Risk Assessment for the Importation of Farmed Elk

to Saskatchewan from Ontario

with respect to

Elaphostrongylus cervi and *Parelaphostrongylus tenuis*

Submitted by:

Canadian Cooperative Wildlife Health Centre
Department of Veterinary Pathology
Western College of Veterinary Medicine
University of Saskatchewan
52 Campus Drive
Saskatoon, SK S7N 5B4

Submitted to:

Saskatchewan Agriculture and Food
Livestock and Veterinary Operations Branch
Room 201 Walter Scott Building
3085 Albert Street
Regina, SK S4S 0B1

Saskatchewan Environment and Resource Management
Wildlife Branch
3211 Albert Street
Regina, SK S4S 5W6

January 1998

Risk Assessment for the Importation of Farmed Elk to Saskatchewan from Ontario

with respect to

Elaphostrongylus cervi and *Parelaphostrongylus tenuis*

Canadian Cooperative Wildlife Health Centre
c/o Department of Veterinary Pathology
Western College of Veterinary Medicine
University of Saskatchewan
52 Campus Drive
Saskatoon, SK S7N 5B4
(306) 966-5099

N. de With, C. Ribble, J.J. Aramini, F.A. Leighton, G. Wobeser

January 1998

Executive Summary

Elk Imports to Saskatchewan from Ontario
Under Proposed *Elaphostrongylus cervi* and *Parelaphostrongylus tenuis* Requirements.

This report presents the results of a risk assessment for the importation of *Elaphostrongylus cervi* and *Parelaphostrongylus tenuis* with live farmed elk from Ontario to Saskatchewan, under proposed import protocols. The import protocol requires, among other things, quarantine in both Ontario and Saskatchewan, and evidence of two negative Baermann beaker tests on feces collected prior to animal movement for all members of a group being proposed for import.

The range of scenarios presented, based upon a mathematical model created for a total importation of 350 animals, indicates that there is a greater than 50% chance of importing a small number (1-6) of elk that test negative but are actually infected with either *E. cervi* or *P. tenuis*. The accuracy of our estimates would improve if better information became available. At this time, a rigorous evaluation of the sensitivity of the Baermann test has not been carried out, a wide range is possible for the prevalence of the two parasites in question in Ontario elk, and the exact number of elk and number of groups of elk being imported is unknown. However, in general, importing multiple small groups would result in a greatly increased probability of importing at least one infected, false negative, elk.

It was not possible to evaluate the effectiveness of the proposed post-entry quarantine. The post-entry quarantine standards are not specifically defined, and details of molluscicide use and effectiveness are lacking. Information is limited on the presence and distribution of the gastropod intermediate hosts in Saskatchewan, although some of the known intermediate hosts for *P. tenuis* do exist in southern areas of the province.

The probability of establishment of *E. cervi* in Saskatchewan is unknown. There is no information to show whether *E. cervi* has or has not become established in eastern Canada. The potential negative impact of *E. cervi*, if it were to become established in Saskatchewan, is unknown. The probability of establishment of *P. tenuis* is considered low for much of Saskatchewan, but could be significantly higher in areas that meet the ecological requirements of the parasite. *P. tenuis* has very well documented negative effects on a variety of species, and the risk of significant negative impact, if the parasite should become established in Saskatchewan, is considered high.

Table of Contents

Risk Assessment

FOR: *Elaphostrongylus cervi* and *Parelaphostrongylus tenuis*

PRELIMINARY RISK PROFILE

Brief Description of Disease to be Assessed

Assess risk of *Elaphostrongylus cervi* and *Parelaphostrongylus tenuis* from live elk (wapiti) imports to Saskatchewan from Ontario, under proposed import protocols.

Brief History & Background of the Request

Prior to 1991, elk were imported to Saskatchewan from Ontario. In that year, red deer imported to Ontario from New Zealand were found to have a positive funnel Baermann test, and were presumptively diagnosed as being infected with *E. cervi*. Saskatchewan then closed its borders to elk from Ontario. It was felt that elk farmed with red deer in Ontario may have contracted the parasite, and that the parasite might be translocated in live elk to Saskatchewan.

In addition to the possibility of *E. cervi* occurring in red deer and elk, Ontario is a known endemic region for *P. tenuis*.

In the ensuing years, a number of Saskatchewan elk farmers bought Ontario elk, and have been keeping these elk in Ontario, anticipating that the trade barrier would be lifted and these animals could be imported to Saskatchewan. The individual producers have requested that the import restriction be re-evaluated, and the Saskatchewan Elk Breeders Association (SEBA) has petitioned Saskatchewan Agriculture & Food (SAF) and Saskatchewan Environment & Resource Management (SERM) on their behalf.

Values Potentially at Risk

- Health of Saskatchewan game-farmed elk.
- Health of Saskatchewan alternate livestock species (sheep, goats, llamas, white-tailed deer, mule deer).
- Health of Saskatchewan wildlife species.

Potential Negative Consequences

- Clinical disease, lowered carcass values, death, and lost inter-provincial and international sales for the game-farmed elk industry if *E. cervi* or *P. tenuis* occurs in Saskatchewan.
- Clinical disease (& death) and lost inter-provincial and international sales for the alternate livestock species if *E. cervi* or *P. tenuis* occurs in Saskatchewan.

- Clinical disease (& death) in wildlife species, with potential negative effects on populations.

Public Perception of the Risks

- Depends on point of view.
- Some game farmers see a benefit to bringing elk from Ontario, while others are concerned about the risk of importing parasites.
- Individuals with wildlife interests are seriously concerned about possible impact on wild animals.

Risk Producer-Beneficiaries

- Primarily the individual game-farmers who wish to import the elk.
- Individual game-farmers, and the industry as a whole, as different genetic stock is imported; however, as Ontario has only a few wild elk, the farmed elk in Ontario were originally introduced from Alberta and Saskatchewan.
- Possibly the industry as a whole, as an increase in the number of elk or access to more animals would allow more people to start producing elk; presently the demand for elk is significantly higher than the supply available for sale, and prices are very high.
- The industry as a whole may benefit in that an increase in the number of farmed elk in Saskatchewan would help to promote a self-sustaining industry.
- Manufacturers of molluscicide would benefit from sales of their product.
- Any group that produces a diagnostic test, for *E. cervi* or *P. tenuis*, would benefit from sales of the test.

Risk Bearers

- Saskatchewan elk farmers bear the risk of diseased livestock, lowered carcass value at slaughter, and loss of export sales.
- Saskatchewan public as a whole bears the risk of disease or death in wildlife species.

Risk-Benefit Distribution

- The risks and benefits are not evenly distributed in the game farm (elk) industry.

Risk Internalization and Voluntary Self-Management Options

- Individual farmers could test elk between intra-provincial farm sales, and could impose controls on intermediate hosts in and around their farms (molluscicide).
- If one or both of the parasites are imported and are detected early, the individual elk producers would likely bear the risk.

COMMODITY PROFILE

What

Elk of all ages, both sexes, from multiple herds, year round (see test and seasonal restrictions below).

An expected 350 to 400 animals would be moved the first year, and 50-75 elk imported on an annual basis thereafter.

Presently, an average of 263 elk are imported into Saskatchewan annually; these animals are imported from British Columbia, Alberta, Manitoba, and the Yukon Territory.

Where

From Ontario to Saskatchewan.

The known areas in Ontario from which elk would be imported are specifically: farms close to Coldwater, Orr Lake, Meaford, and Owen Sound.

The known areas in Saskatchewan to which elk would be imported are specifically: farms close to North Battleford, Marsden, and Yorkton.

How

The following is a summary of the proposed import protocols:

PRE-ENTRY TEST/QUARANTINE:

1. All elk to be permanently marked or identified.
2. Quarantined on farm of origin for minimum of 60 days.
3. Certified as having not been exposed to anthelmintics for at least 30 days prior to quarantine.
4. Quarantined in a mollusc-free environment or only moved from 01 January to 30 April (ie. NOT imported to SK from 01 May to 31 December).
5. Two fecal exams (at least 20 grams feces collected from individual elk) using the Baermann beaker technique from feces collected on day 30 of quarantine, and 15 days (minimum) later.
6. At time of second collection, each elk is to be treated with ivermectin.
7. All animals must test negative for red deer genes.
8. An official certificate of veterinary inspection must accompany each animal movement.
9. All quarantine facilities must meet standards for quarantine of animals for export from Canada, and must be inspected and certified.
10. Necropsy, including histopathology, shall be performed on any animal that dies during pre-entry quarantine, with particular attention and search for evidence of *E. cervi* and *P. tenuis* infection.

TRANSPORTATION:

1. The elk are to be transported by the most direct route to Saskatchewan and are to avoid any cervid rearing or transportation facilities en route.

Known Management Characteristics of the Risk

- There is no evidence to date that *E. cervi* could not become established in Saskatchewan.
- There is some evidence to indicate that *P. tenuis* could not become established in Saskatchewan, as only two cases (one white-tailed deer and one moose) of parelaphostrongylosis have been reported to date. Some ecological factors may play a role in inhibiting the spread of *P. tenuis* into the province by natural movement of white-tailed deer; however, importing a group of animals into western Saskatchewan may allow any natural barrier to be overcome.

POST-ENTRY TESTS/QUARANTINE:

1. All elk imported to SK shall be quarantined in a facility meeting quarantine standards for export of cervids from Canada to a foreign country.
2. All elk shall remain in quarantine until they have been tested for *E. cervi* and *P. tenuis* by SERM-approved sero-diagnostic tests.
3. The quarantine facility must be treated with a molluscicide approved by SERM before animals enter the facility; a 4 m tract around the perimeter of the quarantine area must be treated with a molluscicide once every five days, and within 24 hours of every rainfall of 10 mm or more.
4. Molluscicide must be done by, or directly supervised by, a SAF employee.
5. All elk must be treated with ivermectin or moxydectin at 30 day intervals, beginning 30 days after the pre-entry treatment.
6. Necropsy, including histopathology, shall be performed on any animal that dies during post-entry quarantine, with particular attention and search for evidence of *E. cervi* and *P. tenuis* infection.
7. Offspring from elk in quarantine will be released from quarantine.
8. Herd sires may be introduced into the quarantine area; however, once introduced, they must remain in quarantine and are subject to the same quarantine treatments as imported animals.

When

1. Elk maintained on a certified mollusc-free environment in Ontario may be imported at any time of the year.
2. Elk not maintained on a mollusc-free environment in Ontario may only be imported from the period 01 January to 30 April.

Why

A response to rising costs of buying elk in existing markets.
Potential expansion of the elk farming industry in Saskatchewan.

Familiarity

SAF and SERM are familiar with the inter-provincial importation of live elk.

Trade Agreements

Inter-provincial and international exports may be affected if the importing provinces/countries perceive the proposed changes to import protocols as presenting unacceptable risk to them

NOTE: Discussion with Alberta experts on *P. tenuis* in Nov. 1997 suggested that the province of Alberta would change its requirements for the import of elk, or disallow the import of elk from Saskatchewan entirely, based on the proposed change in Saskatchewan policy.

RISK CHARACTERIZATION

Based on the following evidence, a mathematical model was developed to partially characterize the risk of translocating game-farmed elk from Ontario to Saskatchewan. There are two parts to the risk assessment model: 1) the probability of the parasites *Elaphostrongylus cervi* and *Parelaphostrongylus tenuis* entering Saskatchewan with the movement of game-farmed elk from Ontario, and 2) the probability of the parasites becoming established in Saskatchewan, given the conditions of the proposed import protocol. The methods used are based on the Risk Assessment Models promoted by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA).

Unfortunately, only a portion of the first part, the probability of parasite entry, can be quantified with any degree of confidence, as few hard data are available to numerically evaluate the potential exposure and resultant impact of the parasites on Saskatchewan animal populations. For this reason, only the probability of entry of the parasites can be included in the mathematical model. Computer software (@Risk, Palisade Corporation, Newfield, NY, USA) was used to simulate importation under the proposed protocol.

PROBABILITY OF DISEASE

Probability of parasite infection was assessed in terms of probability of parasite entry into Saskatchewan, and probability of exposure of native animals to the parasites.

Probability of Entry

The probability of entry of the parasites *E. cervi* and *P. tenuis* into Saskatchewan from Ontario is influenced by the number of animals to be imported, the prevalence of the infection among farmed elk, and the sensitivity and predictive values of the Baermann test.

1) Proposed importation numbers

E1-1 The total numbers of game-farmed elk imported to Saskatchewan from all sources over the ten-year period from 1987-1996, was 2631, for an average of 263 animals per year. The minimum was 58 and the maximum in one year was 584 (SAF).

E1-2 The total number of game-farmed elk presently in Ontario, and therefore, available for importation to Saskatchewan, is estimated to be 2500 (2000-3000).

E1-3 The majority (approximately 95%) of elk producers in Saskatchewan belong to the voluntary organization, the Saskatchewan Elk Breeders Association (SEBA). The following information has been obtained from SEBA members and is thought to reflect the current interest in elk importation from Ontario. There are about 6 producers who presently own elk in Ontario, and 6-10 producers with a serious interest in importing elk. In the future, up to 30-40 producers may be interested in importing animals on an annual basis. In the first year, it is estimated that 300-350 elk may be imported. On a subsequent annual basis, the imports are

likely to be relatively small, at about 50 elk per year; previous communication with a SEBA representative indicated that less than 200 elk, most likely between 50-100 head, could be anticipated to be imported yearly.

For the purposes of the model, the numbers used may in certain cases be higher than those anticipated at this time. This increase has been done deliberately in order to err on the side of safety, and to allow for potential increased importation, should movement becomes less restricted.

For this assessment, the number of game-farmed elk to be imported the first year could range from 300-400, with an estimated number of 350 elk.

The number of elk to be imported on an annual basis could range from 50-100, with an estimated number of 75 elk.

The number of groups (collections of animals held together in quarantine in Ontario) to be imported in any year would range from 1-40, with an estimated number of six groups in any one year.

2) Proportion of elk infected with *E. cervi*

E2-1 There is some evidence to suggest that *E. cervi* may have been imported to eastern Canada in red deer (*Cervus elaphus*) from New Zealand. Between 1989 and 1991, an estimated 8925 (8000-10540) red deer were imported into Canada. During this time period, pooled fecal samples were examined for dorsal spined-larvae, and were found to be negative. However, in 1991, the testing protocol was modified to use 20 g of fecal material from each individual animal, and red deer tested positive for dorsal-spined larvae at this time. It is reasonable to infer that the pooled samples did not produce positive test results, even though some of the red deer were infected with *E. cervi*.

E2-2 In 1991, 6 of 1597 red deer imported from New Zealand and held in quarantine in Canada (in Ontario, Quebec, and New Brunswick) were found to be shedding dorsal spined larvae, when tested using 20 g individual fecal samples and the Baermann funnel technique (Gajadhar et al., 1994). This result gives a prevalence of 0.38%.

In all likelihood, this prevalence was lower than the actual prevalence, as the Baermann funnel technique has a maximum sensitivity of 0.36 based on one fecal sample, 0.59 based on two samples, and 0.74 based on three samples (Welch et al., 1991). For the red deer in quarantine, between 1 and 3 tests were performed. If two tests had been performed, then the actual minimum prevalence would be 0.0038/0.59, or 0.64%.

In addition, once one red deer in a group (four groups) had tested positive for dorsal-spined larvae, the rest of the group may not have been examined; therefore, the 6 positive animals may be positive out of fewer than the total of 1597 animals, which would raise the actual prevalence level still further.

For this assessment, the estimated prevalence of *E. cervi* in red deer imported to Canada in 1991 is 1%, with a range from 0.64% to 1.1%.

E2-3 It is believed that sufficient species of snails are present in Ontario that could become infected with the L1 larvae of *E. cervi* to allow the parasite to develop to the L3 infective stage. If infected red deer were in close physical contact with elk, in the presence of suitable snail hosts, elk could also become infected, and the prevalence in elk could be the same as in red deer (an assumption based on the close relatedness of red deer and elk).

Although the elk are to be tested for red deer genes under the proposed import protocols, a test result that showed pure elk would not eliminate the possibility that red deer and elk were held on the same farm and may have been held in very close physical proximity (ie. fence-line contact). In such a case, the genetically pure elk could become infected with *E. cervi*. The test for genetic purity will, therefore, not be discussed further in this report.

For this assessment, the estimated prevalence of *E. cervi* in elk in Ontario is 1%, with a range from 0.64% to 1.1%.

3) Proportion of elk infected with *P. tenuis*

E3-1 Various studies on *P. tenuis* in white-tailed deer that have been carried out in and around the province of Ontario show a prevalence of from 41-82% (Lankester and Anderson, 1968; Anderson, 1972; Bindernagel and Anderson, 1972; Saunders, 1973; Slomke et al., 1995; Wasel et al, manuscript submitted). The prevalence appears to have increased with time.

Many of these studies are based on finding dorsal-spined larvae in the feces of white-tailed deer and likely underestimate the true prevalence. In two studies that examined both white-tailed deer heads (for adult worms) and feces from those same animals (for dorsal-spined larvae), prevalences of *P. tenuis* were 73 and 82%, based on examination of heads, while they were only 44 and 53% respectively based on Baermann fecal exams (Bogaczyk et al., 1993; Slomke et al., 1995).

For this assessment, the prevalence of *P. tenuis* in white-tailed deer in Ontario is thought to range from 65 to 95%, with an estimate of 80%.

E3-2 In order to estimate the prevalence of *P. tenuis* in wild elk in Ontario, the ratio of infection in wild white-tailed deer and in wild elk/ red deer in Pennsylvania was used to generate the ratio of infection in wild white-tailed deer and wild elk in Ontario (Woolf et al., 1977). In Pennsylvania, white-tailed deer have an estimated infection prevalence of 61% (range 42-72%), and elk/red deer have and estimated prevalence of 10% (range 6-18%).

By this approximation, the prevalence of *P. tenuis* in wild elk in Ontario is thought to range from 1 to 24%, with an estimate of 13.5%.

E3-3 It was thought that elk on farms may have a different ecology than elk in the wild, and therefore may have a lower prevalence than wild elk. Information from the related species, *E. cervi*, in feral and in farmed red deer in New Zealand was considered. One report indicated that the prevalence of *E. cervi* in feral red deer may be up to 300 times higher than the prevalence in farmed red deer (Mason and Gordon, 1994). Another source suggested that the prevalence of *E. cervi* in feral red deer was more likely about 16 times higher than in farmed red deer (pers. comm.). Using these available figures, the estimated and minimum values for

the prevalence of *P. tenuis* in wild elk were adjusted; the resultant values for farmed elk may represent a conservative estimate.

For this assessment, the expected prevalence of *P. tenuis* in farmed elk in Ontario is considered to range from 0 to 24% with an estimate of 0.8%.

4) Proportion of non-infected elk which become infected during assembly and transportation

E4-1 The prepatent period for *E. cervi* in elk is 80-137 days, with more extreme values of up to 206 days (Gajadhar et al., 1994). There is a three to five month period in which an elk could be infected but will not shed larvae.

E4-2 The prepatent period for *P. tenuis* in elk is 90-116 days, and may be longer. There is a three to four month period in which an elk could be infected but will not shed larvae.

E4-3 The pre-entry quarantine period is only 60 days. Elk infected with either *E. cervi* or *P. tenuis* shortly before entering quarantine might not become patent until after both fecal samples were collected for Baermann tests.

E4-4 Some evidence suggests that the shedding of L1 larvae is reduced in fall and winter seasons, compared to the spring, so the chances of becoming infected may be lower at these times. It is also known that some terrestrial gastropods will remain active until the mean weekly overnight air temperature falls to about -2° C, and the daytime highs are about 2-3° C (Lankester and Peterson, 1996). So, the potential exists for an elk to become infected late in the fall, or even early winter, depending on annual seasonal temperature fluctuations.

However, it is not known what proportion of elk would become infected during the fall and winter. For this assessment, the proportion of non-infected elk that become infected in fall and winter was considered to be negligible (0%).

5) Proportion of infected elk expected to be shedding L1 larvae

E5-1 L1 larvae traditionally have been detected using the Baermann funnel technique. Using this method, researchers have found dorsal-spined larvae in 36.6% of the total number of red deer fecal samples collected on a daily basis from red deer infected with *E. cervi* (6 red deer--using data from Gajadhar et al., 1994), and 41.4% of total elk fecal samples collected on a daily basis from elk infected with *P. tenuis* (7 elk--using data from Welch et al., 1991).

E5-2 There is evidence that the Baermann funnel technique provides a low estimate of the infection rate. Forrester and Lankester (1997) evaluated the Baermann funnel technique, and showed that only 13-14% of the L1 larvae in a fecal sample would actually be drained off in the liquid examined under the microscope. Elk shedding larvae at low numbers, therefore, have a high probability of not being detected by this technique.

E5-3 The prevalence of dorsal-spined larvae in the feces of white-tailed deer fluctuates with seasons; a higher prevalence is found in spring than autumn and winter (Peterson and Lankester, 1991; Slomke et al., 1995). This finding has implications for the importation of elk

in the winter if elk also follow this pattern of shedding. Infected elk tested for larvae in the winter may be more likely to test negative using the Baermann technique.

Therefore, it is estimated that infected elk would shed L1 larvae about 40% of the time (range 30-50%).

6) Sensitivity, specificity & predictive values of Baermann test for *E. cervi* and *P. tenuis*

E6-1 Unfortunately, there has been only one study (Welch et al., 1991) that has evaluated the Baermann test and tried to determine a sensitivity for the test, using *P. tenuis* infection in elk. This study showed a maximum sensitivity of 0.35 on any one fecal test of sample size 20 g, 0.49 for two samples, and 0.57 for three fecal samples, using the Baermann funnel technique.

E6-2 Elk testing positive or negative for dorsal-spined larvae using the Baermann test could fall into several different categories with respect to infection with *E. cervi*.

Positive Baermann test results could include:

a) elk patently infected with *E. cervi*
b) elk patently infected with *P. tenuis*
c) elk patently infected with another parasite producing dorsal-spined larvae

Negative Baermann test results could include:

a) elk that are free from infection with any parasite producing dorsal-spined larvae
b) elk that are infected with only one sex of a parasite that produces dorsal-spined larvae
c) elk infected with *E. cervi*, but not shedding larvae at the time of fecal collection
d) elk infected with *P. tenuis*, but not shedding larvae at the time of fecal collection
e) elk infected with another parasite producing dorsal-spined larvae, but not shedding larvae at the time of fecal collection
f) elk recently infected with *E. cervi*, in which the infection has not yet become patent (pre-patent period generally ranges from 80 to 137 days)
g) elk recently infected with *P. tenuis*, in which the infection has not yet become patent (pre-patent period generally ranges from 90 to 116 days)
h) elk recently infected with another parasite producing dorsal-spined larvae, in which the infection has not yet become patent

E6-3 It is thought that elk or red deer patently infected with *E. cervi* or *P. tenuis* will shed L1 larvae intermittently. In all cases, the Baermann funnel technique has been used to identify the shedding of larvae. As discussed in evidence point E5-2, there are some difficulties with the funnel technique. The proposed import protocols would use the Baermann beaker technique described by Forrester and Lankester (1997). Using this beaker test, Forrester and Lankester found that 87 % of the larvae in the sample would be collected in the liquid to be examined under the microscope; the Baermann beaker test may be a more sensitive test when larvae are being shed in low numbers. However, as the sensitivity of this new technique has

not been evaluated on a herd basis with known positive and negative animals, the only sensitivity values available at present are those referring to the Baermann funnel test.

Therefore, for the purposes of the assessment model, the sensitivity of the Baermann beaker test is considered equal to that of the Baermann funnel test. In the proposed import protocols, two Baermann tests would be performed using 20 g of feces collected from individual animals. This procedure will have a maximum sensitivity of 0.49.

7) Sensitivity, specificity & predictive values of serodiagnostic test for *E. cervi* and *P. tenuis*

At present, no validated serodiagnostic test, with documented sensitivity and specificity, is available for either parasite.

8) Estimate of probability of entry

To estimate the probability of entry of *E. cervi* and *P. tenuis* into Saskatchewan as a result of the proposed movement of farmed elk from Ontario, a spreadsheet program was built using Microsoft Excel (Microsoft Corporation). Values for the true prevalence of infection, sensitivity of the Baermann test (the proportion of infected animals that test positive), number of groups of animals being imported (up to a maximum of ten groups), and number of animals per group could then be entered into the spreadsheet. Equations derived from simple probability theory were programmed into the spreadsheet model. The above values were used by the program to calculate the probability of at least one infected animal being imported into Saskatchewan, if all animals tested negative twice to the Baermann test in Ontario prior to shipping, and, conversely, the probability that all test negative animals would truly be negative.

Because the only diagnostic test available (the Baermann test) does not allow the user to distinguish among the various species of parasite that produce dorsal-spined larvae, the prevalences for both *Elaphostrongylus cervi* and *Parelaphostrongylus tenuis* were combined. It is important to note that the results presented concern only the groups of imported animals in which all individuals tested negative for dorsal-spined larvae using the Baermann test. The proposed import protocols state that, "If any animal tests positive for dorsal-spined larvae, the entire herd shall have failed quarantine tests..." As such, the only groups of concern are those in which all elk have tested negative, but which may contain animals that are infected with either *E. cervi* or *P. tenuis* (ie. false negatives).

Tables 1 to 12 show the results of a dozen different spreadsheet outcomes. The sensitivity of the Baermann test was set at 49% in Tables 1 to 6; it was reduced to 33% in Tables 6 to 12. In each Table, 10 groups of varying group sizes were selected (between 1 and 250 animals/group) so that one can see the effect of group size on the probability of parasite entry, given the suggested importation protocol. Finally, six different prevalences of infection were selected (0.5, 1, 3, 6, 12, and 24%) for each sensitivity. These prevalences represent the potential range of combined prevalences of infection of *E. cervi* and *P. tenuis* within Ontario herds of farmed elk.

A comparison of Tables 1-6, with test sensitivity set at 49%, with Tables 7-12, with test sensitivity set at 33%, shows that lower test sensitivity results in an increased probability of at least one infected (false negative) animal being imported into Saskatchewan. A specific comparison of column 4 in Table 2 to column 4 in Table 8 shows that at the higher test

sensitivity the probability of importing at least one infected elk in a group of 10 animals at a combined *E. cervi* and *P. tenuis* prevalence of 1% was 4.8%, while at the lower test sensitivity the probability of importing at least one infected elk in a group of 10 animals climbed to 6.3%.

This trend was consistent across all prevalences of infection and group sizes and numbers. In general, the lower the test sensitivity, the higher the probability of importing at least one infected (false negative) elk.

This is an important consideration because the estimate of the sensitivity of the Baermann test (49% for two tests) is based upon its evaluation in a small number (seven) of animals experimentally infected with a moderate to high dose of *P. tenuis*. This evaluation falls far short of that recommended for a diagnostic test, especially one that might be used for making decisions about importation. Normally, diagnostic test evaluation should include its comparison (in a "blinded" fashion) with a gold standard of diagnosis, its evaluation in a reasonably sized animal sample that includes a spectrum of mild to severe cases of disease or infection as well as uninfected control animals, and an evaluation of its reproducibility (precision) and interpretation (observer variation) (Sackett et al, 1991). As a result, the use of 49% as an estimate of the sensitivity of two Baermann tests may be far from the actual sensitivity of the test as used on naturally infected animals. The spreadsheet models indicate that if our estimate of the sensitivity is high, then our estimates of probability of entry are low.

The suggested importation protocol of disqualifying any group of source animals if only one of the individuals within the group tests positive means that prevalence of infection and group size must be examined together for a proper interpretation of their effects.

Examination of the four left columns in Tables 1-3 show that, for small groups of animals, as the prevalence of infection increases the probability of importing at least one infected (false negative) animal also increases. Thus, the probability at prevalence 1% that at least one infected (false negative) animal would be imported is 4.8% (Table 2, row 4); when the prevalence was increased to 3%, this probability climbs to 12.5% (Table 3, row 4). This relationship holds true regardless of the sensitivity of the Baermann test.

However, the relationship reverses itself in the larger groups, such that higher prevalences result in a decreased probability of importing an infected animal (for example, see Grp 10 in Tables 1-3). This occurs as a result of the protocol requirement that any single animal testing positive disqualifies the entire group from importation; a higher prevalence of infection in a large group provides the test with more opportunity to properly identify at least one of the infected animals, disqualifying that group from importation and eliminating the possibility that false negative, infected animals, within the group would be imported.

This finding is important when one considers that a number of groups of different sizes may be imported. Table 1 shows that when the prevalence of infection is low, a single small group of 2-25 animals will have a much lower chance of containing a false negative animal compared to a single larger group of 250 animals. However, this must be examined from the perspective that a total number (say 300) of animals will be imported, and this total will be divided up into a number of groups based upon their farms of origin. They could, for example, be imported as one or two large groups, or as many smaller groups. This will have a major effect on the probability of entry of the parasite into Saskatchewan.

To better investigate how differences in the group sizes that make up a total importation of 300 elk would affect the probability of importing false negative infected animals, we performed a set of simulations with the spreadsheet model in another program called @RISK (Advanced Risk Analysis for Spreadsheets, Palisade Corporation, 31 Decker Road, Newfield, New York, USA 14867). The value of 300 elk was selected because it was the estimated number of elk to be imported during the first year.

The @RISK program allows one to use ranges of numbers where a precise quantity is not known. The computer then selects values, according to a pre-selected distribution, from within that range while a simulation is being run. For the purposes of these simulations, we used a right-skewed beta distribution for the prevalence of infection, with the range set at 0.003 to 24.81% and the most likely prevalence set to 1.48% (Appendix D). The test sensitivity was set at 49%, and the group sizes were set at 1, 2, 10, 25, 50, 100, and 300.

A total of 1000 simulations were run, allowing the prevalence to vary within the pre-selected range for each of the group sizes, to produce a probability of importing all negative animals in one group of each size (Table 13, row 4). The equation presented at the bottom of Table 13 was used to calculate the probability that all test negative animals were truly negative for the number of groups of that size needed to make up a total importation of 300 animals. The last row of Table 13 shows the converse of the row above it: specifically, the probability of importing at least one false negative infected animal when 300 animals are imported in a series of groups of the size indicated in the top of the column.

Table 13. Simulations demonstrating probabilities of importing negative or positive animals in groups of various sizes, which sum to 300 animals.

Group Sizes (Number of Animals/Group)	**1**	**2**	**10**	**25**	**50**	**100**	**300**
Number of Groups	300	150	30	12	6	3	1
Total Number of Animals Imported	300	300	300	300	300	300	300
probability of importing all negative animals ...one group of each size	0.98	0.952	0.86	0.804	0.81	0.862	0.94
probability of importing all negative animals ...to make total of 300 animals [a]	0.002	0.0006	0.011	0.073	0.282	0.641	0.94
probability of importing at least one positive ...when 300 animals are imported[b]	**0.998**	**0.999**	**0.989**	**0.927**	**0.718**	**0.359**	**0.06**

[a]The following formula was used:

$$P_{negative} = (P_{group})^{\#groups}$$

where: P_{group} = the probability of negative animals being imported when only one group of that size is imported
#groups = the number of groups of each size (1-300) to make up a total number of 300 animals
P_{total} = the probability of negative animals being imported when a total of 300 animals is imported

[b]The following formula was used:

$$P_{positive} = 1 - P_{negative}$$

where: $P_{positive}$ = the probability of importing at least one infected animal when all 300 animals are imported

Comparison of the first and last columns of Table 13 shows that the chance of importing one or more false negative infected animals is almost 100% if 300 "groups" of one animal each are imported, compared to only 6% if one large group of 300 is imported using the proposed importation protocol. In general, importing multiple small groups results in a greatly increased probability of importing at least one false negative, infected elk. One could protect against this

problem by testing the entire "source" herd of the small groups and applying the rules of the importation protocol to the entire source herd, rejecting the small group if any of the source herd tested positive.

The above modeling procedures provide a general understanding of how sensitivity, prevalence, and specific single-group sizes affect the likelihood that false negative infected animals will be imported into Saskatchewan. However, they do not provide a precise estimate of number of false negative infected animals that would enter Saskatchewan if 350 animals were to be imported in a number of different group sizes. To achieve this estimate, the @RISK program was again used, this time to vary both the prevalence and the group size.

A set of six different simulations or "scenarios" were run using @RISK. The sensitivity of the Baermann test was set at 49% for one set of scenarios (Figures 1, 2, and 3), and 33% for a second set of scenarios (Scenarios 4, 5, and 6). Test specificity for dorsal-spined larvae, or the proportion of non-infected animals that test negative, was set at 100%. The exact number of elk and number of groups was not available. The number of groups was set at six, and the total number of elk to be imported was set at 350 (Figures 1-6).

In Scenario 1 (Figure 1), six groups of size 1, 5, 10, 50, 100, and 184 animals were selected, and the sensitivity of the test was set at 49%. The results of the simulation show that there was about a 48% probability that all test negative animals were truly non-infected. Conversely, there was a 52% probability that at least one animal was a false negative and was actually infected. Furthermore, the chances were about 15% that two false negative animals would be imported, and about 5% that three false negative animals would be imported.

In Scenario 2 (Figure 2), a more uniform range of group sizes (20, 40, 50, 60, 80, 100 animals) was chosen, with the sensitivity of the test kept at 49%. The probability of all test negative animals being truly non-infected dropped to 32%. Conversely, there was a 68% chance that at least one test negative elk was infected.

Scenario 3 (Figure 3) combined four small groups (1, 2, 5, 22 animals) and two larger groups (150, 170 animals). Again, the test sensitivity was set at 49%. This time, there was about a 56% probability that all animals testing negative were truly non-infected, and a 44% probability that at least one elk was a false negative (and really infected).

For Scenarios 4, 5, and 6, (Figures 4, 5, 6) we used the same group arrangements as for Scenarios 1, 2, and 3 respectively. However, in Scenarios 4, 5, and 6, the test sensitivity was changed to the lower sensitivity of 33%.

In Scenario 4 (Figure 4), the probability of all test negative animals being truly negative was 32%; therefore the probability of importing at least one infected false negative elk was 68%.

In Scenario 5 (Figure 5) there was about a 17% probability that all the elk testing negative were truly non-infected. Thus, there was an 83% probability that at least one animal was an infected false negative.

Finally, in Scenario 6 (Figure 6), the probability that all test negative animals would be truly non-infected animals was 38%, while the probability that at least one test negative elk was an infected false negative was 62%.

Out of these six scenarios, the "best" case was Scenario 3, where 56% of the test negative groups of elk were truly non-infected.

Conclusion regarding probability of entry: **The range of scenarios presented indicate that there is likely a greater than 50% chance of importing a small number (1-6) of elk that test negative but are actually infected with either *E. cervi* or *P. tenuis*.** The accuracy of this prediction would improve if more accurate information became available. At this time, a rigorous evaluation of the sensitivity of the Baermann test has not been carried out, a wide range is suggested for the prevalence of the two parasites in question, and the exact number of elk and number of groups of elk being imported is unknown.

Probability of Exposure

Routes of parasite entry

There are several possible routes by which *E. cervi* or *P. tenuis* could enter Saskatchewan. There is some anecdotal evidence to indicate that larvae may pass unchanged through the gastrointestinal tract of ungulates. If this report is true, then there is the rare possibility that these parasites could be translocated across provinces in the movement of domestic species, such as sheep, goats, and cattle.

P. tenuis could come westward from Manitoba in the natural movements of wild white-tailed deer. However, as will be discussed in more detail later, there is evidence that this westward movement may be blocked by some natural barrier that prevents the parasite from establishing itself. Wasel et al. (manuscript submitted) found a relatively high prevalence of *P. tenuis* (30-60%) in the eastern regions of Manitoba during a 1989-1990 survey of white-tailed deer heads. The prevalence (0-16%) was much lower in the western regions of Manitoba. In Saskatchewan, along the eastern border regions, only one deer (1/258 sampled or <0.4%) was found to be positive for *P. tenuis*. No infected white-tailed deer were found in the remainder of Saskatchewan, although relatively few deer were sampled on the western border regions. This same east to west pattern of declining prevalence of infection was documented for North Dakota in the study.

An additional source for the introduction of *P. tenuis* to Saskatchewan is the importation of game-farmed elk from Manitoba. Manitoba is an endemic area for *P. tenuis*, and *P. tenuis* was confirmed from wild elk in southwestern Manitoba (Spruce Woods Provincial Forest) by Pybus et al. (1989). A total of 40 farmed elk have been imported from Manitoba to Saskatchewan between Jan 1992 and Oct 1997, in groups ranging in size from 1-16 per year (average 6 elk/year). The probability that one or more of these imported elk was infected with *P. tenuis* depends on the prevalence of infection in the region from which they were originally housed in Manitoba, and the nature of the testing and quarantine procedures.

The current testing protocol requires a 60 day quarantine period in Manitoba; the elk must be free of ivermectin treatment, and a Baermann test performed on fecal samples collected on day 45 of the quarantine period. Any test positive animals are to be disqualified from importation and removed from the group; the remainder of the group is to be quarantined for a further 90

days and Baermann tests performed on fecal samples collected on days 30, 60, and 90 of the extended quarantine period.

It is possible, based on the above information, that a small number of wild white-tailed deer or game-farmed elk infected with *P. tenuis* have moved from Manitoba into Saskatchewan. While the probability that *P. tenuis* has recently entered Saskatchewan is low, data regarding the presence or absence of *P. tenuis* in farmed elk in Saskatchewan are lacking, and no systematic surveillance for the parasite is carried out on wildlife in this province.

The final source of possible entry of these parasites into Saskatchewan is the proposed importation of game-farmed elk from Ontario.

Quarantine Effectiveness

In order for a parasite imported from Ontario to become established in Saskatchewan, the parasite would have to "break" quarantine. Some comments will be made about the proposed post-entry quarantine.

1. The post-entry quarantine standards are not directly defined in the proposed import protocols; instead, the quarantine facility must meet quarantine standards for export of cervids from Canada to a foreign country. An official from the Canadian Food Inspection Agency (formerly Agriculture and Agri-Food Canada) was contacted to elucidate precisely what these standards would be; quarantine standards are dependent on what is required by the importing country, and are not consistent among all countries (Dr. Greg Graham, pers. comm.). If the proposed import protocols are adopted, the facility requirements should be clearly detailed to avoid any possible confusion.

2. The elk are to remain in quarantine until they have been tested for *E. cervi* and *P. tenuis* by approved sero-diagnostic tests. Unfortunately, such tests are currently under development and unavailable for commercial application.

At the time of writing, it could not be accurately determined how soon such a test might become available. It should be noted that once the research has been completed, each test needs to be validated and carefully peer-reviewed by other knowledgeable scientists before being released for commercial application.

3. The import protocols specify the application of molluscicide on a regular basis, and after a certain amount of precipitation. As far as could be determined, the only molluscicide approved for use in Canada is metaldehyde. This product is set out as baits which are attractive to the gastropods. Baits cannot be relied upon to eliminate molluscs from an area, a uniformly applied molluscicide will be required.

Copper sulfate was suggested as a spray, but was generally felt by those consulted to be too toxic to the environment to be approved. There is limited literature available on the use, safety, and efficacy of molluscicides. Selection and approval for use of an effective molluscicide will be required if the quarantine protocols are to be implemented. That applies to quarantine in Ontario as well as in Saskatchewan.

Information is limited on the presence, population, and distribution of gastropods in Saskatchewan. There have only been a few studies on snails and slugs. Known intermediate hosts for *P. tenuis* exist in southern areas of the province (Wasel, manuscript submitted). Thus, the potential for establishment of *P. tenuis* exists.

4. The elk are to be treated with ivermectin or moxydectin at thirty day intervals. Neither ivermectin nor moxydectin are approved in Canada for use in elk; efficacy trials and tissue residue studies have not been performed. Ivermectin is approved for use in New Zealand as a pour-on for red deer, but its efficacy against *E. cervi* was not evaluated (Merck Veterinary Bulletin, 1995 - No. 6).

Ivermectin was shown to reduce, but not to eliminate, shedding of larvae of *P. tenuis* in white-tailed deer for 28 days (Kocan, 1985). Samuel and Gray (1988) showed that ivermectin eliminated shedding for a variable time period in white-tailed deer infected with *P. tenuis*. In one animal, the effect lasted only 1.5 weeks. It is not known if *P. tenuis* infected elk respond in the same manner.

If the animals are to be individually handled in order to treat them with anthelminthics, they will need to be restrained in some manner. **In order for quarantine to be effective, any restraints (chute/squeeze) used on quarantined elk would have to be completely separate from facilities used for other animals on the farm.** This requirement would have to be addressed within the quarantine standards as already discussed.

5. Supervision and verification of compliance with all aspects of the quarantine protocol will place heavy demands on personnel of SAF. The period of quarantine within Saskatchewan may be prolonged (1-2 years or more). Rigid adherence to protocol during this time may be difficult to sustain.

Summary of Probability of Disease

Given the range of prevalence of both *E. cervi* and *P. tenuis* in Ontario game-farmed elk, the expected number of animals to be imported, the number of groups to be imported, and the poor sensitivity of the Baermann test, the probability of importing infected elk to Saskatchewan is high. In the scenarios presented, the minimum probability of importing at least one infected elk to Saskatchewan was 44%.

The quarantine protocols are of uncertain effectiveness and sustainability. Many procedural details have yet to be established.

The probability of establishment of *E. cervi* in Saskatchewan is unknown. There is no information that shows that *E. cervi* has become established in eastern Canada; however, no studies have been done to show it has not become established.

The probability of establishment of *P. tenuis* is considered to be low for much of the province, but could be significantly higher in areas that meet the ecological requirements of the parasite.

IMPACT OF THE DISEASE/AGENT

The impact of these two parasites on populations within Saskatchewan is dependent on two factors: the ability of the parasites to establish themselves in the province, and the effect of infection in the various potential host species.

Bindernagel and Anderson (1972) hypothesized that there is a natural barrier that prevents the western spread of *P. tenuis*; ecological factors associated with the grassland biome may inhibit its establishment. There has been one detailed study of *P. tenuis* by Wasel et al. (manuscript submitted), which looked at parameters associated with the presence of the parasite in a region. The specific conditions that had the highest correlation with the presence of *P. tenuis* were high summer and fall precipitation, cold winter and spring temperatures, forest cover between 50 and 75 %, high deer density, and low elevation.

However, if *P. tenuis* were introduced to the westernmost areas or parkland regions of the province, the parasite might thereby "jump" such climate-related ecological barriers by being transported greater distances in game-farmed animals than would occur naturally by movements of wild white-tailed deer.

If *P. tenuis* established itself in the wild-white-tailed deer population, the impact could be far reaching, as the range of white-tailed deer in Saskatchewan overlaps to some degree with the ranges of moose, caribou, wild elk, mule deer, antelope, and the distribution of the major farming regions within the province.

It is not known whether *E. cervi* could establish itself in Saskatchewan.

The effects of *E. cervi* and *P. tenuis* on species of concern in Saskatchewan are variable; they are dependent on both parasite and host species. Each parasite will be discussed individually.

FOR: *Elaphostrongylus cervi*

Unfortunately there are few references that discuss the effect of *E. cervi* in species that are of concern in Saskatchewan; the ones consulted are listed in Table 14.

Table 14. Species known to have been infected with *E. cervi* and selected references for each.

Species	Reference
elk/red deer	Sutherland, 1976 Hollands, 1985 Mason, 1989, 1995 Mason and Gordon, 1994
mule deer	Gajadhar and Tessaro, 1995

The main findings of these studies will be reviewed.

Elk/red deer: Rarely show clinical signs. The main significance is considered to be mild, diffuse, interstitial pneumonia produced by the eggs and larvae as they pass through the alveoli to enter the bronchioles (verminous pneumonia). Heavily infected animals may develop lesions under the skin and between the muscle layers that lessen carcass values.

Mule deer: Experimentally infected mule deer showed neurological signs; the deer first showed posterior ataxia which progressed until the animals could not rise or stand without assistance, and had difficulty maintaining sternal recumbancy.

FOR: *Parelaphostrongylus tenuis*

There is extensive information about *P. tenuis* in various species. The following list (see Table 15) does not represent a comprehensive literature search. These are the articles that were selected for their relevance to this risk assessment:

Table 15. Species known to have been infected with *P. tenuis* and selected references for each.

Species	Reference
white-tailed deer	Anderson, 1965 Bindernagel and Anderson, 1972 Anderson and Prestwood, 1981 Nichols et al., 1986 Carreno and Lankester, 1993
moose	Saunders, 1973 Gilbert, 1974 Lankester, 1974 Whitlaw and Lankester, 1994a, 1994b Lankester and Peterson, 1996 Gogan et al., 1997
caribou/reindeer	Anderson and Strelive, 1968 Anderson, 1971 Trainer, 1973 Dauphine, 1975 Nichols et al., 1986 Pitt and Jordan, 1994
elk	Anderson et al., 1966 Carpenter et al., 1973 Woolf et al., 1977 Olsen and Woolf, 1978, 1979 Pybus et al., 1989 Raskevitz et al., 1991 Samuel et al., 1992
mule deer/black-tailed deer	Anderson et al., 1966 Nettles et al., 1977a, 1977b
sheep	Anderson and Strelive, 1966 Jortner, 1985 O'Brien et al., 1986
goats	Kopcha et al., 1989
cattle	Yamini et al., 1997
llamas	O'Brien et al., 1986
fallow deer	Kistner et al., 1977
sable antelope	Nichols et al., 1986

A brief description of the major findings in each species will be presented. Please note, the relationships between white-tailed deer, *P. tenuis*, and other species are extremely complex and subtle, and should not be simplified.

White-tailed deer: White-tailed deer are considered the natural host of meningeal worm. There have been no reports of clinical disease.

Moose: Neurological disease, commonly called 'moose sickness' was seen for years in moose before *P. tenuis* was discovered to be the probable cause. Moose sickness is thought to have contributed to moose population declines in some regions. Moose population densities were shown to be inversely related to prevalence of *P. tenuis* in white-tailed deer, such that where the prevalence was high, the moose numbers were low. Another study showed that the moose numbers were inversely related to the intensity (or numbers) of dorsal-spined larvae in the feces of white-tailed deer.

However, moose populations can also increase in areas endemic for *P. tenuis* under some circumstances. A theory was proposed that moose have 'refugia', which separate them from white-tailed deer. This separation can be due to spatial, temporal, or behavior conditions. For example, snow depth and crust may limit white-tailed deer movement, while not impeding moose.

Large fluctuations have occurred in North America in both white-tailed deer and moose populations. Generally, moose have persisted in areas with infected deer.

Caribou/Reindeer: Experimentally infected caribou show neurological signs. Translocated caribou or reindeer, introduced into *P. tenuis* endemic regions, consistently die after showing neurological signs. Meningeal worm is considered a major limiting factor in the re-introduction of caribou.

Elk: Experimentally infected elk show a variation in clinical signs dependent on the infective dose of *P. tenuis* given: no clinical disease if given a low dose of infection (<25 larvae), neurological signs and death if infected at a higher dose (>=25 larvae).

There has been evidence of clinical disease and death in wild elk translocated into a *P. tenuis* endemic area. It is thought that elk and infected white-tailed deer can co-exist because habitat selection and foraging behaviours act to spatially separate the two species.

Elk may become patently infected.

Mule deer/ Black tailed deer: Experimental infections produced severe neurological signs with death resulting from paralysis. Black-tailed deer released into areas inhabited by white-tailed deer, and which became infected with meningeal worm, showed neurological disease consisting of inco-ordination, recumbancy, paralysis, and death.

Sheep: *P. tenuis* is considered to be a serious pathogen in sheep on pastures frequented by white-tailed deer. As many as 59% of sheep in flocks can show neurological signs attributed to meningeal worm.

Goats: Goats infected with meningeal worm develop neurological disease.

Cattle: There is one recent report of neurological disease caused by *P. tenuis* in a mixed breed calf. Such infection appears to be rare.

Llamas: Llamas infected with *P. tenuis* develop neurological disease, including urinary incontinence, hind limb paresis, and total paralysis.

Fallow deer: Natural infections produce neurological impairment, including inability to rise, paralysis, lateral recumbancy, and death.

Sable antelope: Natural infection occurred in a zoological park setting; two animals were found to be stumbling, then ataxic and unable to rise.

Summary of Impact

Although *E. cervi* has only been shown to have adverse effects on mule deer, the pertinent studies have not yet been carried out in other species of concern. It can be speculated that *E. cervi* probably does not harm sheep, as there have been no reports of infection in sheep from New Zealand, but its possible effects in other species are unknown at this time. Therefore, the potential negative impact of *E. cervi*, if it were to become established in Saskatchewan, is unknown.

P. tenuis has very well documented negative effects on a variety of species, and the risk of significant negative impact, if the parasite should become established in Saskatchewan, is considered high.

SUMMARY OF RISK AND UNCERTAINTY

The probability of Saskatchewan experiencing a serious negative impact from the presence of the parasite *Elaphostrongylus cervi* is unknown. The probability of Saskatchewan experiencing a serious negative impact from the presence of the parasite *Parelaphostrongylus tenuis* is considered to be moderate to high.

Uncertainty exists in estimates of prevalence because information in game-farmed elk must be extrapolated from information derived from other species or from wild elk.

The most influential factors in the mathematical model included the prevalence of infection in game-farmed elk, the sensitivity of the Baermann test, and the number of groups (and group sizes) of animals to be imported.

Risk Management Considerations

FOR: *Elaphostrongylus cervi* and *Parelaphostrongylus tenuis*, Ontario elk imports

Summary Statement of Agent/Commodity/Pathway Assumptions

Importation of farmed elk from Ontario as per the import protocols proposed, involving the use of the Baermann test to detect animals shedding dorsal-spined larvae, and specific serodiagnostic tests when they become available.

Importation of elk not maintained on certified mollusc free environment only to occur from the period 01 January to 30 April annually.

Summary Statement of Overall Risk

The overall risk to Saskatchewan of the importation of farmed elk, given that the only diagnostic test available at this time is the Baermann test, is considered to be unknown for *E. cervi*, and moderate to high for *P. tenuis*.

Summary of Detection Systems

The analysis was conducted using data from the Baermann test for the detection of dorsal-spined larvae.

Firm data on the sensitivity, specificity, and predictive values of the screening test would improve the accuracy of the mathematical model.

Firm data on the true prevalence of each parasite in farmed elk in Ontario would improve the accuracy of the mathematical model.

Eradication Potential

If either of these parasites were suspected to have been imported to Saskatchewan, it would be difficult to verify their presence since another parasite producing dorsal-spined larvae , *Varestrongylus alpenae* (Gray et al., 1984), already occurs in the province.

If either of these two parasites became established in wildlife species, it would be difficult, if not impossible, to completely eradicate the parasites from the province.

Import Options

1) Maintain the status quo. Unlikely to be accepted by the elk producers.
2) Adopt the proposed import protocols, using the Baermann test as the screening test.
3) Modify the proposed import protocols to test the entire source herd (ie. every cervid on the farm of origin), using the Baermann test.
4) Maintain the status quo until a more sensitive and specific test becomes available.

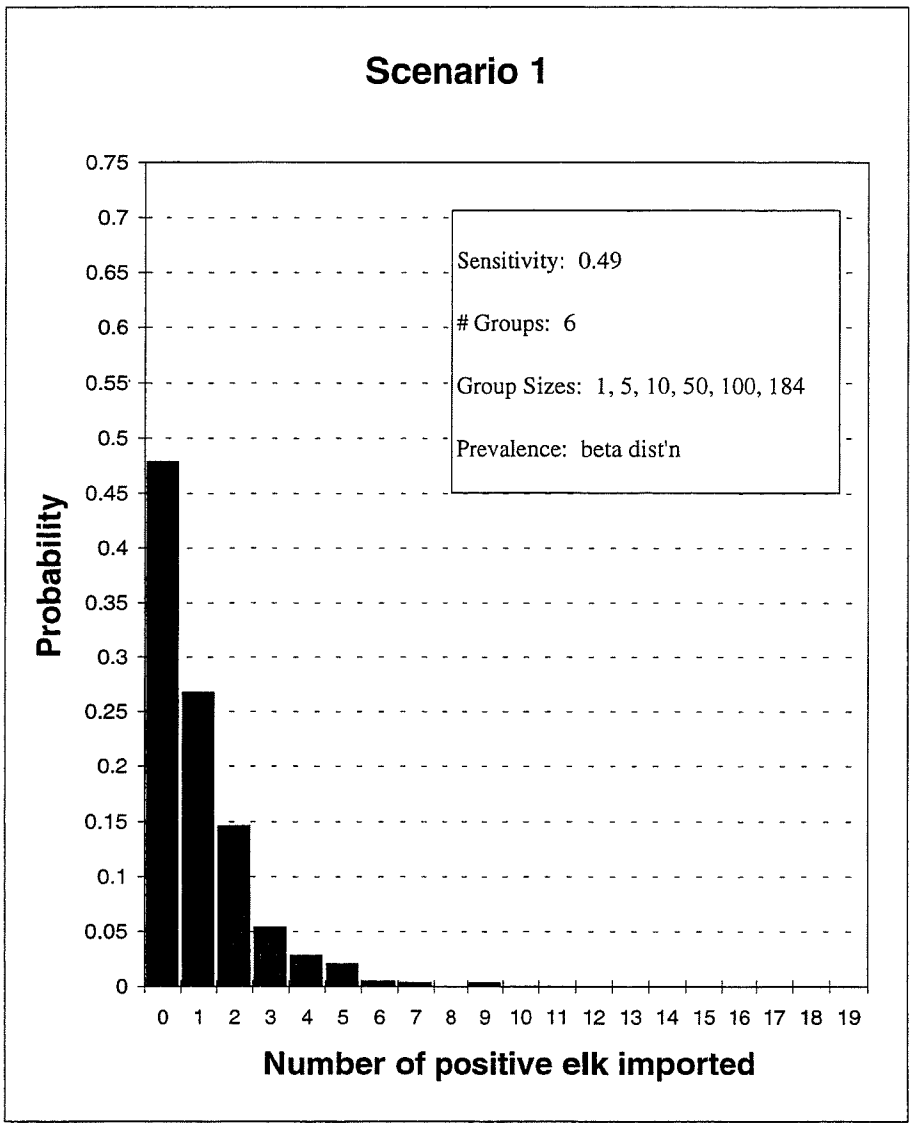


Figure 1. Probability of importing positive elk in test negative groups (Scenario 1)

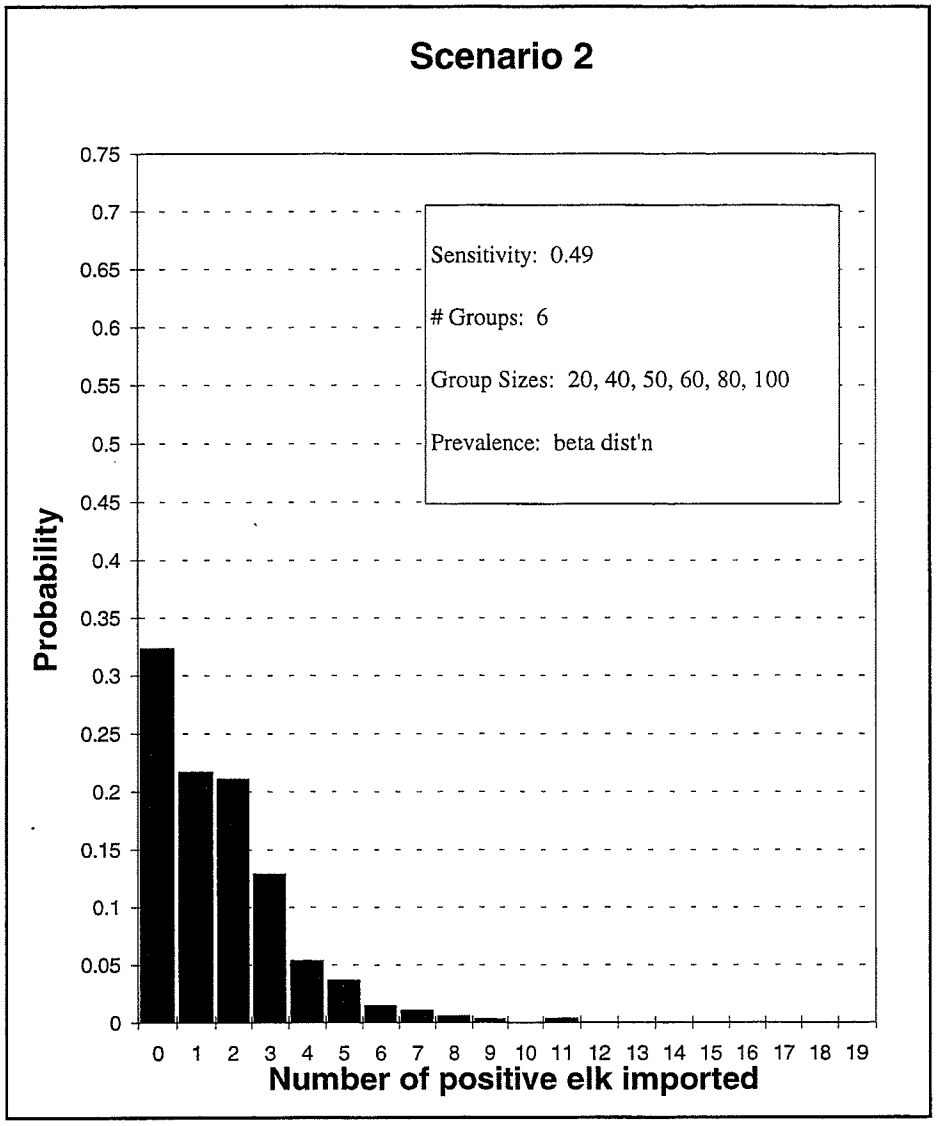


Figure 2. Probability of importing positive elk in test negative groups (Scenario 2)

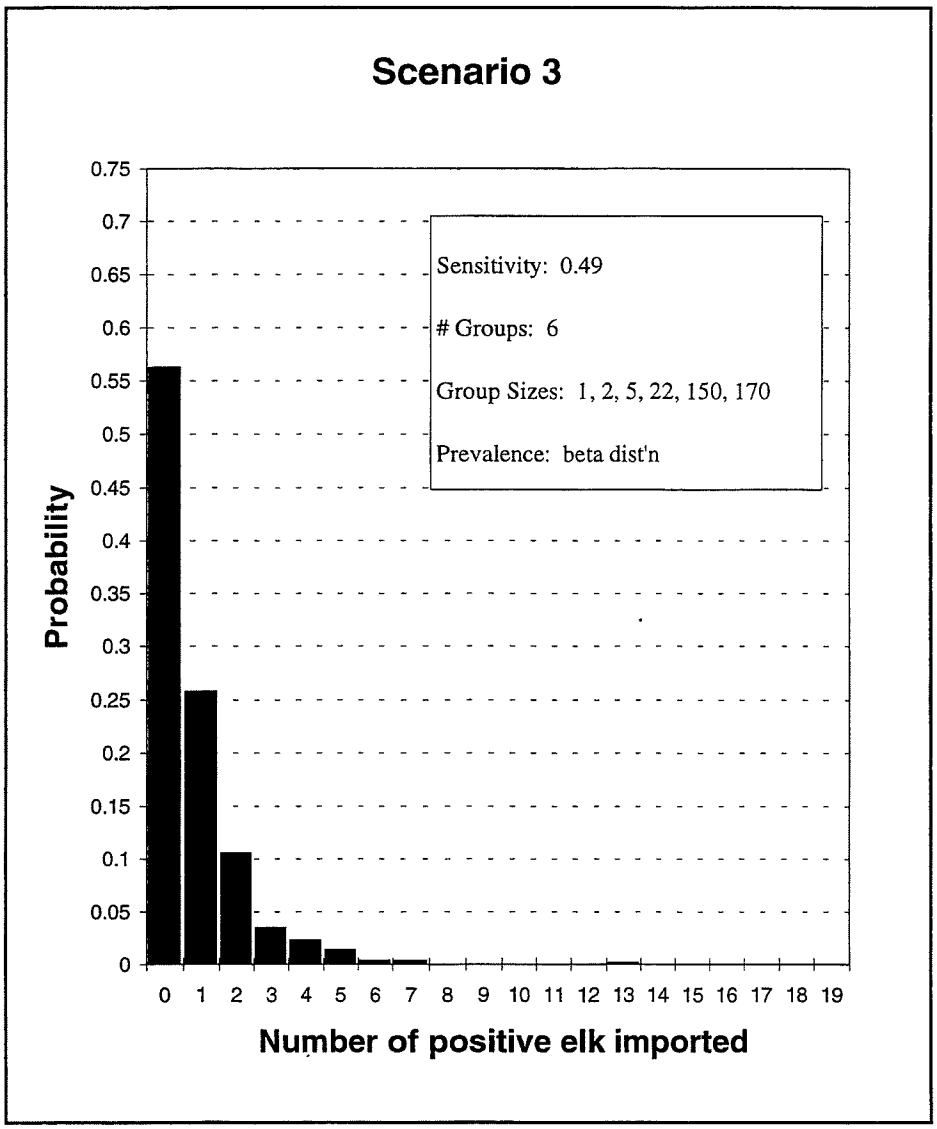


Figure 3. Probability of importing positive elk in test negative groups (Scenario 3)

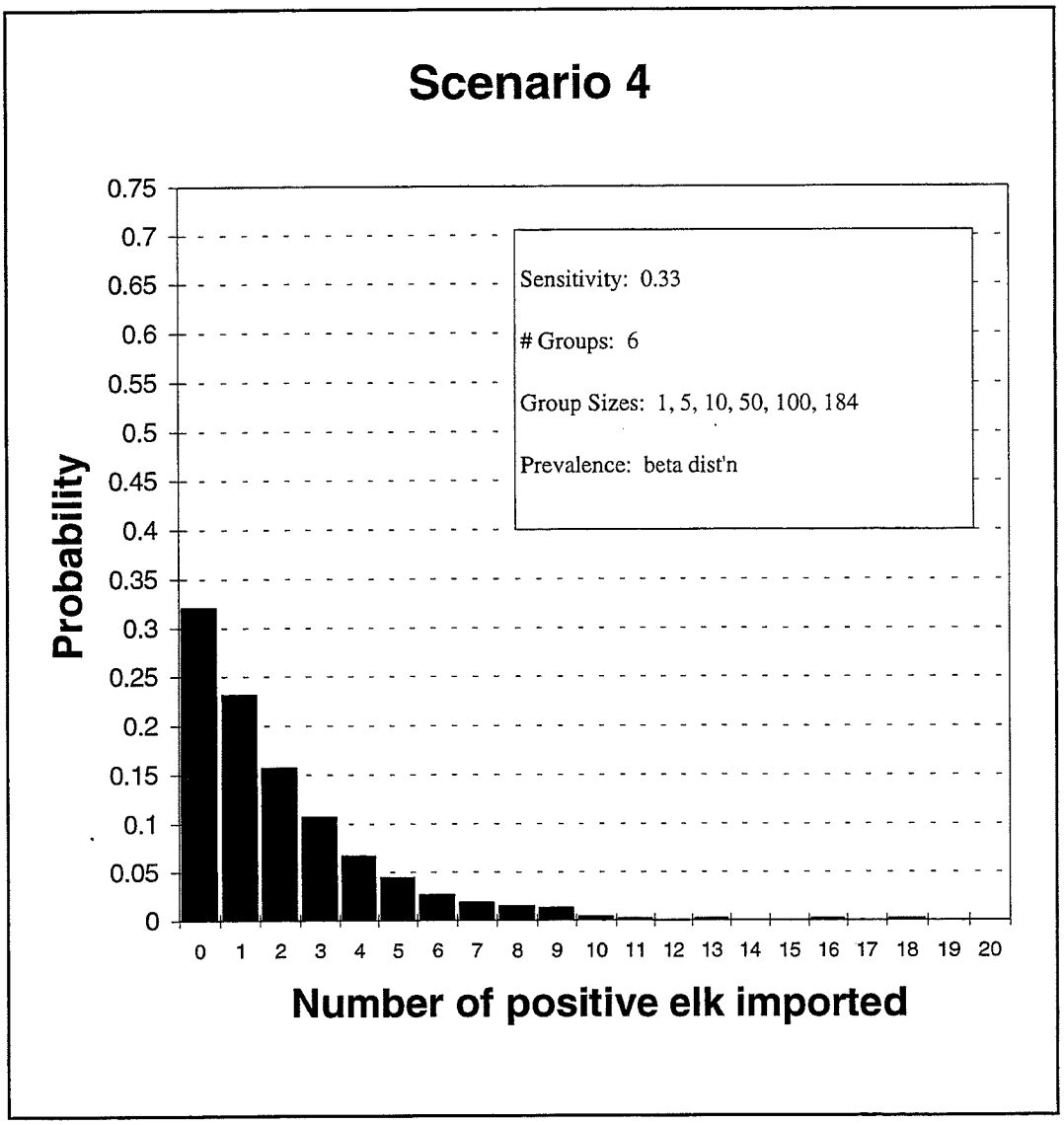


Figure 4. Probability of importing positive elk in test negative groups (Scenario 4)

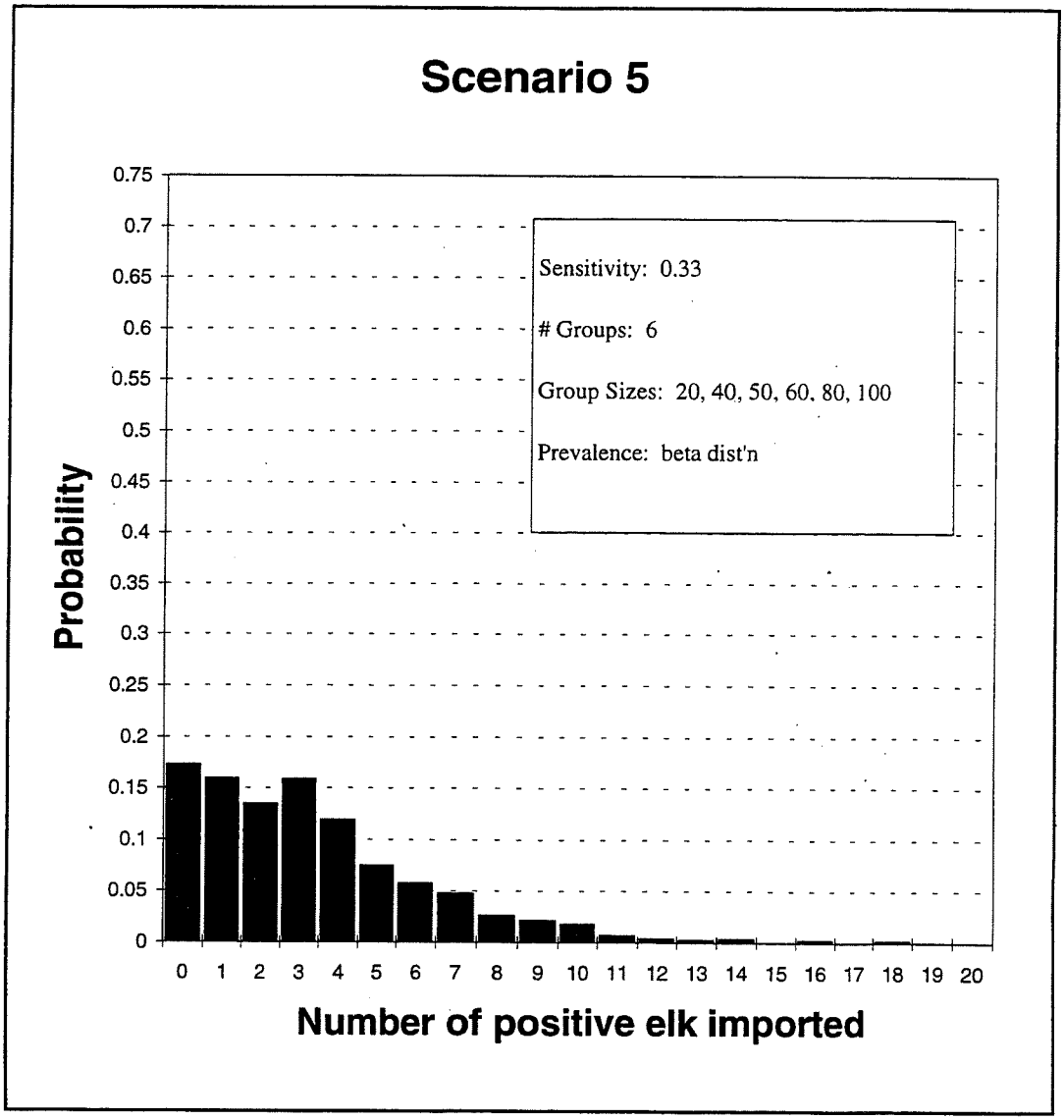


Figure 5. Probability of importing positive elk in test negative groups (Scenario 5)

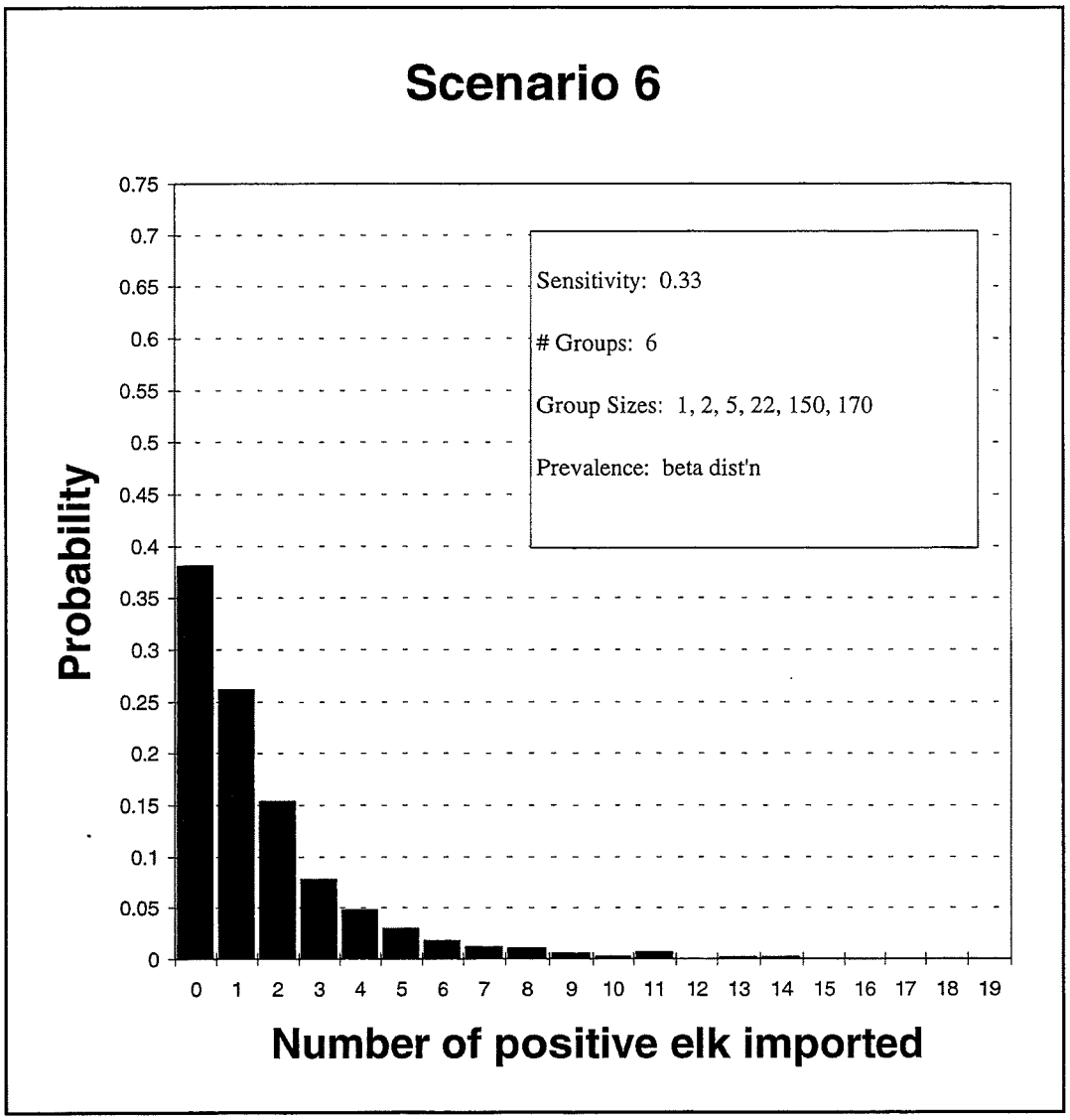


Figure 6. Probability of importing positive elk in test negative groups (Scenario 6)

Table 1. Probability of importing infected animals given a prevalence of 0.005 and a test sensitivity of 0.49

True prevalence of infection 0.005 Sensitivity of test 0.49

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9	Grp 10
# Animals per Group	1	2	5	10	25	50	75	100	150	250
If all animals test negative, the probability of importing:										
...at least 1 infected animal	0.003	0.005	0.013	0.025	0.058	0.106	0.145	0.177	0.221	0.256
...all negative animals	0.997	0.995	0.987	0.975	0.942	0.894	0.855	0.823	0.779	0.744

Table 2. Probability of importing infected animals given a prevalence of 0.01 and a test sensitivity of 0.49

True prevalence of infection 0.01 Sensitivity of test 0.49

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9	Grp 10
# Animals per Group	1	2	5	10	25	50	75	100	150	250
If all animals test negative, the probability of importing:										
...at least 1 infected animal	0.005	0.010	0.025	0.048	0.107	0.177	0.221	0.246	0.257	0.212
...all negative animals	0.995	0.990	0.975	0.952	0.893	0.823	0.779	0.754	0.743	0.788

Table 3. Probability of importing infected animals given a prevalence of 0.03 and a test sensitivity of 0.49

True prevalence of infection 0.03 Sensitivity of test 0.49

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9	Grp 10
# Animals per Group	1	2	5	10	25	50	75	100	150	250
If all animals test negative, the probability of importing:										
...at least 1 infected animal	0.015	0.030	0.070	0.125	0.224	0.259	0.228	0.180	0.098	0.024
...all negative animals	0.985	0.970	0.930	0.875	0.776	0.741	0.772	0.820	0.902	0.976

Table 4. Probability of importing infected animals given a prevalence of 0.06 and a test sensitivity of 0.49

True prevalence of infection 0.06 Sensitivity of test 0.49

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9	Grp 10
# Animals per Group	1	2	5	10	25	50	75	100	150	250
If all animals test negative, the probability of importing:										
...at least 1 infected animal	0.0306	0.0585	0.1275	0.2034	0.2613	0.1796	0.097	0.0485	0.0113	0.0006
...all negative animals	0.9694	0.9415	0.8725	0.7966	0.7387	0.8204	0.903	0.9515	0.9887	0.9994

Table 5. Probability of importing infected animals given a prevalence of 0.12 and a test sensitivity of 0.49

True prevalence of infection 0.12 Sensitivity of test 0.49

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9	Grp 10
# Animals per Group	1	2	5	10	25	50	75	100	150	250
If all animals test negative, the probability of importing:										
...at least 1 infected animal	0.0612	0.1115	0.2109	0.267	0.1789	0.0466	0.0106	0.0023	0.0001	3E-07
...all negative animals	0.9388	0.8885	0.7891	0.733	0.8211	0.9534	0.9894	0.9977	0.9999	1

Table 6. Probability of importing infected animals given a prevalence of 0.24 and a test sensitivity of 0.49

True prevalence of infection 0.24 Sensitivity of test 0.49

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9	Grp 10
# Animals per Group	1	2	5	10	25	50	75	100	150	250
If all animals test negative, the probability of importing:										
...at least 1 infected animal	0.1224	0.201	0.2814	0.2219	0.0428	0.0019	8E-05	4E-06	7E-09	3E-14
...all negative animals	0.8776	0.799	0.7186	0.7781	0.9572	0.9981	0.9999	1	1	1

Table 7. Probability of importing infected animals given a prevalence of 0.005 and a test sensitivity of 0.33

True prevalence of infection 0.005 Sensitivity of test 0.33

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9	Grp 10
# Animals per Group	1	2	5	10	25	50	75	100	150	250
If all animals test negative, the probability of importing:										
...at least 1 infected animal	0.0034	0.0067	0.0165	0.0325	0.0773	0.1424	0.1969	0.242	0.3091	0.3762
...all negative animals	0.9967	0.9933	0.9835	0.9675	0.9227	0.8576	0.8031	0.758	0.6909	0.6238

Table 8. Probability of importing infected animals given a prevalence of 0.01 and a test sensitivity of 0.33

True prevalence of infection 0.01 Sensitivity of test 0.33

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9	Grp 10
# Animals per Group	1	2	5	10	25	50	75	100	150	250
If all animals test negative, the probability of importing:										
...at least 1 infected animal	0.0067	0.0133	0.0326	0.0631	0.1429	0.2427	0.3098	0.3525	0.3876	0.3566
...all negative animals	0.9933	0.9867	0.9674	0.9369	0.8571	0.7573	0.6902	0.6475	0.6124	0.6434

Table 9. Probability of importing infected animals given a prevalence of 0.03 and a test sensitivity of 0.33

True prevalence of infection 0.03 Sensitivity of test 0.33

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9	Grp 10
# Animals per Group	1	2	5	10	25	50	75	100	150	250
If all animals test negative, the probability of importing:										
...at least 1 infected animal	0.0201	0.0394	0.0927	0.1679	0.3128	0.39	0.3723	0.3222	0.2145	0.0826
...all negative animals	0.9799	0.9606	0.9073	0.8321	0.6872	0.61	0.6277	0.6778	0.7855	0.9174

Table 10. Probability of importing infected animals given a prevalence of 0.06 and a test sensitivity of 0.33

True prevalence of infection 0.06 Sensitivity of test 0.33

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9	Grp 10
# Animals per Group	1	2	5	10	25	50	75	100	150	250
If all animals test negative, the probability of importing:										
...at least 1 infected animal	0.0402	0.0772	0.1709	0.2801	0.3936	0.3226	0.2135	0.1333	0.0497	0.0067
...all negative animals	0.9598	0.9228	0.8291	0.7199	0.6064	0.6774	0.7865	0.8667	0.9503	0.9933

Table 11. Probability of importing infected animals given a prevalence of 0.12 and a test sensitivity of 0.33

True prevalence of infection 0.12 Sensitivity of test 0.33

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9	Grp 10
# Animals per Group	1	2	5	10	25	50	75	100	150	250
If all animals test negative, the probability of importing:										
...at least 1 infected animal	0.0804	0.148	0.2893	0.3891	0.3232	0.1309	0.0482	0.0176	0.0023	4E-05
...all negative animals	0.9196	0.852	0.7107	0.6109	0.6768	0.8691	0.9518	0.9824	0.9977	1

Table 12. Probability of importing infected animals given a prevalence of 0.24 and a test sensitivity of 0.33

True prevalence of infection 0.24 Sensitivity of test 0.33

	Grp 1	Grp 2	Grp 3	Grp 4	Grp 5	Grp 6	Grp 7	Grp 8	Grp 9	Grp 10
# Animals per Group	1	2	5	10	25	50	75	100	150	250
If all animals test negative, the probability of importing:										
...at least 1 infected animal	0.1608	0.2703	0.4084	0.3739	0.126	0.0162	0.0021	0.0003	4E-06	1E-09
...all negative animals	0.8392	0.7297	0.5916	0.6261	0.874	0.9838	0.9979	0.9997	1	1

Appendix A

DISEASE AGENT FACTS SHEET

FOR: *Elaphostrongylus cervi*

Disease

Taxonomy/Nomenclature:

Family:	Protostongylidae	
Subfamily:	Elaphostrongylinae	[Boev and Schultz, 1950]
Genus:	*Elaphostrongylus*	[Cameron, 1931]
Species:	*E. alces*	[Steen, Chabaud & Rehbinder, 1989]
	E. cervi	[Cameron, 1931]
	E. rangiferi	[Mitskevitch, 1958]

common name: tissue worm

disease: elaphostrongylosis

Etiology

Life Cycle:

- adult parasite--slender worm, can reach lengths of up to 60 mm, usually 29.0-44.5 mm long x 0.100-0.125 mm wide, and usually found coiled in the connective tissue between muscle blocks or associated with the CNS
- females lay eggs--either hatch *in situ*, or are carried to the lungs in the bloodstream and then hatch
- if hatched in situ, the larvae (L1) pass to the lungs via the bloodstream
- larvae migrate through the lungs and up the air passages, are swallowed, and voided by the host in the mucous coating on the feces
- first stage larvae (L1) are tolerant, and can survive for more than two years in the environment
- L1 must penetrate the foot of a suitable molluscan intermediate host
- develops through L2 to L3 infective stage in the intermediate host in 27-50 days, depending on the ambient temperature and the molluscan species
- L3 can survive in molluscs for up to two years
- a cervid becomes infected when it ingests a mollusc containing L3, the L3 are "released" from the mollusc by digestion in the abomasum
- L3 burrow through the gastrointestinal wall of the host and migrate to their final site, developing into adults along the way
- can migrate to the brain, spinal column, under the dura mater, in the pia mater, ventricles, eyes, connective tissue of the muscles, and the peritoneum

- prepatent period (from ingestion of L3 to shedding of L1) can be up to 4 months (64 days to 125 days, depending on host species and number of L3's used to infect host
- *E. cervi* can produce eggs in the host for at least six years
- host species develop variable immunity to E. cervi--Ab to Ag's on the cuticle of the first stage larvae can be detected; a relationship may exist between stress, immunity and larval output, with two major stressors being identified--calving and rutting
- some host species seem to develop complete immunity to infection

Descriptive Etiology and Host Range

Susceptible hosts:

- parasite considered a generalist
- parasite has been shown to infect:

E. cervi	maral deer (*Cervus elaphus sibiricus*) from USSR mule deer (*Odocoileus hemionus*) in experimental studies in Canada red deer (*Cervus elaphus*) from Scotland, the Netherlands, Czechoslovakia, Austria, New Zealand, Denmark, and Poland roe deer (*Capreolus capreolus*) from Sweden, Austria, and Scotland sika deer (*Cervus nippon*) from USSR wapiti (*C. elaphus canadensis*) from New Zealand

Clinical Findings

<u>4 different reactions</u>:

1. an acute disease characterized by hind limb paralysis, associated with parasite in brain or spinal cord
2. a chronic disease with signs of ill thrift, associated with parasite in connective tissue
3. verminous pneumonia, associated with migration of larvae through the lungs
4. no evidence of clinical disease

Detection and Diagnosis

- Definitive diagnosis is by finding adult worms in the carcass. ["...somewhat akin to finding a needle in a haystack." (Mason, 1989)].
- Presumptive diagnosis is based on finding dorsal-spined larvae in the feces using a Baermann test, in regions where no other parasites producing dorsal-spined larvae are known to occur.
- The Baermann funnel technique has a maximum sensitivity of 57% on individual animals when 3 tests are performed 30 days apart using 20 grams of feces per sample, and is not specific. It will detect dorsal-spined larvae of any parasite species that produce dorsal-spined larvae (DSL). The specificity for DSL also depends upon the experience of the person performing the procedure (ie. must be able the visualize the tiny spine close to the tail of the parasite).

- The Baermann funnel technique will only recover 13-14% of the larvae that have been introduced into the funnel in infected feces, while a beaker technique has been demonstrated to recover 87% of the larvae. It has been shown that a red deer, with a patent infection, will shed the L1 larvae intermittently in the feces, so infected animals may test negative on some fecal samples.

Treatment

- Thiabendazole (in maral deer), mebendazole (in reindeer), and oxfendazole have been used. Also, ivermectin has been used in red deer and wapiti. In each instance, the anthelminthic may reduce larval shedding, but does not eliminate the infection.

Prevention and Control

- In untreated animals, sequential fecal sampling from animals under stress would provide the best chance of demonstrating positive animals.
- Molluscicíding, if it was 100 % effective in killing all the potential intermediate hosts, would provide local control and eliminate spread of the parasite by eliminating the necessary intermediate host in the parasite life cycle.
- Continual treatment of animals with an anthelminthic (as above) may reduce the shedding of L1 larvae needed to complete the life cycle of the parasite. Anthelminthics should eliminate spread of the parasite, if the anthelminthic was 100 % efficacious in eliminating the shedding of the L1 larvae.

(Sutherland, 1976; Mason and Gladden, 1983, Hollands, 1985; Mason, 1989, 1995; Gibbons et al., 1991; Welch et al., 1991; Mason and Gordon, 1994; Gajadhar and Tessaro, 1995; Forrester and Lankester, 1997)

Appendix B

DISEASE AGENT FACTS SHEET

FOR: *Parelaphostrongylus tenuis*

Disease

Taxonomy/Nomenclature

Genus:	*Parelaphostrongylus*	
Species:	*P. andersoni*	[Prestwood, 1972]
	P. odocoilei	[Hobmaier and Hobmaier, 1934]
	P. tenuis	[Dougherty, 1945]
common names:	meningeal worm	
	brain worm	
disease:	cerebrospinal parelaphostrongylosis	
	moose sickness	

Etiology

Life Cycle

- adult parasite, 31-90 mm long -- usually found associated with the subdural space or the cranial venous sinuses of white-tailed deer
- females lay eggs -- usually into the venous circulation; the eggs are then carried to the lungs where they hatch
- alternatively, eggs may be laid and hatch *in situ*; the L1 larvae then pass via the blood stream to the lungs
- larvae migrate through the lungs and into air passages, are swallowed and pass through the digestive system to be voided by the host in the mucous coating around the fecal pellets
- L1 larvae penetrate the foot of a suitable molluscan intermediate host
- the larvae develops through the L2 to the L3 infective stage in the intermediate host (this process takes a minimum of 21 days at an ambient temperature of 20° C)
- the larvae can overwinter in gastropods
- an ungulate becomes infected by ingesting infected gastropods; the L3 larvae are "released" from the mollusc by digestion in the abomasum, penetrate through the gastrointestinal tract wall, cross the peritoneal cavity and migrate along lumber nerves to reach the vertebral canal (takes about 10 days)
- L3 invade the gray matter of the dorsal horns of the spinal cord and develop into subadults (takes about 30 days)
- subadults enter the spinal subdural space and migrate to the cranium, maturing to adulthood along the way

- adult worms remain in the subdural space, imbed in the dura mater, or migrate to the venous sinuses
- the pre-patent period, from ingestion of infective L3 to shedding of L1 larvae, in white-tailed deer in reported to be 82 - 91 days, and in wapiti ranges from 90 - 115 (or more) days

Susceptible hosts

- the natural host is considered to be white-tailed deer
- parasite has been shown to infect:

P. tenuis	white-tailed deer (*Odocoileus virginiansus*)
	wapiti (*Cervus elaphus canadensis*)
	moose (*Alces alces*)
	caribou (*Rangifer tarandus*)
	reindeer (*Rangifer tarandus tarandus*)
	mule deer (*Odocoileus hemionus*)
	black-tailed deer (*Odocoileus hemionus columbianus*)
	domestic sheep
	domestic goats
	domestic cattle
	fallow deer (*Dama dama*)
	llamas
	sable antelope (*Hippotragus niger*)

Descriptive Etiology and Host Range

Prevalence of P. tenuis in wild Canadian white-tailed deer

Ontario:

1. 41 % (1963)
2. 61 % (1968)
3. 63 % (1972)
4. 82 % (1995)

Saskatchewan

1. < 0.005 %

Clinical Findings

Neurological signs in the aberrant host (cerebrospinal parelaphostrongylosis)

- lumbar weakness, ataxia, torticollis, circling, blindness, fearlessness, depression, paresis, paralysis, death

NB: Under field conditions, white-tailed deer rarely exhibit clinical signs that can be determined to be associated with infection with *P. tenuis*.

NB: Elk may show mild to no clinical signs and become patently infected.

Detection and Diagnosis

- Definitive diagnosis is by finding adult worms in the animal on post-mortem examination.
- Presumptive diagnosis is based on finding dorsal-spined larvae in the feces using a Baermann test, in regions where no other parasites producing dorsal-spined larvae are known to occur. (see appendix)
- The Baermann funnel technique has a maximum sensitivity of 57% on individual animals when 3 tests are performed 30 days apart using 20 grams of feces per sample, and is not specific. It will detect dorsal-spined larvae of any parasite species that produce dorsal-spined larvae. The specificity for dorsal-spined larvae also depends upon the experience of the person performing the procedure (ie. must be able the visualize the tiny spine close to the tail of the parasite).
- The Baermann funnel technique will only recover 13-14% of the larvae that have been introduced into the funnel in infected feces, while a beaker technique has been demonstrated to recover 87% of the larvae.
- It has been shown that elk, with a patent infection, will shed the L1 larvae intermittently in the feces, so may test negative on some fecal samples.

Treatment

- Treatment with an anthelminthic may reduce larval shedding, but does not eliminate the infection.

Prevention and Control

- In untreated animals, sequential fecal sampling from animals under stress would provide the best chance of demonstrating positive animals.
- Molluscicide, if it was 100% effective in killing all the potential intermediate hosts, would provide local control and eliminate spread of the parasite by eliminating the necessary intermediate host in the parasite life cycle.
- Continual treatment of animals with an anthelminthic (as above) may reduce the shedding of L1 larvae needed to complete the life cycle of the parasite. Anthelminthics would eliminate spread of the parasite, if the anthelminthic was 100% efficacious in eliminating the shedding of the L1 larvae.

(Anderson, 1965; Anderson and Prestwood, 1981; Nichols et al., 1986; Welch et al., 1991; Carreno and Lankester, 1992; Forrester and Lankester, 1997)

Appendix C

RELATIONSHIP OF PROTOSTRONGYLID PARASITES KNOWN TO PRODUCE DORSAL-SPINED LARVAE

(those genus' and species marked with a double asterisk (**) are known, or suspected, to occur in Canada)

Family: Protostongylidae

Genus: *Cystocaulus*

Genus: *Elaphostrongylus*
Species:
- *E. alces*
- *E. cervi***
- *E. rangiferi***

Genus: *Muellerius*
Species:
- *M. capillaris***

Genus: *Parelaphostrongylus*
Species:
- *P. andersoni***
- *P. odocoilei***
- *P. tenuis***

Genus: *Pneumocaulus*

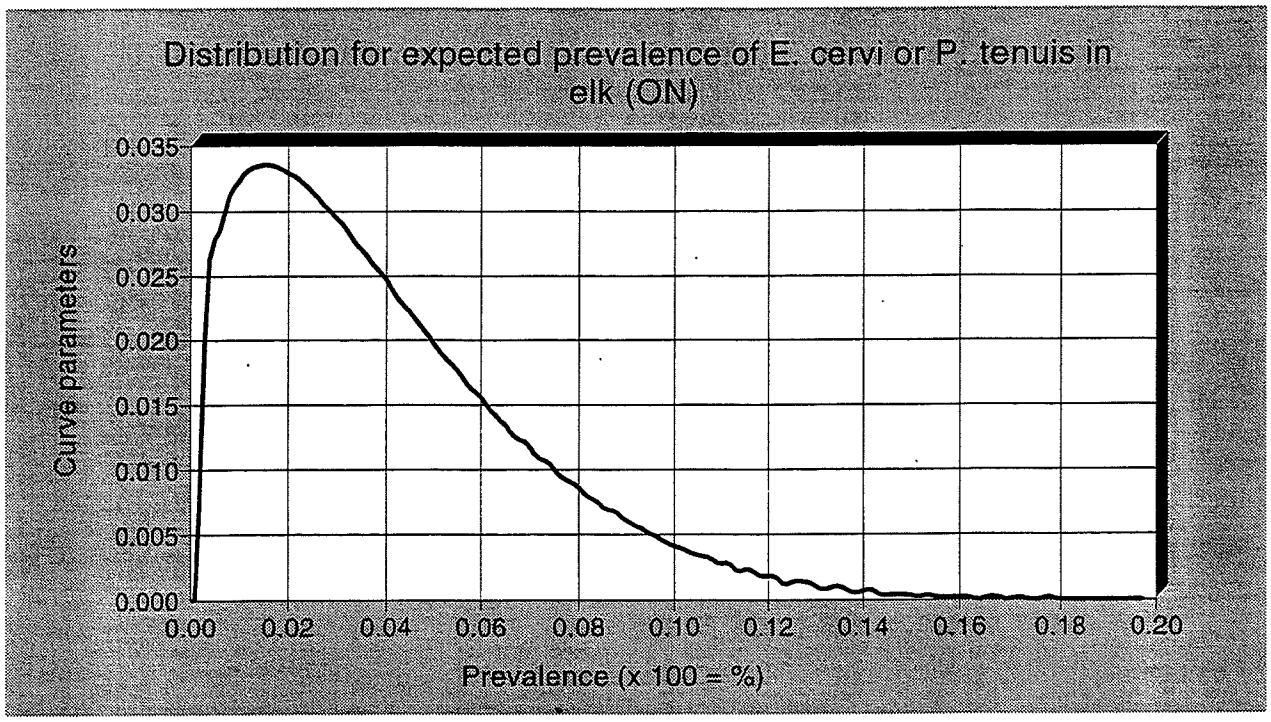
Genus: *Pneumostrongylus*

Genus: *Varestrongylus*
Species:
- *V. alpenae***
- *V. capreoli*
- *V. capricola*
- *V. pneumonicus*
- *V. sagittatus*
- *V. schulzi*

Genus: *Umingmakstrongylus*
Species:
- *U. pallikuukiensis***

(adapted from Mason, 1995)

Appendix D


Distribution for expected prevalence of E. cervi or P. tenuis in elk (ON)
Curve parameters
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
Prevalence (x 100 = %)

REFERENCES

Anderson, R.C., 1965. The development on *Pneumostrongylus tenuis* in the central nervous system of white-tailed deer. Pathol. Vet., 2(4): 360-379.

Anderson, R.C., 1971. Neurologic disease in reindeer (*Rangifer tarandus tarandus*) introduced into Ontario. Can. J. Zool., 49(2): 159-166.

Anderson, R.C., 1972. The ecological relationships of meningeal worm and native cervids in North America. J. Wild. Dis., 8: 305-310.

Anderson, R.C., and Strelive, U.R., 1966. Experimental cerebrospinal mnematodiasis (*Pneumostrongylus tenuis*) in sheep. Can. J. Zool., 44(5): 889-894.

Anderson, R.C., and Strelive, U.R., 1968. The experimental transmission of *Pneumostrongylus tenuis* to caribou (*Rangifer tarandus terraenovae*). Can. J. Zool., 46(3): 503-510.

Anderson, R.C., and Prestwood, A.K., 1981. Lungworms. Diseases and Parsites of White-tailed Deer. Davidson, W.R., Hayes, F.A., Nettles, V.F., et al. (eds). Tallahasee, Fla: Tall Timbers Research Station, pg 266-317.

Anderson, R.C., Lankester, M.W., and Strelive, U.R., 1966. Further Experimental Studies of *Pneumostrongylus tenuis* in cervids. Can. J. Zool., 44: 851-861.

Bindernagel, J.A., and Anderson, R.C., 1972. Distribution of the meningeal worm in white-tailed deer in Canada. J. Wild. Manage., 36(4): 1349-1353.

Bogaczyk, B.A., Krohn, W.B., and Gibbs, H.C., 1993. Factors affecting *Parelaphostrongylus tenuis* in white-tailed deer (*Odocoileus virginianus*) from Maine. J. Wild. Dis., 29(2): 266-272.

Carpenter, J.W., Jordan, H.E., and Ward, B.C., 1973. Neurologic disease in wapiti naturally infected with meningeal worms. J. Wild. Dis., 9: 149-153.

Carreno, R.A., and Lankester, M.W., 1993. Additional information on the morphology of the Elaphostrongylinae (Nematoda: Protostrongylidae) of North American cervidae. Can. J. Zool., 71(3): 592-600.

Dauphine, T.C. (Jr.), 1975. The disappearance of caribou re-introduced to Cape Breton Highlands National Park. Can. Field Nat., 89(3): 299-310.

Forrester, S.G., and Lankester, M.W., 1997. Extracting protostrongylid nematode larvae from ungulate feces. J. Wildl. Dis., 33(3): 511-516.

Gajadhar, A.A., and Tessaro, S.V., 1995. Susceptibility of mule deer (*Odocoileus hemionus*) and two species of North American molluscs to *Elaphostrongylus cervi* (Nematoda: Metastongyloidea). J. Parasitol., 81(4): 593-596.

Gajadhar, A.A., Tessaro, S.V., and Yates, W.D.G., 1994. Diagnosis of *Elaphostrongylus cervi* infection in New Zealand red deer (*Cervus elaphus*) quarantined in Canada, and experimental determination of a new extended prepatent period. Can. Vet. J., 35: 433-437.

Gibbons, L.M., Halvorsen, O., and Stuve, G., 1991. Revision of the genus *Elaphostrongylus* Cameron (Nematoda, Metastrongyloidea) with perticular reference to species of the genus occurring in Norwegian cervids. Zool. Scripta, 20: 15-26.

Gilbert, F.F., 1974. *Parelaphostrongylus tenuis* in Maine: II--Prevalence in moose. J. Wildl. Manage., 38(1): 42-46.

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

Gray, J.B., Samuel, W.M., Shostak, A.W., and Pybus, M.J., 1985. Varestrongylus alpenae (Nematoda: Metastrongyloidea) in white-tailed deer (Odocoileus virginianus) of Saskatchewan. Can. J. Zool., 63: 1449-1454.

Hollands, R.D., 1985. *Elaphostrongylus cervi cervi* in the central nervous system of red deer (*Cervus elaphus*) in Scotland. Vet. Rec., 116(22): 584-585.

Jortner, B.S., Troutt, H.F., Collins, T., and Scarratt, K., 1985. Lesions of spinal cord parelaphostrongylosis in sheep. Sequential changes following intramedullary larval migration. Vet. Pathol., 22: 137-140.

Kistner, T.P., Johnson, G.R., and Rilling, G.A., 1977. Naturally occurring neurologic disease in a fallow deer infected with meningeal worms. J. Wildl. Dis., 13: 55-58.

Kocan, A.A., 1985. The use of ivermectin in the treatment and prevention of infection with *Parelaphostrongylus tenuis* (Dougherty) (Nematoda: Metastrongyloidea) in white-tailed deer (*Odocoileus virginianus* Zimmermann). J. Wild. Dis., 21(4): 454-455.

Kopcha, M., Marteniuk, J.V., Sills, R., Steficek, B., and Schillhorn van Veen, T.W., 1989. Cerebrospinal nematodiasis in a goat herd. JAVMA, 194(10): 1439-1442.

Lankester, M.W., 1974. *Parelaphostrongylus tenuis* (Nematoda) and *Fascioloides magna* (Trematoda) in moose of southeastern Manitoba. Can. J. Zool., 52: 235-239.

Lankester, M.W., and Anderson, R.C., 1968. Gastropods as intermediate hosts of *Pneumostrongylus tenuis* Dougherty of white-tailed deer. Can. J. Zool., 46: 373-383.

Lankester, M.W., and Peterson, W.J., 1996. The possible importance of wintering yards in the transmission of *Parelaphostrongylus tenuis* to white-tailed deer and moose. J. Wildl. Dis., 32(1): 31-38.

Mason, P.C., 1989. *Elaphostrongylus cervi* -- a review. Surv., 16(1): 3-10.

Mason, P., 1995. *Elaphostrongylus cervi* and its close relatives; a review of protostrongylids (Nematoda, Metastrongyloidea) with spiny-tailed larvae. Surv., 22(1): 19-24.

Mason, P.C., and Gladden, N.R., 1983. Survey of internal parasitism and anthelminthic use in farmed deer. NZ Vet. J., 31: 217-220.

Mason, P., and Gordon, D., 1994. Identification of *Elaphostrongylus cervi* lesions at routine meat inspection of deer carcasses. Surv., 21(4): 27-28.

Nettles, V.F., Prestwood, A.K., and Smith, R.D., 1977a. Cerebrospinal parelaphostrongylosis in fallow deer. J. Wildl. Dis., 13: 440-444.

Nettles, V.F., Prestwood, A.K., Nichols, R.G., and Whitehead, C.J., 1977b. Meningeal worm-induced neurologic disease in black-tailed deer. J. Wildl. Dis., 13: 137-143.

Nichols, D.K., Montali, R.J., Lyndsay, G.P., Alvarado, T.P., Bush, M. and Collins, L., 1986. *Parelaphostrongylus tenuis* in captive reindeer and sable antelope. JAVMA, 188(6): 619-621.

O'Brien, T.D., O'Leary, T.P., Leininger, J.R., Sherman, D.M., Stevens, D.L., and Wolf, C.B., 1986. Cerebrospinal parelaphostrongylosis in Minnesota. Minn. Vet., 26: 18-22.

Olsen, A., and Woolf, A., 1978. The development of clinical signs and the population significance of neurological disease in a captive wapiti herd. J. Wild. Dis., 14: 263-268.

Olsen, A., and Woolf, A., 1979. A summary of the prevalence of *Parelaphostrongylus tenuis* in a captive wapiti population. J. Wild. Dis., 15: 33-35.

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

Pitt, W.C., and Jordan, P.A., 1994. A survey of the nematode parasite *Parelaphostrongylus tenuis* in the white-tailed deer, *Odocoileus virginianus*, in a region proposed for caribou, *Rangifer tarandus caribou*, re-introduction in Minnesota. Can. Field-Nat., 108(3): 341-346.

Pybus, M.J., Samuel, W.M., and Crichton, V., 1989. Identification of dorsal-spined larvae from free-ranging wapiti (*Cervus elaphus*) in southwestern Manitoba, Canada. J. Wild. Dis., 25(2): 291-293.

Raskevitz, R.F., Kocan, A.A., and Shaw, J.H., 1991. Gastropod availability and habitat utilization by wapiti and white-tailed deer sympatric on range enzootic for meningeal worm. J. Wild. Dis., 27(1): 92-101.

Sackett, D.L., Haynes, R.B., Guyatt, G.H., and Tugwell, P., 1991. Clinical Epidemiology: A Basic Science for Clinical Medicine. Little, Brown and Company, Toronto, 441 pp.

Samuel, W.M., and Gray, J.B., 1988. Efficacy of ivermectin against *Parelaphostrongylus andersoni* (Nematoda, Metastrongyloidea) in white-tailed deer (Odocoileus virginianus). J. Wild. Dis., 24(3): 491-495.

Samuel, W.M., Pybus, M.J., Welch, D.A., and Wilke, C.J., 1992. Elk as a potential host for meningeal worm: Implications for translocation. J. Wild. Manage., 56(4): 629-639.

Saunders, B.P., 1973. Meningeal worm in white-tailed deer in northwestern Ontario and moose population densities. J. Wildl. Manage., 37(3): 327-330.

Slomke, A.M., Lankester, M.W., and Peterson, W.J., 1995. Infrapopulation dynamics of *Parelaphostrongylus tenuis* in white-tailed deer. J. Wild. Dis., 31(2): 125-135.

Sutherland, R.J., 1976. Elaphostrongylus cervi in cervids in New Zealand 1. The gross and histological lesions in reed deer (Cervus elaphus). NZ Vet. J., 24: 263-266.

Trainer, D.O., 1973. Caribou mortality due to the meningeal worm (*Parelaphostrongylus tenuis*). J. Wildl. Dis., 9(4): 376-378.

Wasel, S.M., Samuel, W.M., and Crichton V., submitted. Meningeal worm, *Parelaphostrongylus tenuis* (Nemotoda), in Manitoba and Saskatchewan (Canada), and North Dakota (USA): Distribution and ecological correlates. Wild. Mon.,.

Welch, D.A., Pybus, M.J., Samuel, W.M., and Wilke, C.J., 1991. Reliability of fecal examination for detecting infections of meningeal worm in elk. Wildl. Soc. Bull., 19: 326-331.

Whitlaw, H.A., and Lankester, M.W., 1994a. A retrospective evaluation of the efficts of parelphostrongylosis on moose populations. Can. J. Zool., 72: 1-6.

Whitlaw, H.A., and Lankester, M.W., 1994b. The co-occurence of moose, white-tailed deer, and *Parelaphostrongylus tenuis* in Ontario. Can. J. Zool., 72(5): 819-825.

Woolf, A., Mason, C.A., and Kradel, D., 1977. Prevalence and effects of *Parelaphostrongylus tenuis* in a captive wapiti population. J. Wild. Dis., 13: 149-154.

Yamini, B., Baker, P.C., Stromberg, C.H., and Gardiner, C.H., 1997. Cerebrospinal nematodiasis and verterbral chondrodysplasia in a calf. J. Vet. Diagn. Invest., 9: 451-454.