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Original papers

Parasitological surveillance in a rat (*Rattus norvegicus*) colony in São Paulo Zoo animal house

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ABSTRACT. *Rattus norvegicus* (Mammalia: Rodentia) is a widespread and synanthropic rodent, broadly used in medical experiments. It can also be used for feeding captive animals in zoos. Parasitological surveys are important to guarantee the health of both the animals and the staff responsible for their management. The aim of this study was to identify intestinal parasites of *Rattus norvegicus* offered as food to captive animals from São Paulo Zoo, and demonstrate the importance of sanitary hurdling, disease control and biosecurity. The identified protozoan parasites were *Eimeria* sp., *Entamoeba* sp., *Spironucleus* sp., *Giardia* sp., *Tritrichomonas* sp., *Chilomastix* sp., unidentified cysts and non-sporulated coccidians oocysts (*Isospora/Eimeria*). The following helminths were found: *Syphacia muris*, *Rodentolepis nana* and *Aspiculuris tetraptera*.

Key words: *Rattus norvegicus*, protozoa, helminth, zoos

Introduction

Rattus norvegicus (Mammalia: Rodentia) is a widespread and synanthropic rodent species broadly used in experimental biomedical research all over the world. In addition, many institutions have their own animal house, to guarantee the quality of the food offered to carnivorous animals, such as snakes, raptors and small mammals [1]. Rat colonies are classified according to their sanitary profile as conventional (animals reared under open cage and unrestricted animal room entry conditions, their microbial burden is relatively uncontrolled); specific pathogen-free (animals are free of a specific type of viral, bacterial or parasitic microorganism) and pathogen-free (animals do not have any pathogen) [2].

Sanitary control is extremely important, not only to ensure that the rats are in good health but also the zoo staff involved in animal management. Rats can harbour different microorganisms and some have unknown zoonotic potential [3–7]. Some microorganisms can be transmitted from the rats when they are consumed, but most of the parasites only infect rodents and will not harm other animals [5,8,9]. The rat parasites can also present a problem in the parasitological surveillance of captive zoo animals, especially because many parasites are considered to be pseudo-parasites and their presence can lead to a misdiagnosis if examined by untrained professionals [5,8,9].

There are many reports of enteric protozoan parasites in *R. norvegicus*, although most of them are considered to be non-pathogenic and have no zoonotic potential. One of the most important is *Giardia muris*, which can cause health problems in chronic infections in rats [3]. Other relevant protozoan is *Spironucleus* sp., which causes health problems and even compromises the growth of young animals [3]. Other protozoans such as *Chilomastix* sp., *Hexamita* sp., *Tritrichomonas* sp., *Chilomaxtix* sp. and *Entamoeba* sp. are relatively common and can be easily found in conventional colonies [3,10]. Coccidian parasites such as *Eimeria miyairii*, *E. nieschulzi*, *E. separata* and *Isospora ratti* may also be found infecting rats [3], causing

diverse pathological effects such as diarrhoea, enteritis, weight loss and even death [3].

Helminths from different groups can also be found in *R. norvegicus* [3]. The most common are the cestode *Rodentolepis nana* and the nematode oxyurid *Syphacia muris* [3]. Rats can also harbour *Strongyloides ratti*, *Hymenolepis diminuta*, *Citellina dispar*, *Heterakis spumosa*, *Aspiculuris tetraptera*, *Syphacia obvelata* and *Trichuris muris*, among others [3,11–13]. Some of these parasites can be highly pathogenic and interfere in the development of the young animals, while others do not offer any risk to infected animals [3].

The aim of this study was to identify the species of intestinal parasites in *Rattus norvegicus* offered as food to captive animals from the São Paulo Zoological Foundation, using different parasitological techniques, and demonstrate the importance of sanitary hurdling, disease control and biosecurity.

Materials and Methods

Study site. São Paulo Zoological Foundation was established in 1958, and, nowadays, is considered the largest zoo in South America. The Foundation maintains approximately 3000 captive animals, including mammals, birds, reptiles and invertebrates. São Paulo Zoo has its own animal house with about 6000 rats, guinea pigs and insects, kept under conventional conditions, which are used as food for other animals. The rats are divided into four storage rooms and two rooms for reproduction purpose. Plastic cages are submitted to regular cleaning with water, soap and sodium hypochlorite, and the substrate is autoclaved before use. Food and water are offered *ad libitum*.

Sample collection. Twenty-one rats, randomly selected, between May-June 2010, were euthanized by cervical dislocation according to ethical procedures [5]. The animals were then divided into two groups: Group 1, composed of 14 animals, and Group 2, composed of seven animals. In Group 1, the content of the rectum was collected and conditioned in clean recipients for posterior coproscopical analysis. In Group 2, the entire intestine was removed and divided into small intestine, caecum, large intestine and rectum; each section was put into a Petri dish and physiological solution was added for immediate analysis [5].

Sample analysis. Coproscopical analyses (Group 1) were executed using three different qualitative methods: 1. direct smear, 2. passive flotation using saturated sodium chloride solution, and 3. Hoffman, Pons and Janer [3,9,14]. All samples were analysed by optical microscopy using an Olympus CX31: the entire coverslip (22×22mm) was scanned, and considered positive when the presence of parasite eggs, larvae or adults were observed.

The digestive tract content analyses (Group 2) were performed in each intestinal section separately. Each sample was opened longitudinally and the content gently scraped into a Petri dish with physiological solution to macroscopically search for helminths. A direct smear was made to detect protozoans (cysts and trophozoites) and helminths (eggs, larvae and adults); all microscopical analyses were performed as described for Group 1. The adults were separated into another Petri dish with physiological solution for posterior fixation [9].

For both groups, the species and developmental stage of the parasite were identified according to available literature based on microscopic morphological analysis [3,9,14].

Results

The coproscopic analyses (Group 1) found only one animal to be negative for parasites, all the others were infected (92.9%) (Table 1). In total, helminths were present in eleven animals (78.6%) and seven of them had protozoan infection (50%). Co-infection was present in eight animals (57.1%), six of them with two parasite species (42.9%) and two with three parasite species (14.3%). The most common parasite group was Coccidia, with a prevalence of 35.7%. *Eimeria* sp. infection was confirmed in two animals. In addition, *Entamoeba* sp. (14.3%), *Spironucleus* sp. (7.1%) and a non-identified cyst (7.1%) were identified. Among the helminths, the superfamily Oxyuroidea was the most common, with a prevalence of 64.3%, followed by eggs of *Aspiculuris tetraptera* (21.4%) and *Syphacia muris* (42.8%). *Rodentolepis nana* was the only cestode found, with a prevalence of 35.7%.

Comparing the different methods used for this group, direct smears allowed the detection of all infections by protozoans and helminths (Table 2). Of all the employed methods, the use of sodium chlorite flotation solution was the most effective for diagnosing helminth infections (*R. nana, A. tetraptera* and *S. muris*). Hoffman, Pons and Janer,

Table 1. Prevalence of parasites in *Rattus norvegicus*, kept in São Paulo Zoo colony

Parasites	Group 1 (n=14)		Group 2 (n=7)	
	N+	P%	N+	P%
Negative	1	7.1%	–	–
non sporulated coccidian (*Isospora/Eimeria*)	3	21.4%	1	14.3%
Entamoeba sp.	2	14.3%	–	–
Spironucleus sp.	1	7.1%	2	28.6%
Giardia sp.	–	–	3	42.9%
Tritrichomonas sp.	–	–	7	100%
Chilomastix sp.	–	–	2	28.6%
Eimeria sp.	2	14.3%	4	57.1%
non identified cyst	1	7.1%	–	–
Syphacia muris	6	42.9%	1	14.3%
Rodentolepis nana	5	35.7%	4	57.1%
Aspiculuris tetraptera	3	21.4%	–	–

n: number of investigated animals; N+: number of positive samples; P%: prevalence

or sedimentation technique was capable of detecting a few infections.

The digestive tract content analyses (Group 2) found all animals to be positive with multiple infections. *Tritrichomonas* sp. was present in all studied animals. *Giardia* sp. was found in 3/7, *Spironucleus* sp. and *Chilomastix* sp. were present in 2/7. Coccidian parasites were also present in 5/7, *Eimeria* sp. had a prevalence of 4/7 and a non-sporulated coccidian (*Isospora/Eimeria*) were present in 1/7. Only two helminth species were found: *Rodentolepis nana* found in 4/7, followed by *Syphacia muris*, present in one of the seven samples.

Table 2. Efficacy of the three methods used in coproscopical analyses

	D	F	S
Non-sporulated coccidian (*Isospora/Eimeria*)	2	1	1
Entamoeba sp. (cyst)	2		
Spironucleus sp. (trophozoite)	1		
Eimeria sp. (coccidian)	2	2	
Non-identified cyst	1		
Syphacia muris (egg)	3	5	1
Syphacia muris (larvae)	2		1
Rodentolepis nana (egg)	3	4	
Aspiculuris tetraptera (egg)	1	2	

D: direct smear; F: fecal flotation with sodium chlorite; S: sedimentation (Hoffman, Pons and Janer)

Discussion

No parasitological study has been performed in rat colonies kept in zoos for feeding purposes; previous studies usually only report cases in laboratory and free-living animals [4,6,11]. All parasites found in this study are commonly reported in conventional *R. norvegicus* colonies [11,12,15]; they have a direct life cycle and can be easily transmitted to other individuals. These animals live in an environment with no contact with other animals that could act as a reservoir or a vector to parasites with an indirect life cycle [16]. Care should be taken while handling the rats to avoid transmission between different rooms.

With regard to the methods used in the coproscopic analysis, direct smears can be considered a sensitive technique to diagnose protozoan and helminth infections. In contrast, the Hoffman, Pons and Janer method was found to have the lowest sensitivity of the three methods: it is recommended for use in diagnosing infections by trematodes and acanthocephalans [9], which was not the case of this study. These parasites are not common in artificially-raised rat colonies, since they have a more complex life cycle requiring an intermediary host to complete their life cycle [9]. Choosing the most appropriate method in parasitological diagnosis must be one of the main concerns for laboratory professionals, and when possible, at least two techniques should be

combined to improve laboratory diagnose [9].

Helminth eggs were frequently found in the analysed rats. The use of different techniques to diagnose parasites increases the probability of diagnosing infections, especially light infections [11]. On the other hand, the analyses of the digestive tract revealed a high prevalence of protozoan infection, this can be related to the ability of some parasites to adhere to the intestinal wall, such as *Giardia* sp. [17]; additionally, some parasites live in the upper portion of the small intestine, and only when it is analysed in its totality can infections be diagnosed.

Data from another parasitological surveillance study conducted in different animal houses in Brazil, demonstrated a higher prevalence of *Giardia muris* [16] than that found in the present study. Besides the lack of identification of *Giardia* sp. to species level in the present study, in the present study the infections are probably due to *Giardia muris,* which is specific to rodents with no zoonotic potential, and present little risk for human health [3]. *Giardia* sp. can be considered a major health problem for rats in conventional colonies. Chronically-infected animals can develop chronic enteritis and even growth problems [3], which could compromise their health and, consequently, the quality and nutritional value of the food offered to captive animals, probably due to weight loss. *Giardia duodenalis* has also been described in a wide variety of animals, and even in humans [17]; this parasite has different genotypes, but only one was described to infect humans and rats [17]. Even if the *Giardia* sp. found in this study does not represent a risk to zoo staff, care should be taken when these animals are managed.

A high prevalence of the coccidian was found in the studied rats at São Paulo Zoo; similar results were described in a study conducted in Brazilian animal houses keeping animals intended for research projects: it was found that 60% harboured coccidians [16]. This parasite can be extremely pathogenic and cause severe disease, but is not common in animal house rat colonies [3,18]. The presence of *Eimeria nieschulzi* and *E. separata* has been reported in rats [3,5]. However, in the present study, the parasite was not identified to species level. Identifying coccidian species can be challenging due to their small size and the necessity to first sporulate them in a sodium dichromate solution [19], a diagnostic method not performed in many laboratories as a routine method.

A small prevalence of *Entamoeba* sp. was found in the present study. This is an uncommon parasite in rats [3], although some studies indicate a high prevalence of around 80% in other rat colonies in Brazil [16,18]. *Entamoeba muris* can be frequently found in colonies, which can be attributes to a lack of water sterilization [18].

Tritrichomonas sp. was present in 100% of the animals whose intestinal tract was analysed. This non-pathogenic flagellated protozoan [20] is commonly found in rat colonies and a high prevalence was already reported [16,18]. *Spironucleus* sp. also had a high prevalence in this group (28.6%). This parasite is considered to be pathogenic, especially in immunodeficient, stressed and young animals [21], demonstrating that it is important to select pathogen-free rats to maintain a healthy colony. *Spironucleus muris* is a common protozoan found in *R. norvegicus* [3,16,18]; this parasite also has a direct life cycle and can be inactivated with disinfectants and heat (45°C for 30 minutes). In immunocompromised animals, it can cause diarrhoea, dehydration, weight loss, apathy and even death [10].

Among helminths, one cestode species, *Rodentolepis nana,* and two different oxyurid nematodes species, *Syphacia muris* and *Aspiculuris tetraptera*, were found. *Rodentolepis nana* and *S. muris* were present in both analysed groups, while *A. tetraptera* was found only in the group on which coproscopic analysis was performed.

The high prevalence of *Rodentolepis nana* in both groups found in the present study differ from other reports in Brazilian animal houses, which report it to be absent [16,18]. When present, prevalence can vary significantly from 8.8% to 56.3% [12,15,16]. This is the only known cestode with a direct life cycle. During the life cycle, the eggs pass through the faeces of the definitive host and are already infective [3]. Its zoonotic potential has been contested, with some studies suggesting that *R. nana* of humans and rodents are morphologically indistinguishable, and should be considered as cryptic species [7]. Despite this, it is recommended that the animals are handled with care [5]. This cestode is one of the most common parasites in children, and can cause enteritis and some complications, such as weight loss, in chronic infections [22].

Syphacia muris is a well-studied oxyurid nematode found in rats [11,23,24] whose prevalence can be as high as 64.6% [12] or even 80% [14]. In

the present study, a prevalence of 42.8% was detected. This parasite can interfere in the development of young animals, causing enteritis and weight loss [11,23,24]. Females lay their eggs in the perianal region, usually in the afternoon hours. The eggs became embryonated and infective within a few hours and are highly resistant to the environment, surviving for long periods at room temperature [25,26]. The eggs are light and can be transported through the air to other rooms. Another important feature of this species is its short pre-patent period, allowing an infective rat to rapidly eliminate eggs into the environment [3,27].

Aspiculuris tetraptera the other oxyurid species found in the São Paulo Zoo rats were detected only in Group 1. This parasite has a direct life cycle, but the eggs eliminated in the faeces only became embryonated and infective in five to eight days at 27ºC. Eggs are resistant to cold, desiccation and disinfection, but are sensitive to high temperatures [3,27]. Infection by *A. tetraptera* affects mainly young animals aged from four to five weeks. Females inhabit the cecum but migrate to the colon to lay their eggs. Older animals have been found to present some physiological processes capable of inhibiting the infection process [27,28].

The high prevalence of parasites observed in the animal house rat samples is probably due to the fact that all the parasites encountered have direct life cycle. Comparing both groups, protozoan parasites had a higher prevalence in Group 2; in this group, the intestinal wall was scratched: this technique removes all the parasites that could be attached in this area, usually protozoans, making identification easier. It is important to choose the best method to perform parasite diagnosis [10,24], and even to use more than one method to evaluate parasitic infections in animals. The use of perianal tape impression, which is a sensitive method for *Syphacia* spp. detection [11,24,29], can be easily implemented in parasite control programs.

Otherwise, infected rats used as a food source for other animals can be vector of different microorganisms, and possibly cause infections and/or false-positive results in parasitological exams of some captive animals. In many cases, these parasites can be found in snake faeces, and interpreted as snake parasites, when the real host is the rat. Training laboratory professionals to deal with these situations and the veterinarians to correctly interpret the findings is essential for guaranteeing the health of all captive animals.

The “gold standard” of pathogen eradication in a rat colony is rederivation of rodents via hysterectomy and cesarian section or embryo transfer, but this approach is expensive and time consuming [30]. Some other protocols are used for the treatment of rat colonies, mostly for research animals. For example, the use of ivermectin in the drinking water has been well documented with short and long courses for the eradication of pinworms [31,32]. For treating protozoan, nematode and cestode infection, it is recommended to use food containing 150ppm of fenbendazole for at least three to seven-day periods over at least five weeks [33]. Nevertheless, it is important to accompany treatment and decontamination with the removal of potentially infective materials and fomites and the use of autoclaved bed material [34].

This work confirms the importance of conducting periodical exams and parasitological control in the animal house population to assure the health of the rats, the animals that feed on them, and the entire zoo staff involved in the process. It is essential to have well established protocols for the quarantine of new animals and quality controls for the environment and hygiene, as well as trained staff [10,24].

In conclusion, in a zoo, it is not necessary to maintain rats as pathogen-free animals. However, it is important to provide potable or sterile water and autoclaved wood shavings. The use of preventive medicine, as well as trained staff and a clean environment can guarantee the health of the animals kept in the animal house, and consequently the quality of the food served to the animals in the zoo: a healthy rat is certainly a better meal than one full of parasites. Rats with parasites usually have poor growth and need more food to gain weight, thus becoming more expensive for zoos. A program to ensure that the rats remain in good condition also directly influences the health of the zoo staff, as some of the parasites may potentially have unknown zoonotic potential. This work emphasises the importance of parasitological surveillance in zoo rat colonies and the importance of the correct parasitological diagnosis performed by laboratory professionals.

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