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R E P O R T

INTRACELLULAR BLOOD PROTISTA WITH
REFERENCE TO IMMUNOPROPHYLAXIS AND CONTROL

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Consultant Report by
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CONSULTING ACTIVITIES REPORT

By

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I. OBJECTIVES OF THE SUB-PROJECT :

The program of this sub-project is targeted towards the development of immuno prophylactic and diagnostic methods for prevention and control of leukocytic and erythrocytic blood protistan diseases transmitted by arthropods, primarily ticks. In addition to food producing animals being the main object of the study draught equine species (e.g. horses, mules, and donkeys) are also highly susceptible to blood protistan agents and suffer severe losses when infected by these organisms. Similarly, dogs which are important within the police and military forces are known to experience severe infections and mortalities due to protistan agents. Major pathogenes responsible for losses in cattle are: Theileria annulata, Babesia bovis, B. bigemina, Anaplasma marginale and Ehrlichia bovis. Those responsible for losses in equine species are: Babesia equi and B. caballi. Losses in dogs are caused by Ehrlichia canis and Babesia canis.

Modern research, including bio-technology and cellular engineering involving various antigens/antibody systems, is needed to develop control measures against above pathogenes. The work on the development of vaccine and field applicable diagnostic test for bovine theileriosis caused by Theileria annulata has made sizeable progress.

In fact, on the worldwide basis India has definitely assumed the leading role in the control of this disease. However, the research on the remaining protista is lacking behind. Over the years the consultant and his research team have developed novel field applicable methods for preventive and epidemiological studies of babesiosis, anaplasmosis, and ehrlichiosis. It is anticipated that by training Indian Scientists on the use of these novel research tools in the U.S. and in India the progress will also be made toward an effective control of diseases caused by the remaining blood pathogenes.

II. PARTICIPATING UNITS AND ADMINISTRATION OF THE SUB-PROJECT :

The study under this sub-project is being conducted at six Indian Institutions. These are: Department of Medicine, Haryana Agricultural University, Hisar; 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, Tamil Nadu Veterinary and Animal Sciences University, Madras; and the most recent member of the sub-project, the Department of parasitology, Bihar Veterinary College, Patna, Bihar.

The Coordinating unit and Central Laboratory for the sub-project are located in Hisar under the direction of Dr. Y. Bhattacharyulu.

III. SYNOPSIS OF MAJOR ACCOMPLISHMENTS :

There have been two major developments in the research of blood protista. Firstly, the Government of India has issued a provisional licence to NDDB for production and commercial marketing of Theileria annulata vaccine. The vaccine represents 150th passage of in vitro propagated T. annulata Schizonts. The vaccine is manufactured at Hyderabad, A.P. by Immunobiologicals, a subsidiary of NDDB. According to information from scientists at NDDB some 100,000 doses of the vaccine have thus far been sold. The input from the field has been generally good. The consultant visited a farm which in the past has experienced severe losses due to theileriosis and is currently using commercial vaccine and found the owner highly satisfied with the results of the vaccination program. Also letters from several other farms came to the attention of the consultant suggesting favorable opinion of the vaccine.

Secondly, scientists at P.A.U. led by Dr. A.S. Grewal developed DOT-ELISA, a field applicable serological test for detection and titration of serum antibodies to T. annulata. The test should be an important tool for epidemiological studies of theileriosis and a follow-up of vaccination trials.

Some other new findings include the occurrence of ehrlichiosis among cattle and military dogs in Tamil Nadu. The disease caused in military working dogs seems to be very virulent causing severe losses, hence requiring immediate attention. Several other stations including Tamil Nadu reported frequent occurrence of bovine anaplasmosis and babesiosis among their cattle. Trypanosomes have been also detected in cattle and horses in many regions of India.

IV. RECOMMENDATIONS :

It is recommended that scientists at NDDB alongwith their colleagues at Hyderabad carefully monitor the results of the efficacy and safety of the commercial vaccine during the next year. The Sub-project Coordinator should actively follow the results and report pertinent developments to the ICAR.

Scientists with advanced research on T. annulata vaccine at IVRI, HAU and PAU should continue with their experimental vaccination studies at laboratory and field levels. Pending favorable results of their studies they should be encouraged to initiate mass vaccination program in their respective states.

With these favorable developments toward control of theileriosis the scientists of the four stations: IVRI, NDDDB, HAU and PAU should initiate more intensive research on babesiosis caused by Babesia bigemina and B. bovis, B. equi, and B. caballi and anaplasmosis caused by Anaplasma marginale.

In view of the fact that two scientists Drs. S. Sarup of HAU and A.K. Mishra of IVRI have been well trained in the U.S. on bovine anaplasmosis and babesiosis, it is recommended that they rapidly develop DOT-ELISA for diagnosis of the above two diseases and then in collaboration with Dr. A.S. Grewal of PAU formulate a triple test useful for simultaneous detection of the three diseases. In the meantime Dr. Grewal should be contracted to produce reagents for T. annulata DOT-ELISA and these standardised reagents should be expeditiously made available to other five stations. Using these test reagents each recipient station should develop a plan for epidemiological study of theileriosis in their respective state. The plan of action should be submitted for approval to the program co-ordinator at Hisar. Quarterly progress reports on epidemiological studies should be made available to the Program Coordinator.

The two stations at Madras and Patna should initiate studies toward elucidation of inter-relation between the vector (tick) and the parasite. In addition, Dr. D.J. Chellappa of Madras Station will be trained in the U.S. on cultural and serological procedures for Ehrlichia canis. Upon his return he should initiate studies toward the use of a serologic test for detection of ehrlichial infections in cattle and dogs.

Finally, trypanosomal infections in cattle and horses seem to be on the rise in most regions of India. Accordingly, scientists at HAU and PAU should initiate studies toward serodiagnostic and chemotherapeutic methods for control of trypanosomal infections.

V. TRAINING FOR 1991 :

Training arrangements beginning mid April of 1990 call for three candidates to be trained for a period of 5 months and two for two weeks at the University of Illinois, at Urbana. The three candidates are: Drs. J. Chellappa of TNVASU, and D.V. Malhotra and R.D. Sharma of HAU. The two week candidates are Drs. Y. Bhattacharyulu and S. Dhar, the Co-ordinator and Epidemiologist of the sub-project at Hisar.

Discussions regarding future training of Indian Scientists were held at Winrock Int., ICAR. Coordinating Centre at Hisar and at all six participating stations. It was generally agreed that the productivity of the training can be enhanced by offering a training program for 1991 at the Coordinating Centre at Hisar. The benefit of such an arrangement would be that greater number of

trainees could be provided with modern technical knowledge of research on blood protista at a lower cost; the organisms used in these typical of India will be used; and laboratory facilities and epidemiologic settings will be representative of those generally found in India. The training would be provided at newly constructed Coordinating Centre Building in Hisar. The Vice-Chancellor of HAU has made firm commitment that the building will be ready for occupancy by August, 1990.

Other matters that must be taken care of if this proposal is to be materialised are:

- (1) Furniture for the building must be available at the time building is completed.
- (2) Needed equipment and supplies must be in place not later than November of 1990. The list of these will be provided.
- (3) Suitable intact and splenectomised animals, approximately 10 of each must be made available by January, 1991. These animals may be 5 to 8 months of age and be fully susceptible to infection with bovine babesias (B. bovis and B. bigemina) and Anaplasma marginale.
- (4) Two technically well trained individuals from the U.S. must take part in this training. One may remain in Hisar for a period of 2 months and the other for 4 months.
- (5) Taking leadership in this training should be Dr. S. Sarup of Hisar and A.K. Mishra of IVRI. Both of these individuals have undergone an intensive 5 month training at Illinois and they are fully versified and competent in all aspects of research on anaplasmosis and babesiosis.
- (6) Hisar has newly constructed housing facilities for International guest - The Vice-Chancellor of HAU assured us that these facilities will be available for the U.S. participants in the training program. This is a must condition for U.S. participants.

The overall responsibility for the organization of this training is in the hands of sub-project Coordinator Dr. Y. Bhattacharyulu. He must, however, be assisted in his efforts by the officials of Winrock, Int. and ICAR. A full compliance with the above described arrangements must take place if the proposed training is expected to be successful. If this initial training in India proved to be satisfactory, it should be continued by ICAR institutions in subsequent years.

Regarding future training of Indian scientists in the U.S. it is proposed that each year 1 to 3 very selected scientists be sent

to the appropriate Universities in the U.S. to take an advanced training in parasitologic immunology. The consultant will be happy to suggest the names of appropriate institutions.

Two more scientists who will be involved in fostering commercial-scale production of the vaccine would undertake short study tours (2 to 3 weeks to the U.S. They should visit commercial enterprises specialised in novel industrial procedures for mass production of vaccines and diagnostic reagents against blood protistant and similar agents. Again the consultant will be happy to suggest the names of such biological houses.

VI. REVIEW OF RESEARCH ACTIVITIES AT PARTICIPATING STATIONS :

1. Punjab Agricultural University (PAU)

a) Findings:

The consultant participated in the last day of the 3-day workshop on DOT-EIA for diagnosis of infections caused by Theileria annulata. The workshop was organised by the Principal Investigator Dr. A.S. Grewal and his staff. Some 25 participants attended this workshop representing the six stations and few other Universities.

DOT-EIA is widely used in the U.S. and some other countries for rapid detection of antibodies to variety of antigens and in this case antibodies of T. annulata. In contrast to the tube method DOT-EIA, the modification of this test developed by PAU researchers, uses comb type multiple spots dip sticks. The advantage of the latter method is conservation of reagents and fast processing of samples for mass screening. The test is simple, specific, sensitive, rapid, and cost effective having potential for application in sero-epidemiological survey, monitoring theileria vaccination, and even screening of hybridoma culture supernatants for antibodies to T. annulata antigens. The antigen used in the test is derived from erythrocytic Theileria piroplasms.

The test will be useful tool in the study of the following aspects of bovine tropical theileriosis: (1) Obtaining information on the incidence/prevalence of the disease primarily in crossbred cattle, (2) Defining the reservoir role of buffaloes and indigenous cattle in the epidemiology of T. annulata, (3) Monitoring vaccination trials at the laboratory and field level, (4) Establishing the rate of occurrence of new infections from season to season, and (5) Correlating the infection rate with the productivity of the animals.

The consultant alongwith the Vice-Chancellor, Dr. K.S. Gill took part in the ceremony at the termination of the workshop by handing over workshop certificates to the participants. In conversation with several participants of the workshop it was learned that all of them were feeling satisfied with personal use of the test. Also all of them indicated great interest in using the test in their own state.

The second aspect of study at PAU is the vaccination of cattle against T. annulata using in vitro cultured laboratory vaccination experiments have been accomplished experiment. For this purpose arrangements have been made with a nearby farm. The experiment will be of limited scope but well controlled. It will utilise some 20 vaccinated and equal number of control animals. All experimental animals will be determined to be susceptible to T. annulata by tests using the newly developed DOT-EIA. After vaccination and for a period of one year all animals will be followed serologically and haematologically in order to determine that the vaccine has established subclinical infection in innoculated cattle and also ascertain whether tick exposure took place in both groups of animals.

This consultant visited laboratories of Dr. Grewal in the Department of Immunology. On that occasion he met with two principal associates of Dr. Grewal, Avtar Singh, Ph.D and Miss. Jyotika Kapur, Ph.D. The latter individual appears to be a very impressive basic scientist well founded in the fields of biochemistry and immunology.

Prior to departure the consultant had one hour conversation with the Vice-Chancellor, Dr. K.S. Gill and Dr. S.S. Gill, Additional Director of Research (Veterinary and Animal Science) at PAU. Present were also Dr. S. Dhar, from Department of Veterinary Medicine, HAU, Hisar and Dr. Grewal. Two major points were subject of discussion. Firstly the issue on the completion of the Tick-borne diseases research building. The Vice-Chancellor was very cooperative and assured us that the building will be ready for occupancy within next 3 months. Secondly, there are several non-filled positions that should be available to the Blood Protista program at PAU. Again, the Vice-Chancellor promised that he will do everything possible that such positions be filled in the near future. Since Dr. Grewal is rather short in technical assistance staff, it was recommended that such persons be hired before more qualified scientists are being located.

b). Recommendations :

The availability of DOT-EIA for detection of antibodies to T. annulata is a major research development towards control of theileriosis. Hence, the test should be made available as soon as possible to all other five participating stations. It is, therefore, recommended that PAU and specifically Dr. A. Grewal's unit be contracted to produce standardized antigens and conjugates in sufficient quantities for use by participating stations. The actual distribution of test reagents should be done through the office of Dr. Y. Bhattacharyulu, Project Coordinator, AICRP on Blood Protista. The office of Dr. Bhattacharyulu should project the scope of epidemiological study within the state of each participating station. The study should be so designed to cover most of the regions of the state. The reports of serologic findings should be quarterly delivered to the office of the coordinator. It is anticipated that findings of such epidemiological studies should render very valuable data regarding the incidence of the disease in respective states. Consequently, such information will be very valuable in future field vaccination studies.

2. Haryana Agricultural University (HAU) :

a) Findings :

The Principal Investigator is Dr. R.D. Sharma. He is assisted by Dr. S. Sarup, Dr. Malhotra and Dr. A.K. Nichani. This station has conducted studies on all three diseases: theileriosis, babesiosis and anaplasmosis.

Theileriosis: These investigators consider young calves less than a week of age, as being most susceptible to infection with T. annulata. Hence, they conducted further testing using a modification of their schizont cell culture vaccine to make it safe and effective when used in very young calves. In a controlled experiment using 6 vaccinated and 2 control calves they demonstrated that following tick sporozoite challenge at 35 days post vaccination all vaccinated animals showed no clinical signs of the diseases while the 2 control calves became very sick and died.

In other studies they examined chemotherapeutic effect of buparvaquone against infections caused by T. annulata. The drug was found effective against all three stages of the disease. However, the prophylactic effect of the drug was very limited.

Babesiosis: Research on bovine babesiosis caused by B. bigemina and anaplasmosis caused by A. marginale are under the direction of Dr. S. Sarup who recently returned from 5-month training at the University of Illinois. In charge of a study on equine babesiosis is Dr. D.V. Malhotra, who is the candidate for the next (April, 1990) training program in the U.S.

Dr. Sarup has initiated in vitro cultivation studies of B. bovis using MASP techniques (Levy-Ristic, 1982) aimed at defining cultural and other properties of Indian B. bovis. Similar efforts are underway using B. bigemina. Antigens derived from cell cultures were utilized to develop DOT - ELISA test for detection and quantification of antibodies to bovine babesias.

Dr. Malhotra has developed capillary tube agglutination (CA) test for detection of antibodies in horses infected with B. equi and B. caballi. The method was based on Ristic's CA test for bovine anaplasmosis. Based on the results of this test out of the total 695 serum samples, 252 (36.25%) were serologically positive for equine babesiosis.

Anaplasmosis: Dr. Sarup has initiated studies towards antigenic definition of Indian A. marginale and the development of DOT-ELISA for detection and quantification of antibodies to A. marginale.

b) Recommendation :

In view of the fact that two of the investigators Drs. R.D. Sharma and D.V. Malhotra will be on a 5-month training at the University of Illinois, it is recommended that: (1) Dr. S. Sarup continue to be fully responsible for research on bovine babesiosis and anaplasmosis. Dr. Sarup is fully capable of developing DOT-ELISA for bovine anaplasmosis and babesiosis. When these two tests are developed and used in conjunction with PAUs DOT-ELISA for T. annulata then a full set of diagnostics will be available for epidemiologic studies of the three diseases. In addition these tests will be valuable in a follow-up of vaccination studies against these three diseases (2) In absence of Dr. R.D. Sharma, Dr. A.K. Nichani should assume full responsibility of follow-up of experimental and field vaccination studies with cell culture derived T. annulata vaccine.

3. National Dairy Development Board (NDDB) :

a) Findings :

The program at this station is under the direction of Dr. D.K. Singh, Sr. Scientist. Associated with him are Drs. P.R.S. Raghav, Scientist-II, M. Thakur, Scientist-I and B.C. Varshney, Scientist-I. The major effort of this station has been the development of T. annulata cell culture vaccine. To this effect appears that a great deal of work has been accomplished. The final outcome of these efforts is a clearance of the NDDB's vaccine by Drug Controller of India for commercial production and marketing under a provisional licence in February of 1989. The vaccine is commercially produced by Indian Immunologicals at Hyderabad, a subsidiary of NDDB. Based on information received some 100,000 doses of the vaccine have thus far been sold. According to Dr. Singh the efficacy and safety of the vaccine has been very good thus far.

The consultant visited one of the dairy farms that is using commercially produced vaccine. The owner of the farm reported that he is fully satisfied with the performance of the vaccine. The consultant also reviewed half a dozen letters from farmers in various regions of India expressing their satisfaction with the performance of the vaccine.

Prior to the commercialization, the vaccine was used experimentally on some 20,000 animals marked both as organized herds as well as individual animals in the villages. These animals live with the indigenous cattle and buffaloes, where moderate to severe T. annulata infective tick challenge is regularly taking place. Categories of animals such as calves from 3-months of age and above, breeding bulls, heifers and calves upto seven months of pregnancy have been successfully test vaccinated. Animals covered, included crossbreds (Bos taurus x Bos indicus) and purebred foreign cattle imported from European countries.

The experimental and commercial vaccines used 150 passage of T. annulata ODE isolate which originate from the region of Anand. Results of cross protection studies against various other isolates such as Dapchari, Hisar, Bhopal etc. indicated significant antigenic overlapping amongst the isolates. On the basis the ODE isolant was selected as the candidate vaccine for control of tropical theileriosis in India. On the world basis the accomplishment by Indian Scientists introduces the first commercial vaccine for theileriosis (T. annulata). In

all other cases such vaccines have been distributed gratis by government agencies.

b) Recommendations :

The scientists at Anand should continue to monitor performance of the commercial vaccine and the ICAR coordinator of the program and his associates should follow developments closely. According to discussions with Dr. Singh and his associates, their future major research efforts will focus on anaplasmosis and babesiosis. According to the results of their epidemiological studies the causative agents of the two diseases are frequently found as single infections or in combination as linked infections with T. annulata.

4. Indian Veterinary Research Institute (I.V.R.I.)

a) Findings :

Division of parasitology headed by Dr. S.C. Srivastava is the participating unit of the program. Earlier to this Dr. N.N. Sharma, the Principal Investigator of the unit was the head of the Division. Other Scientists in the program include Drs. G. Subramanian, R.V.N. Srivastava, A.K. Mishra, G.C. Bansal and M.H. Khan.

Major research effort at IVRI has been on the development of a cell culture vaccine against T. annulata. Most instrumental in these studies are Drs. G. Subramanian and G.C. Bansal. Two types of vaccines have been studied. The first was live attenuated schizont vaccine and the second is the microzoit surface coat protein, a killed vaccine. The live vaccine was first successfully tested on 2-4 week old calves at a laboratory trial and then used in the field on some 2500 cattle representing some 10 regions of India.

With the exception of the region of Parbani (Maharashtra) where the vaccine apparently failed to protect vaccinated animals, the other 9 regions reported satisfactory vaccine performance. This occurrence was followed by isolation of the local Parbani T. annulata isolant. In cross protection studies with the IVRI isolant the protection was found to be incomplete.

The second vaccine is a protein derived from the surface of the T. annulata schizont. This protein was used in purified form at the level of 4 mg. a dose. Several calves received 2 doses of this protein given 20 days apart. At 30 days following the second dose, animals were challenged with sporozoites derived from the homologous strain. Researchers claim that vaccinated animals were protected. However, there was no protection against heterologous isolant of T. annulata.

Other studies included investigation on growth supporting factors isolated from cell culture. These factors have been identified as being 116 kd and 140 kd. An investigation of the type of lymphocyte supporting growth of T. annulata showed that this organism most readily invades and grows in B cell clones. This is in contrast to T. parva which is known to use as a host cell T cell clones.

b) Recommendations :

Two of the investigators will be retiring within next few months. These are Drs. N.N. Sharma and G. Subramanian. It is understood that Dr. G.C. Bansal will assume a leading position in research on T. annulata. To this effect it is recommended that the field vaccination experiments continue and that responses to vaccination (safety and efficacy) be carefully monitored.

One of the investigators, Dr. A.K. Mishra has recently returned from a 5-month training at the University of Illinois. Dr. Mishra has carried out extensive studies on Babesia bigemina and B. bovis including SDS-polyacrylamide gel electrophoresis and Western immunoblotting as well as in vitro culturing of both Babesia species. Moreover, Dr. Mishra has been able to prepare culture derived vaccines against the two babesia's using exoantigen systems (Ristic, 1979). It is, therefore, recommended that Dr. Mishra assumes a leading role in the development of serodiagnostic and immunoprophylactic measures against B. bovis and B. bigemina of Indian origin.

5. Tamil Nadu Veterinary and Animal Sciences University (TNVASU)

a) Findings :

Department of Parasitology headed by Dr. R. Anandan is the participating unit in the program. Dr. Anandan returned from one year assignment at another University. During his absence, incharge of the program was Dr. S.S. Joseph who soon will be replaced by Dr. G. Rajavelu. Another participant in the program is Dr. J. Chellappa who is scheduled to start a 5-month training at Illinois in April of this year. Joining the program by August of 1990 will be Dr. Lalita John.

The consultant had extensive discussions with the Vice-Chancellor, Dr. Richard Masilamony and other officials of the University. Dr. Masilamony, a microbiologist has shown a great deal of interest in the program and has provided assurance that this program will greatly be strengthened during the forthcoming year and that participating personnel will be kept in place.

Because of great prevalence of another leucocytic rickettsia in the blood of cattle in the region, Ehrlichia bovis and a similar widespread occurrence of canine Ehrlichia canis in the region and because of great concern of the latter agent to the Indian Military working dogs, the Vice-Chancellor asked the consultant to make a presentation on Ehrlichia and diseases they cause in man and animals. In a formal ceremony in which among some 100 members of the faculty in addition to the Military Commanding Officer and six of his associates from National Defence Academy, Pune the consultant spent some 3 hours assessing the impact of ehrlichiosis on domestic animals and pointed the way the disease can be diagnosed and controlled. Since 1968 when ehrlichiosis killed more than 500 U.S. Military working dogs in Vietnam, the consultant and his research team have made major accomplishments toward the control of diseases caused by Ehrlichia canis and other ehrlichiae.

Because of this great impact on the health of bovine and canine species caused by ehrlichiae the Vice-Chancellor and the department of Parasitology requested that the trainee from that station, Dr. Chellappa, be familiarized with all aspects of research on ehrlichiosis in addition to other diseases which include cultural and serodiagnostic procedures.

The accomplishment of the past year of this station has focused on epidemiological studies of haemoprotistan diseases in Tamil Nadu. The study revealed presence of four major diseases which include: theileriosis, babesiosis, anaplasmosis and ehrlichiosis, the latter in both cattle and dogs - military and civilian.

b) Recommendations :

The project leader, Dr. R. Anandan is a trained entomologist, hence his interest in the study of vector (ticks and other arthropods) and their interrelationship with the causative agents of the above mentioned four diseases. He and his associates are also interested in the study of optimal methods for chemical and biological control of disease carrying arthropods. Because of the great impact of ehrlichiosis in cattle and military dogs it is recommended that Dr. J. Chellappa initiate studies toward E. bovis and E. canis upon his return from the U.S.

6. Bihar Veterinary College - RAU - Patna, Bihar

a) Findings :

Participating unit in the sub-project is the Department of Parasitology of the College of Veterinary Medicine. Principal investigator is Dr. P.S. Srivastava. He is being associated by Drs. S.N. Samant Roy and S.P.S. Sinha and four Laboratory technicians. The first aspect of study during the past year has been epidemiological investigation of hemoparasitic diseases in various regions of Bihar. A total of 3274 animals have been examined for the presence of Theileria, Babesia and Anaplasma. The incidence of Theileria was 3.39%, Anaplasma 2.35% and Babesia 2.07%. Among tick species detected feeding on the above animals the most common tick was Boophilus microplus. Experimental trials with insecticides proved effective in control of ticks. Finally, limited studies on control of ticks by use of immunization of cattle with various tick homogenates fortified by adjuvants proved relatively effective means for control of tick infestation.

b) Recommendations :

In view of the availability of DOT-ELISA for detection of cattle exposed to T. annulata it is being recommended that this station continue with field epidemiologic investigation of theileriosis using the above test.

According to the principal investigator there is an indication that Boophilus microplus tick may be involved in transmission of T. annulata. Hence, the investigator should attempt to experimentally provide an evidence to this effect. Of particular interest would be to find whether B. microplus transmits T. annulata by transovarial passage or that ticks other than Hyalomma Spp are involved in the transmission of T. annulata.

In order to make all epidemiologic studies and those related to tick investigation more efficient it is recommended that this station be provided with a vehicle and a good microscope. Other equipment is also needed.