Helminth Parasites of Conventionally Maintained Laboratory Mice - II. Inbred Strains with an Adaptation of the Anal Swab Technique

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Worm burdens recovered from inbred mice strains, namely C57Bl/6, C57Bl/10, CBA, BALB/c, DBA/2 and C3H/He, conventionally maintained in two institutional animal houses in the State of Rio de Janeiro, RJ, Brazil, were analyzed and compared, regarding their prevalences and mean intensities.Three parasite species were observed: the nematodes Aspiculuris tetraptera, Syphacia obvelata *and the cestode* Vampirolepis nana. *A modification of the anal swab technique is also proposed for the first time as an auxiliary tool for the detection of oxyurid eggs in mice.*

Key words: inbred mice - helminths - *Aspiculuris tetraptera* - *Syphacia obvelata* - *Vampirolepis nana* - prevalence - mean intensity - anal swab technique

This approach intends to add new data on the helminth parasites of laboratory mice, since these investigations have arised a great interest and also, taking into account that these hosts as experimental animal models, are widely utilized in the evaluation of several biological parameters. Previously, besides the considered Swiss Webster mice, only two inbred strains (C57Bl/6, DBA/2) were investigated for the presence of helminths in Brazil (Pinto et al. 1994).

Thus, the present findings ratify those reported for these strains, as well as complement the evaluation of worm burden in the other commonly referred inbred mice.

The modification of the anal swab technique proposed herein aims to avoid unnecessary necropsies to investigate the spectrum of intestinal parasites in a rodent colony, since oxyurid eggs are of difficult detection during conventional stool examination procedures.

MATERIALS AND METHODS

Two hundred and fifty *Mus musculus* (Linnaeus, 1758) inbred mice from two animal houses in the State of Rio de Janeiro, RJ, Brazil, were divided in two groups (A, B) according to their source. The suppliers were not identified by name due to ethical reasons. In group A, were included mice of the strains C57Bl/6, C57Bl/10, CBA, BALB/c, DBA/2 and C3H/He, with 25 animals each. The group B was represented by the strains C57Bl/6, CBA, BALB/c and C3H/He, with 25 mice each. Mice were male, 30 days old, weighting 20g each.

Mice were sacrificed, helminths were recovered and processed for study as described elsewhere (Pinto et al. 1994).

As for the swab anal procedures, the technique of Hall (1937) was modified and the device consists of a plastic rod (Fig. 1), with appropriate dimensions, for the examination of mice weighting at least 18g. One hundred and twelve mice were tested by this method. Animals are maintained in the proper position (Hofman's position), in order to avoid stress to the animals during the procedure. The rod is moistened in a 0.85% NaCl solution (Fig. 2) and after, introduced into the anus of the mouse, with carefully induced rotatory movements (Fig. 3), to avoid bleeding. The collected feces sample is transferred to a drop (20 µl) of saline physiological solution (0.85% NaCl) on a slide with a coverslip and examined under light microscope.

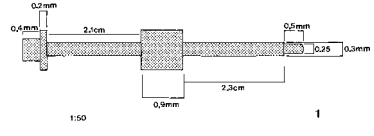

Fig. 1: the anal swab device.

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The classification of the helminths adopted herein is that of Pinto et al. (1994).

Photomicrographies of eggs and larva were obtained in a Zeiss Axiophoto System. Graphics were drawn with the aid of Harvard Graphics software.

Anal swab scraping - Fig. 2: the moistening of the anal swab in NaCl solution. Fig. 3: the introduction of the anal swab in the mouse.

RESULTS

Mice of the studied strains, were parasitized by the nematodes *Aspiculuris tetraptera* (Nitzch, 1821) Schulz, 1927, *Syphacia obvelata* (Rudolphi, 1802) Seurat, 1916 and by the cestode *Vampirolepis nana* (Siebold, 1852) Spasskii, 1954.

In the six strains of group A, a high prevalence of parasitism (68-100%) was observed in the necropsied mice (Fig. 4). In the study regarding the prevalence of species, the strains C57Bl/6, C57Bl/10 and C3H/He showed high percentages (88-96%) for *A. tetraptera*, whereas BALB/c, DBA/2 and C3H/He presented higher prevalence (80-100%) for *S. obvelata*, while the prevalence of *V. nana* was higher (92%) in the C3H/He strain.

Except for this latter strain, *A. tetraptera* was found together *S. obvelata* in mice of the other studied strains. *A. tetraptera* was associated to *V. nana* in C57Bl/6, C57Bl/10 and C3H/He mice. *S. obvelata* appeared with *V. nana* in C57Bl/10 and C3H/He. Except for CBA and DBA/2, association of the three helminth species was observed in mice of the four remaining strains of group A and with high levels in C3H/He (Fig. 5).

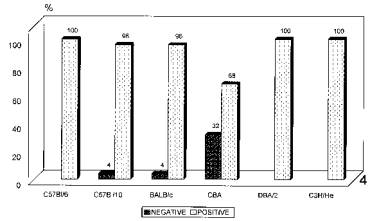


Fig. 4: prevalence of parasitism in mice of group A.

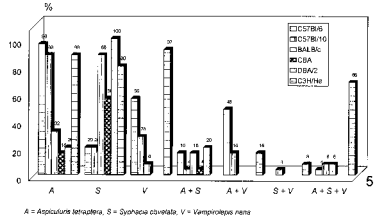


Fig. 5: prevalence of helminth species in mice of group A.

In the analysis of mean intensities, except for the CBA and DBA/2 strains, *A. tetraptera* was represented by 111-139 specimens/strain, the same occurring with the BALB/c and C3H/He strains, concerning *S. obvelata*, with 110 and 152 specimens/strain, respectively, whereas the mean intensity for *V. nana* in the C57Bl/10 strain was of 174 worms (Fig. 6).

Mice of group B, also showed high levels (76-100%) of parasitism (Fig. 7). *A. tetraptera* appeared with the highest prevalence in the C57Bl/6 mice

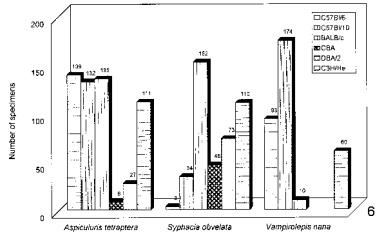


Fig. 6: mean intensity of helminth species in group A.

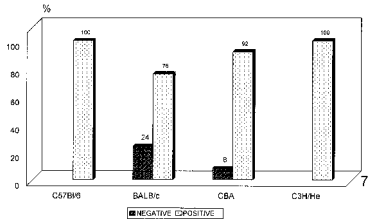


Fig. 7: prevalence of parasitism in mice of group B.

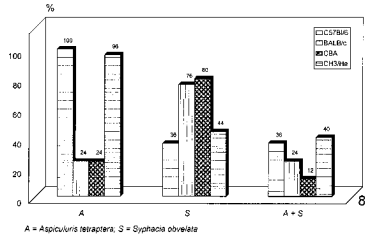


Fig. 8: prevalence of helminth species in mice of group B.

(100%) and all the strains presented the association of the two nematode species. *S. obvelata* appeared with higher percentages (80%) in mice of the CBA strain (Fig. 8).

Vampirolepis nana was absent in mice of group B, in which the animals of the C3H/He strain presented a mean intensity of 118 specimens of *A. tetraptera*, compared to the mean intensity of 41 worms of *S. obvelata* for the CBA strain (Fig. 9).

The test for the presence of oxyurid eggs and larvae was positive in 96 out of the 112 examined mice. In this manner it could be verified the presence of eggs of *S. obvelata* (Fig. 10), eggs and larvae of *A. tetraptera* (Figs 11,12).

In the comparison of the obtained results with the swab anal with those of the necropsies of the 112 animals of the group, it was observed that in

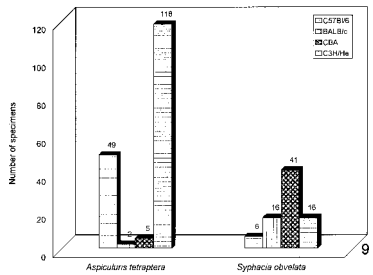
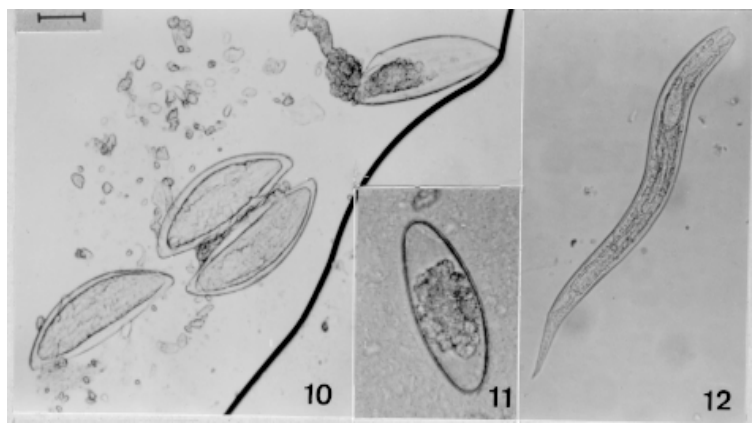


Fig. 9: mean intensity of helminth species in mice of group B.



Results after anal swab scraping - Fig. 10: *Syphacia obvelata* eggs. Fig. 11: *Aspiculuris tetraptera* egg. Fig. 12: *Aspiculuris tetraptera* larva. Same bar for the Figs 10-12. Values are as follow: Fig. 10 = 30 µm; Fig. 11 = 20 µm; Fig. 12 = 10 µm.

25 C57Bl/6 mice, although the test was negative in five animals it was verified the presence of *A. tetraptera* in the five and of *S. obvelata* in one, during necropsies.

In four negative CBA mice, out of the 25 examined, two were positive for *S. obvelata* and two for *A. tetraptera*. In the BALB/c group four negative animals for the test out of the 25 necropsied showed the presence of *S. obvelata*, the same occurring with the two negative animals out of the 25 DBA/2 mice, whereas one out of the 12 mice of the C3H/He group, negative for the swab anal, was found to be parasitized with *A. tetraptera*. In fact, 16 out of the 112 examined mice were negative for the presence of oxyurid eggs, with the employment of the anal swab technique (Table).

DISCUSSION

Animal models have been exhaustively investigated regarding aspects related to their suitability for the development of experimental protocols under laboratory conditions. Nevertheless, in most of the adopted procedures, the prior detection of their ecto and endo parasites are generally overlooked.

Thus, the main purpose of previous accounts (Pinto et al. 1994) and of the present report, is, once more, to emphasize the importance of the proper evaluation of laboratory animals to be used in experiments, concerning their health conditions.

The release of multiple antigens in an already parasitized animal model, further exposed to experimental infections or submitted to vaccination protocols, aiming either the maintenance of life-cycles in laboratory or the obtaintion of specific immune responses, may determine the infeasibility or inaccuracy concerning the expected results, considering that physiological and immunological natural conditions are deeply affected by the presence of undetected parasites (Vyas et al. 1981, Gabrielle et al. 1988, Pinto et al. 1994).

It was verified that the results so far obtained do not differ from those with the SW mice strain, when prevalences of worms burdens are compared. The "crowding effect", due to hyperinfections with the cestode *V. nana*, also occurred in the C57Bl/6, C57Bl/10 and C3H/He strains (Fig. 6) in the manner it was previously observed in the SW mice (Pinto et al. 1994).

This fact is of extreme relevance, taking into account that cestode infections induce very high levels of immunogenic responses in the parasitized animals (Lucas et al. 1980, Ito, 1982). An interesting situation was observed in the inbred mice strains investigated: when the parasitism due to *A. tetraptera* reached high levels, those related to the infection with *S. obvelata* were low and vice-versa (Figs 5, 8). This parameter could be better analyzed during studies of population dynamics of these species in mice (Scott & Gibbs 1986).

Based on our results, we can conclude that among the studied inbred strains, the CBA was that in which the mice were negative for cestodes in group A and also presented the lower prevalence of helminths, whereas, in the C3H/He, conversely, the mice were found to be highly infected with the identified parasite species (Figs 5, 6). When high prevalences are observed it is necessary to provide barriers to reverse or to avoid this situation. Kamiya et al. (1979) were able to compare data on parasitism in mice and rats either conventionally maintained or raised in barrier-sustained colonies, with better results obtained in the latter group.

Saiz-Moreno et al. (1983) call attention to the diagnosis of pathogenic cases on the basis of observation and hypothesis only, since diagnostic procedures must be a matter of concern, mainly in the presence of subclinical and mild undetected infections sufficient to contaminate animal colonies. They insist in this topic, considering that feeding habits, infective and parasitic processes are of concern, since frequent infections may determine a situation of stress and contribute for the development of latent viral or bacterial diseases. In this scope, the modification of the anal swab technique, to be applied in mice, is proposed herein. This procedure if adopted as a routine in the animal houses where mice are raised and maintained, certainly will provide a safe and rapid diagnostic regarding the presence of oxyurid worms in these animal models.

TABLE

Comparison of data on parasitism by oxyurids in mice with the employment of the anal swab technique and posterior necropsy

Strain [a]	Anal swab						Necropsy			
	No. positive [b]				No. negative	(%)	No. positive	(%)	No. negative	(%)
	Eggs	(%)	Larvae	(%)						
C57Bl/6	4	(16)	17	(68)	5	(20)	25	(100)	-	(0)
CBA	13	(52)	8	(32)	4	(16)	23	(92)	2	(8)
BALB/c	10	(40)	7	(28)	4	(16)	19	(76)	6	(24)
DBA/2	20	(80)	10	(40)	2	(8)	25	(100)	-	(0)
C3H/He	3	(25)	10	(83.3)	1	(8.3)	11	(91.6)	1	(8.3)

a: 25 mice/strain, except for C3H/He with 12 animals; *b*: considered the concomitance of eggs and larvae.

The methods commonly adopted in different animal houses consist of those related to coproscopy and necropsies. Stool examination, seems to be suitable for the detection of *V. nana* and *A. tetraptera* but totally inaccurate on what concerns *S. obvelata*, for according to Philport (1955) in Anderson (1992) the presence of eggs in the feces of the host is not common as similarly occur with the human pinworm *Enterobius vermicularis* (L., 1758) Leach,1853.

Considering this fact, Hall (1937) proposed several types of swabs for the detection of oxyurid eggs in man and later, Grahan (1941) modified one of these techniques, namely the cellophane-tape device.

In the present paper another modification of the cotton swab is introduced, since our device lacks the cotton tip and is flexible (Fig. 2). This plastic rod showed to be very appropriate since it is smooth enough and not absorbent, permitting a reliable analysis of the material collected after the scraping of the terminal portion of the large intestine and perianal region (Figs 10, 11, 12).

An interesting fact was to observe also a great number of *A. tetraptera* eggs among those of *S. obvelata* recovered with the plastic anal swab.

The so far obtained results, with the use of the plastic modified device, seemed to be very stimulating, considering that while through coproscopy the positivity for oxyurid eggs or larvae ranges from 5 to 10 %, this percentage increases to 86.3%, when the plastic anal swab is utilized (Table).

Sixteen cases showed to be negative to the anal swab technique and in 11 out of them, mice were parasitized by *S. obvelata*. Nevertheless, these animals showed a very low worm burden (a mean of eight worms/mouse) and the presence of young females, that did not have migrate yet to the perianal region. This observation is in accord to data reported by Chan (1955) in Anderson (1992).The proposition of the anal swab technique for the detection of pinworms eggs in mice is undoubtedly related to *S. obvelata* infections due to the similarity this species shares with *E. vermicularis*, taking into account their biological behavior. The utilization of this device associated to coproscopy procedures provides a reliable diagnosis and also aims to avoid unnecessary sacrifices of those animals randomly chosen as a sampling, to be necropsied for helminths possibly occurring in a colony, or in an experimental group, in order to prevent the loss of individuals that should adequately be treated with no interruption of the protocols under development.

This approach in its main scope, considers a safe diagnostic test for the evaluation of oxiuriasis in mice for the further administration of antihelmintic drugs to these hosts. Moreover, it aims to contribute and is also in agreement with the worldwide spread National Committees and/or Non Governmental Organizations that have been strongly advising against mass sacrifices of experimental animal models maintained in laboratories as well as those endangered species used in scientific research.

According to Weis and Ernst (1981), the administration of anti-helminthic drugs alone, is not sufficient for the obtaintion of acceptable results concerning the eradication of parasites; this procedure should be associated to the adoption of rules for the proper maintenance of animals under appropriate care and health conditions.

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