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# New diagnostic tools for monitoring parasites of sheep

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## Abstract

Resistance of gastrointestinal nematode worms to anthelmintic drenches has led to new tactics for treatment of worms in sheep. Regional, calendar-based drenching programs have been replaced with programs designed for individual farms, which are based on measures of worm burden, drench resistance and genetic resistance of sheep to worms. To support these new worm control strategies, the Australian Sheep Industry CRC is developing improved on-farm and laboratory tests to measure worm burdens and the genera of worms present in parasitized sheep. Automated systems for measuring weight loss caused by worms and for diagnosis of covert blowfly strike are under development by the CRC. A test for diagnosis of *Haemonchus* worm infections on-farm is currently under field trial in the lead up to commercial release in 2006.

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## Introduction

Gastrointestinal nematodes are a major cause of economic loss in Australian sheep production systems. Current control methods include anthelmintics, grazing management and breeding for resistant sheep. Resistance to anthelmintics is widespread and individual strains of gastrointestinal nematodes with selective resistance to each of the anthelmintics introduced since the 1970s have been identified (Besier and Love, 2003). Multiple resistance to combinations of anthelmintics is also common. Major causes of resistance are overuse and inappropriate use of drenches. Appropriate choice of drenches requires information about worm burdens, species present and the drench sensitivity of those species. This information can be gained from faecal worm egg counts, faecal culture followed by morphological identification of larval species, and by faecal egg count reduction tests (Anon, 1989). These laboratory tests take from days to two weeks to complete. These delays are inconvenient to graziers and contribute to the inappropriate use of drenches. The Australian Sheep Industry CRC is therefore investigating a range of new diagnostic technologies that should result in easy on-farm diagnosis of worm infections or quicker laboratory assays. In addition, the CRC is examining a technology for remote diagnosis of covert fly strike and body weight change associated with gastrointestinal parasites.

## Rapid identification of parasitic worm burdens

### *Dipstick assay for Haemonchus contortus infection*

*Haemonchus contortus*, the barber's pole worm, is a blood-sucking parasite that inhabits the abomasum of sheep and cattle. Worms start sucking blood from about day 13 of infection. Blood loss is proportional to the number of worms and is the major cause of lost production and death in infected sheep (Le Jambre, 1995). Worm numbers can build up very rapidly and large numbers of sheep can be lost in outbreaks of haemonchosis. Sheep fed at a good level of nutrition can cope with modest

blood loss of up to 10 mL/d, which is typical of burdens of 100 worms per gram or worm egg counts of 1600 eggs per gram, without loss of body weight or a fall in haematocrit. Blood loss in excess of 10 mL/d results in anaemia and decreased production. A diagnostic test called FAMACHA, which is based on examination of the colour of the ocular mucous membranes, was developed in South Africa to monitor pathology and production losses associated with *H. contortus* infections. This on-farm method allows informed drenching decisions to be made quickly (van Wyk and Bath, 2002). The Australian Sheep Industry CRC has developed tests to monitor the presence of blood in faeces to aid the diagnosis of *H. contortus* infections.

Commercial test-strips for detection of blood were found to provide a sensitive method for detecting the presence of faecal blood associated with *H. contortus* infections, even before worms have matured into adults and commenced laying eggs. A provisional patent has been lodged and an extensive field trial, jointly funded by Meat & Livestock Australia and Australian Wool Innovation, is being undertaken to calibrate the test and determine its specificity for the diagnosis of *H. contortus* infections in ruminants. If the field trial proves successful, the new diagnostic test should be commercially available in the second half of 2006. The test is performed on-farm using small faecal samples from a representative number of sheep; faeces can be collected from the ground behind the sheep as they graze. One limitation of the test is that it will not alert graziers to the presence of worms other than *Haemonchus*. Consequently, it will still be necessary to conduct conventional worm egg counts and larval differentiation tests until on-farm tests are developed for other worm species.

### ***The use of worm-derived molecules in faeces for diagnosis***

As part of our strategy to develop on-farm tests for parasite infections, a CRC project jointly funded by MLA and AWI is examining the presence of antigens released from parasites within the gut. Several tests have recently been developed for identifying gastrointestinal viral infections in humans by detecting viral antigens in faeces. Following these successes, antigens in sheep faeces are being examined in *H. contortus*, *Teladorsagia* (*Ostertagia*) *circumcincta* and *Trichostrongylus colubriformis* infections of sheep.

To date, we have isolated proteins from faeces that are unique to sheep infected with *H. contortus* and have used these to make antibodies that bind the faecal protein. Using these antibodies, we developed a simple laboratory assay for detecting the parasite. We are developing a dipstick version of the assay for use in the field. Once this has been accomplished, large numbers of animals will be tested to confirm the efficacy of the dipstick method under field conditions. It is anticipated that the final form of the test will require a small amount of faeces, which will be mixed with a buffer before application onto the dipstick. A colour change at a detection band on the dipstick will signal a positive reaction within about 10 minutes.

Work has begun on isolating proteins shed in faeces during *Teladorsagia* and *Trichostrongylus* infections. We hope to make these assays available within 12–18 months for field testing and then to combine all three assays into a single dipstick test.

In another approach, we tested a dog's ability to smell parasites in sheep faeces. A dog has been trained using techniques employed by customs and police services to teach dogs to detect drugs or explosives. The project has been very successful and the dog can now reliably detect the presence of each of the common nematode parasites and mixtures of them in samples of sheep faeces. This development could change the diagnosis of parasites completely, as infections could be detected in individual sheep, not just in a sample of the flock. We are now attempting to train the dog to identify individual sheep that are infected with parasites. If this is successful, it may be possible to use a dog to monitor parasite infections when sheep are yarded and only treat those animals that the dog identifies. However, for on-farm use we would need to devise a way of allowing the dog to smell sheep quickly and with a minimum of handling.

The success of the dog research provides strong impetus to develop "electronic noses" for detecting parasites, which may be more reliable than dogs. We have already started such work by analysing the

odour of faeces during infection and comparing this with that of faeces from uninfected controls. We hope to use the dog to assist in determining which odour molecules are important for parasite detection and then to develop an electronic nose that can recognise these odours on sheep.

## **Rapid identification and quantification of worm egg genera**

### ***Quantitative estimation of the number of worm eggs in faeces***

Worm egg counting is a simple technique and has been successfully used for decades (Whitlock, 1948). The technique requires only a saturated salt solution, a special counting chamber and a microscope. Some training for the recognition of eggs is necessary, but the skill is easily acquired. However, the technique is time consuming, especially when large numbers of samples have to be examined. With the trend away from calendar-based drenching schedules, there is increasing reliance on worm egg counts for estimating parasite burdens, pasture contamination and for identification of parasite-resistant sheep. The amount of faecal samples that are processed by laboratories for estimating worm egg counts is therefore increasing rapidly.

Attempts have been made to automate worm egg counting (Mes et al., 2001), but the method is unsuitable for samples collected in some parts of Australia during the drier months of the year because of the high fibre content of faeces at this time (D.G. Palmer, personal observation).

We have therefore developed antibody-based technologies to make worm egg counts amenable to automated high-throughput testing in the laboratory. Worm-egg-specific antibodies are used to detect worm eggs extracted from faecal material. Eggs in faecal material are extracted with a cell disruptor and then detected in the supernatant by an antibody capture assay. The quantitative assessment of egg material captured and detected by the antibodies correlates well with the number of eggs present in faeces. All steps in the test can be automated and are designed for high-throughput screening. The test can detect 50 eggs/mL of sample, and work on the rapid concentration of eggs from faecal material is in progress. The test can be conducted in the presence of faecal material without requiring further egg purification prior to testing. The test detects eggs from *H. contortus*, *Trichostrongylus spp.* and *Teladorsagia spp.* Calibration of the assay for each of these worm genera is in progress.

Although the test is designed for high throughput testing in dedicated parasitology laboratories, it has potential to be developed into a rapid semi-quantitative test for on-farm use.

### ***Rapid identification of worm egg genera***

On-farm treatment decisions for gastrointestinal nematodes depend not only on worm egg counts, but also on the worm genera involved. The presence of *H. contortus* is of particular significance but the identification of other worm genera is becoming increasingly important. Because drench resistance is worse in some worm genera than others, drench strategies need to be tailored to the worm genera present and their drench resistance status. This will result in an increasing demand for worm egg differentiation. Currently, differentiation of worm eggs relies on identification of third-stage larvae after eggs have been allowed to hatch. This delays the diagnosis by seven days, which is not compatible with modern farm management practices. Furthermore, the differentiation of larvae requires skill and experience that can only be acquired after considerable training.

We have shown in previous work that barber's pole eggs can be identified by staining with fluorescent-labelled lectins (Palmer and McCombe, 1996; Colditz et al., 2002). Within the sheep CRC project, we have extended this technique and identified three lectins that either alone or in combination allow the differentiation of eggs from *H. contortus*, *Trichostrongylus spp.*, *Teladorsagia spp.* and *Chabertia ovina* (large-mouthed bowel worm). The test can be completed on the same day as sample collection. Egg differentiation with lectin staining promises to be far more accurate than the larval differentiation technique because it reduces the variability of larval culture. Lectin-based egg differentiation can be conducted in any laboratory equipped with a fluorescence microscope.

However, peroxidase-labelled lectins could be used for colour-based detection without the need for a fluorescence microscope.

### **Weight change as an index of the effect of parasites**

As an alternative to diagnostic tests aimed at quantifying worm burdens, assessment of the effects of parasites on sheep may prove to be a useful index of whether treatment is necessary. Two different responses of sheep to parasitism are central to this concept: resistance (the ability to limit the size of worm burdens) and resilience (the ability to maintain production despite worm infection). Considerable genetic variation exists between sheep in resilience to the effects of worms, and for at least some worm species, resistance and resilience are independent traits (Bisset and Morris, 1996).

A resilience-based approach represents a novel method for parasite management. Whereas whole-flock treatment decisions for parasite burdens are based on the results of tests conducted on a relatively small proportion of a flock, resilience assessments must differentiate between individual animals so that only those that are significantly affected by parasites receive treatment. The benefits of this concept include both the efficiency of treatment (lower treatment costs) and the provision of a source of worm “refugia” in the form of resilient sheep that have not been drenched (a tactic for management of anthelmintic-resistant worms; Besier and Love, 2003).

This approach forms the basis of the successful FAMACHA system discussed previously. For the scour worm genera, chiefly *Teladorsagia spp.* and *Trichostrongylus spp.*, the proposed index of resilience is short-term weight change, which reflects the effects of these parasites on nutritional efficiency and feed intake. Comparative growth rates of sheep subject to various parasite challenges are under investigation as a selection index for lamb breeding enterprises in New Zealand (Bisset et al., 2001). In Australia, research in progress through the Sheep CRC aims to develop assessment protocols applicable to various environments and parasite species. Using electronic ear tags for the identification of individual sheep in an automated weighing system, body weight at a particular time can be compared with previous records. Drafting gates linked to the database can then direct animals failing to meet preset weight change criteria to pens for closer inspection, drenching or nutritional treatment. The goal is a fully automated system that obviates the need for direct operator involvement.

Research is needed on the effectiveness of targeted treatment regimens vs. traditional whole-flock treatment regimens and on whether the effects of parasites can (or should) be distinguished from those of nutrition. Managing parasites on an individual sheep basis rather than a flock basis is likely to contribute to maximising the efficiency of sheep enterprises as well as assisting in the sustainability of parasite control.

### **Remote diagnosis of covert blowfly strike**

Diagnosis of fly strike currently relies on close and regular examination of sheep during periods of susceptibility. The CRC is examining technologies for remote, automated diagnosis of fly strike as a component of the e-sheep concept. Sheep reduce voluntary intake of feed and rapidly lose weight when infected with blowfly larvae (Colditz et al., 2005). In addition, characteristic odours released from sheep with dermatitis (such as fleece rot) precede most cases of fly strike. These odours assist female flies to locate sheep that have sites suitable for egg laying (Emmens and Murray, 1983). An electronic-nose apparatus is currently being evaluated in combination with remote, automated electronic weighing to determine whether the early stages of blowfly strike can be detected in the field. The ability of the system to detect the presence of predisposing conditions such as dermatitis and dags will also be investigated.

Future work will focus on delivery of on farm technologies for diagnosis and treatment of parasite infections, with an emphasis on individual animal management.

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