Prevention THE BEST CURE

For more than two decades ACIAR has sponsored research to advance knowledge about livestock diseases in Indonesia, to discover controls that are cheap to implement and acceptable to farmers, **Janet Lawrence** reports

The Jembrana disease research group, meeting at Murdoch University.

Smallholder producers rely on healthy livestock and poultry for income and food. But a sick animal is a worthless animal. So it is far better to prevent animals getting sick in the first place, either by selecting those with a genetic resistance to disease or by developing vaccines. ACIAR is adopting both approaches to managing diseases, including one found only in Indonesia.

Jembrana is a viral disease first detected in Bali and since found in Kalimantan, Java and Sumatra. Another viral disease, Gumboro (infectious bursal disease of poultry), causes high mortality levels, while surra is a disease caused by a blood parasite similar to that which causes African sleeping sickness.

Another costly animal health issue is the tropical parasitic fluke worm *Fasciola gigantica*, which has been the target of considerable ACIAR investment. This work was profiled in the September 2004 issue of *Partners*, and a plan of action is now under way to prevent cattle becoming infected.

New approach to parasite liver fluke control

In looking at the fundamental question of why animals become infected with liver fluke, the basis of natural immunity to *fasciola* in Indonesian thin tail (ITT) sheep has been identified.

In July 1992 an ACIAR-sponsored collaboration looked to see if extracts of antigens from regular liver fluke (*Fasciola hepatica*), which had been shown to have a protective role in vaccines for ruminants in Australia and Europe, would be effective against *F. gigantica*. However, the two trials that were conducted did not induce significant protection.

This prompted changes to the approach. In addressing the possible 'virulence' of *F. gigantica*, the research team confirmed that many ITT sheep were resistant to it. This demonstrated that *F. gigantica* was susceptible to attack and that resistance was probably due to a single major dominant gene, or a very few of them – a unique situation in terms of known resistances to large parasites.

The ITT sheep also demonstrated partial resistance to the gut worm *Haemonchus contortus*. Study of these animals thus promised to deliver a greater understanding of resistance to two very different forms of parasites, a fluke and a flatworm.

The scientists believed these results had considerable potential in the search for vaccine antigens and selective breeding, and in a follow-on project, 'Genetic and immunological characterisation of high resistance to internal parasites in Indonesian thin tail sheep', they sought to identify resistance genes in the ITT sheep.

The scientists crossed parasite-resistant ITT sheep with parasite-susceptible Merinos. From these hybrids they produced a backcross flock where they identified resistant and susceptible individuals – and which ITT sheep had the strongest parasite-resistance genes.

The DNA from these animals was analysed to find the location of the parasite-resistance genes on the chromosome. By the end of the project, markers (DNA sequences) were consistently found in animals showing resistance, enabling identification of some candidate resistance genes.

At the same time, scientists studied the ITT sheep to find out exactly how their immune systems kill the parasites. By selectively 'knocking out' arms of the immune system, they tried to identify the part responsible for conferring resistance.

A major scientific outcome has been the identification of three chromosomal regions containing genes linked to the expression of *H. contortus* resistance and nine chromosomal regions associated with resistance to *F. gigantica* infection, including genes which control eosinophil activity. This is important, as the immunological work has suggested that eosinophils (a type of white blood cell) are a major vehicle of resistance in ITT sheep.

It also seems that ITT sheep, but not Merinos, have the ability to produce more oxygen radicals which eliminate early fasciola stages.

With a little more work the researchers hope to identify the exact genes responsible for this resistance.

A by-product of the project has been the identification of gene loci important to wool growth and carcass traits such as growth and composition. This information has significant potential value for both the Indonesian and Australian livestock industries.

Improved Jembrana vaccine

In Indonesia, smallholder farmers throughout the country have received Bali cattle (*Bos javanicus*) in several development programs. While Bali cattle have a number of advantages under Indonesian conditions, a major problem is their unique susceptibility to Jembrana disease – a viral disease peculiar to Indonesia, and endemic in Bali, Kalimantan, Java and Sumatra.

Its origin is unknown, but it was first detected in 1964 in Bali cattle (on the island of Bali itself), where thousands of animals died.

Since then, it has led to a ban on inter-island cattle trade which has affected food production, especially in the eastern islands.

Research supported by ACIAR demonstrated the feasibility of a Jembrana-disease vaccine derived from tissues of infected animals. However, despite its efficacy, there were constraints to its use – it was expensive and unstable with quality control problems.

ACIAR supported further research addressing these issues through testing three possible approaches to an improved vaccine. Of the three, the most promising to emerge involved the use of viral proteins produced by recombinant DNA technology.

Consequently, an improved vaccine for Jembrana disease is a step closer. The project team produced several recombinant virus proteins. Two proteins, when used as vaccines during laboratory trials, significantly decreased the severity of the disease that occurred after vaccinated animals were challenged with live virus. The recombinant protein vaccines will now be tested under field conditions, and methods of large-scale production of the recombinant protein vaccine in Indonesia are being investigated.

Serious poultry disease

Infectious bursal disease (IBD), or Gumboro, is the most serious viral disease of poultry in Indonesia after Newcastle disease. The virus infection suppresses the animal's normal immune response, meaning that other infections can readily take hold. Even when not fatal, infections can greatly reduce growth or egg-laying. The economic impact of IBD in Indonesia ballooned after the appearance in 1991 of very virulent strains of the virus (referred to as vvIBDV). These vvIBDV strains cause up to 30 per cent mortality in broilers and 60 per cent in layers, and surviving birds are severely debilitated.

Unfortunately, most smallholders and backyard chicken farmers do not vaccinate against vvIBDV in spite of frequent heavy losses, because of the vaccine price and the packaging sizes available. Also, many village chicken owners are not aware of the importance of the disease. They tolerate poor growth and deaths, unaware they could be easily controlled.

Australia is one of the few countries free of vvIBDV, but it could be introduced from Indonesia. For this reason Australia and Indonesia have studied the virus together for some years, and it was a natural extension for Indonesia's Balitvet to approach ACIAR for support in developing a vaccine that would be cheaper, with packaging tailored in dosages to suit the flock size of smallholders.

The first project task was to render the Indonesian vvIBDV strains less virulent, by a process known as attenuation. Scientists selected a suitably diluted strain for development as a vaccine, and then undertook field testing. The Australian Animal Health Laboratory selected one vvIBDV clone that is safe and able to produce immunity for vaccine development, then produced a master seed.

Having developed a protective master seed vaccine clone in the laboratory, the vaccine is now ready to be field-tested on kampung and smallholder chickens.

Looking to control surra

Both Indonesia and the Philippines would like to reduce the impact of surra on livestock productivity. But before any control programs can be designed, more needs to be known about the impact of *Trypanosoma evansi* on livestock and the determinants of clinical disease. So an ACIAR project has been developing accurate diagnostic tests for surra and looking for more effective controls.

The project began with a training workshop in Indonesia to improve surveillance along with further study of the parasite among project partners. Then experimental work started with screening trypanocidal drugs for activity against the parasite, to find the most efficacious and cost-effective drug.

The scientists tested the pathogenicity of the parasite in rodents to identify strains with high and low virulence, which they then studied to determine the genetic differences and to find markers for the pathogenicity. The next stage was to use molecular techniques to create highly sensitive and specific diagnostic tests for the presence of *T. evansi* in host blood and tissues.

Project scientists have found key diagnostic features of the pathology and clinical signs of *T. evansi* infection. They have provided training in epidemiology so that participants could evaluate and interpret the results of diagnostic tests.

All partner institutions have increased their capacity to undertake serological diagnosis, helped by improvements to their laboratories.

The Indonesian scientists have used their knowledge and skills to train scientists in the Philippines, where surra is the most significant cause of livestock mortality.

Balitvet technician Fester Politedy demonstrates techniques to scientists from the Philippines and Papua New Guinea at the surra workshop in Indonesia.