

ARTÍCULO ORIGINAL

Human infection by *Trichostrongylus* spp. in residents of urban areas of Salvador city, Bahia, Brazil

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Introduction: Nematodes of *Trichostrongylus* genus are mainly parasites of herbivores, although sporadic human infections have been reported in many countries.

Objective: To describe the frequency and seasonality of *Trichostrongylus* spp. infection in individuals attended at a public clinical laboratory.

Materials and methods: Fecal samples of 9,283 individuals were evaluated by spontaneous sedimentation (Lutz) in the Parasitology Laboratory of the Pharmacy College, Federal University of Bahia, Brazil, from January of 2006 to May of 2008. The positive samples for either *Trichostrongylus* spp. or hookworms were further examined to evaluate the morphometry of nematode eggs.

Results: One-hundred and ten patients (1.2%) were confirmed to be infected by *Trichostrongylus* spp. The positive cases were significantly more frequent in females (1.6%; $p < 0.05$), with higher distribution in the age group between 11-20 years (1.9%), compared to those aged 51-60 (0.8%) and older than 60 years (0.9%) ($p < 0.05$), independent of gender. *Trichostrongylus* spp. infections were more common from March to May (40 cases) and showed a homogeneous distribution over the other periods of the year (21-25 cases). The hematological analyses of 60 *Trichostrongylus*-infected patients showed normal levels of eosinophils in most of the positive cases.

Conclusions: The data reveal that the occurrence of infection by *Trichostrongylus* spp. in residents of Salvador is more frequent than those reported in other urban regions and that it is essential to distinguish the parasite from other nematodes in routine parasitological examination.

Key words: *Trichostrongylus*, infection; diagnosis, differential; eosinophilia, seasons.

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Infección humana por *Trichostrongylus* spp. en residentes de zonas urbanas de la ciudad de Salvador, Bahia, Brasil

Introducción. Los nematodos del género *Trichostrongylus* son parásitos principalmente herbívoros, aunque se hayan descrito en muchos países infecciones humanas esporádicas.

Objetivo. Describir la frecuencia y estacionalidad de la infección por *Trichostrongylus* spp. en individuos atendidos en un laboratorio clínico público.

Materiales y métodos. Las muestras fecales de 9.283 individuos fueron analizadas mediante sedimentación espontánea (Lutz) en el Laboratorio de Parasitología de la Facultad de Farmacia de la Universidad Federal de Bahía, Brasil, desde enero de 2006 a mayo de 2008. Las muestras positivas para *Trichostrongylus* o *Ancylostoma* fueron reexaminadas para evaluar la morfometría de los huevos de nematodos.

Resultados. La infección por *Trichostrongylus* spp. se confirmó en 110 pacientes (1,2 %), con mayor frecuencia en el sexo femenino (1,6 %; $p < 0,05$). La frecuencia fue mayor en el grupo de edad de 11 a 20 años (1,9 %), independientemente del sexo, en comparación con las personas de 51 a 60 (0,8 %) y con los mayores de 60 años (0,9 %) ($p < 0,05$). Las infecciones por *Trichostrongylus* spp. fueron más frecuentes entre marzo y mayo (40 casos) con una distribución homogénea en otros periodos del año (21 a 25 casos). Los análisis hematológicos de 60 pacientes infectados con *Trichostrongylus* spp. presentaron niveles normales de eosinófilos en la mayoría de los casos positivos.

Conclusiones. Los datos revelan que la incidencia de la infección por *Trichostrongylus* spp. en individuos residentes en Salvador es más frecuente que las descritas en otras áreas urbanas y, por ello, es esencial distinguir dicho parásito de otros nematodos en los exámenes parasitológicos de rutina.

Authors' contributions:

Márcia Cristina Aquino Teixeira designed the study.

Márcia Cristina Aquino Teixeira, Robson Paixão Souza and Neci Matos Soares wrote the manuscript.

Robson Paixão Souza and Leda Maria Alcântara analyzed the results.

Joelma Nascimento Souza and Joelma Figueiredo Menezes performed the laboratory work.

Palabras clave: *Trichostrongylus*, infección, diagnóstico diferencial, eosinofilia.

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Nematodes of the genus *Trichostrongylus* are ubiquitous parasites of the digestive tract or respiratory system of several animals, with large distribution among the herbivores (1-3). Abebe and colleagues showed that sheep and goats are important reservoirs of these parasites (3). Moreover, studies conducted in the forest of Ulu Gombak, Kuala Lumpur, showed that the helminth *Trichostrongylus*, along with *Taenia* and *Hymenolepis*, accounted for roughly 41% of infection in rodents (4). The parasite has also been observed in chickens, with a prevalence of 4% (5).

Parasites of the family Trichostrongylidae have a tendency to colonize sites such as the abomasum, stomach, or small intestine of ruminants, horses, and swine, depending on the species. The life cycle takes place when eggs are passed in the feces of infected animals. Subsequently, first-stage larvae (L₁) hatch from mature eggs and develop into the second stage (L₂) and then into the third stage (L₃) infective larvae, protected by environmental variations by an impermeable sheath. The L₃ form may survive winter or dry periods due to hypobiosis and produce clinical outbreaks in ruminants in early spring or after rainy periods by re-hydrating (6).

Epidemiological studies indicate a global distribution and low prevalence of infections in humans (7-13). Accidental infections by *T. orientalis* have been reported in Korea, China, Japan and Armenia (9). Other species of *Trichostrongylus* associated with infections in humans have also been described, such as *T. orientalis*, *T. colubriformis*, *T. vitrinus*, *T. axei*, *T. capricola*, *T. probolurus* and *T. skrjabin*. Nevertheless, the species *T. axei*, *T. colubriformis* and *T. orientalis* are the most common parasites in human infections; most frequently acquired through contact with herbivorous animals (8-12).

The weather is one of the major factors influencing the transmission of the parasite, which usually occurs in regions that have warm temperatures (22 °C to 26 °C) and high humidity (80-100%). Thus, inter-tropical humid areas offer the most conducive conditions, while arid environments present the

least conducive conditions for larval development. Sandy soil and large-size grasses also contribute to the spread of *Trichostrongylus*, by facilitating the movement of larvae and their survival, respectively (2,14). The transmission of the parasite to humans is often related to the ingestion of water or unwashed vegetables contaminated with infective larvae, especially where manure is used for soil fertilization (7-9,15).

Trichostrongylus spp. human infections are usually asymptomatic. However, in the most severe cases, the hematophagism of the L₄ larvae and adult forms can cause inflammation of the bowel mucosa and tissue damage. The clinical symptoms are cramping, abdominal pain, flatulence, nausea and diarrhea. Infections with a heavy worm load can progress to eosinophilia and anemia (12,13,16). Treatment is similar to that used for other helminthes: albendazole, mebendazole, pyrantel pamoate or ivermectin (15-19).

The diagnosis of *Trichostrongylus* infection is mainly performed by detecting eggs in stool examinations using spontaneous sedimentation or formalin-ether methods (15,17,20). While there is a similarity between *Trichostrongylus* and hookworm eggs, the former are longer and elongated (6,15,20). Despite the vast literature in veterinary medicine on parasites of ruminants, scientific reports on the prevalence of *Trichostrongylus* spp. in humans are scarce. Reported frequencies after 1990's are usually very low, ranging from 0.01% to 2.6%, usually associated with rural workers who work directly with cattle, goats and sheep (13,21-23). In Brazil, there are very few reports of the frequency of *Trichostrongylus*, especially in the northeastern region of the country. This is most likely due to difficulties in distinguishing the parasite eggs from other nematodes, such as hookworms. Therefore, the aim of this study was to evaluate the frequency and seasonality of *Trichostrongylus* infection in individuals living in urban areas of the city of Salvador in northeastern Brazil and to discuss the importance of its differential diagnosis.

Materials and methods

Samples and parasitological analysis

A total of 9,283 fecal samples from patients seen at the Clinical Analysis Laboratory of the Pharmacy College, Federal University of Bahia, were analyzed from January 2006 to May 2008

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using spontaneous sedimentation (Lutz method). Approximately three grams of feces were dissolved in water and filtered through gauze into a 200 ml tapered glass cup for spontaneous sedimentation. After two hours, the supernatant was discarded and the pellet was suspended in water. The sedimentation process was repeated twice to clarify the sediment. For each sediment sample, three slides were examined under light microscopy at 100x and 400x magnification, using an iodine stain. Positive samples for hookworms, *Trichostrongylus* spp or *Meloidogyne* spp. eggs were re-analyzed using an ocular micrometer lens to measure the eggs. *Trichostrongylus* eggs are elongated and range in size from 75-95 μm long by 40-50 μm wide. They are similar to hookworm eggs, however, the eggs of hookworms are 60-75 μm long by 35-40 μm wide and have a shorter length:width ratio. Hookworm eggs also show a clear space between the morula and egg shell, decreasing over the egg maturation. The eggs of *Meloidogyne* spp are ovoid or ellipsoid, measuring 82-120 μm in length and 24-43 μm in width. They may present rounded or oval corpuscles, which resemble the drops of fat found between the morula and the shell.

Analysis of data

Data referencing age, gender, address, physical complaints, levels of blood eosinophilia of *Trichostrongylus*-infected individuals and month of fecal analysis were collected from patient laboratory records. Comparisons of frequencies of *Trichostrongylus*-positive cases, according to

the age and gender of patients and the month of detection of infection were performed using the chi-square test. Values of $p < 0.05$ were considered significant. We evaluated the *Trichostrongylus* infection seasonality by distributing the diagnosed cases into four periods of three months: September to November, December to February, March to May and June to August (with the June to August period corresponding to the rainy season in Salvador).

Ethical considerations

All adult individuals and parents or legal guardians of minors with confirmed *Trichostrongylus* infection were required to sign an informed consent upon receiving laboratory results. This work was approved by the Ethics Committee of the Gonçalo Moniz Research Center, Oswaldo Cruz Foundation.

Results

The parasitological examination of 9,283 fecal samples detected 110 (1.2%) *Trichostrongylus*-infected patients. All cases were confirmed by further analysis of the size and shape of eggs in order to differentiate them from other smooth, thin-shelled, ellipsoidal nematode eggs found in human feces (figure 1). The positive cases were significantly ($p < 0.05$) more frequent in females (1.6%) than in male patients (0.7%) as seen in table 1. The age group between 11-20 years old was the most affected by *Trichostrongylus* infection (1.9%). The positivity observed in this cluster was significantly higher than those found in people aged 51-60 and above 60 years ($p < 0.05$) (table 1).

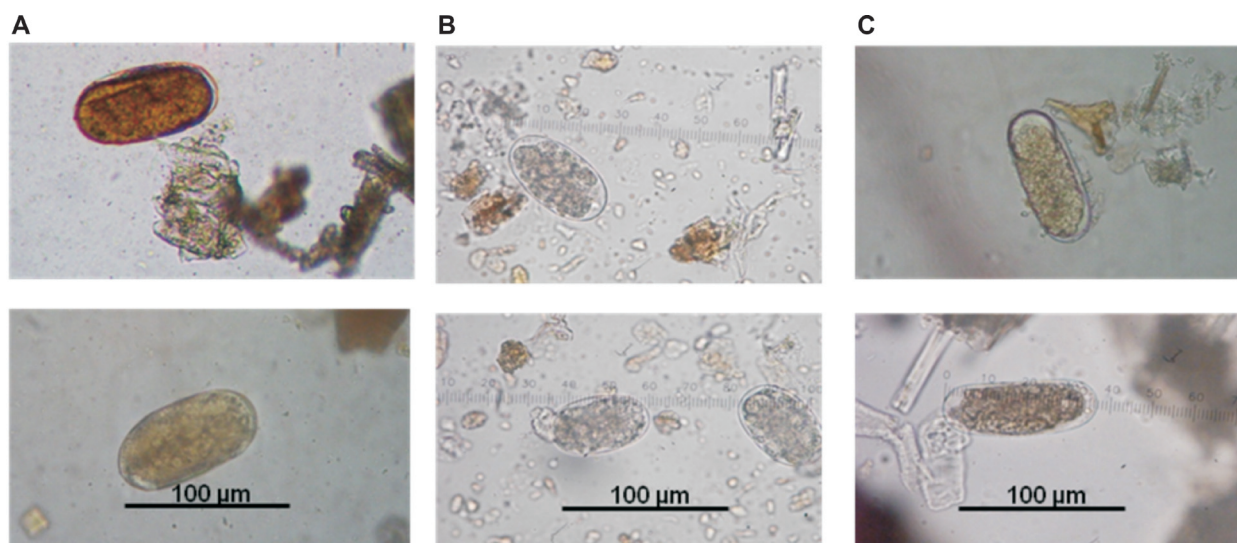


Figure 1. Photomicrographies of nematode eggs in iodine wet mount of fecal samples showing *Trichostrongylus* (A), hookworm (B) and *Meloidogyne* sp. (C), a plant-parasitic nematode, rarely found in human feces.

Table 1. Frequency of *Trichostrongylus* human cases according to gender and age-group of individuals living in urban areas of Salvador, Brazil and attended in a public Clinical Laboratory.

Age groups (years)	Male		Female		Total	
	Tested n	Positivity n (%)	Tested n	Positivity n (%)	Tested n	Positivity n (%)
0-10	351	3 (0.8)	397	6 (1.5)	748	9 (1.2)
11-20	386	5 (1.3)	513	12 (2.3)	899	17 (1.9)*
21-30	1,071	8 (0.8)	1,310	25 (1.9)	2,381	33 (1.4)
31-40	536	2 (0.4)	557	9 (1.6)	1,093	11 (1.0)
41-50	514	3 (0.6)	632	11 (1.7)	1,146	14 (1.2)
51-60	628	3 (0.5)	706	8 (1.1)	1,334	11 (0.8)*
>60	807	5 (0.6)	875	10 (1.1)	1,682	15 (0.9)*
Total	4,293	29 (0.7)	4,990	81 (1.6)*	9,283	110 (1.2)

* $P < 0.05$; statistical difference of frequencies of infection between males and females and among age groups, chi-square test.

Table 2. Distribution of 60 *Trichostrongylus*-infected patients according to their eosinophilia levels and the presence of other intestinal parasites.

Eosinofilia level (%)	<i>Trichostrongylus</i> monoinfection n (%)	<i>Trichostrongylus</i> co-infection with other helminths n (%)	Total n (%)	Helminth related to co-infection (n° of infected patients)
1-6	45 (75)	3 (5)	48 (80)	Hookworm (1) <i>Trichuris trichiura</i> (1) * <i>Trichuris trichiura</i> and <i>Schistosoma mansoni</i> (1)
7-9	5 (8.3)	2 (3.3)	7 (11.7)	<i>Trichuris trichiura</i> (1) <i>Strongyloides stercoralis</i> (1)
10-12	4 (6.6)	1 (1.7)	5 (8.3)	<i>Strongyloides stercoralis</i> (1)
Total	54 (90)	6 (10)	60 (100)	

*Patient with polyparasitism, infected with *Trichostrongylus*, *Trichuris trichiura* and *Schistosoma mansoni*.

The majority of *Trichostrongylus*-infected patients (62.7%; 69/110) were mono-infected. Co-infection with other helminths included: *Trichuris trichiura* (n=4), *Strongyloides stercoralis* (n=2), *Schistosoma mansoni* (n=2), hookworm (n=1) and *Enterobius vermicularis* (n=1). The hematological analyses of 60 *Trichostrongylus*-infected patients showed normal relative levels of eosinophils (1-6%) in 48 individuals and discrete to intense eosinophilia in the 12 remaining cases (table 2). This final group had *Trichostrongylus* associated with *Trichuris trichiura* (n=1) and *Strongyloides stercoralis* (n=2) co-infection (table 2).

Trichostrongylus spp. human infection in Salvador was more common from March to May (40 cases) and showed a homogeneous distribution over the other periods analyzed, with 21 cases from December to February, 25 cases from June to August and 24 cases from September to November, as shown in table 3. However, no statistical difference was found in the distribution of *Trichostrongylus*

infection in this population according to the period of year.

Most *Trichostrongylus*-infected individuals (67 cases) lived in urban areas close to the laboratory location, which is considered a middle-class neighborhood. The other 43 positive cases resided in distant urban boroughs (more than 10 Km distance from the laboratory) of lower socioeconomic characteristics.

Discussion

Human infection by *Trichostrongylus* spp. varies according to the population and geographical region studied, with rates of prevalence higher in rural areas (9). In addition to greater contact with animals, the socioeconomic conditions and hygiene in rural zones tend to be lower than those found in urban areas (11-13).

In comparison with the urban network of other Brazilian cities, Salvador is considered a national metropolitan center. Salvador has 2.6 million

Table 3. Frequency of *Trichostrongylus* positive cases according to the month period of diagnosis of infected subjects.

Three-month period	Parasitological analysis of individuals	
	Tested n (%)	Positivity n (%)
December to February	1,826 (19.7)	21 (1.1)
March to May	2,872 (30.9)	40 (1.4)
June to August	2,132 (23)	25 (1.2)
September to November	2,453 (26.4)	24 (1.0)
Total	9,283 (100)	110 (1.2)

inhabitants, making it the third most populous city in Brazil and the eighth most populous city in Latin America. In this study, all subjects evaluated were from boroughs of Salvador or metropolitan areas, all with basic urban amenities: treated potable water supply, sewage system, rubbish disposal, public lighting, and sidewalks. Nevertheless, other epidemiological risk factors for *Trichostrongylus* infection, such as a past history of visiting rural areas was not collected or analyzed in this study.

The prevalence of 1.2% of *Trichostrongylus* infection in this study was higher than that reported in humans living in urban areas in South Africa (0.1%) (24), Egypt (0.2%) (21) and Brazil (0.2% and 0.05%) (25), and lower than those reported in an irrigation zone in Peru (2.1%) (26), in a livestock-breeding area of a southeast Brazilian city (2.1%) (20), and in patients attending the Mansoura University Hospital in Egypt (2.6%) (21). The heterogeneity in environmental, socioeconomic and personal hygiene habits and characteristics of the populations studied may account for the differences observed in *Trichostrongylus* infection frequencies.

The source of *Trichostrongylus* spp. human infection in urban areas is uncertain. Disturbances in water reservoirs for human consumption caused by contamination with feces of ruminants, rodents or even humans might be implicated (9). Furthermore, water used for irrigation of leaves of lettuce, green onions, parsley, and other greens frequently used to prepare salads and sandwiches, might be contaminated with infective larvae.

Although *Trichostrongylus* spp. infection occurs across all ages and in both genders, women were significantly the most affected group with 1.9% of cases with age between 11-20 years. This may suggest an influence of dietary habits in this cluster (higher intake of fruits or other raw vegetables) predisposing them to infection (27,28), with the

development of resistance leading to reduction of the prevalence in older people, as observed herein. Nevertheless, there is no published data about the role these factors play in the susceptibility of *Trichostrongylus* infection. This is most likely due to the low prevalence of this parasite in humans.

In this work, a higher number of *Trichostrongylus* cases were diagnosed between March and May, the months immediately preceding the rainy season, which corresponds with the autumn months. Salvador has a tropical rainforest climate with no discernible dry season. Temperatures are relatively constant throughout the year, with hot and humid weather conditions. The driest month of the year is January and the rainy season is mainly between May and July, with 789 mm of rainfall. In another study conducted in Brazil, most of the L₃ larvae of *Trichostrongylus colubriformis* were recovered from the forage apex of the *Panicum maximum* (*Aruana* grass) during autumn months, while in springtime, the biggest L₃ recovery occurred at the 21-28 cm stratum from both *Aruana* and *Brachiaria decumbens* (14). The results of this study suggest that the migration of *T. colubriformis* larvae is more influenced by weather conditions than by forage species. The increased vertical migration of larvae on vegetation during March to May might have favored the spread of the parasite among animals and then on to areas with water reservoirs or vegetable plantations intended for human consumption in Salvador. In fact, in tropical and subtropical areas, clinical infections in animals with weight loss and diarrhea can be observed right after the summer, during periods of rain due to rehydration of larvae in hypobiosis (1,2,6).

Concerning the differentiation of the species of the genus *Trichostrongylus*, this usually can be accomplished by evaluating the morphology of larvae, by analyzing the spicules and copulatory bursa of male adults, or by molecular techniques (10,15). In this study, the only evolutionary forms detected in fecal samples were eggs. Therefore, it was not possible to determine the species involved in the positive cases since it would have been necessary to either analyze the morphology of infective larvae (L₃) obtained from fecal culture, or the morphology of adult worms, which are rarely found in feces.

Studies on the epidemiology and diversity of the genus *Trichostrongylus* in human infections are still very limited. The real prevalence may be underestimated because most of the patients are

asymptomatic or develop mild gastrointestinal disorders (13,16). Patients infected with *Trichostrongylus* may be asymptomatic, with normal eosinophilia. Conversely, a high worm burden can lead to marked eosinophilia and development of symptoms, including epigastric pain and diarrhea (12). Most *Trichostrongylus* human cases diagnosed in our study had no complaints of gastrointestinal symptoms and presented normal levels of blood eosinophils and low worm burden, as observed by the few parasite eggs detected in feces. These data suggest that *Strongyloides stercoralis* co-infection was the likely etiology of eosinophilia in two patients with discrete and intense blood eosinophil levels, respectively, as the correlation between asymptomatic persistent eosinophilia and strongyloidiasis is well documented (29). Furthermore, the parasite eggs may be confused with those from hookworms, and, therefore, the use of an ocular micrometer to measure the eggs for a parasitological diagnosis is necessary. In fact, in a recent study conducted in a rural village in Laos, 93.5% (43 from 46) of parasitological cases initially diagnosed as hookworm were later confirmed as *Trichostrongylus colubriformes* by analyzing adults worms eliminated after treatment with albendazole (30). These results show a high transmission of *Trichostrongylus* in the Laos Province and point to a misdiagnosis of the nematode.

Further studies on the *Trichostrongylus* spp. prevalence in human hosts should be carried out using more sensitive diagnostic techniques, such as the agar plate culture, which also permits the identification of L₃ infective larvae. Finally, the appropriate differentiation between hookworms and the genus *Trichostrongylus* is crucial in order to adopt specific measures to control widespread parasitic infections.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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