

COMPARISON OF WORM MIGRATION AND SALINE INCUBATION AS METHODS FOR RECOVERING *NIPPOSTRONGYLUS BRASILIENSIS* FROM THE SMALL INTESTINE OF RATS.

E.L. ORTOLANI

Departamento de Clínica Médica - Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Av. Corifeu de Azevedo Marques, 2720, V. Lageado, Cep 05340-900, São Paulo S.P. (Brazil).

SUMMARY: Incubation in saline solution at 37°C was compared with worm migration through a double layer of gauze to a phosphate buffered saline (PBS) solution as a quantitative method for recovering adult worms of *Nippostrongylus brasiliensis* from the intestinal mucosa of rats. Thirty worm-free, 12 week-old Wistar rats were used. They were infected with 3,300 infective larvae and killed seven days later. The whole length of the small intestine was opened longitudinally and distributed randomly in each treatment. For counting the number of worms that could not migrate after the worm retrieval the intestines were incubated in a solution of 1% hydrochloric acid and 1% pepsin, in both methods. Higher number of male, female and total parasite counts ($p < 0.05$) were recovered by saline incubation than the worm migration method. Less than 1% of worms were recovered in the digests of the intestine after the saline incubation retrieval. However, more than 20 % of the worms were trapped inside the intestine or the gauze in the worm migration method. It was concluded that saline incubation is more efficient method for recovering worms of *Nippostrongylus brasiliensis* than the established worm migration method.

KEY-WORDS - *Nippostrongylus brasiliensis*, methods, recovering, adult worm, rat, comparison.

INTRODUCTION

Nippostrongylus brasiliensis is an intestinal nematode of some rodents and has been used, for decades, as a reproducible animal model to study the host-parasite relationship and the inflammatory response of the host. Several reasons explain the widespread use of this parasite in parasitological research: the life cycle of the parasite resembles those many species of nematodes that inhabit the intestinal tract and are of veterinary and medical significance; the development of the parasites is fast, an intermediate host is not required; this nematode develops in inexpensive hosts (rats, mice etc) used in experimental parasitology and finally the parasite is easy to handle and count (KASAI, 1982 ; MILLER, 1984).

There are many techniques used to estimate the worm burden counts of *Nippostrongylus brasiliensis*. Although the

most used technique nowadays is that one described by NAWA & MILLER (1978). This technique applies the principle of worm migration out of the intestine to a preheated solution similar the technique used to recover *Dictyocaulus viviparus*. According to NAWA & MILLER (1978) five to 15% of the worms recovered by their technique remained trapped in the intestine and gauze while for KASSAI (1982) the percentage of trapped worms was less than 10%.

In a preliminary procedure of a previous experiment ORTOLANI *et al.* (1992), where rats were infected with *Nippostrongylus brasiliensis*, the amount of trapped worms was variable and many cases exceeded the percentage reported elsewhere. JACKSON *et al.* (1984) developed a saline incubation technique to recover *Ostertagia circumcincta* larvae from abomasum where the mucosa surface was incubated in direct contact with the medium without any obstacle, for instance, gauze. They concluded that the larvae actively migrated out to the physiological saline and the worm

countings were as efficient as the established pepsin digestion method.

The object of this trial was to compare the efficiency for recovering *Nippostrongylus brasiliensis* worms of the saline incubation method after JACKSON et al. (1984) and the established worm migration method of NAWA & MILLER (1978).

MATERIALS AND METHODS

Animals: 30 worm-free Wistar rats, of both sexes, 12 weeks-old were used in this experiment.

Parasitological Techniques: A strain of *Nippostrongylus brasiliensis* that has been cultured at the Moredun Research Institute, Edinburgh, Scotland, for several years was used. Infective larvae (L₃) were obtained by the technique described by NAWA & MILLER (1978). Larval suspensions were adjusted to have 3300 L3/dose and were inoculated subcutaneously into the flank region of the rats. After seven days from infection the rats were killed and whole length of the small intestine opened longitudinally and placed with all its contents in a plastic pot.

Randomly, the pots were divided in two equal groups. In the first half it was used to retrieve *N. brasiliensis* by the classical technique according to NAWA & MILLER (1978). The whole content of the pots was transferred and enveloped in a double layer of surgical gauze, suspended in preheated (37°C) phosphate buffered saline solution (PBS), and incubated at 37°C for three hours. During this time the worms could migrate out through the gauze to the PBS solution.

The other half was processed by a slight modification of the method described by JACKSON et al (1984) to retrieve *Ostertagia circumcincta* from abomasum of sheep. The whole content of the pots was placed in a kilner jar which was filled with physiological saline at 37°C and incubated at this temperature for three hours with vigorous shaking for 15 seconds at every hour. After this incubation time the strips of the intestine were slightly rubbed in order to remove the worms occasionally adhered to the mucosa.

After thoroughly shaking, a 10% aliquot of fluid, from all samples, was collected and fixed with formalin.

In both techniques all pieces of small intestine, which remained from the worm migration and saline incubation, were incubated in a solution of 1% hydrochloric acid and 1% pepsin for four hours at 37°C. This operation was performed to count the number of worms that remained retained in the small intestine. The worms trapped on the surgical gauze were counted and added to the larvae found after digestion of the intestine.

The aliquot of material was stained with Gram's iodine and

counted under a stereomicroscope at X 20 magnification. The number of males, females and total parasites was counted plus the worms retrieved after the digestion. As the different worm counts presented a normal distribution the treatments were compared by analysis of variance with application of Duncan's multiple-range test.

RESULTS

The number of worms recovered from the small intestine by each treatment is shown in Table 1. Higher number of male, female and total parasite were recovered by the saline incubation technique than the NAWA & MILLER's worm migration technique.

When total parasites recovered by digestion and trapping were added to the worms counted in larvae migration there was no difference between the total counting of the techniques tested. About 85% of worms recovered after the larval migrations were held in the intestine loops.

Very few worms were recovered from the intestine digestion in the saline incubation method.

Table 1 - Estimates number of worms recovered from small intestine with different methods.

METHOD	NUMBER OF WORMS		
	MALE	FEMALE	TOTAL
WORM MIGRATION (WM)	933±390 ^b	1363±239 ^b	2296±554 ^b
WM+ DIGEST	1122±322 ^{ab}	1603±272 ^a	2725±546 ^a
SALINE INCUBATION (SI)	1290 ±185 ^a	1641±278 ^a	2931±351 ^a
SI+ DIGEST	1299 ±180 ^a	1641±278 ^a	2940±348 ^a

a > b > c at the same column (p < 0.05)

DISCUSSION

The present results (table 1) clearly show that the established technique of NAWA & MILLER (1978) to recover *Nippostrongylus brasiliensis* worms might underestimate by more than 20% the total worm counting. About 85 % of the trapped worms were confined in the internal loops or intestinal folds in the middle of the enveloped intestinal segments. Thus, these worms were unable mechanically to migrate to the surrounding area of the gauze. This observation shows that the gauze is not the principal obstacle for successful worm migration. It was observed as well that the worms placed around the gauze migrate out easily through this obstacle when the migration process had started.

The saline incubation method proved to be very ease to perform and very accurate. As the strips of the intestine were opened and in direct contact with the medium practically all the worms were free to migrate out, principally after the shaking and rubbing. Less than 1% of the worms were recovered after the digestion. The only disadvantage of the saline incubation method concerns with the counting. When the intestine rubbing is not made very slightly some debris can supervene with the worms interfering with their counting. This problem can be easily overcome with appropriate rubbing.

It was concluded that saline incubation method is more efficient for recovering *Nippostrongylus brasiliensis* worm burdens than the established worm migration method. Thus, every time that a worm burden must be estimate with precision, the saline incubation method should be adopted.

ACKNOWLEDGMENTS

The author express his appreciation to Anne Mckellar, Frank Jackson, Robert Coop, Elisabeth Jackson and Neville Suttle for technical support and the Moredun Research Institute for providing the facilities to carry out this experiment.

SUMÁRIO

Foi comparada, neste experimento, a eficiência de recuperação de vermes adultos de *Nippostrongylus brasiliensis*, em ratos, pelos métodos de incubação do intestino em solução salina e de migração de vermes do intestino para uma solução tamponada salina fosfatada, separado por uma dupla camada de gaze. Utilizaram-se trinta ratos Wistar, de 12 semanas de idade, os quais foram criados em condições livres de infecção por helmintos. Os animais foram inoculados, pela via subcutânea, com 3600 larvas infectantes (L3) e sete dias após foram sacrificados. Os intestinos foram retirados e abertos longitudinalmente e em seguida distribuídos ao acaso para um dos dois tratamentos citados acima. Para estimar o número de vermes que permaneceram no intestino, após a realização dos métodos, este órgão foi digerido com solução de ácido clorídrico e pepsina. Foi recuperado maior número de vermes machos, fêmeas, assim como da carga parasitária total ($p < 0,05$) com o método de incubação em solução salina, do que com a migração através da gaze. Menos de 1% dos vermes foram recuperados após a digestão em intestino incubados em solução salina. Porém, na técnica de migração através de gaze esta recuperação foi bem maior, ao redor de 20%. Concluiu-se que o método de incubação em solução salina é

um método mais eficiente na recuperação de vermes adultos de *Nippostrongylus brasiliensis*, do que a migração pela gaze, tradicionalmente utilizada até hoje.

PALAVRAS-CHAVE - *Nippostrongylus brasiliensis*, métodos, recuperação, vermes adultos, rato, comparação.

REFERÊNCIAS

- JACKSON, F.; JACKSON, F. & SMITH, W.D. (1984). Comparison of saline incubation and pepsin digestion as methods for recovering *Ostertagia circumcincta* larvae from the abomasum of sheep. *Res. Vet. Sci.* 36, 380-381.
- MILLER, H. R. P. (1984). The protective mucosal response against gastrointestinal nematodes in ruminants and laboratory animals. *Vet. Immun. Immunopath.* 6, 167-259.
- KASAI, T. (1982) Handbook of *Nippostrongylus brasiliensis*. Budapest: CAB & Akademia Kiado. 257p.
- NAWA, Y. & MILLER, H.P.R. (1978) Protection against *Nippostrongylus brasiliensis* by adoptive immunization with immune thoracic duct lymphocytes. *Cell. Immun.* 37, 51-60.
- ORTOLANI, E.L.; KNOX, D.P.; NEWLANDS, E.F.J. & SUTTLE, N.F. (1992) Effects of dietary molybdenum supplementation or depletion on *Nippostrongylus brasiliensis* infection in the rat. *Proc. Nutr. Soc.* 3, 143 A.

(Received 19 November 1996, Accepted 21 January 1997)