RUMEN FLUKES *(Paramphistomum spp.)* IN MOOSE OF NORTHWESTERN ONTARIO

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Abstract: Rumen flukes *(Paramphistomum spp.)* were found in 86% of 160 moose examined from northwestern Ontario throughout the year. The number of flukes per moose ranged from 16 to 28,262 (median = 1,135). The intensity of infection did not vary with age with the exception of calves (0.5-1.4 years) which had fewer flukes. Adult rumen flukes were found in dense aggregations in the rumenus atrium where they caused loss of papillae at the site of attachment. Small, newly acquired flukes were first seen in the rumen of calves and older moose in October. No intestinal lesions due to migration of flukes were seen.

Few gravid flukes were found in moose during the winter. The proportion of worms with eggs increased in March and April and 100% were gravid from May to July. Thereafter, the proportion gravid declined, reaching 0.5% in November. Changes in the numbers of eggs in the feces of moose followed the same seasonal pattern as the proportion of gravid worms.

The seasonal maturation of rumen flukes is considered an adaptation that synchronizes the production of eggs with their access to water. Paramphistome eggs were killed by freezing and desiccation. Eggs held at 11°C did not develop. When the temperature was raised to 19°C, eggs began to hatch after 26 days.

Rumen flukes of the genus *Paramphistomum* Fischoeder, 1901 have been reported from moose *(Alces alces)* throughout much of North America (Anderson and Lankester 1974, Lankester *et al.* (1979). Specimens from this host, generally, have been referred to *P. cervi* (Zeder 1790) and those from white-tailed deer *(Odocoileus virginianus)* to *P. liorchis* Fischoeder, 1901 (Prestwood *et al.* 1970). This practice implies strict host specificity but the presence of both species in other wild and domestic ruminants (Price and McIntosh 1944, Sey 1980) suggested otherwise. Recently, a careful examination of specimens from moose in northwestern Ontario demonstrated that both *P. cervi* and *P. liorchis* occur, often concurrently, in this northern cervid (Kennedy *et al.* 1985). Because infections were mixed, it was not feasible to re-analyze the data on a species basis. Preliminary examination of the data suggested that the developmental stages of both *P. cervi* and *P. liorchis* were similar in moose. Consequently, for the purpose of the work reported here, the rumen flukes are referred to as *Paramphistomum spp.*

A preliminary study by Lankester *et al.* (1979) revealed that most rumen flukes from moose in winter (November-February) were small and contained no eggs. Those from moose during summer (July and August) were larger and all were gravid. Calves became infected in their first summer of life. This evidence suggested that rumen flukes were picked up by moose during summer, possibly while feeding on aquatic vegetation. Worms did not become gravid until the following spring. Because this prepatent period (6-7 months) is much longer than that reported for paramphistomes in other ruminants (56-130 days) (Kraneburg and Boch 1978, Horak 1971, Sey 1982) it was suggested by Lankester *et al.* (1979) that the maturation of the worms in moose may be delayed until some environmental cue, such as change in diet, signalled

favorable spring conditions. It was also hypothesized that rumen flukes acquired over summer by moose probably died in September and October the following year.

The purpose of this study was to confirm observations made by Lankester *et al.* (1979) and to collect additional data which would test their hypotheses regarding the life cycle of rumen flukes in moose. Information from 60 moose collected between 1976 and 1979 and reported by Lankester *et al.* (1979) are included in this study. Another purpose of this study was to assess the pathogenicity of rumen flukes in moose.

METHODS AND MATERIALS

The rumens from 160 moose were examined for flukes during the period 1976-1981. Most samples were obtained from moose killed by hunters or by vehicles. All moose came from an area of Ontario along the north shore of Lake Superior extending west to Turtle Lake (48°50'N., 92°40'W.), east to Lake Superior Provincial Park and north to Stevens (49°32'N., 85°49'W.). The sex of each animal was recorded. Age was determined by tooth eruption patterns (Passmore *et al.* 1955) or cementum annuli counts (Sergeant and Pimlott 1959).

Rumens were opened mid-dorsally and the inner surface and contents inspected for flukes. Rumen flukes were stored in 10% formalin or in a 10% solution of glycerin in 70% alcohol. In 1980 and 1981, the lining and contents of the omasum and abomasum were also searched for flukes. When present, the proximal 20 cm of the duodenum was opened and searched for immature flukes and lesions. The tissue was either pressed between glass plates and held to the light or inspected directly.

Estimates were made of the total number of flukes present in the rumens of 60 moose in 1980 and 1981. The volume of the contents of the rumen and reticulum were measured in a graduated polyethylene container. The contents were then placed in a 200-L drum. Water was added to achieve a total volume 3 times that of the original rumen plus reticulum contents to facilitate thorough mixing. The material was stirred with a paddle and while stirring, 4, 1-L samples were taken by dipping a 1-L beaker. Each sample was washed in a sieve and stored in 70% alcohol. The subsamples of rumen material were later examined for flukes. The material was spread on gridded white enamel trays, immersed in water and carefully searched. Flukes recovered were counted and stored in 70% alcohol. Chi-square tests on the number of flukes in each of the 4, 1-L samples indicated that the samples were drawn randomly.

An estimate of the total number of flukes was determined by the product of the number of rumen flukes counted in the subsamples times the volume sampling rate. Rumen flukes collected off the wall of the rumen prior to subsampling were added. To determine the ratio of gravid to non-gravid flukes, up to 70 worms were arbitrarily selected from 160 counted rumen subsamples. Samples of less than 10 flukes were not used in analysis. Flukes were dehydrated and cleared in graded alcohol and xylene series respectively and examined with a Bausch and Lomb dissecting microscope at 20 X magnification. If a fluke had one or more eggs in its uterus it was considered gravid.

Fecal material deposited by free-ranging moose and fecal material from vehicle-killed and hunter-killed moose was examined for rumen fluke eggs using a sedimentation technique. Feces were examined while fresh or after

being frozen and thawed. Twenty grams of feces were macerated in 0.5 L of water and poured through a No. 80 Endicott sieve to remove larger particles. More water was added to the sieve and the material stirred until a 2-L graduated cylinder was filled. The paramphistome eggs, which easily passed through the sieve, were allowed to settle to the bottom of the cylinder. A tube with an opening of 1 mm diameter at its end was lowered to the bottom of the cylinder. Water flowed through the tubing from an elevated header tank that could be adjusted in height to provide a flow of about 100 ml per minute. Light material floated up and out of the cylinder but the heavier eggs did not. Each sample was gently washed in this way for 4 to 8 hours before allowing eggs to settle and decanting the cylinder to a volume of 200 ml. The 200 ml sample was agitated and 3, 2 ml subsamples were removed with a pipette and examined in a gridded syracuse glass using a dissecting microscope at 20 X magnification. The number of eggs per gram of feces was calculated from the mean egg counts of these 3 subsamples.

The accuracy of the sedimentation technique was assessed by dividing a single fecal sample into 9, 20 g subsamples and 3, 1 g subsamples. The 9, 20 g subsamples were put through the sedimentation process. The estimated mean of the 9 subsamples was 372 eggs/g with a range of 232 to 470 and a standard deviation of 76.2 eggs/g. The 3, 1 g subsamples were each mixed in enough water to make it possible to count the eggs on a gridded syracuse glass. The counts obtained were 429, 519 and 402 eggs. Differences between the 3 direct counts and the 9 sedimentation estimates was not significant ($t = 1.59$, $df = 10$, P 0.10).

The effect of temperature on development of fluke eggs was investigated using eggs from an experimentally infected moose. Eggs were divided into 9

finger bowls (15 cm diameter) with each bowl containing several hundred eggs. Three finger bowls were kept at each of three temperatures (11°C, 19°C and 27°C) under a light regime of 12 hours of light and 12 hours of dark. A sample of 20 to 30 eggs in each was examined periodically with a dissecting microscope and the stage of development and the proportion hatched were noted. Examination was completed quickly and the bowls returned to their respective locations to minimize temperature changes. One of the 3 finger bowls held at 11°C was transferred to 19°C on the 57th day of the experiment to test their viability. To examine egg viability after an extended period of time, a large sample of paramphistome eggs from an experimentally infected moose was kept in the dark at 11°C from August 18, 1981 to May 19, 1982. Thereafter the sample was kept at room temperature (18°C) on a natural light regime. Eggs were examined periodically for development and hatching.

To study the effect of freezing, eggs were removed from an experimentally infected moose and were divided into 6 equal samples in distilled water. The control was placed in a finger bowl on the laboratory bench at approximately 22°C. Five samples were placed in plastic bottles and frozen at -4°C to -5°C for 24 hours, 2, 4, 5 and 8 days, thawed at room temperature and placed in finger bowls at control conditions. Samples returned to room temperature were checked regularly for miracidial development and hatching.

To test the resistance of rumen fluke eggs to desiccation, a fecal sample from an experimentally infected moose was divided into 4, 20 g subsamples. Eggs were removed from the control subsample and placed in water at approximately 22°C. The other 3 fecal subsamples were placed in open Petri dishes in a growth chamber at 19°C. After varying periods of

desiccation, fluke eggs were removed using the sedimentation technique, placed in water at 22°C and checked at regular intervals for egg development and hatching. All statistical analyses followed Zar (1974) and the minimum level of significance chosen at $P \leq 0.05$.

RESULTS

Prevalence and intensity of rumen flukes in moose

The rumens of 160 moose were examined from 1976 to 1981; 137 (86%) contained rumen flukes. Of 140 aged animals none of 8 newborns (< 2.5 months) was infected (Table 1). Seventy-two percent of 36 calves (defined herein as animals 0.5-1.4 years) and 86% of 37 yearlings (1.5-2.4 years) and all moose older than 2.4 years (n = 59) were infected. There was no significant difference in the prevalence of infection between calves and yearlings ($\chi^2 = 2.27$, $P > 0.10$), but yearlings had a significantly lower prevalence of infection than adult moose ($\chi^2 = 8.60$, $P < 0.005$). The prevalence of infection in males and females was not significantly different ($\chi^2 = 0.11$, $P > 0.50$).

The number of rumen flukes was estimated in 56 infected wild moose older than 0.4 years. Infected moose had a mean of 3,435 ± 5,650 (1 S.D.). The number of flukes was not normally distributed about the mean but showed a negative binomial distribution as shown in Fig. 1. A large proportion of the moose had few flukes while 2 individuals had over 20,000 each. The strong departure from a normal distribution made the use of parametric statistics invalid. Non-parametric tests were used instead. The median number of flukes was 1,135 (16 - 28,262; n = 56) (range followed by sample size).

TABLE 1. Prevalence of rumen flukes *(Paramphistomum spp.)* in moose of northwestern Ontario

	Age of moose (yrs.)[1]				
	< 2.5 mths.	0.5-1.4 yrs.	1.5-2.4 yrs.	≥ 2.5 yrs.	Total no.
Males	0%(3)[2]	84%(19)	81%(21)	100%(25)	68
Females	0%(3)	56%(16)	93%(15)	100%(34)	68
Males and females combined[3]	0%(8)	72%(36)	86%(37)	100%(59)	140

[1] The birth date of all moose is assumed to be June 1st. Animals 2.5 months are referred to as newborns, age 0.5-1.4 yrs. as calves and age 1.5-2.4 yrs. as yearlings.

[2] Percent of the moose infected followed in brackets by the number examined.

[3] The sex was not recorded for 4 moose which are included in this category. In addition, several moose not aged are omitted from this table.

A Kruskal-Wallis test indicated significant differences in rumen fluke numbers (Table 2) among moose of various ages. Calves had significantly fewer flukes than yearlings ($U = 173$, $P < 0.002$) and all older moose ($Z = 3.51$, $P < 0.001$) (Table 2) (Larger samples excluded use of the Mann-Whitney test. The normal approximation of the test justified calculation of the Z value). Older moose (4.5+) had significantly fewer flukes ($U = 44$, $P = 0.002$) than moose aged 3.5-4.4 years (Table 2).

There were no differences in the numbers of flukes between sexes for calf moose ($U = 21$, $P > 0.20$) or for moose older than calves ($U = 155$, $P > 0.20$). The effect of time of year on fluke numbers was examined by dividing the year into 3 seasons from January 1st to April 30th, May 1st to September

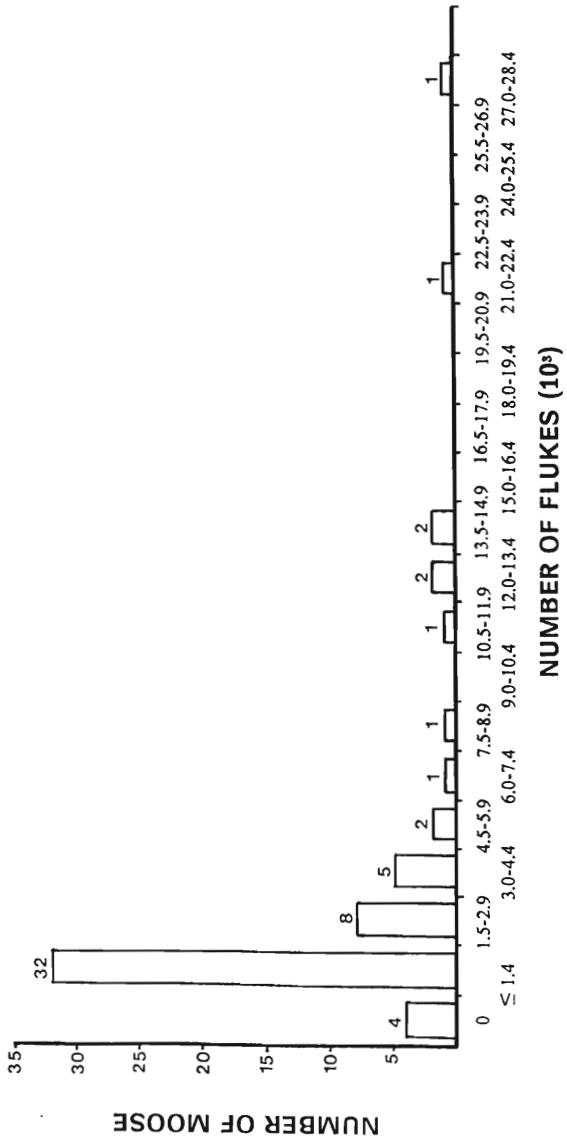

Figure 1. Dispersed distribution of rumen flukes from moose. Numerals represent sample size.

30th, and October 1st to December 31st. There were no differences in the numbers of flukes between seasons for calf moose or for moose older than calves (Kruskal-Wallis test).

TABLE 2. Estimates of intensity of rumen flukes *(Paramphistomum spp.)* in moose of northwestern Ontario

Age of moose	No. examined[1]	No. infected	Median no. of rumen flukes	Range in no. of rumen flukes
0.5-1.4	15	14	202	16-2,297
1.5-2.4	17	15	2,127	166-13,553
2.5-3.4	9	8	2,881	262-14,987
3.5-4.4	5	5	4,102	480-21,227
4.5+	9	9	812	73-28,262

[1] Five moose that were not aged are omitted from this table.

Rumen flukes were found only in the rumenus atrium portion of the rumen (Fig. 2). They were aggregated and attached to the lining by drawing the base of a rumen papilla into the acetabulum. The distal two-thirds of the rumen papilla was gone, leaving the basal stub to which worms were attached. The surrounding mucosa was normal in colour. Attached flukes were only found associated with damaged papillae in patches up to 5 x 12 cm. Patches of damaged papillae were found with no attached flukes. Presumably flukes had released themselves from these locations some time after the death of the moose since freshly killed moose still had the rumen flukes attached to the rumen wall. In heavy infections an estimated 10% of the papillae in the rumenus atrium were damaged. No lesions attributable to immature papamphistomes were found in the duodenum of 10 moose killed in July and

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August or in 33 moose killed at other times of the year.

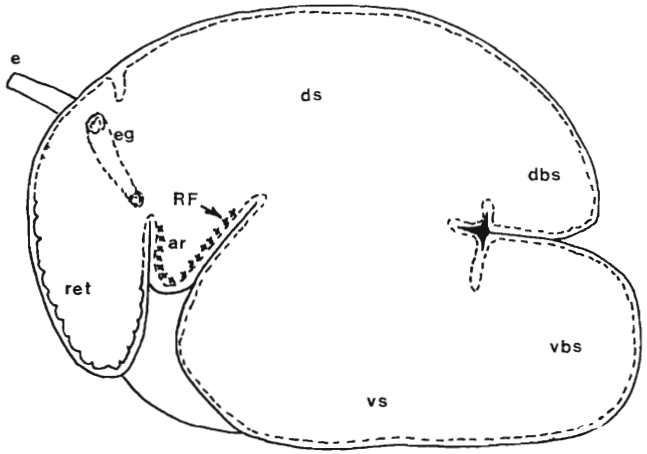

Figure 2. Location of rumen flukes shown in left lateral view of a moose rumen. RF = rumen flukes, ret = reticulum, ra = rumenus atrium, e = esophagus, eg = esophageal groove, ds = dorsal sac, dbs = dorsal blind sac, vs = ventral sac, vbs = ventral blind sac.

Seasonal maturation of rumen flukes

Few paramphistome eggs were found in feces of moose from November to February (Fig. 3). The numbers of eggs began to increase in March, reaching a mean of over 100 eggs/g in July. By September and October few eggs could be found. The proportion of gravid flukes from moose rumens rose from 8% in March to 47% in April (Fig. 4). Almost 100% were gravid from May to July. Thereafter, the proportion of gravid flukes declined, reaching 0.5% gravid in November.

Migration of rumen flukes in moose

No migrating flukes were seen in the duodenum, abomasum or omasum of the 3 calves examined on June 4th, June 27th and August 1st. Four of 8

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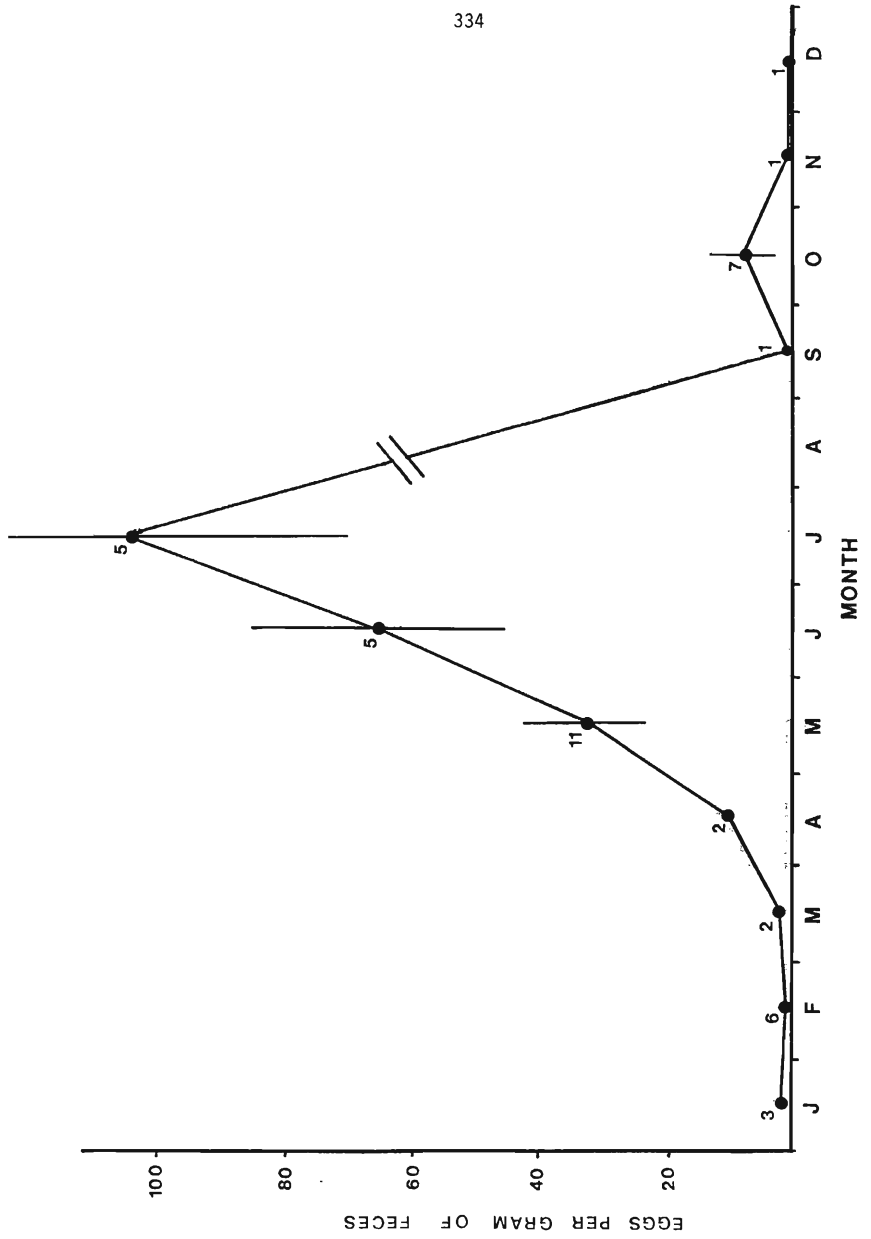

Figure 3. Seasonal variation in mean number (± 1 S.E.) of paramphistome eggs in feces of adult moose. Numerals represent sample size.

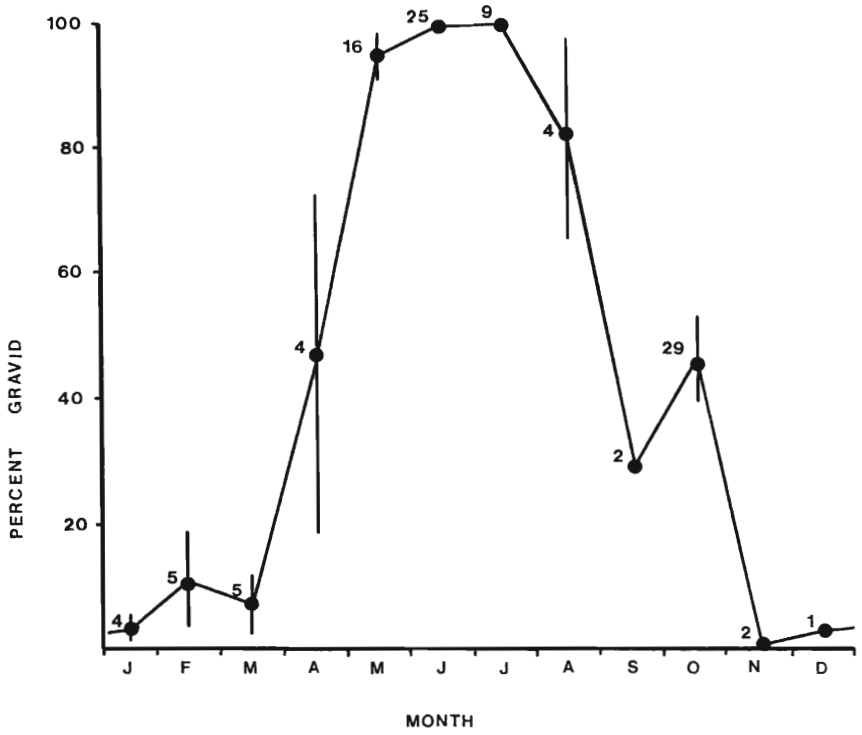

Figure 4. Seasonal variation in mean proportion of gravid rumen flukes (±1 S.E.) taken from moose. Numerals represent sample size.

calves collected in October had flukes in the rumen. These specimens were 2.5 to 3.5 mm in length. The duodenum, abomasum and omasum were examined in 4 of these October calves and 2 small migrating paramphistomes, less than 3.5 mm in length, were found in the omasum of one.

No migrating flukes were found in the duodenum, abomasum or omasum of older moose during summer. A 9.5-year-old female collected October 18th had 20 small, thin, pink paramphistomes in its duodenum from 1.5 to 3.5 mm long, and 2 in its omasum. Small, thin flukes (< 3.5 mm long) resembling those found in the omasum and duodenum were found in the rumen of some moose. They were first seen in the rumen of 19 of 28 moose collected in October and were found in 2 calves as late in the year as April. The small, thin flukes were absent in all moose examined May through September.

Factors affecting the development and viability of rumen fluke eggs

Fluke eggs kept at 27°C began to hatch after 10 days; by 19 days 80% had hatched (Fig. 5). Eggs kept at 19°C began to hatch after 26 days and by 49 days 80% had hatched. No eggs kept at 11°C had hatched after 57 days nor could any development within the eggs be detected at this time. All eggs held at 11°C for 57 days hatched within 21 days when placed at 22°C. A second sample of eggs in water and total darkness at 11°C had not hatched nor developed when checked after 274 days. When placed at 20°C, 87% of these eggs had hatched within 43 days.

Fecal material from an experimentally infected moose was divided into 20 g subsamples and placed in open Petri dishes at 19°C to test the resistance of fluke eggs to desiccation. Only 3% of the eggs subjected to 8 days of desiccation hatched, while no eggs hatched after 13 and 21 days of

desiccation. Eighty-three percent of the eggs in the undesiccated control sample hatched.

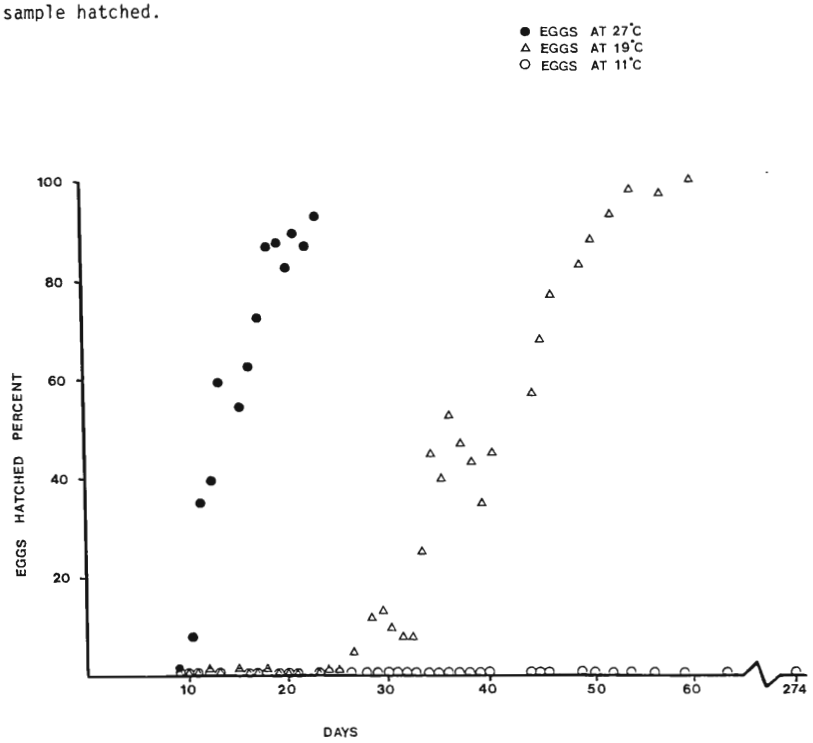

Figure 5. Effect of temperature on hatching of rumen fluke eggs.

To test their resistance to freezing, fluke eggs were removed from feces of an experimentally infected moose, placed in water and frozen at -4°C to -5°C for varying lengths of time up to 8 days. Ninety-three percent of eggs in the unfrozen control sample hatched. Only 10% and 3% hatched in samples frozen for 24 hours and 8 days respectively.

DISCUSSION

In this study 86% of 160 moose had rumen flukes, which is higher than previously reported for moose. Olsen and Fenstermacher (1942) found 30% (n = 30) of moose in Minnesota infected, Threlfall (1967) reported a 5% (n = 109) prevalence of infection in Newfoundland moose, Peterson (1955) reported a 12% (n = 25) prevalence of infection in moose examined from northwestern Ontario, and Samuel *et al.* (1976) reported a prevalence of infection of 2% (n = 47) in moose examined from Alberta. The localized distribution of flukes in the rumenus atrium makes them difficult to detect and may explain the lower prevalence reported in some areas.

In results reported here the youngest infected animals were calves killed in October, however, no calves were examined from the latter part of August or September. Seventy-two percent of calves examined in October or later were infected, 86% of yearlings and 100% of adult moose were infected. The high prevalence of infection in moose of all ages indicates that most moose feed in aquatic areas at a time suitable for transmission of this parasite and that by their third summer, all moose have ingested metacercaria.

Counts of the number of flukes in the rumen of 56 infected moose ranged from 16 to 28,262. The number of rumen flukes are reported only from moose of the USSR where one heavily infected moose had 40,000 *P. cervi* (Aleksandrova 1962). The number of flukes in each rumen was overdispersed and resembled a negative binomial distribution as has been described for many parasites (Anderson 1974). In this type of distribution most host individuals contain only a few parasites while a large proportion of the parasite's suprapopulation (in sense of Kennedy 1977) is concentrated in a

few hosts. These few heavily infected hosts are of particular value to the parasite suprapopulation because they presumably contain a large portion of the species' reproductive capacity. In this study the 6 most heavily infected moose contained 54% of all flukes present in a sample of 56 infected moose. This overdispersion could result if some lakes contained more infected snail intermediate hosts than others.

There were no seasonal differences in numbers of flukes in the rumen of moose. Apparently, infection occurs in summer, probably while moose feed on aquatic vegetation. By October, small, thin flukes have migrated from the duodenum into the rumen. Most continue their development to adult size but some remain small and can still be found in moose collected in late winter (April).

Paramphistomiasis in domestic ruminants is characterized by outbreaks of acute gastroenteritis with high morbidity and mortality, particularly in young animals (Horak 1971). Such consequences were not observed in wild moose during this study despite the high prevalence of infection in the population. The loss of rumen papillae at the site of attachment by paramphistomes has been reported previously in moose of the USSR (Aleksandrova 1962) and for roe and red deer in Europe (Graubmann *et al.* 1978)

The maturity of rumen flukes in moose varies seasonally. Nearly all are non-gravid from November through March and nearly all have eggs from May through August. The numbers of paramphistome eggs in the feces of moose changed correspondingly throughout the year. These results substantiate the initial findings of Lankester *et al.* (1979). Several studies found no seasonal variation in paramphistome egg output (Horak 1967, Bouvry and Rau

1983, and Karubaev and Amangaliev 1964). In contrast, Rodonaya (1960) found that *P. skryabini* infecting cattle in the USSR had low fecal counts in November and peak egg counts in June. In India, Gupta *et al.* (1984) found that *P. cervi* infecting sheep were gravid from April to August and immature from September to March. The peak egg production was during the monsoon season of July and August, coinciding with the availability of the intermediate snail host, *Indoplanorbis exustus*.

Seasonal changes in the diet of moose initially were thought to be linked with the observed cyclic maturation pattern seen in rumen flukes by Lankester *et al.* (1979). Moose have a major shift in diet from leafy vegetation in summer to a diet of woody twigs after leaf-fall in the autumn and back to leafy plants after leaf-out in the spring (Stewart *et al.* 1977). However, leaf-fall generally occurs between October 7th and October 18th (n = 10) in Northwestern Ontario (unpublished data). This occurs after fecal egg counts and the proportion of worms gravid have started to decline. Similarly, average leaf-out is May 21st (n = 13) (unpublished data) when eggs are already present in moose feces and nearly 100% of the flukes are gravid. Environmental and/or physiological rhythms rather than changes in diet may be responsible for seasonal change in egg production.

Rumen fluke eggs were killed by relatively moderate freezing or desiccation. Eggs developed and hatched more slowly at low temperatures. When water temperature was reduced to 11°C no development occurred but eggs remained viable. These hatching requirements impose stringent ecological limits on the parasite. Egg production in winter results in freezing and destruction of eggs. Egg production in late summer and fall during a period of lower lake temperatures results in lengthened or arrested development.

Conditions are optimum for development from May through August when egg production is highest.

Aquatic feeding by moose is most prevalent from mid-June through July (Fraser *et al.* 1982, and Cobus 1972). Factors affecting the development of paramphistomes in aquatic snails and subsequent transmission to moose feeding on aquatic plants will be the subject of a future publication.

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