OCCURRENCE OF *Cryptosporidium* sp IN SINANTROPIC *Rattus* (FISCHER, 1803) AND *Mus* (LINNAEUS, 1758) CAPTURED IN THE URBAN AREA OF UMUARAMA, PR, BRAZIL

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ABSTRACT: Sinantropic rodents, mainly of the genus *Rattus* and *Mus*, can be used as indicators of environmental contamination by *Cryptosporidium* oocysts, responsible for high rates of infection in humans and animals, and for economic losses and poor health of individuals. In this work, in order to measure the frequency of *Cryptosporidium* in rodents in the urban area of Umuarama, PR, fecal samples of these animals were collected and analyzed using the Kinyoun staining method to determine the absolute and relative frequency of the parasite. From 50 samples collected, 26 showed rounded reddish structures, with the presence of parasitic forms inside, compatible with oocysts of *Cryptosporidium* sp. Higher frequency (P-value <0.05) is found in *Rattus*, with no difference between sexes and origin of animals. The measurement of oocysts suggests the occurrence of zoonotic species *Criptosporidium parvum*.
KEYWORDS: Cryptosporidiosis; Rodent; Frequency; Zoonosis.

OCORRÊNCIA DE *Cryptosporidium* sp EM SINANTROPIC *Rattus* (FISCHER, 1803) E *Mus* (LINNAEUS, 1758) CAPTURADO NA ÁREA URBANA DE UMUARAMA, PR, BRASIL

RESUMO: Os roedores sinantrópicos, principalmente dos gêneros *Rattus e Mus,* podem servir como indicadores da contaminação ambiental por oocistos de *Cryptosporidium,* parasito celular obrigatório, responsável por elevadas taxas de infecção no ser humano, e animais, e por perdas econômicas, e na saúde dos indivíduos. Neste trabalho, verificou-se a frequência de *Cryptosporidium* em roedores na área urbana de Umuarama, PR. Para tanto, foram coletadas amostras fecais dos roedores, que foram analisadas pelo método de Kinyoun, determinando a frequência absoluta e relativa do parasito. Das 50 amostras coletadas, 26% apresentaram estruturas arredondadas, com presença de formas parasitárias em seu interior e coloração avermelhada, compatíveis com oocistos de *Cryptosporidium* sp. Maior frequência (P>0.05) foi encontrada em *Rattus* sem diferença entre os sexos e a origem dos animais. A mensuração dos oocistos sugere a ocorrência da espécie zoonótica *Criptosporidium parvum.*
PALAVRAS-CHAVE: Criptosporidiose; Roedores; Prevalência; Zoonose.

OCURRENCIA DE *Cryptosporidium* sp EN SINANTROPIC *Rattus* (FISCHER, 1803) Y *Mus* (LINNAEUS 1758) CAPTURADO EN LA ZONA URBANA DE UMUARAMA, PARANÁ, BRASIL

RESUMEN: Roedores sinantrópicos, principalmente del género *Rattus* y *Mus*, pueden servir como indicadores de contaminación ambiental por ooquistes de *Cryptosporidium*, parásito celular obligatorio, responsable por altas tasas de infección en el ser humano y animales, y para la salud de las personas y pérdidas económicas. En este estudio se verificó la frecuencia de *Cryptosporidium* en roedores de la zona urbana de Umuarama, PR. Fueron recogidas muestras de heces de los roedores, que fueron analizadas por el método Kinyoun, determinándose la frecuencia absoluta y relativa del parásito. De las 50 muestras recolectadas, 26% presentaban estructuras redondeadas, con presencia de formas parasitarias en su interior y coloración rojiza, compatibles con ooquistes de *Cryptosporidium* sp. Mayor frecuencia (P<0.05) se encontró en *Rattus*, sin diferencia

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entre los sexos y el origen de los animales. La medición de los ooquistes sugiere la ocurrencia de la especie zoonótica *Cryptosporidium parvum*.
PALABRAS CLAVE: Criptosporidiosis; Roedores; Prevalencia; Zoonosis.

Introduction

The genera *Cryptosporidium* is an intracellular protozoa parasites that infect epithelial cells of intestinal microvilli membranes in all classes of vertebrates, including man and domestic animals, occurring throughout the world. The pathogenic effects of infection vary according to the species of the parasite and the immune status of the host, however can range from asymptomatic infection to severe acute or chronic pictures with a tendency to become self-limiting (FAYER; XIAO, 2007).

Current evidence indicate that the main reservoirs of zoonotic Cryptosporidiosis are livestock animals, however, genotypes associated with dogs and cats, with less frequency, and deer and Lagomorphs also has been incriminated as potential infective to humans, which justifies the demand for research of new hosts in different geographical areas (THOMPSON; SMITH, 2010).

In Brazil, studies with *Cryptosporidium* and criptosporidiosis are mainly focused on birds, cattle, dogs, cats, and humans, with some work in wild birds and rodents, as well as in *Cavia porcelus* (MEIRELES, 2010). Despite the importance of sinantropic rodents in the chain of transmission of several zoonoses (BRASIL, 2002), few brazilian studies have evidenced the occurrence of *Cryptosporidium* in these species.

The present work had as objective to verify the occurrence of infection by *Cryptosporidium* spp. in sinatropic rodents in the urban area of Umuarama, Paraná, Brazil.

Material and Methods

The sinantropic commensal rodents were captured in Sherman and cage traps, in locations with reports or signs (stains, feces) from the presence of these animals, such as irons hoods, households and schools. In each trap was placed as bait a small fragment of raw corn. The traps were checked every day, and once they had captured rodents, they were transported to the laboratory, where they were submitted to the collection of samples and laboratory tests.

In the laboratory, the trapped animals were anesthetized in a chamber saturated with halothane vapour; and to achieve the anesthetic plan, euthanasia by inoculation of potassium chloride solution via heart inoculation. The species and sex were determined according to BRASIL (2002). Then the animal was fixed in Styrofoam plate in supine position, and the peritoneal cavity open with the aid of scissors and tweezers. Then, the digestive tube was removed and all of its contents transferred to a 15 mL Falcon tube. To this tube was added the MIF (Merthiolate-Iodine-Formaldehyd) fixative, in volume of five times the volume of intestinal contents recovered. The sample was stored under refrigeration until the examination for detection of *Cryptosporidium*. During all procedures for specimen collection and handling of these animals, biosecurity measures have been taken, using chemical and physical barriers, such as individual protection equipment (lab coat, gloves and gas barrier masks) and chemical (formalin solution, disinfection with sodium hypochlorite), as recommended by BRASIL (2002).

The detection of *Cryptosporidium* by Kinyoun method was performed by WHO (1994), with approximately 0.5 mL of each stool sample. The stained slides were examined in optical microscope under 100x objective. Were considered positive smears that have typical oocysts represented by structures with compatible size, presence of reddish esporocistys containing esporozoytes on the bluish background. The probable species was determined by measurement of equatorial and polar axes of oocysts (BOMFIM; LOPES, 1995), in images captured in digital system attached to the microscope.

Were calculated the absolute and relative frequency, as well as 95 confidence intervals for the detection of *Cryptosporidium*. The influence of species, sex and origin of the samples on the frequency detection of *Cryptosporidium* was evaluated by Pearson χ^2 test with calculation of the odds ratio and its 95 confidence interval. P values less than 0.05 was considered significant. The origin of the samples are divided in three areas: 1, samples collected in houses; 2, samples collected in an high school campus; and 3, samples collected in a veterinary hospital.

Capture of rodents and sample collection were submitted and approved by institutional committee on animal use on research (14707/2009).

Results

From 50 captured rodents, all samples were submitted for Kinyoun staining. From these, 13 samples (26.0%; CI95% 14.6-40.3) presented rounded structures, with the presence of reddish parasitic forms compatible with oocysts of *Cryptosporidium*.

The positive frequency was 5.8 (CI95% 1.13-29.9) higher in *Rattus Rattus* (37.9; CI95% 22.6 -56.1) than in *Mus musculus* (9.5%; CI95% 2.9 -29.2), with P-value=0.0531. Regarding sex, there was no significant difference (p=0 .4161), with 19.2 (CI95% 8.6-38.1) and 33.3 (CI95% 17.9-53.5) positive females and males, respectively.

According to the origin of the animals, in the area 1, 5 animals were captured, being 1 positive; in the area 2, 7 animals were captured and 2 were positive; and in the area 3, 38 animals were captured, 10 were positive, with no significant difference between the areas (p-value =0.9420).

Measurement are taken from 138 oocysts, being 21 from samples of *Mus* and 77 from *Rattus*. For *Mus* equatorial diameter (ED) was 4.6±0.8 mm and polar diameter (PD) was 3.6±0.6 mm; for *Rattus* ED=4.9±0.9 mm and PD=3.9±0.7 mm. From the evaluation of the data obtained, regardless of the species examined, it turns out that the diameters obtained allow characterizing the oocysts found as being species *Cryptosporidium parvum*, in accordance with the classification suggested by Bonfim and Lopes (1995).

Discussion

Cryptosporidium positive rodent indicates the risk of infection to other animals, and for Bajer et al. (2008), sinantropic rodents also are part of the chain of transmission of cryptosporidiosis, acting as a source of infection for humans and animals, and these authors identified the species *Rattus norvegicus* rodents as sources of water contamination by *Cryptosporidium*.

In this study, we detected no significant difference in the frequency of positive between *Mus* and *Rattus*. This effect can be strongly related to the origin of animals. *Rattus* were more often found in area 3, a Veterinary Hospital, located in a suburban area, where there were several species of animals living so close, in varied environmental conditions. LV et al. (2009) points out that the environmental conditions in which live animals are important in determining the rate of infection by *Cryptosporidium*, which determines different rates in rodent species evaluated. However, individual factors related to the interaction between the parasite and the host cannot be discarded, since in the long field study period, Bajer et al. (2001) reported a higher frequency of parasite in the species *R. norvegicus*, between the three species evaluated.

In isolation, gender is not a factor associated with the rate of infection with this parasite in rodents. As in this work, LV et al. (2009) found no differences, regardless of species evaluated, and when Bajer et al. (2008) reports difference between the sexes, this is only checked when associated to the information age or place of capture, depending on rodent species examined.

The environmental conditions of the capture locations may influence infection rates of rodents. In this work, the areas 1 and 2 were characteristically urban, and the 3 area, suburban, with opportunity of interaction of rodents with different species of animals, including ruminants and monogastric herbivores. In this last area the highest rate of infection of animals was found, similarly to reported by Paparrini et al. (2012). These authors found higher rates of infection in *R. rattus* in suburban areas, where the coexistence with other animals and the environmental characteristics were varied.

The possibility of interaction with animals or with environments used by cattle is important in determining the rate of infection of rodents, which eventually become reservoirs of the parasite for cattle (STURDEE et al., 2003) and also determines that the rodents will host parasite genotypes found most often in cattle, and of zoonotic potential (HAJDUSEK et al., 2004).

In Brazil, few works have been published with data regarding *Cryptosporidium* species prevalent in rodents. Bonfim and Lopes (1995) do not report the rate of infection of six *R. rattus* found in a pig farm, but compare the oocysts obtained in these species with those obtained from feces of pigs, concluding, by morphological parameters to be *C. muris*, a species that can affect both rodents and pigs. In a study conducted in Londrina-PR (RUFFOLO, 2008), 181 *Rattus Rattus* and one *Mus musculus* were captured. Of these, 26 animals (14.2) samples showed oocysts of *Cryptosporidim* sp; 14 of the species *C. muris*, 11 of the species *C. parvum* and one with both.

Using the morphological classification criterion (BOMFIM and LOPES, 1995) all parasites measured in this work belong to the species *C. parvum*. Phylogenetic studies on this species, with isolates derived from different animals and man, however, suggest more genotypes adapted to one or another species, which need to be checked to be inferred about the actual zoonotic potential of parasites found in a given animal species.

LV et al. (2009) contrast morphological and molecular data, determining similarity in the rankings: *C. muris* (non zoonotic) showing larger oocysts, while genotypes from *Rattus* feature smaller oocysts, compatible with *C. parvum* and potentially zoonotic. It is noteworthy that in this research, even the *Cryptosporidium* from mice had compatible size with *C. parvum*. Ajjampur et al. (2007) describe the discover of *C. parvum* genotypes in murine in children with diarrhoeal illness, but in lower prevalence than the antroponotics genotypes of the parasite. In this way only molecular studies allow discrimination of genotypic parasite can properly inform about the zoonotic potential of parasites found in this work. These studies are in progress.

Conclusion

Rodents were found positive for oocysts of *Cryptosporidium*, which allows to infer that these animals are members of the transmission chain of the parasite, and can serve as reservoirs for other animals and eventually to humans.

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