**TITLE**: *Angiostrongylus cantonensis* in urban populations of rats and terrestrial gastropod mollusks in slum communities in Brazil

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**ABSTRACT**

**Background:** *Angiostrongylus cantonensis* is the most common causative agent of eosinophilic meningitis in humans, a disease endemic to Asia and the Americas. The life cycle of the nematode depends on terrestrial gastropods as intermediary hosts and rats as definitive hosts, as well as specific environmental conditions that influence host abundance and distribution, especially in urban favelas. In this study, we identify the risk factors associated to the presence and abundance of terrestrial gastropods – potential intermediate hosts of *A. cantonensis* – and the frequency of *A. cantonensis* infection in *Rattus norvegicus*, in an urban slum area in Brazil.

**Methods:** we identified and quantified the occurrence of terrestrial gastropods in a slum community with previous records of *A. cantonensis* infected rats. Zero-inflated model was applied to associate possible environmental risk factors to the presence and abundance of gastropods. In addition, we associated the individual rodent characteristics, as well as environmental factors, to the probability and intensity of the *A. cantonensis* infection.

**Results:** Eight species of mollusks (577 individuals) were identified, five of which were positive to *A. cantonensis*. The prevalence of *A. cantonensis* in 168 individuals of *R. norvegicus* captured was high (33.3%) across sampling campaigns and seasons; younger rats presenting higher intensity of *A. cantonensis* infection. The relevant environmental risk factors associated to both mollusk abundance and intensity of *A. cantonensis* infection in rats were presence of water (running water, puddles, sewage, moist environments and accumulated rain) and the accumulation of construction materials.

**Conclusions:** Our study indicates that the environmental conditions of poor urban areas contribute concomitantly to the distribution and abundance of terrestrial gastropods, as well as infected rats, contributing to the maintenance of the *A. cantonensis* transmission cycle in the areas. The mollusk species richness can expand the distribution of the nematode, contributing to the susceptibility to *A. cantonensis* and other parasites with zoonotic potential transmitted by mollusks and/or rodents.

**KEYWORDS:** Helminth;Snail; Slung;*Rattus norvegicus*; Angiostrongyliasis; Zoonosis diseases; One Health; Urban slum; Risk factors.

**BACKGROUND**

The rat lungworm *Angiostrongylus cantonensis* (Chen, 1935) (Nematoda: Metastrongyloidea) is the parasitic nematode that causes cerebral angiostrongyliasis, which causes eosinophilic meningitis, with severe and fatal outcomes [[1–3]](https://paperpile.com/c/vdxkIc/bnljR%2BtkXQY%2BGUqaU). This nematode can also cause ocular angiostrongyliasis, which develops in 1.2% of the patients diagnosed with cerebral angiostrongyliasis [[4]](https://paperpile.com/c/vdxkIc/9UQa5). Humans are incidental hosts, with infection occurring after ingestion of terrestrial gastropods (slugs and snails) infected by third-stage larvae, or through raw or undercooked foodstuffs contaminated by the gastropod’s mucous trail [[5]](https://paperpile.com/c/vdxkIc/sIJo1). Neuroangiostrongyliasis has expanded from southeastern Asia and Pacific Islands, where the disease is considered endemic, to countries and islands around the globe [[2]](https://paperpile.com/c/vdxkIc/tkXQY), advancing from tropical to temperate zones [[6]](https://paperpile.com/c/vdxkIc/1fMXJ). Currently, there are cases in the Americas [[7]](https://paperpile.com/c/vdxkIc/jqIQN) and, in Brazil, the disease is emerging and has been reported since 2007 in the North [[8]](https://paperpile.com/c/vdxkIc/8VhUP), Northeastern [[9,10]](https://paperpile.com/c/vdxkIc/DckSs%2BLPxys) and Southeastern regions [[11,12]](https://paperpile.com/c/vdxkIc/UR8Ut%2BNwKr0), despite natural intermediary and definitive hosts occurring in almost all regions of the country [[9,11,13–18]](https://paperpile.com/c/vdxkIc/UR8Ut%2BDckSs%2BLVdJ6%2Bg3juD%2BTMj5c%2BazgE3%2BmGktM%2BizPeM).

Gastropods are the intermediary host of *A. cantonensis*, with rodents as definitive hosts [[2]](https://paperpile.com/c/vdxkIc/tkXQY). Rats eliminate first-stage larvae through their feces, which infect mollusks either by penetrating the body wall, respiratory pore or by ingestion [[5]](https://paperpile.com/c/vdxkIc/sIJo1), where it develops up into its third stage. These larvae can infect rodents when infected mollusks or other contaminated foodstuffs are ingested, as well as by ingestion of infected paratenic hosts [[2]](https://paperpile.com/c/vdxkIc/tkXQY). Then, the larvae develop into adults and complete the cycle.

In urban slums in the tropics, both gastropods and rats find highly favorable conditions to thrive. The lack of adequate sanitation and refuse collection offer harborage and food, contributing to the abundance of hosts for *A. cantonensis*, such as *Rattus norvegicus* (Berkenhout, 1769) – one of its main definitive hosts [7,19–21]. In this environment, the maintenance of the life cycle of *A. cantonensis* occurs due to its low specificity on intermediary hosts [[19]](https://paperpile.com/c/vdxkIc/l6qgk) – such as the case with the introduced (and successful) colonizer snail species *Achatina fulica* (Bowdich, 1822), and the slug *Sarasinula marginata* (Semper, 1885) [[11,15]](https://paperpile.com/c/vdxkIc/UR8Ut%2BTMj5c). Environmental characteristics and the variety of host species are likely to facilitate infection by this nematode in residents of urban slums already affected by several other zoonotic agents [[18,20,21]](https://paperpile.com/c/vdxkIc/k7KVV%2BUsdQg%2BizPeM).

Recent studies indicate *A. cantonensis* prevalence of around 40% in a urban population of *R. norvegicus* in a Brazilian slum, indicating that the residents of the region are exposed to risk of infection by this nematode [[18]](https://paperpile.com/c/vdxkIc/izPeM). However, such exposure risk cannot be quantified as information on the prevalence of *A. cantonensis* in the diversity of intermediary host species is scarce [[22]](https://paperpile.com/c/vdxkIc/7fqZ4).

Thus, the main objective of this study was to identify and evaluate the distribution of terrestrial gastropods and the risk factors associated to the occurrence of, among the recorded species, potential intermediary hosts for *A. cantonensis* in an urban slum with previous records of the nematode in rodents. In parallel, we evaluate the risk factors for *A. cantonensis* infection in *R. norvegicus*. Reaching these objectives could indicate potential areas and maintenance factors for this helminth’s cycle, and thus passive of intervention. To achieve that, we, (i) identified and estimated the frequency and abundance of terrestrial mollusks that are confirmed and potential intermediary reservoirs for *A. cantonensis*; (ii) verified the environmental risk factors associated to the presence of terrestrial mollusk reservoirs; and (iii) evaluated the environmental, demographic and body condition risk factors, as well as coinfection with other helminths, associated to the presence and intensity of *A. cantonensis* infection on *R. norvegicus*, using long-span monitoring data.

**METHODS**

1. *Study Area*

We conducted our study in the neighborhood of Pau da Lima in the municipality of Salvador-Bahia (northeastern Brazil), in an area geographically stratified in three valleys, with total area of 0.17 Km2, with approximately 3,717 inhabitants [[23]](https://paperpile.com/c/vdxkIc/mN1f) (Fig. 1a-b). The area presents precarious inhabitation and sanitation infrastructure [[18,23]](https://paperpile.com/c/vdxkIc/mN1f%2BizPeM), cwith cases of zoonotic diseases documented [[24,25]](https://paperpile.com/c/vdxkIc/O31Ep%2Bmedqi), such as leptospirosis, which presents asymptomatic cases in a frequency of 35.4 per 1.000 people/year and severe cases in 19.8 per 100.000 people/year [[25,26]](https://paperpile.com/c/vdxkIc/9R9lK%2Bmedqi).

1. *Study Design*

Within 108 points selected by a previous study developed in the area [[23]](https://paperpile.com/c/vdxkIc/mN1f), we randomized, stratified by the three valleys, 40 points for gastropod sampling and 45 *Rattus norvegicus* sampling points, 17 of which coinciding. In each geotagged point, a buffer with a 15-meter radius was established as the sampling area to sample both taxa.

A single mollusk sampling campaign was performed between November 2016 and January 2017, occurring posteriorly to the rodent sampling campaigns. However, the time interval has no effect in our comparative approach, given that rat abundance has no seasonal variation in tropical urban slums [[23]](https://paperpile.com/c/vdxkIc/mN1f). Similarly, terrestrial mollusk populations follow the same temporal pattern, not varying in abundance in time [[27–29]](https://paperpile.com/c/vdxkIc/cZLZE%2BWy4zJ%2Bb5JAZ).

1. *Terrestrial gastropod sampling*

Mollusk sampling was performed by active hand search, following the protocol developed by the Brazilian Ministry of Health [[30]](https://paperpile.com/c/vdxkIc/EwqMo), during two or three days, in the early morning (08:00-10:00), given that the animals are more active and exposed during this period of time. We performed visual scans in each sampling point, and captured individuals were counted by morphotype and added to plastic bags with humid gauze to assure the survival of individuals during transport to the laboratory. In each point, sampling lasted around 20 minutes and, in parallel, an environmental survey was performed to record data to test the possible risk factors for presence and abundance of terrestrial mollusks. Variables were selected taking into account biotic and abiotic conditions referring to the physiology of these invertebrates (e.g.: humidity, water, soil and vegetation) (see Additional file 1: Table S1) [[31,32]](https://paperpile.com/c/vdxkIc/Th9k0%2BVzvbX). Identification was performed using morphological characteristics, based in literature [[33–37]](https://paperpile.com/c/vdxkIc/IhBeX%2BhLuS5%2Bj6fEg%2BKRYNP%2BkDbkc).

*3.1 Processing of terrestrial mollusk samples and larval extraction*

Larval extraction was segregated by sampling point, were individuals of the same species, with size inferior to 2cm were pooled for analysis [[15]](https://paperpile.com/c/vdxkIc/TMj5c), while bigger individuals were dissected and analyzed in separate. The animals (or pools) were macerated and digested following Wallace e Rosen (1969)[[38]](https://paperpile.com/c/vdxkIc/LG4Tk), modified by Instituto Adolfo Lutz (IAL), with the inclusion of adaptations from the technique described by Rugai (1954) [[39]](https://paperpile.com/c/vdxkIc/tg6Z4).The mollusk macerate was divided in small portions of approximately 3 grams, laid in six layers of cloth, and distributed in sedimentation calyxes with peptic solution (4% pepsin 0.7% Hydrochloric acid, in de-chlorinated water) heated to 42ºC. the calyxes were identified and incubated at 37ºC for two hours. After incubation, the sediment in the calyxes was aspirated using a pipette, transferred to a Petri dish and observed in a binocular stereoscopic microscope in 40x. All larvae found were counted and fixed in ethanol 70%, with a subsample stored at -20ºC for molecular tests.

*3.2 Morphometrics and identification of A. cantonensis larvae by PCR-RFLP*

The larvae selected for morphometric analysis were chosen by their morphology, mainly tail conformation [[40]](https://paperpile.com/c/vdxkIc/U8wVU), including only larvae with similar characteristics to the metastrongilid group. Thus, the analyses were performed by observing specific measurements in each specimen: total body length, length of esophagus, distance between excretory pore and anterior extremity, distance between genital primordium and tail, length of genital primordium and distance between anus and tail. These measurements were compared to the measurements from reference isolates of *A. cantonensis* and *A. costaricensis*, kept in IAL, with results obtained by percentage of similarity. According to the results, the samples were sent for RFLP (Restriction Fragment Length Polymorphism) PCR for molecular confirmation of species identification between *A. cantonensis*, *A. costaricensis* or *A. vasorum*, following the method by Caldeira et al. [[41]](https://paperpile.com/c/vdxkIc/1iY57).

1. *Rat sampling and A. cantonensis infection identification*

Rat data was obtained from four sampling campaigns spanning 2014 a 2016: two campaigns in the rainy season – March-July/2014 and March-April/2016 – and two in the dry season – October-December/2014 and November-December/2015. Capture, processing and sample collection followed previously established protocols, as well as local environmental data collection [[18,20,23,42–44]](https://paperpile.com/c/vdxkIc/rkFuc%2Bk7KVV%2BEMfXD%2BLqRsz%2BmN1f%2BizPeM).

Daily pluviometry data was obtained from stations localized in the study area, managed by the Institute of Environment and Hydric Resources of Bahia (INEMA). During animal processing, demographic data (sex, age, reproductive status) and body condition (presence and level of wounds and fat deposits, as well as the Scaled Mass Index [SMI] [[45]](https://paperpile.com/c/vdxkIc/4JaPp) – which accounts for the animal’s age). Feces were collected directly from the large intestine and stored in formaldehyde 10% for posterior identification of the target nematode.

Fecal sample analysis were divided in qualification and quantification stages, with sample processing following Carvalho-Pereira et al. [[18]](https://paperpile.com/c/vdxkIc/izPeM). During the qualitative stage, we employed an adapted sedimentation method [[46]](https://paperpile.com/c/vdxkIc/cgyJH), with larval identification occurring based in morphologic characteristics observed in optic microscopy, where the suspicion of occurrence of metastrongilid nematodes was indicated by observing the tail conformation, a particular characteristic of this parasite group [[2,47–49]](https://paperpile.com/c/vdxkIc/l0QIX%2By0PIA%2BfzmtR%2BtkXQY). We also identified eggs of other parasites based in literature [[50–52]](https://paperpile.com/c/vdxkIc/i2y8q%2BFBHzF%2Bd8P8W). To confirm species identifications, we verified the occurrence of adult worms by collecting target tissues from the rodents captured during one of the campaigns, such as intestines, stomach, and lungs, fixated in AFA at 50°C and stored at 4°C. This allowed for the confirmation of occurrence of *A. cantonensis* in captured *R. norvegicus* lungs, as well as the other helminth species included in the study (see Additional file 2: Table S2). The quantitative analysis followed the qualitative, consisting in an adapted flotation method [[53]](https://paperpile.com/c/vdxkIc/ApcMT), using a saturated Zinc Sulphate solution with density of 1.18g/cm³ [[54]](https://paperpile.com/c/vdxkIc/snrjS) – for *A. cantonensis* counting in larvae per gram of feces (LPG) and for the other helminth eggs identified in eggs per gram of feces (EPG).

1. *Statistical analysis*

Descriptive statistics were employed to present the occurrence and abundance of mollusk species, as well as the prevalence and intensity (number of larvae) in each mollusk species identified. Posteriorly, the prevalence of *A. cantonensis* in rats was estimated for each sampling campaign and period, with comparisons made by chi-squared tests.

We fitted models to verify the determinants for occurrence and quantity of gastropods for the species confirmed to be intermediary hosts for A. cantonensis, according to the PCR-RFLP. To model the risk factors associated to the occurrence of terrestrial mollusks in the study are, we used Zero-inflated negative binomial models using *pscl* [*[55,56]*](https://paperpile.com/c/vdxkIc/Ga1A%2Bahsz), with α= 0.05. Univariate analyses were performed with each environmental variable, with their inclusion in the multiple models when were considered at least marginally associated (p ≤ 0.1) (see Additional file 3: Table S3).

To identify risk factors associated to the presence and intensity of *A. cantonensis* in rats, we developed regression models for the groups of independent variables. Those being environmental, demographic and of co-infection (see Additional file 2: Table S2). We ran zero-inflated generalized models with *Gamma* distribution and response variables in base 2 logarithm to facilitate result interpretation [[18]](https://paperpile.com/c/vdxkIc/izPeM). Modelling followed three steps: (1) univariate analysis of the environmental variables, including in the multiple model the variables with marginal association to *A. cantonensis* infection (p ≤0.1); with the multiple model using α= 0.05; (2) Selected variables were inserted in the new model that considered the contribution of demography and body condition of rats susceptible to *A. cantonensis*; (3) Finally, *a posteriori* to the comprehension of how environment and individual characteristics could influence the rate of infection in rodents, we modelled how the richness of coinfected helminths or the probability and intensity of other helminth species coinfections, individually, are associated with the presence and intensity of *A. cantonensis* infestation. All models were performed using the *pscl* package onR [[57]](https://paperpile.com/c/vdxkIc/eJ2Op).

Final models were defined for terrestrial mollusks and rats using model selection criteria based on Akaike’s Information Criterion corrected for small samples (AICc) [[58,59]](https://paperpile.com/c/vdxkIc/AxtCP%2BUGyvv), with the help of the *MuMIn* package, which ranks models by AICc based in the importance of each explanatory variable included [[60,61]](https://paperpile.com/c/vdxkIc/a7kCC%2Bj5VhM). Thus, it was possible to identify all plausible models (ΔAICc ≤ 2.00) and the most parsimonious, this being the model with the least explanatory variables within plausible models (see Additional files 4-5: Tables S4 and S5).

**RESULTS**

1. *Terrestrial mollusks*

We collected 577 gastropods, widely distributed in all valleys of the area studied, representing eight species from seven families. Most common species were *Subulina octona* (Bruguière, 1789) (47.5%), *Sarasinula marginata* (30%), *Achatina fulica* (25%) and *Bulimulus* sp. (22.5%), with *S. octona* and *A. fulica* (278 and 96 individuals respectively) were the most abundant in the samples. The co-occurrence of the four species was common in the sampled areas, and only this group of species presented larval infection (Fig. 1b). *Bradybaena similaris* (Férrusac, 1821), *Drymaeus papyraceus* (Mawe, 1823), *Helicina* sp. and a representant of the family Streptaxidae had lower occurrence and abundance, presenting an average of five individuals per sample point.

The larvae morphologically identified as part of the metastrogilidae were sent for *PCR-RFLP*, allowing to estimate the specific prevalence of *A. cantonensis* of each collected gastropod species by sampling point. *A. fulica* (33%), *Bulimulus* sp. (11%), *S. marginata* (8%), *S. octana* (5%). *A. fulica* presented the highest number of larvae per individual, with an average of 22.66±σ22.54. the remaining species presented lower levels of infection, with only one individual of *S. marginata* infected with four larvae. *Bulimulus* sp. and *S. octona*, normally analyzed in pool, presented proportion of 0.5 and 0.3 larva per individual, respectively.

Occurrence and abundance of mollusk species in the area of study were associated to different local environmental variables, specially to proxies to water presence (see Additional files 3-4: Tables S3 and S4). Amongst the predictors, the humidity and accumulated rain presented positive associations with the abundance of *A. fulica* and *S. octona,* respectively. Presence of construction materials was positively correlated to the number of *S. marginata* individuals sampled. Also, for this species, valleys 2 and 3 presented significantly smaller abundances compared to valley 1. In univariate models (see Additional file 3: Table S3), the presence of vegetation is the predictor to the abundance of *S. octona*, making areas with herbaceous vegetation favorable to high numbers of *S. octona* individuals*.* On the other hand, areas with shrub vegetation presented higher *Bulimulus* sp. numbers. The model including solely sampling effort (EA) – our control variable – was significantly associated to the presence of *Bulimulus* sp*.,* indicating that a 3-day sampling effort was associated with a higher chance of finding individuals of *Bulimulus* sp., compared to two-day efforts (Table 1). Given the reduced number of samples infected by *A. cantonensis* (n = 7) it was not possible to analyze the risk factors associated to the presence and intensity of infection by the helminth in the collected gastropod species. All 17 points sampled both for rodents and mollusks were positive for both taxa. Of those, 45% presented rats positive for *A. cantonensis* (Fig. 1b).

1. *Rodents*

A total of 168 *R. norvegicus* were trapped in the four sampling campaigns. Amongst them, sub-adults (103; 61.3%) were captured more frequently than the young (35; 20.8%) and adults (30; 17.9%) [[20]](https://paperpile.com/c/vdxkIc/k7KVV), with more females (90; 53.9%) than males (77; 46.1%); and a similar number of captures between dry (85; 50.6%) and rainy seasons (83; 49.4%). First-stage *A. cantonensis* larvae were detected in 56 (33.3%) individuals, with no significant difference between sampling campaigns (p = 0.3603). Besides *A. cantonensis*, another seven helminth species already recorded by Carvalho-Pereira et al. [[18]](https://paperpile.com/c/vdxkIc/izPeM) were identified, of which, the soil-transmitted nematode *Strongyloides* spp. (160; 95%) and *Nippostrongylus brasiliensis* (Travassos, 1914)(52; 30.9%)were the most prevalent.

Among environmental risk factors, those related to water and shelter (construction materials) were associated to the probability and intensity of *A. cantonensis* infection in rats (Table 2). Accumulated rainfall in the last two weeks, as well as the presence of construction materials, were positively associated to the increase of *A. cantonensis* in *R. norvegicus*, relations that were maintained when added to the demographic and body condition variables model (Table 2 - step 2; accumulated rain: Rate 1.000, IC95% 0.999 - 1.001; construction materials: 1.333, IC95% 1.056 - 1.693). In this model, body condition of the individuals was associated to the probability of infection: rats with larger SMI presented significantly smaller chances of being infected by *A. cantonensis*. Contrarily to what was expected, however, the increase of age (in days) was associated negatively to the infection intensity(Table 2). The final model (step 3), including coinfection variables, kept body condition as the sole significantly associated factor linked to probability of infection. However, in terms of infection intensity, besides the accumulated rain, which stayed positively associated to an increase of *A. cantonensis* intensity, co-infecting nematode species presented significative associations.

**DISCUSSION**

This study allowed to observe that environmental conditions that contribute to the occurrence of terrestrial gastropods (intermediary hosts) are also indicators that rats (definitive hosts) are infected with the parasite *A. cantonensis* in urban slums. The establishment and maintenance of the transmission cycle of *A. cantonensis* were expected, as said conditions (available water and accumulation of inorganic matter such as construction materials) create microhabitats that favor mollusk abundance, which can represent a food source for rats in shared environments. Also, the occurrence of different mollusk species in most of the studied area, and not the seasonal difference in rat prevalence throughout two years, suggest that the cycle of the target helminth occurs in an ample and continuous fashion. The presence of the *A. cantonensis* reproductive cycle alerts to the necessity of preventive action, given the severity of human neuroangiostrongyliasis in spite of the lack of recorded cases of the disease in the community at this moment. The lack of notification could be linked to the sub diagnosis, given that the main symptoms (e.g.: headache, pain in the nape of the neck and fever) [[3]](https://paperpile.com/c/vdxkIc/GUqaU) can be easily mistaken with other infirmities that co-occur in urban slums with elevated abundance of rats and mollusk richness.

Several mollusk species are described in literature as susceptible to *A. cantonensis* infection, as well as being capable of transmission. However, usually few species are considered as the main intermediary hosts in a given region [[62]](https://paperpile.com/c/vdxkIc/Ay5LE). In the study area, we detected high species richness and occurrence of mollusks compared to other localities in Brazil and the world [[15,32,63]](https://paperpile.com/c/vdxkIc/TMj5c%2BVzvbX%2Bvo4Ws). The presence of the species *S. octana, A. fulica*, *S. marginata*, *Bulimulus* sp. and *Bradybaena* *similaris* (all characterized as *A. cantonensis* hosts) indicate the occurrence of more contamination foci, given that these species occupy different habitats, which is consistent with the findings of Kim et al. (2014) [[32]](https://paperpile.com/c/vdxkIc/VzvbX). This is the first record of the genus *Bulimulus* as a natural intermediary host of *A. cantonensis* in the state of Bahia. Specimens of *B. similaris*, although collected and previously detailed as hosts [[15,64,65]](https://paperpile.com/c/vdxkIc/TMj5c%2BWBunu%2BHakla), had no positive results *A. cantonensis* infection in this study. On the other hand, we can highlight the diversity of positive intermediary host species, together with the co-occurrence with more than one species infected by *A. cantonensis* in a same sampling point, could favor the development of larvae for the infectant phase, which could promote continuous dissemination.

The selected environmental variables considered risk factors for mollusk abundance reflect the physiologic and ecologic needs of this invertebrates. However, local small-scale landscape characteristics (e.g.: temperature, soil porosity and pH) can influence the presence of these species. This set of characteristics can proportionate microclimates that favor better adaptation, survival and reproduction of terrestrial mollusks, as well as providing favorable areas for rodent populations, as those compose favorable climate and food supply [[66–68]](https://paperpile.com/c/vdxkIc/jPaRK%2BhXKDT%2Byi0Ie). This set of variables related to environment and climate can modulate the distribution patterns of host species and parasite positivity, such as what is observed in the spatial distribution of highly infested rats and the presence of positive mollusks, although geospatial analyses are still needed. Different non-measured characteristics of each measured valley contribute to the non-proportional distribution of mollusk species. Compared to valleys 2 and 3, valley 1 presented (visually) more precarious conditions and a higher presence of potential faunal harborage (e.g.: construction material), which can contribute to the abundance of *S. marginata* in the environment. A micro-habitat analysis could be a more suitable approach to understand the life-history of the recorded mollusk species, as the populations vary and/or depend on microclimates and nutrients, due to their low mobility and high susceptibility to predation [[32,69]](https://paperpile.com/c/vdxkIc/Cxw86%2BVzvbX).

The identification of risk factors for the presence and abundance of terrestrial mollusks in the study area could be limited by the sampling size, given that the variable “sampling effort”, initially used as a control, highlighted that a 3-day sampling protocol guarantees higher odds of finding individuals compared to two-day samplings. Despite its apparent obviousness, this finding needs to be stressed, as it could significantly influence the diagnostics of the presence of intermediary *A. cantonensis* hosts documented so far, which do not discriminate sampling effort. This phenomenon could be exemplified by the presence of *Bulimulus* sp., as the increase in sampling effort could increase the chances of finding individuals of this species. Other factors that could have limited the identification of environmental risk factors for the evaluated mollusk species include the fact that some of the identified species are generalists, occupying several habitats that vary from humid environments to dry and hot areas; as well as some species not being present in the entire study area, probably given to specific variables that were not measured [[70–73]](https://paperpile.com/c/vdxkIc/WVlQ0%2BJ3F44%2BlzHED%2BPJ5lz). *A. fulica*, one of such generalist species, was the most abundant and presented the highest prevalence of *A. cantonensis*, as previously seen in literature [[63]](https://paperpile.com/c/vdxkIc/vo4Ws). This fact could be associated to the fact that *A. fulica* is an invasive species in Brazil, without a specific niche and capable of exploring different habitats. However, humid habitats contribute to its increased abundance, which can increase the chances of infection by *A. cantonensis* [*[32]*](https://paperpile.com/c/vdxkIc/VzvbX). Amongst the species with specific characteristics, we highlight *Sarasinula marginata,* who depends on permeable soil (as determined in this study, see Additional file 3: Table S3)), and *Bradybaena similaris*, with arboreal habits, and thus commonly associated to this type of vegetation. Thus, variables such as temperature, relative humidity or type of soil can be good predictors to determine the presence of terrestrial mollusks. It is possible that the choice of variables in a finer environmental scale, more appropriate to each species of interest, could be sensitive enough to identify their risk factors, something only achievable after identifying the species that occur in the area.

The prevalence of *A. cantonensis* recorded in our samples of the *R. norvegicus* population (33% of 116 captured specimens) was relatively larger than compared to other countries in Asia and the Americas, such as China (21% of 351 examined rats) and the United States (21% of 94 sampled rats) [[62]](https://paperpile.com/c/vdxkIc/Ay5LE). When comparing to other localities in Brazil, the prevalence was higher than in the state of Pará (11% of 19 examined rats) [[17]](https://paperpile.com/c/vdxkIc/mGktM) but smaller than in Rio de Janeiro (71% of 114 examined rats) [[74]](https://paperpile.com/c/vdxkIc/orcd0). Nonetheless, when comparing a study performed in the same area of Salvador (40% of 299 captured rats in two seasons of the same year) [[18]](https://paperpile.com/c/vdxkIc/izPeM), the prevalence observed in this study was relatively smaller, although stable, taking in consideration that our captures were performed in the span of two years. Also, the prevalence values were not statistically different between campaigns or seasons. This seasonal equity detected indicates towards a continuous infestation of *A. cantonensis* on the environment through rat feces, and potential year-round transmission, corroborating with the results of Simões et al. (2014), in a two-year longitudinal study in São Gonçalo, state of Rio de Janeiro [[74]](https://paperpile.com/c/vdxkIc/orcd0). The climate of our study area is tropical, the city presents an annual average temperature of 25.2ºC, with maximum and minimum temperatures around 31ºC and 22ºC, respectively; its rainy season spans from March to August, and the dry season from August to February. Although one season is considered the “dry” season, there is significant rainfall throughout the year, with an annual precipitation average of 1781 mm [[75]](https://paperpile.com/c/vdxkIc/UTyf). This scenario relatively similar to what is observed in São Gonçalo, where a higher prevalence of *A. cantonensis* was recorded [[74]](https://paperpile.com/c/vdxkIc/orcd0), despite a considerably smaller sampling and no analysis evaluating the associations between environment and the presence of *A. cantonensis* in rodents.

Regarding the demographic and body condition analyses, the negative association between age or the rodent and intensity of *A. cantonensis* infestation indicate that younger rats present higher rates of infection than older individuals (age in days). As the present study uses cross-sectional data, a possibility is that older rats have been previously exposed to the nematode, and, consequently, have developed the capacity to modulate the parasite load when re-infected, presenting a smaller infection rate [[76]](https://paperpile.com/c/vdxkIc/ARqER). Lower body condition, represented by the SMI, was associated to an increase in the probability of infection by *A. cantonensis*. Thus, rats with lower body condition had higher odds of being infected, either by the reduction of immune response in adipose tissue, given the reduced bodily fat stores, affecting their response to the pathogen [[77,78]](https://paperpile.com/c/vdxkIc/SsTTa%2BQXtUN). Alternatively, coinfections with other parasites can influence body condition, contributing indirectly to the increase of *A. cantonensi*s infection.

The ubiquity of rats in urban slums, and their lack of seasonal variation in abundance [[23]](https://paperpile.com/c/vdxkIc/mN1f) make these animals highly susceptible to parasite (co)infections [[18,79]](https://paperpile.com/c/vdxkIc/izPeM%2BxFowG). The excretion of said parasites in the environment can be controlled by intra and interspecific interactions that regulate the presence and intensity of a parasite species in relation to another [[79]](https://paperpile.com/c/vdxkIc/xFowG). The positive association on the coinfection of *Strongyloides* sp. and *A. cantonensis*, and the negative association between the intensity of *N.* *brasiliensis* and *A. cantonensis* in the rat population were stable in time [[18,79]](https://paperpile.com/c/vdxkIc/izPeM%2BxFowG). However, when analyzing rats stratified by sex, the pattern observed changes in females, with an observed positive association between the intensity of *N. brasilienses* and *A. cantonenis*. Pregnant or lactating females can allocate less resources to fend off parasites, thus being more prone to infections and coinfections [[80–82]](https://paperpile.com/c/vdxkIc/AoNDO%2Bd8Wy4%2BbIdX3).

This study was carried in an urban slum Community, which presents an environment characterized by precarious sanitation services and lacking urban infrastructure such as pavement, and the presence of points of construction materials [[23,83]](https://paperpile.com/c/vdxkIc/OOfHF%2BmN1f). Although the accumulation of construction materials was not kept in the final model (that includes coinfection) for *A. cantonensis* infection in rats, this was considered a risk factor for mollusk abundance, probably by fostering the formation of microhabitats and harboring the gastropods. The absence of pavement was not characterized as a risk factor for rat infection or for the abundance of mollusks, despite it being previously described as an important factor for the establishment of synanthropic rodent populations, given their habit of digging burrows in soil [[84]](https://paperpile.com/c/vdxkIc/nlNxh). The absence of pavement can also contribute to the accumulation of Rainwater in the soil, a risk factor detected by our study. The accumulated rain of the previous two weeks was an important predictor for intensity of infection by *A. cantonensis* in rats, as well as being considered a risk factor for the abundance of mollusks, allowing for humid, propitious environments for the mollusks. Regardless the seasonal equity found in this study, factors associated to water seem to be related to the cycle of *A. cantonensis*, given that accumulated rain in the previous weeks was important to predict the intensity of infection in rats. This could be related to a potential contribution of this phenomenon to the viability of *A. cantonensis* larvae in the environment, and favor infection in intermediary hosts (by contact with water contaminated with rodent feces) as well as in rats (by ingestion of contaminated water or infected mollusks).

The integration of ecological mollusk and rodent data can be the key to understand the establishment and transmission of *A. cantonensis* in human populations. The differences in environmental conditions within the urban landscape, especially in areas with precarious sanitation, living and infrastructure conditions which facilitate the existence of microhabitats [[68,85]](https://paperpile.com/c/vdxkIc/xj3RP%2Byi0Ie), affecting the terrestrial mollusk assemblage on its composition, abundance and distribution. Microhabitats that favor an elevated abundance of mollusks can contribute to the increase of the invertebrate hosts get in contact with the parasite, and consequently increase the number of infected rats. Contrastingly, if environmental changes are promoted, the tendency is to upset the equilibrium of the microhabitats, and consequently, the target animal populations [[86]](https://paperpile.com/c/vdxkIc/tO0B1), likely resulting in reduced *A. cantonensis* transmission. This highlights the importance of carrying structural and sanitation interventions to help prevent the transmission of *A. cantonensis* between hosts and to the inhabitants of the area, with both local and short-span measures (e.g.: cleaning yards and empty lots, reduction of the construction material) and long-span actions (e.g.: structural urban interventions that reduce water accumulation in the environment, such as street pavement) can disrupt the parasite cycle already established in areas defined by such characteristics.

**CONCLUSIONS**

Our study indicates that the transmission cycle of the rat lungworm *A. cantonensis* is well established in an urban slum community in Brazil, given the evidences of its presence in definitive hosts and five species of intermediary hosts. In this context, the prevalence of the nematode in Brown rats was relatively high, with no significant differences in prevalence between seasons. This is the first records of *A. cantonensis* in mollusks from the genus *Bulimulus* in the state of Bahia. Regarding the risk factors associated to the abundance of mollusks, as well as the environmental factors associated to the presence and intensity of *A. cantonensis* infection in rats, water presence (running water, puddles, environmental humidity and accumulated rain) and the accumulation of construction materials. These conditions promote the establishment and maintenance of the parasite in the environment, given the elevated abundance of susceptible mollusks and rats. Our findings indicate the urgent necessity of the involvement of government entities in the execution of structural and sanitation interventions, as well as the involvement of public health and zoonosis control agencies that can inform and guide the residents with educational and participative action about the risks associated to *A. cantonensis* and to the potential of mollusks and rats as hosts of an array of potential zoonotic parasites. Thus, not only neuroangiostrongyliasis would be prevented, but other relevant zoonosis, such as leptospirosis (transmitted by rat urine) and schistosomiasis (transmitted by snails).

**LIST OF ABBREVIATIONS**

**IAL:** Instituto Adolf Lutz

**PCR-RFLP:** polymerase chain reaction-restriction fragment length polymorphism

**Smi:** scaloned mass index

**DECLARATIONS**

**Ethics approval and consent to participate**

The study related to the rodents sampling used the protocol 003/2012, which was approved by Ethical Committee of the Animal Use (CEUA), of the Gonçalo Moniz Institute (IGM) – Oswaldo Cruz Foundation (Fiocruz). The license for the collection of terrestrial mollusks in an urban area of Salvador-Bahia was provided by the Chico Mendes Institute for Biodiversity Conservation (ICMBio), a federal agency linked to the Brazilian Ministry of the Environment. None of the collected specimens belong to threatened or protected species.

**Consent for publication**

Not applicable.

**Availability of data and materials**

Data supporting the conclusions of this article are included within the article and its Additional files 1, 2, 3, 4 e 5. Furthermore, the datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Contributions**

FNS, MAS and DAA performed mollusks sampling in the field and environmental data. FNS, TCP and ACP performed rodents sampling in the field and environmental data. LCVM, DJGM, RG and PLSP provided the modified methodology for processing mollusks samples and performed *A. cantonensis* identification in mollusks. FNS, MAS, DAA conducted the processing of mollusk and rodent samples. FNS, MAS, DAA e TCP performed helminths identification in rodents, with supervision of TCB. FNS, TCP, TCB, FC, CGZ, MGR, AIK, MB e PLSP contributed to the design and definition of data analysis. FNS, MAS e TCP analyzed the data. FNS e MAS drafted the manuscript. TCP, FC, TCB, LCVM, DJGM, CGZ, MGR, AIK e MB critically reviewed the manuscript. All authors read and approved the final manuscript.

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**SUPPLEMENTARY INFORMATION**

**Additional file 1: Table S1**. Environmental and biotic (demography and body condition of rats) variables collected during trapping of animals.

**Additional file 2: Table S2.** Univariate Zero-Inflated models by stages for the presence and counting of Angiostrongylus cantonensis in rats.

**Additional file 3: Table S3**. Univariate Zero-Inflated models for the presence and count of terrestrial mollusks associated with environmental conditions.

**Additional file 4: Table S4**. Selection models of risk factors associated with infection of Angiostrongylus cantonensis infection in terrestrial mollusks.

**Additional file 5: Table S5**. Selection of multilevel models of risk factors associated with Angiostrongylus cantonensis infection in Rattus norvegicus.