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Parasitic and fungal infections in synanthropic rodents in an area of urban expansion, Aracaju, Sergipe State, Brazil

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ABSTRACT. This study analysed the prevalence of parasitic and fungal infections in rodents in an area of urban expansion, Aracaju, Brazil. Traps were placed in the area from December 2011 to January 2013. Blood samples, faeces and hair were collected from the animals. We collected a total of 47 rodents; 44 were *Rattus rattus*, and 3 were *Mus musculus*. Parasitological evaluation revealed the cestode *Hymenolepis diminuta* infection in both rodent species. The nematodes *Aspiculuris tetraptera* and *Syphacia obvelata* were found in *M. musculus*, and the commensal *Entamoeba coli* was found in *R. rattus*. We observed that 69.2% of the *R. rattus* and 33.3% of the *M. musculus* were infected with the haemoparasite *Babesia* sp. The differential leukocyte count revealed normal (72.3%), neutrophilic (15.9%) and lymphocytic (11.4%) profiles. The evaluation showed the following species of fungi in the rodents: *Aspergillus* sp. (77.1%), *Penicillium* sp. (28.6%), *Cladosporium* sp. (14.3%), *Mucor* sp. (14.3%), *Curvularia* sp. (8.6%), *Acremonium* sp. (8.6%), *Chrysosporium* sp. (2.9%), *Syncephalostrum* sp. (2.9%), *Alternaria* sp. (2.9%), *Trichophyton* sp. (2.9%) and *Scopulariopsis* sp. (2.9%). The parasites and fungi found in rodents are potentially zoonotic, and the presence of these household animals demonstrates their potential role as reservoirs and disseminators of fungal and parasitic infections.

Keywords: synanthropy, intestinal parasites, disseminators of fungal diseases, *Rattus rattus*, *Mus musculus*.

Infecções parasitárias e fúngicas em roedores sinantrópicos coletados em área de expansão urbana, Aracaju, Estado do Sergipe, Brasil

RESUMO. Este estudo objetivou analisar a prevalência de infecções parasitárias e fúngicas em roedores em área de expansão de Aracaju, Brasil. Foram colocadas armadilhas com iscas no período de dezembro de 2011 a janeiro de 2013. Os animais coletados foram submetidos à coleta de amostras sanguíneas, fecais e de pelos. Foram coletados 47 roedores sendo 44 *Rattus rattus* e três *Mus musculus* e a avaliação parasitológica revelou infecção pelo cestódeo *Hymenolepis diminuta* em ambas as espécies, pelos nematódeos *Aspiculuris tetraptera* e *Syphacia obvelata* em *M. musculus* e pelo enterocomensal *Entamoeba coli* em *R. rattus*. Observou-se também que 69,2% de *R. rattus* e 33,3% de *M. musculus* estavam infectados pelo hemoparasita *Babesia* sp. A contagem diferencial dos leucócitos revelou perfil normal (72,3%), neutrofílico (15,9%) e linfocitário (11,4%). A avaliação micológica apresentou variabilidade qualitativa de fungos: *Aspergillus* sp. (77,1%), *Penicillium* sp. (28,6%), *Cladosporium* sp. (14,3%), *Mucor* sp. (14,3%), *Curvularia* sp. (8,6%), *Acremonium* sp. (8,6%), *Chrysosporium* sp. (2,9%), *Syncephalostrum* sp. (2,9%), *Alternaria* sp. (2,9%), *Trichophyton* sp. (2,9%) e *Scopulariopsis* sp. (2,9%). Os parasitas e fungos encontrados nos roedores são potencialmente zoonóticos sendo que a presença destes animais no peridomicílio evidencia o seu papel como reservatórios e disseminadores de infecções parasitárias e fúngicas.

Palavras-chave: sinantropia, parasitas intestinais, disseminadores de doenças fúngicas, *Rattus rattus*, *Mus musculus*.

Introduction

Human exploitation of nature favours the proliferation of rodents. Although most species of wild rodents inhabit wild environments, some of these animals have adapted to urban conditions and can be considered synanthropic rodents. These species, especially the murid omnivores, are different from wild rodents and live close to humans where they find water, shelter and food (grains) to survive. In this context, these rodents can cause economic losses to humans, such as damage to food for human consumption and the destruction of grains and seeds in planting areas and storage facilities (BRASIL, 2004).

In addition to the economics losses, rodents can also damage the health of humans or domesticated animals because they serve as vectors for a number of diseases, such as fungal and parasitic infections. Rats and mice belong to the family Muridae, subfamily Murinae and are considered synanthropic because their environments are affected by human action. Among the commensal synanthropic species, the water rat (*Rattus norvegicus*), the black rat (*Rattus rattus*) and mice (*Mus musculus*) have a particularly cosmopolitan distribution and are responsible for most of the damage caused to the economy and public health.

It is estimated that more than 1.5 million fungal species have been identified, and 150 of these species are pathogenic to mammals (CRUZ, 2010). Three fungal genera are keratinophilic dermatophytes in animals and humans. In wild animals, depending on the causal state, the dermatophytosis can be extremely pathogenic and contagious for both animals and humans. According Tomaz (2011), approximately 20 to 25% of the world population has superficial fungal infections.

Rodríguez et al. (2009) cited a case of infection with *H. diminuta* in a child caused by the constant presence of rodents in and out of homes and emphasised the relationship this case and the sanitary deficiency of the family residence. This report confirmed the assertions of Russomando et al. (2008) that infection with *H. diminuta* affect more children than adults, spreads easily from person to person and is very common in populations with limited hygiene, low social status, houses near sanitary landfills, and houses with an infrastructure deficit. A few cases of human infection with *Hymenolepis diminuta* have been reported, confirming data from the World Health Organisation estimating that the world would have 3.8 billion patients infected by intestinal worms.

Rapid and unplanned urbanisation in the expansion zone of Aracaju, the capital of Sergipe State, may enable the movement of rodents between wild and anthropogenic environments. Therefore, this study aimed to analyse the potential risk for fungal and parasitic infections in the rodents to spread to humans in the expansion area of Aracaju, Sergipe State, Brazil.

Material and methods

Twenty Tomahawk and Shermann traps were baited with a mixture of banana, corn meal, peanuts and vanilla and placed in the expansion area of Aracaju, Sergipe State, northeast Brazil (11° 01' 39,08" S e 37° 06' 16,11" O) between December 2011 and January 2013. The captured animals were submitted to biometrics, and blood, faecal and hair samples were collected. The taxonomic identification was based on body length (CC), foot length (CP) and ear length (CO) (BONVICINO et al., 2008). Australian-type markings were made for recapture control, and the animals were released into their natural habitat.

The hairs were cultured on mycosel agar, potato agar, Lactrimel agar and Saboraud agar and incubated at 37°C for seven days to isolate and identify fungi at the Central Laboratory of Public Health of Sergipe, Parreira Horta Institute (LACEN). The isolated fungi were identified based on typical macroscopic and microscopic characteristics after treatment with blue cotton (LACAZ, 1991).

The faeces were collected in a plastic bulkhead placed under the traps and were evaluated using the spontaneous sedimentation technique (SAMPAIO, 2006). Blood samples were collected with a syringe containing 0.10 μL of 3.8% sodium citrate, and blood smears were prepared for differential leukocyte counting and the identification of haemoparasites using panoptic staining.

Pearson's correlation coefficient was calculated to assess the relationship between the total body length and number of species (richness) of fungi present in the Rattus rattus specimens. The statistical procedure was performed with the software Statistica 7.0 and was applied with a confidence interval of 5%.

The collection and handling of animals followed the principles established by COBEA (Brazilian College of Animal Experimentation), and our protocol was authorised by the Ethics Committee of Animal Use (CEUA) under the number 040811/2011. After the biological samples were collected, the animals were released in their natural habitats.

Results

A total of 47 rodents were collected, 44 *Rattus rattus* (average total length = 30.5 ± 2.9 cm) and 3 *Mus musculus* (average total length = 23.8 ± 2.0 cm). The evaluation of the stool samples revealed the presence of the cestode *Hymenolepis diminuta* (Figure 1) in both rodent species, the nematodes *Syphacia obvelata* and *Aspiculuris tetraptera* (Figure 2) in *Mus musculus* and the cysts of the enterocommensal *Entamoeba coli* in *Rattus rattus*.

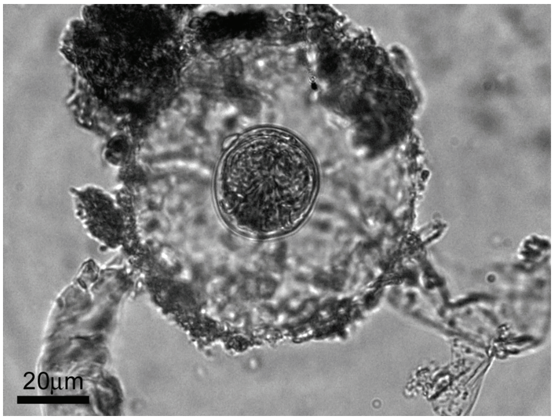

Figure 1. Egg of *Hymenolepis diminuta*.

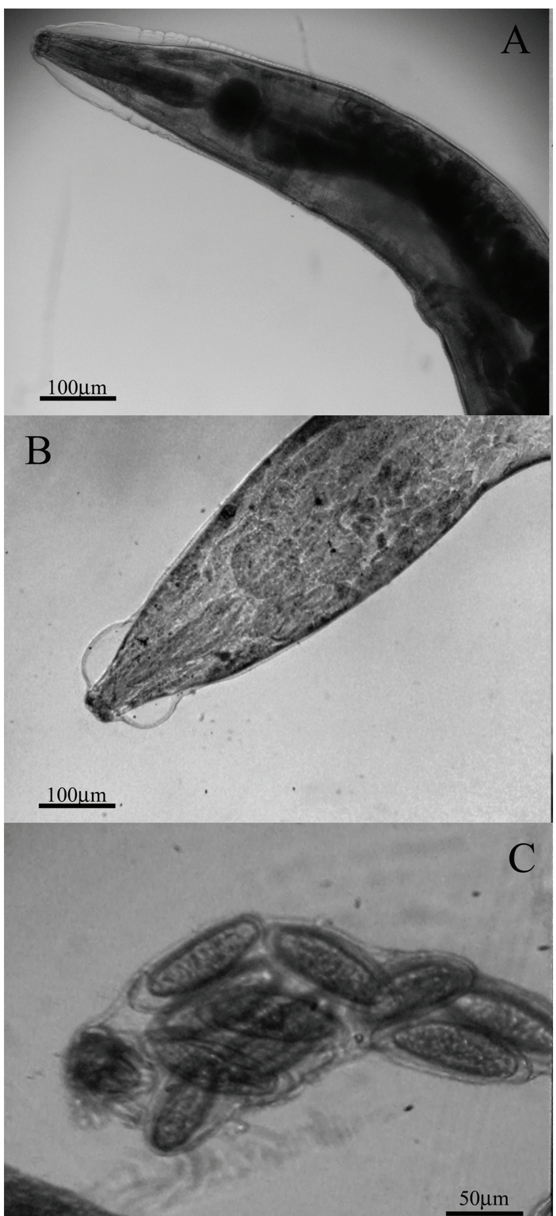

Figure 2. (A) female *Aspiculuris tetraptera* nematode; (B) Gravid *Syphacea obvelata* female; (C) Eggs of *Syphacea obvelata*.

The blood smears showed that *R. rattus* and *M. musculus* were infected with the blood parasite *Babesia* sp. The prevalence of this parasite and others in the captured rodent species are shown in Table 1.

Table 1. Parasites and enterocommensals found in rodents collected in the area of expansion of the city of Aracaju, Sergipe State.

	Entamoeba coli	*Hymenolepis diminuta*	*Aspiculuris tetraptera*	*Syphacea obvelata*	*Babesia* sp.
Rattus rattus	2.3%	52.3%	-	-	69,2%
Mus musculus	-	66.7%	66.7%	33.3%	33.3%

The differential haematological counting of leucocytes revealed the following values in 100 cells: bands 0 – 2; segmented 7 – 61; eosinophils 0 – 3; lymphocytes 37 – 90 and monocytes 0 – 4. This profile shows increased numbers of neutrophils and lymphocytes (Figure 3).

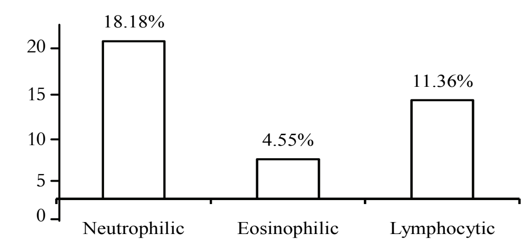

Figure 3. Haematological profile of rodents collected in the expansion area of Aracaju, Sergipe State, 2011-2013.

Eleven genera of fungi, including six keratinophilic genera and one dermatophyte, belonging to nine families were isolated from the 42 hair samples of the rodents (Table 2 and Figure 4). The highest genera diversity was observed for the families Trichomonaceae and Pleosporaceae.

Table 2. Frequency of fungi isolated according to the taxonomic families.

Families	Frequency (%)
Trichomonaceae	
Aspergillus sp.	77.1
Penicillium sp.	28.6
Pleosporaceae	
Curvularia sp.*	8.6
Alternaria sp.*	2.9
Onygenaceae	
Chrysosporium sp.*	2.9
Clavicipitaceae	
Acremonium sp.*	8.6
Davidiellaceae	
Cladosporium sp.*	14.3
Syncephalastraceae	
Syncephalastrum sp.	2.9
Mucoraceae	
Mucor sp.	14.3
Arthrodermataceae	
Trichophyton sp.**	2.9
Microascaceae	
Scopulariopsis sp.*	2.9

*keratinophilic; **keratinophilic and dermatophyte.

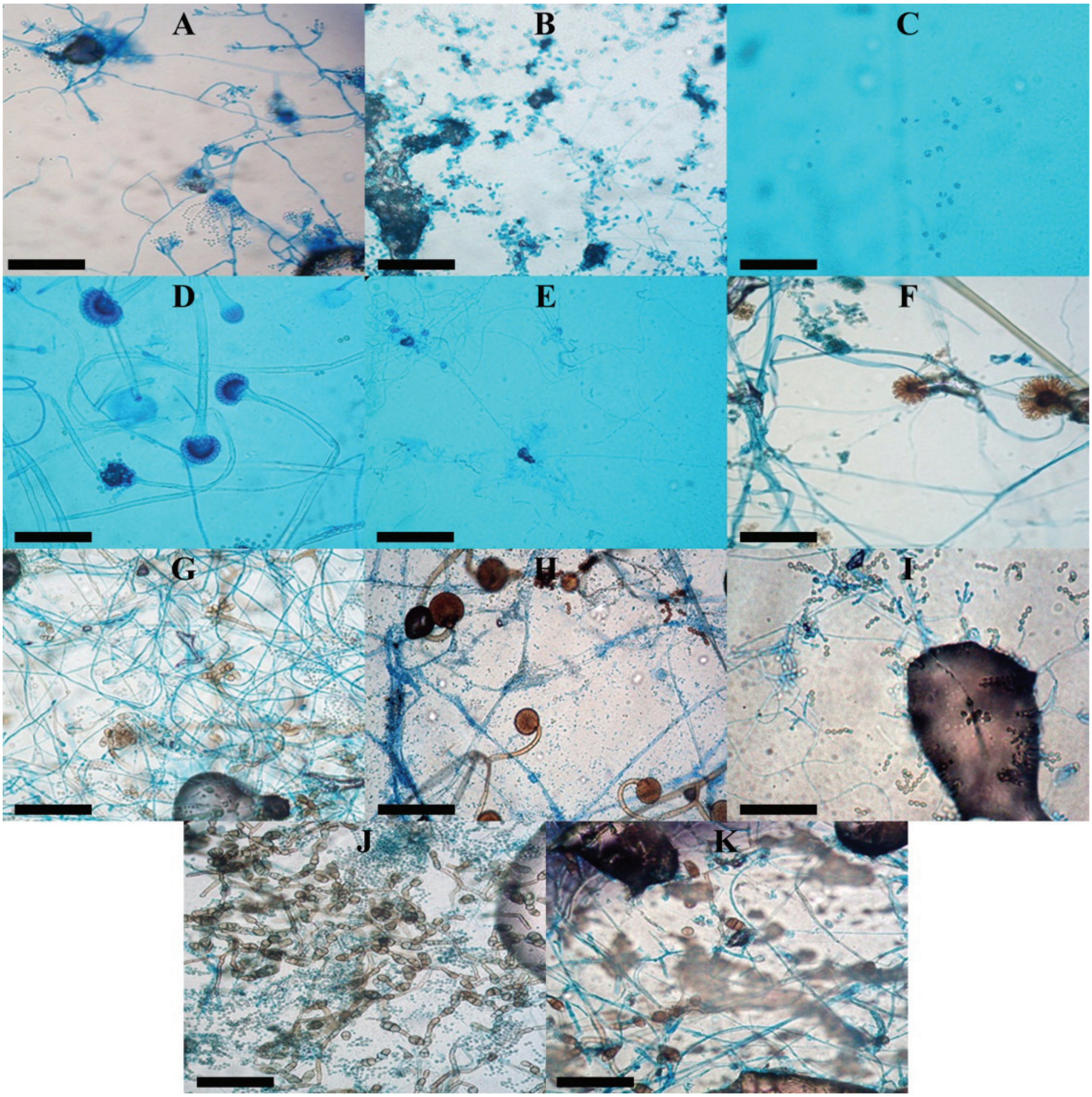

Figure 4. Fungi identified in rodent hairs collected in the expansion area of Aracaju, SE. *Penicillium* sp. (A), *Chrysosporium* sp. (B), *Acremonium* sp. (C), *Aspergillus* sp. (D), *Syncephalostrum* sp. (E), *Cladosporium* sp. (F), *Curvularia* sp. (G), *Mucor* sp. (H), *Scopulariopsis* sp. (I), *Alternaria* sp. (J), and *Trichophyton* sp. (K). (Bar = 50 μm).

All *Rattus rattus* specimens examined showed at least one species of fungus; 43.75% had one species, 46.9% had two species, 6.3% had three and 3.1% had four. The total body length correlated positively but not significantly with the species richness of fungi present in the animal's body ($p = 0.085$, $r = 0.359$; Figure 5).

Discussion

Rodents are a group of mammals that have become increasingly important in the transmission of various diseases to humans in recent decades. In this study, the parasites *Hymenolepis diminuta* and *Babesia* sp. found in the rodents (Table 1) are potentially zoonotic and are considered a serious threat for the health of humans, especially for children under 10 years of age because to present immunities in early stages of consolidation.

More variability in the species of helminth parasites was observed in rodents from a mountainous area in Iran in a study that found 13 species of parasites in *Meriones persicus* and 7 species in *Microtus socialis* (KIA et al., 2010). These authors also identified the 3 species of intestinal helminths that we found in the rodents collected in Sergipe.

Among the species of helminth parasites found in the rodents in this study, *Hymenolepis diminuta* and

Syphacia obvelata are considered zoonotic (ASHFORD; CREWE, 2003); the prevalence of these species in the present study is shown in Table 1.

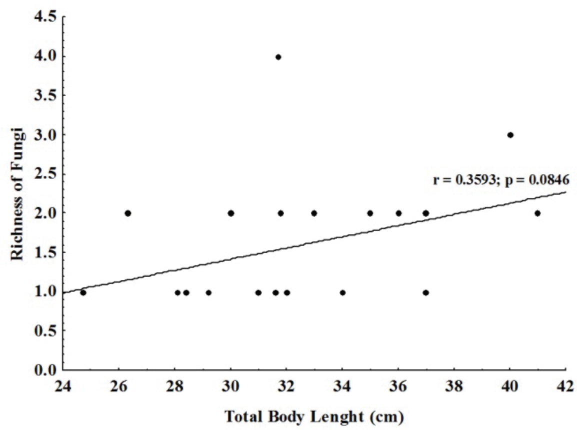

Figure 5. Pearson's correlation coefficient between the total body length and number of species of fungi present in the body of *Rattus rattus* collected in the expansion zone of the city of Aracaju, Sergipe State.

The helminth *Hymenolepis diminuta* may occasionally parasitise humans, but its habitual hosts are rodents, as observed in this study (Table 1). Human infection is established when the coprophilic arthropods that become intermediate hosts by ingesting the eggs of cestodes eliminated in the faeces of small rodents (Figure 1) are accidentally ingested by humans. This parasitic infection usually occurs in children (TENA et al., 1998; MARANGI et al., 2003; RODRÍGUEZ et al., 2009) and can be asymptomatic. In symptomatic patients, the most common symptoms are gastrointestinal disorders such as abdominal pain, diarrhoea, irritability and pruritus (TENA et al., 1998; MARANGI et al., 2003).

Despite the low prevalence rates of hymenolepiasis, which range from 0.5 to 4% as determined by epidemiological studies with children in Latin America (MACHADO; COSTA-CRUZ, 1998; IANNACONE et al., 2006), we observed a frequency of 60% in the rodents collected in Aracaju (Table 1). Thus, there is a high occurrence of *Hymenolepis diminuta* in in the examined rural-urban environment. The zoonotic transmission of this parasite of rodents to humans, due to the low prevalence of human infections, is not considered to be a major public health problem.

The infection of rodents with *Aspiculuris tetraptera* or *Syphacia obvelata*, which are considered to be less pathogenic oxyurids, (Table 1) can be asymptomatic but can also lead to the delayed development of the host and changes in their behaviour (LUCA et al., 1996). According to Jacoby and Fox (1984), infections with *S. obvelata* usually occur in young animals because adult animals seem to be more resistant to the parasites. Ashford and Crewe (2003) argued that human infections with *S. obvelata* may occur but at a lower prevalence.

Infection with *A. tetraptera* affects older rodents because they lose the resistance obtained from the mother after the first weeks after birth (TAFFS, 1976). The resistance associated with age in rats may be due to increased mucus production and increased activity of the immune system, however, no specific immune response has been reported.

According to Anderson and Gordon (1982), the aggregation of helminths in host populations is common both in laboratory conditions and in the field (Table 1). These aggregates are heterogeneous due to differences in the susceptibility of mice to helminth infections because of behavioural aspects, genetic load, immunological status or the concentration of infective eggs in the environment (SCOTT; GIBBS, 1986); these factors make it difficult to predict the risk of accidental infections of humans and rodents.

The observed infections with intestinal helminths in rodents collected in the field (*Mus musculus* and *Rattus rattus*) presented in this study are unique and relevant (Table 1) because most studies on natural and experimental helminth infections have used laboratory animals (BAZZANO et al., 2002; GONÇALVES et al., 1998; SILVA et al., 2007). In the case of mice, their chance of infection and the potential accidental transmission of infection to man is low because their range is small, rarely exceeding 3-5 m. However, these animals are more intimately associated with human dwellings (neophilia). On the other hand, rats have a range approximately ten times larger (60 m) but preferentially associate with deposits of food for domestic animals (chicken, ostrich, goats, peacocks). These rodents avoid intimate contact with the human habitat (neophobia) (BRASIL, 2002).

A study of gastrointestinal parasites in the fauna of the Atlantic Forest on the island and mainland in Santa Catarina State, Brazil, (KUHNEN et al., 2012) revealed that four species of wild rodents contained ten groups of parasites, including *Hymenolepis* sp., *Syphacia* sp. and Oxyuridae, as observed in the rodents collected in the present study. The distinct spatial patterns of the different zoonotic parasites was most likely due to differences in the distribution, abundance and habitat use of their definitive hosts. In Switzerland, a study of 658 rodents in urban environments revealed infections shared with intermediate hosts or paratenic carnivores (foxes and cats). These shared infections included *Echinococcus multilocularis*, *Taenia taeniaeformis*, *Toxocara* sp. and *Toxoplasma gondii*, and can serve as valuable indicators of the occurrence, level of environmental contamination and the potential for infection with these parasites. It is noteworthy, then, that rats have potential value as indicators of zoonotic parasitic infections.

Regarding the low estimate of intestinal helminths species richness in this study (2 species of nematodes and one cestode), Poulin (1990) demonstrated that usually one or two species are not detected when fewer than 40 hosts are examined. This phenomenon can be explained by the low prevalence of helminths in mammals; helminths normally occur in less than 5% of the host population. Therefore, the rate of parasitic infections determined by the sample size of rodents in the micro-area of Aracaju can be considered representative of the local transmission dynamic. The local richness of species can be correlated with the size of the habitat ($\approx$35.600 m^2), body size ($\bar{x}$=16,24±2,51 cm) and the geographic coverage of the host (POULIN; MORAND, 2000).

Infections with *Babesia* sp. are not directly transmitted from rodent to man; instead, the susceptible host has to be bitten by a tick carrying the protozoan. In human infections, the disease is potentially fatal for individuals with low immunity, such as splenectomised individuals, carriers of HIV or lymphoma or patients who use immunomodulators to control autoimmune diseases.

The results obtained in this study showed that the number of fungi present in the collected hosts was directly related to the body length of the rodent (Figure 5). Kataranovski et al. (2007) asserted that the body structure of rodents allows for the growth of infectious organisms, especially fungi, and thus, the zoonotic risk of these animals cannot be ignored in the development of emerging zoonotic diseases and fungal infections (THOMAS et al., 2012).

Some of the fungi identified in this study (Figure 4) are considered pathogenic both for humans and animals. *Aspergillus* sp. are found in environments rich in oxygen, often develop in plants and trees and produce a toxin called aflatoxin that can cause liver damage, cutaneous mycoses and subcutaneous infections in humans, especially in those that are immunocompromised (OLIVEIRA, 1999). Infections due to *Aspergillus* sp. in humans can range from allergic alveolitis to allergic bronchopulmonary aspergillosis, aspergilloma, and invasive aspergillosis with mixed syndromes (NUCCI; MARR, 2005).

Of the eleven genera collected, seven are classified as keratinophilic (Table 2), meaning that they require a keratinous substrate for their development. One of the collected keratinophilic species was also a dermatophyte. Most species of keratin-decomposing fungi are truly saprophytic, but some species are potentially pathogenic for warm-blooded vertebrates (HUBALEK, 2000).

The species of dermatophyte fungi of the genus *Trichophyton* are primarily responsible for the increased prevalence of skin and nail infections in humans and animals around the world (WOODFOLK, 2005). These zoophilic species are pathogenic to animals and often appear in only one or another very limited group of host species. Some zoophilic species of *Trichophyton* can cause dermatophytosis in humans, and anthropophilic species cause dermatitis in animals. (SIMPANYA, 2000). The frequency of *Trichophyton* in rodents collected in this study (3.6%) did not differ from the average occurrence for this fungus (4.1%) found in India by Thomas et al. (2012) in *Rattus norvegicus* collected in a rural area. What draws attention, however, is that in our study the Rattus rattus specimens were collected at the peridomicile and thus represent an increased risk of transmission to the human inhabitants.

Species of the genus *Chrysosporium* were observed in only one of the rodents collected in this study. These organisms are common soil inhabitants (PAPINI et al., 2008). This fungus is keratinophilic and has an important role in nature as a keratin digester. By comparing the occurrence of keratinophilic fungi in birds and mammals, Hubalek (2000) demonstrated that *Chrysosporium* is one of the genera most commonly found in wild vertebrates, primarily in mammals.

Fungi that belong to the genus *Acremonium* are also keratinophilic and are also commonly found in soil. Human infections with this fungus are rare and are usually associated with immunosuppression (TUON et al., 2010).

Species of filamentous fungi are currently being described frequently as causing cutaneous mycoses mainly onychomycosis, especially the genera *Alternaria* and *Scopulariopsis* (GODOY et al., 2004; TAKAHASHI et al., 2011).

According to Pinheiro et al. (1998), mice and rats must maintain their internal environments and strictly control the limits of its variation to remain healthy. The evaluation of this homeostasis can reveal modifications induced by pathological processes, such as those caused by fungi and parasites. In this study, we observed a relationship between the standard lymphocyte immune response in rodents infected with *Aspergillus* sp. and the neutrophilic hematologic profile of those infected by *Hymenolepis diminuta*.

The effects of rapid urbanisation without following the guidelines of a government plan can increase the number of rodents circulating in the human environment. Because these animals can serve as reservoirs of parasitic and fungal infections, they may disseminate these infections to humans in areas of urban expansion.

Conclusion

The area sampled in the region of Aracaju has a greater diversity of fungal species than parasites in synanthropic rodents. We found a direct relationship between the length of the animal and the species richness of fungi present and identified species that are potentially pathogenic to humans. We emphasise the need to implement control measures to prevent transmission of disease to humans.

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