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Full Length Research Paper

Morphometric and quantitative analysis of the intestine of *Rattus rattus* infected by *Strongyloides* spp.

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Strongyloidiasis is caused by *Strogyloides* spp., which occurs when the parasite reaches the intestines. It is estimated that more than 370 million people throughout the world are infected with this disease. The aim of this study was to analyze the morphometric alterations and variation in the count of goblet cells of the jejunum of sinantropic rodents infected by *Strongyloides* spp., and to compare with non-infected rodents. 157 rodents of the species of *Rattus rattus* were captured. After identifying the intestinal parasite, 20 rodents were selected. The criteria used were: animals between 20 and 25 cm (n=10), 5 females and 5 males infected by *Strongyloides* spp. and 5 females and 5 males (n=10) without the parasite. Sedimentation technique was used to identify the parasite. Morphometric and quantitative analysis of the intestinal wall was done through histological processing. In male and female infected rats, the followings were observed: smaller villus height, crypt depth increased, smaller villus area and a larger number of goblet cells in the crypts of the jejunum. The morphologic alterations of the jejunum wall may impair nutrient absorption process.

Key words: Goblet cells, crypts, jejunum, sinantropic rodents, villi.

INTRODUCTION

The order Rodentia is the largest mammal group alive, with 2,277 known species, comprising 42% of the mammal biodiversity in the world (Don and Reeder, 2005). Rodents are divided into 34 families (Musser and Carleton, 2005); in Brazil there are three species of the rodent family Muridae: *Rattus rattus*, *Rattus norvegicus*

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and *Mus musculus*. Most rodent species live in wild environments, in nature, being part of the food chain of predators such as prey birds, snakes, and lizards. However, some species adapt better to manmade environmental conditions. These are the species considered as synanthropic (BRAZIL, 2006).

The proximity of such animals to humans has become a public health issue, since they are carriers of several infectious and parasitory diseases (BRASIL, 2006). Research made in different states of Brazil, including Paraná (PR), Sergipe (SE) and Minas Gerais (MG) has observed prevalence of intestinal parasites in synanthropic rodents (Porta et al., 2014; Guimarães et al., 2014; Mati et al., 2014). This study surfaces the importance of synanthropic rodents in the transmission chain of different parasite related illnesses to humans and other animal species.

The Strogyloide nematode belongs to the Strongyloidae family (Rey, 2001) and comprises more than 52 nematodes that parasite vertebrates (Costa et al., 1997). The disease known as strongyloidiasis is a parasitosis. It is estimated that 370 million people throughout the world are infected with it (Buonfrate et al., 2014). In Brazil, it is one of the most prevalent parasitic diseases (Sudré et al., 2006). It infects farmers and farm workers, immigrants and travelers who visit endemic areas, and also children exposed to contaminated soil (Costa et al., 1997).

This parasite infects humans through skin penetration, and has a complex life cycle involving 2 stages of development: a phase of free-living that occurs in the environment, good conditions of temperature and humidity, and the other stage as a parasite, which occurs within the host (Paula and Costa-cruz, 2011).

Infection rate varies according to the studied region and method chosen for the diagnosis (Costa et al., 1997). The clinical characteristics of strongyloidiasis are connected to the degree of parasite infection, nutritional status of the host and absence of other gastrointestinal parasitosis (Machado, 2003).

The presence of parasites inside the gastrointestinal tract may cause reduction of villi and microvilli. This reduction can cause decreased absorption and malnutrition. The jejunum is a portion of the small intestine whose function is to regulate motility and absorption of nutrients. Another factor responsible for the adequate growth of the animal is the quality of food provided, as well as the capacity to use these nutrients. It is well known that malnutrition affects several organs and is correlated to low body weight (Torrejais et al., 1995; Natali et al., 2000; Zanim et al., 2003).

Morphological alterations caused by malnutrition have been extensively assessed, demonstrating a reduction of thickness of the intestinal wall layers, reduction of the tunica mucosa (Rodrigues et al., 1985; Brandão et al., 2003; Hermes et al., 2008), villi length (Firmansyah et al., 1989; Gurmini et al., 2005), crypt depth (Firmansyah et al., 1989; Hermes et al., 2008), and tunica muscularis (Torrejais et al., 1995; Brandão et al., 2003; Azevedo et al., 2007).

Within this context, the present study aimed to assess the morphometric alterations and variation in goblet cells of the jejunum of synanthropic rodents infected with *Strongyloides* spp. compared to non-parasite infected rodents.

MATERIALS AND METHODS

Ethical aspects

This study was approved by the Research Ethics Committee for Animal Experimentation (Comitê de Éticaem Pesquisa Envolvendo Experimentação Animal, CEPEEA) from Universidade Paranaense (UNIPAR), following the rules of the National Council for Animal Experimentation (Conselho Nacional de Controle de Experimentação Animal, CONCEA), under protocol no. 24469/2013. It was approved in 12/10/2012.

Rodent capture and sampling

The species chosen for the study was *Rattus rattus* identified in accordance with Brazil (2002). The capture of the rodents was accomplished by using galvanized wire traps (Tomahawk) mounted at dawn in places that had vestiges of rodents. They were collected the next morning (Araujo et al., 2010) at different locations of the urban and surrounding area of the city of Umuarama, from December 2012 to December 2013.

After capturing, the rodents were separated by sex, placed in appropriate boxes for transportation and sent to the Preventive Veterinary Medicine and Public Health Laboratory (Laboratório de Medicina Veterinária Preventiva e SaúdePública) at UNIPAR for further identification, analgesia, euthanasia, collection of intestinal segment, and parasitological and histological procedures.

Euthanasia, identification and collection of biological samples

The captured rodents were placed in halothane vapor saturated chamber before euthanasia procedures were done (Araujo et al., 2010), and then identified (BRASIL, 2002). The animals were measured using a regular measuring tape and soon after necropsy with an abdominal mid-line incision was done. The intestine (jejunum) segments were removed from each animal, fixated in Bouin solution for 24 h and then transferred to 70°C ethanol until histological processing.

Subsequently, feces from the large intestines (colon and rectum) was collected and stored in individual plastic recipients with formalin of 10%; it was kept in appropriate storage at ambient temperature until the moment of coproparasitological exams.

Enteroparasite identification

The technique described by Hoffman et al. (1934) was used for the identification of enteroparasites, and the slides were analyzed through microscope (Nikon® Eclipse E-200) with 40x magnification.

Inclusion criteria and grouping

After identification of enteroparasites, 20 *R. rattus* were selected.

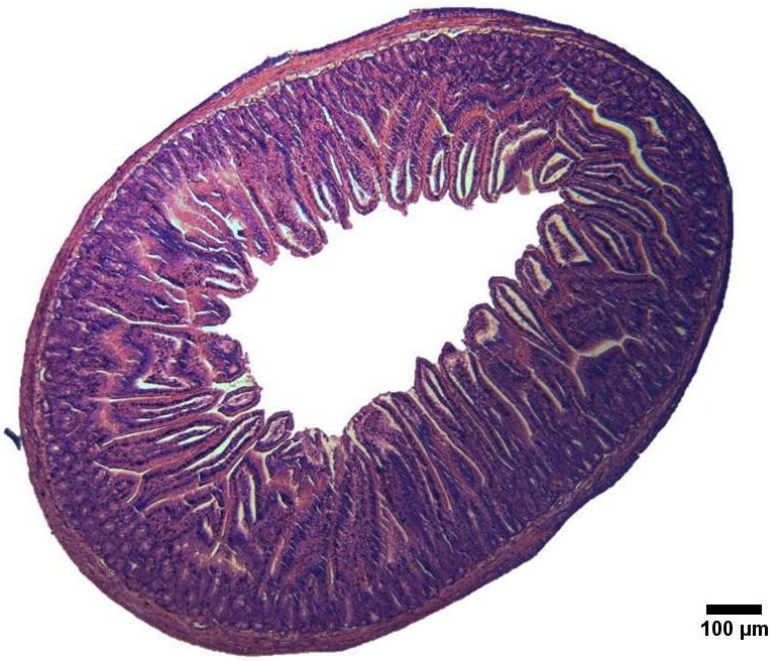

Figure 1. Histological cut of jejunum of Rattus rattus without endoparasites stained with HE (hematoxylin eosin) x20.

The criteria used were: animals with length of body and tail between 20 and 25 cm; animals infected by *Strongyloides* spp. and animals without parasite infection. The measure of the length of the body and tail was used to standardize samples.

The animals were divided into two groups: positive group PG (n=10), with five males and five females infected by *Strongyloides* spp., and a negative control group NCG (n=10), with five males and five females without the presence of intestinal parasites.

Histological processing

A ring of two centimeters of each jejunum collected was fixated in Bouin solution, dehydrated in ascending series of ethanol, diaphanized in xylol and included in paraffin to obtain posterior transversal semi-sequential slices, of 4 µm thickness (Junqueira and Junqueira, 1983).

The morphometric analysis of the intestinal wall was done from images of transversal cuts dyed in Hematoxilin-Eosin (HE) and observed in optical microscope with 20x objective, used to measure villi length (base to apex), crypt depth, villi area and crypt area from the jejunum of infected and non-infected rodents (Gumini et al., 2005). Histological sections of jejunum of Rattusrattus without endoparasites (Figure 1) and those infected by *Strongyloides* spp. are analyzed (Figure 2). 50 measures for each parameter were done and distributed through the whole intestinal circumference, in 250 intestinal segments per experimental group.

To quantify the goblet cells, images captured from slides treated by the method of Alcian Blue pH 2.5 (Junqueira and Junqueira, 1983) were used, under 20x objective. 10 crypts of each infected and non-infected animals were analyzed, for each jejunum segment, in a total of 100 crypts per group. All the images of the transversal cuts were captured through a digital camera (Moticam 2000, 2.0 megapixel) coupled to a microscope with trinocular light (MOTIC B5). All the measurements were done using the software MoticImagens Pro-Plus, version 2.0.

Statistical analysis

The data were analyzed using the statistics software Bioestat 5.3 (Ayres et al., 2007). The parameters (animal length, intestine total length, villi length, crypt depth, villi area, crypt area, and goblet cell count) studied underwent variance analysis, analyzing between groups of rodents of the same sex. T test was used to make comparison between the averages. There was a level of significance of 5% ($p<0.05$).

RESULTS

A total of 157 rodents were captured and identified as *R. rattus*. From these, 50.95% were males and 49.04%, females. As seen in Table 1, there was no statistically significant difference ($p>0.05$) between the groups for animal length (AL) and intestine total length (IL) between male and female *R. rattus*, infected or not by *Strongyloides* spp.

In the morphometric analysis of the villi and crypts of the jejunum, considering the averages obtained (villi length/crypt depth) it was observed a smaller length of villi and a greater depth of crypt in the male and female rodents parasite by *Strongyloides* spp. (Table 2). Considering the morphometric analysis of the villi and villi crypt areas in the jejunum, a statistically smaller area of in the male and female rodents infected by *Strongyloides* spp. was observed; considering the crypt area, this difference was only significant in the females of the parasite group, according to data presented on Table 3.

Concerning the goblet count by crypt in the jejunum, a

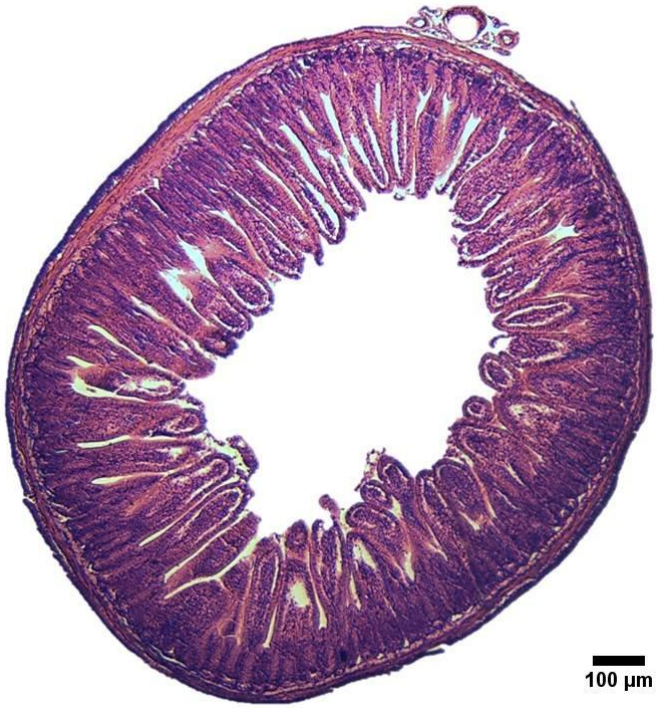

Figure 2. Histological cut of jejunum of Rattus rattus parasitized by Strongyloides spp stained with HE (hematoxylin eosin) x20.

Table 1. Average ± standard error of the animal length (AL) and total intestine length (IL), intestinal villi length and intestinal crypt depth, intestinal villi and crypt areas and the number of goblet cells per intestinal crypt of male and female *Rattus rattus* infected or non-infected by *Strongyloides* spp.

Groups	Animal length (cm)	Total intestine length (cm)
Males – negative control	22.60±0.75	39.40±5.48
Males - infected	22.60±0.50	46.20±3.83
Females – negative control	22.20±0.73	31.00±3.82
Females - infected	21.40±0.51	40.40±2.89

No statistical difference (p>0.05) was observed among groups.

Table 2. Average ± standard error of intestinal villi length and intestinal crypt depth of male and female *R. rattus* infected or non-infected by *Strongyloides* spp.

Groups	Villi lenght (µm)	Crypt depth (µm)
Males – negative control	193.62±11.20[a]	40.53±0.88[a]
Males - infected	164.38±3.52[b]	49.35±0.72[b]
Females – negative control	175.82±3.83[a]	35.20±0.86[a]
Females - infected	129.25±1.82[b]	44.77±0.81[b]

Different letters represent statistical difference (p<0.05) among groups.

statistically higher number of goblet cells in male and female rodents infected by *Strongyloides* spp. was observed (Table 4).

DISCUSSION

In the present experiment, there was no significant

Table 3. Average ± standard error of intestinal villi and crypt areas of male and female *R. rattus* infected or non-infected by *Strongyloides* spp.

Groups	Villi area (μm^2)	Crypt area (μm^2)
Males – negative control	7185.77±208.13^a	910.41±28.26
Males - infected	6376.06±160.68^b	965.43±22.71
Females – negative control	7807.15±209.0^a	577.13±17.73
Females - infected	5023.74±105.42^b	734.46±17.68^b

Different letters represent statistical difference (p<0.05) among groups.

Table 4. Average ± standard error of the number of goblet cells per intestinal crypt of male and female *R. rattus* infected or non-infected by *Strongyloides* spp.

Groups	Number of gobletcells per crypt
Males– negative control	4.90±0.19^a
Males- infected	7.94±0.26^b
Females– negative control	4.66±0.17^a
Females- infected	5.78±0.22^b

Different letters represent statistical difference (p<0.05) among groups.

difference (p>0.05) in relation to animal length (AL) and intestine total length (IL) between the groups, but despite no significant difference, the rodents infected by *Strongyloides* spp. have shown an increase in IL.

The absorption processes are completely dependent on the mechanisms occurring in the intestinal mucosa, being the small bowel (duodenum, jejunum, and ileus) function essential to the digestive process, particularly to the absorption of nutrients. According to Reimer et al. (2010), the diet restriction may induce an increase in intestine length on the long term.

In young rodents with high parasite load, there is a delay on growth, weight loss, and intestinal occlusion, possibly inducing death. Moderate infections provoke a small inflammation of the intestinal mucosa (Negrão, 2001; Silveira et al., 2002).

Considering the averages obtained at the morphometric analysis of the villi and crypts in the jejunum (villi length / crypt depth), it was observed smaller villi and deeper crypts in the male and female rodents infected by *Strongyloides* spp. The size of the villus depends on the number of cells it comprises, thus, the higher the number of cells, the bigger the villus, and consequentially, the larger the area for nutrient absorption (Furlan et al., 2004).

The results obtained in this study are in accordance with thatv of Blankenhaus et al. (2011), that induced the infection of mice by *Strongyloides ratti*, observing moderate alterations in the small intestine, such as: slight reduction of villi and hyperplasia of crypts. In research performed by Mati et al. (2014) on small bowel of the *Callithrix penicillata* experimentally infected with *Strongyloides stercolaris*, were observed in histological analysis, alterations characterized for atrophy of the intestinal villi.

However, Zaman et al. (1980) did not observe alterations in intestinal villi of *Rattus novergicus* infected by *S. ratti*, the same was observed by Dawkins et al. (1981), who analyzed the small intestine of mice infected by *S. ratti*. Comparing the villus and crypt areas, it was observed a statistically smaller villi area in male and female rodents infected by *Strongyloides* spp. Concerning only crypt area, the difference was only significant for females. The desirable relation between intestinal villus and crypts occurs when the villi are long and the crypts are shallow, because the higher the relation between villus length / crypt depth, the better will be the nutrient absorption and smaller will be the energy losses by cellular renewal (Li, 1991; Nabuurus, 1995).

In the quantitative analysis it was observed a statistically higher amount of goblet cells in the crypts of the jejunum of male and female rodents infected by *Strongyloides* spp., this higher number of mucous cells may be related to the protection of the intestinal mucosa facing pathogens such as *Strongyloides* spp. These results are in accordance to Shi et al. (1995) that observed hyperplasia of goblet cells in hamsters (*Mesocricetus auratus*) infected by *Strongyloides venezuelensis* at 43 days of age. However, Mimori et al. (1982) observed no alteration on the number of goblet cells in the intestine of rodents infected by *S. ratti*.

In the intestinal epithelium, goblet cells are responsible by production and excretion of mucines responsible for the lubrication and protection against pathogens (Miller,

1987; Deplancke and Gaskins, 2001). Alterations in goblet cells and mucus constitution are observed in response to parasite and microorganisms infection (Deplancke and Gaskins, 2001; Banks et al., 2005; Lievin -le Moal and Servin, 2006), as well as the hyperplasia in response to helmints infection (Onah and Nawa, 2000) and the hypersecretion induced by enterotoxins from *Escherichia coli* and coleric toxin (Banks et al., 2005).

Dawkins et al. (1983), when studying *R. novergicus* infected by *S. ratti*, using electronic microscopy, observed that adult Strongillus remained between enterocytes. The Strongillus were in intimate proximity to these cells. The intestine epithelium cells were distorted, showing the action of these parasites on the intestinal mucosa, such organisms keep their presence in the jejunum mucosa, impairing nutrient absorption and being responsible by malabsorption syndrome.

In the present study the jejunum of rodents infected by *Strongyloides* spp. suffered alterations in the mucosa; it was found smaller villi length, greater crypt depth, and an increase in the number of goblet cells in the jejunum crypts. These morphofunctional alterations impair the absorption capacity of the intestine, consequently they may promote a reduction on the growth and physical development of the host animal.

Conflict of Interests

The authors have not declared any conflict of interests.

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