MOOSE EXPERIMENTALLY INFECTED WITH GIANT LIVER FLUKE (*FASCIOLOIDES MAGNA*)

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ABSTRACT: Moose (*Alces alces*) are abnormal, dead-end hosts of the giant liver fluke *Fascioloides magna*. The worms migrate extensively in moose causing considerable hepatic tissue damage before eventually dying. Few reach sexual maturity and eggs are seldom, if ever, passed in feces. Occurrence of the parasite in moose depends on the presence of a competent definitive host and suitable aquatic snail intermediate hosts of the genus *Lymnaea.* There is no clinical evidence that *F. magna* kills moose although the considerable tissue pathology seen in some heavily infected livers is suggestive that they do. In this study, 2 farm-reared moose calves (2 months old) and a yearling moose (15 months old) were given 50, 110, and 225 *F. magna* metacercariae, respectively, and observed for 12.5-16 months. No outward signs of disease were observed. The livers of the 2 animals infected as calves were swollen and contained bloody tracks, extensive fibrosis, and capsules; 1 and 11 immature flukes were recovered. The liver of the animal infected as a yearling had 3 large, thick-walled capsules but no flukes. Weight gain and behaviour of all were similar to those of uninfected farm-reared moose. Known aspects of the biology of this parasite and our experimental results suggest that *F. magna* is unlikely to have been a major factor in the recent moose decline in northwestern Minnesota.

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The giant liver fluke (*Fascioloides magna)* develops normally in white-tailed deer (*Odocoileus virginianus*), wapiti (*Cervus elaphus canadensis*), and caribou *(Rangifer tarandus caribou*), but in moose (*Alces alces*) migrates extensively causing considerable damage to liver tissue (Pybus 2001). Moose are an abnormal dead-end host with few worms reaching maturity, and eggs are likely prevented from leaving infected livers by the resulting fibrosis and thick-walled, closed capsules unconnected to bile ducts (Lankester 1974). Infection in moose depends on continuous cohabitation with normal cervid hosts and appropriate conditions for transmission, including persistent aquatic habitat for the required, intermediate snail hosts of the genus *Lymnaea*.

The extensive and noticeable tissue damage seen in some moose has led to speculation that infection may cause death, particularly in nutritionally stressed animals (Pybus 2001, Lankester and Samuel 2007). Based on counts of flukes and liver cysts in dead moose, Murray et al. (2006) concluded that the giant liver fluke was a significant mortality factor responsible for a marked decline in the moose population in northwestern Minnesota. However, no clinical evidence exists that links the presence of flukes in livers with death of moose. To better understand any such impact on moose, we administered metacercariae of the giant liver fluke to 3 captive, hand-reared moose, observed their behaviour and weight gain following infection, and performed subsequent necropsies.

METHODS

Moose were acquired in 1977 and 1978 as orphaned calves (0.5-2 months old) in the vicinity of Thunder Bay, Ontario where *F. magna* is unreported in deer. Two calves were infected shortly after capture; a third, obtained the previous year, was infected when 15 months old (Table 1). They were housed in a compound without access to any infected water body from the date of capture. Initially they were bottle-fed a formulated milk diet (385 mL of Carnation milk with an equal volume of whole milk and 2 egg yolks) supplemented with pelleted beet pulp, alfalfa hay, and commercial dairy ration (Nutrena Sweetflow-16; Lankester et al. 1993). Animals were weaned at 16-18 weeks of age and maintained thereafter on dairy ration and alfalfa hay supplemented sporadically with fresh browse.

Metacercariae were obtained from Baldwin Enterprises (Monmouth, Oregon, USA) and their viability on arrival was confirmed in a subsample by microscopic examination of flame cell movement. Both free and still-encapsulated metacercariae were counted and immediately administered in water by stomach tube to moose lightly anaesthetized with xylazine hydrochloride (Rompun, Haver-Lockhart Laboratories, Mississauga, Ontario, Canada).

Moose were observed daily and any clinical signs noted. They were eventually euthanized using T-61 (Hoechst Canada Inc., Montreal, Quebec, Canada), bled by cutting neck vessels, and hung to obtain whole weights; visceral organs were removed and inspected grossly. The liver was weighed, its volume measured by water displacement, and sliced at thickness of 1-1.5 cm. Migrating and encapsulated flukes were dislodged from liver tissue by gently agitating the slices in a pail of warm physiological saline, and were recovered by pouring the saline through a 2 mm mesh screen. Flukes were pressed gently between 2 glass microscope slides and examined for eggs in the uterus as an indication of maturity. They were measured to the nearest mm after formalin fixation. Protocols were approved by the Animal Care Committee of Lakehead University.

RESULTS

Both moose infected as calves showed depressed appetite for 7-10 days after infection, but thereafter resumed normal feeding and behaviour until they were euthanized 12.5-14 months later. A female yearling moose raised from a calf, but not infected, died at 14 months of age of accidental trauma and was considered a control; its liver weighed 3.3 kg and was 3,050 cc in volume.

Calf #1 was given 50 metacercariae when 2 months old and euthanized 12.5 months later; its liver weighed 4.9 kg with volume of 4600 cc and had a rounded marginal edge with a single 3 cm long immature fluke (Table 1). Seven capsules were present (4 were 3.5-4.5 cm and 3 were 1.0-1.5 cm in diameter), most in the vicinity of the hilus, with one protruding from the surface just beneath the serosa. Capsules had thickened, fibrous walls with incorporated black pigment. They were filled with brown-black, pasty material and the larger had a thin, stoney inner lining. Small, scattered patches (1-3 mm diameter) of black pigment were visible on the surface of the liver and in the omentum adjacent to the liver.

Calf #2 was given 35 and 75 metacercariae at 2 and 3 months old, and was euthanized 14 months after the first infection. Its liver appeared somewhat enlarged (6.1 kg and 5,650 cc) with rounded marginal edge. Eleven immature flukes (1.5-2.0 cm long) were recovered; 2 were in narrow (3-4 mm diameter) blood-filled tracts while the precise location of the others could not be determined. About 30-40% of the liver volume was comprised of thick-walled capsules and diffuse fibrosis. Ten capsules (3-4 cm in diameter) had thick, fibrous walls and 15 smaller capsules (1-1.5 cm) had a grey-black inner lining (not stoney) and were filled with grey-black (clay-coloured)

Table 1. Description of captive moose infected experimentally with metacercariae of the giant liver fluke (*Fascioloides magna*). All animals were captured as calves (0.5-2.0 months old; assumed born 15 May) in an area without *F. magna* and held in captivity without access to infected water until euthanasia.

Calf	Sex	Age (months) at infection	# of metacercariae	Infection duration (months)	Body weight (kg)	Liver weight (kg)	Liver volume (cc)	# flukes	# capsules
1	♂	2	50	12.5	291	4.9	4600	1	7
2	♀	2, 3	35, 75	14	218	6.1	5650	11	25
3	♂	15	225	16	334	3.8	3600	0	3

pasty material. Meandering tracts with fibrous walls (1-2 mm thick) were filled with dark red blood. The antero-dorsal surface of the liver was covered with whitish fibrinous tags and adhered firmly to the diaphragm. Diffuse accumulations of black pigment were visible on the surface of the liver, in omental fat, and in mesentery and lymph nodes around the lower colon.

Moose #3 was given 225 metacercariae when 15 months old and euthanized 16 months later. It exhibited vigorous rutting behaviour and had developed average-sized antlers in the fall of its 3rd year. There was a whitish fibrinous coating over 25% of the diaphragmatic surface of the liver, but no areas of adhesions were seen nor was black pigment visible in the mesenteries. The liver (3.8 kg, 3600 cc) had a sharp marginal edge. Three whitish capsules (1.7-6.0 cm diameter) were visible as raised areas on the surface of the liver. They had fibrous walls 4-6 mm thick and were filled with pasty to hardened grey-black matter; no flukes were recovered.

DISCUSSION

Interpreting the results of parasitic infections reproduced experimentally is often difficult. In nature, the impact of infection on individuals commonly depends on dosage, or numbers of infective forms acquired, and over what time period (Samuel et al. 1992, Lankester 2002). These natural acquisition rates usually are unknown and experimenters may be tempted to exaggerate doses to ensure infection. Seldom is it practical to administer repeated small doses or "trickle infections" over a period of time, as would more closely approximate what probably occurs in nature (Prestwood and Nettles 1977). The outcome of infection may also depend on host age at first exposure and whether the initial exposure induces a degree of protection against further infection (i.e., concomitant immunity; Lankester 2002). The varying effects of dose on the outcome of *F. magna* infection are evident from experimental infection of mule deer, a host not commonly infected in nature (Butterworth and Pybus 1993). Mule deer fawns given 250-500 metacercariae all died within 163 days of infection, whereas 3 of 4 fawns given 50 metacercariae survived; flukes matured and eggs passed in their feces (Foreyt 1992, 1996).

In our experiment, *F. magna* was relatively efficient in reaching the liver of 2-month-old moose given doses presumed moderate. Considerable liver damage resulted but no worms reached sexual maturity; the longest was 3 cm but mature flukes can be 8 cm long (Pybus 2001). The parasite had lower success in reaching the liver of the moose infected at 15 months old and all flukes were dead 16 months after infection. Having to traverse the large functioning rumen of an adult moose may, in part, explain the lower recovery. Whether the diet provided to captive, experimental animals may have altered their response to fluke infections cannot be judged.

Cattle, like moose, are dead-end hosts of *F. magna,* yet commonly become infected in enzootic areas. Infections are generally

sub-clinical and go undetected until slaughter (Wobeser et al. 1985, Pybus 2001). For example, 12 domestic calves given 1000 metacercariae each and monitored for 26 weeks were described as healthy but had conspicuous liver damage. Relatively few flukes were recovered (1-32) and weight gain of infected animals was similar to that of controls (Conboy and Stromberg 1991).

Weights of our infected calves were similar to those of calves raised at the facility in subsequent years (Lankester et al. 1993) and to those reviewed by Broadfoot et al. (1996) including weights of their 11-month-old farm-reared animals (206 kg for females and 228 kg for males); mean liver weight for both sexes was 4243 g (range = 3800-5500). Whole weights of wild, 2.5-year-old moose in Manitoba were higher (288 and 332 kg; Crichton 1979) but calves reared in captivity often weigh less than wild animals due to several factors including captive diets and digestive disorders (Addison et al. 1983, Welch et al. 1985).

In nature, calf and yearling moose are less likely to be found with giant liver fluke infection than older animals (Karns 1972, Pybus 1990, 2001). Is this because they are particularly susceptible to infection and die unnoticed, or are they somewhat refractory, either physiologically or because their feeding habits reduce the likelihood of infection? Our results suggest that calves are not unusually vulnerable to hepatic disease, but their feeding habits probably reduce exposure. Moose encounter fluke metacercariae while feeding on aquatic plants, and Lepitzki (1998) found that trematode metacercariae (presumably *F. magna*) appeared on aquatic vegetation in greatest numbers during June and in mid-August-early September in the marshes of Vermilion Lakes, Alberta. Adult moose consume submerged and floating aquatic plants in greatest amount from mid-June to mid-July (Cobus 1972, Fraser et al. 1982), but calves rarely forage in a similar manner at such a young age. The rumen fluke *Paramphistomum* spp. (although non-pathogenic in moose) is acquired similarly by moose ingesting metacercariae encysted on aquatic vegetation; moose <1.4 years old had fewer rumen flukes than older animals and calves <2.5 months had none in a study in Sibley Provincial Park, Ontario (Snider and Lankester 1986).

Poor recruitment is a feature of moose declines occurring in the past 15-20 years in areas west of Lake Superior (i.e., northwestern Ontario, southeastern Manitoba, and northwestern Minnesota; Lankester 2009). However, our results indicate that moderate doses of *F. magna* do not kill young moose and suggest that poor recruitment of young is unlikely explained by undetected calf mortality due to *F. magna*. An inference (Murray et al. 2006) that liver flukes elicit high moose calf mortality has been attributed to Karns (1972), but we could not confirm this interpretation. On the contrary, Karns (1972) reported that the net productivity of moose was greater in northwestern Minnesota where the prevalence of *F. magna* was 87%, than in the northeast where only 17% of moose were infected; this difference in productivity would seem related to factors other than liver fluke. Recently, Maskey (2008, 2011) concluded that a low and apparently declining prevalence of *F. magna* (<20%) was probably not the cause of a moose decline in North Dakota occurring simultaneously with a decline in adjacent northwestern Minnesota (Murray et al. 2006).

It is noted that the number of flukes (intensity of infection) in some moose in northwestern Minnesota was high (Murray et al. 2006). Moose classified as likely to have died of fluke infection were defined as those with "signs of severe pathological damage to tissue and organs and no other overt cause of death" or those where "liver flukes were abundant." Not surprisingly, this group had more flukes (48.1 ± 9.6, $n = 23$) than animals considered dying of non-fluke related causes (14.2 ± 2.2, $n = 69$). However, the high prevalence of infection (89%, $n = 100$) was similar to that

(87%, n = 128) found >30 years earlier when the population was robust and moose hunting was reinstated (albeit intensity data were not reported, Karns 1972). Lankester (1972) examined a much smaller sample of moose from neighbouring southeastern Manitoba and found 64% of livers with signs of fluke infection; 1-5 immature flukes were recovered from 3 of 7 animals with liver damage. Butterworth and Pybus (1993) found flukes (16.7 ± 7.3, range = 5-30) in 52% of moose (n = 22) in Banff National Park in Alberta, and 2.7 ± 0.3 flukes (range = 2-3) in 63% of moose (n = 9) in Kootenay National Park in British Columbia. Pybus (1990) found 4% of adult moose (n = 191) with 3 ± 1 (range = 2-5) flukes in the foothills region of Alberta, and Shury (1995) found flukes (x = 8) in 40% of moose >6 years old (n = 10) from Banff National Park.

There are several other reasons why *F. magna* is unlikely a major factor in moose declines. This parasite has a disjunct distribution across North America and has never occurred in some areas where moose declines are known (Lankester 2009). As well, the prevalence of giant liver fluke infection increases with age of the host and reaches a plateau in older animals (Lankester and Luttich 1988, Pybus 2001). But the mean intensity of infection is similar within infected age classes, suggesting the development of an immunological resistance as infection accumulates (Pybus 2001). Results reported here, and the observations of Pybus (1990) suggest that moose react strongly to worms in the liver and liver hypertrophy accompanying infection may eventually mitigate some of the hepatic tissue damage. Lastly, liver fluke infections have an aggregated distribution in definitive host populations. Most individuals have relatively low-moderate numbers of worms while a few heavily infected animals carry the majority of the parasite population (Addison et al. 1988, Lankester and Luttich 1988, Mulvey and Aho 1993). Thus, even if the heaviest fluke infections can be shown to cause the death of moose, the greatest impact of the disease would be expected in only a relatively small portion of the population.

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