

hexose was galactose (40.9) with smaller proportions of glucosamine and galactosamine (24.6 and 21.9 respectively). Fucose (12.6) approached a significant decrease in *H. contortus* infected (6.3) and *O. circumcincta* infected sheep (8.3). Galactosamine was lower in infected animals than in worm-free sheep. There was no difference in the proportion of galactose between uninfected and *H. contortus* infected animals (40.2) whereas it increased in those *O. circumcincta* infected (62.9). The study showed that parasitism caused changes in the ratio of hexoses and hexosamines in gastrointestinal mucins of sheep.

Recovery of L3 *Haemonchus contortus* larvae from grass samples—where do they go?

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A common problem with estimating the contamination of pasture with sheep parasitic larvae is obtaining an accurate estimate of the population from a plot sample. Even if the plot correctly represented the whole pasture, recovery of worms using standard methods can vary from 20 to 80%. Experiments were carried out to assess the loss of worms at each step through the process of larval recovery from standardised grass samples. A counted number (100, 500 or 1000) of fluorescently-labelled *Haemonchus contortus* larvae (L3) was incubated on grass overnight and then recovered by baermannisation for 4–48 h. The baermannising solutions included water, bleach 0.13%, Triton X100 (1% or 0.5%) or Pyroneg 0.4%. To identify points within the technique where larvae might be lost, larvae remaining in pipettes, beakers and different layers of the baermannising solution were counted. The highest recovery (50%), while

maximising consistency, was with 500 larvae and baermannisation for 48 h in water. Higher recovery, but greater variability (30–90%) was obtained using the bleach solution. Treatments such as adding detergents Triton X100 or Pyroneg had little effect (20–45%) on improving recovery. Flushing pipettes with water after each handling process of the larvae reduced the loss by only 1% and siliconising the glassware to avoid adhesion to the glass surface by 2%. The 40% not recovered are likely to be left on grass. Overall the most consistent method for worm recovery was baermannising using siliconised glassware, for 48 h at room temperature.

Effect of excretory/secretory products of abomasal parasites on epithelial tight junctions

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The presence of abomasal parasites is thought to be associated with an increase in the permeability of the gastric epithelium. Epithelial permeability is regulated by junctional complexes between adjacent cells. The most apical component of this junctional complex is the tight junction which functions as a paracellular diffusion barrier. Any disruption of tight junctions results in impaired barrier function and an associated increase in epithelial permeability. To investigate the effect of abomasal parasites on the integrity and barrier function of epithelia, Caco-2 cell monolayers were exposed to the excretory/secretory products (ES) of adult *Ostertagia (Teladorsagia) circumcincta* and *Haemonchus contortus*. Changes in epithelial barrier function were monitored by measuring transepithelial electrical resistance (TEER) and tight junction integrity was visualised using immunofluorescence localisation of the tight junction-associated proteins, occludin and zonula occludens-1 (ZO-1), by confocal microscopy. Under control conditions, occludin and ZO-1 were localised to a continuous pericellular ring around individual cells when viewed from the apical sur-

face. In cells exposed to ES for 24 h, staining of this pericellular ring was diminished in intensity and corresponded with an increase in the presence of punctuate, intracellular staining. Exposure to ES was also shown to interfere with tight junction integrity, which was detected as a decrease in TEER from $678 \pm 10 \Omega\text{cm}^2$ (control) to $526 \pm 8 \Omega\text{cm}^2$ (ES-treated) ($n = 12$) in 6 h. These alterations in TEER, along with intracellular changes in occludin and ZO-1 distribution, suggest that parasite ES disrupts tight junctions, leading to an increase in epithelial permeability which may be of importance in the pathology of abomasal parasitism.

Abamectin toxicity in farm dogs

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Macrolide antiparasitic agents including abamectin, are used extensively in New Zealand. Although the sensitivity of collie and collie-crosses with the MDR1 gene mutation to macrolide parasiticides is well documented, abamectin toxicity in other breeds of dogs without this mutation is not well known. Cases of serious toxicity and deaths in farm dogs exposed to abamectin pour-on products highlight the risks of exposure in working dogs.

Clinical signs of toxicity include dilated pupils, blindness, tremors, ataxia, vomiting, hypersalivation, depression, coma and death. There is no antidote for macrolide antiparasiticides, but symptomatic treatment will result in a favourable outcome in many dogs. The prognosis depends on the amount of compound ingested and the severity of the clinical signs. If the dog becomes comatose, recovery may take several weeks. Blindness is usually reversible.

A farmer treated his cattle with an abamectin pour-on. The following day one farm dog was found dead in its kennel and four other dogs showed a range of signs from ataxia to depression. A blood sample from one of the more severely affected dogs had a plasma abamectin concentration of 0.149 mg/litre. In comparison, beagles given a topical exposure of 6 mg/kg had a peak plasma level of 0.022 mg/litre. The LD_{50} of orally dosed abamectin in dogs is reported as approximately 8 mg/kg. Farmers using pour-on products, which contain 10 mg/ml of abamectin, need to be aware of the danger to their working dogs.

Marketing problems for New Zealand's hydatid vaccine

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A vaccine to prevent sheep getting hydatid disease was invented at Wallaceville Animal Research Centre. It was patented in 1993 in New Zealand, Australia, Mongolia, Bulgaria, Hungary, Brazil, Ukraine, Europe, Japan, USA, Chile, Uruguay and Russia. Yearly renewals are very costly so most protection was relinquished by 2000. Marketing problems included the following:

- (1) Interest was expressed mainly from Third-World Countries where patents were not recognised, including Argentina, Peru, Bolivia, Paraguay, all the Eastern Russian "Stan" countries, China, all of the Middle East, and all of Africa.
- (2) Before 1990, the necessary molecular biology skills were not available in New Zealand, so cloning of the New Zealand-identified protective molecule was done by Melbourne University. For this service they have always insisted on 50% of any royalties that may be generated.
- (3) The Eg95 vaccine was transformed into *E. coli* and expressed as inclusion bodies, which facilitated purification of large amounts of protein. After production technology capable of scaling-up to 1000 litre level was developed in New Zealand, we began technology transfer to an Argentine firm (1997). This move coincided with the crash of the Argentinian economy, and the project was dropped. Another Argentinian firm is now (2008) starting to make the vaccine.
- (4) China was interested in the vaccine, and 3 years of field trials using the New Zealand vaccine were successful (1999). A factory was built in Beijing and scientists trained at Wallaceville (2001) and we supervised the production of an excellent batch of vaccine (2003). Extensive safety trials were then required and were completed in 2005. The registration document was then assembled and registration was granted in June 2007. The factory then had to be moved from a centre of habitation, so it is being built again. Chinese authorities concerned with hydatid control are now calling for the vaccine, but it is still not available.