TRANSMISSION OF *ANGIOSTRONGYLUS CANTONENSIS* THROUGH THE GIANT AFRICAN SNAIL *ACHATINA FULICA*: AN EXPERIMENTAL STUDY

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Abstract. Observations on transmission of the rat lung worm, *Angiostrongylus cantonensis*, from rats to the snail intermediate host, *Achatina fulica*, in a vacant lot in Bangkok are described. The prevalence of *A. cantonensis* increased with snail age until 200 days of age when it attained a plateau of 50-60%. The overall prevalence was 53%. The worm burden slowly rose with age until 200 days of age beyond which it remained relatively constant. The highest mean worm burden of 5,478 was observed in the oldest age group. The parasite distribution in the snail population was highly aggregated both within each age class and in the overall population. Experiments on susceptibility of snails to laboratory infection revealed that worm recovery was dependent on dose of first stage larval infection but was independent of snail size in the range of 4-8 cm. The percent worm recovery of third stage larvae was negatively correlated with dose of infection, and no density-dependent effects of worm burden on worm size were observed.

INTRODUCTION

Angiostrongylus cantonensis, or rat lung worm, is a nematode parasite known to cause eosinophilic meningoencephalitis in man in several tropical countries. Both rodent and molluscan hosts are required to complete the life cycle and hence transmission of the parasite to man. One of the snail intermediate hosts is the giant African snail, *Achatina fulica*, a common pest occurring extensively in tropical areas. Alicata (1966) has postulated that *A. fulica* might play a crucial role in the spread of the rat lung worm since this mollusc is present in most areas where *A. cantonensis* is endemic. In some areas, following the introduction of *A. fulica*, eosinophilic meningitis has been subsequently found.

Ecological studies of *A. cantonensis* infection in *A. fulica* in the natural environment are essential for understanding the population dynamics of the parasite. Previous studies have revealed that *A. fulica* is more susceptible to infection and harbors higher worm burdens of *A. cantonensis* than other land slugs in the same habitat (Margono and Ilahude, 1974) and aquatic snails (Lim and Ramachandran, 1979). However, the average prevalence and intensity of infection are greatly affected by age of the snail host, as demonstrated by Chen (1979), Wallace and Rosen (1969) and Noda *et al* (1987).

In this study the prevalence of infection and abundance of *A. fulica* of *A. cantonensis* in relation to size or age of snail were examined in a small population of *A. fulica*. To assess the influence of parasite and host attributes upon the susceptibility of *A. fulica* to rat lung worm infection, effects of dose of infection and snail size on size and number of worms recovered following primary infection with *A. cantonensis* were also investigated.

MATERIALS AND METHODS

Study area

The study area inhabited by *A. fulica* was a vacant lot 20 × 60 meters off Soi 4, Sukhumvit Road, Bangkok. It was fenced off by the adjacent occupied houses. Local residents discarded garbage, plant litter and other refuse in one end. The area was covered with growing vegetation, mainly grass and vines forming a dense cover over the ground surface and one large tree (*Ficus religiosa*)

provided shade in the corner near the garbage pile.

Snail collection

Snails were collected by walking about and searching for several consecutive mornings for active snails in the lot. The collections were done after the completion of the study of the natural growth rate of snails in which a growth curve was constructed from marked and recaptured snails (Sithithaworn, 1979). The snails were brought to the laboratory and their shell length and width were measured with either a Vernier caliper or dissecting microscope. The approximate age of individual snails was read from the growth curve.

Determination of infection and worm burden

The presence of *A. cantonensis* infection were determined after the snails were shelled and the mantle dissected since this organ is the major lodging site for the parasite (Brockelman *et al*, 1976). The nematode could be readily seen when the mantle was spread between two heavy glass plates and examined over an inverted microscope. The snails found to be infected either naturally or artificially were further dissected for recovery and counting of third stage larval burden as described of organ dissection, maceration and digestion with acid-pepsin and finally concentration in a Bearman's funnel.

Laboratory infection of snails

A. cantonensis was originally obtained from Dr Manoon Bhaibulaya, Faculty of Tropical Medicine, Mahidol University. The nematode had been cycled through the snail *A. fulica* and white Wistar rats as previously described (Tiengamol and Brockelman, 1982). Fresh fecal pellets of infected rats were collected and mixed with a small volume of distilled water. The fecal suspension was then placed on a coarse sieve in a glass funnel filled with distilled water. First stage larvae were allowed to concentrate, the larval sediment was withdrawn and the density of worms was estimated by volumetric counting on a Scott slide using an inverted microscope (Brockelman and Jackson, 1974). The larval suspension was adjusted to the desired concentration using fecal suspension from an uninfected rat as diluent. One-half ml of first stage larval suspension was dropped carefully onto finely blended lettuce in the infecting bowls, each containing one snail. The snails were allowed to feed on this moist mixture for 12 hours. If the lettuce in the infecting bowl was not all consumed the snail was discarded. Infected snails were kept for 40-50 days at a room temperature of 24-26°C in earthenware bowls 7-18 liters in volume containing sterilized soil. They were fed daily with fresh lettuce *ad lib* and sodium alginate was added as a food supplement.

Experimental design

Uninfected *A. fulica* used for laboratory infection were collected from non-endemic areas in Chon Buri Province. The snails were separated into 4 groups according to shell length: 4-5, 5-6, 6-7 and 7-8 cm. Smaller snails (< 3 cm) have limited consumption capacity and could not be reliably infected with accurate numbers of first stage larvae. The number of first stage larvae employed for infections varied from 750 to 24,000 per snail, and 7 doses were used (Table 2). Eight snail replicates were allocated for each treatment; thus a total of 224 snails were used in this experiment. Worm burden as determined 40 to 50 days after infection and samples of worms were kept in glycerin alcohol for measurement using camera lucida.

Statistical analysis

Analysis of variance (ANOVA) was employed to assess the relationship between snail size and dose of infection on the number, percent and mean size of worms recovered. Data transformation using log (x + 1) was applied to normalize worm recovery data for statistical tests. Estimations of the parameter k of the negative binomial distribution of worm burden in each age class were performed by the maximum-likelihood method (Bliss and Fisher, 1953). The parameter k represents the index of dispersion and its value varies inversely with the degree of clumping of worms in hosts. The overall parasite distribution was also examined using a power function (Taylor, 1961). Chi-square test for goodness of fit was used to assess the difference between the observed and predicted frequency distribution.

RESULTS

Natural infection

The age specific prevalence of *A. cantonensis* infection in *A. fulica* is shown in Fig 1. The youngest snail found infected was 1.15 cm in length, about 55-60 days old. The young snails picked up infections rapidly and prevalence reached a plateau beyond age 200 days (approximately 3.8 cm in shell length). Above this age prevalence was relatively constant, except in the last age group where more than 80% of snails were infected. The age of these large snails could not be estimated accurately, and many were probably older than estimated from the growth curve.

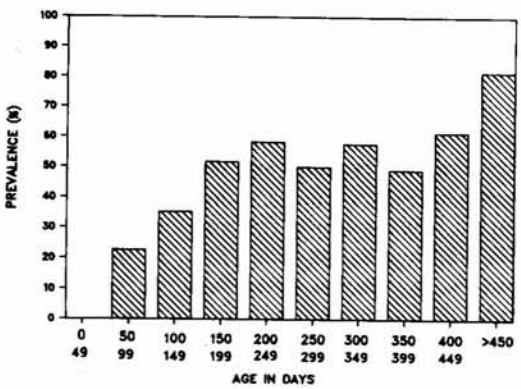

Fig 1—Age-specific prevalence of *A. cantonensis* infection in the snail intermediate host, *A. fulica*, in a vacant lot, Bangkok. The infection was determined by the presence of third stage larvae in the mantle of the snail (n = 481).

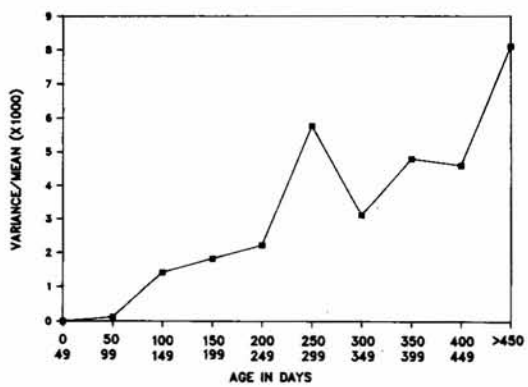

Fig 2—The variance to mean ratio (s^2/m) of *A. cantonensis* (third stage larvae) for different age classes of *A. fulica*.

The mean number of third stage larvae in young snails increased slowly with age. In snails older than 250 days the worm burden increased little before it rose sharply to more than 5,000 worms per snail in the oldest age group (Table 1).

The frequency distribution of *A. cantonensis* in each age class of the snail host was characterized by a highly aggregated distribution and the negative binomial model agreed with the observed data in age groups older than 200 days (chi-squared test, $p > 0.05$) but not in younger groups (Table 1). The variance (s^2) to mean (m) ratio of worm burden in each age class was much greater than unity, and the degree of over-dispersion increased with host age (Fig 2). The relationship between variance and mean of parasite count is well described by the power function (Taylor, 1961)

$$s^2 = am^{-b}$$

$$\text{or} \quad \log s^2 = \log a + b \log m$$

where a and b are parameters which reflect the size of sampling unit (in this case a single host) and the degree of aggregation, respectively. The slope of the regression line, b, also represents an index of dispersion which varies from zero for a regular distribution to infinity for a highly dispersed distribution. When $a = b = 1$ then $s^2 = m$ and the distribution is random. The calculated slope was 2.05 which is significantly greater than unity (*t*-test, $p < 0.05$).

Experimental infection

A. fulica size (4-8 cm) had no significant influence on worm recovery following exposure to primary infections (ANOVA, $p > 0.05$). As shown in Table 2, the number of third stage larvae recovered increased with increasing dose of infection (ANOVA, $p < 0.05$) but the percent recovery was negatively correlated with dose of infection (ANOVA, $p < 0.05$) (Fig 3). At doses of infection of 750 to 6,000 larvae per snail, the mean recovery was 45% while a lower mean recovery, 33%, was obtained with higher doses from 9,000 to 24,000. The average recovery rate, regardless of dose of infection, was 32%.

Worm load, which reached more than 10,000 larvae per snail, showed no significant effect on body length or width of the third stage larvae

Table 1

Age-related distribution and abundance of *A. cantonensis* (third stage larva) in *A. fulica*. Zero worm counts were included in the calculation of mean and standard deviation (SD) of worm burden. k represents the clumping index of the negative binomial distribution.

Age (days)	Size (cm)	No. of snails	Worm burden		k
			mean	SD	
0- 49	0.5 -1.12	14	0	0	
50- 99	1.13-2.04	66	8.2	31.7	0.048*
100-149	2.05-2.94	62	113.9	403.3	0.089*
150-199	2.95-3.79	60	276.4	722.5	0.087*
200-249	3.80-4.59	45	487.7	1,042.3	0.111*
250-299	4.60-5.29	36	1,609.8	3,049.1	0.083
300-349	5.30-5.99	41	1,363.5	2,065.9	0.117
350-399	6.00-6.39	27	1,084.4	2,280.7	0.062
400-449	6.40-6.59	18	1,967.1	3,009.3	0.156
>450	>6.60	112	5,478.3	6,701.8	0.262
Total		481	1,767.0	4,104.3	

Chi-square test, *$p < 0.05$

Table 2

Recovery of third stage larvae of *A. cantonensis* (mean and SD) from *A. fulica* exposed to varying numbers of first stage larvae.

Dose of infection	Worms recovered		
	mean no.	SD	%
750	369.7	90.9	49.3
1,500	711.4	220.0	47.4
3,000	1,100.3	314.9	36.7
6,000	2,817.3	613.3	47.0
9,000	2,763.9	1,210.8	30.7
12,000	4,507.4	1,448.5	37.6
24,000	7,283.6	2,286.1	30.4

recovered (Table 3). The average size of third stage larvae was 0.500 and 0.025 mm in length and width, respectively.

DISCUSSION

The fact that prevalence and intensity of *A. cantonensis* infection varied with age of the snail suggests that the duration of infection or age may regulate the pattern of infection. However, not all of the snails have equal chance of infection since the prevalence did not reach 100%, but stabilized at 50-60%. Several factors might account for this observation. Firstly, the snails varied in mobility on hence degree of exposure to rat feces. The highly mobile group occurring more often on the

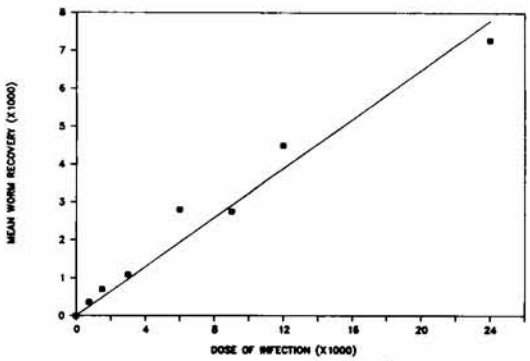

Fig 3—Relationship between dose of first stage larval infection and third stage larval recovery measured at 40-50 days post infection. Each point represents observed values of mean worm recovery for 32 snails and the line is the best fit regression model constrained through the origin, with the slope of 0.32 ($r^2 = 0.76$).

Table 3

Comparison of body size of third stage larva of *A. cantonensis* obtained from *A. fulica* haboring different worm loads. Values of mean and SD were calculated from sample of 30 larvae.

Worm load	Length (mm)		Width (mm)	
	mean	SD	mean	SD
1-1,000	0.4992	0.0138	0.0254	0.0015
1,000-2,000	0.5042	0.0128	0.0254	0.0012
2,001-6,000	0.5013	0.0144	0.0255	0.0013
6,000-10,000	0.5000	0.0139	0.0258	0.0012
>10,000	0.4907	0.0124	0.0248	0.0014

ground surface would represent the proportion of population at risk. Less active snails lie under the ground and have less chance of infection. This subpopulation hypothesis was originally proposed for another land snail, *Helix pomatia* (Lomnicki, 1969). Secondly, there was heterogeneity in distribution of rat feces in the area. Rats occupied only part of the area and the snails living outside the territories of rats were less likely to become infected. Thirdly, some snails might have fed preferentially on rat feces and hunted for it. The fact that rat feces can prolong survivorship of the first stage larvae (Brockelman *et al,* 1979) also facilitates transmission to snails.

Worm load also increased with snail age. There was no evidence for innate resistance associated with age since in the laboratory medium to large (4-8 cm). Snails were equally susceptible to infection. At doses of infection less than 6,000 larvae per snail the percent worm recovery was similar to that reported by Brockelman *et al*(1976). However, at higher doses (9,000 to 24,400 larvae per snail), percent worm recovery was lower which suggests a crowding effect of the larvae during infection. Under natural conditions where worms are acquired more slowly, crowding due to competition in penetrating the snail intestinal tract may be greatly reduced. This could explain why it is not unusual to find snails haboring several thousand worms and as many as 90,800 in a single snail have been recorded (Wallace and Rosen, 1969). The relatively large body size, high consumption capacity and long survival time permit individual snails to accumulate heavy worm burdens. Although some crowding effects may occur during the process of infection in the snail intestine, no clear upper limit of worm load was evident, the load probably being determined by opportunities for acquiring new worms. It is likely that population control of *A. cantonensis* occurs mainly during the adult stage in the rat definitive host rather than in the snail intermediate host (Kwong and Dobson, 1982; Noda *et al,* 1987).

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