Future challenges

Despite the complexities of *T. parva* and the immune responses to it, a subunit vaccine for this parasite is now on the horizon. The demonstration that cattle can be protected against *T. parva* sporozoites suggests that ILRAD's approach to a vaccine against *T. parva* may profitably be applied to research on other economically important tick-borne parasites, such as *Theileria annulata.* This parasite is the cause of Mediterranean (or tropical) theileriosis in northern Africa, southern Europe and Asia. The sporozoite form of *T. annulata* has a surface antigen whose amino acid sequence is very similar to that of *T. parva* p67. It is possible, therefore, that a recombinant *T. parva* p67 antigen can be made to stimulate immune responses against *T. annulata* as well as *T. parva.*

With tools now available to manufacture parasite molecules and to test their ability to protect animals against subsequent parasite infection, the challenge for the future is .to design a vaccine that will immunize cattle in a single dose against all the important tick-borne diseases—anaplasmosis (gall sickness), babesiosis (redwater or tick fever) and cowdriosis (heartwater) as well as theileriosis (East Coast fever and Mediterranean theileriosis). Genes encoding antigens of the organisms that cause these diseases could all be expressed as components of a single live vaccine that would protect animals from all tick-borne diseases.

The most sophisticated technologies and expertise of modern biology will be needed to bring this research to fruition. The rewards will be lasting. Development of a novel vaccine against tick-borne parasites will lift some of the burden of animal disease from Third World farmers. In addition, because no broadly effective recombinant vaccine against a protozoan parasite has yet been developed, such an achievement would also be a major biological breakthrough, ushering in a new era of human as well as veterinary medical care.

This article is based on recent work by ILRAD scientists Tony Musoke, Declan McKeever, Vish Nene, Evans Taracha and Subhash Morzaria. The Editor thanks Declan McKeever for greatly improving many drafts of the article.

ILRAD's links with global animal disease research

Through ILRAD's links with research institutions in regions where trypanosomiasis and tick-borne diseases are a problem, the Laboratory is ensuring that results of its research gain currency among all scientists endeavouring to control global livestock diseases. The following are examples of ILRAD's collaborations.

Trypanosomiasis

Mechanically transmitted trypanosome parasites, which are spread by means other than the tsetse fly, occur over vast areas in and outside sub-Saharan Africa. In Asia, trypanosomiasis due to *Trypanosoma evansi* kills horses and pigs and is a severe constraint on the productivity of cattle and working buffalo. This disease severely affects camels in western Asia and northern Africa and cattle and horses in Latin America and the Caribbean. In the latter region, trypanosomiasis due to *T. vivax* also adversely affects cattle raising.

To improve detection of trypanosome parasites in their animal hosts and insect vectors, ILRAD scientists have developed enzyme-linked immunosorbent assays (ELISAs) based on monoclonal antibodies that specifically recognize molecules unique to given species of trypanosomes. These tests are being used to diagnose *T. evansi* infections in Asia and *T. vivax* infections in South America.

The tests were first used at ILRAD on parasites and sera from infected animals in these regions. ILRAD then transferred the diagnostic technology to laboratories in Thailand (National Animal Health and Production Institute, Bangkok), Indonesia (Balitvet, Bogor), French Guyana (Institute for Animal Husbandry and Veterinary Medicine in Tropical Countries [IEMVT] Laboratory, Cayenne) and Argentina (Centro de Diagnostico Veterinarias, Formosa). ILRAD staff has provided these laboratories with reagents and training in their use and makes periodic visits to the laboratories for hands-on instruction and problem solving.

Isolates of *T. vivax* from cattle in Colombia have been subjected to a range of molecular and serological analyses at ILRAD to determine similarities among parasite isolates from South America, similarities between South American and African parasites, and the possible contribution of characterized differences to cattle diseases in both regions. Scientists from around the world are kept abreast of developments in research on *T. evansi* by paying visits to ILRAD to conduct further research and to attend workshops on diagnostic antigens and chemotherapy for trypanosomiasis.

ILRAD has given training in general diagnostic technologies to staff of the Indian Council of Medical Research and has provided laboratories in India with monoclonal antibodies for categorizing bovine cells and isotypes of serum antibodies in infected cattle.

Although trypanosomiasis is not endemic to Japan, ILRAD has introduced Japanese scientists to research methodologies appropriate for tackling problems in trypanosomiasis research and control in developing countries. The trypanosomiasis research program at ILRAD has been greatly assisted by Japanese-funded research staff working at the Laboratory. These scientists return to their home institutions to continue work of relevance to ILRAD's trypanosomiasis program.

Tick-borne diseases

Tick-borne diseases of livestock—principally theileriosis, babesiosis, anaplasmosis and cowdriosis—are widespread in the tropical, subtropical and temperate regions of the world. *Theileria* parasites severely constrain livestock productivity in Africa and Asia. *Babesia* and *Anaplasma* parasites occur globally. *Cowdria ruminantium,* the causative agent of cowdriosis (heartwater), is found in African and Caribbean countries.

ILRAD keeps in close contact with scientists and research institutes in India, where the parasite *Theileria annulata* causes tropical theileriosis. ILRAD staff members have exchanged visits with senior Indian scientists from the National Dairy Development Board, in Anand, Gujarat, and with staff from the Punjab Agricultural University, Ludhiana. Discussions with the latter institute have concentrated on development of new methodologies for immunizing calves against theileriosis and simple tests for diagnosing theilerial infections.

In Japan, where theileriosis due to *Theileria sergenti* and *Theileria orientalis* constrains livestock production, ILRAD enjoys close links with two institutes—The Tropical Agricultural Research Centre and the National Institute for Animal Health. Several scientists from these institutes have conducted research at ILRAD for periods of up to two years. Japanese scientists have played key roles in ILRAD's development of monoclonal antibody markers and protein analytical techniques used to define antigenic molecules of theilerial parasites. Employing the markers and techniques on return to their home institutes, these scientists have helped to identify antigens with vaccine and diagnostic potential for the disease caused by *T. sergenti.*

A recent comparison was made between Japanese and African *Theileria* parasites using species-specific diagnostic DNA probes. Results of this work have helped to clarify the species complexity of *Theileria* parasites in both regions. Japanese scientists have also helped ILRAD staff to establish the technology needed to express parasite antigens in a baculovirus expression system for experimental vaccine research.

ILRAD's collaborative links with universities in the US and research laboratories in Australia help to integrate research on tick-borne disease problems in South America, the Caribbean, Asia and the Pacific islands.

For more information, contact Dr. Peter Gardiner, Information & Planning officer.

ILRAD Research Updates

Improved technology for labelling DNA probes

ANIMAL DISEASE control agents and researchers in developing countries have long needed improved methods of accurately detecting parasite infections in animals and precisely identifying the infecting organisms. Vector-borne parasitic protozoa endemic in the tropics have traditionally been detected and identified by staining and microscopic examination of specimens from infected host animals and insect vectors, by propagating parasites in tissue culture and experimental animals and by characterizing the organisms using isoenzymes and serological reagents. Each of these methods has drawbacks, making diagnosis and parasite identification problematical and often unreliable.

THESE TRADITIONAL methods may now be complemented, and in many instances replaced, by new methods employing recombinant DNA reagents. Some of the latter also exploit the polymerase chain reaction, to amplify copies of a given DNA fragment, and hybridization of single strands of DNA with their complementary strands. DNA probes, consisting of single strands of parasite DNA fragments, have now been made to identify many important parasitic protozoa. The probes are conventionally labelled with radioisotopes to detect their binding to parasite DNA. By screening field samples of parasites with a panel of DNA probes for different species and subspecies, laboratory workers are identifying parasites with unequalled precision.

IN MOST THIRD WORLD laboratories, however, use of radioisotopes is constrained by lack of technical expertise and expensive equipment. The latter includes intensifying screens and special refrigerators for amplifying the radioisotope signal at very cold temperatures (–80 °C) and darkrooms for processing the exposed x-ray films. A search was therefore undertaken at ILRAD to find alternative, safer and more convenient methods for labelling DNA probes. Several commercially available kits were evaluated for their ability to reveal parasites in crude samples prepared from infected mammalian hosts and insect vectors. Of the kits tested, the most valuable proved to be one employing digoxigenin molecules with an antibody visualization system (Boehringer Mannheim Biochemica Company, Germany).

Diagnostic tests using this technology require relatively simple laboratory support: signals from the probe bound to parasite DNA are visualized by a change of colour on filter