Faecal Antigens For Parasite Diagnosis: Preliminary Findings of Proposed Research

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Introduction

Lungworms and gastrointestinal nematodes are common infections of red deer (*Cervus elaphus*) in New Zealand. They are responsible for considerable losses in production of farmed deer. Surveys have shown that the most common gastrointestinal nematodes occurring in red deer in New Zealand are *Ostertagia*, *Spiculopteragia*, *Skrjabinagia* and *Apteragia* species (Andrews 1964; Mason, 1977). Although, gastrointestinal nematodes rarely cause clinical disease in wild red deer, they may cause clinical disease and loss of production in farmed deer when large numbers are present in the abomasum. This may occur when climatic conditions and the epidemiology of gastrointestinal parasitism may influence an increase in the prevalence of nematodes resulting in clinical disease (Mason, 1977). This may also occur when all the gastrointestinal parasites are not eliminated because of the use of ineffective anthelmintics, or when they are used improperly. Both type I and II ostertagiasis have been reported to occur in red deer (Mason, 1983; Connan, 1997), and in Canadian Elk (*Cervus canadensis*, Mason, 1984). Chronic-ill thrift or "fading elk syndrome" can occur in elk and elk crosses and this condition is often associated with the presence of *Ostertagia*-type larval infections in the abomasum (Orr et al., 1990; Mackintosh and Orr, 1990; Conway, 1990; Waldrup and Mackintosh,1992). It is believed that the pathogenesis of this condition involves large numbers of adults accumulating in the lumen of the abomasum and immature or inhibited larvae invading the abomasal mucosa, damaging the gastric glands, leading to loss of function and a rise of abomasal pH (Waldrup and Mackintosh, 1993). This results in poor digestion, loss of protein, cachexia and weight loss. Use of topical anthelmintics effective against *Ostertagia*-type larvae, such as avermectins and milbemycins, can control the occurrence of this condition.

Faecal egg counts

Diagnosis of gastrointestinal parasite infections is important to maintain effective control of parasitic disease for maximum production of farmed deer. Diagnosis based on detection of parasite eggs in the faeces of suspected animals is unreliable. Faecal egg counts in red deer are poorly correlated with parasite burdens (Mackintosh et al.,). Passage of parasite eggs in faeces is inconsistent and differential because worm fecundity is not constant and may be affected by several factors including host immunity and parasite species. The techniques are labour intensive, cannot be automated and require a trained person to effectively diagnose the infections. Presence of arrested larvae or

worms during the prepatent period of the life cycle may give false negative results and later give rise to clinical disease.

Immunological tests

Immunological tests offer an alternative to examinations of faeces for parasite eggs and larvae. These tests can be developed as simple kits (e.g. a dip stick assay), so that samples can be tested in the field by lay personnel. These tests can be cheap, specific and can be automated. Results can therefore be obtained within a short time and without sophisticated equipment. Most immune assays are based on the detection of antibodies in serum, but they do not differentiate current infections from past exposure. This drawback can be overcome by designing tests to detect the presence of antigens specific to the infecting organism. It has also been shown that antigen detection tests are more reliable as a diagnostic strategy. This report outlines studies underway at AgResearch Invermay designed to detect parasite antigens voided in the faeces of infected red deer.

Materials and methods

Experimental design

In this experiment we propose to develop a diagnostic test utilising secretory excretory proteins (SEP) of abomasal nematode parasites of red deer in an antigen capture ELISA, a hypothesis tested in a laboratory model of *Heligmosomoides polygyrus* in mice (Johnson and Behnke, 1996) and for *Fasciola hepatica* in cattle (Dumenigo et al., 1996). This study requires establishment of infections of deer with specific abomasal parasites. These infected deer act as donors of both adult parasites and faecal samples containing parasite antigens. The steps involve; a) the isolation and characterisation of deer specific abomasal parasite SEP proteins, b) the vaccination of rabbits with purified SEP antigen to generate polyclonal antibody, and c) the isolation of antibody from the rabbit serum which is then used to develop the antigen capture ELISA test.

Establishment of deer specific parasite cultures is a lengthy process therefore the study was designed to be conducted in 2 parts

1. A preamble study using a parasite of sheep, *Ostertagia circumcinta*, whose infective doses as pure cultures were available. This parasite has been reported to infect deer and was used as a model to determine if the proposed ELISA can be developed for deer
2. Establish infective doses of deer specific abomasal parasites and repeat the above to establish the ELISA.

This paper reports on the preamble study of experimental infections of *O. circumcinta* in red deer and on the studies conducted so far on establishment of deer specific abomasal parasite cultures.

A. *Ostertagia circumcinta* in red deer:

Four, four-month-old fawns raised in parasite free environment were each challenged with an infective dose of 5000 L3 of *O. circumcinta* (kindly provided by Vlassoff, A., AgResearch Wallaceville). A challenge dose of 5000 L3 was chosen because in previous experience infections have been established with 3000 L3 of deer-origin abomasal parasites. The doses were warmed up to room temperature for 30 minutes and a sample tested for larval motility before they were given to the deer via a stomach tube. The use of stomach tube was to avoid the oesophageal reflex ensuring that the L3 larvae were administered intraruminally to model a naturally acquired infection. Body weights, faecal samples and blood samples were collected from each deer on a weekly basis. From day 14 onwards the faecal samples were collected every day to document the prepatent period of this parasite in deer.

B. Deer-specific *Ostertagia* type parasite infections

Deer-specific *Ostertagia* type cultures were established by culturing faecal material collected from a centinel deer fawn grazing with a mob on an infected pasture. The challenge dose will be prepared for deer raised in a parasite free environment.

Results

A. *Ostertagia circumcinta* in red deer

Patent infections were not detected until the sensitivity was increased from 100 epg to 50 epg on day 28 post-infection (PI), when egg counts were observed in 2 of 4 deer. The fawns were euthanaised on days 36 to 43 PI. No visible lesions were seen on the abomasum wall of any of the 4 deer. One fawn had zero worms and another had 2 worms detected after sifting through half of their abomasal contents. Of the remaining two deer, one had 10 worms and the other had 98 worms after the whole abomasal content was sifted. These worms were not active or mobile, and some worms were disintegrating. The worms were collected and incubated in media to harvest SEPs. After dialysis, concentration, and protein determination of the SEP it was observed that the 24hr. cultures did not yield enough protein to conduct any tests.

B. Deer-specific *Ostertagia* type parasite infections

A challenge dose of 14,000 L3s was established and used to challenge 2 parasite naive deer at an infective dose of 7000 L3 each. The dose consisted of 95% *Ostertagia*-type larvae and 5% *Trichostrongylus*-type L3's. At the moment these deer are being monitored for patency of infection.

Discussion

Ostertagia circumcinta is a parasite of sheep which has been reported in deer on several occasions (Anonymous, 1979; Mason, 1994). Mackintosh et al. (Pers. Ccomm.) observed *O. circumcinta* in 2 of 20 deer at 9% of the total abomasal counts. These deer were from one of five properties examined, where deer were grazing pastures previously grazed by sheep.

O. circumcinta is a well studied parasite whose somatic protein profiles and surface antigen profiles have been characterised (Baker and Gershwin, 1993; Wedrychowicz et al., 1994). Wedrychowicz (1986) identified 2 SEP antigens from adult *O. circumcinta* which were immunologically reactive. Immune response of sheep to specific antigens of this parasite have been determined by ELISA and western blot analysis (Wedrychowicz et al., 1994). This study showed that surface antigens of fourth-stage larvae and adult *O. circumcinta* induced high levels of serum IgG antibodies which were stage-specific. In this study a 63 kDa polypeptide shed from adult nematode surface was identified in addition to 10 or 11 SEP antigens. These polypeptides or glycopeptides are passed out in the faeces and can be identified using polyclonal or monoclonal antibodies specific to these antigens.

This study showed that it is difficult to establish infections with *O. circumcinta* in 4 month old deer naive to parasite infections. Red deer are probably not a natural host for *O. circumcinta* and may require high challenge doses, or are naturally resistant to the parasite, or the infective strain of *O. circumcinta* used in this experiment does not establish infections in red deer. Examination of the larva immediately prior to inoculation showed that 100% of the L3's in the infective dose were actively mobile, suggesting that they were viable and had been warmed sufficiently to rejuvinate them from cold storage. Resistance of red deer to experimental infections of *O. circumcinta* was also observed by Johnstone et al (1984) at a dose of 30000 L3. They infected 4 month old stag fawns which were not raised in parasite free environment and could have developed immunity preventing establishment of infections. The current trial with deer infected with a mixture of deer-specific abomasal parasites is still underway.

Acknowledgements

The authors would like to thank Alex Vlassoff, AgResearch Wallaceville for the infective cultures of *Ostertagia circumcinta*. We would also like to thank Barbara Masters, Sue McGregor, for assistance in the care of animals.

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