GIANT LIVER FLUKE IN NORTH DAKOTA MOOSE

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ABSTRACT: The giant liver fluke (*Fascioloides magna*) is a parasite of white-tailed deer (*Odocoileus virginianus*) and wapiti (*Cervus elaphus*) that can cause extensive and conspicuous liver damage in moose (*Alces alces*), a dead-end host. The implication of *F. magna* as a factor in the long-term decline of moose in northwestern Minnesota has raised concern that a concurrent decline in moose in northeastern North Dakota may also be linked to this parasite. I reviewed data collected from moose hunter check stations in1977-1984 and necropsy reports of non-harvested animals examined in 1983-1992 to estimate past prevalence of *F. magna* in moose in North Dakota. I also collected livers from harvested moose in 2002 and 2003 to investigate the current occurrence of this parasite. I also surveyed 78 wetlands at 12 sites in 2003-2006 to examine the potential for *F. magna* transmission based on the occurrence of aquatic snail intermediate hosts. Flukes or signs consistent with fluke infection were observed in 19.6% of harvested moose (n = 158) in 1977-1984, and in 18.8% of moose necropsied (n = 32) in 1983-1992. *Fascioloides magna* was not recovered from any of the 78 moose livers collected in 2002 and 2003. However, lymnaeid snails were found at 10 of 12 sites in the aquatic gastropod surveys indicating that the intermediate hosts for this parasite occur widely throughout the range of moose in North Dakota. While this represents the first known report of *F. magna* in North Dakota, this parasite occurs at relatively low prevalence, and there is no evidence that it has been an important factor in recent moose declines, nor that it noticeably impairs the health of moose in North Dakota. Transmission may be limited by the transient availability of wetlands capable of supporting the life cycle of *F. magna*.

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White-tailed deer (*Odocoileus virginianus*) are the normal host for 2 parasites that may cause fatal disease in moose (*Alces alces*). The best-known of these is the meningeal worm (*Parelaphostrongylus tenuis*), a nematode long implicated as a limiting factor of moose populations (Lankester 2001, 2010). The other is the giant liver fluke (*Fascioloides magna*), a large trematode that occurs in pairs or groups within fibrous capsules in the liver parenchyma of its normal hosts, white-tailed deer and wapiti (*Cervus elaphus*) (Pybus 2001). *Fascioloides magna* has an indirect life cycle, requiring aquatic snails in the family Lymnaeidae (hereafter lymnaeid snails) to serve as intermediate hosts (Pybus 2001). In dead-end hosts such as moose, juvenile flukes migrate much more extensively than in normal hosts before becoming encapsulated, and as a result, cause considerable destruction of liver tissue. Extensive fibrosis of the migratory tracts and capsules containing adult flukes can damage 50-90% of the liver, and sometimes be suspected of causing death of the host (Pybus 2001). Recently, *F. magna* was implicated in the long-term decline of moose in northwestern Minnesota (Fig. 1) where 89% of moose examined in 1995-2000 were infected with *F. magna* (Murray et al. 2006).

The North Dakota Game and Fish Department (NDGF) conducts annual winter aerial surveys of moose populations in 3 survey areas (Turtle Mountains, Drift Prairie, and Pembina Hills; Fig. 1). Survey data collected

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over the past decade indicate that while moose populations appear to be stable to increasing in the Turtle Mountains and Drift Prairie areas, moose have declined considerably in the Pembina Hills (Johnson 2002, 2007; Fig. 2). During this same period, white-tailed deer in the state have increased considerably, suggesting increased transmission potential of deer parasites (namely *F. magna* and *P. tenuis*) to moose (Jensen 2007, Smith et al. 2007). Additionally, because the Pembina Hills area is adjacent to the declining moose population in northwestern Minnesota, concern existed that the North Dakota decline also may be related to *F. magna* infection. This study addressed this concern by 1) examining historical data to estimate past prevalence of this parasite in the moose in North Dakota, 2) investigating the current occurrence of *F. magna* infection in moose, and 3) determining whether suitable intermediate hosts for this parasite occur in the state.

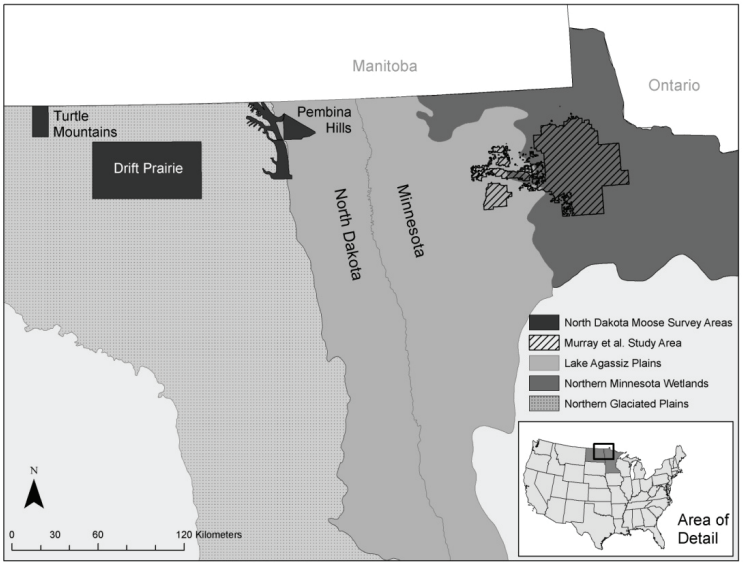

Fig. 1. Moose aerial survey units in North Dakota (Turtle Mountains, Drift Prairie, and Pembina Hills) and study areas of Murray et al. (2006) in adjacent northeastern Minnesota (Agassiz National Wildlife Refuge, Red Lake Wildlife Management Area, Thief Lake Wildlife Management Area, Beltrami Island State Forest).

METHODS

To estimate the historical prevalence of *F. magna* in North Dakota moose, I reviewed 2 data sets collected previously by NDGF. The first data set consisted of hunter check-station records for 158 moose harvested in 1977-1984. During these first 8 years of the moose season, hunters were encouraged to bring entire carcasses to check stations where the animals were weighed and the viscera examined to assess reproductive status and parasitic infection. The second data set was historical necropsy reports of non-hunting related deaths. These included full necropsies on 32 such moose conducted by the NDGF wildlife veterinarian in 1983-1992 as part of targeted surveillance for wildlife diseases.

I reviewed check station data sheets and necropsy reports for evidence of liver fluke infection based on the recovery of flukes from liver tissue or comments in reports that suggested fluke infection including unspecified cysts or capsules in the liver, fibrous areas, migratory tracts, detritus, necrosis, congestion or "bad" or "questionable" livers. Additionally, examination of the necropsy reports from targeted surveillance allowed me to compare the relative frequency of *F. magna* infection with that of other pathogens. A Clopper-Pearson binomial confidence interval was calculated for the historical estimate of *F. magna* prevalence obtained from the check station and necropsy data (Rosza et al. 2000).

In addition, I estimated the current occurrence of *F. magna* infection in moose by examining 78 moose livers collected from hunters during the 2002 and 2003 moose seasons. Livers were sectioned into approximately 2-cm wide slices and examined for the presence of adult or juvenile *F. magna* and signs associated

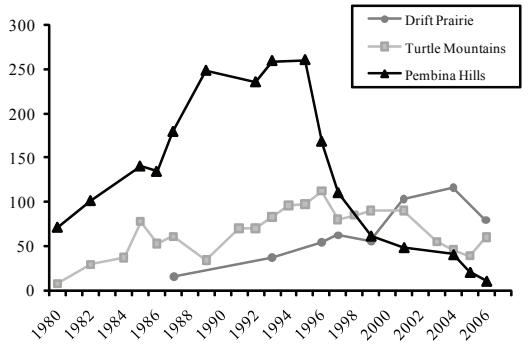

Fig. 2. Number of moose observed within 3 survey units in North Dakota by the North Dakota Game and Fish Department during winter 1980-2006. Winter surveys were not completed in the Drift Prairie area prior to 1987. Data are from Johnson (2002, 2007).

with *F. magna* infection such as fibrous tissue, migratory tracts, detritus, liver necrosis, and congestion (Lankester 1974).

I also investigated the occurrence of intermediate hosts for *F. magna* by sampling permanent and semi-permanent wetlands, small lakes, and streams for lymnaeid snails during 4 summer periods (2003-2006). I sampled for the presence of lymnaeid gastropods in 78 wetlands that included small lakes and streams at 12 sites (11 in northeastern North Dakota, 1 in northwestern Minnesota; Fig. 3). Each site was sampled by a series of 10 1-m sweeps with a dip net approximately every 10 m within 1-2 m of shore. After each sweep the contents of the net were examined for aquatic gastropods; lymnaeid snails observed floating on the surface were collected opportunistically. Snails were placed in 70% ethanol or frozen, and were subsequently identified to species using the criteria of Clarke (1973) and Cvancara (1983).

RESULTS

Based on the review of check station records and necropsy reports, the past prevalence of *F. magna* infection in North Dakota moose during the period 1977-1992 was 19.5% (95% C.I., 14.1-25.8%, $n = 190$). There was evidence of *F. magna* infection in 31 of 158 (19.6%) harvested moose (Table 1). Liver flukes were recovered from 18 (11.4%) of these moose, while signs suggesting possible *F. magna* infection were observed in the remainder (8.2%; 6 with unspecified cysts, 5 with bad livers, 2 with fibrous tracts). Only the northeastern area of North Dakota (Unit M1C, Fig. 3) was open to moose hunting from 1977-1982, thus 138 of 158 harvest samples originated from this area. In M1C, liver flukes were recovered from 16 (11.6%) moose, and signs consistent with *F. magna* infection were observed in the remainder (9.4%). Hunting for moose was initiated in Units M4-M10 in 1983; liver flukes were recovered from 2 of 20 moose harvested in Units M4-M10 in 1983 and 1984 (Table 1).

Six of the 32 (18.8%) non-hunting related fatalities (1983-1992) exhibited pathology

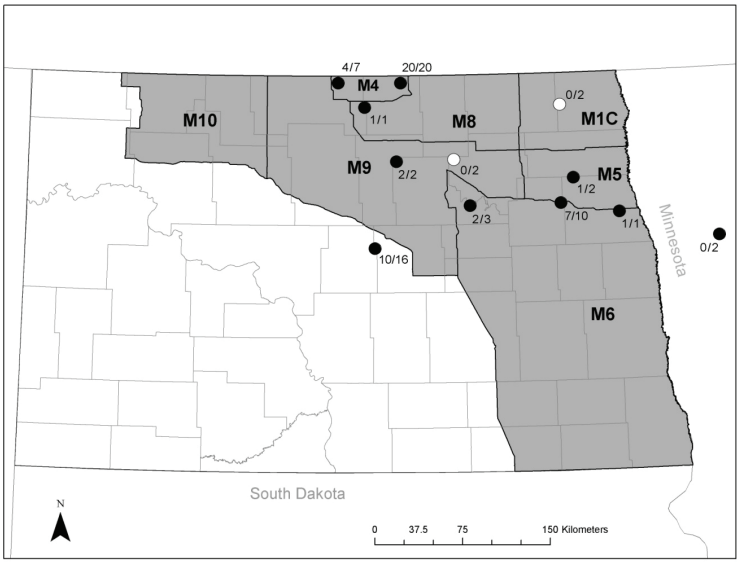

Fig. 3. North Dakota moose hunting units (M1-10) and sites sampled for lymnaeid snails (● = lymnaeids present; o = absent). Numerals represent number of wetlands from which snails were recovered/ number of wetlands sampled at each site.

Table 1. Incidence of *F. magna* infection in harvested moose and unharvested moose in 7 management units in North Dakota.

	Harvested 1977-1984		Unharvested 1983-1992		Harvested 2002-2003	
Unit	Examined	Infected	Examined	Infected	Examined	Infected
M1C	138	29 (21.0%)	6	2 (33.3%)	4	0
M4	4	0	1	0	22	0
M5	6	0	2	1 (50%)	1	0
M6	5	0	21	3 (14.3%)	4	0
M8	4	2 (50.0%)	0	0	16	0
M9	1	0	0	0	7	0
M10	0	0	0	0	2	0
Unknown	0	0	2	0	22	0
Total	158	31 (19.6%)	32	6 (18.8%)	78	0

suggesting *F. magna* infection (2 with liver congestion, 1 with liver unspecified infection, 1 with fibrosis of the liver, and 1 with fibrous capsules; Table 1). Only a single moose was believed to have died as a result of *F. magna* based on the amount of liver damage caused by the fluke infection. Flukes or signs of infection were not seen in any of 78 moose livers collected in 2002 and 2003. The moose hunting unit of origin was known for 56 of the samples; however, unit of origin was not available for 22 samples (Table 1).

A total of 418 lymnaeid snails representing 3 species (*Lymnaea caperata*, *L. palustris*, and *L. stagnalis*) were recovered from 10 of the 12 sites, and 55 of 78 wetlands sampled (Table 2, Fig. 3). The 2 sites where snails were not found were represented by only a single wetland sampling area. All 3 species collected are known hosts for *F. magna* (Swales 1935, Foreyt and Todd 1978, Laursen and Stromberg 1993); *Lymnaea palustris* was the most common occurring at 9 sites, and *L. stagnalis* and *L. caperata* were found at 5 and 4 sites, respectively (Table 2).

DISCUSSION

To my knowledge, this study represents the first report of *F. magna* in moose in North Dakota. However, because the historical data were collected by previous investigators, they were subject to a degree of interpretation. First, I assumed that flukes collected by past investigators were actually *F. magna*, as this fluke has been recovered from moose in adjacent northeastern Minnesota (Karns 1972, Murray et al. 2006), and the only other large liver fluke in North America, *Fasciola hepatica*, has not been reported in North Dakota (Pybus 2001). Second, I interpreted all signs suggestive of *F. magna* infection as actually being caused by this parasite; however, certain described signs may have been due to injury (Lankester and Samuel 1998), bacterial infection (Leighton 2001), *Echinococcus granulosis*, or *Taenia hydatigena* cysts (Jones and Pybus 2001). As a result, the true prevalence of *F. magna* in moose in eastern North Dakota may have been lower than the 19.5% estimated from check station records and necropsy reports. Unfortunately, data from white-tailed deer were not available to corroborate the presence

Table 2. Survey data for lymnaeid snails in North Dakota and northwestern Minnesota, 2003-2006.

Species	No. collected	Sites (present/sampled)*
Lymnaea caperata	48	4/12
Lymnaea palustris	271	10/12
Lymnaea stagnalis	99	5/12
Combined total	418	10/12

*Includes 78 wetlands at 11 sites in North Dakota and 1 wetland in northwestern Minnesota (see Fig. 3).

of *F. magna* in North Dakota. Nonetheless, *F. magna* appears to be enzootic in moose in eastern North Dakota, although at a much lower prevalence than in nearby northwestern Minnesota. For example, Murray et al. (2006) reported 89% prevalence of *F. magna* in moose in northwestern Minnesota in the late 1990s, and Karns (1972) reported 87% prevalence in the same region in the 1970s.

The failure to detect *F. magna* in 2002 and 2003 may have been due to the geographic distribution of my sampling. While the majority of historical reports of *F. magna* infection originated from Unit M1C (Fig. 3), my ability to sample this area was limited. Only 10 moose tag were issued annually in this area in 2002 and 2003, compared to 150 tags issued in 1977-1984; I obtained only 4 samples from unit M1C (Table 1). Nonetheless, my recent data confirm that *F. magna* is not highly prevalent in North Dakota moose suggesting that the parasite has not experienced a marked increase in prevalence since prior surveys.

For example, based on binomial probability, I had a 95% chance of detecting *F. magna* in Unit M1C with only 4 samples if the current prevalence was at least 53%, and a 90% chance if the current prevalence was at least 44%. Also, given my sample size of 25 moose in the 4 units where historical data suggests *F. magna* occurs (M1C, M5, M6, M8), I had a 95% probability of detecting this parasite even if it occurred at a moderate prevalence (11.5%). In addition, all of the 22 samples from unknown locations were also negative for signs of *F. magna* infection. Because several of these unknown samples were received in November when only units M5 and M6 (Fig. 2) remained open for hunting, it is probable that a substantial proportion of these livers originated from the eastern part of the state, and *F. magna* likely infects a relatively small proportion of moose in eastern North Dakota. The 2002-2003 hunter returns provided a more complete sampling of the western part of moose range in the state (M4, M9, M10; n = 31), and these results suggest that prevalence of *F. magna* is low in these areas as well.

While my surveys for lymnaeid snails were by no means exhaustive, results indicate that at least 3 species of suitable intermediate hosts for *F. magna* are widespread within the primary range of moose in the state. Natural or experimental infections with *F. magna* have been reported in *L. caperata*, *L. stagnalis*, and *L. palustris* (Foreyt and Todd 1978, Lausen and Stromberg 1993, Pybus 2001).

Although white-tailed deer, the normal host for *F. magna*, are abundant in North Dakota and at least 3 species of intermediate hosts for *F. magna* appear to be widely distributed in the state, my results suggest that actual transmission of *F. magna* to moose is currently limited. This may be due to lack of available wetlands. In central and north-central North Dakota (hunting units M4, M8, M9, and M10) the range of moose lies within the larger "prairie pothole" region of the Great Plains (USFWS 1955) where wetlands are abundant but subject to seasonal dry down and long-term drought cycles (Todhunter and Rundquist 2004). Thus on a seasonal or annual basis, environmental conditions may limit the availability of intermediate hosts or aquatic vegetation, prevent embryonation and hatching of eggs, and reduce survival of metacercariae (Swales 1935, Pybus 2001). And based on my data, a large part of the primary range of *F. magna* in moose in North Dakota (Units M1C, M5, and M6) is within the northern Red River Valley that is part of the Lake Agassiz Plain Ecoregion (U.S. Environmental Protection Agency 1996; Fig. 1). This area includes a number of permanent rivers and streams associated with the Red River that are known to support lymnaeid snails (Clarke 1973, Cvancara 1983). However, compared to the prairie pothole region of central and north central North Dakota, the Red River Valley has relatively few permanent or semi-permanent

wetlands. Because riparian habitats and suitable wetlands make up a relatively small proportion of the overall landscape, they may not be capable of sustaining high levels of *F. magna* infection in cervids.

Since recent conditions in eastern North Dakota apparently support only a moderate level of *F. magna* transmission, the parasite is unlikely to represent a major source of mortality in moose. The historical data reviewed in this study were collected during a period of moose population growth, and the prevalence of *F. magna* certainly has not increased since that time. Since the completion of the current study, there has been only a single report of an *F. magna* infected moose in North Dakota, a sick adult cow moose collected in unit M6 (NDGF 2004, unpublished); this moose was also infected with *P. tenuis*. Additionally, while only 18.8% of moose necropsied as part of targeted surveillance showed signs of *F. magna* infection, 75.0% were infected with *P. tenuis* (manuscript in preparation), suggesting that other mortality factors may be more important than *F. magna*.

Although Murray et al. (2006) concluded that *F. magna* was the major source of mortality and morbidity in the declining moose population in northwestern Minnesota, it should be noted that the prevalence of *F. magna* had not increased since the pre-decline period (Karns 1972). Further, because the nearly simultaneous decline of 2 moose populations in close proximity to each other in northwestern Minnesota and in northeastern North Dakota cannot both be attributed to *F. magna*, other factors common to both areas presumably play a larger role in influencing these populations. As a result, future investigations in North Dakota and Minnesota should consider how other stressors or pathogens such as *P. tenuis* affect moose population dynamics in the region.

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