**Article / Artigo**

# DNA de *Strongyloides venezuelensis* é detectável por PCR a partir de fezes de ratos experimentalmente infectados

# *Strongyloides venezuelensis* DNA is detectable through PCR from feces of experimentally infected rats

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## Abstract

Experimental infections with *Strongyloides venezuelensis* are used as a model for studying human strongyloidiasis, mainly for testing new diagnostic tools. We evaluated the effectiveness of PCR for the detection of *S. venezuelensis* DNA in samples of blood, serum and feces from experimentally infected rats. *S. venezuelensis* DNA was amplified in fecal samples. There was no amplification observed in blood or serum samples. It is concluded that PCR can be used in fecal samples for the detection of *Strongyloides* infections, and can be applicable as a diagnostic test in strongyloidiasis.
**Key words:** molecular diagnosis, *Strongyloides*, Polymerase chain reaction, helminths.

## Resumo

Infecções experimentais por *Strongyloides venezuelensis* são utilizadas como modelo para o estudo da estrongiloidíase humana, principalmente para testar novas ferramentas diagnósticas. Avaliamos a eficácia da PCR para a detecção de DNA de *S. venezuelensis* em amostras de sangue, soro e fezes de ratos infectados experimentalmente. O DNA de *S. venezuelensis* foi amplificado nas amostras fecais. Não foi observado amplificação especifica nas amostras de sangue ou soro. Conclui-se que a PCR pode ser utilizada em amostras de fezes para a detecção da infecção por *Strongyloides*, e pode ser aplicável como um teste diagnóstico na estrongiloidíase.
**Palavras-chave:** Diagnóstico molecular, *Strongyloides*, reação em cadeia da polimerase, helmintos.

## INTRODUCTION

Parasitic nematodes, including *Strongyloides*, are important pathogens, capable of infecting both animals and humans [1, 2]. *Strongyloides stercoralis* species causes human strongyloidiasis, an important neglected parasitic disease. This helminthiasis has high prevalence in different parts of the world and is considered potentially fatal in cases of hyperinfection and/or disseminated disease [3].

The standard method used for the detection and diagnosis of *S. stercoralis* infection is the parasitological examination of fecal samples. However, these techniques are not appropriate due to their low sensitivity and high rates of false negative results [4]. Alternative diagnostic methods with higher sensitivity include serological techniques. Although serology is an excellent option with high sensitivity, some difficulties related to obtaining antigens and reactivity after treatment must be considered [5, 6].

To overcome these problems and facilitate the identification of intestinal parasites quickly, molecular techniques have been developed [6]. Conventional polymerase chain reaction (PCR) techniques and real-time PCR have been frequently used for the detection of *S. stercoralis* in fecal samples [7-9]. However, molecular techniques are rarely used in laboratory routine.

In this context, experimental models represent alternatives for the evaluation of potential diagnostic tools of human strongyloidiasis, mainly throughout the experimental infection [1, 2]. It has been shown that in experimental infections, infective larvae of *Strongyloides venezuelensis* travel the pulmonary pathway before establishing in the host duodenal mucosa, similarly to *S. stercoralis* [2]. Given the advantages of using PCR in the diagnosis of strongyloidiasis, our objective in the present study was to evaluate a PCR-based technique for the detection of *S. venezuelensis* DNA in feces, blood and serum samples from experimentally infected animals.

## MATERIALS AND METHODS

### *Experimental design*

This study was approved by the Ethical Committee from the Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo (protocol CPE-IMT 2012/152). Male Wistar rats (*Rattus norvegicus*), with 4-6 weeks of age and weighing 150-175g were subcutaneously infected with $4x10^3$ *S. venezuelensis* infective larvae (iL3) that were obtained from charcoal cultures of infected rat. Rats were divided into the experimental groups of 5, 8, 13, 21, 28, 35, 49 and 60 days post-infection (dpi). For infection, iL3 larvae were washed in 0.01 M phosphate-buffered saline (PBS), pH 7.2, and counted. The animals were kept in collective cages, in the amount of 3 animals/cage, separated according to the experimental groups, at a room temperature of approximately 25ºC, with 12 hours light/dark cycle, receiving ration and water *ad libitum*. Three animals that were not infected were used as negative controls.

At the end of the experimental time, the animals were with anesthetized with 20 µL ketamine 10% and 10 µL xylazine 2% intraperitoneally, cardiac puncture was performed and the animals were euthanized. An aliquot of the blood samples was directly stored at -20°C and the remainder were centrifuged to obtain the serum, which was stored at -20ºC for further analysis.

Throughout the experimental time, animal feces were collected. The dynamics of infection was determined by counting the number of eggs per gram of feces (EPG) using a modified McMaster method [10]. The EPG counting was performed in triplicate

on the daily pool of fecal samples. Results obtained were expressed as mean±SEM. The same procedures mentioned above were performed with control animals.

***DNA extraction and Polymerase chain reactions (PCR)***

For DNA extraction from feces, samples from three animals in each experimental group were pooled. Approximately one gram of feces sample was mixed with approximately 1 mL of 70% ethanol and shaken vigorously. The mixture was centrifuged and the pellet washed twice with PBS. DNA from fecal samples was extracted from the pellet by using the QIAamp DNA Stool Mini kit (Qiagen, Hilden, Germany).

For blood and serum samples, 200 µL of each sample were used to extract DNA, according to the QIAamp DNA mini kit (Qiagen), following the manufacturers´ instructions. The DNA was eluted using 100 µL of elution buffer and quantified in a NanoDrop ND-1000 UV-VIS spectrophotometer v.3.2.1 (NanoDrop Technologies, Wilmington, Delaware, USA).

PCR reactions were performed by using a primer pair (forward, 5'-AAAGATTAAGCCATGCATG-3' and reverse, 5'-GCCTGCTGCCTTCCTTGGA-3') designed to amplify a 340 base-pair (bp) fragment coding small ribosomal subunit *S. venezuelensis* DNA, as described by Marra et al. [11]. For control testing of extracted DNA, all samples without amplification were tested using universal primers (forward: 18SEUDIR 5′-TCTGCCCTAACTACTTTCGATGG-3′ and reverse: 18SEUINV 5′-TAATTTGGCCTGCGCCTG-3′) that amplify a 140-bp region of the eukaryotic 18S ribosomal RNA gene, as described by Fajardo et al. [12].

PCR reactions were performed in a 10 µL volume containing ~50 ng/µL of DNA, according Marques et al. [13]. The PCR products were loaded on a 2% agarose gel containing Sybr safe (Invitrogen™, Thermo Fisher Scientific Corporation, Waltham, USA) and visualized under ultraviolet light. Positive and negative controls (DNA from iL3 *S. venezuelensis* and PCR mix without DNA template, respectively) were included in each round of amplification. Each sample was tested in triplicate. In addition, DNA from adult worms of *Toxocara canis* and *Schistosoma mansoni*, was used as controls of PCR specificity.

## RESULTS

The egg count per gram of feces demonstrated experimental kinetics, with 5 dpi (13,800 ± 1,414), 8 dpi (63,100 ± 1,598), 13 dpi (19,350 ± 1,342), 21 dpi (5,883 ± 765), 28 dpi (6,600 ± 265) and 35 dpi (90 ± 36).

*S. venezuelensis* DNA was detected in feces samples throughout all the experimental infection times, especially from day 5 to 35 dpi and at lower staining intensity in agarose gel at 49 dpi. (Figure 1). There was no amplification of the 340bp fragment in the serum or blood samples. It is important to observe that the DNA obtained from all samples was submitted to PCR using a universal primer for eukaryotic 18S ribosomal subunit, showing specific amplification (~141bp) in all samples. In addition, there was no amplification of the target using DNA from adult worms of *T. canis* and *S. mansoni*, which reinforces the high specificity of PCR (data not shown).

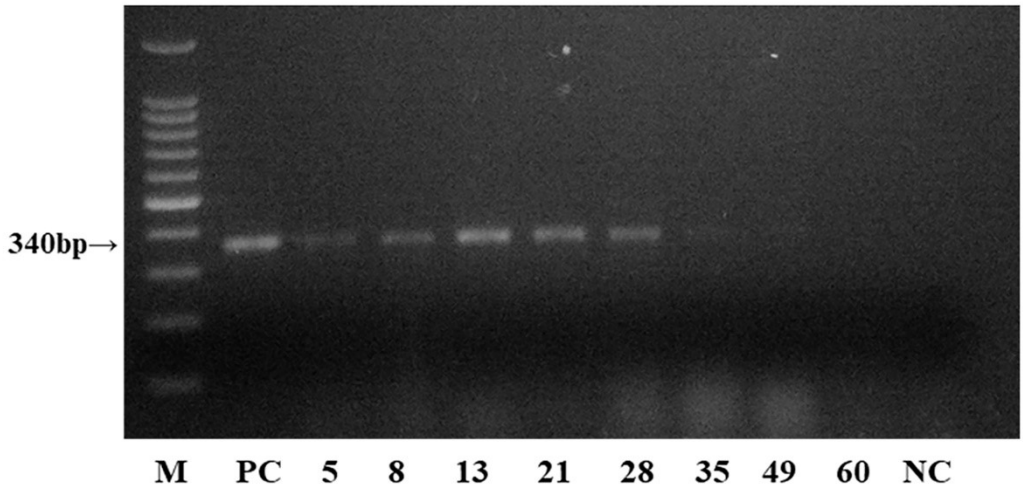


**Figure 1.** PCR products from fecal DNA of rats infected with *S. venezuelensis* (5, 8, 13, 21, 28, 35, 49 and 60 dpi.); positive control (PC - *S. venezuelensis* larvae DNA) and control/uninfected rats (NC). M (100 bp ladder).

## DISCUSSION

The optimization of strongyloidiasis diagnosis is a crucial step in order to prevent its serious clinical complications. Classic parasitological methods show an insufficient sensitivity, even for agar plate culture [14]. Serological tests have shown increased sensitivity in comparison to parasitological tests, however, immunological methods for *S. stercoralis* diagnosis remain challenging, mainly due to the difficulty of obtaining sufficient quantities of larvae for use in assays. Instead, heterologous antigens from other species (e.g., *Strongyloides ratti* and *Strongyloides venezuelensis*) are frequently used, yielding promising results [15,16]. For this purpose, methods for detecting the parasite in low quantities are increasingly indicated, such as molecular methods.

Molecular identification through DNA in fecal samples is now feasible and is becoming increasingly used for the diagnosis of strongyloidiasis [17,18]. In present study, PCR with fecal samples proved to be an efficient method for detection of *S. venezuelensis* during the experimental infection. This has been also shown by different reports using experimentally infected animals [8, 13, 19, 20]. In our findings we observed that the DNA amplification occurred up to 35 dpi, representing the egg count throughout the experimental infection. In addition, it can be seen on the agarose gel that the amplification of the target occurred up to 49 dpi, which can be especially interesting in human infection, given the elimination of larvae is small and irregular. These results reinforce the high positivity of PCR in relation to parasitological techniques (EPG), that is, it was possible to amplify the target fragment in the absence of eggs in the feces.

*S. venezuelensis* does not perform the autoinfection process, which is apparently a unique feature of human *S. stercoralis* infection [1]. Nevertheless, it would be expected that during the acute phase of experimental infection (especially from 5 to 13 dpi), when larval migration is possible, the presence of circulating DNA in serum and/or blood samples could be detected. Marra et al. [19] demonstrated the presence of *S.*

*venezuelensis* DNA in tissue samples from infected animals for up to 120 hours, which would be explained by any parts of the parasite left in the tissue during migration. However, we could not detect amplification of *S. venezuelensis* in DNA samples extracted from peripheral blood and serum. One possible explanation is that the present study performed PCR on DNA extracted from total peripheral blood, and the high background of host DNA in these samples may inhibit the amplification of the *S. venezuelensis* DNA. The use of specific protocols for cell-free DNA extraction or more accurate detection methods, such as real-time or digital droplet PCR, may yield different results. Of note, Javani et al. [20] reported a greater detection of *S. stercoralis* DNA in human serum when compared to standard parasitological tests, but we cannot forget the real possibility of autoinfection in human strongyloidiasis.

Recently, the LAMP technology was shown to be sensitive for the detection of *S. venezuelensis* DNA in an experimental infection model using Wistar rats in both stool and urine samples [21]. Even so, we reinforce the search for *Strongyloides* DNA in different samples as a fast, sensitive and specific alternative tool for the diagnosis of human strongyloidiasis, mainly in view of the possibility of hyperinfection and/or disseminated disease.

In conclusion, the present study demonstrates that the detection of *Strongyloides* DNA in fecal samples was effective for diagnosis, even in the late stage of *S. venezuelensis* experimental infection. Our findings may help in the diagnosis of human strongyloidiasis, especially in chronic infections common in endemic areas.

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**CONFLICT OF INTEREST**

None.

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