NSW DEPARTMENT OF PRIMARY INDUSTRIES

TURNING THE WORM

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Edited by Stephen Love, Veterinarian/State Worm Control Coordinator, NSW Department of Primary Industries, Armidale[1]

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Welcome

to this issue of TTW. The main purpose of this informal newsletter is to share information with those particularly interested in the management of endoparasites of farmed animals, including sheep, goats and cattle.

wormboss - update

The WormBoss Project Team met in Melbourne in November. The content of WormBoss and the development of the website (and CD) are in the final stages.

What is WormBoss?

Here is a recent statement from the Sheep Cooperative Research Centre:

'WormBoss is a computer-based decision tree, developed by the Sheep CRC with significant assistance from Australian Wool Innovation, to

[1] NSW DPI, PO Box 991 Armidale, NSW, AUSTRALIA. Email: <stephen.love@agric.nsw.gov.au>

assist with decisions on parasite management. It is likely to be a very valuable product for producers as well as re-sellers and consultants. It will be launched jointly by the CRC and AWI […early in 2005].'

Multiple drench resistance in goat worms

West and others recently wrote on multiple drench resistance in *Trichostrongylus* and *Teladorsagia* (*Ostertagia*) species in goats in the New Zealand Veterinary Journal

The authors reported the results of investigations into resistance in goat worms on a NZ farm. Before arriving at the farm, the goats were 'quarantined drenched' with levamisole (LEV), a benzimidazole (BZ), and moxidectin (MOX). The mean faecal egg count (FEC) 6 days after arrival was 665 eggs per gram (*Trichostrongylus* and *Ostertagia*).

[It should be noted that, in Australia, the only anthelmintics registered for use in goats are certain BZ-, morantel- and triclabendazole-based drenches. Drenches licensed for use in goats are also limited in NZ].[2]

The goats were treated again with these three anthelmintics (LEV, BZ, MOX), at 1.5 times the recommended dose rate for sheep. There was no reduction in FEC at days 7 and 14 post-treatment.

The wormboss Team with Scott Williams from AWIL. Melbourne 2004. L-R: Arthur Le Feuvre[1], Scott Williams, Ian Carmichael[2], Rob Woodgate[3] and Brown Besier[4], Noel Campbell[5], Stephen Love[6]. Absent: Andrew Bailey[7].

[1]Senior Extension Officer, Queensland Dept of Primary Industries and Fisheries

[2]Chief Veterinary Parasitologist, South Australian Research and Development Institute

[3]Veterinary Officer, Department of Agriculture, Western Australia

[4]Principal Veterinary Parasitologist, Department of Agriculture Western Australia, Manager, Sheep CRC parasitology Sub-Program

[5]Senior Parasitologist, Department of Primary Industries, Victoria

[6]Veterinarian/State Coordinator-Internal Parasites, NSW Department of primary Industries (incorporating NSW Agriculture)

[7]Department of Primary Industries, Water and Environment, Tasmania

A group of goats were treated with the same drenches and dose rates, but on two occasions 24h apart. There was a 63% FEC reduction 7 days post-treatment.

A further group of 10 were treated with the organophosphate (OP) compound trichlorphon (Neguvon® (Bayer)), at the sheep dose rate. Based on FEC and larval cultures, *Ostertagia* were reduced by 74% and *Trichostrongylus* by 0%. However, it should noted that such OPs have only ever been incompletely effective against these genera

[2] Neguvon is not actually registered for use in goats, but it can be used by way of a permit which applies in most Australian states and allows it to be used for treatment of *Haemonchus* in goats.

The authors' key concern is that veterinarians need to be aware that such multiple resistant worms exist on farms in NZ.

The situation is not a whole lot different in Australia. Veale (2002) for example reported ivermectin and MOX-resistant *Trichostrongylus* sp and *Ostertagia* sp in goats in north-eastern Victoria, Australia.

More recently Le Jambre and others (2004) characterised MOX-(and avermectin-) resistant *Haemonchus contortus* (described as Hc-MOX-R) and *Trichostrongylus colubriformis* (Tc-MOX-R) from goats in the Gold Coast hinterland, Queensland. Selection pressure was extreme, with some age groups being treated eight times a year with MOX. Both naphthalophos (NAP) and LEV were effective against Hc-MOX-R, with use of a NAP+LEV being suggested as a good control option. LEV and especially NAP were somewhat less effective against Tc-MOX-R. An avermectin (abamectin) was also tested and, as expected, was even less effective against these strains than MOX. The authors discuss the results of their characterisation study and differences between avermectin- and milbemycin (eg MOX) resistance. They note also that *H. contortus* and *T. colubriformis* join *O. circumcincta* from New Zealand as being able to survive MOX treatment as adult resident worms.

Rolfe and others (1994) reported multi-drug (including avermectin/moxidectin) resistance in *Ostertagia* spp from goats in southern New South Wales imported from New Zealand. Treatment of the goats before export (ivermectin) and after export (MOX as well as LEV and fenbendazole) failed to be highly efficacious. Subsequent (faecal egg count reduction (FECR)) testing with albendazole (ABZ), MOX, or MOX with morantel indicated resistance to these drugs. Subsequent slaughter studies in sheep and goats confirmed these findings. However, three treatments with ABZ at 12 hourly intervals were highly effective based on FECR, but failed to remove the worms (egg output suppression). (P. Rolfe personal communication). This may have been an isolated even highly unusual case, however it is still noteworthy.

Macrocyclic lactones

The macrocyclic lactone (ML) group of broad-spectrum drenches includes

- Avermectins eg abamectin, doramectin, ivermectin
- Milbemycins eg moxidectin

ML sheep products in Australia contain abamectin, ivermectin or moxidectin. There are no ML drenches registered for use in goats in Australia.

What are the practical implications?

- Resistance to all broad-spectrum groups (including avermectins/milbemycins) in worms of small ruminants is becoming more common.
- Farmers and advisers need to be more aware of the importance of effective quarantine procedures.
- Treatment with three, preferably four unrelated actives is necessary.
- The efficacy of the quarantine treatment should be checked, with imported animals being held in an area which allows for remedial action to be taken with respect to any highly resistant worms that are found to have survived quarantine drenching.

For more information, see the NSW DPI Agnote on this subject (Love 2004).

SL.

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Love S (2004). Sheep worms: don't import resistance. Agnote. www.agric.nsw.gov.au/reader/sheep-internal

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West DM, Pomroy WE and Leathwick DM (2004). Multiple resistance in *Trichostrongylus* and *Teladorsagia* (*Ostertagia*) in goats to oxfendazole, levamisole and moxidectin, and inefficacy of trichlorphon. New Zealand Veterinary Journal 52(5), 298-299.

Resistance to moxidectin and abamectin in *Ostertagia circumcincta* in New Zealand

McKenna and others recently reported on their investigation of resistance to macrocyclic lactone (ML) anthelmintics by *Ostertagia circumcincta* in lambs on a sheep and cattle property in the North Island of New Zealand.

Groups of lambs received one of various treatments at recommended dose rates: oral moxidectin, oral abamectin, an albendazole-levamisole combination, and an albendazole-levamisole-ivermectin combination. Post mortem worm counts were undertaken 7 days after treatment to determine the efficacy of each anthelmintic.

Results (% reduction of *O. circumcincta* worm burdens) were:

albendazole + levamisole	100%
albendazole + levamisole + ivermectin	100%
moxidectin	72%
abamectin	29%

The authors concluded: 'These results clearly demonstrated the occurrence of resistance to MLs by *O. circumcincta*. Although this is not the first occasion where resistance to this anthelmintic family has been detected in this parasite in sheep in New Zealand, it is the first instance that resistance to either moxidectin or abamectin has been reported."

[Obviously these results need to be viewed in context. Different farms –whether in NZ or Australia – might yield entirely different results.]

The authors' introductory comments provide interesting background information on the current situation in NZ:

"Over the last few years, resistance to (ML) or milbemycin/avermectin anthelmintics has been reported with increasing frequency in ruminants in New Zealand. While most of these reports have involved parasites of goats and cattle, a few cases in sheep have also begun to emerge. In cattle, ML resistance has mainly involved infections of *Cooperia oncophora* (Vermunt et al 1995; Loveridge et al 2002) and, more recently, *Trichostrongylus longispicularis* (Loveridge et al 2002). In goats, it has been found in *Ostertagia circumcincta* (Badger and McKenna 1990; Pomroy et al 1992; Watson et al 1996) and *Trichostrongylus colubriformis* (Gopal et al 1999), while in sheep it has been reported in *O. circumcincta* (Leathwick et al 2000; Mason et al 2001; Vickers et al 2001) and *Haemonchus contortus* (Vickers et al 2001). In the latter two hosts, and in sheep in particular, these cases of resistance have generally involved ivermectin only, and the remaining two MLs

Home » Farm management » Farm chemicals » Pesticide record keeping »

Keeping Records of Pesticide Application

11 July 2002

In NSW 'drenches' are not 'pesticides' (unless applied externally for the purposes of controlling ectoparasites [I kid thee not]), but the information at < www.agric.nsw.gov.au/reader/record/keeping-records.htm > may be of interest to some readers of TTW.

registered for use in sheep in New Zealand, namely moxidectin and abamectin, have continued to demonstrate a high therapeutic efficacy against them (Leathwick et al 2000; Vickers et al 2001).

We report here the first instance of resistance to moxidectin and abamectin by a sheep parasite in New Zealand." (The first (published) report in Australia, in 2003, was in *Haemonchus* (Love and others 2003).

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Novel approaches to worm control

[The following is of interest, although there is nothing new, and the statement that the FAMACHA system is 'directly and immediately applicable to all regions where *H. contortus* is a problem' is perhaps optimistic. –Ed]

Miller JE, Waller PJ, Thamsborg SM, Larsen M, Knox MR, Peter R, Molento MB, Beriajaya, Hood B (2004). Novel approaches to control of parasites - a workshop. Veterinary Parasitology. 125(1-2 Special Issue SI):59-68, 2004 Oct 28.

Abstract
'With the advent of helminth parasite populations that have developed resistance to anthelmintics over the last decade or so, especially in small ruminants, sustainable productivity has been threatened. This workshop on novel approaches to control was held at the 19th international Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP) at New Orleans, LA, USA, during 10-14 August 2003.

The workshop was organized and chaired by J.E. Miller and P.J. Waller.

Novel or alternative approaches to control have been the focus of research (basic and applied) in many parts of the world. The objective of the workshop was to discuss where we have been and what direction(s) appears to be viable for both the short and long term future. In the long term, all represented regions at the workshop have promulgated programs where breeding for resistance may be the best approach as genes for resistance can be fixed in host populations. However, it does take many years to achieve results and the question of trade-off concerning alteration of production traits needs further evaluation. Vaccination, especially against *Haemonchus contortus*, has been a thrust of laboratories in Scotland and Australia where natural "hidden gut" antigens have shown promise, but recombinant products have yet to be developed. In Europe, North and South America, Australia, South Africa and Asia, biocontrol using the nematode-trapping fungus *Duddingtonia flagrans* has been shown to be effective under experimental conditions, but some field evaluations have been disappointing. Most recently, the FAMACHA system was

Some recent DPI 'parasite' Agnotes

Internal Parasites

Cattle worm control - the basics 209kb pdf
<www.agric.nsw.gov.au/reader/cattlehealth>

For sheep worm Agnotes, go to
<www.agric.nsw.gov.au/reader/sheep-internal>

External Parasites
<www.agric.nsw.gov.au/reader/sheep-external>

Chemicals registered to treat lice and flystrike on sheep, September 2004 150kb pdf
(More information: contact the author [Ian Evans]).

developed in South Africa. This system is directly and immediately applicable to all regions where *H. contortus* is a problem. Although not a new or novel approach, copper-oxide wire particles have been revived as a means to control *H. contortus.* Work being done in Europe, North and South America, South Africa and Australia have shown very encouraging results and can probably be considered the best short term approach available. However, caution needs to be considered in sheep to avoid potential copper toxicity problems. Work in New Zealand, Scotland and the US with forages and feeds containing condensed tannins have shown some limited control. Many laboratories have demonstrated that adequate and balanced nutrition programs are also important to maintain mechanisms that combat infections.

Overall, no one approach alone is the answer. Approaches that are integrated, including "smart" use of anthelmintics, are necessary.'

National sheep project to survey farmers

From 'UNE News and Events'
September 29, 2004

'Thousands of sheep farmers are being asked to help with a $2.7 million, nation-wide project that could contribute to the "greening" of the Australian sheep industry.

The University of New England is surveying more than 6,300 producers in five States, seeking information about their use of chemicals to control parasites of sheep.

Lyndal Thompson, the UNE researcher conducting the survey and from UNE's Institute for Rural Futures, said the project aimed to demonstrate that farmers could maintain production while reducing the frequency of chemical treatments. This would have several beneficial outcomes, she said. "For example, less frequent drenching would help producers meet standards required for 'organic' and 'ecologically certified' labelling of their products. At the same time, it would slow the parasites' development of resistance to the chemicals. While parasite problems cost the Australian sheep industry more than $550 million a year at the moment, this figure could rise to more than $1 billion as resistance increases."

The "Integrated Parasite Management (Sheep)" project, managed and funded by Australian Wool Innovation Ltd, has appointed 24 "demonstration farms" throughout the country, where modified regimes of parasite control, including less frequent drenching, will be tried. "We hope to be able to demonstrate the benefits of reducing the number of drenches by one or two a year," Mrs Thompson said. "This would show farmers that there are simple and effective ways of handling problems such as parasites' resistance to chemicals."

The project is concerned with the management of both internal parasites such as barber's pole worm and liver fluke, and external parasites such as blowfly larvae and lice. It involves researchers at the Universities of New England and Melbourne, the West Australian Department of Agriculture, and the Queensland Department of Primary Industries and Fisheries.

Mrs Thompson said the survey form would be posted, at the beginning of October, to sheep farmers in New England, south-west Queensland, southern NSW, and the sheep-growing regions of Victoria, South Australia and Western Australia. "It's basically a benchmarking survey," she said. "The idea is to get a picture of what farmers are doing now to control parasites, and when and why they're doing it. Another survey in two years' time will reveal how much these practices have changed as a result of the demonstration-farm trials." She is asking farmers to return the survey within three weeks of receiving it.

Mrs Thompson's work is part of the socio-economic component of the Integrated Parasite Management project. Six other UNE researchers are involved in the project as rural scientists. As

well as assisting with the trials on demonstration farms and assessing the results, they are conducting research on the effect of climatic conditions on the proliferation of worms on pastures, and a promising method of controlling the worms biologically. The coordinator of the UNE research team, Dr Andrea Crampton from the University's School of Rural Science and Agriculture, said the method involved a fungus that was able to trap and kill worms on the pasture before they were able to infect stock.

Media contact: Lyndal Thompson, Institute for Rural Futures, UNE (02) 6773 5144 or Lydia Roberts, Public Relations Manager, UNE (02) 6773 2779.
Posted by Lydia Roberts at September 29, 2004 10:45 AM. '

Serological testing for liver fluke in cattle: comparison of a commercial antibody ELISA kit with the NSW DPI-developed assay

Gareth W Hutchinson and Catherine Fitzgibbon

Parasitology Section, Elizabeth Macarthur Agricultural Institute,
NSW Department of Primary Industries, Menangle, NSW 2568

Background
Liver fluke, *Fasciola hepatica*, is a snail transmitted parasite of sheep and cattle in Australia which has been estimated to result in 5% average loss of production and some $20 million annual costs of chemical treatments [1]. Current parasitological tests to detect liver fluke by finding parasite eggs in faeces are insensitive, time consuming and can only detect infections some 12-14 weeks after infection. By this time considerable damage to the liver has already been caused. Approximately 20,000 tests are performed annually in Australia for diagnosis of infection, monitoring of control programs and certification for movement of livestock to fluke-free areas (Western Australia).

Serological (blood) tests have been developed which are capable of detecting antibodies produced in response to infection. These tests are useful as herd tests but not for individual animals. They are good at confirming freedom from disease (high specificity) but less reliable in accurately assessing infection (relatively low sensitivity) [2]. Antibodies persist for months after fluke have been killed or died naturally.

An antibody-based test (ELISA) has been developed by a French research company Institut Pourquier [3], and is now available commercially in Australia [4]. The test is claimed to be highly specific and sensitive in detecting liver fluke infection in cattle. However, limited validation of the test under Australian conditions has been conducted [5], particularly in areas endemic with paramphistomes (conical/stomach flukes)

This project was designed to further assess the usefulness of the Pourquier test for use in Australian cattle.

Objectives
The aims of the project were to use the Institut Pourquier ELISA kit to determine:

- The sensitivity and specificity of the test in a range of experimental and natural infections;
- Whether the test can be used to distinguish between infections with liver fluke and stomach flukes (paramphistomes);
- The concentration of antibodies over time following the termination of liver fluke infections by chemotherapy;
- The degree of cross-reactions in the test with other parasite infections in cattle;
- The time course of antibody responses following infection, and the sero-conversion in prepatent infections;
- And if possible to determine the relationship between antibody levels and fluke burdens or faecal egg counts.

The effectiveness of the kit was compared with an existing in-house antibody ELISA used by NSW Agriculture.

Activities

A series of studies were conducted using available serum banks from experimental and natural infections of cattle held by NSW Agriculture. Additional batches of sera were obtained from cattle believed to be free of liver fluke from a non-endemic region of Western Australia, and from cattle infected with a related parasite, *F.gigantica* from Indonesia. Over 1500 individual sera were tested. Some studies involved experimental infection of fluke-free calves maintained parasite free and away from possible reinfection for periods up to 18 months. The effects of chemotherapy on antibody levels were observed in some animals, and others were from epidemiological projects involving tracer calves exposed to natural infections of paramphistomes [7]. Numerous sera from dairy cattle naturally infected with liver fluke from coastal areas of Southern NSW were also investigated.

The sera were tested with the Pourquier ELISA according to the manufacturer's instructions and the results compared with values produced by the NSW Agriculture in-house ELISA. This latter test uses standards of high fluke-positive sera and known paramphistome infected sera and nematode (worm) infected as negative controls. In some instances, historical data were also available on the infection status of the cattle as determined by fluke faecal egg counts or adult fluke counts.

Major Outcomes

The Institut Pourquier ELISA kit for liver fluke in cattle has a high specificity (95-98%) and sensitivity (99%). It appears to be substantially more sensitive than the NSW DPI-developed ELISA. Using the manufacturer's recommended positive cut-off (30% S/P against the kit positive control 1:150 IHA) gives adequate sensitivity.

Presence of paramphistome infection does not appear to significantly interfere with the accuracy of the test, but further studies with experimental stomach fluke infections in parasite-naïve calves would be required to confirm this.

The kit detects seroconversion within a few weeks (2-4 weeks) of infection. The length of persistence of antibody following effective treatment (in the absence of natural re-infection) requires better-controlled studies to determine the exact length of period, provided it is possible to ensure that flukes are killed by the treatment.

Previous nematode infection did not result in false positive results, nor did such infections, when chemically abbreviated; restrict the ability of the ELISA kit to detect subsequent liver fluke infection. Concurrent nematode infections in natural fluke infections were not directly examined. Incidental findings are that they do not cause cross-reactions.

Limited studies with serum from animals infected with the related tropical liver fluke *Fasciola gigantica* indicate that the Pourquier ELISA kit may be used to detect this parasite in overseas countries.

The pattern of serological responses in cattle experimentally infected with *F.hepatica* was similar whether using the Pourquier Kit or the NSW Agriculture ELISA. IgG antibodies were detected earlier with the kit, and reached maximum levels sooner. Persistence of positive titres continued to at least 18 months in the absence of reinfection.

There was no apparent increase in antibodies detected by the kit that could be attributed to the maturity of adult liver fluke at the time of patency (production of eggs).

There was no relationship between positive kit results and either numbers of adult flukes or counts of fluke eggs in faeces.

Recommendations and Benefits to the Meat Industry

- The Pourquier ELISA kit is easy to use and is suitable for diagnostic parasitology

laboratories equipped with an ELISA microplate reader. It is suitable for use in Australia, and would be a good additional test to be offered by both government and private veterinary laboratories, which currently do not have access to serological tests for liver fluke.

- The test proved to be only marginally more expensive than existing in-house ELISA and is now offered as a commercial service by NSW DPI (current prices as at November 2004 are $17.65 for a single sample, $11.70 each for >1 sample). Good standardisation and quality control of reagents makes it an attractive alternative test, particularly as it can also be use to detect liver fluke antibodies in milk (provided adequate further validation is conducted).
- The test is also being used by QDPI for epidemiological studies on liver fluke in SE Queensland and for use on bulk milk samples [7].
- Limitations of the Pourquier test are characteristic of all IgG antibody ELISAs: primarily the inability to differentiate between active and previous infections, and potential for cross-reactions with other parasites. The kit, in its present form, is not suitable for crush/race-side detection or as a barrier exclusion test.
- There are still grounds to support the development of alternate diagnostic tests for liver fluke, such as antigen-detection methods or molecular procedures.

Acknowledgements

Thanks are due to Dr Peter Rolfe, Paul Young and former staff at EMAI and Glenfield Veterinary Research Station for access to serum samples and details of animal histories; to Dr Dieter Palmer, Department of Agriculture WA for sera from fluke-free cattle and testing samples; and Dr David Piedrafita, Centre for Animal Biotechnology, University of Melbourne for sera from *Fasciola gigantica* infected sheep and cattle. Ms Sonia Whittle, Product Manager, Laboratory Diagnostics Pty. Ltd., Bankstown kindly supplied kits at discounted prices. Funding for the IP kit evaluation was provided by Meat and Livestock Australia.

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[7]. Anderson G and Molloy J (2004). Detection and distribution of liver fluke in Queensland. Program and Abstracts, 46th Annual Scientific meeting, Australian Society for Parasitology Inc. Fremantle, WA Sept 26-30, C14

Note: Full details of the results of this study are available from Meat & Livestock Australia Ltd. as the report Validation of French Antibody ELISA for Liver Fluke by GW Hutchinson, Project number AHW.021 (July 2003) by contacting MLA by email to <eleung@mla.com.au>.

SHEEP CRC PARASITE PROJECTS

The following is from the Australian Sheep industry CRC website < www.sheepcrc.org.au>. There may have been further developments with some of the projects since this was posted to the website, but it gives an overall view of what is happening nonetheless. – Ed.

SUB-PROGRAM 1.4
PARASITE MANAGEMENT

Sub-program leader:
Dr Brown Besier, Department of Agriculture Western Australia

The Sheep CRC is coordinating national research and communication efforts into the central issue in parasite management: how to achieve effective and efficient parasite control while ensuring long-term sustainability and consumer acceptance? Major challenges include resistance to anthelmintics and insecticides, chemicals residues in sheep produce, and the need for ethical production practices.

This Sub-program will focus on two major parasite management themes: minimum-chemical approaches and individual sheep assessment and treatment. Research into improved technologies for parasite diagnosis will support the objective and cost-effective assessment of worm burdens and anthelmintic resistance, to ensure that treatments are given only where necessary and with appropriate control agents. New non-chemical approaches include biological worm control and nutritional strategies to enhance worm immunity. The new diagnosis and control strategies will be integrated into the concept of "targeted treatment", utilizing electronic identification and individual weight assessment, so appropriate treatments are given according to need rather than on a routine flock basis.

Further parasite management research involves genetic and immunological approaches to the control of blowfly strike, to reduce the reliance on chemical treatments and mulesing.

The Sub-Program will also facilitate rapid access to individually-relevant parasite management advice, by developing a decision aid and worm information website and CD, with national technical agreement and industry-wide support

Sub-program 1.4 is being delivered through nine projects:

Project 1.4.1A On-farm detection of parasite eggs in faeces

Project 1.4.1B PCR detection of sheep nematode parasites in pasture samples

Project 1.4.1C Diagnosis of sheep nematodes by a faecal antigen ELISA

Project 1.4.1D Improving the DrenchRite test for detection of nematode resistance to macrocyclic lactones

Project 1.4.2A Genetic resistance to blowfly strike

Project 1.4.2B Development of strategies for the immunological control of blowfly strike in sheep

Project 1.4.3 Nutritional strategies to optimize control of internal parasites in meat and wool production systems

Project 1.4.5 On-line decision support for sheep worm management wormboss

For more information on the individual projects, go to < www.sheepcrc.org.au>.