COMPARATIVE ASPECTS OF *CRYPTOSPORIDIUM MURIS*, TYZZER, 1907 (APICOMPLEXA: CRYPTOSPORIDIIDAE) OOCYSTS ON *RATTUS NORVEGICUS* FROM TWO DIFFERENT HABITATS.

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SUMMARY: One hundred and seventy three brown rats were captured randomly from two habitats: a urban area in the municipality of Nova Iguaçu, and rural area in the municipality of Seropédica, both located in the metropolitan area of Rio de Janeiro, Brazil. These animals were grouped according to origin, and within groups according age. *Cryptosporidium* oocysts were found in feces of animals from both habitats, and through morphometric characterization and their location in the intestinal tract, they were classified as *C muris*. Twenty nine animals were parasitized (16.77 %), and the majority of them had a body weight greater than 201 g, being then considered as adults. The aim of the present paper was to compare the incidence of *C. muris* infections in populations of rats from two different habitats.

KEY WORDS: *Cryptosporidium muris*, oocysts, *Rattus norvegicus*, infection.

INTRODUCTION

Since TYZZER (1907) discovered protozoan parasites of the genus *Cryptosporidium* in mice, this infection has been reported in several vertebrate hosts around the world.

The major trait that distinguishes this parasite from the great majority of other coccidia is the lack of specificity for its mammal hosts (TZIPORI *et alii*, 1980a and MOON & BEMRICK, 1981). TZIPORI *et alii*, in cross-infection experiments with farm animals and man, verified that *Cryptosporidium* could be considered the only species of this genus, although domestic animals could act as reservoirs for humans. ARCAT *et alii* (1995) infected all vertebrate classes (mammals, birds, reptiles, amphibians and fishes) with a human isolate of *C. parvum*. These results reinforce the lack of host specificity by parasites of the *Cryptosporidium* genus.

Nevertheless, some studies on parasites of the *Cryptosporidium* genus, considered also the species *C. parvum* and *C. muris* for mammals (UPTON & CURRENT, 1985; CIIRISP *et alii*, 1990) and *C. baileyi* for birds (CURRENT *et alii*, 1986).

Parasites of this genus have been observed in several domestic and wild mammals, generally with a intestinal localization (GOEBEL & BRANENDLER, 1982 and JOKIPPI *et alii*, 1985). Non-intestinal parasitism was reported by PAVLÁSEK (1984) in mice experimentally infected with a calf isolate of *C. parvum*. ARCAY *et alii* (1995) made the same report for mammals, birds, reptiles, amphibians and fishes using a human isolate of *C. parvum*.

KELSIUS *et alii* (1986) found a infection rate of 30 % for *Cryptosporidium* among 115 wild mice captured in cattle pens. These authors proposed that this cycle could occur naturally: cattle infecting each other and mice, as well as mice infecting each other and also cattle.

New-born wild rats presented the same susceptibility to *Cryptosporidium* infections than new born laboratory mice (FAYER & UNGAR, 1986).

Some experimental infections using oocysts derived from other mammals have been reported in rodents. POHJOLA & LINDBERG (1986) and ERNEST *et alii* (1986) were successful in infecting rodents with oocysts obtained from cattle, RESSE *et alli* (1982) did the same with human-derived oocysts.

SHEWOOD *et alli* (1982) demonstrated that *Cryptosporidium* isolates caused subclinical infections in eight breeds of pathogen-free mice.

Some prevalence surveys in *R. norvegicus* identified *Cryptosporidium* as a normal occurrence for this host. ISEKI (1986) captured 61 specimens of *R. norvegicus* and 3 *R. rattus*,

and observed that their oocysts presented two different sizes, with the following diameters: mean 5.3 x 4.9 µm (range 5.0-6.0 x 4.0-5.5) and mean 8.4 x 6.3 µm (range 7.5-9.8. x 5.5-7.0). This author found a prevalence of 14.8 % for *R. norvegicus* but did not find any *R. rattus* infected.

YAMAURA *et alii* (1990) captured 175 *R. rattus* and 48 *R. norvegicus* specimens, founding a prevalence of 17.7 % and 2.1 %, respectively. The oocysts presented the following diameters: 3.7 ± 0.22 x 4.8 ± 0.33 µm. MIYAJI *et alli* (1989) found prevalences of 48.5 % among 171 *R. norvegicus* and 21.3 % among 47 *R. rattus*. The aim of the present paper was to compare the incidence of *C. muris* infections in populations of rats from two different habitats.

MATERIALS AND METHODS

One hundred and seventy-three rats (*R. norvegicus*) were captured from two different areas, with wire traps containing assorted baits.

Two habitats located in the Grande Rio area (FIBGE, 1985), Rio de Janeiro State, Brazil, were chosen for animal capture. The first was located in rural areas from the municipality of Seropédica, and the second was a urban area in the municipality of Nova Iguaçú.

Habitat A: This area was considered an open environment, with pastures located near to livestock breeding facilities, where there were feedstuffs, of both plant or animal origin, available.

The rodent population in this area was considered abundant, and many burrows were found in the ground. At night, rats were constantly found in the facilities, looking for food. From this environment 61 animals of different ages were captured.

Habitat B: In this second environment, considered as a closed area, the rodents barely needed to look around for food, since the place had a huge reserve of both plant and animal derived feedstuffs.

Insects were commonplace in this area. Pigeons and cats (introduced to control the huge rat population) were also found.

Around this environment a garbage deposit was found, as well as a human population with poor subsistence conditions. Many families raised pigs and poultry, with free access to the area. Cats and dogs were also common.

The environment was closed by brick walls, but a network of burrows with connections to the neighboring human and domestic animal populations was observed. It could be concluded that different rodent colonies co-existed: one resident of this place, and outside colonies which came to this environment looking for food.

In the same way as in the first environment, rats were usually found at night, but outnumbering the population of habitat A. From this environment, 112 animals of different ages were captured.

Captured animals: When rats were found inside the traps, they were anesthetized with sulfuric ether and then placed in plastic boxes (two animals per box at most), where water and *ad libitum* feed (Purina®, São Paulo, Brazil) was provided. These animals were transported to the laboratory of the Experimental Station for Parasitological Research W.O. Neitz, (Department of Animal Parasitology, Biology Institute, Universidade Federal Rural do Rio de Janeiro). The animals were kept in the boxes for at most one week, being then necropsied.

Laboratory procedures: Rodents were killed with sulfuric ether. After death animals were separated by sex and age. Age was determined according the animals weight, as stated by CALHOUN (1962): animals under 100 g were considered as young, from 101 until 200 g as adolescent, and above 201 g as adults. (Table 1).

Fecal samples were collected both before and during necropsy. They were macerated, filtered through gauze, poured into vials with potassium dichromate (2,5 %) in a proportion of 1:3, and then kept at room temperature for a week. After this time, examinations for *Cryptosporidium* oocysts were made using the floating - centrifugation method (FIGUEIREDO *et alii*, 1984).

Whenever *Cryptosporidium* oocysts were found, fecal samples were centrifuged several times with distilled water in order to remove all potassium residues. Blood smears were then made and stained by the modified Ziehl-Neelsen technique, that allows a clear view of the oocysts.

Stomach and intestinal samples were fixed with formalin (10%), embedded in paraffin and sectioned at 3µm, and stained with haematoxilin-eosin (HE) for the observation of parasite developmental stages in the tissues. This procedures follow BEHMER *et alii* (1976).

Table 1 - Number of animals captured from habitats A and B, assorted by weight and sex.

Age	Habitat				Total
	A		B		
	Male	Female	Male	Female	
Young (under 100g)	10	8	11	6	35
Adolescent (101-200g)	6	8	32	21	67
Adult (above 201g)	19	10	25	17	71
Total (sex)	35	26	68	44	-
Total (habitat)	61		112		173

Measurements and Photomicrography: A Leitz microscope (model H.M. Luz, Leitz Wetzlar, Germany) was used for oocyst measurement, together with micrometric lens (K-15 PZO, Poland).

Tissues photographs were made with the help of a INAI.H microscope (Mexico), using FOTOVIX (Mexico) and a video

EDITOR II (Mexico), and a Color Video Printer Mavigarph with a color TV (Sony Corporation, Japan).

The histological preparations were photographed using a three lens microscope (Dialux 20EB, Leitz Wetzlar, Germany) and a MPS51 camera (Leitz Wetzlar, Germany), using Kodacolor URG Kodak, 35 mm, 100 ISSO. Exposures were measured with the help of an automatic photometer (Wild MPS 55, Leitz Wetzlar, Germany). Another three lens microscope, JENAPOL model, with a mf-AKS 24x36 Automatic-2 camera (Carl Zeiss/ Jena, Germany).

Statistical Analysis: Morphometric data was analyzed with the Minitab program using an analysis of variance and the Tukey test (SCHAFFER & ANDERSON, 1989). Linear regression calculations were performed with Microsoft Excel (FONSECA *et alii*, 1995; LAPPONI, 1995).

RESULTS AND DISCUSSION

No clinical signs, such as diarrhea or the softening of feces, were observed in any of the animals captured in either environment, and positive for *Cryptosporidium* at fecal examination. It is known however, that diarrhea is one of the common symptoms of cryptosporidiosis in humans and domestic animals (CURRENT *et alii*, 1983; FAYER & UNGAR, 1986 and STEHR-GREEN *et alii*, 1987). ISEKI (1986) found such clinical symptomatology among naturally infected brown rats. Conversely, YAMAURA *et alii* (1990) did not observe clinical symptoms (diarrhea) neither in black nor in brown rats, both captured from their wild habitats and naturally infested. In the same way, no clinical signs were found in cats positive for *Cryptosporidium* (ARAI *et alii*, 1990).

It is not known if clinical signs present some degree of variation for different hosts of this protozoa. Only when man or animals present some organic imbalance or infectious disease, can the opportunist agent develop easier and cause clinical symptomatology (SNYDER *et alii*, 1978; CLERIHEW, 1980; LOPES & BOMFIM, 1993; BOMFIM & LOPES, 1995a; BOMFIM & LOPES, 1995b; SOUZA & LOPES, 1995), or even reinforce the zoonotical characteristics of species in this genus, which can attack non-humans with acquired immune deficiency syndrome (AIDS), and those involved with activities related with animals (ANDERSON *et alii*, 1982; RESSE *et alii*, 1982 and TZIPORI, 1983).

Characteristics of oocysts, after flotation - centrifugation in saturated sugar solution: With this methodology, the oocysts were characterized as spheroid or sub-spherical, shiny structures, presenting one or more black spots inside, that correspond to the sporozoites nucleus and to the residue oocyst (Figure 1-A). The morphological descriptions of these oocysts are similar to those presented by RESSE *et alii* (1982); WILLSON & ACRES (1982); BOMFIM (1989) and BOMFIM & LOPES (1995b).

Several other structures could be observed by using saturated sugar solution, which could lead to an incorrect diagnosis. Yeast are abundant in the feces and must be differentiate from oocysts, since they are morphologically very similar. They are usually seen as round-shaped or slightly egg-shaped structures, with a dense mass inside, and with buds in some forms (Figure 1-B).

Oocysts characteristics when stained by the modified Ziehl-Neelsen method: Protozoa stained by this method are very visible, with spherical or sub-spheroid structures, and a color ranging from reddish to pale pink in a green field vision (Figure 1-C). Some times, four elongated structures could be seen inside the oocysts, corresponding to sporozoites (Figure 1-D). Such staining characteristics agree with the observations of HENRICKSEN & POHLENZ (1981) and BONFIM (1989). In this method, yeast-like bacteria and other structures are not stained with fucsin, being then easily differentiated from protozoa.

Morphometric characteristics of oocysts from brown rats of A and B habitats: The mean diameter of oocysts from animals of the two different habitats, measured both with or without staining, are presented in Table 2. After analysis it could be concluded that significant differences between habitats and between the techniques used, existed with a higher frequency. Certain degree of polymorphism between coccidia was observed by LONG & JOYNER (1984). The morphometric indexes are presented in Table 3, and a sub-spherical characteristic can be observed, which can be considered as an important specific character of coccidia species (LONG & JOYNER, 1984), since oocysts do not change their spatial form whether spherical or ellipsoid, despite significative changes in polar or equatorial diameters, caused by internal or external factors (FAYER, 1980).

Table 2 - Comparative and morphometric characteristics of *Cryptosporidium muris* oocysts (µm) collected from *Rattus norvegicus* (habitats A and B), and stained either by modified Ziel-Nielsen and shiny filed methods[a].

Methods	Measurements[b]			
	Habitat A		Habitat B	
	Large diameter	Small diameter	Large diameter	Small diameter
Ziel-Nielsen	7.01 ± 0.45^{ye}	6.44 ± 0.50^{ya}	6.98 ± 0.33^{ye}	6.63 ± 0.40^{yb}
Shiniy field	7.34 ± 0.36^{zf}	7.13 ± 0.38^{zc}	7.27 ± 0.34^{zg}	6.95 ± 0.33^{zd}

[a] Measurements done in one-hundred oocyst for each method and habitat. Values shown are $X \pm S_{(x)}$.
[b] Different letters are significant at $P \leq 0.001$

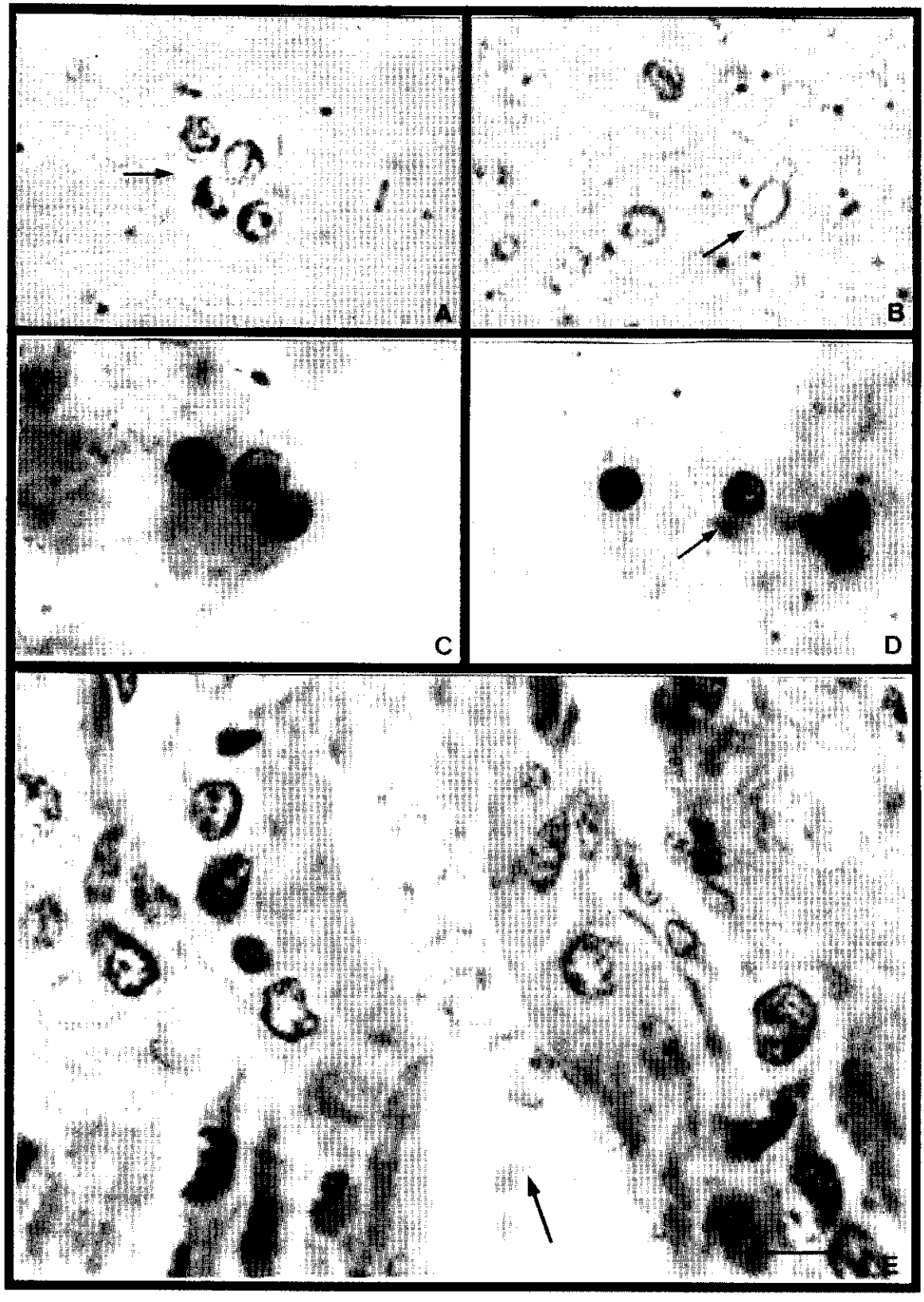

Fig. 1 - *Cryptosporidium muris*: A) oocysts and B) yeast, modified floating-centrifugation in saturated sugar solution on a shiny field (→); C) oocysts and D) oocysts with sporozoites, modified Ziehl-Neelsen (→); E) oocysts (→) inside the gastric glands of brown rats, H.E. (— = 10μm).

By comparing the regression lines of oocysts from habitats, with and without staining (Figures 2 A,B and 3 A,B), a more regular spatial distribution of the concordance points between greater and lower diameters. Such result suggest that only one species is involved, despite the significant differences that existed in oocysts sizes.

Two common species are parasites of mammals. Based upon the morphology of oocysts isolated from cattle, UPTON & CURRENT (1985) named those with greater size as *C. muris* (mean diameter from 7.0 to 8.4 μm), and the other smaller species as *C. parvum* (mean diameter from 4.0 to 5.3 μm). ISEKI (1986) confirmed also that among the oocysts isolated from brown and black rats, the bigger ones had a mean diameter ranging from 8.4 to 6.3 μm, and the smaller ones presented values ranging from 5.3 to 4.8 μm: they were *C. muris* e *C. parvum*, respectively. YAMAURA *et alii* (1990) found oocysts with mean diameters of 3.7-4.8 μm in black rats, and classified them as small size type. In the present work, area related means were found (Table 2) and considered as near to those of large size oocysts, being than classified as *C. muris*.

Table 3 - Evaluation of the morphometric index of oocysts from *Criptosporidium muris* collected from *Rattus norvegicus* (habitats A and B).

	Morphometric index	
Methods	Habitat A	Habitat B
Ziel-Nielsen	1.09 ± 0.04	1.05 ± 0.03
Shiniy field	1.03 ± 0.04	1.05 ± 0.02

Prevalence of *Cryptosporidium muris* oocysts among brown rats from habitats A and B: Among the 173 animals captured from the two habitats, 29 (16.77 %) were positive for *C. muris*. When separated by sex, a higher prevalence was found among males weighing more than 201 grams, considered adults. Among females, the highest prevalence was found for animals weighing between 101 and 200 grams, and thus considered adolescents (Figures 4 and 5).

In a total of 61 animals from the habitat A, 5 (8.20 %) were parasitized. For habitat B, of 112 animals, 24 (21.43 %) were positive for *Cryptosporidium* oocysts.

Experimental trials have shown that younger animals are more susceptible to *Cryptosporidium* infections than older animals (SHEWOOD *et alii*, 1982 and TZIPORI *et alii*, 1983). YAMAURA *et alii*, (1990) in a infection in 31 black rats, found a higher prevalence in the group of animals with a higher body weight. MIYAJI *et alii* (1989) demonstrated that the incidence of *Cryptosporidium* infections in black rats decreased as animal weight rose.

In the present work it was observed that the highest prevalence occurred among animals weighing more than 201 grams, and thus considered as adults.

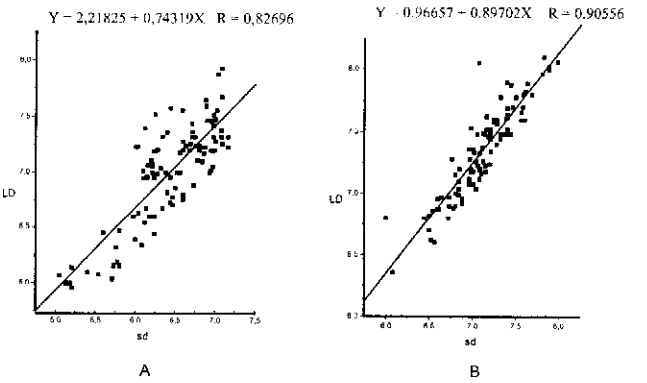

Fig. 2 - Linear regressions of the larger (LD) and smaller (sd) diameters of *Cryptosporidium muris* oocysts collected from habitat A: A) Stained by modified Ziehl-Neelsen and B) Shiny field/ saturated sugar solution.

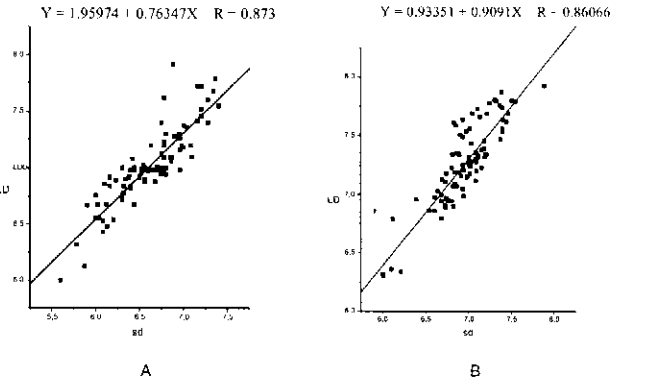

Fig. 3 - Linear regressions of the larger (LD) and smaller (sd) diameters of *Cryptosporidium muris* oocysts collected from habitat B: A) Stained by modified Ziehl-Neelsen and B) Shiny field/ saturated sugar solution.

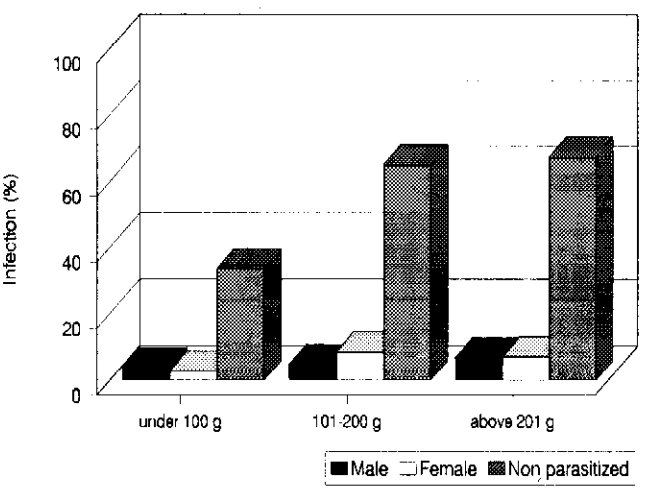

Fig. 4 - Prevalence of *Cryptosporidium* genus according age and sex, observed in the feces of *Rattus norvegicus* captured from habitats A and B.

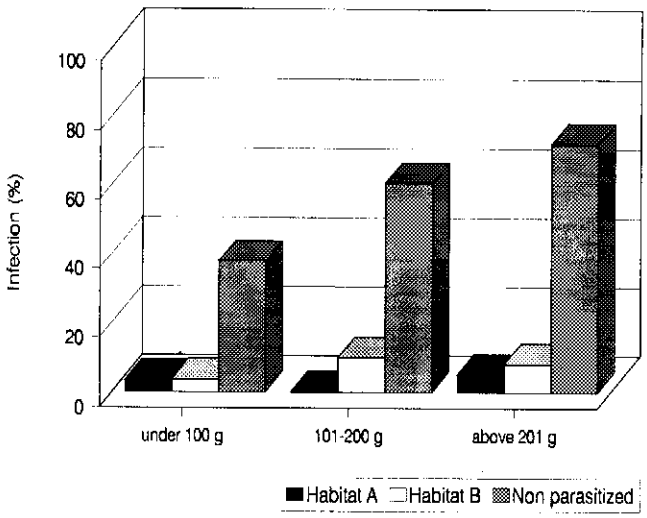

Fig. 5- Prevalence of *Cryptosporidium* genus, observed in the feces of *Rattus norvegicus* captured from habitats A and B.

The role of brown rats as source of *Cryptosporidium* infections: Experimental transmission from sheep, calves and pigs to mice was possible during the mouse nursing period (TZIPORI *et alii*, 1981 and 1983; SHEWOOD *et alii*, 1982 and BOMFIM & LOPES, 1995a), and also for adult rats (BRASSEUR *et alii*, 1988) and hamsters (ROSSI *et alii*, 1990), using immune suppressed animals.

YAMAURA *et alii* (1990) observed that young and adult black rats presented a high susceptibility to infection with oocysts naturally isolated from black rats, and that immune suppression was not necessary for the establishment of infection.

Immune competent adult humans are also susceptible to infection with *Cryptosporidium* (FAYER & UNGAR, 1986). Adult guinea-pigs (CHRISP *et alii*, 1990) and wild mice (KLESIUS *et alii*, 1986) have been easily infected with oocysts derived from guinea-pigs and wild mice, respectively, without showing any clinical sign of disease.

It is reasonable to suppose that brown rats could be responsible for the spread of *Cryptosporidium* oocysts both in urban and rural areas, being an important source of infection. Observations made by FAYER & UNGAR (1986) indicate that *Cryptosporidium* derived from rats did not present host specificity, and can easily infect calves and humans.

The possibility of cross infection between rodents and humans for this parasitic disease exists, due to the lack of specificity for mammal host species. Hence, in this work, based upon data from habitats A and B, brown rats can be considered as infection sources for humans and domestic animals both in the rural and urban areas, regardless their age and sex.

Histopathology: Histological cuts of gut and stomach presented endogenous forms only in the stomach, located in the gastric glands (Figure 1-E).

TYZZER, in 1907 observed endogenous forms of a protozoan in histological sections from the gastric mucosa of laboratory mice, and called it *C. muris*. By 1910, this author described the same organism in further detail, a proposed that it should be classified as a new species : *C. parvum*, differentiated from *C. muris* by its localization at the gut and due its smaller diameter. If it was assumed that each species has its own site of parasitism, and the morphometric data from the oocysts was considered, the protozoan observed in the gastric glands of brown rats can be classified as *C. muris*.

SUMÁRIO

Ratazanas em número de 173, foram capturadas aleatoriamente em dois habitats: um de característica urbana, no município de Nova Iguaçu e outro rural, no município de Seropédica, ambos localizados na microrregião homogênea Fluminense do Grande Rio, no estado do Rio de Janeiro. Estes animais foram agrupados considerando o seu habitat e, dentro dele, de acordo com a idade. Oocistos de *Cryptosporidium* foram observados em fezes dos animais nos dois habitats e, através da caracterização morfométrica e localização no trato gastrointestinal, foram considerados como *C. muris*. Observou-se uma freqüência de 29 (16,77%) de animais parasitados nos dois habitats, sendo que a maior freqüência foi atribuída aos animais com peso corporal superior a 201 g, considerados adultos.

PALAVRAS-CHAVE: *Cryptosporidium muris*, oocistos, *Rattus norvegicus*, infecção.

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