete academic requirements toward their graduate degree in the U.S. and then follow with their thesis research at cooperating Indian institutions. This inter-institutional cooperation frequently leads to intelectual interactions and eventually submission of joint research grant proposals to national and international research granting agencies.