

BRIEF COMMUNICATION

PARASITOLOGICAL AND MOLECULAR DIAGNOSIS IN EXPERIMENTAL *Strongyloides venezuelensis* INFECTION

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SUMMARY

Strongyloides venezuelensis is a parasitic nematode of rats which is frequently used as a model to study human and animal strongyloidiasis. The aim of this study was to evaluate the correlation between parasitological and molecular diagnosis in *Strongyloides venezuelensis* infection. PCR assays were used to detect *S. venezuelensis* DNA in fecal samples obtained from experimentally infected *Rattus norvegicus*. The results showed a higher sensitivity of the PCR assay in detecting the infection compared to parasitological methods.

KEYWORDS: *Strongyloides venezuelensis*; Parasitological diagnosis; Molecular diagnosis.

Strongyloidiasis is a helminthic infection that affects 30-100 million people in the world, with a greater occurrence in areas located in tropical and subtropical regions⁹. This helminthiasis can occur with several clinical aspects: without symptoms, as a potentially fatal hyperinfection or as a disseminated infection³. Strongyloidiasis is difficult to diagnose because usually the parasite load is low and the larval output is irregular⁴, which leads to an underestimation of infection rates. The development and validation of more sensitive assays to detect light helminth infections are therefore required¹¹.

Strongyloides venezuelensis is a parasitic nematode of rats that has been used as a model to study the host-parasite relationships in human and/or animal infections, molecular aspects during infection, the efficacy of new therapies and many immunological events related to strongyloidiasis^{5,6}. This nematode has also been used to standardize new immunological techniques to optimize the diagnosis of human strongyloidiasis².

Polymerase chain reaction (PCR) has been considered a highly sensitive method to detect pathogens in light infections¹⁴. As DNA detection becomes increasingly utilized in the diagnosis of parasite infections, this method might have the potential to overcome the problems in diagnosing strongyloidiasis¹¹. The PCR assay could be a useful alternative to the commonly used parasitological method, offering an increase in the detection rate¹³.

In the present study, we evaluated the performance of molecular

diagnosis in parallel with parasitological techniques, using an experimental model of strongyloidiasis.

Rats were handled in compliance with the ethical guidelines adopted by the Comissão de Ética no Uso de Animais (CEUA) of the Instituto de Medicina Tropical da Universidade de São Paulo (IMT/USP). The experiments were conducted in accordance with animal ethics guidelines and were approved by the local Ethical Committee (protocol CPE-IMT 2011/126).

Five male *Rattus norvegicus* (Wistar) were infected subcutaneously with 2,000 third-stage infective larvae (L3) of *S. venezuelensis*. Fecal samples were collected 0, 1, 4, 6, 8, 11, 13, 15, 18, 20, 25, 29, 32, 36, 40, 53, 60 and 68 days after infection. Each fecal sample was divided into two aliquots: one was processed by parasitological methods and the other was stored at -20 °C, to be utilized for molecular diagnosis.

Parasitological diagnosis was performed by direct fecal examination and charcoal culture. For direct fecal examination, a suspension of 2 g of fecal sample in saline was used, followed by an optical microscopy examination of smears stained with lugol. To the charcoal culture, 10 g of fecal samples were mixed with distilled water and charcoal, and incubated at 28 °C for two days. After being concentrated by the RUGAI method¹⁰ it was analyzed using the optical microscope.

Approximately 200 mg of fecal samples were used for DNA extraction using the QIAamp DNA stool minikit (QIAGEN, Hilden,

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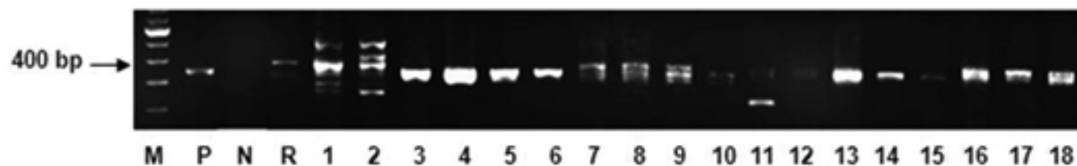


Fig. 1 - *Strongyloides venezuelensis* PCR amplification from fecal samples of infected rats. Lane M: 100 bp ladder (Life Technologies); PCR controls; Lane P: 340 bp (positive control, *S. venezuelensis* L3 DNA); Lane N: negative control (without DNA); Lane R: 420 bp (fecal sample of *Rattus norvegicus* no *S. venezuelensis* infection), 380 bp (corresponding to the *Syphacia muris*); Lanes 1-18: DNA fecal samples (collected 0, 1, 4, 6, 8, 11, 13, 15, 18, 20, 25, 29, 32, 36, 40, 53, 60 and 68 days after infection) 340 bp, 380 bp, 200 bp (unknown band) and 420 bp.

Germany), following the manufacturer's instructions. The QIAamp DNA mini kit (QIAGEN, Hilden, Germany) was used to extract DNA from *S. venezuelensis* L3 larvae, and then it was employed as a positive control in all PCRs. DNA was eluted with 100 µL AE buffer and quantified by nanoDrop ND-1000 UV-VIS spectrophotometer v.3.2.1 (NanoDrop Technologies, Wilmington, DE).

PCR reactions were performed using the genus primer pair (forward 5'-AAAGATTAAGCCATGCATG-3' and reverse 5'-GCCTGCTGCCTTCCTTGGA-3') to amplify a 340 bp target in the small subunit ribosomal RNA gene. MARRA *et al.*⁷ described this method as more sensitive to experimental infection by *S. venezuelensis* in Lewis rats. The reaction was carried out in a 50 µL volume containing 10 mM dNTPs, 20 µM of each primer, 1.5 mM MgCl₂, 50 mM KCl pH 8.4, 0.5 U of Platinum Taq DNA polymerase (Invitrogen by Life Technologies CA, USA) and 5 µL of DNA (100 ng). The cycling conditions comprised an initial denaturation step at 95 °C for five min, 40 cycles of 95 °C for 30 s (denaturation), 60 °C for 30 s (annealing) and 72 °C for 30 s (extension). PCR were performed in the Master cycler ep gradient S thermocycler (Eppendorf, Hamburg, Germany). The resulting amplification products were loaded on 2% agarose gel and submitted to electrophoresis in 1X TAE buffer.

Our results showed that parasitological diagnosis was positive for the first time on day 6 after infection, became negative on day 32 after infection in direct examination and on day 40 after infection in the charcoal culture (Table 1). These data were similar to those presented by NAKAI & AMARANTE⁸, which refer to the peak larval elimination on days 6 and 7 after infection. The higher sensitivity of culture techniques is therefore evident in comparison to conventional methods for the diagnosis of strongyloidiasis³.

S. venezuelensis DNA was detected for the first time on day 4 after infection (Table 1 and Fig. 1). A band of 380 bp was detected in fecal samples collected indicating the possible contamination of animals with *Syphacia muris*, a parasite often found in the gut of laboratory rodents. In some samples, unknown bands of 200 bp and 420 bp were detected. The appearance of several bands could have occurred because this primer amplified a ubiquitous region of the small ribosomal subunit for PCR amplification in mixed samples⁷. Moreover this primer was originally employed for analyzing species within the genus *Strongyloides*¹.

These results disclose a higher rate of positivity using PCR in fecal samples collected at the beginning and after 40 days post-infection, when the parasitological diagnostic methods did not show *S. venezuelensis* in fecal samples. This methodology could be applied in association with

Table 1
Results of parasitological and molecular diagnosis of *Strongyloides venezuelensis* infections in rats

| Days post-infection | Parasitological diagnosis | | Molecular diagnosis |
|---------------------|---------------------------|------------------|---------------------|
| | Direct fecal smears | Charcoal culture | |
| Day 0 | - | - | - |
| Day 4 | - | - | + |
| Day 6-29 | + | + | + |
| Day 32-40 | - | + | + |
| Day 42-68 | - | - | + |

+ Positive results, - Negative results.

fecal examination in epidemiological studies to improve the diagnosis of strongyloidiasis.

RESUMO

Diagnóstico parasitológico e molecular na infecção experimental por *Strongyloides venezuelensis*

Strongyloides venezuelensis é um nematódeo parasita de roedores frequentemente utilizado como modelo experimental da estrogiloidíase humana e animal. O objetivo deste estudo foi avaliar a correlação entre o diagnóstico parasitológico, efetuado por meio de exame direto e cultura das fezes em carvão e técnicas moleculares na infecção experimental por *S. venezuelensis*. A reação de PCR foi utilizada para detectar DNA de *S. venezuelensis* em amostras fecais obtidas de *Rattus norvegicus* infectados com este nematódeo. Os resultados demonstraram a maior sensibilidade da reação de PCR em detectar a infecção em relação aos métodos parasitológicos.

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