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SHORT REPORT
Factors affecting carriage and intensity of infection of *Calodium hepaticum* within Norway rats (*Rattus norvegicus*) from an urban slum environment in Salvador, Brazil

R. WALKER[1,2], T. CARVALHO-PEREIRA[1,2,6], S. SERRANO[1], G. PEDRA[1,2], K. HACKER[1,3], J. TAYLOR[1,2], A. MINTER[2], A. PERTILE[1,6], A. PANTI-MAY[1], M. CARVALHO[1], F. N. SOUZA[1], N. NERY JR.[1], G. RODRIGUES[4], T. BAHIENSE[7], M. G. REIS[1,3], A. I. KO[1,3], J. E. CHILDS[3], M. BEGON[2] AND F. COSTA[1,2,3,5]*

[1] *Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Ministério da Saúde, Salvador, Brazil*
[2] *Institute of Integrative Biology, University of Liverpool, Liverpool, UK*
[3] *Department of Epidemiology of Microbial Disease, Yale School of Public Health, New Haven, CT, USA*
[4] *Centro de Controle de Zoonoses, Secretaria Municipal de Saúde, Ministério da Saúde, Salvador, Brazil*
[5] *Instituto de Saúde Coletiva, Universidade Federal da Bahia, UFBA, Salvador, Brazil*
[6] *Instituto de Biologia, Universidade Federal da Bahia, UFBA, Salvador, Brazil*
[7] *Instituto de Ciências da Saúde, Universidade Federal da Bahia, UFBA, Salvador, Brazil*

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SUMMARY

Urban slum environments in the tropics are conducive to the proliferation and the spread of rodent-borne zoonotic pathogens to humans. *Calodium hepaticum* (Brancroft, 1893) is a zoonotic nematode known to infect a variety of mammalian hosts, including humans. Norway rats (*Rattus norvegicus*) are considered the most important mammalian host of *C. hepaticum* and are therefore a potentially useful species to inform estimates of the risk to humans living in urban slum environments. There is a lack of studies systematically evaluating the role of demographic and environmental factors that influence both carriage and intensity of infection of *C. hepaticum* in rodents from urban slum areas within tropical regions. Carriage and the intensity of infection of *C. hepaticum* were studied in 402 Norway rats over a 2-year period in an urban slum in Salvador, Brazil. Overall, prevalence in Norway rats was 83% (337/402). Independent risk factors for *C. hepaticum* carriage in *R. norvegicus* were age and valley of capture. Of those infected the proportion with gross liver involvement (i.e. >75% of the liver affected, a proxy for a high level intensity of infection), was low (8%, 26/337). Sixty soil samples were collected from ten locations to estimate levels of environmental contamination and provide information on the potential risk to humans of contracting *C. hepaticum* from the environment. Sixty percent (6/10) of the sites were contaminated with *C. hepaticum*. High carriage levels of *C. hepaticum* within Norway rats and sub-standard living conditions within slum areas may increase the risk to humans of exposure to the infective eggs of *C. hepaticum*. This study supports the need for further studies to assess whether humans are becoming infected within this community and whether *C. hepaticum* is posing a significant risk to human health.

Key words: Brazil, *Calodium hepaticum*, infection intensity, prevalence, *Rattus norvegicus*.

Conditions within tropical urban slums, characterized by low-level infrastructure, inadequate sanitation, and poor access to safe water, contribute to the transfer of

* Author for correspondence: Dr F. Costa, Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, Ministério da Saúde, Salvador, Brazil, 40296-710.
(Email: fcosta@gmail.com)

zoonoses to humans. Within Brazilian slums, these conditions have been shown to contribute to the spread of harmful zoonoses such as leptospirosis [1]. Many other infectious organisms exist within these urban areas, capable of posing substantial risk to the health of the resident human population. 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 [1]. Tropical urban areas are therefore important environments in which to study zoonoses, in an attempt to identify and quantify their risk to humans, and to develop approaches that prevent and control the spread of disease to inhabitants of urban slums.

Calodium hepaticum (Brancroft, 1893) (syn. *Capillaria hepatica, Tricocephalus hepaticus, Hepaticola hepatica*) is a globally distributed, zoonotic nematode that causes hepatic capillariasis in humans [2] and is understudied in tropical urban environments. Most research concerning *C. hepaticum* has been restricted to temperate areas [1–4]. *C. hepaticum* has more than 180 documented mammalian hosts, with the Norway rat (*Rattus norvegicus*) considered to be the most important [5]. Prevalence of *C. hepaticum* has been reported as high as 87·4% in Baltimore, USA, and >50% within populations of *R. norvegicus* sampled in Salvador, Brazil [6]. *C. hepaticum* infects the liver of its host and requires the death of the host to release the unembryonated ova into the environment [3]. With sufficient time and suitable conditions of temperature, oxygen tension and humidity, eggs embryonate after 5–7 weeks [3]. Infection then occurs when contaminated soil, water, or vegetation are ingested [2].

The result is hepatic infection, where *C. hepaticum* 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 [2]. Over 70 documented cases of human hepatic infection have been reported worldwide, a figure widely believed to be an underestimate, with most cases reported in children [2].

Studies of *C. hepaticum* in Norway rats from urban areas in the tropics have served to determine the prevalence within rat populations, or focused on histopathological effects of the parasite on its host [6–8]. Thus, the present study systematically evaluates how environmental and demographic factors affect the carriage and level of infection intensity of *C. hepaticum* 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 rats and contamination of the environment. This information will be used to inform risk areas within urban slum environments, in which transmission of *C. hepaticum* to humans may be possible.

From May 2013 to December 2014, Norway rats were captured from the neighbourhood of Pau da Lima in Salvador, Brazil (13° 32′ 53·47″ S; 38° 43′ 51·10″ W). This area, which is occupied by communities already participating in an active leptospirosis surveillance programme [1], has three distinct valleys (1, 2, 3). This site is typical of a slum community, and ~30% of the populations from Salvador (and Brazil) reside in similar social and environmental conditions. Live trapping of rats using Tomahawk® traps (40·6 × 12·7 × 12·7 cm) (Tomahawk Live Traps, USA) took place in all three of the valleys, with traps being set at sunrise and collected at sunrise the following day. Methods for transporting animals to the outdoor processing site, handling and obtaining tissues were as described previously [1]. Animal-specific factors including weight, body length, sex and the number of open wounds were recorded. Recorded weights of the animals were converted to ages (days) using the von Bertalanffy equation [9] and previously published data. Moreover, body condition was estimated using a scaled mass index based on mass and body length, while accounting for the effect of age [10]. Environmental variables including location of capture, the presence of an open sewer within 10 m of the capture location, the number of trees at the capture location and number of alternative hosts of *C. hepaticum*, were recorded at the time of live capture. Data regarding the number of trees and the number of alternative hosts was recorded per household in cases when the capture location was inside the resident's grounds. Trees were recorded within a 10-m radius from the trap location and both cats and dogs were recorded as alternative hosts of *C. hepaticum*.

The presence and absence of *C. hepaticum* were evaluated through macroscopic examination of the liver during necropsies, with the presence of *C. hepaticum* being noted based on visible yellowish-white lesions on the liver surface and scored as described

Table 1. *Unadjusted and adjusted logistic regression predicting carriage and intensity of infection of* Calodium hepaticum *within individuals of* Rattus norvegicus

Variables	*C. hepaticum* carriage in *R. norvegicus* individuals ($n = 402$)		Intensity of *C. hepaticum* infection in *R. norvegicus* individuals ($n = 402$)	
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age (days)	1·03 (1·02–1·05)**	1·03 (1·02–1·05)**	1·003 (0·997–1·009)	
Scaled mass index	0·998 (0·994–1·006)		1·003 (0·998–1·008)	
Sex	1·04 (0·56–1·93)		0·814 (0·760–1·008)	
Season	1·11 (0·6–2·06)		0·995 (0·572–1·736)	
Valley				
2	2·17 (0·81–1·21)	2·32 (0·84–6·42)	0·308 (0·152–0·617)**	0·318 (0·157–0·640)**
3	0·53 (0·77–1·17)	0·54 (0·21–1·26)	0·308 (0·152–0·617)**	0·255 (0·118–0·539)**
No. of cats	0·95 (0·89–1·03)		0·242 (0·113–0·507)	0·662 (0·293–1·043)
No. of dogs	1·35 (0·98–1·03)		1·125 (0·944–1·331)	
No. of trees	0·95 (0·89–1·03)		1·042 (0·969–1·118)	
Presence of sewer within 10 m	1·97 (1·01–3·83)*		0·810 (0·422–1·609)	
Cumulative rainfall 60 days before capture	0·99 (0·99–1·00)		0·998 (0·996–1·001)	

OR, Odds ratio; CI, confidence interval.
* $P < 0·05$, ** $P < 0·001$.

previously [3, 4, 8] 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 (75–100%) liver involvement.

Additionally, soil samples were evaluated for evidence of environmental contamination of *C. hepaticum*. Using a cross-sectional design, ten locations previously used to trap rodents were randomly selected using a random number generator, and six samples of soil (30 g) were collected at each location during August 2015. Those 10 locations represented ~10% of the total number of capture sites and were homogeneously distributed across the study area. All ten locations were in close proximity to places of residence and were easily accessible by humans, making them potential risk areas for humans. Furthermore, given that monthly rainfall and temperatures are consistently high year round, environmental factors are not limiting egg embryonation at capture locations. Samples were processed as described previously [11].

A logistic regression model was performed using R v. 2.11.1 (R Foundation, Austria), to examine the relationship between carriage of *C. hepaticum* and explanatory variables. An ordinal logistic regression model was used to examine the relationship between the infection level of *C. hepaticum* and the explanatory variables. For multivariate analysis, variables associated in univariate models with $P < 0·2$ were included and the models were then judged based on Akaike's Information Criterion (AIC). This allowed for the selection of a statistical model that best fitted the data available.

The carriage of *C. hepaticum* was assessed for 402 synanthropic rats. The carriage level was 83% (337/402) in the Norway rat. In univariate analyses, carriage tended to increase with age, location (valley) of capture, proximity (within 10 m) 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 (valley) of capture. This model formula produced an AIC score of 228·5, of which was the lowest AIC score relative to the other models that were assessed (Table 1, Supplementary Table S1). This model was therefore the best fit to the available data. The age of an individual was a significant predictor of whether or not an individual was carrying *C. hepaticum*, after controlling for the location of capture, i.e. valley [unadjusted odds ratio (OR) 1·03, 95% confidence interval (CI) 1·02–1·05)]. Of individuals that were sexually mature, i.e. aged ⩾65 days, 91% (235/261) of the individuals were infected compared to 69% (97/141) of juvenile rats. The increased probability of infection in older individuals may be due to higher levels of activity and therefore an increased likelihood of coming into

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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' lifetime [3]. In valley 1, 81% (65/80) of *R. norvegicus* individuals were infected compared to 92% (144/157) captured in valley 2 and 75% (123/165) captured in valley 3. Univariate analysis showed the presence of an open sewer within 10 m of the capture location, to be a significant predictor of carriage of *C. hepaticum* within individuals (unadjusted OR 1·97, 95% CI 1·01–3·83) (Table 1). Open sewers run along the bottom of valleys and it is possible that heavy rainfall, typical in the region between the months of April and July, could wash infective ova into low-lying areas, increasing the environmental concentration of ova and therefore the probability of infection. However, the inclusion of this variable in the multivariate analysis was not strongly supported (Table 1, Supplementary Table S1).

Regarding intensity, of the infected individuals 71% (237/337) were classified as having a slight level of infection (<25% liver involvement) and only 8% (26/337) had gross liver involvement. Multivariate analyses supported the inclusion of valley, i.e. location of capture and cats, an alternative host of *C. hepaticum.* This model formula was shown to be the best fit for the data available, producing an AIC score of 371·48, the lowest score relative to the other evaluated models (Table 1, Supplementary Table S1). Multivariate analyses showed location of capture, i.e. valley, to be a significant predictor of infection intensity after adjusting for the number of cats recorded at the residence where capture points were located (Table 1). The odds of individuals possessing higher levels of infection intensity were significantly greater in valley 1 compared to those in valley 2 (adjusted OR 0·318, 95% CI 0·157–0·640) and valley 3 (adjusted OR 0·255, 95% CI 0·118–0·539) (Table 1). This difference between valleys has no obvious explanation. Previous work has shown higher levels of *C. hepaticum* carriage within populations of *R. norvegicus* to be associated with higher the levels of infection intensity [3]; however, carriage level did not show a significant difference between valleys in either the univariate or multivariate models (Table 1). It is possible that unexamined factors, including the effects of co-infection with other parasites and the health of the individual hosts could be contributing to the observed result.

Data regarding environmental contamination of *C. hepaticum* 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. 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 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 may also be of benefit.

This study demonstrates the role of specific demographic and environmental factors in predicting both the carriage and intensity levels of *C. hepaticum* in *R. norvegicus* in an urban slum. Additionally, the confirmation of environmental contamination suggests that humans could be at some risk of becoming infected with *C. hepaticum*. The extent of which, however, is unknown without further investigation. This is the first published report of *C. hepaticum* prevalence in *R. norvegicus* from Salvador since 1976 when carriage in captured rats was 57% [6]. 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%) [8] and Porto Velho (2%) [7]. This is potentially due to the high host density and year round conditions suitable for egg embryonation. There is a lack of epidemiological studies of *C. hepaticum* in humans and such studies are needed given the high levels of *C. hepaticum* carriage within the rat population. Further study is required to assess whether humans are becoming infected within this community and whether *C. hepaticum* is posing a significant risk to human health.

The Institutional Animal Care and Use Committees at the Oswaldo Cruz Foundation, Salvador, Brazil (003/2012) and Yale University in the United States (2012–11 498) approved all animal procedures and methods.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268816002259.

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DECLARATION OF INTEREST

None.

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