

## Detection of *Strongyloides stercoralis* in Tierralta, Colombia using four parasitological methods

### Detección de *Strongyloides stercoralis* en Tierralta, Colombia, utilizando cuatro métodos parasitológicos

MSc. Mario Javier Olivera Rivero,<sup>I</sup> MSc. Mayra Raciny Alemán,<sup>II</sup>  
MSc. Myriam Consuelo López,<sup>II</sup> MSc. Ligia Moncada,<sup>II</sup> MSc. Patricia Reyes  
Harker<sup>III</sup>

<sup>I</sup> Institute of Clinical Research, Faculty of Medicine, Universidad Nacional de Colombia, Bogotá, Colombia.

<sup>II</sup> Córdoba Microbiological and Biomedical Research Group, Universidad de Córdoba, Montería, Colombia.

<sup>III</sup> Public Health Department, Faculty of Medicine, Universidad Nacional de Colombia, Bogotá, Colombia.

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#### ABSTRACT

**Introduction:** soil-borne helminth *Strongyloides stercoralis* is one of the most neglected among neglected tropical diseases. A study was conducted of the presence of *S. stercoralis* in a village from the department of Córdoba, Colombia, with the purpose of comparing the effectiveness of several diagnostic methods.

**Methods:** stool samples from 262 persons were evaluated. Each sample was examined with four parasitological techniques: direct examination, agar plate culture (APC), the modified Baermann method, and the Harada-Mori technique.

**Results:** *S. stercoralis* was detected by at least one of the techniques in four of the 262 samples: the Harada-Mori technique detected 2 cases, APC 1 case and direct examination 1 case. The modified Baermann method did not detect any case. No significant differences were found when comparing the techniques.

**Conclusions:** results show that *S. stercoralis* is not endemic in the village of Córdoba, and that parasitological techniques should be used in combination to improve the quality of diagnosis.

**Key words:** *Strongyloides stercoralis*, diagnosis, laboratory techniques and procedures, diagnostic techniques and procedures.

## RESUMEN

**Introducción:** el helminto transmitido por el suelo, *Strongyloides stercoralis* es uno de los más olvidados entre las enfermedades tropicales desatendidas. Estudiamos la presencia de *S. stercoralis* en un pueblo en el departamento de Córdoba, Colombia, y evaluamos comparativamente el desempeño de diferentes métodos diagnósticos.

**Métodos:** se evaluaron muestras de heces tomadas de 262 personas; cada muestra fue examinada usando cuatro técnicas parasitológicas: examen directo, método de agar en placa (APC), la técnica de Baermann modificado y el método de Harada-Mori.

**Resultados:** *S. stercoralis* se detectó en cuatro de las 262 muestras evaluadas por al menos una de las técnicas utilizadas; el método de Harada-Mori detectó 2 casos, APC 1 caso y el examen directo 1 caso, mientras que la técnica de la Baermann modificado no detectó casos. No hubo diferencias significativas al comparar las técnicas.

**Conclusiones:** estos resultados permiten concluir que *S. stercoralis* no es endémico en el pueblo de Córdoba y que las técnicas parasitológicas deben ser combinadas para mejorar el diagnóstico.

**Palabras clave:** *Strongyloides stercoralis*, diagnóstico, técnicas y procedimientos de laboratorio, técnicas y procedimientos de diagnóstico.

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## INTRODUCTION

*Strongyloides stercoralis* is an intestinal parasite which has special biological characteristics; it can cause infection to persist, self-infection and the development of chronic disease.<sup>1</sup> It has been estimated that 30-100 million people are infected around the world by this geohelminth, especially in tropical and subtropical regions.<sup>2</sup> It often occurs in low socioeconomic level areas, where soil conditions and environmental humidity favour the parasite's development.<sup>3</sup> The consequences on health caused by *S. stercoralis* infection differ according to whether one is dealing with an immunocompromised or immunocompetent host, infections ranging from asymptomatic to chronic symptomatic ones. The outcome is often fatal.<sup>4,5</sup>

In South and Central-America, the range of infection rates in the communities varies from 1.0 % in Haiti, while in Colombia reports a prevalence of 30 % and Peru the infection rate is as high as 75.3 %.<sup>6</sup>

Several parasitological techniques have been used for detecting this parasite's larvae in faecal samples; culture methods would include Arakaki's agar,<sup>7</sup> plate culture method,<sup>8,9</sup> Baermann's larvae concentration method and its variations,<sup>10</sup> the Harada-Mori technique<sup>11</sup> and sediment concentration method.<sup>12</sup>

Given that examining faecal material samples using conventional techniques, such as direct fecal smear or Kato-Katz, are not very sensitive<sup>5,13</sup> and that there is no gold standard test for diagnosing Strongyloidiasis, then it could be thought that this parasite's prevalence has been underestimated to date.<sup>1</sup> *S. stercoralis* infection is one of the most difficult infections to diagnose.<sup>5</sup>

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It was thus proposed to make a search for *S. Stercoralis* and comparatively assessed the performance of four different parasitological techniques.

## METHODS

### *Stool samples*

This consistency study used stool samples collected from people living in Tierra alta. This municipality is located in the extreme Southwest of the Córdoba department, covering 5,079 km<sup>2</sup>, having both urban and rural populations and small Indian communities. The main economic activities are agriculture, cattle farming, logging and fishing. This is a region where the rural population lacks sewerage systems and drinking water, these being predisposing factors for intestinal parasitism.

Minimum inclusion criteria for the study involved being 2 to 60 years old, not having been deparasitised during the last three months and supplying a sufficient amount of stool sample for diagnosis by all the tests used. Clean Kraft paper and screw-topped jars were distributed to patients to obtain suitable and appropriate samples one day before collecting them. Instructions were given regarding how to take the sample.

The stool samples were sent to the Universidad de Córdoba's microbiological and biomedical research laboratory within three hours of having been collected for processing. The final suspensions were stored with formalin and then sent to the Universidad Nacional de Colombia's parasitology laboratory to be read and their final diagnosis, 15 days after their preparation.

### *Stool examination*

Each stool sample was examined by APC,<sup>8</sup> modified Baermann's technique,<sup>10</sup> the Harada-Mori method<sup>11</sup> and direct exam using saline solution.

Modified Baermann's technique involved placing 2 g of faecal material in a plastic-tipped 1,000 µL 16 x 100 mm test tube containing 8 mL 85 % physiological saline solution (tube 1); tube 1 was inverted and placed over tube 2 which was in a water bath containing 6 ml 85 % physiological saline solution. Once two hours had elapsed, tube 2 was centrifuged at 1,500 xg for 10 min and observed by light microscope.

The Harada-Mori method involved placing 2 g homogenised faecal material on a strip of filter paper, leaving the edges free. The sown strip was then placed in tube containing 5 mL sterile distilled water and incubated at 20 °C for 72 hours; 0.5 mL 10 % formalin was then added and the tube was centrifuged at 500 xg for 5 min. Sterile Petri dishes containing nutritive agar were used for culturing. They were incubated at 25 °C to 33 °C for 48 hours. The agar surface was then washed with 10 % formalin and centrifuged at 500 xg for 5 min and observed under a light microscope.

Trained personnel having experience in detecting *S. stercoralis* then participated for confirming the diagnosis.

The efficiency ratio (dividing the total number of cases of *Strongyloidiasis* detected by the number of cases proving positive by each technique) was determined to ascertain whether there were any significant differences between the techniques' effectiveness.

*Anti-helminth treatment and ethical considerations*

The current recommendations for research involving human beings were followed and the protocol was approved by the Universidad Nacional de Colombia's Medical Faculty's Ethics Committee. All the participants were informed about the study. Written consent was obtained from each participant when collecting their sample. Individuals having a positive result for *S. stercoralis* were given antihelminth treatment with ivermectin (200 µg/kg weight, single dose) at the end of the study. All study participants were given instructions about how to prevent and avoid *S. stercoralis* infection.

**RESULTS**

In total, 262 individuals fulfilled the inclusion criteria and provided a suitable amount of stool sample during the study period (41.2 % females and 58.8 % males).

*S. stercoralis* was present in 4 of the 262 samples analysed by at least one of the techniques used [Table](#); 3 cases were detected in males (16,17 and 55 years old) and 1 in a female (17 years old). The 4 infections diagnosed as being due to *S. stercoralis* were considered light.

**Table.** Number of cases of *S.stercoralis* detected in 262 stool samples using four diagnostic methods. Tierralta, Clombia, 2010

| Tecniq <u>e</u> used | Cases detected | Ratio of cases detected | Efficiency ratio |
|----------------------|----------------|-------------------------|------------------|
| Harada-Mori          | 2              | 2/4                     | 0,50             |
| Agar plate culture   | 1              | 1/4                     | 0,25             |
| Direct exam          | 2              | 1/4                     | 0,25             |
| Modified Baermann    | 0              | 0/4                     | 0,00             |

The efficiency ratio did not reveal a significant difference regarding the efficacy of the parasitological techniques used.

**DISCUSSION**

Few parasitological and/or epidemiological investigations have been related to *S. stercoralis* in Colombia.<sup>14,15</sup> Existing studies concerning diagnostic methods for *S.*

*stercoralis* have given conflicting results, thereby showing the lack of current knowledge about the functioning of different approaches to diagnosis in this field.

In the present study, infection was observed with *S. stercoralis* in 1.52 % of patients, a relatively low prevalence of infection compared with previous studies in Colombia with prevalence rates in Cali 14 % and Buenaventura 16 %.<sup>14</sup> These results also contrast with the prevalence found in northern Ghana 10.6 %<sup>16</sup> and 91.8 % in Gabon.<sup>6</sup> In South East Asia, considered another endemic region have reported infection rates of 17.5 % in Cambodia, Thailand 23.7 % and Lao PDR 26.2 %.<sup>6</sup>

In general, information about this parasitosis is scarce, and other research suggests a high underreported, because no studies focusing on *S. stercoralis*.<sup>6</sup>

It is likely that the frequency observed in this study was underestimated and such observation may be justified by the following points: first, the frequency with which larvae migrated from stool samples or remained on the agar surface could not be determined in the absence of a gold standard, larvae may not yet have reached tube 2 when the water was filtered in the modified Baermann technique and some larvae may not have descended into the water and may have remained on the strip of filter paper when using the Harada-Mori method;<sup>13</sup> second, parasites were not uniformly distributed in the stool samples;<sup>17</sup> third, delay in collecting human stool samples and their processing could have negatively influenced *S. Stercoralis* diagnosis sensitivity<sup>13</sup> and fourthly, these may have been chronic infections in which larvae were only present in very small amounts, thereby making their diagnosis more difficult.<sup>18</sup>

The stool samples used in this study were collected in compliance with the inclusion criteria of a line of research which was being conducted at the same time which may have imposed some limitations which could have influenced the results and which should be born in mind when interpreting the findings. Memory-type bias could have been introduced into the information as exposure variables were based on self-reporting; however, the influence of chance cannot be discarded from the results nor can the results be extrapolated to the rest of the population.

The diagnostic tests used in the present study detected cases similar to those reported by Kaminsky.<sup>19</sup> She compared three diagnostic methods and found that the Baermann method was most effective (detecting 7.7 % of cases), followed by agar plate culture and direct exam (6.5 % and 2.1 %, respectively). None of the three methods detected all the infected cases; all the techniques used detected different cases.

It was found that the Harada-Mori method detected more cases than the rest of the techniques used; such results agreed with those found by Mahdi<sup>20</sup> who reported this test's superiority (100 % sensitivity) compared to direct exam and the formalin ether concentration technique (FECT). However, the effectiveness found in Mandhi's study has not been observed by other researchers.<sup>21,22</sup> The Harada-Mori technique detected 29 % of cases when compared to direct exam, agar plate culture and FECT. Other studies have reported less than 60% case detection rate for this technique.<sup>8,23</sup>

Many studies have demonstrated that agar plate culture was a test having high sensitivity (higher than 90 %) for *S. stercoralis* diagnosis.<sup>7,21</sup> However, this technique only detected one case in the present study.

The Baermann method used in this study had been modified.<sup>10</sup> The results observed by the aforementioned researchers showed that this modified technique was equally as sensitive as the standard Baermann test; however, no cases were detected by the modified Baermann method in the present study.

One of the cases was detected by the direct exam method using saline solution and not by any other test; this could have been attributed to several factors such as non-uniform parasite distribution in stool samples and intermittent laying of *S. stercoralis* larvae.

Even though the results obtained in this study did not show a significant difference between the efficacies of the parasitological tests used, it did provide evidence that using several diagnostic methods increased detection regarding the number of cases caused by this nematode. It is thus considered that using different techniques can increase sensitivity when diagnosing *S. stercoralis* infection.

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*Mario Javier Olivera Rivero*. Carrera 30 No. 30 - 45, Universidad Nacional de Colombia, Facultad de Medicina. Edificio 471, Oficina 302, Bogotá, D.C., Colombia.  
Tel.: +57 1 3165000 ext. 15033, fax: +57 1 3165405.  
Correo electrónico: [mjoliverar@unal.edu.co](mailto:mjoliverar@unal.edu.co), [molivera@ins.gov.co](mailto:molivera@ins.gov.co)