DIAGNOSING PARELAPHOSTRONGYLOSIS IN MOOSE (*ALCES ALCES*)

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ABSTRACT: Thirty-six moose (*Alces alces*) reported as acting abnormally were examined in northwestern Ontario and adjacent northeastern Minnesota in 1986 – 2000. Thirty-four typically had little fear of humans, remained in an area for some time, and showed clinical signs of neuromotor incoordination including walking in circles, showing weakness and difficulty in rising, head tilted to one side, or standing with legs positioned wide apart. A definitive diagnosis of parelaphostrongylosis was confirmed in 15 (44%) of these by finding small numbers (2.5 ± 0.6; 1 – 9) of adult meningeal worms, *Parelaphostrongylus tenuis*, within the cranium; the meninges of 12, (excluding 3 unsuitable for examination), were cloudy in appearance. An additional 5 clinically abnormal animals had no visible *P. tenuis* but presented with cloudy inflammation of the meninges. No evidence of infection other than typical neurological signs was found in 14 more, but examination was impossible or incomplete for 9 of these. One, however, had *P. tenuis*-like, dorsal-spined larvae in its feces and another tested positive for *P. tenuis* using the newly developed serological test (ELISA). Female animals predominated in the sample (21/34) and 10 were judged underweight. The remaining 2 moose in the sample, although aggressive toward humans, had no worms visible in the cranium and neither showed neuromotor signs or cloudy meninges; 1 tested using the ELISA was negative for *P. tenuis*. Moose with adult *P. tenuis* in the cranium were younger (1.8 ± 0.5 yr) than those abnormal animals without worms (5.2 ± 1.2 yr) ($U = 20$, $P = 0.006$). Five of 15 moose with adult worms in the cranium were passing small numbers of dorsal-spined larvae in their feces (0.1 – 2.8 larvae/gm). Sixty-five percent of animals exhibiting typical neuromotor clinical signs of moose sickness showed post-mortem evidence of parelaphostrongylosis. The diagnostic reliability of clinical signs would have been further increased by wider use of the *P. tenuis* ELISA. This is a convenient, commercially available test and potentially a valuable tool for investigating the level of *P. tenuis* exposure experienced by moose populations sharing range with infected white-tailed deer.

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Parelaphostrongylosis is a disease in moose (*Alces alces*) and other ungulates caused by a neurotropic nematode, *Parelaphostrongylus tenuis*, spread by white-tailed deer (*Odocoileus virginianus*) which is the parasite's normal host (see review by Lankester 2001). In extreme cases, infected moose may show a pronounced posterior weakness and have difficulty rising while those less affected may lack fear of humans or show only slight, transitory signs such as unsteady gait or stumbling over obvious obstacles. From experimental work (Lankester 2002), the severity of clinical signs is associated with

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parasite burden, the age of the infection, and possibly the host's immunological familiarity with the parasite. In the wild, only the most severely affected animals are likely to be reported. Yet, because of their large size, careful post-mortem examination of animals showing signs is a daunting task and may be abandoned. However, the extent to which a typical suite of recognizable neuromotor signs accurately predicts *P. tenuis* infection in moose has not been examined thoroughly.

The causative agent of parelaphostrongylosis has been known for many years but important aspects of its pathogenesis and impact on moose populations remain unclear. An early experiment demonstrated that when large numbers of infective larvae are given to calf moose, *P. tenuis* can cause a rapidly advancing, acute neurological disease (Anderson 1964). As well, naturally infected moose showing similarly severe signs were sometimes found to have only a single worm in the cranium, making it tempting to think that moose were particularly susceptible. The prevailing idea was that moose could survive only where they were almost totally isolated from contact with the parasite. However, study of contemporary moose populations sympatric with white-tailed deer confirm that the impact of parelaphostrongylosis on moose is likely more subtle and complex (Whitlaw and Lankester 1994a,b).

Moose currently persist in many areas of eastern North America where deer densities are held at modest levels by regulated hunting (Whitlaw and Lankester 1994b). When deer densities remain below 4 – 5/km^2, *P. tenuis* may cause only low, and marginally limiting mortality (Karns 1967, Lenarz and Kerr 1987, Whitlaw and Lankester 1994a, Dumont and Crete 1996, Gogan et al. 1997). However, there remains good reason to believe that *P. tenuis* played a significant role historically in marked moose population declines (Whitlaw and Lankester 1994a) and could again with warming climate favoring increased deer numbers. The current and future impact of parelaphostrongylosis on moose populations may be underestimated because of our limited ability to conveniently and reliably identify exposed animals.

This paper describes the clinical manifestations of sick moose observed over a 14-year period in northeastern Minnesota and northwestern Ontario and the available diagnostic procedures and tools used to determine which had parelaphostrongylosis.

METHODS

Moose exhibiting abnormal behaviour and/or neuromotor impairment were observed by the authors (Murray Lankester and Wm. Peterson) or by field personnel over a period of 14 years (1986 – 2000). A description of moose behaviour was recorded and live video taken on occasion. When possible, the age of animals was estimated by tooth eruption and wear. On the basis of their size alone, 7 animals whose teeth could not be examined were simply categorized as adults (i.e., > 2 years). A cursory field examination was conducted and the head, feces, and serum (in 4 instances) collected and frozen until examined. The brain was removed and the entire surface inspected using a stereomicroscope at 16X. Within the cranium, the inner surface of the dura was examined for worms and all meningeal venous blood sinuses, including the cavernous and intercavernous sinuses in the floor of the cranium, were opened and searched for adult *P. tenuis* using the method of Slomke et al. (1995). For the last 8 animals examined (#29 – 36; see Table 1), the cranium was liberally flushed and the collected water placed in a settling flask, repeatedly topped up and decanted, and the sediment examined for dorsal-spined larvae. Feces, when available, were also examined for dorsal-spined larvae, initially using the classical Baermann funnel technique and in later years (after 1994), the improved Baermann-beaker method (Forrester and Lankester 1997). Numbers of

Table 1. Clinically abnormal moose examined or observed in northwestern Ontario and northeastern Minnesota, 1986-2000.

No.	Date examined	Location[1]	Sex	Age (years)	Abnormal signs	*P. tenuis*	Grossly visible meningitis[2]	Larvae in feces
1[3]	9/22/1986	MN	F	10.5	Yes	?	?	0
2	12/7/1986	MN	F	2.5	Yes	0	?	?
3	5/6/1987	MN	M	1	Yes	0	?	0
4	9/24/1987	MN	F	1.5	Yes	1F	?	0
5	11/17/1988	MN	F	3+	Yes	1M, 2F	Yes	0
6	12/26/1988	FF	M	0.6	Yes	1M, 1F	?	2.8/g
7	1/10/1989	TB	F	A	Yes	0	Yes	?
8	2/7/1989	MN	F	11.5	Yes	0	Yes	0
9	6/9/1989	TB	M	1+	Yes	2M, 3F	Yes	2.2/g
10	2/3/1991	FF	F	A	Yes	1M, 1F	Yes	0.3/g
11[4]	3/26/1991	FF	M	2+	Yes	?	?	0
12	5/28/1991	MN	M	1	Yes	1F	?	0
13	6/18/1991	MN	M	5	Yes	0	Yes	0
14[5]	12/2/1991	TB	F	A	Yes	0	Yes	0
15[5]	2/13/1992	FF	F	8+	Yes	0	?	0
16	2/17/1992	MN	M	A	Yes	1M	Yes	0
17[4]	3/16/1992	MN	?	A	Yes	?	?	0
18	3/19/1992	MN	M	0.8	Dead	2F	Yes	0
19	9/7/1992	MN	F	7.5	Yes	1F	Yes	0
20[5]	10/24/1992	MN	F	6.5	Yes	0	No	0
21	12/3/1992	FF	F	3+	Yes	0	No	0
22	12/9/1992	FF	F	A	Yes	0	?	0
23[6]	3/19/1993	MN	F	2	Atypical	0	No	0
24[3]	1/1/1994	MN	M	0.6	Yes	?	?	0
25	4/18/1994	KE	F	1+	Yes	2M, 2F	Yes	0.2/g
26	??/06/94	SGPK	F	A	Yes	0	No	?
27[6]	6/18/1994	MN	F	4	Atypical	0	No	?
28	7/4/1994	MN	F	14	Yes	0 (cord only)	Yes	0
29	7/7/1994	FF	F	1+	Yes	5M, 4F	Yes	0 (cranium+)

[1]TB = Thunder Bay District, ON; FF = Fort Frances District, ON; KE = Kenora District, ON; SGPK = Sleeping Giant Provincial Park, ON; MN = Grand Marais, Minnesota.

[2]? = organ not available or unsuitable for examination.

[3]Animal shot but not available for examination.

[4]Animal observed but not shot; feces collected.

[5]Lens or cornea of 1 or both eyes opaque.

[6]Animal aggressive.

[7]Serologically positive using ELISA.

Table 1 (continued). Clinically abnormal moose examined or observed in northwestern Ontario and northeastern Minnesota, 1986-2000.

No.	Date examined	Location[1]	Sex	Age (years)	Abnormal signs	*P. tenuis*	Grossly visible meningitis[2]	Larvae in feces
30	8/3/1994	MN	F	4	Yes	2F	Yes	0
31[3]	8/20/1994	KE	M	1+	Yes	?	?	0.12/g
32	10/19/1994	SGPK	M	1.5	Yes	1F	Yes	0
33[7]	3/30/1995	MN	M	0.9	Yes	1F	Yes	0 (cranium+)
34[5]	7/27/1995	KE	F	1.5+	Yes	0	No	0
35[7]	6/14/1999	TB	F	1	Yes	1M, 2F	Yes	0.7/g
36[5, 7]	1/6/2000	MN	F	7.5	Yes	0	No	0

[1]TB = Thunder Bay District, ON; FF = Fort Frances District, ON; KE = Kenora District, ON; SGPK = Sleeping Giant Provincial Park, ON; MN = Grand Marais, Minnesota.
[2]? = organ not available or unsuitable for examination.
[3]Animal shot but not available for examination.
[4]Animal observed but not shot; feces collected.
[5]Lens or cornea of 1 or both eyes opaque.
[6]Animal aggressive.
[7]Serologically positive using ELISA.

larvae recovered were expressed per gm of fresh fecal material. An enzyme-linked immunosorbent assay (ELISA) was conducted on serum from 4 clinically abnormal moose and on 4 control moose from an area without *P. tenuis*. The method was similar to that previously used to diagnose experimental and natural parelaphostrongylosis in moose (Ogunremi et al. 2002a) and white-tailed deer (Ogunremi et al. 1999a, b; Ogunremi et al. 2002b) and relied on the availability of an anti-IgG conjugate produced according to Ogunremi et al. (2002a). Mean ages and mean number of adult worms found include their respective standard errors of the means. Statistical analysis of mean ages was carried out with the Mann-Whitney (U) test.

RESULTS

A total of 36 moose behaving abnormally was examined from the vicinities of Grand Marais, Minnesota (n = 20); Thunder Bay, Ontario (n = 6); and Fort Frances-Kenora, Ontario (n = 10) in 1986 – 2000 (Table l). Thirty-five animals observed while alive exhibited a variety of abnormal neurological or behavioural signs; 1 was found dead. Two animals (#23 and 27) were described as being unusually aggressive toward onlookers, but otherwise showed normal gait and coordination. Females predominated (23/36) (Table 1) and 10 of the 36 were judged to be underweight or undersize for their age. The mean age of 29 animals for which tooth-age estimates were available was 3.6 ± 0.7 (0.6 – 14 years). Moose exhibiting abnormal behaviour were reported in all months of the year including 18 in winter (November – March) and 18 in the remaining seasons.

Clinical neuromotor signs observed by the authors or described by field personnel in 34 moose included 1 or more of the following: lack of fear upon human approach (13 moose), remaining in an area for an extended period (13, including the 1 found dead), walking or swimming in circles (8), inability to stand (incoordination) (7), partial paralysis manifested as difficulty in rising (paresis) (5), head tilted to one side (4), head and neck turned posteriorly (torticollis) (6), standing with legs

positioned wide apart (wide base stance) or weight shifted forward over front legs (3), eyes twitching (nystagmus) (3), unsteady gait (2), knuckling of lower limb joints or stumbling over conspicuous objects (2), weakness in the hind quarters (1), or excess salivation (1). Two moose observed exhibiting one or more of these signs were not collected for further examination. Of 13 animals described as remaining in the same area for extended periods, most were observed for 1 – 2 weeks beyond the initial sighting while one was reportedly present in the area for up to 10 months.

Adult *P. tenuis* were found in the cranium of 15 moose providing a definitive diagnosis of parelaphostrongylosis. An additional animal (#31, Table 1) whose head was not available for examination had dorsal-spined larvae in its feces identical to those of *P. tenuis*. The mean age of 14 animals with adult *P. tenuis* in the head or larvae in feces was less (1.8 ± 0.5 years) than that of abnormal moose that had no evidence of worms (5.2 ± 1.2 years) ($U = 20$, $P = 0.006$).

Worms in the cranium were most frequently located on the surface of the brain or on the inner surface of the overlying dura. In 3 animals, portions of worms penetrated into brain tissue. Worms in 2 moose were located in the cavernous or intercavernous sinuses beneath the dura in the floor of the cranium. The mean number of adult *P. tenuis* found was 2.5 ± 0.6 (range = 1 – 9). Five of the 15 moose with adult worms in the cranium were passing small numbers of dorsal-spined larvae in their feces (0.1 – 2.8 larvae/gm); an additional 2 had larvae in washes of the cranium but none in feces.

All moose with adult *P. tenuis* in the cranium (except 3 unsuitable for detailed examination) had grossly visible inflammatory changes in the meninges near vessels and across the surface of the pia-arachnoid covering the brain and particularly over sulci. The piarachnoid had a whitish cloudy appearance and loose patches of yellowish exudate were often seen on the brain surface. An additional 5 abnormal moose had a similar cloudy meningitis but neither adult worms nor larvae were found.

At necropsy, 5 animals had opaque lenses in one or both eyes (Table 1). All heads had been frozen and thawed before examination. Histologically, the eyes of #36 were considered normal (T. Bollinger, Canadian Cooperative Wildlife Health Centre, Saskatoon), despite the lens of one eye appearing enlarged and opaque. The eyes of the remaining 4 moose were not examined histologically.

Serum for ELISA testing was available from only 4 moose (#23, 33, 35, and 36). All had neurogical signs typical of *P. tenuis* infection except #23 that was killed because it was unusually aggressive. Three had an antibody titre indicating positive *P. tenuis* exposure. The highest titre (1,140 units occurred in a 10-month-old calf (#33) with 1 adult worm in the cranium and larvae free in the cranium. A 1-year-old animal (#35) had 3 adult worms in the cranium, but a lower titre (140). Its serum sample was contaminated with ingesta which may have affected the ELISA. A third animal observed circling (#36) but in which no worms could be detected, also had a positive titre (170). The unusually aggressive animal (#23) tested negative as did 4 control moose (titre < 10 units).

DISCUSSION

Thirty-four of the animals examined here showed a reasonably consistent set of clinical signs not unlike those described by some of the earliest workers studying moose sickness (Thomas and Cahn 1932, Lamson 1941, Benson 1958). Variation in the severity of observed clinical neuromotor signs is due in part to the number of developing worms in the central nervous system and to the age or stage of infection (Anderson 1964, Lankester 2002). Animals recently infected with only 1 or 2 worms, or having overcome worms, can appear lethargic, walk with occasional

knuckling, stumbling, or an unsteady gait. However, when startled, some may flee as if unaffected. Other animals, presumably with more worms and/or worms still developing in the spinal cord, can show more severe manifestations of *P. tenuis* infection. Signs include marked paresis or weakness of the hind-quarters, wide-base stance needed to maintain balance, circling, head tilted or turned posteriorly, reluctance to move, difficulty rising, or being unable to stand. Total paralysis is not seen; recumbent animals may continue to move their legs in an effort to stand or run. Nystagmus, or twitching of the eyes, often seen in these more severely affected animals may suggest a balance disorder. Animals showing such severe signs will not likely recover. The clinical signs observed here in wild moose are similar to those seen in other cervids with cranial nematode infections, for example, moose in Sweden with *Elaphostrongylus alces* and caribou in Newfoundland with *E. rangiferi* (see review by Lankester 2001). Overly aggressive behaviour toward humans is not typical of parelaphostrongylosis. Interestingly, an ELISA test done on 1 of 2 aggressive moose in our sample was negative for *P. tenuis*.

On the basis of typical neuromotor signs, parelaphostrongylosis might tentatively be diagnosed in 34 of the 36 moose. However, this diagnosis could be corroborated in only 22 of the 34 (65%) based on recovery of adult *P. tenuis* and cloudy meningitis (15), just cloudy meningitis (5), *P. tenuis*-like larvae in feces (1; no head examined), and no worms or cloudy meningitis but a positive ELISA (1). The remaining 12 moose with typical neuromotor signs either showed no additional evidence of infection on examination (3) or examination was incomplete (9). These results are comparable to those of Smith and Archibald (1967) and Gilbert (1974) who both found worms in the cranium of 80% of moose showing signs of parelaphostrongylosis in Nova Scotia and New Brunswick, and in Maine, respectively. It is impossible to determine whether their samples included more severely affected moose with a greater likelihood of recoverable *P. tenuis*.

A definitive diagnosis of parelaphostrongylosis in moose of North America is possible when long slender nematodes can be located within the cranium. Male worms can be identified to species and none other than *P. tenuis* is expected in that location (*E. rangiferi* in moose of Newfoundland is an exception). But conducting a reliable cranial examination requires appropriate necropsy tools and facilities and some experience on the part of the investigator. Finding dorsal-spined larvae in the cranium or in feces is also strong evidence of meningeal worm infection. However, the muscle worm, *P. andersoni*, also becomes patent in moose and produces similar larvae in feces (see review by Lankester 2001). This species is not likely to have been present in moose of the study area since it is not known in deer of northeastern Minnesota (Peterson and Lankester 1991). Furthermore, dorsal-spined larvae of *P. tenuis* can now be distinguished from those of *P. andersoni* (and other related species) using molecular diagnostic methods (PCR-polymerase chain reaction and SSCP-single-strand conformation polymorphism) (Chilton et al. 2005, Huby-Chilton et al. 2006).

Inflammation of the meninges due to the accumulation of lymphocytes, plasma cells and eosinophils is a known feature of *P. tenuis* infection (Anderson 1964, Smith et al. 1964, Smith and Archibald 1967, Lankester 1974) but can only be confirmed by histological examination. Thus, brain tissue must be in reasonable condition and ideally fixed fresh in formalin or quickly frozen. Animals found dead in the warmer months, or heads left for periods in the sun before freezing, will be unsuitable. Pronounced meningitis, however, is visible grossly as a cloudy discoloration (white to yellowish) of the meninges, especially in the pia-arachnoid membrane against the brain tissue. It is particularly evident over sulci,

the grooves or furrows on the brain surface. Excessive wetness of the membranes and/or the presence of loose, yellowish-red accumulations of viscous exudate may also be evident in the subdural space, on top of, or beneath the brain. Meningitis is most easily appreciated grossly if the brain is examined fresh. Freezing and thawing and any degree of post-mortem change decreases the likelihood of recognizing it. Meningitis resulting in cloudiness of the membrane over the cerebrum was found in all examined moose with *P. tenuis* in the cranium (3 noted exceptions) and in 5 additional, abnormal moose in which no worms were located. Either worms were missed in the latter group or they were overcome and died. Moose are known to develop an immune response capable of killing worms in the cranium and abnormal signs can persist after all gross evidence of worms is gone (Lankester 2002, Ogunremi et al. 2002a). Histological confirmation of an eosinophilic meningoencephalitis along with typical signs could have strengthened the diagnosis of parelaphostrongylosis in the 5 animals.

The serum ELISA test is specific for *P. tenuis* antibodies and sensitive enough to detect infection with fewer than 6 developing worms (Ogunremi et al. 2002a). Used here, it correctly identified 2 moose with 1 and 3 adult worms present in the cranium. Of equal interest was the positive test on 1 moose observed circling but in which no adult worms or other evidence of *P. tenuis* could be found. Without the ELISA test, a positive *P. tenuis* diagnosis could only have been suggested based on the presence of typical clinical signs. Although atypical, the aggressive moose might also have been suspected, but the ELISA test was negative. Had the test been available at the beginning of this study, infections in more clinically suspect moose might have been confirmed. The *P. tenuis* ELISA is currently available through Prairie Diagnostics Services, Regina, SK (contact: gail.krohn@pds.usask.ca).

Only a small volume of serum (approximately 1 ml) is required for the ELISA. Although serum ideally is separated from whole blood by centrifugation, field collection is relatively easy even when no specialized equipment is available. Killing shots should be directed at the chest to cause bleeding into the thoracic cavity (and to spare the brain for examination). Vehicle-killed animals may also bleed into the chest cavity. An incision between the ribs on the "down side" of the animal will release the pooled blood that can be drained into a container (plastic bag or jam jar). Otherwise the chest cavity may have to be sawn open or accessed through the diaphragm from the abdominal cavity. Clotted or liquid blood free in the abdominal cavity can also be scooped into a container, although samples badly contaminated with rumen material are problematic. Fresh blood should be kept at room temperature for a few hours allowing a clot to form and then chilled in a refrigerator over-night to settle before gently pouring or pipetting the straw-coloured serum into small vials or bottles for freezing. If contaminated by red blood cells, the serum will be red and "bloody" and the contaminating red blood cells have to be either centrifuged out of the serum or allowed to settle and the watery, yellowish supernatant fluid (serum) removed and frozen. However, lysis of red cells may occur during sample collection and preparation in which case the serum sample will be red or reddish. As long as such samples were promptly frozen after preparation, testing can still be successfully carried out. Whole blood put directly into the freezer may not be used in the ELISA.

Results reported here contribute to our understanding of *P. tenuis* infection rates in moose populations. The frequency of first-stage, dorsal-spined larvae in field-collected feces was used by Lenarz and Kerr (1987) to estimate that only 2.7% of northeastern MN moose were infected with *P. tenuis*. Their calculations were based on a previous study in Minnesota by Karns (1977) who found

larvae in feces of 29% of moose with worms in the head. For their estimation, Lenarz and Kerr (1987) arbitrarily chose a more conservative value (20%) for larval shedding by the infected moose. In our study, determining the frequency of infected animals passing larvae depends on the value used as denominator. Six abnormal animals had larvae in feces. If all moose exhibiting typical neuromuscular signs are considered to have been infected, 17.6% (6/34) were passing larvae. This value is not appreciably different from that chosen by Lenarz and Kerr (1987). However, if moose with less conspicuous, or sub-clinical signs, were also included, the larger sample denominator would further lower the expected frequency of larvae passed by infected moose and thereby increase the predicted rates of *P. tenuis* infection in the population. In addition, it is important to recognize that these are "point in time" estimates that ignore the importance of turnover rates of infection that may be short but ongoing. This could further increase estimates of annual morbidity and mortality.

Accurate infection rates are even more difficult to predict from field-collected fecal samples because of the habit of sick animals staying for long periods in one area. Thirteen of 36 abnormal moose examined here were reported to have been in the area for at least 1 – 2 weeks. This can bias fecal collections. The relatively high frequency of larvae in field-collected feces reported by Clark and Bowyer (1986) in Maine (31%) and by Thomas and Dodds (1988) in Nova Scotia (13%) probably reflect over-representation of a positive animal, especially since their results could not be confirmed by subsequent studies in the same areas (Upshall et al. 1987, McCollough and Pollard 1993). Animals with parelaphostrongylosis often spend considerable time in a limited area depositing pellets and make representative sampling difficult. How long an infected animal passes larvae and whether shedding occurs more in one season than another will also affect the predictive value of this statistic.

Other parasitic diseases are known to be important to moose health but the pattern of their epizootics differs from that expected of meningeal worm. Ticks (*Dermacentor albipictus*) kill moose, usually in late winter and die-offs are signaled by extensive late winter hair loss and numerous carcasses being found following a "ticky" winter (Samuel 2004, 2007). The severity of tick infestations is dependent on moose densities and on weather favourable for tick survival. Effects may last only 1or 2 winters and are not dependent on densities of co-habiting deer. In contrast, historical moose declines in which *P. tenuis* was thought to play a role, were characterized by relatively low annual numbers of reported cases of parelaphostrongylosis (1.5 – 10/year) over an extended period while deer numbers peaked (Whitlaw and Lankester 1994a). The giant American liver fluke (*Fascioloides magna*) is a parasite whose impact on moose is not fully understood but has recently been implicated in a moose decline in northwestern Minnesota (Murray et al. 2006). In contrast to ticks, the frequency of liver-fluke infection in moose will be related to densities of co-habiting deer. The fluke does not mature in moose, but instead requires deer or wapiti (and the presence of appropriate aquatic snail, *Lymnaea* spp.) for dissemination. They are very conspicuous parasites that could not go unnoticed if present and do not occur in Nova Scotia, New Brunswick, Maine, and New Hampshire where moose declines have historically been observed (Pybus 2001).

Although some progress has been made in recognizing meningeal worm infections in moose, measuring the specific role of parelaphostrongylosis relative to that of predators and other diseases such as winter ticks in limiting moose numbers, continues to be a challenge. Results reported here demonstrate that the display of a typical set of neuromotor clinical signs is a reasonably good indication

of infection. Improved certainty of this diagnosis, and the possible detection of sub-clinical animals, can be expected using the *P. tenuis* ELISA. Only by accurately identifying all moose compromised by infection will the true impact of this disease on moose populations be understood. Work of this type has begun in Minnesota (DonCarlos et al. 2002).

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REFERENCES

Anderson, R. C. 1964. Neurologic disease in moose infected experimentally with *Pneumostrongylus tenuis* from white-tailed deer. Veterinary Pathology 1:289-322.

Benson, D. A. 1958. "Moose sickness" in Nova Scotia. Canadian Journal of Comparative Medicine 22:244-248.

Chilton, N. B., F. Huby-Chilton, M. W. Lankester, and A. A. Gajadhar. 2005. A method for extracting genomic DNA from individual elaphostrongyline (Nematoda: Protostrongylidae) larvae and differentiation of *Elaphostrongylus* spp. from *Parelaphostrongylus* spp. by PCR assay. Journal of Veterinary Diagnostic Investigation 17:585-588.

Clark, R. A., and R. T. Bowyer. 1986. Occurrence of protostrongylid nematodes in sympatric populations of moose and white-tailed deer in Maine. Alces 22:313-322.

DonCarlos, M., R. O. Kimmel, J. S. Lawrence, and M. S. Lenarz. 2002. Summary of wildlife research findings 2002. Unpublished Report, Section of Wildlife, Minnesota Department of Natural Resources, St. Paul, Minnesota, USA.

Dumont, A., and M. Créte. 1996. The meningeal worm, *Parelaphostrongylus tenuis,* a marginal limiting factor for moose, *Alces alces,* in southern Quebec. Canadian Field-Naturalist 110:413-418.

Forrester, S. G., and M. W. Lankester**.** 1997. Extracting protostrongylid nematode larvae from ungulate feces. Journal of Wildlife Diseases 33:511-516.

Gilbert, F. F. 1974. *Pneumostrongylus tenuis* in Maine. II – prevalence in moose. The Journal of Wildlife Management 38:42-46.

Gogan, P. J. P., K. D. Kozie, E. M. Olexia, and N. S. Duncan. 1997. Ecological status of moose and white-tailed deer at Voyageurs National Park, Minnesota. Alces 33:187- 201.

Huby-Chilton, F., N. B. Chilton, M. W. Lankester, and A. A. Gajadhar. 2006. Single-strand conformation polymorphism (SSCP) analysis as a new diagnostic tool to distinguish dorsal-spined larvae of the Elaphostrongyline (Nematoda: Protostrongylidae) from cervids. Veterinary Parasitology 135:153-162.

Karns, P. D. 1967. *Pneumostrongylus tenuis* in deer in Minnesota and implications for moose. Journal of Wildlife Management 31:299-303.

_____. 1977. Deer-moose relationships with emphasis on *Parelaphostrongylus tenuis*. Minnesota Wildlife Research Quarterly 37:40-61.

Lamson, A. L. 1941. Maine moose disease studies. M.Sc. thesis, Department of Biology, University of Maine, Orono, Maine, USA.

Lankester, M. W. 1974. *Parelaphostrongylus tenuis* (Nematoda) and *Fascioloides magna* (Trematoda) in moose of southeastern Manitoba. Canadian Journal of

Zoology 52:235-239.

_____. 2001. Extrapulmonary lungworms of cervids. Pages 228-278 *in* W. M. Samuel, M. J. Pybus, and A. A. Kocan, editors. Parasitic Diseases of Wild Mammals. Second edition. Iowa State University Press, Ames, Iowa, USA.

_____. 2002. Low-dose meningeal worm (*Parelaphostrongylus tenuis*) infections in moose (*Alces alces*). Journal of Wildlife Diseases 38:789-795.

LENARZ, M. S., and K. D. KERR. 1987. An evaluation of *Parelaphostrongylus tenuis* as a source of mortality of moose. Annual Report of the Minnesota Department of Natural Resources, Populations and Research Unit, Grand Rapids, Minnesota, USA.

MCCOLLOUGH, M. A., and K. A. POLLARD. 1993. *Parelaphostrongylus tenuis* in Maine moose and the possible influence of faulty Baermann procedures. Journal of Wildlife Diseases 29:156-158.

MURRAY, D. L., E. C. COX, W. B. BALLARD, H. A. WHITLAW, M. S. LENARZ, T. W. CUSTER, T. BARNETT, and T. K. FULLER. 2006. Influence of pathogens, nutritional deficiency, and climate change on a declining southern moose population. Wildlife Monographs 166.

OGUNREMI, O., M., W. LANKESTER, S. DERGOUSOFF, and A. A. GAJADHAR. 2002a. Detection of anti-*Parelaphostrongylus tenuis* antibodies in experimentally infected and free-ranging moose (*Alces alces*). Journal of Wildlife Diseases 38:796-803.

_____, _____, and A. A. GAJADHAR. 2002b. Immunodiagnosis of experimental *Parelaphostrongylus tenuis* infection in elk. Canadian Journal of Veterinary Research 66:1-7.

_____, _____, J. KENDALL, and A. GAJADHAR. 1999a. Serological diagnosis of *Parelaphostrongylus tenuis* infection in white-tailed deer and identification of a potentially unique parasite antigen. Journal of Parasitology 85:122-127.

_____, _____, S. LORAN, and A. GAJADHAR. 1999b. Evaluation of excretory-secretory products and somatic worm antigens for the serodiagnosis of experimental *Parelaphostrongylus tenuis* infection in white-tailed deer. Journal of Veterinary Diagnostic Investigation 11:515-521.

PETERSON, W. J., and M. W. LANKESTER. 1991. Aspects of the epizootiology of *Parelaphostrongylus tenuis* in a white-tailed deer population. Alces 27:183-192.

PYBUS, M. J. 2001. Liver flukes. Pages 121-149 *in* W. M. Samuel, M. J. Pybus, and A. A. Kocan, editors. Parasitic Diseases of Wild Mammals. Second edition. Iowa State University Press, Ames, Iowa, USA.

SAMUEL, W. M. 2004. White as a Ghost: Winter Ticks and Moose. Federation of Alberta Naturalists, Edmonton, Alberta, Canada.

_____. 2007. Factors common to epizootics of winter ticks and die-offs of moose. Alces 43:39-48.

SLOMKE, A. M., M. W. LANKESTER, and W. J. PETERSON. 1995. Infrapopulation dynamics of *Parelaphostrongylus tenuis* in white-tailed deer. Journal of Wildlife Diseases 31:125-135.

SMITH, H. J., and R. M. ARCHIBALD. 1967. Moose sickness, a neurological disease of moose infected with the common cervine parasite, *Elaphostrongylus tenuis*. Canadian Veterinary Journal 8:173-177.

_____, _____, and A. H. CORNER. 1964. Elaphostrongylosis in maritime moose and deer. Canadian Veterinary Journal 5:287-296.

THOMAS, J. E., and D. G. DODDS. 1988. Brainworm, *Parelaphostrongylus tenuis* in moose *Alces alces*, and white-tailed deer *Odocoileus virginianus* of Nova Scotia. Canadian Field-Naturalist 102:639-642.

THOMAS, L. J., and A. R. CAHN. 1932. A new disease in moose. I: Preliminary report.

Journal of Parasitology 18:219-231.

UPSHALL, S. M., M. D. B. BURT, and T. G. DILWORTH. 1987. *Parelaphostrongylus tenuis* in New Brunswick: the parasite in white-tailed deer *(Odocoileus virginianus*) and moose (*Alces alces*). Journal of Wildlife Diseases 23:683-685.

WHITLAW, H. A., and M. W. LANKESTER. 1994a. A retrospective evaluation of the effects of parelaphostrongylosis on moose populations. Canadian Journal of Zoology 72:1-7.

_____, and _____. 1994b. The co-occurrence of moose, white-tailed deer and *Parelaphostrongylus tenuis* in Ontario. Canadian Journal of Zoology 72:819-825.