Prevalence of intestinal parasites in children of an invasion community in a municipality of Colombia

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Abstract—According to World Health Organization, (WHO), intestinal parasites are present in one in four people, estimated that these agents constitute the 15% burden of disease in the Americas caused by neglected infectious diseases. To determine the prevalence of intestinal parasites in the “La Carrilera” invasion community Tuluá-Valle del Cauca. Cross-sectional study, the included boys and girls were part of an induced demand program using a sample of 150 children. The parasitological analysis was performed with saline solution, Lugol, formalin-ether concentration and Graham's method. For the assessment of the nutritional status, standards of the WHO were taken as reference with the use and interpretation of the Anthro and Anthro Plus software. The overall prevalence of enteroparasites 93.3%. The cases of monoinfection and polyparasitism were higher percentages of pathogenic parasites Entamoeba Complex 93.3%, and the Pan American Health Organization, estimates that IPs constitute 15% of the burden of disease in the Americas [4], Likewise, intestinal parasitism is a medico-social issue that affects not only the Third World countries, but also, the more developed ones [5], its importance is derived both by its high relative frequencies of occurrence and by the characteristics of the population that currently suffers. IPs are considered by the United Nations as markers of underdevelopment [6], increasing the socio-economic depression of the population, perpetuating risk factors in developing countries, and also constitute a public health problem, with rates of prevalence up to 90%, a figure that intensifies as the socioeconomic level of affected populations deteriorates [2,7].

According to estimates of the World Health Organization (WHO), PIs are present in one in four people, represent 45% of deaths in poor countries in Asia and Africa, 63% of deaths in children aged 0 to 4 years [6] In addition to being endemic in developing countries, they are an important cause of morbidity and mortality in many countries and affect one billion people worldwide [8] with 155,000 deaths per year [9] and the Pan American Health Organization, estimates that IPs constitute 15% of the burden of disease in the Americas caused by neglected infectious diseases [10]. With such figures, it is necessary to investigate the type of parasites that affect the population in general [11]. Colombia is no stranger to this reality, being one of the 17 countries with the highest number of preschool and school children at risk of intestinal infection IP [12]. In the Pacific

Keywords— Intestinal parasites, malnutrition, community participation, children, learning, vulnerable, risk factors.

I. INTRODUCTION

Intestinal parasites (IP) or enteroparasites are classified into two groups: protozoa and specifically geohelminths helmiths, disease-producing widely scattered throughout the world, in Latin America and the Caribbean (LAC), causing "intestinal parasitic infections" (IPI) collective term which includes infections caused by these groups [1].

Children of preschool age (1 to 4 years, 11 months to 29 days) and schoolchildren (5 to 14 years, 11 months to 29 days) are among the highest risk age groups for IPI [2] due to the presence of factors such as, the immature immune system, malnutrition, poor hygiene, geophagy, close contact with pets, water intake inappropriate sources and contact with dirty objects, which are introduced to the mouth favoring transmission of intestinal parasites [3], which makes them more vulnerable. In them intestinal helminths affect their nutrition, growth, physical development, learning, school performance with consequences that may persist throughout their lives, affecting their labor productivity and consequently their ability to generate income. Intestinal protozoa are frequent causative agents of acute diarrheal disease, digestive disorders, malabsorption, and malnutrition, loss of appetite, intestinal mucosal lesions and extraintestinal manifestations.

It is estimated that there are more cases of intestinal parasite infections than cases of HIV / AIDS, tuberculosis and malaria combined. In fact, scientists have estimated that children lose an average of 3.75 IQ points for each intestinal parasite infection. Some experts have called intestinal parasites "the most common infections among the poor people of the Americas" [4].

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region, specifically in Tuluá (Valle del Cauca); space-population where the present investigation was carried out, there are no known studies that relate the prevalence of enteroparasites. However, in nearby regions such as the city of Cali [13] and regions nationwide belonging to the departments of Caquetá [14], Boyacá [15], Atlántico, Risaralda [16] high prevalences of protozoa are reported. Despite its high prevalence, few efforts have been made to characterize specifically the profiles of intestinal polyparasitism, referencing the first study that is done in the country in a community of the Colombian Amazon [17].

Data on the prevalence of intestinal parasitic infections (IPI, for its acronym in Spanish) in children in Colombia are limited, especially for the category of preschool children, which impedes public health efforts to target the appropriate subpopulation, these data being essential to identify local vulnerabilities and adapt public health programs [1].

In Colombia according to the National Survey of intestinal parasitism in the 2012-2014 school population, it was found that 29.62% of the studied population was infected with some geohelminth, Trichuris trichiura the most prevalent (18.4%) followed by Ascaris lumbricoides (11.3%). Another intestinal helminth, such as Enterobius vermicularis with prevalence of 6.4%. The most prevalent enteroparasite was Blastocystis. (60.3%), followed by Entamoeba Complex (48%) and Giardia duodenalis (37.3%). In all the biogeographic regions, different commensal protozoa were found, an important fact since it indicates exposure to fecal-oral contamination, however, the infected individuals may also have been exposed to organisms that can cause disease. Therefore, some authors consider the presence of non-pathogenic intestinal protozoa as an epidemiological and non-clinical variable.

In recent years, parasitic diseases have changed their classical course with the improvement of sanitary measures. Macroscopic parasites have been eradicated as a cause of intestinal disease and protozoa have been increasing significantly. In addition, other parasitic elements called emerging parasites commonly investigated such as Blastocystis have emerged, which have produced a conceptual change of practical application in the epidemiology and pathogenesis, clinical syndrome and treatment of diseases [18-20], however, this persists This is why it is essential to mold it empirically, above all, in areas where the vulnerable population lives [16].

From the above it can be inferred, then, that the frequency of occurrence of IPs is associated with poverty, thus being a marker of development, representing a heavy but little recognized burden for poor children and their families, as well as for communities, the industry, and the economic development of developing countries [12]. For LAC this situation is problematic because the occurrence of the problem has remained unchanged for more than 60 years.

The population and, within it, the community with which they shared academic experiences and social development, was found in the department of Valle del Cauca -specifically in the municipality of Tuluá. – Invasion Community can be classified as highly vulnerable, according to the high proportion of dysfunctional families with more than three unsatisfied basic needs. There, the Unidad Central Del Valle del Cauca (UCEVA, for its acronym in Spanish) in cooperation with the Municipality of Tuluá, headed by the Ministry of Health and the Municipal Hospital Rubén Cruz Velez, carry out activities aimed at the construction of capacities for self-management of problems and the development of social value. In the present study, it was proposed to advance an active search of potential IP cases through microscopic stool analysis using the Ritchie technique, rectal smear by Graham's method, assessment of nutritional status and completion of a survey about clinical conditions. In children from 1 to 14 years of age.

In this context, the objective of this study was to determine the prevalence of intestinal parasites in the “La Carrilera” invasion community, Tuluá-Valle del Cauca.

II. MATERIALES AND METHODS

The present study is descriptive transversal. It was carried out in the “La Carrilera “invasion community of the municipality of Tuluá (Valle del Cauca, Colombia), accompanied by the Unidad Central Del Valle del Cauca, (UCEVA); between the years 2015 to 2017.

Sampling was non-probabilistic at convenience, in such a way that the children included in the study were part of a program of induced demand and active community search (BAC) of this population at risk, which was performed by the UCEVA, as an integral part of its academic training practices in the Collective Interventions Plan, and that were framed in Law 1438 defined by the Ministry of Health and Social Protection and whose purpose is the strengthening of the General System of Social Security in Health through a model of provision of the public health service, which within the framework of the strategy Primary Health Care allows coordinated action by the State, institutions and society for the improvement of health and creation in a healthy and healthy environment, which provides services of greater quality, inclusive and equitable, where the center and objective of all efforts are the residents in the country “and through the resolution 0518 of 2015 “Whereby provisions are issued in relation to Public Health Management and guidelines are established for the execution, monitoring and evaluation of the Public Health Plan for Collective Interventions - (PIC,for its acronym in Spanish).

In this investigation, a total of 150 participating children were included voluntarily in the study, after completing the informed consent by their mother, family member or legal representative and informed consent in those children who were older than 5. Years, a survey referring to the clinical conditions, hygienic habits, and anthropometric parameters was applied. We excluded children with antiparasitic treatment performed in the last 6 months or during the study, or those who were under 2 years old or older than 10 and / or did not reside in the aforementioned community.

Previously contacted community leaders by teachers and students, a meeting was scheduled with assistance from mothers, parents or legal representative, in which detailed
information was given on the scope of the project and educational session on intestinal parasitism with which, they were invited to participate in the study, each procedure was clearly explained and then the work was categorized as minimal risk research.

To obtain the samples, a bottle for the storage of fecal material was supplied, these specimens were refrigerated. The samples were processed in the Microbiology Laboratory of the UCEVA; the method of concentration of formalin ether was performed on each coprological [11], as well as direct fecal smear with saline and iodine solution [21]. The analysis of the samples was only performed microscopically.

For the collection of the samples a bottle for the storage of the fecal material was supplied, said specimens were refrigerated. The samples were processed in the Microbiology Laboratory of the UCEVA; each of the samples was processed by 2 different parasitological techniques: direct coprological with saline and iodine and coprological solution by concentration of formalin ether [21] the analysis of the samples was performed microscopically.

Children who presented with anal itching were voluntarily submitted -with informed consent completed by their mother, family member or legal representative- to a sampling of the perianal region in the morning hours and without previous routine cleaning. (three times), for the diagnosis of Enterobius vermicularis by means of the Graham technique or technique of the adhesive tape according to reference method to demonstrate the presence of eggs with their typical D shape with the help of a microscope according to Botero's specifications, et to [21].

For the assessment of nutritional status, the weight was taken using a medical grade digital scale (RICE LAKE - 440HH, precision 1g). The height was measured with a wooden stadiometer with moving part and measurements in centimeters with an accuracy of one millimeter. The indicators were calculated: weight for age (W / A), height for age (H / A), weight for height (W / S) and BMI. The standards of the World Health Organization (WHO) and the Pan American Health Organization (PAHO) were taken as reference through the use and interpretation of the Anthro software for children under 5 years of age and Anthro Plus for those over 5 years of age [22].

The study was endorsed by the Ethics Committee of the Faculty of Health Sciences and the Vice-Rector for Research of the UCEVA, the study was classified as a minimum risk. The identity of the study subjects is kept confidential, which is recorded in the institutional files.

The anthropometric parameters were classified using the Anthro software of WHO /PAHO, the data obtained in the application of the surveys and the information of the intestinal parasites found were organized in electronic tables of the Excel program (Microsoft XP 20013). The variables were summarized according to their nature-means for continuous variables; proportions and ratios of proportion for categorical variables. The analysis was developed through the SPSS program version 23 (SPSS Inc., USA).

### III. RESULTS

A total of 150 children participated in the study, shown in table 1, in the demographic description. The predominance of females was 55.3% (83/150). The average age was 5.61 years, with a minimum age of 1 year and a maximum of 10 years. The distribution of the age groups was done in the following way to guide the analysis according to the current regulations for childhood policies in Colombia: the first one of children from 1 to 5 years old and the second one from 6 to 10 years. The predominant group was that of children from 6 to 10 years of age with 30.56%.

Within this information where the ages range from 1 to 10 years; 45.3% (n = 68) with age between 1 to 5 years and 54.7% (n = 82) with age between 6 to 10 years. 55.3% (n = 83) were girls and 44.7% (n = 67) were boys. Of the population of children, they reported abdominal pain (78.7%), anal itching (52.7%), diarrhea (58%), hand washing before eating 52.7%, walking barefoot 67.3%, and eating nails 40%.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>%</th>
<th>IC 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to 5 years</td>
<td>68</td>
<td>45.3</td>
<td>36.7</td>
</tr>
<tr>
<td>6 to 10 years</td>
<td>82</td>
<td>54.7</td>
<td>46.7</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>83</td>
<td>55.3</td>
<td>46.7</td>
</tr>
<tr>
<td>Male</td>
<td>67</td>
<td>44.7</td>
<td>36.7</td>
</tr>
<tr>
<td><strong>Symptom</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequent diarrhea</td>
<td>87</td>
<td>58.0</td>
<td>50.0</td>
</tr>
<tr>
<td><strong>Risk factor's</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anal itching</td>
<td>79</td>
<td>52.7</td>
<td>44.7</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>118</td>
<td>78.7</td>
<td>72.0</td>
</tr>
<tr>
<td>Washing hands before eating food</td>
<td>79</td>
<td>52.7</td>
<td>44.7</td>
</tr>
<tr>
<td>Walk barefoot</td>
<td>101</td>
<td>67.3</td>
<td>60.0</td>
</tr>
<tr>
<td>Bite nails</td>
<td>60</td>
<td>40.0</td>
<td>32.7</td>
</tr>
</tbody>
</table>

Table 2, the general prevalence of enteroparasites was 93.3%, according to the type of infection in relation to age. Among the pathogenic parasites in both groups, Blastocystis and Giardia duodenalis predominated. In the group comprised of 6 to 10 years, higher percentages were found both in the finding of pathogenic and nonpathogenic parasites, Entamoeba Complex 19.5% (n = 16), Ascaris lumbricoides 15.9% (n = 13), Enterobius vermicularis 15.9% (n = 13), Trichuris trichiura 6.1% (n = 5) and Iodamoeba bütschlii 18% (n = 22), Entamoeba coli 24.4% (n = 20), Endolimax nana 30.5% (n
Infection due to:
- a parasite 57 38
- Multiparasite 83 55
and by age range

In the age group from 1 to 5 years, *Giardia duodenalis* predominated 23.5% (n = 16) as pathogenic protozoan, as non-pathogenic, *Endolimax nana* 26.5% (n = 18) predominated and was predominant as helminth *Enterobius vermicularis* 11.8% (N = 8). There were 57 cases of monoinfection (38%) and 83 cases of polyparasitism (55.3%) due to exclusive pathogens.

In relation to risk factors, symptoms and presence of parasites, there were 57 cases (38%) of monoinfection and 83 (55.3%) poliparasitism. In the total of the subjects the pathogenic parasite that predominated was *Blastocystis* with 43.3% (n = 65) and the nonpathogenic parasite that predominated was *Endolimax nana* with 38.6% (N = 57).

No statistically significant associations were found between parasitic infection (presence of any of the parasites) and the risk factors analyzed. In the analysis of the infection of each parasite individually and the risk factors and symptoms were found the following associations statistically significant (p <0.05): it was found associated to bite nails with *Giardia duodenalis* infection (OR: 2.042, p: 0.048) and *Trichuris trichiura* (OR: 8.091, p: 0.038), barefoot walking associated with *Blastocystis* infection (OR: 2.550, p: 0.011), frequent diarrhea associated with *Blastocystis* (OR: 2.938, p: 0.002) and *Enterobius vermiculares* (OR: 3.582, p: 0.022), anal pruritus (OR: 3.352, p: 0.020) and abdominal pain (OR: 6.327, p: 0.046) associated with *Enterobius vermiculares*.

According to the nutritional assessment in the two age groups in relation to the indicator weight for height (W / H), it was found that 57.35% (n = 86) of children had adequate weight for height, 7, 35% (n = 11) were overweight, 2.94% (n = 4) obesity, 29.41% (n = 44) some risk of acute malnutrition and 2.94% (n = 4) malnutrition.

In weight for age (W / A), 41.11% (n = 6) of the children were eutrophic, 47.05% (n = 71) were overweight or obese. With regard to malnutrition, 8.81% (n = 13) of children at risk of global malnutrition were found.

The Body Mass Index (BMI) indicator showed that 45.58% (n = 69) of the children were eutrophic, 39.7% (n = 60) at risk of malnutrition, 13.2% (n = 20) with Undernourishment 2.9% (n = 4) with overweight.

For the group older than 5 years, the BMI indicator was taken into account, which showed that 53.65% (n = 80) of the children were eutrophic, 35.36% (n = 53) with overweight, 1.21 % (n = 2) with obesity and 9.75% (n = 15) with risk of malnutrition.

### Table 2. Prevalence of parasites in the entire population

<table>
<thead>
<tr>
<th>Infection</th>
<th>Total n</th>
<th>%</th>
<th>1 to 5 years n</th>
<th>%</th>
<th>6 to 10 years n</th>
<th>%</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entamoeba Complex</td>
<td>20</td>
<td>13</td>
<td>4</td>
<td>5.9</td>
<td>16</td>
<td>19.5</td>
<td>0.016</td>
</tr>
<tr>
<td>Giardia duodenalis</td>
<td>44</td>
<td>29</td>
<td>16</td>
<td>23.5</td>
<td>28</td>
<td>34.1</td>
<td>0.207</td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td>15</td>
<td>10</td>
<td>2</td>
<td>2.9</td>
<td>13</td>
<td>15.9</td>
<td>0.012</td>
</tr>
<tr>
<td>Blastocystis</td>
<td>65</td>
<td>43</td>
<td>32</td>
<td>47.1</td>
<td>33</td>
<td>40.2</td>
<td>0.414</td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>6</td>
<td>4.0</td>
<td>1</td>
<td>1.5</td>
<td>5</td>
<td>6.1</td>
<td>0.222</td>
</tr>
<tr>
<td>Enterobius vermicularis</td>
<td>21</td>
<td>14</td>
<td>8</td>
<td>11.8</td>
<td>13</td>
<td>15.9</td>
<td>0.637</td>
</tr>
<tr>
<td>Iodamoeba bütschlii</td>
<td>23</td>
<td>15</td>
<td>5</td>
<td>7.4</td>
<td>18</td>
<td>22</td>
<td>0.021</td>
</tr>
<tr>
<td>Entamoeba coli</td>
<td>29</td>
<td>19</td>
<td>9</td>
<td>13.2</td>
<td>20</td>
<td>24.4</td>
<td>0.099</td>
</tr>
<tr>
<td>Endolimax nana</td>
<td>57</td>
<td>38</td>
<td>18</td>
<td>26.5</td>
<td>25</td>
<td>30.5</td>
<td>0.717</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The prevalence of parasites was higher than the global prevalence of intestinal parasites reported in the National Survey of parasitism in the school population, Colombia, 2012-2014 and that reported by the Pan American Health Organization.

Although the percentage of parasites found in the study is high, other studies carried out in South American countries, in children, report prevalences of parasites with frequencies ranging from 67 to 87% [23].

In Argentina, *Blastocystis* has been reported as one of the most frequent intestinal parasites in children with values between 23% and 51%, which indicates that it could be considered emergent in this country (24). In Veracruz-Mexico, a study conducted on 100 schoolchildren between 6 and 14 years of age, collected fecal samples processed by direct technique and by concentration, reported 80% prevalence [18].

The prevalence found in this study was (93.3%). In a study
carried out in children in Argentina [7], a prevalence of 69.2% was observed. In Honduras (2014) [9] they conducted research on intestinal parasitism and anemic syndrome in preschool and school children with a prevalence of 61%.

In another investigation carried out by Roberto [25] in Cuba 51.4% of the children were infected by some species of PI and of them more than half were parasitized where Blastocystis and Giardia duodenalis were the most notified parasitic species. In contrast to the results of this research where the protozoan, specifically the Giardia duodenalis and the enteric chromatin Blastocystis, also predominated in Saudi Arabia [26], in a retrospective study they determined intestinal parasites and found in Asia the highest prevalence 59.8% and the most common parasite Blastocystis with 78.9%, followed by Giardia duodenalis. In the Northeast Region of Brazil, [27] intestinal parasitic infections in preschool children where almost 30% of children were infected with more than one intestinal parasite and the most common protozoan Giardia duodenalis parasite.

Study in Medellin-Colombia, [28] in a marginal community on frequency of intestinal parasites, the overall frequency of parasites, according to the examination by concentration, was 74.4%; the evaluation was made for general parasitism and for the protozoan, helminth, both commensal and pathogenic categories, with high frequency with method and results similar to those found in the study.

In Dosquebradas Colombia [16], in research on the prevalence of intestinal parasites in 258 children from two communities with an average age of 4.8 years and a prevalence of 37.2% reported Blastocystis as the most frequent parasite.

Study in Caquetá-Colombia [14], in intestinal parasitosis and risk factors in children from subnormal settlements, with prevalence of 90% and poliparasitism of 53%.

As in Boyacá-Colombia by [29], in a study on the prevalence of intestinal parasites and risk factors in 50 schoolchildren between 7 and 12 years of age, they collected fecal samples processed by direct technique and reported a prevalence of 96% where the most frequent parasites were Blastocystis and Giardia intestinalis. In another study in the Colombian Amazon, [17] profiles of poliparasitism in 300 children from one to 15 years old were 84%, reporting Blastocystis and Giardia duodenalis as more frequent parasites.

In relation to the BMI indicator in the study, 2.9% were found to be malnourished and 39.7% were at risk of malnutrition; the same population differed in reported data; in Western Africa, undernutrition 29.4% [11] Angola, Portugal population prevalence study of 16 schools it was higher than 10% with severe malnutrition, [30]. The association of parasites with nutritional status in children under 5 was determined with 29.41% risk of malnutrition, 2.94% malnutrition, 7.35% overweight and for the group over 5 years with 9.75% with risk of malnutrition, 35.36% with overweight and 1.21% with obesity, this comparing with other studies there are similar figures as the one carried out in Brazil by Pires et al [8].

Regarding the living conditions of the children of the community studied, it is important to highlight the precarious conditions in which their community lives, where there were significant associations between parasitosis, frequent diarrhea, anal itching, abdominal pain, hand washing before ingesting food, walking barefoot and eating nails, as well as dwellings with dirt floors, lack of access to drinking water, poor hygiene habits such as washing hands after going to the bathroom and before consuming food risk factors that allow transmission of the infection.

Although in this study water consumption was not a variable studied, it is worth mentioning that the total population consumes water from a stream belonging to the Tuluá River, which is transported in unhygienic containers.

The high percentage of infection by Blastocystis in the subjects, is associated with the poor conditions of hygienic habits, precarious conditions of environmental sanitation, elimination of garbage, lack of vaccination of pets, lack of latrines and adequate drainage which interferes in the dissemination of parasites by vectors, a factor that could have influenced the high prevalence obtained.

The high percentage of poliparasitism found 55.3%, the decrease in BMI with risk of malnutrition and malnutrition are factors that reflect the poor sanitary conditions in the community and if non-pathogenic parasites are also found, such as Iodamoeba bütschlii 15.3%, Entamoeba coli 19.3% Endolimax nana 38.6%, considered commensals and whose finding is an indicator of faecal contamination of food including water, which implies a significant increase with the nutritional deterioration of the subjects under study.

100% of the children were not assigned to growth and development controls, or to a monitoring program of anthropometric indicators and psychosocial development of early childhood, and second childhood, and of the young man who is currently the policy of the Colombian state.

Among the limitations of the study, many children were detected who did not want to participate in the research, nor is it possible to eliminate the conglomeration effect of the children, for which information bias may exist.

Some strengths of this research include the application of previous treatment results, the goal of coverage of the guidelines of the World Health Organization [31] was taken into account, for which two days of antiparasitic administration were carried out to the total of the population, in cooperation with the Municipality of Tuluá headed by the
Ministry of Health, the Municipal Hospital Rubén Cruz Vélez and the research team, in addition on the part of the medical team, dentistry and nurses were made cytologies, revision and dental treatments and immunization to the general population.

V. CONCLUSION

A high prevalence was found (93.3%), surpassing that found in other studies in children in the same age group, with a higher percentage of infections by protozoa than by helmints, highlighting the chromatist Blastocystis, an emerging enteric protist which has been generating a conceptual change of practical application in the epidemiology and pathogenesis, clinical syndrome and treatment at this level, which is why it is important to approach this population with intervention at the educational level to promote awareness of hygienic habits to improve risk factors.

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AUTHOR’S CONTRIBUTIONS

Giraldo-Ospina B, conceived and participated in the study design, coordinated the research, was responsible for laboratory work and participated in the analysis, discussion and final reporting. Fontal-Vargas PA, participated in the study design, was responsible for sample collection, and final reporting. Giraldo-Ospina B, conceived and participated in the study design, was responsible for sample collection, laboratory work and participated in the analysis, discussion and final reporting. Flórez-Salazar DE, participated in the analysis, discussion and final reporting. Gómez-C were responsible for samples collection final participated in the analysis, discussion and final reporting. and approved the version finally submitted.

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