Diagnosis of *Elaphostrongylus cervi* infection in New Zealand red deer (*Cervus elaphus*) quarantined in Canada, and experimental determination of a new extended prepatent period

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Abstract

A modified Baermann assay was used to recover dorsal-spined, first stage larvae of *Elaphostrongylus cervi* from feces and lungs of red deer (*Cervus elaphus elaphus*) from three of four herds imported from New Zealand into Canadian quarantine facilities. Tests done on a series of fecal collections showed that larval output from infected red deer was low and sporadic, casting doubt on the efficacy of the Baermann assay to detect all infected individuals in the herds. The animals had passed repeated pre-embarkation Baermann tests for *E. cervi* in New Zealand. Seven larvae recovered from these red deer were used to establish a patent infection in a naive red deer. The prepatent period was 206 days and larval shedding was intermittent. *Elaphostrongylus cervi* is a foreign animal parasite in continental North America, which could become irrevocably established if it were introduced. The data reported indicates that there is currently no reliable method for the detection of *E. cervi* infection.

Résumé

Le diagnostic d'une infection à *Elaphostrongylus cervi* chez les chevreuils de la Nouvelle-Zélande gardés en quarantaine au Canada et détermination expérimentale d'une nouvelle période prépatente prolongée

Une épreuve Baermann modifiée a été utilisée pour récupérer les larves de premier stade d'*Elaphostrongylus cervi* des matières fécales et des poumons de chevreuils provenant de 3 troupeaux de la Nouvelle-Zélande. Les épreuves effectuées sur une série d'échantillons de matières fécales ont démontré que les larves étaient excrétées sporadiquement et en faible quantité, ce qui mettait en doute l'efficacité de l'épreuve de Baermann à déceler tous les animaux infestés. Les animaux avaient été soumis à plusieurs épreuves de Baermann pour déceler *E. cervi* en Nouvelle-Zélande. Sept larves provenant de ces chevreuils ont été utilisées pour induire l'infection chez un chevreuil indigène du Canada. La période prépatente a été de 206 jours et l'excrétion des larves s'est faite de façon intermittente. *Elaphostrongylus cervi* est un parasite non indigène de l'Amérique du Nord qui pourrait s'établir irrévocablement s'il y était introduit. Les données indiquent qu'il n'existe présentement aucune méthode fiable pour déceler l'infection à *E. cervi*.

(Traduit par Dr Thérèse Lanthier)

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Introduction

Elaphostrongylus cervi is a protostrongylid nematode that invades the central nervous system and skeletal musculature of a variety of cervids, including red deer, marals, and wapiti (*Cervus elaphus* subspp.), sika deer (*C. nippon*), and roe deer (*Capreolus capreolus*) (1). Deer become infected when they ingest terrestrial snails or slugs containing the third stage larvae of the parasite. After a lengthy prepatent period of at least three months, eggs or newly hatched dorsal-spined larvae are carried via the bloodstream to the lungs, where the larvae penetrate the alveoli and enter the bronchial tree. They are coughed up, swallowed, passed through the gastrointestinal tract, and excreted with feces. These first stage larvae penetrate the foot of gastropods and develop into infective third stage larvae within two months.

The dorsal-spined larvae of *E. cervi* cannot be reliably differentiated from those of other species in the subfamily Elaphostrongylinae, which includes *Parelaphostrongylus tenuis, P. andersoni,* and *P. odocoilei* (1). Therefore, recovery and identification of the adult nematodes are necessary for a definitive diagnosis in regions where more than one of these parasites occur. In geographic locations where other elaphostrongyline nematodes of deer do not occur, the presence of dorsal-spined larvae in the feces of cervids can be considered diagnostic of *E. cervi* infection.

The occurrence and intensity of disease caused by *E. cervi* vary according to several factors, including the availability of suitable intermediate hosts and the susceptibility and age of host species. Elaphostrongylosis in cervids has been observed in three forms: a) nervous disorders such as ataxia, paralysis, and blindness caused by the presence of worms in the central nervous system; b) verminous pneumonia resulting from migration of large numbers of larvae through the lungs; and c) chronic ill-thrift (1).

Elaphostrongylus cervi, originally found only in Eurasian countries, has been translocated in red deer to other parts of the world. The parasite is now enzootic in New Zealand, and has been discovered in imported red deer in Australian quarantine (2). We report herein the diagnosis of *E. cervi* in imported New Zealand red deer in Canadian quarantine. The temporary incursion was terminated in quarantine.

Materials and methods

Four groups of red deer, numbering 250, 975, 280, and 92, were imported from New Zealand and held under Canadian federal government quarantine in Barrie, Ontario; Fredericton, New Brunswick; Lanark, Ontario; and Saint-Hyacinthe, Quebec, respectively. These red deer had passed one to three Baermann tests for *E. cervi* in preembarkation quarantine in New Zealand.

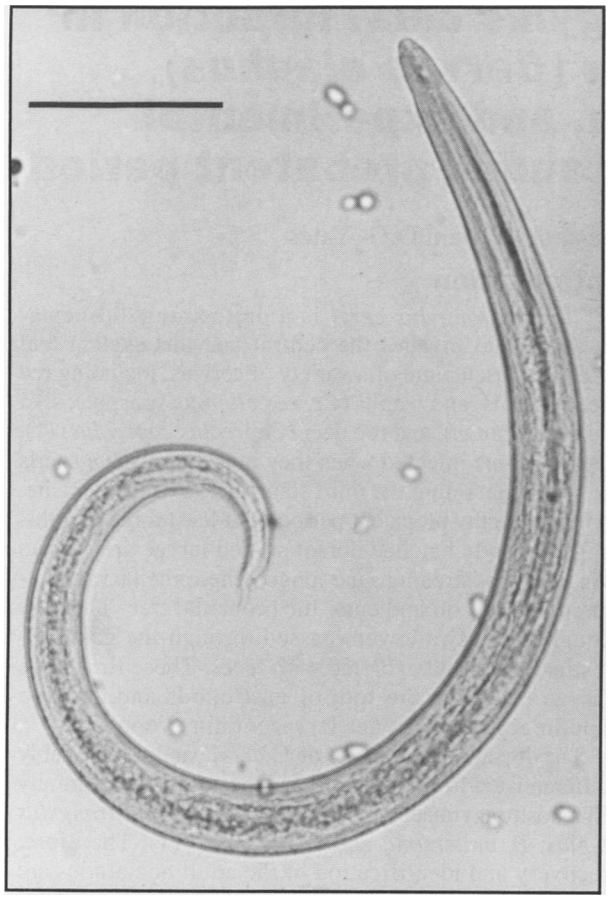

Figure 1. Dorsal-spined, first-stage larva of *Elaphostrongylus cervi* recovered from the feces of an imported red deer. Bright field illumination. Bar = 50 μm.

Within 30 days of arriving in Canada, feces were collected per rectum from each animal by Agriculture Canada personnel. Up to four additional fecal samples were collected in instances where none of the deer in a group tested positive for dorsal-spined larvae. Precautions were taken to ensure that samples did not come in contact with the ground, and that contamination between samples did not occur. Samples were individually labelled, placed on ice, and shipped by overnight courier to the Health of Animals Laboratory in Saskatoon for testing.

Samples were stored at 4°C, and within three weeks, they were tested by a modified Baermann assay for the presence of dorsal-spined larvae. Twenty grams of feces were placed on a single layer of cheese cloth (grade No. 50) that was supported on a tea strainer in a 15 cm glass funnel containing tap water. The temperature of the room was maintained at 23 ± 1°C. After 20–24 h, approximately 50 mL of fluid was collected from the stem of the funnel and centrifuged in a swing-out rotor at 250 × g for 10 min. Most of the supernatant from the microcentrifuge tube was removed, leaving approximately 50–100 μL with the sediment. The sediment was resuspended, placed on a glass slide, and examined under a compound microscope for the presence of dorsal-spined larvae. Larvae were examined and photographed using an Olympus BHT microscope equipped with Nomarski interference contrast optics (Olympus Inc., Tokyo, Japan). A drawing tube attached

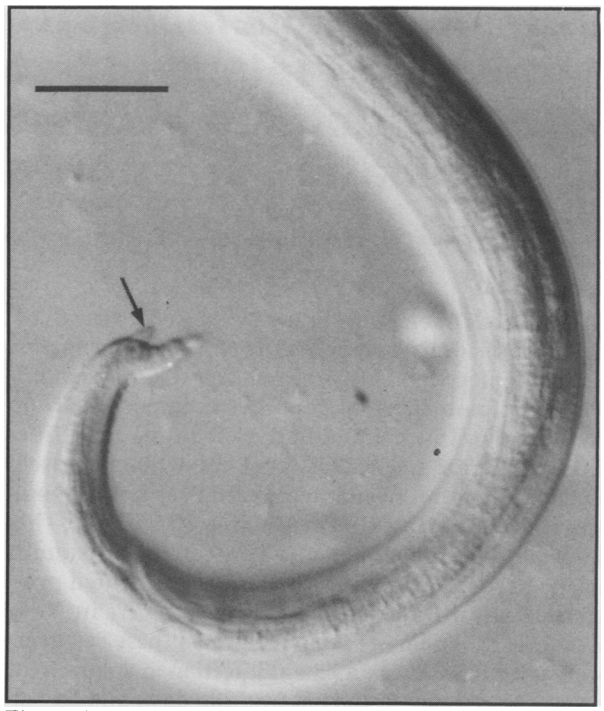

Figure 2. The posterior end of a first stage larva of *E. cervi* recovered from the feces of an imported red deer showing the kinked, concentrically creased, tail and dorsal spine (arrow). Nomarski interference contrast. Bar = 20 μm.

to the microscope and calipers were used to make measurements of dorsal-spined larvae.

Animals found to be passing dorsal-spined larvae were isolated immediately and slaughtered within a few weeks. Whenever possible, multiple fecal samples were collected from these animals prior to slaughter and tested by the modified Baermann method to monitor the shedding of dorsal-spined larvae. Complete necropsies were performed on all slaughtered red deer, and thorough gross and histological examinations were made of the musculature and central nervous system. The entire content of the rectum from each animal was collected and processed by the modified Baermann method for detection of dorsal-spined larvae. A sample of lung from each animal was cut into small pieces, homogenized in a blender, and tested by the modified Baermann method as described for feces.

Laboratory-reared snails (*Triodopsis multilineata*) were exposed to dorsal-spined larvae recovered from feces of quarantined red deer. After 42 days, the snails were digested in pepsin HCl solution and seven

Table 1. Results of Baermann tests for the detection of dorsal-spined larvae in fecal samples collected from four groups of red deer in Canadian quarantine

Group	No. of animals sampled	No. of collections	No. of positives
New Brunswick	975	1	3
Ontario–1	236	1	2
Ontario–2	280	3	1
Quebec	92	5	0

Table 2. Results of Baermann tests done on repeat fecal collections from six red deer found to be positive for dorsal-spined larvae. Each number followed by a comma represents an individual test result

Day of fecal collections	Number of dorsal-spined larvae recoverd					
	New Brunswick			Ontario–1		Ontario–2
	#1	#2	#3	#1	#2	#1
0	1	180	1	1,0	4	1
7				0		
8				0		
13	0,0					
14	0,0,0,0					
25		>20				
26		>20				
27		>20				
28		>20,>20		0,2	3	
29						0,0
30			0,0,0,0			0,0
31			0,0,0,0,0			0
32			0,0			0,0
33			0,0,0,0,0			0
44			0,0,0,0			
45			0,0			
46			0,0,0			
47			0,0,0			
48			0,0			

third-stage larvae were recovered and used to orally inoculate a yearling female red deer. Prior to inoculation this animal had been negative on five Baermann tests conducted over a period of five weeks. For the first 60 days postinoculation a single daily Baermann test was done. Thereafter, Baermann tests were done on the total daily fecal output, and the numbers of larvae were recorded. The red deer was euthanized 250 days postinoculation, and a complete necropsy was performed to recover the adult nematodes.

Results

Dorsal-spined larvae were recovered from the feces of a total of six red deer in three of the four quarantine facilities (Table 1). Individuals in two groups of animals were found to be excreting typical first stage larvae of *E. cervi* on the first collection of feces, and an individual in another group was found to be infected on the third set of samples. No dorsal-spined larvae were found in the smallest group of animals after five sets of fecal samples had been collected and tested. The Baermann assay did not consistently detect the parasite in repeated collections of fecal samples from each patent red deer (Table 2). However, demonstration of the larvae in samples collected at necropsy was more reliable: Baermann examination of the lung and entire rectal content revealed dorsal-spined larvae in five of the six red deer. Gross and histological examination of the carcasses failed to locate the adult nematodes.

The larvae recovered from these red deer were usually coiled at the posterior end, and had a distinctive cuticular spine on the dorsal surface near the tip of the tail (Figures 1 and 2). A lateral ala was present along each side of the larvae. The mean length of dorsal-spined larvae recovered from feces collected per rectum was 432.4 ± 3.1 μm (range 395.5–457.0 μm; N = 33). Many of the fecal samples contained first stage larvae of *Dictyocaulus sp.* which were readily distinguished from *E. cervi* larvae by their smaller size and lack of a dorsal spine.

Dorsal-spined larvae were first recovered from the feces of the experimentally inoculated red deer on day 206 postinoculation. Larval output ranged from 155 to 18,309 larvae per day over 44 days, but the pattern of shedding was extremely irregular. At necropsy, although dorsal-spined larvae were recovered from the lungs and colonic feces, the adult nematodes were not found.

Discussion

The diagnosis of *E. cervi* was made on the basis of larval detection for several reasons: the animals were from New Zealand, where *E. cervi* is enzootic in red deer and is the only species of elaphostrongyline nematode known to occur in that country (1); the period between arrival and testing of the red deer in Canada was months shorter than the prepatent period of indigenous elaphostrongyline nematodes, thereby eliminating the possibility that the infection occurred in Canada; rigorous quarantine conditions, including fenced and defoliated buffer zones, intensive mollusc control, concrete flooring, and elimination of all vegetation, which greatly reduced the possibility of infection by indigenous nematodes; the presence of the same first stage, dorsal-spined larvae in the lungs of infected animals confirmed that the larvae previously found in feces collected per rectum were the product of a patent infection in those deer, not larvae that had been picked up from the environment and simply passed through the gastrointestinal tract. Failure to find the adult nematodes in the infected animals is not unexpected. Mason (1) described the difficulty of finding adult worms in a deer carcass as being "somewhat akin to finding a

needle in a haystack." In the present case, the level of infection was extremely low, the parasite is only 29–44.5 mm long × 0.1–0.125 mm wide, its color is difficult to distinguish from host tissues, and it can occur anywhere within the central nervous system and musculature.

Our experimental study showed that the larvae recovered from these red deer do infect snails, mature to the third stage, and produce patent, subclinical infection in orally exposed, naive red deer. This further restricts the possibility of the parasite being anything other than *E. cervi.* This experiment also showed that larval shedding by red deer can be intermittent; therefore, infection is not always detectable by the Baermann method. It is notable that only seven dorsal-spined larvae were sufficient to produce a patent infection. The extremely small number of parasites present was likely a major factor in our inability to recover the adult nematodes.

Reports of the prepatent period of *E. cervi* being 86–125 days have been based on experimental studies where large numbers of larvae were given to red deer. The results of our study showed that the prepatent period can extend to at least 206 days. The small number of larvae used in our study was probably more representative of a natural infection. Determination of the prepatent period of a parasite is critical for establishment of proper quarantine and testing protocols.

Even when it was modified to maximize the recovery of larvae under stringent conditions, the Baermann assay was not reliable in detecting red deer infected with *E. cervi*, as evidenced by the poor repeatability of the assay. Our results suggest that animals with low level infection may not be detected by the Baermann test. This is more likely due to biological characteristics of the parasitosis than the mechanics of the assay. Intermittent shedding of larvae, the long and variable prepatent period, and the ability of anthelmintics to temporarily suppress larval output without reliably terminating the infection (3), limit the usefulness of the Baermann assay in assuring that red deer are free of *E. cervi.* Because the dorsal-spined larvae of *E. cervi* cannot be differentiated from those of other elaphostrongyline nematodes of Cervidae, the Baermann assay does not allow a definitive diagnosis when used to evaluate deer from regions where more than one species of elaphostrongyline parasites exist. A more sensitive and specific test is needed for the accurate diagnosis of *E. cervi* infection in deer.

Numerous, extensive surveys and related research have not found *E. cervi*, or other members of the genus, in Cervidae in continental North America (4). Two reports suggested that the parasite might occur in barren ground caribou (*Rangifer tarandus groenlandicus*) in the Northwest Territories and woodland caribou (*R. t. caribou*) in Labrador, Ontario, and Manitoba (5,6), but the larvae in question were subsequently shown to be *Parelaphostrongylus andersoni* (7). *Elaphostrongylus cervi,* or more likely *E. rangiferi*, does occur in woodland caribou on the island of Newfoundland, as a result of an importation of infected European reindeer (*R. t. tarandus*) in 1908 (4).

Research and experience in Eurasia and New Zealand have shown that wapiti and several species of molluscs, which are common and widely distributed in North America, can serve as hosts for *E. cervi* (3,8,9). Therefore, it is likely that this foreign parasite could readily become established on this continent. If it were introduced into free-ranging populations of wapiti, *E. cervi* would be impossible to control. It is not known whether the parasite can produce patent infections or disease in other species of North American cervids. However, it can cause lesions and clinical disease in primary host species (10–14) and, like the closely related parasites *E. rangiferi* and *Parelaphostrongylus tenuis*, it may be capable of causing severe neurological disease in a wide variety of wild and domestic ruminants that occur in North America (6,15–20). Consequently, it would be unwise to permit the introduction of *E. cervi* into continental North America when the risk to native cervids and other wildlife has not been determined. Because of these epizootiological concerns and the poor reliability of the Baermann assay, these herds of red deer were depopulated and further importation of cervids into Canada, from countries where *E. cervi* is known or suspected, was stopped.

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Answers to Quiz Corner/Les réponses du Test Éclair

1. a

2. d — Ruminal acidosis in feeder lambs is commonly a result of diets high in grain.
   d — L'acidose du rumen est souvent le résultat, chez les agneaux à l'engraissement, d'une diète riche en grains.

3. d — Toxoplasmosis can cause abortions and birth defects.
   d — La toxoplasmose peut causer des avortements et des anomalies à la naissance.

4. b

5. b — The cow with abomasal displacement and relatively minor complications would have the best prognosis.
   b — La vache souffrant d'un déplacement de la caillette accompagné de complications mineures devrait avoir le meilleur pronostic.

6. a — Bile acid concentrations are very useful for detecting liver disease in cats.
   a — La concentration des acides biliaires est très utile, chez les chats, pour déceler une maladie hépatique.

7. c — Myasthenia gravis has most commonly been associated with thymoma as a paraneoplastic syndrome.
   c — La myasthénie grave, comme syndrome paranéoplasique, a le plus souvent été associée à la présence d'un thymome.

8. a — The esophagus is a muscular tube; therefore, diseases that affect muscular tone may affect the esophagus. Causes of lower motor neuron disease are important causes of acquired esophageal dysfunction. These dogs very rarely recover from this disease spontaneously. Cimetidine and prophylactic antibiotics are not useful in this disease.
   a — L'œsophage est un tube musculaire; par conséquent, les maladies qui affectent le tonus musculaire peuvent affecter l'œsophage. Les causes des affections du neurone moteur inférieur sont des causes importantes de la dysfonction acquise de l'oesophage. Ces chiens recouvrent très rarement la santé spontanément. La cimétidine et la prophylaxie aux antibiotiques ne sont pas utiles pour cette maladie.

9. b — This is why vaccination of breeder flocks is routinely used to control this disease.
   b — Ceci est la raison pour la vaccination de routine des troupeaux d'élevage afin de contrôler cette maladie.

10. d