Prevalence of ecto and endoparasites in mice and rats reared in animal houses

Prevalência de ecto e endoparasitas em camundongos e ratos criados em biotério

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SUMMARY

During five years (1986-1991), 229 mice and 128 rats of both sexes, aged 01 to 02 months, from different breeding colonies in the city of São Paulo, Brazil, were examined for parasitological control. Four conventional colonies (2 of rats and 2 of mice) and one mice barrier colony (control) were analyzed. Semi-annual sampling made use of 5% of the animals in the rooms. The population of each room comprised over 100 animals. In the general examination, abdominal distention and diarrhea were observed in the mice and rats from conventional colonies, but not in animals from the control colony. Mild alopecia and bristly hairs were detected in all animals from the evaluated colonies. Ectoparasites such as *Myobia musculi*, *Myocoptes musculinus*, *Radfordia affinis*, *R. ensifera* and *Poliplax spinulosa* were identified in the conventional colonies, in multiple and single infestations. *Myobia musculi* is described for the first time in rats reared in breeding colonies. Endoparasites such as *Hymenolepis nana*, *Syphacia* sp, *Aspiculuris tetraptera*, *Tritrichomonas muris*, *Spironucleus muris*, *Giardia muris*, and *Eimeria* sp were observed in the conventional colonies. However, through necropsy results, in the control colony *Syphacia* sp and *Aspiculuris tetraptera* were the only parasites found. Necropsy confirmed the indication of the anal swab method for detection of *Syphacia* sp rather than the Willis method, and revealed the degree of infection by intestinal protozoa and *Syphacia* sp. *H. nana* and *Aspiculuris tetraptera* were efficiently detected by Willis method.

UNITERMS: Ectoparasites; Endoparasites; Mice; Rats; Animal house.

INTRODUCTION

Type and degree of infections caused by endo and ecto parasites can represent severe problems for animal breeding, interfering with the productivity of colonies as well as with the results of biologic tests and research[8]. Thus, in order to obtain healthy animals, factors such as prophylactic rules and sanitary control, by means of a variable number of barriers in laboratory animal facilities, become extremely important[11].

Several studies describe endoparasites as causing harmful effects on the host, besides being responsible for alterations in the immune system, interfering with the response to heterologous antigens [13,17,19]. Although endoparasites found in laboratory animals are considered mildly pathogenic and infections are asymptomatic, some factors such as temperature variation, high density in the cages and stress can increase or favor the parasitism degree of endoparasites, resulting in rectal prolapse, mucoid enteritis, intussusception and stunted development of the host [5,9,19]. Ectoparasites are epidemiologically important and act like vectors for pathogenic microorganisms, able to modify the behavior of other experimental infections as a result of competition among parasites [1,6]. Infestation by ectoparasites can be unapparent, although clinical signs, such as alopecia, chronic dermatitis, itching, ulcerations and hyperkeratosis can be observed during the infection course. The degree of the lesions is directly associated to the number of ectoparasites [2,16,20].

Studies developed in conventional mice breeding colonies in the United States, Europe and Asia exhibited a high level of infection by endo and ectoparasites [3,4,20], as opposed to parasite-free colonies obtained by cesarean derivation or aseptic hysterectomy, and maintained in animal houses with barrier systems and/or isolators [5,12,18].

The aim of this study was to assess the prevalence of ecto and endoparasites from several conventional and barrier colonies of mice and rats in the city of São Paulo. The efficacy of diagnostic methods was also evaluated.

MATERIALS AND METHODS

ANIMALS

The animal population under examination included 229 mice and 128 rats, both sexes, with ages between 1 and 2 months.

These animals proceeded from 2 conventional mice breeding colonies, 2 conventional rat breeding colonies and 1 mice breeding colony with barriers, used as control, all of them in the city of São Paulo. The study was carried out for a period of 5 years (1986-1991), and the laboratory exams were performed in the Department of Parasitology Biomedical Science Institute, University of São Paulo, Brazil.

The semi-annual random sampling was made up by 5% of the population in the rooms that held more than 100 animals each, from the several colonies studied. Colony A - 82 mice, colony B - 77 mice, colony C - 53 rats, colony D - 75 rats, and Control colony - 70 mice.

In the conventional breeding colonies included in the research, animals were kept in polypropylene cages with wood shavings as bedding material. The hygiene of the cages was accomplished by two weekly changings. Filtered water and commercial feed were given "*ad libitum*." The rooms were cleaned twice a week with a 0.1% sodium hypochlorite solution and cages, nipples and drinkers were rinsed with a biodegradable tensoative detergent kept in a 5% sodium hypoclorite solution during 2 hours, then washed in water.

For hygienization of the barrier colony, besides the same methods employed on the conventional colonies, cages, wood shavings, lids, drinkers and nipples were sterilized at 121° C for 30 minutes, and water and feed at 121° C for 60 and 15 minutes, respectively. Caretakers were required to shower and wear sterilized uniforms, masks, caps, gloves and shoe protectors, before entering the colony. The control colony also had a mechanical ventilation system with high efficiency filters (85%), on the inlet and outlet of ducts in each room.

EXAMINATION OF THE ANIMALS

Each animal was identified and submitted to general examination, observing the fur, presence of nasal and ocular secretions, diarrhea and abdominal distention.

In order to carry out the Willis method [21], a fecal sample was obtained from each animal, and material was also collected from the perianal region for the anal-swab method [7]. After euthanasia by cervical displacement, all animals were examined under stereomicroscope for collection and setting-up of ectoparasites in Hoyer's solution, for later identification.

After abdominal incision, intestines were removed and subdivided into four sections (jejunum-ileum, caecum and large intestine). Sections were opened longitudinally and samples of intestinal mucous membrane were obtained by scrapping. The material was placed on a slide with coverslip, with a drop of saline solution, to search for protozoa under the optical microscope. Petri dishes containing the sections were taken to the stereomicroscope for searching and collecting of helminths, which were placed in acetic formalin (10%) as fixative, for later identification.

STATISTICS TEST

Results obtained by these different parasitological methods and necropsy were compared by using the Kappa index to verify concordance among them [10].

RESULTS

During the general examination, abdominal distention and diarrhea were observed in 1.4% of animals from colony A, 5.2% of animals from colony B, 2.8% of rats from colony C and 3.2% from colony D. These alterations, however, were not observed in mice from the control colony. Some alopecia and bristly hairs were detected in all evaluated colonies. The percentage of these alterations observed in mice was 23.17%, 29.87% and 17.14% in the animals from colonies A, B and control respectively, and 7.55% in the rats from colony C and 25.33% from colony D (Table 1).

In the general examination to search for and collect ectoparasites, it was observed a positivity for mice of 86.58% in colony A, 92.20% in B, and for rats 33.96% in colony C and 58.67% in colony D. No ectoparasites were found in the control mice colony. The following ectoparasites were identified: *Myobia musculi*, *Myocoptes musculinus*, *Rhadfordia affinis*, *Rhadfordia ensifera* and *Poliplax spinulosa*. Multiple infestations were seen in several animals.

The percentages of ectoparasite species identified in the various colonies are shown in Table 1. *Myobia musculi* and *Myocoptes musculinus* were found in mice from colonies A and B and infestations by *R. affinis* was also detected in some mice from colony B. *Poliplax spinulosa* infestations was observed in rats from colony C and D, as well as some animals with *Rhadfordia ensifera* and *Myobia musculi*, from colonies C and D, respectively.

Mice from colonies A and B had a higher incidence of *M. musculinus* in the dorsal region and *M. musculi* in the eyelids and nose. In rats from colonies C and D, *Myobia musculi* was homogeneously distributed throughout the animal's body, while *Poliplax spinulosa* was mainly located on the dorsal-lumbar region.

Multiple infestations by *M. musculi* and *M. musculinus* were observed in mice from colonies A and B in 52.11% and 23.94% animals, respectively. Association of *M. musculi* and *P. spinulosa* was observed in 9.09% of rats from colony D. Rats from colony C showed single infestation by *R. ensifera* or *P. spinulosa*.

Through the Willis and the Anal-swab methods, presence of the following helminths was diagnosed: *Hymenoleps nana*, *Aspiculuris tetraptera* and *Syphacia* sp. Frequencies of animals positive to helminth eggs found during the fecal exams are shown in Table 2. When compared to necropsy data, the Willis method revealed 61.3% of infections by *H. nana*, 91.7% by *A. tetraptera* and 34.0% by *Syphacia* sp, whereas the Anal-swab method revealed 66.2% of infections by *Syphacia* sp (Table 4).

By means of necropsy, *H. nana* was identified in the animals from the four conventional colonies, and percentages of infection are shown in Table 3. *Syphacia* sp and *Aspiculuris tetraptera* were identified in the animals from the five colonies.

Specimens of *H. nana* were found in the small intestine. *Syphacia* sp and *Aspiculuris tetraptera* specimens were mainly found in the cecum and large intestine, but when the infection was severe they were also present in the small intestine.

It was observed that the Willis method was practically as efficient as necropsy for diagnosing parasitism by *H. nana* and *Aspiculuris tetraptera*, the calculated Kappa index being, respectively, 0.742 and 0.940, meaning significant and almost perfect concordance. On the other hand, for *Syphacia* sp, the Willis

method, according to the same criterion, supplies results that did not agree with those of necropsy and the calculated Kappa index, in this case, was 0.273, which means "slight concordance". Use of anal swab for diagnosing infections by *Syphacia* sp or *Aspiculuris tetraptera* produced different results when compared to necropsy. Calculated Kappa indexes were 0.591 for *Syphacia* sp and 0.058 for *A. tetraptera*, respectively indicating moderate and no concordance .

The flagellates *Tritrichomonas muris* and *Spironucleus muris* were found in animals from colonies A, B, C and D; *Giardia muris* in colonies B, C and D and *Eimeira* sp in colony. The presence of these protozoa was not detected in the control animals (Table 3).

The intestinal protozoa *S. muris* and *Eimeira* sp were found mainly in the jejunum-ileum section, *Giardia muris* in the duodenum and *T. muris* in the cecum and large intestine. In severe infections, it was observed that *S. muris* was located in the duodenum section and *T. muris* in the distal portion of the jejunum-ileum.

DISCUSSION AND CONCLUSIONS

Abdominal distention and diarrhea were seen during the general examination of mice and rats. This picture can be related to infection by helminths such as *H. nana* and *Syphacia* sp found in almost all colonies under evaluation, responsible for several intestinal disorders, or by protozoa as *Giardia muris* and *Eimeria* sp, causing chronic enteritis mainly in young animals[4].

In most cases, alopecia observed in animals from the evaluated colonies was due to the high ectoparasite infestation in mice from colonies A and B, but it was smaller in rats from colonies C and D. However, it should be emphasized that some cases of diffuse alopecia, without the presence of ectoparasites, such as those seen in mice from the control colony, can be related to genetic factors, present in some inbred strains such as C57BL/6, or even to nutritional deficiencies[15].

The ectoparasites found in the colonies were not pathogenic. However, depending on the degree of infestation they may be stressful to the animals, or act as vectors for microorganisms able to modify experimental infections, such as *Eperythrozoon coccoides* and *Tiphus murino*, both transmitted by *Poliplax* sp[1,3,15].

Multiple infestations by *M. musculi* and *M. musculinus* and by *M. musculi* and *P. spinulosa* were observed in mice from colonies A and B and in rats from colony D. This kinds of infestations in mice are in agreement with reports in the literature[3,15].

The ectoparasite *M. musculi* is being described for the first time in rats raised in breeding colonies[14], although infection by *M. musculi* in wild and synanthropic rats has already been described[13].

Analysis of the parasitological methods demonstrated that the anal-swab method is more efficient than the Willis method in detecting *Syphacia* sp eggs and not adequate for the detection of *Aspiculuris tetraptera* and *H. nana* eggs. Differences in the results for *Syphacia* sp and *Aspiculuris tetraptera* are due to the specific characteristics of the oviposition of these oxyurids, eggs of *Syphacia* sp being more adherent to the peri-anal region than eggs of *Aspiculuris* sp.

By comparing results obtained from the Willis and the anal-swab methods with those from necropsy, it was concluded that both methods should be used in the evaluation of sanitary conditions of the breeding colonies, and that they can be used to verify the efficacy of anthelmintic treatments in the parasitological control of these colonies.

In general, rodent protozoa do not present resistant forms that can be detected by the coproparasitologic methods used in these studies, exception made to *Eimeria* sp and *G. muris*. Thus, in order to evaluate their presence, as well as the infection degree, in laboratory animals, necropsy is recommended.

Most endo and ectoparasites found in the studied colonies were introduced through wood shavings contaminated by wild rats or insect vectors, in the sawmills. Dissemination occurs by contaminated feces, coprophagy, or grooming; permanent contact within the breeding cages and later move of animals - after weaning - to stock cages facilitate dissemination of endo and ectoparasites throughout the whole animal housing.

The presence of *Syphacia* sp and *Aspiculuris* sp in the barrier colony possibly is due to the fact that their eggs can be carried to other compartments, through the air conditioning system.. Therefore, their eradication from breeding colonies is very difficult by means of the usual control programs.

All parasites detected in this study are considered to have low pathogenicity and are very common in conventional colonies.

According to the results, it is possible to conclude that the Willis and the anal-swab methods are adequate for routine diagnosis of helminths, but necropsy is recommended for detection of gastrointestinal protozoa.

Table 1
Percentage and positivity of infestations, by ectoparasites, in mice and rats from the breeding colonies studied.São Paulo, 1986-1991

Animals	Breeding colonies	Number of animals	Animals alopecia or with bristly hair (%)	Positive animals	Ectoparasites (%)				
					Myobia musculi	*Myocoptes musculinus*	*Radfordia affinis*	*Rhadfordia ensifera*	*Poliplax spinulosa*
Mice	Control	70	17.14	0	0	0	0	0	0
	A	82	23.17	71	53.66	71.95	0	0	0
	B	77	29.87	71	87.01	20.78	7.79	0	0
Rats	C	53	7.55	18	0	0	0	26.41	16.98
	D	75	25.33	44	12.00	0	0	0	42.67

Good prophylaxis can be achieved by[1] sterilization of the wood shavings, feed, cages and drinking water[2]; disinfection of the breeding rooms; and[3] air conditioning systems with positive pressure filters. The number of cages per room, as well as the number of animals per cage, should also be evaluated.

Such procedures prevent the introduction of contaminants and parasite dissemination into the breeding areas. These can also be controlled by therapy with specific drugs. Thus, foster mothers can be obtained and kept in isolators, for later use on the nursing of clean youngsters - obtained by hysterectomy or cesarean derivation - that will re-populate breeding areas.

Table 2
Frequency of helminth eggs found by the use of Willis and anal-swab methods in parasitological examination of mice and rats. São Paulo, 1986-1991

		WILLIS						ANAL-SWAB					
		Hn		Sy		Ap		Hn		Sy		Ap	
Animals	Number of animals	F	%	F	%	F	%	F	%	F	%	F	%
Mice	229	10	4.3	32	14.0	89	38.9	0	0	64	27.9	4	1.7
Rats	128	09	7.0	41	32.0	0	0	0	0	79	61.7	0	0
Total	357	19	11.3	73	46.0	89	38.9	0	0	143	89.6	4	1.7

Table 3
Percentage of infection, by endoparasites found at necropsies of mice and rats from the breeding colonies studied. São Paulo, 1986-1991

Animals	Breeding colonies	Number of animals	Endoparasites (%)						
			Hymenolepis nana	*Syphacia sp*	*Aspiculuris tetraptera*	*Tritrichomonas muris*	*Giardia muris*	*Spironucleus muris*	*Eimeria sp*
	Control	70	0	42.86	17.14	0	0	0	0
Mice	A	82	1.22	64.63	65.85	40.24	0	64.63	8.53
	B	77	11.68	50.64	35.06	72.72	68.83	88.31	0
Rats	C	53	20.75	81.13	1.88	93.22	58.49	72.24	0
	D	75	13.33	65.33	4.00	80.00	61.33	0	0

Table 4
Number of positive animals detected during necropsies and results of the corresponding fecal exams performed by Willis and anal-swab methods. São Paulo, 1986-1991

PARASITES	NUMBER OF POSITIVE ANIMALS TO NECROPSY	WILLIS		ANAL-SWAB	
	N	N	%	N	%
Hymenolepis nana	31	19	61.3	0	0
Syphacia sp	216	73	34.0	143	66.2
Aspiculuris tetraptera	97	89	91.7	4	4.1

n= number of positive animals

RESUMO

Durante um período de 5 anos (1986-1991) foram examinados 229 camundongos e 128 ratos, de ambos os sexos, com idade entre 1 a 2 meses, provenientes de 4 biotérios convencionais (2 de camundongos e 2 de ratos), e de um biotério provido de barreiras sanitárias (camundongos controle). A amostragem semestral aleatória consistiu em 5% da população das salas com mais de 100 animais cada. No exame clínico geral observaram-se distensão abdominal e diarréia nos camundongos e ratos dos biotérios convencionais, o que não foi constatado nos animais do biotério controle. Discreta alopecia e pêlos eriçados foram detectados em todos os animais das colônias estudadas. Infestações por *Myobia musculi, Myocoptes musculinus, Radfordia affinis, Radfordia ensifera* e *Poliplax spinulosa* foram identificadas nas colônias convencionais, em infestações múltiplas ou simples. *Myobia musculi* foi descrito pela primeira vez em ratos criados em biotérios. Infecções por endoparasitas *Hymenolepis nana, Syphacia* sp, *Aspiculuris tetraptera, Trichomonas muris, Spironucleus muris, Giardia muris* e *Eimeria* sp foram observadas nas colônias convencionais. Entretanto, através dos resultados da necrôpsia, na colônia controle de camundongos somente foram encontrados *Syphacia* sp e *Aspiculuris tetraptera*. A necrópsia confirmou que o uso do método de anal-swab para detecção de ovos de *Syphacia* sp é preferível ao de Willis, e revelou o grau de infecção de protozoários intestinais. *Syphacia* sp, *Hymenolepis nana* e *Aspiculuris tetraptera* foram eficientemente detectados pelo método de Willis.

UNITERMOS: Ectoparasitas; Endoparasitas; Camundongo; Rato; Biotério

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