

A serological survey of agents causing leptospirosis and toxoplasmosis in *Rattus rattus* in the city of Umuarama, northwest Paraná, Brazil

Inquérito sorológico de agentes causadores de leptospirose e toxoplasmose em *Rattus rattus* da cidade de Umuarama, Noroeste do Paraná, Brasil

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Abstract

Synanthropic rodents, present in both urban and rural areas, are responsible for the zoonotic transmission of several diseases to humans as well as for significant economic losses. They act as reservoirs for important viral diseases, bacterial diseases such as leptospirosis, and parasitic diseases such as toxoplasmosis and leishmaniasis. The aim of the present study was to assess the seropositivity of synanthropic rodents in Umuarama city, located in the northwestern region of Paraná State, Brazil, to agents causing leptospirosis and toxoplasmosis. The microscopic agglutination technique (MAT) was used to detect anti-*Leptospira* antibodies, and the indirect immunofluorescence reaction (IIFR) was used to detect anti-*Toxoplasma gondii* antibodies. Of 178 animals collected, four (2.24%) were seropositive for *Leptospira* spp. and ten (5.62%) for *Toxoplasma gondii*. Ninety-five (53.38%) of the collected animals were male and 83 (46.62%) were female, and two (1.23%) originated from urban areas while 176 (98.87%) originated from peri-urban areas. Serological results showed that the synanthropic rodents examined here had low seroreactivity for agents causing both leptospirosis and toxoplasmosis, in both urban and peri-urban regions of Umuarama city. This could be associated with the high Human Development Index for the study area. However, preventative measures must continue to be observed, as rodents are important reservoirs for, and disseminators of, disease causing agents.

Key words: *Leptospira* spp. Rats. Serological diagnosis. *Toxoplasma gondii*. Zoonosis.

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Resumo

Os roedores sinantrópicos determinam grandes prejuízos econômicos, e são responsáveis pela transmissão de várias zoonoses ao homem tanto em áreas urbanas quanto nas rurais, atuando como reservatórios de importantes doenças que incluem a leptospirose, toxoplasmose, leishmaniose e algumas de etiologia viral. O presente trabalho investigou a sororeatividade para leptospirose e toxoplasmose em roedores sinantrópicos da cidade de Umuarama, cidade localizada na região noroeste do estado do Paraná, Brasil. A técnica Soroaglutinação Microscópica (SAM) foi utilizada para a detecção de anticorpos anti-*Leptospira spp* e a Reação de Imunofluorescência Indireta (RIFI) para a detecção de anticorpos anti-*Toxoplasma gondii*. Dos 178 animais examinados, quatro (2,24%) foram reagentes para *Leptospira spp.* e 10 (5,62%) para o *Toxoplasma gondii*. Destes animais, 95 (53,38%) eram machos e 83 (46,62%) fêmeas, dois (1,23%) eram provenientes da área urbana e 176 (98,87%) da área periurbana. Os resultados obtidos demonstraram que os roedores sinantrópicos examinados apresentaram baixa frequência de sororeatividade para as duas zoonoses investigadas, tanto na região urbana como na periurbana da cidade de Umuarama, fato que pode estar associado ao alto Índice de Desenvolvimento Humano encontrado, contudo, as medidas preventivas devem ser mantidas, pois os roedores são importantes elos na cadeia epidemiológica das doenças analisadas.

Palavras-chave: Diagnóstico sorológico. *Leptospira spp.* Ratos. *Toxoplasma gondii*. Zoonoses.

Introduction

Synanthropic rodents, present in both urban and rural areas, are responsible for the zoonotic transmission of several diseases to humans as well as for significant economic losses. They act as reservoirs for important viral diseases, bacterial diseases such as leptospirosis, and parasitic diseases such as toxoplasmosis and leishmaniasis. Control of these rodents is therefore considered important for public health. As humans have invaded or destroyed their natural habitat these animals have undergone a synanthropic process, which has increased their contact with humans and thus the risk of disseminating the diseases they harbor (BRASIL, 2002; FAINE et al., 1999; GERÔNIMO et al., 2014; PORTA et al., 2014).

Rodents are important reservoir hosts for *Leptospira spp.*, and disseminate the etiological agent through contaminated urine, water, soil, and food. Additionally, they increase the susceptibility of human beings and other animal species to further *Leptospira* infection (BRASIL, 2002, 2010a; LEVETT, 2001; MORIKAWA, 2010).

Leptospirosis is a broadly disseminated, contagious disease that is thought to be one of the most widespread zoonotic diseases worldwide

(PALANIAPPAN et al., 2007). In recent decades this disease has been widely neglected, in large part because its symptoms are nonspecific and easily confused with those of other diseases, such as influenza, leading to incorrect diagnoses and fewer notifications (HARTSKEERL et al., 2011; SOCOLOVSKI et al., 2011). However, leptospirosis is considered to be a re-emergent disease with serious public health implications (HARTSKEERL et al., 2011).

The prevalence of leptospirosis in rodents, as determined by the level of antibodies raised in response to different serovars, ranges from 3.33% to 30.2% in different countries (AGUDELO-FLÓREZ et al., 2009; FELT et al., 2011; GAROUSSI et al., 2006; HALLIDAY et al., 2013; MARTINS; LILENBAUM, 2013; MGODE et al., 2014; MOHAMED et al., 2010; SPOHR, 2009; WANG; HE, 2013). In Brazil, leptospirosis positivity rates of 9.3% and 17% were detected in Paraná (PR) (SPOHR, 2009) and Rio de Janeiro (MARTINS; LILENBAUM, 2013), respectively.

Leptospirosis is not the only disease transmitted by rodents. In some cases, rodents infect other animal species, which then become definitive hosts and disseminators of the infectious agent.

This is evident in the case of toxoplasmosis, a disease caused by *Toxoplasma gondii* (*T. gondii*), which infects mammals, birds, and warm-blooded vertebrates (HERRMANN et al., 2012). Rodents participate in the chain of infection by acting as the source of infection for several animal species, mainly felines, which become definitive hosts for *T. gondii* (COLA et al., 2010; DUBEY; FRENKEL, 1998; HERRMANN et al., 2012).

The seroreactivity of rodents to *T. gondii* has been investigated in several countries, with seropositivity ranging from 1.96% to 27.50% (COLA et al., 2010; JITTAPALAPONG et al., 2011; MERCIER et al., 2013; RUFFOLO, 2008; VUJANIĆ et al., 2011). In Paraná, Brazil, 8.80% and 4.94% seropositivity to *T. gondii* was reported by Ruffolo (2008) and Cola et al. (2010), respectively.

Considering the absence of regional data and the importance of rats as transmitters of infectious-parasite diseases to humans and also other animal species, the purpose of this paper was to assess the seroreactivity to leptospirosis and toxoplasmosis in synanthropic rodents in the city of Umuarama, in the Northwestern Mesoregion of the State of Paraná, Brazil.

Material and Methods

Study area

Synanthropic rodents were captured in the urban and peri-urban areas of Umuarama city (23°45'59"S; 53°19'30"W), and blood samples were collected. This region has an average altitude of 442 m and an area of 1232.5 km², and is located in the northwestern mesoregion of Paraná state (IPARDES, 2012).

Rodent Capture and Sample Preparation

The rodents were captured between October 2012 and October 2013, using galvanized wire

traps (Tomahawk) measuring either 30x14x14cm or 45x22x22cm in which the triggering mechanism was activated by placing bait within the trap. The baits used were raw corn, banana, sausage, cheese, and feed based on sardines, peanuts, corn flour, and banana, which was invented by the project researchers. The traps were assembled in the evening at locations where the presence of rodents was indicated by feces, trails, or fat stains, and were then collected the following morning (ARAUJO et al., 2010; RUFFOLO, 2008). The captured rodents were then transported to the Laboratory of Preventive Veterinary Medicine and Public Health at the Universidade Paranaense, Brazil, for investigation.

The rodents were desensitized in a halothane-saturated vapor chamber (ARAUJO et al., 2010), identified using the criteria described in the Manual for Rodent Control (*Manual de Controle de Roedores*, BRASIL, 2002), and guillotined. Blood samples were centrifuged at 1500 rpm for 10 minutes, and the sera were aspirated, transferred to 2 ml tubes, and stored at -20°C until use.

Diagnostic Tests

Microscopic Seroagglutination test (MAT)

Anti-*Leptospira* spp. antibodies in the serum samples were measured at the Laboratory of Leptospirosis, the Department of Preventive Veterinary Medicine (DMVP), the State University of Londrina (UEL), using a MAT assay with live leptospire as antigens. Twenty-three reference *Leptospira* serovars were used: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Whitcomb, Cynopteri, Fortbragg, Grippotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Sentot, Tarassovi, and Ballum. Leptospire were maintained at 28 °C for 5-10 days in Difco™ *Leptospira* Enrichment

medium (EMJH, DIFCO®-USA)⁹ supplemented with rabbit serum (ALVES, 1996). Rat serum samples were serially diluted to 1:25 for use in the MAT assay (RIEDEMANN et al., 1994; SPORH, 2009).

Sera that agglutinated at least 50% of the leptospires were considered seropositive. Two-fold serial dilutions of positive samples were tested in order to determine the titer (the maximum dilution that gave a seropositive result). The serovar with the highest titer was considered dominant for that sample (VASCONCELLOS et al., 1997). In serum samples where more than one serovar presented the highest titer, *Leptospira* spp. was considered dominant (ALMEIDA et al., 1994).

Indirect Immunofluorescence Reaction (IIFR)

The IIFR technique was performed at the Laboratory of Zoonoses and Public Health, DMVP, UEL, as described by Camargo (1973). The sera were serially diluted four-fold, starting from a 1:16 dilution, until the maximum titer was reached. Samples with a titer greater than or equal to 16 were considered seropositive.

Results

A total of 178 rodents of various ages were captured, and all were identified as *Rattus rattus*. Of these, 95 (53.38%) were male and 83 (46.62%) were female, and 2 (1.23%) originated from urban areas while the remaining 176 (98.87%) originated from peri-urban areas.

Four of the 178 samples were determined by MAT assay to be seropositive for leptospires, with antibodies against the following serovars

being detected at a 1:25 dilution: Hardjo (2/4), Copenhageni (1/4), and Pyrogenes (1/4). Two (50%) of the seropositive rats were male, and two (50%) were female.

Ten of the 176 samples were determined by IIFR assay to be seropositive for toxoplasmosis, with titers of 16 (7/10), 64 (2/10) or 1024 (1/10). Of these ten rats, eight were male and two were female.

Discussion

Previous studies that aimed to understand the epidemiology of leptospirosis and toxoplasmosis identified anti-*Leptospira* spp. and anti-*T. gondii* antibodies in rodents from different worldwide locations (COLA et al., 2010; HALLIDAY et al., 2013; JITTAPALAPONG et al., 2011; MARTINS; LILENBAUM, 2013; MERCIER et al., 2013; MGOODE et al., 2014; RUFFOLO, 2008; SPOHR, 2009; VUJANIĆ et al., 2011; WANG; HE, 2013).

When human beings settle in a defined location and develop their own infrastructure and living conditions, commensal relationships develop between humans and rodents, and synanthropic processes are initiated (BRASIL, 2002). In urban centers, deficiencies in basic sanitation are critical factors in the dissemination of rodents and, consequently, the dissemination of zoonoses that use rodent hosts during their epidemiological cycle (MORIKAWA, 2010).

The seropositivity of synanthropic rodents for leptospires reported here, 2.27%, was lower than that reported previously, wherein *R. rattus* in Londrina (PR) (SPOHR, 2009) and *Rattus norvegicus* in Rio de Janeiro (MARTINS; LILENBAUM, 2013) had seropositivity rates of 9.3% and 36.2%, respectively, for anti-*Leptospira* spp. antibodies. These serological differences might reflect differences in the seroreactivity of animal leptospirosis in the respective locations, or differences in the study length, with infection being less likely in a shorter study period.

⁹ DIFCO – Becton, Dickinson and Company 7 Loveton Circle Sparks, MD 21152 USA 800-638-8663 www.bd.com/ds ATCC is a trademark of the American Type Culture Collection. Difco is a trademark of Difco Laboratories, Inc., subsidiary of Becton, Dickinson and Company. BD and BD Logo are trademarks of Becton, Dickinson and Company. © 2011 BD.

The low prevalence of leptospirosis observed here could be due to multiple factors, including periods of hydric deficiency (ROSEGHINI et al., 2000), and the prevalence of soil containing more than 70% sand in the northwestern region of Paraná state where the study takes place. This soil composition is the reason for the local name for the region – Arenito Caiuá, or Sandstone Caiuá (EMBRAPA, 2006). Such sandy soil is very water permeable and thus very dry as water quickly dissipates, which could impede the survival of *Leptospira*, as these organisms require humidity for the maintenance of active infection in animals (DREER, et al. 2013; FAINE et al., 1999).

The most prevalent serovar in this study was the Hardjo serovar, which was present in 50% of the samples. The antibody against this serovar was raised in cattle (MINEIRO et al., 2007). There is only a short distance between the perimeter of the peri-urban region where the rodents were captured and some rural properties, and the people and animals residing in these properties are susceptible to infection. Individuals working directly with animals, such as farmers, veterinarians, rural workers, and slaughterhouse workers, can reportedly acquire leptospiral infection through contact with contaminated urine, milk, aborted fetuses, carcasses, or placentas (WASIŃSKI; DUTKIEWICZ, 2013; GONÇALVES et al., 2013). Indeed, Clazer (2016), studied the same micro-region as this study and observed the presence of antibodies against the Hardjo serovar in the sera of 50% of university students enrolled on a veterinary medicine course.

In a report published in 2016 by Brazil's Ministry of Health (BRASIL, 2016), the Copenhageni serovar was associated with the most severe human cases of leptospirosis. A wide variety of susceptible animals can host this serovar (FORSTER et al., 2013; LANGONI et al., 2016; RODRIGUES et al., 2015). Maciel et al. (2008) investigated seropositivity to the Copenhageni serovar in 269 residents of peripheral neighborhoods in the city of Salvador in Bahia, Brazil, and found that 30%

were seropositive, with titers ranging from 25 to 400. Additionally, a study of bacteria isolated from the sera of ten sick individuals as well as rodents in the São Miguel parish of Lisbon showed that 70% were seropositive for the *Leptospira interrogans* species of the Copenhageni serovar (GONÇALVES, 2009). Additionally, a further study investigating *R. rattus* in a peripheral neighborhood of Barranquilla, Columbia, described the presence of the Copenhageni serovar and noted that, while infection did not present as an acute condition in rodents, the animals could act as reservoirs, leading to more severe infection in humans (ROMERO-VIVAS et al., 2013).

The first report of seropositivity to the Pyrogenes serovar in Brazil was in 1980, in sera from *Nectomys squamipes*, a wild mammalian species (SANTA ROSA et al., 1980). This serovar is now routinely included in serological assays for infection with *Leptospira* for several animal species, and recent studies have shown its prevalence in goats and sheep (HIGINO; AZEVEDO, 2014), dogs (FONZAR; LANGONI, 2012; MASCOLLI et al., 2016; SAMIR et al., 2015), and humans (FONZAR; LANGONI, 2012; SAMIR et al., 2015; BRASIL, 2010b).

The Copenhageni and Pyrogenes serovars detected in this study can reportedly infect humans (GONÇALVES, 2009; FONZAR; LANGONI, 2012). In Brazil, there were 1,827 deaths from leptospirosis between 2011 to 2016 according to the National Medical Service System (SINAM, c2016), and so epidemiological studies to investigate patterns of infection and establish the dominant causative serovars are crucial for disease prevention and public health education measures.

In 2013, Dreer et al., reported a 20% seroprevalence of anti-*Leptospira* spp. antibodies in stray dogs in Umuarama city, a higher prevalence than is reported in this study, demonstrating the importance of stray dogs as probable sources of infection for other domestic animal species as well as rodents. It is also important to emphasize that

antibodies against the same serovars (Hardjo & Pyrogenes) were detected in stray dogs by Dreer et al. (2013) and in rodents in this study.

Considering toxoplasmosis, cats are considered to be definitive hosts for the infection, and are thought to contract the disease by ingesting the sporulated *T. gondii* oocysts that are usually present in the intestinal villi of infected rodents (PIZZI, 1997). Once infected, cats can eliminate approximately 360 million oocysts in a single day. Such oocysts are extremely resistant to harsh environments and can sporulate, allowing them to survive in water for several months. Stray cats are therefore critical to the epidemiological cycle of the disease (ARAÚJO et al., 2010; DUBEY; FRENKEL, 1998; PINTO et al., 2009).

In this study, 5.68% of the samples analyzed were seropositive for *T. gondii*, a higher proportion than was detected by Cola et al. (2010) in Londrina (PR) (4.94%), Mgode et al. (2014) in Dar es Salaam, Tanzania (2.17%), and Mercier et al. (2013) in Niamey, Nigeria (1.96%).

The epidemiological impact of toxoplasmosis makes this disease a significant public health concern. Transmission of *T. gondii* to humans can occur via congenital infection or through the ingestion of cysts in undercooked meat or oocysts in contaminated water (ABREU et al., 2001; CLAZER, 2016). Clazer (2016) reported a *T. gondii* seroprevalence of 29.29% in humans. This is in contrast to the 5.68% seroprevalence in rodents reported here, even though the two studies considered the same geographical region. It is therefore possible to incorporate both species into the epidemiological disease cycle in this region, with rodents being potential disseminators of the disease.

Other important sources of *T. gondii* transmission are contaminated water and meat. From December 2001 to January 2002, an outbreak occurred in the city of Santa Isabel do Ivaí (PR) when a water reservoir became contaminated with the protozoan, most

likely through contaminated cat urine (ALMEIDA et al., 2011). This led to the conclusion that the water supply system was particularly vulnerable to protozoan oocytes contamination. This city is located just 117 km from the city of Umuarama, and thus belongs to the same mesoregion that was considered in the present study. Furthermore, the ingestion of tissue cysts in meat from infected animals, either by humans eating undercooked meat or by carnivorous animals, can contribute to disease spread. In this case, rodents are an important part of the disease cycle.

The region studied in this report, particularly the city in which the rodents were captured, had one of the highest human development index for municipalities (HDI-M) scores in the northwestern mesoregion of Paraná from 2000 to 2010 (TOMÉ; LIMA, 2014). During this period, longevity, income, and education significantly improved, leading to consequent improvements in lifestyle and health. The quality of sanitation improved concurrently with the human development index, reducing the dissemination of the zoonotic diseases analyzed and thus the potential for exposure, which could be a factor in the lower prevalence of both *Leptospira* and *T. gondii* in rodents in the study region.

Conclusion

The rates of infection with the agents causing leptospirosis and toxoplasmosis recorded in the study region were lower than the expected rate, due to unfavorable soil composition and programs for the collection of stray animals reducing the potential for dissemination. Synanthropic rodents, which act as natural reservoirs for a variety of zoonoses, presented a low seroprevalence for *Leptospira* and *T. gondii* in both the urban and peri-urban regions of Umuarama city. This low prevalence could be associated with zoo-sanitary measures, but action to prevent disease dissemination must continue to be observed, as rodents are known to be reservoirs for the agents causing these diseases.

Few studies investigating leptospirosis, toxoplasmosis, or other infectious parasitic diseases in rodents have been published, as capturing these animals is difficult. For this reason, further studies into the infection of rodents with pathogenic agents are vital for a full understanding of their role in zoonotic transmission in a given geographical area.

Ethics Committee

Animals were captured and handled according to the principles established by the National Council for the Control of Animal Experimentation (CONCEA). The study was approved by the Ethics Committee in Research Involving Animal Experimentation (CEPEEA) at the Universidade Paranaense (UNIPAR, project protocol no. 21822/2012).

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