TOWARDS A SERODIAGNOSTIC TEST FOR *Elaphostrongylus cervi* INFECTIONS

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Abstract

In recent years, translocation of cervids, both native and non-native species, have become quite common and concern has been expressed (Samuel 1987) regarding the potential introduction of parasites along with translocated animals. The undesireable nature of these parasites necessitates a reliable method of diagnosing these infections.

An Enzyme-linked immunosorbent assay (ELISA) was developed for the detection of *Parelaphostrongylus tenuis* in white-tailed deer to assess the potential of this procedure in diagnosing a similar parasite, *Elaphostrongylus cervi*, in red deer. The procedure was sucessful in the diagnosis of *P. tenuis* and experiments are underway for a similar ELISA in detecting *E. cervi* in red deer.

Introduction

Elaphostrongylus cervi is a nematode parasite which inhabits the connective tissues and fascia of the skeletal muscles of red deer. The parasite is not known to adversely affect the red deer. There have been recent concerns (Samuel 1987) regarding the translocation of cervids and possible introduction of parasite species to areas where they are non-endemic. *E cervi*, as yet, has not been confirmed from mainland Canada (Lankester & Fong 1989). There is no known effective anthelminthic treatment for *E. cervi* infections (Mason 1994). In 1991, three shipments of red deer arrived in Canada from New Zealand. During the quarantine period, which is required for disease testing, 1-2 animals from each shipment were diagnosed as passing larvae of *E cervi* in their faeces. The animals were eventually slaughtered but adult *E. cervi* were not recovered from the "infected" deer. *E. cervi*, like many other protostrongylid nematodes, produces dorsal spined larvae. Specific identification of infections is therefore extremely difficult by faecal examination alone. Some species can be differentiated by size, but intraspecific and interspecific variation can add to the confusion. A specific diagnosis of infection requires recovery of adult worms at necropsy. One additional problem is that of diagnosing prepatent infections, a period of around 120 days for *E. cervi* (Mason 1994)

E cervi is closely related to *Parelaphostrongylus tenuis*, a nematode which parasitizes the meninges of the central nervous system in white-tailed deer. *P. tenuis*, for the most part, is not known to adversely affect white-tailed deer. In a variety of other ungulates, including moose (*Alces alces*), caribou (*Rangifer tarandus*), fallow deer (*Dama dama*) mule deer (*Odocoileus hemionus hemionus*), North American elk (*Cervus elaphus*) and black-tailed deer (*Odocoileus hemionus columbianus*), *P. tenuis* infections are responsible for causing neurologic disease (Samuel *et al.* 1992). Concerns exist (Samuel 1987) over the potential danger to wildlife with the introduction of *P. tenuis* to non-endemic areas within Canada. The similar concerns regarding the potential introduction of *E. cervi* and *P. tenuis* to non-endemic areas

stem from the developmental phase within the central nervous system (CNS) common to both parasites Gajadhar and Tessaro (1994) reported that mule deer, experimentally infected with *E. cervi*, became severely crippled.

The aforementioned concerns have lead to the desire for a reliable diagnostic procedure for the detection of these parasites. Current diagnostic methods are neither accurate nor sensitive enough and are unable to detect infections which have not reached patency.

Materials and Methods

Canadian Studies.

In 1992, two white-tailed deer fawns were obtained as orphans from the wild at about one week of age. They were reared without any exposure to the infective stage of *P. tenuis*, initially on a dirt floor, in an out-of-doors enclosure for several months prior to transfer to cement-floor pens.

First-stage larvae (L_1) of *P. tenuis* were obtained from faecal pellets of adult captive white-tailed deer, infected with *P. tenuis*, using a modified Baermann technique (Samuel & Gray 1982). The larvae were concentrated and placed on moistened filter paper. Laboratory reared gastropods (*Zonitoides* sp.) were then exposed to the larvae for 24 hours on the filter paper. The snails were maintained in plastic terraria for at least 30 days after exposure to the larvae to allow development of the L_1 to the L_3. Infective third-stage larvae (L_3) were recovered by subjecting snail tissue to pepsin digestion modified from Anderson (1965). Twenty larvae were inoculated by intubation directly into the stomach of each of the two, white-tailed deer fawns. Three guinea pigs were also administered 20 *P. tenuis* L_3's by oesophageal intubation and serum samples obtained at euthanasia.

Serum and faecal samples were taken from the fawns 242, 98, and 7 days prior to inoculation and at least every 2 weeks post exposure. Faecal samples were parasitologically examined using the modified Baermann technique, as well as by sugar flotation (Foreyt 1986).

Adult *P. tenuis* were collected in New Brunswick from road-killed white-tailed deer during the summer and from hunter-killed animals in the fall. Worms were cleaned of host tissue, washed several times in 0.01 M PBS (pH 7.2), rinsed in dH_20 and lyophilized. A crude soluble extract was prepared by grinding these worms in 0.01 M PBS using a Ten Broeck tissue homogenizer, followed by ultrasonic vibration of several short bursts in an ice water bath. The solution was centrifuged for 15 minutes at 31000 x g and the supernatant retained. The soluble protein concentration in the supernatant was determined using the Lowry method (Lowry *et al.* 1957)

Serum samples were analyzed using an ELISA developed for *P. tenuis* in white-tailed deer (for details see Duffy *et al.* 1994).

New Zealand Studies

In 1994, thirty red deer calves were reared at Invermay with their dams under conditions so as to prevent the acquisition of nematode parasite infections. A further 12 calves were reared under natural farm conditions at Invermay on a paddock with their dams. Following weaning, the 30 calves were transferred to gravel pads so as to allow for controlled parasite infections

being maintained. The remaining 12 calves were kept under natural farm conditions following separation from their dams

First stage larvae (L_1) of *E. cervi* were obtained from faecal pellets of red deer, infected with *E. cervi* using the modified Baermann technique. The larvae were concentrated and placed on moistened filter paper. Laboratory reared gastropods (*Deroceras panormitanum*) were exposed to the larvae for 24 hours on the filter paper The slugs were maintained in plastic terraria at 23°C for at least 35 days post-exposure (dpe) to the larvae Slugs were euthanized and squashed between two glass plates to allow for enumeration of infective larvae. Following enumeration of larvae, slugs were placed in modified 20 ml syringes with 10 ml PBS (0.01M, pH 7.2) and administered orally to 3 groups of 6 red deer calves (Table 1). First-stage larvae of *Muellerius capillaris* (the goat lungworm) were obtained from faecal pellets of kid goats, infected with *M capillaris*, using the modified Baermann technique. The larvae were cultured and administered as for *E. cervi* to 3 groups of 6 red deer calves (Table 2). First-stage larvae of *Dictyocaulus viviparus* were obtained from faecal pellets of red deer, infected with *D. viviparus*, using the modified Baermann technique. The larvae were cultured in 500 ml flasks, containing 200 ml water and 30 g of activated charcoal and aerated by aquaria-type aerator pumps, for 8 days at 23°C Third stage infective larvae were enumerated and administered orally in 10 ml of water using a 20 ml syringe to two groups of 6 red deer (Table 1).

Weekly serum and faecal samples were obtained from the calves beginning at 6 weeks prior to experimental infections. Faecal samples were parasitologically examined using the modified Baermann technique, as well as by salt flotation (Soulsby 1968). Adult *E. cervi* were collected from tissue of hunter-killed feral animals in the "wapiti block" of Fiordland National Park. Adult *M. capillaris* were collected from lung modules of slaughtered goats Adult *D. viviparus* were obtained from the lungs of slaughtered red deer. Worms were cleaned of host tissues, washed several times in 0.01 M PBS (pH 7.2) and frozen. Larvae, L_1 and L_3, of the above species were also obtained, cleaned and frozen as above. All parasite material was retained for use as soluble protein extract for later use in ELISA and Western blotting techniques.

Serum samples were obtained from two red deer hinds which were identified as harbouring infections of *E. cervi* at necropsy. Serum samples were obtained from two kid goats which were identified as harbouring infections of *M. capillaris* at necropsy.

Fifteen guinea pigs were utilised in experimental parasite infections. Infective larvae of *E. cervi* and *M. capillaris* were recovered by subjecting the slugs to pepsin digestion, modified from Anderson 1965 These larvae along with *D viviparus*, were administered as per Table 2, via oesophageal intubation. Serum samples were obtained at the time of infection and at 26 dpe.

Results

The white-tailed deer showed a substantial antibody response to *P. tenuis* 75 days post-inoculation as indicated by an increase in the optical density reading of serum samples taken at this time and subsequently (Figure 1).

ELISA readings from nine control positive (*P. tenuis* infected) and four control negative (*P tenuis* free) individuals are included for comparison (Figure 2) The positive blood samples were recovered from road-killed animals within one hour of death, followed by necropsy and

P. tenuis recovery. The negative blood samples were recovered from captive fawns which were less than three months old and free from *P. tenuis* infection.

The guinea pigs were euthanized at 28, 55 and 77 days post-exposure to *P. tenuis* One animal exhibited a posterior paralysis and upon serum analysis was the only one to show a positive reaction using the ELISA. One sub-adult *P tenuis* was recovered from the cervical region of the spinal cord of this animal.

The infections detailed in Tables 1 and 2 were initiated recently and will require monitoring until patency or the appearance of adverse effects due to the infections. Half of the red deer in Groups 4 and 5 (Table 1) were found to have patent infections at 23 dpe and the remainder were patent by 30 dpe (the next sampling period.).

Discussion

The Canadian studies showed that the ELISA was capable of detecting *P. tenuis* specific antibodies from control positive serum samples and as well from the two inoculated animals 11 weeks post exposure. The antibody response detected in the infected fawns 75 days post exposure to infective larvae, represents a time at which the worm would be maturing to an adult. The results of this study suggest that the antibody response which was detected, was due to the antibody response to a stage-specific antigen found only in the adult worm. A soluble extract of the infective stage larvae (L_3) should enable detection of *P. tenuis* specific antibodies at a much earlier time post- exposure.

First stage larvae were not detected in either of the artificially infected animals by 113 days post-exposure. This may be due to the fact that the infection consists of only a single worm, or of same-sex individuals.

At Invermay, the experimentally infected red deer will continue to be monitored weekly, through examination of blood and faecal samples At the time of writing (30 dpe), none of the deer experimentally infected with *E. cervi* or *M. capillaris* had passed larvae with their faeces. The prepatent period for *E cervi* in red deer is approximately 120 days (Mason 1994) Patent infections of *M. capillaris* have not previously been documented in red deer but the prepatent period is around 38 - 48 days in sheep and goats (Anderson 1992). The experimental *D. viviparus* infections in red deer of Groups 3 and 4 (Table 1) were all patent by 30 dpe, or earlier as animals were previously sampled at 23 dpe. The serum samples and parasite material will be used in an ELISA similar to that described for *P tenuis* in white-tailed deer, to evaluate its potential. Western blotting will also be performed to identify proteins which are specific to *E. cervi* alone, yet still elicit a strong antibody response. The procedure described herein using the ELISA provides a good base on which to develop a reliable serodiagnostic test for *P tenuis* in white tailed deer and *E. cervi* in red deer.

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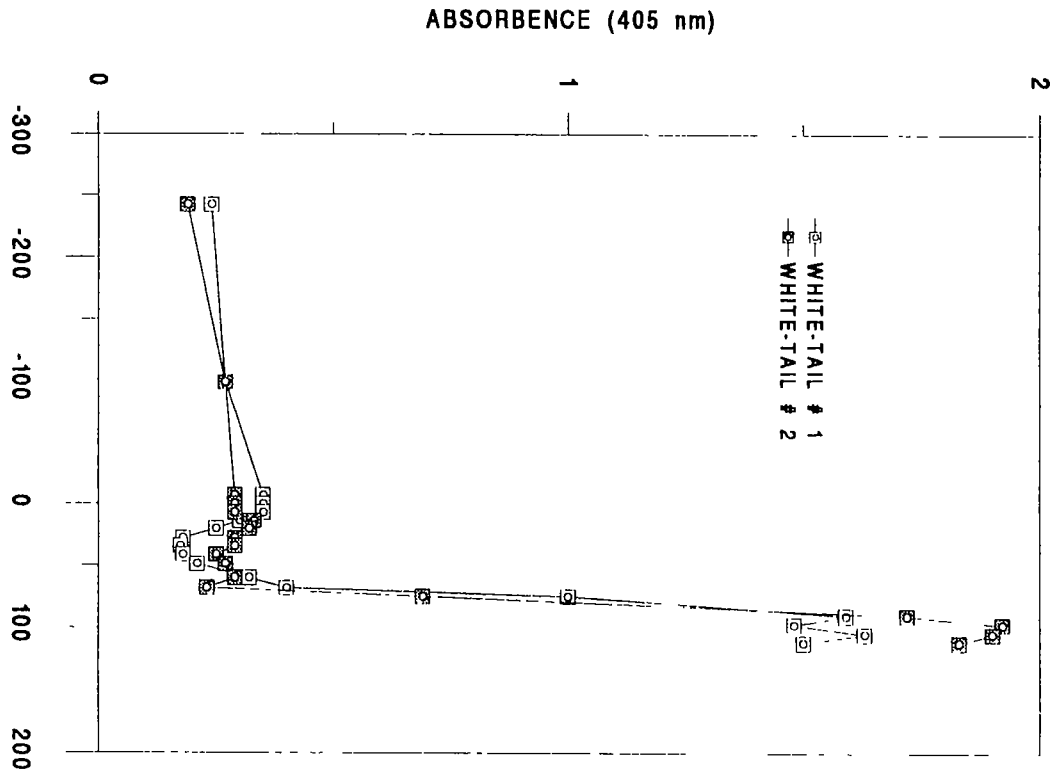

FIGURE 1. ELISA READINGS FOR WHITE-TAILED DEER SERA (Parelaphostrongylus tenuis exposed)

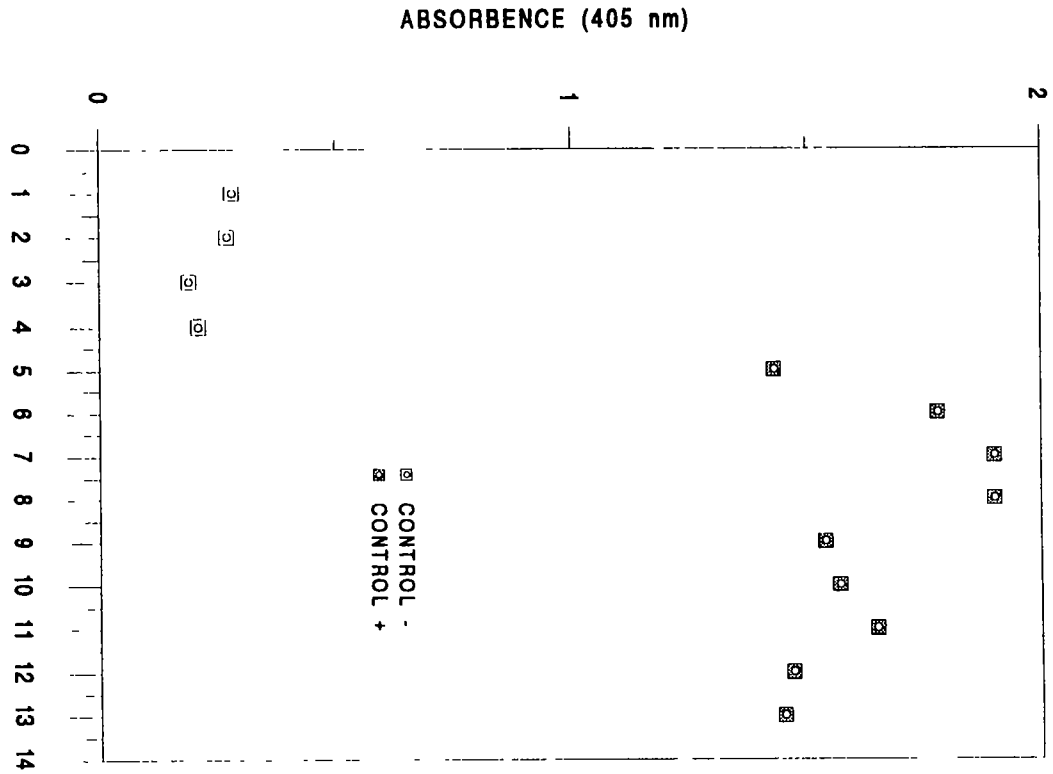

FIGURE 2. ELISA READINGS FOR WHITE-TAILED DEER SERA (control positive and control negative samples)

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Literature Cited

Anderson, R.C. 1965. The Development of *Pneumostrongylus tenuis* in the central nervous system of white-tailed deer. Pathologia Veterinaria. 2: 360-379.

Anderson, R.C. 1992. Nematode parasites of vertebrates. Their development and transmission. C.A.B. International, Wallingford, U.K. pp. 159-160.

Duffy, M.S., C.E. Tanner & M D.B. Burt 1994. Serodiagnosis of *Parelaphostrongylus tenuis* in white-tailed deer (*Odocoileus virginianus*). Proceedings of the International Union of Game Biologists, XX1 Congress. Halifax, Nova Scotia, Canada. August 15-19, 1994.

Foreyt, W.J. 1986. Recovery of nematode eggs and larvae in deer: Evaluation of faecal preservation methods. Journal of the American Veterinary and Medical Association. 189(9): 1065-1067

Gajadhar, A. & S V Tessaro. 1994. Research on *Elaphostrongylus cervi* at the Health of Animals Laboratory, Agriculture and Agri-Food Canada, Saskatoon Wildlife Health Update (Feb. 2).

Lankester, M.W. & D. Fong. 1989. Distribution of Elaphostrongyline nematodes (Metastrongyloidea: Protostrongylidae) in Cervidae and possible effects of moving *Rangifer* spp. into and within North America. Alces 25: 133-145.

Lowry, O.H., N.J. Rosebrough, A.L. Tarr & R.J. Randall. 1957. Protein estimation with the folin phenol reagent. Journal of Biological Chemistry. 193: 265-274.

Mason, P. 1994. Parasites of deer in New Zealand. New Zealand Journal of Zoology. 21: 39-47.

Samuel, W.M. & J.B. Gray 1982 Evaluation of the Baermann technique for recovery of lungworm (Nematoda, Protostrongylidae) larvae from wild ruminants. Biennial Symposium of the North American Wild Sheep and Goat Council 3: 232-243.

Samuel, W.M. 1987. Moving the zoo or the potential introduction of a dangerous parasite into Alberta with its translocated host. Pages 85-92 in L.A. Renecker ed. Focus on a new industry Proceedings of the Alberta Game Growers' Association Conference.

Samuel, W.M., M J. Pybus, D.A. Welch & C.J. Wilke. 1992. Elk as a potential host for meningeal worm: implications for translocation Journal of Wildlife Management 56(4): 629-639.

Soulsby, E.J.L 1968. Helminths, arthropods & protozoa of domesticated animals. 6th ed. Williams and Wilkins company, Baltimore, Maryland, USA.

Table 1. Experimental parasite infections in red deer

GROUP (6 animals per group)	# of *E cervi* L_3's administered	# of *M. capillaris* L_3's administered	# of *D. viviparus* L_3's administered	parasite exposure on pasture
1	0	0	0	No
2	20-40	0	0	No
3	0	0	100	No
4	20-40	40-50	100	No
5	0	40-50	0	No
6	20-40	40-50	pasture	Yes
7	0	0	pasture	Yes

Table 2. Experimental parasite infections in guinea pigs

Guinea pig #	# of *E. cervi* L_3's administered	# of *M. capillaris* L_3's administered	# of *D. viviparus* L_3's administered
1	0	0	992
2	0	0	995
3	0	0	991
4	0	0	415
5	31	0	0
6	29	0	0
7	29	0	0
8	30	0	0
9	0	57	0
10	0	44	0
11	0	56	0
12	0	53	0
13	0	0	0
14	0	0	0
15	0	0	0