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Application of the Midi Parasep® SF technique for the detection of L1 larvae of *Angiostrongylus cantonensis* in faeces of infected rats

M. Teresa Galán-Puchades[1], S. Sáez-Durán[1], M. Gómez-Samblás[2], A. Osuna[2], R. Bueno-Marí[1,3] and M.V. Fuentes[1]

[1]Parasites & Health Research Group, Department of Pharmacy, Pharmaceutical Technology and Parasitology, Faculty of Pharmacy, University of Valencia, Burjassot-Valencia, Spain; [2]Laboratory of Biochemistry and Molecular Parasitology, Institute of Biotechnology, University of Granada, Granada, Spain and [3]Laboratorios Lokímica, Paterna, Valencia, Spain

Short Communication

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Corresponding author:
M. Teresa Galán-Puchades;
Email: mteresa.galan@uv.es

Abstract

We investigated parasitic zoonoses caused by protozoans and helminths in urban and peri-urban rat populations (*Rattus norvegicus* and *R. rattus*) in Spanish cities. Rats were trapped and then dissected to remove adult helminths, and the contents of the large intestine were retrieved for the study of parasitic forms. The Midi Parasep® solvent free (SF) technique was used to concentrate the parasites in the intestinal contents. Some of the rats studied (n = 8) were infected by the rat lungworm, *Angiostongylus cantonensis*, whose first stage larvae (L1) are shed in rat faeces. After the concentration technique, L1 larvae were found in the sediment of 6 of the 8 positive rats. The two negative sediment samples were due to the presence of either only adult females or, in addition to males, only young females in the lungs of the rats. In view of our results, Midi Parasep® SF turned out to be a simple, rapid, inexpensive, and sensitive method to detect nematode larvae, such as the L1 larvae of *A. cantonensis* (or *A. costaricensis*), in natural and experimentally infected rats.

Introduction

In the context of the "One Health" concept, our research group analysed the parasitic zoonoses present in urban and peri-urban rat populations. Specifically, we investigated both protozoan and helminthic zoonoses in Norway rats, *Rattus norvegicus*, and black rats, *R. rattus,* in Spanish cities (Galán-Puchades *et al.* 2018, 2019, 2021, 2022). In this context, the scarcity of surveys investigating zoonotic parasites of rats, particularly helminths, in cities in developed countries is rather surprising (Gliga *et al.* 2020).

We are currently studying rat populations in the city of Valencia in cooperation with the pest control company Laboratorios Lokímica, commissioned with the task by the Pest Control Section of the Health Service of Valencia City Council. As a result, among other helminth and protozoan zoonoses (unpublished data), the rat lungworm, *Angiostrongylus cantonensis* – the causative agent of eosinophilic meningitis in humans – was found in eight rats, i.e. five *R. norvegicus* and three *R. rattus* (Galán-Puchades *et al.* 2023). In the adult stages, the parasites live in the pulmonary arteries of rats. Eggs are shed by the females, mature and hatch in the lungs, and the first stage larvae (L1) are swallowed and finally appear in the faeces of the rats. These L1 larvae are infectious to snails and slugs (first intermediate hosts), wherein they develop into L3 larvae, becoming, in turn, infectious to both rats and other mammals including humans.

For the identification of parasitic forms in the intestine, the contents of the large intestine of the studied rats was collected and concentrated using the Midi Parasep® solvent free (SF) technique (Apacor Ltd., Wokingham, UK), which has the advantage of separating, thanks to its filters, large faecal debris and the fatty content of the sediment, without the addition of solvents (Tenorio-Abreu *et al.* 2012).

Our research aimed to test the efficacy of the Midi Parasep® SF technique in finding *Angiostrongylus cantonensis* L1 larvae in the faeces of the infected rats since, to our knowledge, this technique has never been used before to detect them.

Material and Methods

The trapped rats were preserved at -20 °C until parasitological study (Galán-Puchades *et al.* 2023). After thawing, rats were dissected to remove adult helminths, and the contents of the large intestines – including those of the eight infected by *Angiostrongylus cantonensis* – were collected, filtered, and concentrated by centrifugation (2500 rpm/5 min) in Midi Parasep® SF (Figure 1). Part of the concentrated sample, destined for DNA extraction for polymerase chain reaction

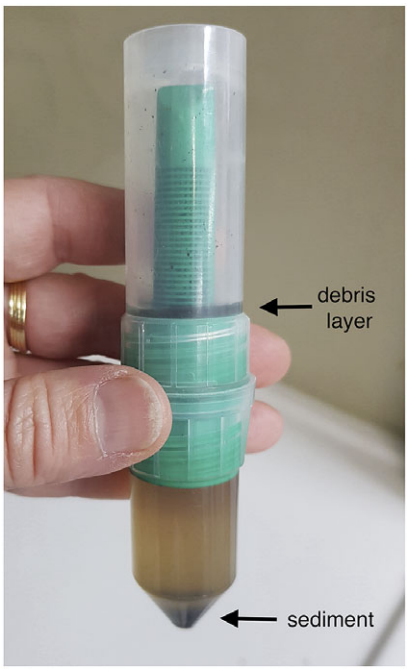

Figure 1. Midi Parasep® SF after the centrifugation process showing the layer of debris and the sediment.

testing for protozoan identification, was kept at -80 °C (Galán-Puchades *et al.* 2023). Another part was placed in 10% formalin for subsequent preparation of direct wet mounts that were observed under a microscope between slide and coverslip, with the aid of a drop of Lugol's iodine, to carry out the morphological identification of protozoan cysts, helminth eggs, and larvae.

Results

Angiostrongylus cantonensis L1 larvae were found in six of the eight infected rats (Figure 2). Apart from one rat that was only infected by *A. cantonensis*, the other seven were coinfected by other helminth parasites, either trematodes, cestodes, nematodes, or acanthocephalans. However, only *A. cantonensis* sheds larvae in faeces, while none of the other nematode species has been found do so.

The study of a single drop of sediment was sufficient to detect the presence of the larvae, regardless of the number of adult males and females present in the lungs, although, as expected, those rats harbouring a high number of adults showed the highest number of L1 larvae/drop (up to 75 larvae/drop).

The first stage larvae (n = 30) measured 272.25 µm (189.80 – 329.00) in length and 14.29 µm (12.65 – 17.71) in width. The larva was slender with a typical sharp posterior end (Figure 2 a–c).

In two of the rat sediment samples, we also found certain ovoid, thin-shelled, non-operculated, and transparent eggs measuring 55-60 x 30-31 µm, likely to correspond to *A. cantonensis* (Figure 2-c,d). The rats, in addition to the lungworm, were also infected with the intestinal cestode *Hymenolepis diminuta* (Figure 2b), the acanthocephalan *Moniliformis moniliformis,* and/or the hepatic nematode *Calodium hepaticum.* However, the morphology and size of the eggs found did not correspond to any of these other helminths, otherwise coinciding with those of *A. cantonensis* (Yousif & Ibrahim 1978). We found eggs both with the embryo inside (Figure 2c) and less developed eggs (Figure 2d).

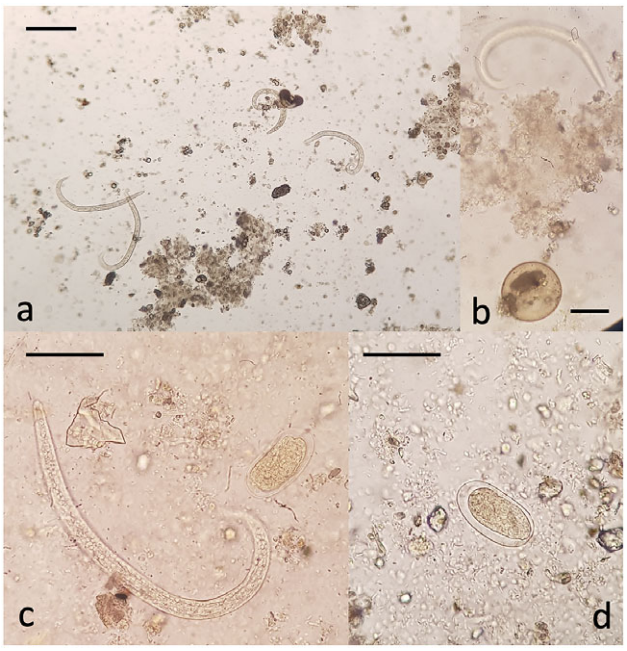

Figure 2. First stage larvae (L1) of *Angiostrongylus cantonensis* in the sediment of faeces of *Rattus* spp. (a) Four L1 larvae (scale bar: 150 µm); (b) A L1 larva and an egg of *Hymenolepis diminuta*; (c) A L1 larva and a worm-shaped egg of the rat lungworm; (d) A less developed egg of *A. cantonensis* in which the worm-like form is not yet visible (scale bars b, c, d: 50 µm).

Discussion and Conclusions

Regardless of the host under study, for the morphological identification of intestinal parasites as well as for those parasites whose resistant forms are eliminated by faeces – mainly liver trematodes or as in this case, lung parasites – the use of concentration techniques in faecal samples, prior to microscopic observation, improves sensitivity, since the excretion of parasitic forms to the exterior can sometimes be not only intermittent but scarce. Such a diagnostic technique should be economical, simple, rapid, and highly sensitive.

In this context, formalin-ethyl acetate, or formalin-ether concentration techniques (FECT) are standard methodologies frequently used to remove the faecal debris and concentrate the parasitic forms (cysts, oocysts, eggs, and larvae) by sedimentation. FECT requires the use of toxic solvent reagents (ethyl acetate/ether). In addition, these techniques require several centrifugation-decantation steps, with an average time consumption of 45 min for processing.

The Midi Parasep® SF technique has proved to be a simpler, less toxic, rapid, and sensitive concentration technique for parasites in faeces (Adugna *et al.* 2017; Tenorio-Abreu *et al.* 2013). In fact, the technique proved to be more sensitive than FECT in the detection of *Strongyloides stercoralis* larvae in human faeces (Kopolrat *et al.* 2022). In addition, once properly cleaned, the device can be reused, making it even more economical.

In terms of sensitivity, we detected two false-negative results in our study. However, the absence of L1 larvae in the sediment samples of these two infected rats was not the result of a failure of the technique. In one case, it was a consequence of the presence of only two females in the lungs, and in the other, it was due to the fact that, although males were present, all females were non-gravid. Both the presence of only females in most cases of dioecious helminths or when the males/females are still young are frequent causes of false negative results, regardless of the concentration technique used since neither eggs nor larvae appear in the faeces.

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Owing to this technique we also detected eggs consistent with those of *A. cantonensis*. Probably due to the cough induced by the presence of the parasites (adults, larvae, and eggs) in the lungs, not only larvae but also eggs can be swallowed and finally appear in faeces. To our knowledge, this is the first time that unhatched eggs of *A. cantonensis* have been found in rat faeces.

In view of our results, and acknowledging the limited number of samples, Midi Parasep® SF turned out to be a rapid, easily applicable, inexpensive, and sensitive technique for the detection of *A. cantonensis* (and could also apply to *A. costaricensis*) first stage larvae both in natural and experimentally infected rats.

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Conflict of interest. The authors declare none.

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