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CONSULTING REPORT

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CONSULTING REPORT
by

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Project: "Intracellular Blood Protista with Particular Reference to Immunoprophylaxis and Control."

I. General Background:

During his visit in January of 1985 this consultant provided a comprehensive report on the development and implementation of immunoprophylactic and diagnostic measures designed to control three major tick-transmitted blood protistan diseases of food producing animals such as cattle, sheep, and goats. The three diseases are bovine theileriosis caused by *Theileria annulata* babesiosis caused by *Babesia bovis* and *B. bigemina* and anaplasmosis caused by *Anaplasma marginale*. In addition to food producing animals, draught equine species, e.g., horses, mules and donkeys are also highly susceptible to infections with *Babesia* parasites such as *Babesia equi* and *B. caballi* and suffer severe losses when affected by these agents. Similarly, dogs which are important within the police and military forces are

known to experience severe infections and mortalities due to babesial agents.

The study under this subproject will be conducted at five Indian institutions. These are: The Department of Medicine, Haryana Agriculture University, Hissar; The Division of Parasitology, Indian Veterinary Research Institute, Izatnagar, UP; The Animal Disease Diagnostic Laboratory, National Diary Development Board, Anand, Gujarat; The Department of Veterinary and Animal Sciences, Punjab Agricultural University, Ludhiana, Punjab; and the Department of Parasitology, Tamil Nadu Agricultural University, Madras Veterinary College, Madras. The projec. is directed by the ICAR in consultation with the officers of the USAID and Winrock, International.

II. Current Visit:

In a two-day workshop held at ICAR, the consultant met with principal investigators and those associated with the four stations (IVRI group was absent) and was briefed on the research progress made at each location. As a follow-up of this meeting the consultant visited research stations at Anand, IVRI and Hissar. These arrangments provided an ample opportunity to meet

participants of the program, assess the standard of their work and inspect their laboratories and animal facilities.

At Haryana Agricultural University (HAU) there are two research groups engaged in the study of bovine theileriosis. One group uses lymphocyte cell culture-derived schizonts for induction of vaccinal immunity while the second group focuses on the use of tick-derived sporozoites as immunogens. The latter method requires that sporozoite infected animals be immediately treated to avoid clinical disease and mortality. Very little, if any, work is underway on bovine babesiosis and anaplasmosis. Some serodiagnostic work on B. equi is being attempted. This station has expressed strong interest in acquiring Babesia and Anaplasma vaccine technology from the University of Illinois.

At the Indian Veterinary Research Institute (IVRI) the work on blood protistan diseases is limited to lymphocyte schizont cell culture of T. annulata under the leadership of Dr. Subramanian. The work on Babesia and Anaplasma, conducted by a previous member of the Division of Parasitology, Dr. M. Malhotra, has been

discontinued. The Head of the Parasitology Division, Dr. N.N. Sharma, is greatly interested in transferring bovine babesiosis and anaplasmosis vaccine technology from the University of Illinois to his Division.

At the National Dairy Development Board (NDDB) the focus of work is on utilization of cultured T. annulata schizonts for immunization against bovine theileriosis. The organism maintained over long periods of time in lymphocyte cell cultures is used for this purpose. The station and its administrative leadership have a strong interest in implementing Babesia cell culture system for immunization against bovine babesiosis. Administrative leadership of NDDB has made it very clear to this consultant that they wish ~~"at any price"~~ to rapidly acquire Babesia technology from the University of Illinois for the purpose of producing a commercial bovine babesiosis vaccine for use in cattle under their jurisidction.

Investigations at Punjab Agricultural University (PAU), under the leadership of Dr. K.S. Gill include the use of tick-derived sporozoites for the induction of T. annulata infection in susceptible cattle followed by the administration of tetracycline and other drugs in order to suppress the development of clinical

signs in inoculated cattle. It is hypothesized that animals so preimmunized should be resistant to subsequent natural infections. A new member of this group, Dr. A.S. Grewal, appears to be well-trained abroad in the field of immunochemistry. The availability of Dr. Grewal, coupled with the recruitment of two additional well-trained scientists (now in progress), will make the PAU team a strong scientific contributor to this subproject.

At the workshop, and by a subsequent return visit with this consultant, Dr.Grewal expressed a very strong interest for the development of a joint research venture between the PAU and the University of Illinois on the transfer and implementation of Babesia vaccine technology from Illinois to PAU.

At Tamil Nadu Agricultural University (TNAU) the work on blood protista is at a very early stage. The focus of this preliminary study is on entomology with special reference on the tick as a vector of T. annulata. The aim of this group is to establish a bank of well-characterized infected tick stabilates.

III. Critical Summary Review.

With very minor exceptions, the research in the above five stations is basically limited to the

development of preventive measures against bovine theileriosis. For this purpose two principal approaches are being used. In the first approach T. annulata schizonts are being propagated for variable periods of time in lymphocyte cultures and then utilized for induction of protective immunity in susceptible cattle. Using this method workers at IVRI, HAU and NDDB have obtained promising, although inconclusive, results.

There is a need for the following improvements of this methodology. There must be clear evidence that experimental animals are free of subclinical theileriosis prior to their use in vaccination experiments. For this purpose, a well-controlled and easy to read IFA test must be developed. There was no evidence of a functional serologic test for T. annulata at any of the stations visited. Once such a serologic test becomes established it must be used to monitor immune responses following vaccination and challenge of experimental animals. The pattern of immune response obtained by experimental challenge should then be related to data generated under natural exposure.

In the second approach for prevention of bovine theileriosis susceptible young animals 1 to 2 weeks of

age are exposed to tick-derived sporozoites and immediately chemotherapeutically treated. This infection and treatment procedure allows for the development of transitory protective immunity. These preimmunized animals are then given a higher dose of sporozoites approximately 3 to 5 weeks later. A recovery from the latter challenge exposure provides treated animals with a sufficient degree of protective clinical immunity against future natural tick-induced infection.

While the infection and treatment method of immunization against theileriosis may not be a desirable procedure for a massive field use it may serve a useful purpose to compare protective effects induced by it with those using cell culture-derived schizonts.

Other strictly practical and economic aspects that question the suitability of both vaccinal methods for massive wide-scale use are: (1) lymphocyte cell culture vaccine must be transported and stored in liquid nitrogen which may not be too suitable under tropical field conditions; (2) the cost of drugs and labor needed for prophylactic treatment using infection and treatment method may be prohibitive and possibly limited to exceptional circumstances.

IV. Request for Transfer of Blood Protistan Technology.

During the workshop at ICAR, in subsequent visits to various stations and in special meetings at the Ashok Hotel with principal investigators and administrative staff of participating institutions the consultant was addressed with the following requests:

1. Dr. M.P.G. Kurup of NDDB has requested that a special joint venture be established between the University of Illinois and his institution for the purpose of transfer of *Babesia* vaccine technology to NDDB. He explained that this is an urgent matter because NDDB associated dairy cattle suffer severe losses due to an infection with *B. bovis* and *B. bigemina*. In specific terms, he proposed that NDDB is in a position to rapidly generate needed funds to cover all expenses associated with the transfer of technology at the University of Illinois and at NDDB. The same request was strongly reiterated in subsequent discussions with Drs. Patel and Kurian.

2. Dr. R.D. Sharma, the Head of the Department of Medicine at HAU requested assistance from the University of Illinois for the transfer of vaccinal technology for bovine anaplasmosis and babesiosis to his university. In addition, he requested that *Babesia* cell culture

developed at Illinois be implemented for the cultivation of equine Babesia species and eventual use of culture generated antigens for the development of equine anti-Babesia vaccines.

3. Drs. K. S. Gill and A. S. Grewal of PAU requested that a special joint research project be established between the University of Illinois and PAU for the purpose of transfer of cultural, immunochemical and other vaccinal knowledge of babesiosis to their university.

4. Drs. P. N. Bhat and N.N. Sharma of IVRI requested that the personnel of the Department of Parasitology be trained in the acquisition of all knowledge and techniques pertinent to the immunodiagnosis and immunoprophylaxisis of bovine babesiosis and anaplasmosis.

V. Blood Protista Technology at the University of Illinois.

Over the last 20 years scientists at the University of Illinois using modern cellular engineering techniques have developed serodiagnostic test procedures and vaccines for the prevention and control of bovine and canine babesiosis and bovine anaplasmosis.

For the control of babesiosis, cell culture systems

have been developed for continuous *in vitro* propagation of *B. bovis*, *B. bigemina* and *B. canis*. Organism-free soluble antigens termed "exoantigens" derived from the above cultures are being utilized as immunogens in the development of effective inactivated vaccines. The University of Illinois holds two patent rights on the use of exoantigens and cell culture systems for the production of *Babesia* vaccines. On a royality basis, the university has assigned some of these rights to the Rhone Merieux, a large pharmaceutical company in Lyon, France. Using this technology Rhone Merieux produced the first commercial blood protozoan vaccine for immunization against *B. canis*, the causative agent of canine babesiosis. The vaccine is manufactured in lyophilized form and sold in France and other countries under the trade name "Pirodog." Two years of field application of the vaccine produced excellent results (please see enclosed information).

For epidemiologic studies of babesiosis, the University of Illinois scientists have developed a latex-based field screening test and an indirect fluorescent antibody test for confirmatory diagnosis and for quantitation of antibodies.

For the control of bovine anaplasmosis the University of Illinois has developed an attenuated *A. marginale* vaccine by selecting a mutant strain through adaption of the organism to sheep. The organism now used for vaccination purposes represent the 125 consecutive passage of the organism in sheep. This vaccine is licensed and commercially used in the State of California over the last 8 years with excellent results. Moreover, this vaccine is being used with equal success in many Latin American countries.

For field sc eening diagnosis of anaplasmosis the University of Illinois has developed the capillary tube agglutination (CA) test and plate latex agglutination (PLA) test. For laboratory confirmatory diagnosis the IFA test has been developed.

Prompted by the successes with babesiosis and anaplasmosis and recognizing biologic similarities between babesiosis and malaria, the USAID and the Institute Merieux have provided some 3 million dollars to the University of Illinois under the leadership of this consultant to explore the application of findings in babesiosis toward the development of a vaccine for human malaria. The research on malaria vaccine is directed

toward the use of molecular biology (Recombinant DNA approach) for the isolation, aminoacid sequencing of immunogenic polypeptide exoantigens, production of DNA probes, cloning of genes encoding for protective exoantigens, and expression of these genes in various vectors including vaccinia virus. Our collaborators at the Institute Merieux are highly specialized in the use of vaccinia virus as a delivery system and have recently successfully used this system for immunization against rabies using viral glycoprotein that is perpetuated by the vaccinia delivery system.

This large biotechnology malaria vaccine program which involves 5 molecular biologists and other scientists, including immunochemists, clinical immunologists, biochemists and cell pathologists, is now gradually transferring their expertise on the molecular biology of malaria, toward similar studies in babesiosis and anaplasmosis.

VI. Recommendations.

1. Training: Future successes in the development of *T. annulata* vaccine will greatly depend upon the improvement of currently used immunoserologic and immunochemical techniques for detection of antibodies in

experimental animals and similar identification of T. annulata schizont and other antigens in cell cultures. Accordingly, those involved in the cell culture system for T. annulata should be provided with additional training in the above fields. For the development, standardization and immunochemical studies of the IFA test and the mechanism of protective immunity the best available training is being offered at the University of Illinois. Further there is a need to develop monoclonal antibodies to certain major T. annulata antigens for a specific follow-up of the development of the parasite in cell culture as well as for optimization of the vaccinal dose. This phase can best be accomplished by a collaboration program now in progress between the Ohio State University and the University of Illinois.

Entomologic training focused on the study of pathogenesis of a blood protistan agent in the tick vector can best be offered at the Oklahoma State University.

Training in cellular engineering including in vitro cultivation of blood protista and all related work on serodiagnostic tests and vaccines is available at the University of Illinois.

For training in molecular biology, comprehensive theoretical and practical programs are available at the Center for Molecular Biology at the University of Illinois. This center has recently received a 60 million dollar grant from Dr. Beckmann, a graduate of the University of Illinois and a major manufacturer of sophisticated Beckmann Instruments. For this type of training, one or a maximum of two, most qualified Indian scientists should be considered. They should possess a good biochemistry background and then take additional molecular biology coursework in the above center.

2. Collaborative Research.

All stations expressed a serious concern with *Babesia* and *Anaplasma* problems in India and requested that a joint research arrangement be made between their respective station and the University of Illinois for the purpose of transferring modern diagnostic and immunoprophylactic technology to their respective stations. Based on the survey of potential scientific power, administrative structure, and physical facilities at each station and, in consideration of the actual experience involved in such an undertaking, it is proposed that:

there should be two collaborative research projects on Babesia and Anaplasma vaccines and immuno-diagnostic technology.

The first project should be with NDDB. This 3-year project will be designed to transfer Babesia vaccinal and diagnostic technology from the University of Illinois to NDDB for the purpose of protecting dairy cattle affiliated with NDDB from losses and mortalities due to bovine babesiosis. NDDB has agreed to procure necessary funds for a contractual arrangement with the University of Illinois. Under this project the working relationship will be directly between the University of Illinois and NDDB, however, ICAR will serve as a supervisory agency of the project.

The second project will be within the frame of the current Blood Protista subproject involving ICAR, USAID, Winrock International, the University of Illinois, HAU and PAU. The specific objectives of the project will be to provide HAU with the vaccinal and diagnostic technologies for anaplasmosis and babesiosis and to make it possible for PAU to conduct immunochemical aspects of culture-derived babesial antigens. The latter study should provide the scientific basis for future biotechnology studies on

babesiosis and other blood protistan agents. Also, this joint project should be designed as a 3-year venture with the option of continuation pending results obtained.

VII. Summary.

The principal objective of the program is to provide additional specialized training for selected Indian scientists in order to ensure more efficient research toward the development of prophalactic and serodiagnostic methods for control of the three principal blood protistan diseases.

With reference to theileriosis, those individuals involved in the development of lymphocyte culture-derived schizont vaccine need to be trained in immunochemical methods with special emphasis on quantitation of vaccinal doses and more efficient use of serodiagnostic methods such as the IFA test. A full understanding of these aspects is essential in order to determine that animals used for experimental vaccination studies are fully susceptible to an infection with *T. annulata* as well as to be able to monitor immune responses following vaccination and artificial or natural challenge.

The investigators interested in the use of tick-derived sporozoite vaccine for the infection and treatment

principle as an immunoprophylactic method, also need further training in the use of immunological methods for standardization of their vaccinal material and for monitoring the immune response in inoculated animals. These investigators should also be provided with additional knowledge regarding the development of the agent in the tick and the means of detecting various parasitic antigens in this vector.

With reference to the transfer of vaccinal technology of culture-derived immunogens of B. bovis and B. bigemina and the use of attenuated and inactivated A. marginale vaccines only selected aspects can be offered through short-term training. The full transfer of these technologies for use under Indian field conditions, however, must be accomplished by specific joint research programs with NDDB for babesiosis and HAU-PAU for babesiosis and anaplasmosis. NDDB indicated that they are able to generate special funds needed for this venture while support for a similar program for HAU-PAU must be provided within the ongoing program of ICAR-AID-Winrock, International.

The University of Illinois holds patent rights on several of these products. On one of the Babesia species, B. canis, the University has assigned the patent rights to Rhone Merieux of Lyon, France. Using this technology, the company has developed the first commercial blood protozoan vaccine. The vaccine is manufactured and marketed under the trade name "Pirodog." Over the last two years, hundreds of thousands of dogs have been vaccinated with Pirodog with excellent results. Some information regarding this vaccine is enclosed.

This consultant successfully trained and guided a number of Indian graduate students during the 1960's. Over this period he has developed a deep feeling and understanding for Indian scientists and their needs. Thus, it is his hope that with a continuous mutual understanding and goodwill, we will be able to jointly develop effective measures for the control of the three diseases in India, thereby making a significant contribution by increasing availability of milk and other animal products for the population of that country.

It is the sincere desire of this consultant to do everything possible to see that the blood protista project produces specific accomplishments within the next 3 years and in that way set an example for other India-USA projects.