**BRIEF COMMUNICATION**

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**Factors affecting carriage and intensity of *Calodium hapaticum* within Norway rats (*Rattus norvegicus*) from an urban slum environment of Salvador, Bahia, Brazil**

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Abstract

Urban slums environments in the tropics are conducive to the proliferation and the spread of rodent-borne zoonotic pathogens to humans. *Caloduim hapaticum* (Brancroft, 1893) is a zoonotic nematode known to infect a variety of mammalian hosts, including humans. There is a lack of studies systematically evaluating the role of demographic and environmental factors in determining carriage of *C. hapaticum* in rodents from urban slum areas within tropical regions. Carriage and intensity of *C. hapaticum* in Norway rats (*Rattus norvegicus*) were studied over a two-year period in an urban slum in Salvador, Bahia, Brazil. We evaluated 402 Norway rats and 5 black rats (*R. rattus*). Overall prevalence in Norway ratsand black rats was 83% (337/402) and 80% (4/5), respectively. Independent risk factors for *C. hapaticum* carriage in *R. norvegicus* were age-group and location of capture. Of those infected the proportion with gross liver involvement, over 75% of the liver affected, was low (8%; 26/337). Soil samples (6/10) were contaminated with *C. hapaticum* at the sites. High carriage levels of *C. hapaticum* within the Norway rats and sub-standard living conditions within slum areas increases the risk to humans of exposure to the infective eggs of *C. hapaticum*. Norway Rats are a major species harbouring *C. hapaticum* within Salvador and are therefore a useful species to inform estimates of the risk to humans living in urban slum environments.

**KEYWORDS:** *Rattus norvegicus; Calodium hepaticum*; Prevalence; Infection intensity; Brazil

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Within tropical urban slums, characterized by low-level infrastructure, inadequate sanitation, and poor access to safe water, conditions contribute to the transfer of zoonoses to humans. The global numbers of slum dwellers is expected to reach 1.4 billion by 2020, with Latin America and the Caribbean having 110.7 million (23.5%) of its population living in these sub-standard conditions. Tropical urban areas are therefore important environments in which to study zoonoses, in an attempt to quantify the risk zoonoses pose to humans, and to develop approaches that prevent and control the spread of disease to inhabitants of urban slums.

Within the tropics of Brazil, the contribution of inadequate living condition to the spread of harmful zoonoses, has already been well documented by studies of leptospirosis (Costa et al., 2014). Many other infectious organisms, however, exist within these urban areas, capable of posing substantial risk to the health of the residing human population. *Calodium hapaticum* (Brancroft, 1893) (syn. *Capillaria hepatica*, *Tricocephalus hepaticus*, *Hepaticola hepatica*) is a globally distributed (Singleton et al., 1991), zoonotic nematode that causes hepatic capillariasis in humans (Fuehrer et al., 2011) but is understudied in tropical urban environments. Most research concerning *C. hapaticum* has been restricted to temperate areas (Farhang-Azad, 1977a; Farhang-Azad, 1977b; Childs et al., 1988). *Calodium hapaticum* has over 180 documented mammalian hosts, with the Norway rat, *Rattus norvegicus* considered to be the most important (Fuehrer, 2013a; Fuehrer, 2013b). Prevalences of *C. hapaticum* have been reported as high, 87.4% in Baltimore, USA and > 50% within populations of *R. norvegicus* sampled in Salvador, Brazil (Galvão, 1976). *Calodium hapaticum* infects the liver of its host and requires the death of its host to release the unembryonated ova into the environment (Farhang-Azad, 1977a). With sufficient time and suitable conditions of temperature, oxygen tension and humidity, eggs embryonate after five to seven weeks and then are infectious when consumed (Farhang-Azad, 1977b) by ingesting contaminated soil, water, or vegetation (Fuehrer et al., 2011).

The result is hepatic infection, where *C. hapaticum* invades the hepatic parenchyma causing visible inflammatory lesions along with necrosis and fibrosis of the liver. Humans usually present with a characteristic triad of symptoms; high fever, liver enlargement and severe eosinophilia. In serious cases infection can be fatal. Unembryonated eggs are passed through the digestive tract and excreted in the faeces. Both infection types in humans are associated with unsanitary living conditions and high prevalence of *C. hepaticum* in synantrophic rat populations (Fuehrer et al., 2011). Over 70 documented cases of human hepatic infection have been reported worldwide, a figure widely thought to be underestimated. Most cases are reported in children (Fuehrer et al., 2011).

Studies of *C. hepaticum* in Norway rats from urban areas in the tropics have served only to determine the prevalence within rat populations, or focused on histopathological effects of the parasite on its host (Galvão 1976, Rocha 2015, Simões 2015). Systematically evaluating the role of interactions of demographic and environmental factors in determining infection of *C. hapaticum* within Norway rats is necessary to evaluate the risk of infection for humans living in urban slum environments. Thus, the present study investigates how such factors affect the carriage and intensity level of infection of *C. hapaticum* in Norway rat populations from an urban slum environment of Salvador, Brazil. Additionally, levels of environmental contamination were assessed in an attempt to better understand the relationship between carriage of *C. hepaticum* in ratsand environment contamination.

Methods and Materials

From 2013-2014 Norway rats were captured from the neighborhood of Pau da Lima in Salvador, Bahia, Brazil (13°32’53.47’’ S; 38°43’51.10’’ W). The area has three distinct valleys (1, 2 and 4). These, and the methods of live trapping of rats using Tomahawk® traps (40.6 x 12.7 x 12.7 cm) were as previously described in Costa et al.( 2014). Recorded weights of the animals were converted to ages (days) using the von Bertalanﬀy equation (Burthe *et al*. 2010) using data in Colhoun (1962). Also, we estimated body condition using a ‘Scaled mass index’ (Smi) based on mass and body length, whilst accounting for the effect of age (Peig and Green, 2009). Environmental variables including location, proximity to open sewage, number of trees and number of alternative hosts of *C. hapaticum*, were recorded at each trap location at the time of live capture.

The presence and absence of *C.hepaticum* were evaluated through macroscopic examination of the liver during necropsies with the presence of *C. hapaticum* beingnoted based on visible yellowish-white lesions on the liver surface and scored as previously described (Farhang-Azad, 1977b; Childs et al., 1988). Percentage of the liver surface covered with lesions was used as a proxy for intensity level of infection and was classified as slight (1–25%), low (26–50%), heavy (51–75%) or gross liver involvement (75–100%). The Institutional Animal Care and Use Committees at the Oswaldo Cruz Foundation, Salvador, Brazil (003/2012) and Yale University in the United States (2012–11498) approved all animal procedures and methods.

Additionally, soil samples were evaluated for evidence of environmental contamination of *C. hapaticum*. Ten locations previously used to trap rodents were randomly selected and six samples of soil (30g) were collected at each location during August 2015. Samples were processed as previously described in Sudhakar et al. (2013).

Statistical Methods

Logistic regression models were performed using R 2.11.1, to examine the relationship between carriage of *C. hepaticum* and explanatory variables. An ordinal logistic regression was used to examine the relationship between infection level of *C. hepaticum* and the same variables. For multi-variate analysis, variables associated in univariate models with a p<0.2 were included and the models were judged based on Akaike information and all models with AIC scores within two of the lowest score (Crawley2007), evaluated and discussed- (see supplementary tables S2 and S3).

Results and Discussion

The carriage of *C. hepaticum* was assessed for 407 synanthropic rats. The carriage level was 83% (337/402) in Norway ratsand 80% (4/5) in black rats. In univariate analyses, carriage tended to increase with age, location (valley) of capture, proximity (within 10m) to an open sewer and the number of dogs recorded at households next to capture locations. Multivariate analysis supported strongly the inclusion of age and location, some support for proximity to a sewer but only weak support for the presence of dogs (Table 1, Supplementary Table S2). Of individuals that were sexually mature i.e. 65 days and over (Clark & Price 1981) 91% (235/261) of the individuals were infected compared to 69% (97/141) who had not yet reached sexual maturity. The increased probability of infection in older individuals may be due to higher levels of activity and therefore increased likelihood of coming into contact with infective ova, but may simply be the result of cumulative exposure. Once the parasite has infected the hepatic parenchyma infection remains for the duration of the individuals’ life time (Farang-Azad 1977a). In valley 1, 81% (65/80) of individuals of *R. norvegicus* were infected compared to 92% (144/157) captured in valley 2 and 75% (123/165) in valley 4.

In the univariate analysis, the odds of being infected with *C. hepaticum* for individuals captured within 10m of an open sewer, was 1.97 times that of individuals captured at a distance greater than this (Table 1.). Open sewers run through the bottom of valleys and it is likely that heavy rainfall, typical in the region between the months of April-July, could wash infective ova into low lying areas, increasing the environmental concentration of ova and therefore the probability of infection. The number of dogs within households next to capture locations increased the odds of carriage within individuals of *R. norvegicus* (Unadjusted OR (95% CI) 1.35 (0.98–1.85)). With dogs being an alternative host of *C. hepaticum*, an increase in their numbers would increase overall host density, and this may be contributing to the maintenance of higher carriage levels at these capture locations. (Fuehrer 2013b)

Turning to intensity, of the infected individuals, 71% ( 237/337) were classified as having a low infection level (<25% liver involvement) and only 8% (26/337) had gross liver involvement. Multivariate analyses showed the numbers of cats and location of capture to be related to infection intensity. Higher numbers of cats corresponded with lower levels of infection intensity in *R. norvegicus,* suggesting cats are acting as a protective factor within this system (Supplementary Table S1.), potentially reducing the density of rats through predation. The odds of individuals possessing higher levels of infection intensity was significantly greater in valley one compared to those in valleys two (Adjusted OR (95% CI) 0.318 (0.157–0.640)) and four (Adjusted OR (95% CI) 0.255 (0.118–0.539)) (Supplementary Table S1). This difference between valleys has no obvious explanation. Previous work has shown higher levels of *C. hapaticum* carriage within population to be associated with higher the levels of infection intensity (Farhang-Azad 1977a), but carriage level was not significant different between valleys in either the univariate or multivariate models. It is possible that unexamined factors, including the effects co-infection with other parasites and the health of the individual host,s could be contributing to the observed result.

Soil sampling at six locations at 10 sites each revealed the proportion of samples contaminated with *C. hepaticum* was low (10%, 6/60) as were the number of eggs found in positive samples (mean 3.25, range 1-7). These results suggest *C. hepaticum* is broadly distributed within the area but at low environmental concentrations. With regards to this environmental contamination, a more systematic evaluation of the environmental levels of *C.hepaticum*, would be necessary to determine how the prevalence of *C. hepaticum* in rats translates to the levels in the environment, thus providing a better indication of the risk to humans of acquiring this infection. It is also possible that infected rats are eaten by other animals (dogs, cats, etc) prior to decomposition, affecting the distribution of environmental *C. hepaticum*. Therefore additional evaluation of alternative and intermediate hosts would also be of benefit.

Conclusions

This study demonstrates the role of specific demographic and environmental factors in determining both the carriage and intensity levels of *C. hepaticum* in *R. norvegicus* in an urban slum Additionally the confirmation of environmental contamination supports the theory that this parasite is posing a risk to human health. This is the first published report of *C. hepaticum* prevalence in *R. norvegicus* from Salvador since 1976 when carriage in captured rats was at 57% (Galvão 1976). The carriage level of 83% determined by this study is higher than the most recent documentations of *C. hepaticum* in *R. norvegicus* from cities in Brazil; Rio de Janerio- 47% (Simões *et al.* 2014), Porto Velho -2% (Rocha *et al.* 2015).

There is a lack of epidemiological studies of *C. hepaticum* in humans and such studies are needed given the high levels of *C. hapaticum* transmission within the rat population. High population level carriage of *C. hepaticum* within *R. norvegicus*, combined with the suitable environmental conditions for egg embryonation and living conditions that are conducive with the exposure of humans to infective eggs, means *R. norvegicus* are likely serving as an important source of zoonotic disease within this community and pose a significant risk to human health.

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REFERENCES

Burthe SJ, *et al.* Individual growth rates in natural field vole, *Microtus agrestis*, populations exhibiting cyclic population dynamics. *Oecologia*, 2010 **162**:653–661

Calhoun, JB. 1962 *The ecology and sociology of the Norway rat*. Public Health Service, Bethesda, Md..

Costa F, *et al.* Infections by Leptospira interrogans, Seoul virus, and Bartonella spp. among Norway rats (Rattus norvegicus) from the urban slum environment in Brazil. *Vector borne and zoonotic diseases (Larchmont, N.Y.)* 2014, **14**: 33–40.

Childs JE, Glass GE, Kroch JR. The comparative epizootiology of *Capillaria hepatica* (Nematoda) in urban rodents from different habitats of Baltimore, Maryland, *Canadian Journal of Zoology* 1988, **66**: 2769-2775

Clark BR, Price EO. Sexual maturation and fecundity of wild and domestic Norway rat (*Rattus norvegicus)*. *The Journal of Reproductive fertility* 1981*,* **63**: 215-220

Crawley M J.*The R Book*. 1st. ed. Chichester: John Wiley & Sons, Ltd., 2007

Farhang-Azad A. Ecology of Capillaria (Bancroft Mechanisms II. Egg- releasing). *The Journal of Parasitology* 1977a, **63**: 701–706.

Farhang-azad A. Hepatica of Capillaria ecology among of infection of the Balitmore Norway rat population Maryland. *The Journal of Parasitology* 1977b, **63**: 117–122.

Fuehrer HP, Igel P, Auer H. Capillaria hepatica in man-an overview of hepatic capillariosis and spurious infections. *Parasitology Research* 2011, **109**: 969–979.

Fuehrer HP . An overview of the host spectrum and distribution of Calodium hepaticum (syn. Capillaria hepatica): Part 1 - Muroidea. *Parasitology Research* 2013a, **113**: 641–651.

Fuehrer HP. An overview of the host spectrum and distribution of Calodium hepaticum (syn. Capillaria hepatica): Part 2 - Mammalia (excluding Muroidea). *Parasitology Research* 2013b, **113**: 641–651.

Galvão VA. Capillaria hepatica, estudo da incidência em ratos de Salvador, Bahia, e dados imunopatológicos preliminares. *Revista da Sociedade Brasileira de Medicina Tropical* 1976, **10**: 333–338.

Hardy AR, Quy RJ, Huson WL. Estimation of Age in the Norway Rat (*Rattus norvegicus* Berkenhout) from the Weight of the Eyelens, *Journal of Applied Ecology* 1983*,* **20**: 97

Peig J, Green AJ. New perspectives for estimating body condition from mass/length data: The scaled mass index as an alternative method. *Oikos* 2009,**118**: 1883–1891.

Rocha JE, *et al.* Study of the prevalence of *Capillaria hepatica* in human and rodents in an urban are of the city of Porto Velho, Rondônia, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo* 2015, **57**: 39–46.

Singleton GR, *et al.* (1991) The geographic distribution and host range of Capillaria hepatica (Bancroft) (Nematoda) in Australia. *Hodgson, International Journal for Parasitology*. **21**: 945 - 957

Simões RO, *et al.* Prevalence of *Calodium hepaticum* (SYN. Capillaria hepatica) In *Rattus norvegicus* in the urban area of Rio de Janeiro, Brazil. *Revista do Instituto de Medicina Tropical de São Paulo* 2015, **56**: 455–457.

Sudhakar NR, *et al.* (2013) Prevalence of toxocara species eggs in soil samples of public health importance in and around Bareilly, Uttar Pradesh, India. *Veterinary World*, **6**: 87–90.

Table 1. Unadjusted and adjusted logistic regression predicting carriage of *C. hepaticum* within individuals of *R. norvegicus*.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variables | Unadjusted OR (95% CI) | P | Adjusted OR (95% CI) | P |
| Age (days) | 1.03 (1.02–1.05) | < 0.001 | 1.03 (1.02–1.05) | < 0.001 |
| Scaled mass index | 0.998 (0.994–1.006) | 0.96 |  |  |
| Sex | 1.04 (0.56–1.93) | 0.90 |  |  |
| Season | 1.11 (0.6–2.06) | 0.73 |  |  |
| Valley  2  3 | 2.17 (0.81–5.8)  0.53 (0.23–1.21) | 0.12  0.13 | 2.31 (0.81–6.28)  0.54 (0.21–1.26) | 0.10  0.17 |
| Number of cats | 0.95 (0.77–1.17) | 0.63 |  |  |
| Number of dogs | 1.35 (0.98–1.85) | 0.07 |  |  |
| Number of trees | 0.95 (0.89–1.03) | 0.21 |  |  |
| Presence of sewer within 10 m | 1.97 (1.01–3.83) | 0.046 |  |  |
| Cumulative rainfall 60 days before capture | 0.99 (0.99–1.00) | 0.35 |  |  |

Supplementary material.

Supplementary Table S1. Unadjusted and adjusted ordinal logistic regression models predicting intensity level of carriage of *C. hepaticum* within individuals of *R. norvegicus*.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variables | Unadjusted OR (95% CI) | P | Adjusted OR (95% CI) | P |
| Age (days) | 1.003 (0.997–1.009) | 0.36 |  |  |
| Scaled mass index | 1.003 (0.998–1.008) | 0.27 |  |  |
| Sex | 0.814 (0.760–1.008) | 0.47 |  |  |
| Season | 0.995 (0.572–1.736) | 0.99 |  |  |
| Valley  2  3 | 0.308 (0.152–0.617)  0.242 (0.113–0.507) | < 0.001  < 0.001 | 0.318 (0.157–0.640)  0.255 (0.118–0.539) | < 0.001  < 0.001 |
| Number of cats | 0.558 (0.251–0.981) | 0.09 | 0.662 (0.293–1.043) | 0.15 |
| Number of dogs | 1.125 (0.944–1.331) | 0.17 |  |  |
| Number of trees | 1.042 (0.969–1.118) | 0.25 |  |  |
| Presence of sewer within 10 m | 0.810 (0.422–1.609) | 0.54 |  |  |
| Cumulative rainfall 60 days before capture | 0.998 (0.996–1.001) | 0.18 |  |  |

|  |  |  |
| --- | --- | --- |
| Model formula | AIC | ∆AIC |
| Age+Valley+Sewer | 232.26 | 0 |
| **Age+Valley** | **232.32** | **0.06** |
| Age+Valley+Dogs | 232.63 | 0.37 |
| Age+Valley+Sewer+Dogs | 233.01 | 0.75 |

Supplementary Table S2- Logistic model formulas predicting carriage of *C. hepaticum*

Supplementary Table S3- Ordinal logistic model formulas predicting intensity levels of *C. hepaticum*

|  |  |  |
| --- | --- | --- |
| Model formula | AIC | ∆AIC |
| **Valley+Cats** | **371.48** | **0** |
| Valley+Cats+Rainfall | 372.21 | 0.73 |
| Valley | 372.42 | 0.94 |
| Valley+Cats+Dogs | 372.67 | 1.19 |
| Valley+Rainfall | 372.92 | 1.44 |
| Valley+Cats+Rainfall+Dogs | 373.51 | 2.03 |