J. Parasitol., 79(6), 1993, p. 922–929

EFFECTS OF SOURCE OF METACERCARIAE ON EXPERIMENTAL INFECTION OF *ZYGOCOTYLE LUNATA* (DIGENEA: PARAMPHISTOMIDAE) IN CD-1 MICE

Allen W. Shostak, Shem Dharampaul, and Miodrag Belosevic
Department of Zoology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9

ABSTRACT: Outbred (CD-1 strain) mice were infected experimentally with metacercariae of *Zygocotyle lunata* obtained from naturally infected *Helisoma trivolvis*. Growth, survival, and fecundity of the parasite and gross pathology in the host were evaluated. Metacercariae from 5 snail sources produced 3 patterns of growth and survival: significantly smaller worms that survived less than 4 wk and did not mature, significantly larger worms that matured but survived less than 6 wk, and worms of intermediate size that matured and lived at least 19 wk. Parasite maturation (at 3–4 wk) and egg production (14 eggs·worm^{-1}·hr^{-1}) were similar among infections from different sources. Ceca of mice with infections at least 4 wk old were enlarged with localized mucosal alteration at the site of parasite attachment, but there was no evidence of splenomegaly. The differences in infection due to the source of metacercariae within 1 strain of final host were similar to differences reported among other species of final host. These results suggest that variation associated with the intermediate host may confound studies of specificity to the definitive host.

Zygocotyle lunata (Diesing, 1836) occurs naturally in waterfowl and ungulates associated with aquatic habitats (McDonald, 1969), and it has been studied experimentally in a variety of avian and mammalian hosts (Willey, 1941; Huffman et al., 1991; Sey, 1991). During the summer of 1990, attempts to identify cercariae shed from a naturally infected snail led us to infect CD-1 mice, a strain we use for routine maintenance of various species of parasites. Adult flukes were recovered and identified as *Z. lunata*. The parasites were unusually large and were expelled more rapidly in comparison to experimental infections in other species of host (Willey, 1941; Fried, 1970; Fried and Nelson, 1978; Huffman et al., 1991) and to other strains of mice (Huffman et al., 1991; Etges, 1992). Accordingly, a larger sample of naturally infected snails was collected, and further experimental infections were done to characterize CD-1 mice as a laboratory host for *Z. lunata*. The present study reports on the effects of metacercariae originating from different snails on the course of infection in CD-1 mice.

MATERIALS AND METHODS

Animals

Five naturally infected snails, *Helisoma trivolvis* Say (Pulmonata: Planorbidae), were the sources of metacercariae. One snail (source A) was collected on 2 June 1990 from Lyle Lake, Alberta (55°12′N, 112°29′W); the other 4 snails (sources B–E) were collected on 28 July 1992 from a beaver impoundment (56°31′N, 111°19′W) near Fort McMurray, Alberta. Snails were maintained individually in 50-ml plastic vials, in dechlorinated water at 20–22 C, and fed daily with flaked fish food. Metacercariae encysted on the vial were removed daily and stored in half-strength Locke's solution at 4 C (Fried and Wilson, 1981).

Outbred (CD-1 strain) mice (Charles River Canada, St. Constant, Quebec), were 6–8 wk old when infected by gavage with 2–4-wk-old metacercariae, pretreated with 3% $NaHCO_3$ (Fried, 1970). Mice were provided food (Wayne Rodent BLOX™, Harlan Sprague Dawley, Madison, Wisconsin) and water ad libitum.

Experimental design

Experiment 1 was started in July 1990 and was done primarily to recover and identify adult parasites. Source A metacercariae were given to 29 female mice. Mice given 10 metacercariae were killed 10 days (n = 1), 20 days (n = 1), 35 days (n = 1), or 46 days (n = 2) postinfection (PI); mice given 15 metacercariae were killed 5 days (n = 1), 17 days (n = 1), or 63 days (n = 8) PI; mice given 20 metacercariae were killed 10 days (n = 1), 20 days (n = 1), 35 days (n = 1), or 49–63 days (n = 6) PI; mice given 30 metacercariae were killed at 10 days (n = 1), 20 days (n = 1), 35 days (n = 1), or 55–63 days (n = 2) PI.

Experiment 2 was started in September 1992. It evaluated establishment, growth, and early egg production for source B–E worms. Twenty female and 20 male mice were each given 20 metacercariae from 1 of the 4 sources (5 female and 5 male mice per source). One female and 1 male per source were killed weekly for 5 wk.

Experiment 3 was started in October 1992. It evaluated long-term survival and egg production of source B and C worms, which were the only 2 from experiment 2 that lived to maturity. Six males received 20 metacercariae each from source B, and 2 males and 5 females received 20 metacercariae each from source C. Two males (source B worms) and 3 males (source C worms) were killed 60 days PI, and 3 males (source B worms) and 5 females (source C worms) were killed 133 days PI.

Received 19 May 1992; revised 3 August 1993; accepted 6 August 1993.

Feces were collected from groups of 1–5 mice placed in a wire-bottom cage, usually for a 3–4-hr period commencing 0800 hr. Water on the cage bottom prevented drying of feces. Eggs were recovered by repeated sedimentation in dechlorinated water. Egg numbers were estimated from counts in replicate subsamples and standardized to a rate per hour. Samples of eggs were maintained at 20–24 C to assess viability.

Processing of samples

Mice were killed by cervical dislocation. Fresh body and spleen weights were taken within 15 min of host death in experiments 2 and 3. The intestine and cecum were removed and examined immediately. The lumen was exposed and rinsed with phosphate-buffered saline, and worms were counted.

The fixation protocol for experiment 1 was designed to render noncurled specimens for identification. Worms were transferred to dechlorinated water, refrigerated (4 C) overnight, and fixed under coverglass pressure in 70 C alcohol–formalin–acetic acid (AFA) (method 1). The worms were identified as *Z. lunata*. They were substantially larger than measurements in several published studies. However, those studies used the method of Fried (1970), in which worms were pipetted directly into hot AFA without coverslip pressure (method 2). This may have caused the differences in worm size. The 2 fixation methods were incorporated into experiments 2 and 3 to allow comparison with results of experiment 1 and with published studies. Up to 5 worms from heavily infected hosts were selected at random and fixed by method 1. The remaining worms from those hosts, and all worms from the lightly infected hosts, were fixed using method 2. Worms were stained with Semichon's acetocarmine, cleared in xylene, and mounted in synthetic resin.

Drawings of mounted worms were made with the aid of a drawing tube, and morphometric measurements (lengths, widths, and measured areas of the outlines of structures) were made using a digitizing tablet (SummaSketch Plus™, Summagraphics Corp., Fairfield, Connecticut) and software (SigmaScan™, Jandel Scientific, Corte Medera, California). Maximum body length and width, and relative area (length × width) were used for comparison with other studies. The length, width, measured area, proportional area (as % measured body area), and position (as % of body length to the anterior margin) of various structures were recorded to evaluate effects of fixation on morphometry.

Representative specimens are deposited in the Canadian Museum of Nature Parasite Collection, Ottawa (catalogue numbers CMNP 1992-0035–1992-0037) and the University of Alberta Parasite Collection (UAPC) (catalogue numbers UAPC 11413–11463). Adult *Z. lunata* in the UAPC, from naturally infected hosts, were measured for comparative purposes. The specimens were from moose *Alces alces* (UAPC 9686, n = 3), snow goose *Chen hyperborea* (UAPC 106 and UAPC 107, n = 2), and coot *Mareca americana* (UAPC 101 and UAPC 7986, n = 3).

Data analysis

Data from the 3 experiments were pooled for graphical presentations. Statistical analysis used SYSTAT 5 software (SYSTAT, Inc., Evanston, Illinois). Data from different experiments were analyzed separately unless stated otherwise. Continuous data were analyzed by analysis of variance (ANOVA) or Kruskal–Wallis tests. Multiple pairwise comparisons were done using the Tukey–Kramer method. Categorical data were evaluated using G-tests. Statistical significance was determined using $\alpha = 0.05$.

RESULTS

Recovery of parasites

Recovery of parasites declined over time (Fig. 1) but at different rates from different parasite sources. Source A worms had intermediate survival. They were recovered from 10 of 11 mice examined 5–35 days PI and 0 of 18 mice examined 46–63 days PI. Source B and C worms were long lived. They were recovered from all 25 mice examined 7–35 days PI (experiment 2) and 60 days PI (experiment 3). At 133 days PI (experiment 3), infections were present in all 3 mice infected with source B worms and 3 of 5 mice infected with source C worms. Infections with source D and E worms were lost rapidly. Source D worms were recovered from 5 of 6 mice examined 7–21 days PI, but 0 of 4 mice examined 28–35 days PI. Source E worms were recovered from 2 of 2 mice examined 7 days PI, but 0 of 8 mice examined 14–35 days PI.

Worms were found in the cecum or in the upper colon. More worms were in the cecum in the 60–133-day-old infections of experiment 3 (86%) than in the 7–35-day-old infections of experiment 2 (53%) ($P < 0.001$). Within experiments, the proportion of worms in the cecum was similar among ages of infection (experiment 2, $P = 0.073$; experiment 3, $P = 0.888$).

The parasite caused damage to the host that increased with the age of the infection, but the damage tended to remain localized. Up to 21 days PI, worms were loosely attached and no damage to the host was observed. The cecum was small and its contents pasty in consistency, typical of uninfected mice. From 28 days PI onward, the cecum of exposed, uninfected mice was normal in appearance, but the cecum of infected mice was enlarged and the contents were more fluid. In addition, the mucosa was raised at the attachment site of the acetabulum, and the vasculature was prominent at about ⅓ of those sites. Qualitative observations in 1990 suggested splenomegaly in infected mice. Quantitative observations in 1992, on spleen weights (as % body weight), were evaluated by multiple regression: spleen weight in males did not vary with intensity

($P = 0.455$) or age ($P = 0.384$). Spleen weight in females increased with time ($P = 0.011$) but not with intensity ($P = 0.291$).

Effects of fixation

Fixation effects were evaluated by comparing the mean measurement of specimens from the same host, fixed by methods 1 and 2. The worms were 14–60 days old and came from sources B and C. The 10 mice for which this was done provided 10 paired observations. A ratio was formed from each pair: the mean of specimens fixed using method 1 divided by the mean of specimens fixed using method 2. Confidence limits (CL) were calculated from the 10 ratios. If the CL for a measurement included the ratio 1.0, we concluded that the 2 fixation methods did not significantly affect that measurement.

Most measurements were not affected by fixation methods. These included the length, measured area, and proportional area of the oral sucker, testes, and ovary; length and measured area of the acetabulum; and the position of the testes, ovary and acetabulum. Most importantly, body length did not differ (mean ratio, 1.01; 95% CL, 0.97–1.05). The narrow ranges of the CL indicate that any effect of fixation was probably small (≤4%).

Most measurements that involved body width were significantly greater using method 1: body width (mean ratio, 1.24; 95% CL, 1.17–1.31), body width as % body length (1.25; 1.18–1.31), measured body area (1.17; 1.04–1.31), relative area (1.26; 1.15–1.37), and width of the acetabulum (1.05; 1.01–1.09). The proportional area of the acetabulum was significantly smaller using method 1 (0.92; 0.88–0.96).

These results suggest that fixation by method 2 did not alter the length of specimens, but it increased their width and area. We pooled data from the 2 fixation methods for graphical presentation. Body lengths did not differ and were pooled without correction. The relative areas of specimens fixed by method 1 were, on average, 1.26× larger than by method 2, so they were divided by 1.26 prior to pooling. We wanted to be more conservative for the statistical analyses and analyzed only specimens fixed similarly.

Parasite size

The oldest specimens from the present study (133 days PI, all fixed using method 2) had a relative area (mean ± SE) of 27.1 ± 0.1 mm^2.

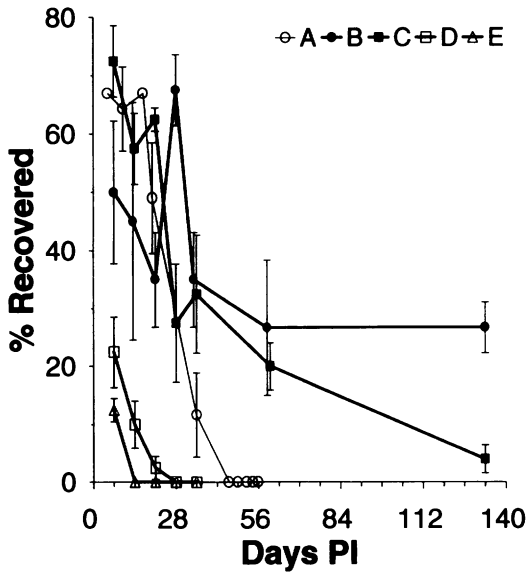

FIGURE 1. Proportion of *Zygocotyle lunata* recovered at various days postinfection (PI) from CD-1 mice given 10–30 metacercariae per os from 5 sources (A–E). Data are presented as mean ± 1 SE.

Measurements of museum specimens were considerably smaller: from moose, 14.4 ± 0.6 mm^2; from geese, 21.6 ± 1.7 mm^2; from coots, 17.0 ± 2.9 mm^2.

Body size of *Z. lunata* in CD-1 mice increased with age throughout the course of experimental infection. Body length (Fig. 2a) and relative area (Fig. 2b) of specimens up to 21 days old were typical of values reported in the literature. Older specimens equalled or exceeded the size of specimens of comparable age from experimental infections of other rodents and of birds. Discontinuities were apparent in the pooled body size data of specimens from CD-1 mice (Fig. 2). Because the pooling combined specimens from various combinations of host sex and parasite source on the different dates of examination, the effect of these factors on parasite body size was examined in greater detail.

Host sex did not affect parasite size. We compared median body length from 11 male hosts in experiment 2 with median body length from 11 female hosts infected similarly (same source of metacercariae, same age of infection, and same method of fixation). There was no tendency for worms from 1 sex of host to be consistently larger than worms from the other sex (paired t-test, $P = 0.94$). Host sex was not incorporated as a factor in subsequent analyses.

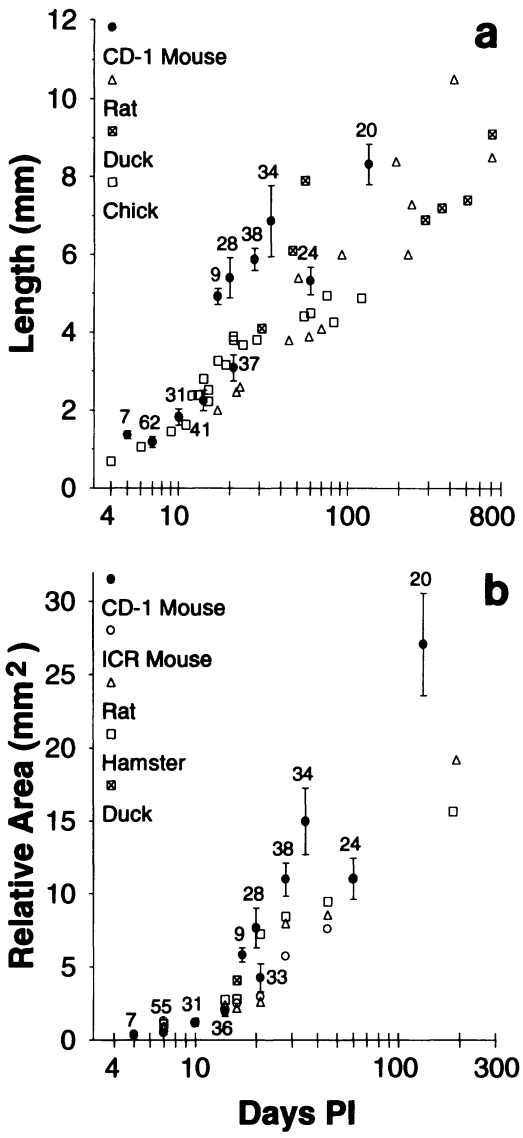

FIGURE 2. Size of *Zygocotyle lunata* at various days postinfection (PI) in experimental infections of different hosts. **a.** Maximum body length. **b.** Relative body area (maximum length × maximum width). Data on CD-1 mice are pooled data from present study (presented as mean ± 1 SD, with sample size). Data on other hosts are means from published studies: lengths from rats and ducks are from Willey (1941); lengths from chicks are from Fried (1970); relative areas from ICR mice, rats, hamsters, and ducks are from Huffman et al. (1991).

Parasite source had a major effect on parasite size. We compared body length of specimens of the same age, fixed similarly. A mixed-model ANOVA design was used. Parasite source was treated as a fixed effect, with the different mice receiving metacercariae from the same source and the different worms recovered from each mouse treated as nested factors within each source. These tests were conducted on 3 groups of specimens fixed by method 1 and on 7 groups of specimens fixed by method 2 (Table I). There was a significant effect of parasite source on body length in 6 of 10 tests. The 4 tests in which there was no significant effect included only source B and C specimens. With few exceptions, multiple comparison tests (Table I) showed that source A worms were larger than source B or C worms, that source B and C worms did not differ, that source B and C worms were larger than source D and E worms, and that source D and E worms did not differ.

The differences in parasite body size among sources could not be attributed to differences in the number of parasites recovered. We calculated mean length of *Z. lunata* per host, pooling specimens fixed by the 2 methods. Then, for each age of infection, the effect of source on parasite length was analyzed by ANOVA using the number of parasites as a covariate. The covariate was not significant ($P > 0.10$) in any case.

Parasite size differed occasionally among host individuals. Significant differences in body length among worms from different mice receiving worms from the same source were detected in 3 of 10 multiple comparison tests (Table I). No single cause was evident. In the 14- and 28-day-old specimens, the hosts were of different sex, but, in the 133-day-old specimens, the different mice receiving worms from each source were of the same sex.

The results provide preliminary evidence of correlated life history traits in the parasite. Worm sources giving similar patterns of recovery also gave similar patterns of growth. Source B and C worms were long lived and intermediate in body size. Those from sources D and E were short lived and also the smallest. Worms from source A lived for an intermediate duration but were the largest at a given age.

Fecal egg counts

Eggs first were observed in the uterus of *Z. lunata* at 17 days PI and in the feces at 25 days PI. Frequent estimates of egg production were made for mice harboring worms from sources B and C (Fig. 3). Egg output per host increased rapidly between 25 and 35 days PI, then continued at a relatively constant average level (but with considerable day-to-day variability) for sev-

TABLE I. Statistical evaluation of body lengths of *Zygocotyle lunata* in CD-1 mice infected with metacercariae from sources A–E.

Fixation	Age (days)	ANOVA effects* Source	ANOVA effects* Mouse	Multiple comparison tests			
Method 1							
	21	<0.001	0.287	C (3.06)†	A (5.37)‡		
	28	0.843	0.499	B (5.26)†	C (5.77)†		
	35	<0.001	0.055	B (6.04)†	C (6.15)†	A (8.32)‡	
Method 2							
	7	<0.001	0.611	E (0.88)†	D (0.98)†	C (1.21)‡	B (1.25)‡
	14	0.002	0.004	D (1.95)†	B (2.18)†‡	C (2.36)‡	
	21	<0.001	0.277	D (2.55)†	C (2.80)‡	B (3.42)‡	
	28	0.067	0.011	B (5.82)†	C (6.06)†		
	35	0.001	0.086	B (6.26)†	C (6.79)‡		
	60	0.147	0.110	B (8.22)†	C (5.51)†		
	133	0.625	0.030	B (8.22)†	C (8.56)†		

* P values from nested ANOVA on body length. Effects are those among different sources, and among different mice receiving metacercariae from each source.

†‡ Tukey–Kramer multiple comparison test. Values are parasite source, with mean body length (mm) in parentheses. Body lengths for the same fixation method and age do not differ significantly ($P > 0.05$) if they have the same symbol.

eral months. The moving average for egg production by worms from source C was generally lower than those from source B, and production by source C worms declined rapidly during the last week of observation. These differences between sources corresponded qualitatively to changes in average numbers of worms per host (Fig. 1). A more detailed analysis was done in which the only data considered were fecal egg counts done on hosts within 5 days of necropsy. Under the assumption that the number of worms present at necropsy was the same as for the previous 5 days, fecal egg counts could be corrected for the number of worms. Data were available from infections from source A, B, and C worms, examined 35, 60, and/or 133 days PI, from 1 or 2 hosts at each time, and from 3–5 fecal examinations done within 5 days of necropsy. Analysis of eggs·worm^{-1}·hr^{-1}, using a nested ANOVA, revealed no difference among sources ($P = 0.286$), among dates of examination within a source ($P = 0.709$), or among host individuals examined each date ($P = 0.621$). More than 70% of the variation in egg counts was explained by the error term (day-to-day variation in egg output of each host). Pooling all data produced an estimated rate of egg output of 13.7 ± 7.3 eggs·worm^{-1}·hr^{-1} (mean ± SD).

Eggs recovered from feces and maintained at 20–22 C hatched after 30–35 days if the water was changed every 1–2 days and after 35–45 days if the water was changed every 3–5 days. Miracidia usually emerged 8–12 hr after the lights were turned on, and they lived about 8 hr.

DISCUSSION

Infections with *Z. lunata* in avian and mammalian hosts produce several phenomena of interest to parasitologists: resistance to reinfection (Willey, 1941; Huffman et al., 1991), damage to the host (Joyner and McDaniel, 1970; Fried and Nelson, 1978), a crowding effect (Willey, 1941; Fried and Nelson, 1978), and patterns of growth

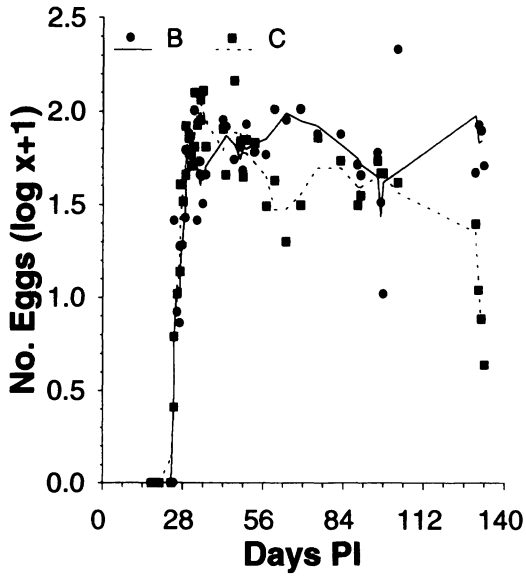

FIGURE 3. Fecal egg counts of *Zygocotyle lunata* at various days postinfection (PI) from CD-1 mice infected experimentally with metacercariae from sources B and C. Points represent eggs·host^{-1}·hr^{-1}. Lines represent 3-point moving averages.

and development that vary among species of definitive host (Huffman et al., 1991). Indeed, Huffman et al. (1991) suggested *Z. lunata* in rodent hosts as a cost-effective experimental system to study cecal amphistomiasis specifically and trematode interactions generally. Our study adds a new dimension to the interpretation of host specificity of *Z. lunata* by showing clearly that differences in survival and growth of the adult parasite are not necessarily consequences of the choice of definitive host species or strain.

It was not surprising that metacercariae from different naturally infected snails produced different infections in the definitive host. First, the phenotype of metacercariae from different sources likely has a strong genetic component. If each infection originated from a single miracidium, cercariae from the same snail would be genetically uniform, whereas cercariae from different snails would reflect the greater genetic variation present within the population of miracidia. Second, the phenotype may also reflect environmental effects: the interaction of each population of rediae with its snail host. The magnitude of variation in infection characteristics in the definitive host that could be attributed to the source of metacercariae was surprising.

Variation in the percentage of recovery was the most striking difference among sources. Worms from 2 sources exhibited high recovery for many months, which is characteristic for *Z. lunata* in many experimental hosts (Willey, 1941; Fried, 1970; Huffman et al., 1991). At the other extreme, those from 2 sources could be recovered only within a few weeks of infection. We did not determine the number of worms that established initially, but those that established did not survive long enough to mature. There was also striking variation among sources in the body sizes attained by *Z. lunata* of comparable ages, indicating differences in growth rates. Indeed, source A worms were as large after 1 mo as those reported from much older experimental infections of ducks, chicks, mice, and rats (Willey, 1941; Huffman et al., 1991).

We recognize that the interpretation of results from source A worms is problematic. Their snail host was collected at a different locale and time than the snails infected with source B–E worms. However, recovery of source A worms was within the range established for source B–E worms. Also, the comparative study of fixation methods on source B–E worms suggests that the large size of source A worms was not a fixation artifact.

Several features of the infection in the definitive host appeared independent of the source of metacercariae and typical of reports in the literature. For example, worms from the 2 longest-lived sources attained maximum sizes that approximated the maximum reported by Willey (1941) for *Z. lunata.* Similarly, egg production did not vary detectably among sources, and our estimate of about 14 eggs·worm^{-1}·hr^{-1} in the feces is similar to the average of 18 eggs·worm^{-1}·hr^{-1} determined in vitro by Etges (1992). The gross pathological response was similar among the 4 sources and confirmed reports of raised, reddened foci at the site of attachment (Joyner and McDaniel, 1970; Huffman et al., 1991) and of a reduction in cecal debris (Joyner and McDaniel, 1970; Fried and Nelson, 1978). Huffman et al. (1991) reported that mice infected with *Z. lunata* exhibit no overt signs of disease, and the absence of splenomegaly in mice infected with worms from sources B–D is consistent with that conclusion. The interpretation of splenomegaly from infections with source A worms was based on qualitative observations and was not confirmed using worms from other sources. However, because the pattern of survival of source A worms was also unique among the sources examined, there may be systemic effects of infection with *Z. lunata* that occur only when certain host and parasite phenotypes interact.

We do not know the extent to which the results of our study can be generalized. Many studies (Willey, 1941; Bacha, 1964; Fried, 1970; Joyner and McDaniel, 1970; Fried and Nelson, 1978; Huffman et al., 1991; Etges, 1992) have used naturally infected snails as a source of metacercariae for experimental infection of definitive hosts. However, these studies did not provide descriptions of the number of infected snails or the manner by which metacercariae from different snails were allocated for infections. Our results suggest 4 ways in which these considerations may influence the outcome and interpretation of experimental studies.

First, the use of a single snail with a monomiracidial infection as the source of metacercariae for a given experiment may produce consistent results because of the low genetic variability of the parasite. However, the results may not be typical of the parasite species or even the population from which the material was obtained. The source A *Z. lunata* that initiated our study is an example. We used a strain of mice that had not been used previously. The unusually rapid

growth, large size, and short lifespan of the parasite led us to hypothesize initially that this was caused by the choice of definitive host. Only our subsequent infections, using different sources of parasite in the same strain of mice, suggest that worms from source A simply were a deviant parasite phenotype. Similarly, the use of different snails as sources of metacercariae in different parts of an experiment may produce results that erroneously suggest a treatment effect.

Second, the pooling of metacercariae from many snails would increase the chance that the results would reflect the average characteristics of the parasite population or species. A greater range of variation might be expected, but bias due to the occasional deviant phenotype would be reduced. However, other problems may be introduced. If worms that differ in survival rates are pooled, young infections may reflect average characteristics of the parasite, but older infections would reflect the characteristics only of those that survived. Of a more general nature, interesting phenomena may simply go undetected. The conclusion that *Z. lunata* may live as long as the rodent host (Huffman et al., 1991) may be a correct generalization, but our results suggest that individual parasites do not have equal probabilities of surviving. Finally, the use of pooled worms from different sources of metacercariae for different parts of an experimental study also runs a risk of producing erroneous treatment effects, although this risk would be much lower than when using different single sources of parasite.

Third, conclusions regarding host specificity of parasites must rely on comparison of studies in the literature when comparative experiments cannot be done. Although failed infections can provide evidence for unsuitability of a host species or strain, they can also result if the source of infective material is limited. Our sample of 5 naturally infected snails contained 3 that produced parasites with poor survival in CD-1 mice. If only those 3 snails had been used, this might have led to the conclusion that CD-1 mice were unsuitable hosts.

Fourth, improved methods (Etges, 1992) for infecting snails experimentally with *Z. lunata* may reduce reliance on naturally infected snails, if these methods prove suitable for other natural sources of this parasite. This would facilitate increasingly sophisticated experimental studies. However, our results suggest that considerable care should be taken in choosing the initial natural source(s) of infective material and in developing protocols for laboratory maintenance of the parasite that do not unintentionally select for or against particular parasite phenotypes.

Our conclusion that survival and growth in the definitive host were partially determined by the time the metacercarial stage was attained and the preliminary evidence of correlated life history traits in the parasite suggests that the phenomenon of host specificity in *Z. lunata* is considerably more complex than thought previously. On the one hand, given an ability to reliably complete the entire life cycle experimentally, the *Z. lunata*–rodent–*Helisoma* sp. relationship might be a suitable system for experimental studies of life history interactions between hosts and parasites. On the other hand, the diversity of parasite attributes that we detected among a sample of only 5 naturally infected snails suggests that naturally infected hosts will continue to be an important source of material for many areas of parasitological investigation.

ACKNOWLEDGMENTS

This study was funded by a Boreal Alberta Research Grant (Canadian Circumpolar Institute) to A.W.S., and an operating grant from the Natural Sciences and Engineering Council of Canada to M.B. Comments from C. P. Goater improved the manuscript greatly.

LITERATURE CITED

BACHA, W. J., JR. 1964. Effect of salt solutions on the establishment of infections of the trematode *Zygocotyle lunata* in white rats. Journal of Parasitology **50:** 546–548.

ETGES, F. J. 1992. *Zygocotyle lunata*: Laboratory maintenance in snails and mice. Journal of the Helminthological Society of Washington **59:** 22–24.

FRIED, B. 1970. Infectivity, growth, development, excystation and transplantation of *Zygocotyle lunata* (Trematoda) in the chick. Journal of Parasitology **56:** 44–47.

———, AND P. D. NELSON. 1978. Host–parasite relationships of *Zygocotyle lunata* (Trematoda) in the domestic chick. Parasitology **77:** 49–55.

———, AND B. D. WILSON. 1981. Survival of metacercariae of *Zygocotyle lunata* (Trematoda) in half-strength Locke's solution under refrigeration. Proceedings of the Helminthological Society of Washington **48:** 96–97.

HUFFMAN, J. E., C. SABOL, AND B. FRIED. 1991. Infectivity, growth, survival, and pathogenicity of *Zygocotyle lunata* (Trematoda) in experimental rodent hosts. Journal of Parasitology **77:** 280–284.

JOYNER, S., AND J. S. MCDANIEL. 1970. Histological observations on mice infected with *Zygocotyle*

lunata. Journal of the Elisha Mitchell Scientific Society **86:** 184.

McDONALD, M. E. 1969. Catalogue of helminths of waterfowl. Special Scientific Report, U.S. Fish & Wildlife Service, Wildlife No. 126, Bureau of Sport Fisheries and Wildlife, Washington, D.C., 692 p.

SEY, O. 1991. CRC handbook of the zoology of amphistomes. CRC Press, Inc., Boca Raton, Florida, 480 p.

WILLEY, C. H. 1941. The life history and bionomics of the trematode, *Zygocotyle lunata* (Paramphistomidae). Zoologica **26:** 65–88.

MAILING DATES FOR VOLUME 79, 1993

No. 1, February ..... mailed 3 March 1993
No. 2, April ..... mailed 5 April 1993
No. 3, June ..... mailed 8 June 1993
No. 4, August ..... mailed 3 August 1993
No. 5, October ..... mailed 29 October 1993
No. 6, December ..... mailed 22 December 1993