Angiostrongyulus cantonensis infection in molluscs in the municipality of São Gonçalo, a metropolitan area of Rio de Janeiro, Brazil: role of the invasive species Achatina fulica in parasite transmission dynamics

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The aim of this study was to analyse the infection dynamics of Angiostrongyulus cantonensis in its possible intermediate hosts over two years in an urban area in the state of Rio de Janeiro where the presence of A. cantonensis had been previously recorded in molluscs. Four of the seven mollusc species found in the study were exotic. Bradybaena similaris was the most abundant, followed by Achatina fulica, Streptaxis sp., Subulina octona, Bulimus tenuissimus, Sarasinula linguaeformis and Leptinaria unilamellata. Only A. fulica and B. similaris were parasitised by A. cantonensis and both presented co-infection with other helminths. The prevalence of A. cantonensis in A. fulica was more than 50% throughout the study. There was an inverse correlation between the population size of A. fulica and the prevalence of A. cantonensis and abundance of the latter was negatively related to rainfall. The overall prevalence of A. cantonensis in B. similaris was 24.6%. A. fulica was the most important intermediary host of A. cantonensis in the studied area and B. similaris was secondary in importance for A. cantonensis transmission dynamics.

Key words: cerebral angiostrongyliasis - Bradybaena similaris - helminths’ larvae - intermediate hosts

Angiostrongyulus cantonensis (Chen, 1935), the rat lungworm, is a parasitic nematode discovered in the pulmonary arteries and hearts of domestic rats in China (Wang et al. 2008). It can parasite the central nervous system of humans causing eosinophilic meningitis. The disease is known as cerebral angiostrongyliasis (Hsu et al. 1990, Ismail & Arsura 1993) or, commonly, rat lungworm disease (RLD). The first record of angiostrongyliasis caused by A. cantonensis infection was reported in 1945, by Nomura and Lin, based on the observation of a nematode in the cerebrospinal fluid of a patient (Prociv & Carlisle 2000). Since then, RLD, which is endemic in Southeast Asia and the Pacific islands, has been reported in more than 30 countries worldwide, especially in tropical and subtropical regions (Kim et al. 2014). Among 2,800 cases of RLD, 77% occurred in Southeast Asia, China and Japan (Wang et al. 2008). In Brazil, RLD is an emerging disease first reported in 2006 and infected hosts were observed in the states of Rio de Janeiro (RJ), Espírito Santo (ES), Pernambuco (PE), São Paulo, Rio Grande do Sul and Paraná (Caldeira et al. 2007, Lima et al. 2009, Espírito-Santo et al. 2013, Morassutti et al. 2014).

The life cycle of A. cantonensis is complex and involves rats as definitive hosts, molluscs as intermediate hosts and crustaceans, frogs, fishes and other invertebrates as paratenic hosts. Several gastropod species from various families have been reported as intermediate hosts of A. cantonensis (Kim et al. 2014), including Achatina fulica, Browdich, 1822, popularly known as the African giant snail (Mead 1961, Alicata 1966, Tsai et al. 2013, Morassutti et al. 2014). A. fulica was included on a list of 100 of the worst invasive species in the world (Lowe et al. 2004). In Brazil it was introduced for commercial purposes in the 1980s and currently is found in 25 of the 26 Brazilian states (Thiengo et al. 2007), usually forming dense populations. A. fulica and other molluscs naturally infected with A. cantonensis have been described in various regions of Brazil by Thiengo et al. (2010), Maldonado Jr et al. (2010), Carvalho et al. (2012) and Moreira et al. (2013).

In Brazil, the emergence of RLD cases associated with the introduction and spread of A. fulica requires a better understanding of RLD transmission dynamics. The aim of this study was to analyse infection dynamics of A. cantonensis in its possible intermediate hosts in an urban area in RJ where the presence of A. cantonensis in molluscs has been recorded.

MATERIALS AND METHODS

Study area - This study was carried out in Trindade locality (22°49’37”S 43°03’14”W), which is a district of the municipality of São Gonçalo (Fig. 1), the second most populous city in RJ with approximately one million inhabitants (IBGE 2010). The climate is warm humid tropical with hot humid summers and mild dry winters.
The annual average temperature is 25ºC. Maximum average temperature during the study period was 30.9ºC in February 2010 and the minimum average temperature was 21ºC in June 2010 and July 2011. The annual average rainfall is 120 mm and varied during the study period from 13.9 mm in September 2011 to 341.2 mm in March 2010 (data obtained from São Gonçalo Urban Climatological Station from Geosciences Laboratory, Rio de Janeiro State University).

The study site comprised an area of 75 m² divided into three plots of 5 x 5 m in grassy vegetation with a predominance of razor grass and a few bushes in the backyards of human dwellings where the presence of A. cantonensis had been recorded (Maldonado Jr et al. 2010). The plots were bordered by a pavement way, a dwelling wall, a small stream and a very narrow strip of grass vegetation continuing along the backyards of the dwellings. In two plots, vegetation covered the ground completely and the third plot had open areas with exposed clay soil.

**Field and laboratory methods** - Molluscs were sampled on 11 occasions in all seasons from January 2010-October 2011. On each occasion, specimens were collected manually on one day, always in the morning. Specimens were taken to the laboratory and kept in a terrarium (20 x 19 x 30 cm) with 3 cm of autoclaved moist clay on the bottom, under controlled temperature (23 ± 2ºC) and fed fresh lettuce leaves every other day. Mollusc identification followed Boffi (1979), Araújo et al. (1960) and Thomé and Gomes (2006).

Larvae of helminths were recovered from the molluscs using a 0.7% HCl artificial digestion method (Graeff-Teixeira & Morera 1995). Larvae were fixed in ethanol alcohol 70%, formaldehyde 40% and acetic acid ≥ 99.85% glacial and identified using a light microscope according to Anderson et al. (2009) and Ash (1970). Larvae recovered from each mollusc specimen were counted only for A. fulica. To confirm identification of A. cantonensis specimens, recovered worms were administered orally.
to rats (*Rattus norvegicus*) in captivity. After 35 days, adult worms were recovered from the pulmonary arteries of the rats and identified by morphometry according to Maldonado Jr et al. (2010) and by molecular techniques according to Monte et al. (2012). This procedure was carried out following the Ethical Committee on Animal Use of Oswaldo Cruz Foundation (license 100/2011).

**Data analysis** - Mollusc abundance was defined as the number of specimens of each species collected. Mollusc species richness was defined as the overall number of species sampled. The prevalence of each helmint species was calculated for each month for all mollusc species and prevalence was defined as the proportion of infected molluscs for a given helmint species in relation to the total number of molluscs analysed for a given mollusc species, according to Bush et al. (2001). To investigate any possible immediate relationship between climatic variables and helmint prevalence, the influence of climatic variables on prevalence of *A. cantonensis* in *A. fulica* and in *Bradybaena similaris* (the molluscs species in which *A. cantonensis* was found) was investigated using multiple linear regressions, with a one-month time lag. To investigate if mollusc abundance and *A. cantonensis* prevalence varied in similar or opposing ways, simple correlations were carried out between these parameters.

Abundance and intensity of *A. cantonensis* in *A. fulica* were calculated for each month according to Bush et al. (2001). Abundance was defined as the number of helmints recovered divided by the total number of molluscs analysed. Intensity was defined as the number of helmints recovered divided by the number of infected molluscs. The dispersion index for *A. cantonensis* in *A. fulica* was calculated as the variance to mean ratio of parasite abundance. We used simple correlations to investigate whether *A. fulica* abundance and *A. cantonensis* abundance and intensity varied in similar ways through time. To verify possible influence of *A. fulica* shell size on abundance and prevalence of *A. cantonensis* we used simple linear regressions. Shell size was defined as length of the mollusc from the apex to the shell opening.

Data on mollusc abundance, parasitological parameters and climatic variables were tested for normal distribution using the Shapiro-Wilk test. Significance level in all analyses was considered as $\alpha < 0.05$. Tests were performed using PASW Statistics v.18 and PAST v.2.10.

**RESULTS**

**Mollusc community** - The mollusc community was composed of seven species (overall species richness) and it did not vary among plots. Monthly species richness varied from one-six, with highest values during the winter months. In total, 467 individuals were collected. *B. similaris* was the most abundant species, with 245 individuals, representing 52.4% of all molluscs collected. *A. fulica* was the second most abundant (153 individuals, 32.7%). Other species occasionally present were *Streptaxis* sp. (22 individuals, 4.7%), *Subulina octona* (11 individuals, 2.3%), *Bulimulus tenuissimus* (d’Orbigny, 1835) (9 individuals, 1.9%), *Sarasinula sinula linguaeformis* (3 individuals, 0.6%) and *Leptinaria unilamellata* (d’Orbigny, 1835) (2 individuals, 0.4%).

*A. fulica* was the only species present in all samples. Its abundance decreased during the study period, except for in April 2011, and it did not exhibit a clear annual pattern of abundance (Fig. 2). *B. similaris* abundance was highest in September and November 2010 (Fig. 3). There was a negative correlation between *A. fulica* and *B. tenuissimus* abundances ($r_s = -0.619, p = 0.042, n = 11$).

**Helminth parasitism on molluscs** - Helminth presence was assessed in 270 mollusc specimens. Three nematode species were found: *A. cantonensis*, *Rhabditis* sp. and *Strongylurus* sp. *A. cantonensis* had an overall presence of 78.7% in *A. fulica* and an overall prevalence of 24.6% in *B. similaris*. *Rhabditis* sp. was present in all mollusc species, except *L. unilamellata*, and had an overall prevalence of 14.2% in *A. fulica*, 68.6% in *B. similaris*, 20% in *S. octona*, 31.8% in *B. tenuissimus*, 33.3% in *Sarasinula* sp. and 9.5% in *Streptaxis* sp. *Strongylurus* sp. occurred only in *A. fulica* and in *B. similaris* with overall prevalence of 13.5% and 2.12%, respectively.

![Fig. 2: A. fulica abundance (right axis) and A. cantonensis prevalence in this host (left axis) through time in Trindade, municipality of São Gonçalo, state of Rio de Janeiro, Brazil.](image)

![Fig. 3: B. similaris abundance (right axis) and A. cantonensis prevalence in this host (left axis) through time in Trindade, municipality of São Gonçalo, state of Rio de Janeiro, Brazil.](image)
A. cantonensis infection in molluscus

- Prevalence of A. cantonensis on A. fulica was always more than 50% (Fig. 2) and showed a negative correlation with A. fulica abundance ($r_s = -0.801$, $p = 0.003$, $n = 11$). Monthly mean abundance of A. cantonensis in A. fulica was 124.3 larvae per host varying from 14 in February 2011 to 416.8 in January 2010. Monthly mean intensity of A. cantonensis in A. fulica was of 220.5 larvae per infected host, varying from 14 in February 2011 to 833.6 in January 2010. A. cantonensis abundance and intensity were not correlated with A. fulica abundance ($r_s = 0.201$, $p = 0.535$, $n = 11$; $rs = 0.420$, $p = 0.198$, $n = 11$, respectively). However, A. cantonensis abundance was related negatively with rainfall ($R = 0.788$, $b = -0.951$, $p = 0.007$). Prevalence and intensity were not related with any climatic variable. Distribution of A. cantonensis was highly aggregated among A. fulica individuals with a dispersion index of 952.81 and the highest absolute abundance in one mollusc was 3,108 larvae.

Overall prevalence of A. cantonensis in B. similaris was 24.6%, varying from 0-100%. This hemlnth was found in B. similaris only in July 2010, September 2010, April 2011 and July 2011 (Fig. 3). There was no relation between prevalence and any climatic variable ($R = 0.369$, $p = 0.294$). There was no correlation between A. cantonensis prevalence and B. similaris abundance ($r_s = 0.035$, $p = 0.918$, $n = 11$) nor between A. cantonensis prevalence in A. fulica and A. cantonensis prevalence in B. similaris ($r = 0.276$, $p = 0.412$, $n = 11$). Abundance and intensity in B. similaris were not calculated because the snails were pooled for digestion and larvae were not counted.

Shell size of the A. fulica specimens collected varied from 1-9 cm, with a mean of 4.35 cm and a standard deviation of 1.67. There was no significant correlation between A. fulica shell size and hemlnth abundance ($R = 0.029$, $p = 0.727$, $n = 142$), nor between monthly median shell size and A. cantonensis monthly prevalence ($R = 0.499$, $p = 0.118$, $n = 10$).

**DISCUSSION**

Four of the seven mollusc species found in the study are considered exotic (A. fulica, B. similaris, L. unilamellata and S. octona) and three are native (B. tenissimum, S. linguaeformis and Streptaxis sp.) (Simone 2006). These species are widely distributed and some play a role in transmission of helminth parasites of medical and veterinary importance (Souza & Lima 1990). The invasive species A. fulica stands out for its recent introduction and for rapidly spreading throughout Brazil (Thiengo et al. 2007, Zanol et al. 2010), including RJ (Carvalho et al. 2012). In the present study, abundance of A. fulica and B. similaris were higher than in other studies conducted in A. cantonensis transmission areas in Brazil. Carvalho et al. (2012) studied mollusc fauna in nine Brazilian ports, finding S. octona to be most abundant, followed by A. fulica and B. similaris. The same was found by Caldeira et al. (2007) in ES. Thiengo et al. (2010) reported a predominance of L. unilamellata, Sarasinula marginata and A. fulica in PE and did not find B. similaris.

A. fulica and B. similaris, originating in Africa and Asia, respectively, have been introduced to many regions of the world by human activities. In Brazil, they occur in nearly all states (Oliveira et al. 1990, Zanol et al. 2010). They spread easily to various environments and can cause major health and economic effects, either as agricultural pests or as intermediate hosts of helminths. In addition, they are able to establish readily in urban and periurban areas, invading gardens, vegetable gardens and wastelands. These characteristics may have resulted in higher abundance of A. fulica and B. similaris compared to other mollusc species in the area studied. Exotic invasive species tend to dominate the natural communities where they establish (Simone 2006).

We observed a marked increase in B. similaris abundance in spring and summer. These results are according to Araújo (1989), who found seasonal occurrence of this species with activities after periods of heavy rainfall, restricting its appearance to relatively short periods of the year. Leahy (1980, 1983) found high resistance to environmental changes by B. similaris and ability to survive to desiccation for up to 24 days and to return to normal activity when replaced in moist environments with food. In general, terrestrial pulmonate molluscs tend to be more active in the rainy season, when relative humidity of air and soil are higher (Pérez et al. 2008). Mollusc species richness did not vary among plots, but varied over time, increasing in the winter months, especially due to the accidental species Streptaxis sp., L unilamellata and S. linguaeformis moving from adjacent vegetation, which seemed to be dependent on seasonal variations.

The present study is the first report of the occurrence of A. cantonensis in B. similaris in RJ, although B. similaris has been identified as an intermediate host of this nematode elsewhere (Caldeira et al. 2007, Carvalho et al. 2012). Results of the present study indicate A. fulica and B. similaris as the most important intermediate hosts of A. cantonensis in Trindade, with much higher prevalence in A. fulica than in B. similaris. In addition, results indicate an important role of A. fulica in the transmission cycle of A. cantonensis, because this species was found infected throughout the year at high prevalence rates. A. fulica seems to be susceptible to various parasite species, which corroborates its importance as a helminth intermediate host. Furthermore, its body size and high abundance may favour parasite occurrence. Larger hosts may offer more space and diversity of microhabitats and they are able to support a greater richness of parasite species.

Prevalence and abundance of A. cantonensis increased most during and at the end of the dry season. In November 2010, as A. fulica abundance decreased, the prevalence of A. cantonensis dropped. At this point, B. similaris abundance was highest and in both 2010 and 2011, the highest prevalence of A. cantonensis on this host species occurred during the first months of A. fulica abundance decrease. Moreover, even with lower abundance of A. fulica in 2011, prevalence of the nematode in this host always was high. These observations suggest B. similaris as the second most significant host for the A. cantonensis transmission cycle. Although this mollusc was the most abundant in the present study, A. cantonensis infection was observed only during dry months. Carvalho et al. (2012) showed that, in Brazilian port areas, the rate of infection of A. cantonensis in B. similaris was
100%, in S. marginata, 84%, in S. octona, 76% and in A. fulica, 66%. They concluded that B. similaris had importance equal to or greater than A. fulica in the parasite transmission cycle. In the present study, it is possible that B. similaris acted as an auxiliary intermediate host for the parasite transmission. It is important to point out that the present study was carried out over nearly two years, while Carvalho et al. study (2012) was less protracted.

The dispersion of parasites is of great importance to understanding parasite-host dynamics (De Jong 1976, Dobson & Roberts 1994). We observed a highly aggregated distribution pattern of A. cantonensis in A. fulica population, indicating that unequal exposure of hosts and differences in individual susceptibility to infection may have influenced this pattern and resulted in many hosts harbouring few or no parasites and a few hosts harbouring the bulk of parasites as expected for most parasite populations (Poulin 2007).

Although the highest mean parasite load of A. cantonensis in A. fulica was observed in the older age group, we did not observe a significant relationship between A. fulica shell size and abundance of A. cantonensis, suggesting that helminth larvae infection did not depend on mollusc shell size. Sithithaworn et al. (1991) demonstrated that A. cantonensis prevalence may increase with A. fulica age and hosts of up to 200 days reached a 50-60% infection level, with total prevalence of 53% at this age and size. Thus, in the present study, the microenvironment may have influenced infection more than shell size, due to high degree of spatial aggregation of parasites in the A. fulica population.

Recent studies have reported A. cantonensis infection in Brazil in Rattus rattus and R. norverigicus, with both acting as definitive hosts (Simões et al. 2011, Moreira et al. 2013). In the studied area, infected R. norverigicus were observed to have high prevalence and abundance of A. cantonensis and to contribute to dispersing the parasite to new areas (Simões et al. 2014).

In the present study, A. fulica played an important role as intermediate host in the A. cantonensis transmission dynamics, due to its high abundance and high infection rates, regardless of season. B. similaris was a second most important to A. cantonensis transmission dynamics, mostly when population abundance of A. fulica was low. The high abundance of these molluscs observed associated with high prevalence of A. cantonensis and the presence of infected rodents may enable transmission of this nematode in the locality throughout the year.

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A. cantonensis in molluscs • Ana PM Oliveira et al.

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