***Enterobius vermicularis* in children at daycare centers**

**Título resumido em inglês:** Enterobiasis in Preschoolers

***Enterobius vermicularis* em crianças de creches**

**ABSTRACT**

**Introduction:** *Enterobius vermicularis* is a common parasite in children and its main symptom is anal itching. It is easily transmitted by directly anal-oral route, associated with unconsolidated hygiene habits. The frequency of enterobiasis is underestimated due to the use of inappropriate techniques. Based on this context, the aim of this study was to evaluate the frequency of enterobiasis in children attending daycare centers in Niterói, RJ, Brazil, using different parasitological techniques, and to correlate its frequency with socioeconomic factors and clinical manifestations. **Methods:** Children from three community daycare centers were asked to fill out a questionnaire and collect perianal region material, by means of the Graham technique, and total feces, to be processed using the techniques of Faust *et al*., Ritchie modified and fine-mesh screening. **Results:** *Enterobius vermicularis* was detected, exclusively by Graham technique, in 12% (11/92) of the children. The age of four to six years was a risk factor for acquiring enterobiasis (OR = 1.256). There were no associations between positivity for *E. vermicularis* and family income, number of children in the house or the parents’ education level, among other variables. Use of two slides for the Graham technique showed substantial/excellent agreement, which indicates that this is an appropriate strategy for diagnosing enterobiasis, thus resulting in lower costs and less discomfort for children and their guardians. **Conclusion:** *E. vermicularis* was the most common parasite among children in Niterói, which indicates the importance of including the Graham technique in studies on the prevalence of intestinal parasites.

**Keywords:** Enterobiasis. Diagnosis. Preschoolers. Frequency. Graham Technique.

**RESUMO**

**Introdução:** *Enterobius vermicularis* é um parasito comum em crianças e seu principal sintoma é a coceira anal. É facilmente transmitida por via diretamente anal-oral, associada a hábitos de higiene não consolidados. A frequência da enterobiose é subestimada devido ao uso de técnicas inadequadas. Com base nesse contexto, o objetivo deste estudo foi avaliar a frequência de enterobiose em crianças frequentadoras de creches de Niterói, RJ, Brasil, por meio de diferentes técnicas parasitológicas, e correlacionar sua frequência com fatores socioeconômicos e manifestações clínicas. **Métodos:** Crianças de três creches comunitárias foram solicitadas a preencher um questionário e coletar material da região perianal, por meio da técnica de Graham, e fezes totais, para serem processadas pelas técnicas de Faust *et al*., Ritchie modificada e tamisação em malha fina. **Resultados:** *Enterobius vermicularis* foi detectado, exclusivamente pela técnica de Graham, em 12% (11/92) das crianças. A idade de quatro a seis anos foi fator de risco para aquisição de enterobiose (OR = 1,256). Não houve associações entre positividade por *E. vermicularis* e renda familiar, número de filhos na casa ou escolaridade dos pais, entre outras variáveis. A utilização de duas lâminas para a técnica de Graham apresentou concordância substancial/excelente, o que indica que esta é uma estratégia adequada para o diagnóstico de enterobiose, resultando em menores custos e menor desconforto para as crianças e seus responsáveis. **Conclusão:** *E. vermicularis* foi o parasito mais comum entre crianças de Niterói, o que indica importância da inclusão da técnica de Graham nos estudos de prevalência de parasitos intestinais.

**Palavras-chave:** Enterobiose. Diagnóstico. Preescolares. Frequencia. Técnica de Graham.

**INTRODUCTION**

*Enterobius vermicularis*, also known as pinworm, is a small nematode that inhabits the large intestine, causing enterobiasis, characterized by intense anal itching1. This parasite is the helminth with the greatest geographic distribution on the planet and more than 30% of children worldwide have the parasite2. It is common in people of low socioeconomic status, families with infected individuals or institutionalized groups such as orphanages, psychiatric institutions and daycare centers3. The highest frequency of this parasite is observed among preschoolers and schoolchildren, associated with the habits of scratching the perianal region and anus-finger-mouth contact, along with practices of unsupervised hygiene care and inadequate hand washing, in addition to placing toys and objects used for writing in the mouth, and biting nails4.

In Brazil, the prevalence of *E. vermicularis* infection varies from 0.1 to 72.1%, associated with age, ethnicity, geographic area and diagnostic technique5. According to Fantinatti & Da-Cruz5, no national study focusing on enterobiasis has been conducted. The majority of cases have been detected through coproparasitological techniques, which makes determining its prevalence challenging, especially because notification is not mandatory.

The adhesive tape technique6 is universally used for diagnosing enterobiasis7, although other procedures can also be applied8. These include the fine-mesh screening technique, which has the aim of detecting adult parasites in feces. In the Brazilian literature, few studies have focused on diagnosing enterobiasis or use of the Graham technique to evaluate the prevalence of intestinal parasites. Using Graham's technique, Tashima & Simões9 detected *E. vermicularis* in 1.9% of children with clinical complaints in Presidente Prudente, SP. Carvalho, Carvalho & Mascarini10 found a positivity rate of 10.48% among children aged zero to six years in Botucatu, SP, and *E. vermicularis* was the most common helminth among those children.

Based on this context, the aim of this study was to evaluate the frequency of enterobiasis in preschoolers, with comparison between different parasitological techniques, and to correlate positivity with socioeconomic factors and symptoms.

**METHODS**

The study was carried out from March to October 2023 in three community daycare centers located in Niterói, RJ. The study population consisted of children aged two to six years old whose participation had been authorized by their guardians and, thus, was a convenience sample study. Each participant was asked to complete an epidemiological questionnaire and to provide samples of total feces and perianal material.

Each participant received two new plastic collectors, with a volume of 400 ml, in which to collect total feces on two different days; and a kit consisting of three microscopy slides with pieces (60 X 20 mm) of transparent adhesive tape, on which to collect perianal material on three different days. Guidance was given that the collection of perianal material should be carried out in the morning, when the child woke up, without prior cleaning of the perianal region.

The biological samples were sent to the Environmental Bioagents Laboratory at UFF. Perianal samples were processed using the Graham technique6 and fecal samples were processed using the techniques of Faust *et al.*11, Ritchie12 as modified by Young *et al*.13 and fine-mesh screening.

All participants received the test results in an individual report and those who were found to be positive were treated under medical supervision. The overall results of the study were presented to the community through a feedback meeting.

The results were analyzed descriptively. Statistical analysis between positivity and associated variables was performed using Fisher's exact test, considering P values ​​less than or equal to 0.05 to be significant. Variables with a significant association were subjected to bivariate analysis, with calculation of odds ratios (OR) to analyze possible risk or protective factors for enterobiasis. Agreement between parasitological techniques was analyzed using the agreement index (Kappa)14 and McNemar's test.

This study was approved by the Research Ethics Committee of the Faculty of Medicine of the Fluminense Federal University (UFF) under opinion no. 5,930,062, in March 2023.

**RESULTS**

The overall positivity for enteroparasites was 20% (19/95) and all parasitized children had monoparasitism. Among the 88 children who provided total fecal material, the material from 86/88 (97.7%) was subjected to the fine-mesh screening technique. All of these samples were negative for *E. vermicularis*.

Parasitism by *Giardia duodenalis* (2/88; 2.3%), *Entamoeba coli* (5/88; 5.7%) and *Trichuris trichiura* (1/88; 1.1%) was demonstrated using the techniques of Faust *et al*. and Ritchie as modified by Young *et al*. In one child, there was no concordant diagnosis using these two coproparasitological techniques (Table 1).

Among all the participants, 92 children (96.8%) provided material for the Graham technique, totaling 255 slides. One slide was provided by 3/92 children (3.3%), two slides by 15/92 (16.3%) and three slides by 74/92 (80.4%). *E. vermicularis* was detected in 12% of these children (11/92) (Table 1). Among these 11 children, in the cases of 3/92 (3.3%) who provided two slides, only one slide was positive. In the cases of the other 8/92 (8.7%) who provided three slides, in 2/92 (2.2%) all slides were positive, in 1/92 (1.1%) two were positive and in 5/92 (5.4%) only one was positive.

Use of a single slide for diagnosing *Enterobius vermicularis* was less efficient than use of three slides in combination, which showed substantial agreement (K = 0.641 and K = 0.748). An association between two slides, from two different days, resulted in an increase in efficiency, with substantial to excellent agreement (K = 0.748 and K = 0.926) (Table 2).

Analysis on the association between socioeconomic information and positivity for *E. vermicularis* showed that there was a statistically significant association with age between four and six years, which thus represented a risk factor for parasitism (OR = 1.256; CI = 1.097-1.437). However, in the present study, there were no significant associations between parasitism and the child’s sex, number of residents in the house, number of children in the house, mother or father’s education level, mother, family income or perianal itching (Table 3).

**DISCUSSION**

A positivity rate for enteroparasites of 20% (19/95) was obtained among the three participating community daycare centers. This rate was lower than previous findings from community daycare centers in Niterói, RJ: 55% (120/218) by Uchôa *et al*.15 and 51.6% (192/372) Uchôa *et al*.16. Thus, a marked reduction in the frequency of intestinal parasitism was observed in the present study, in relation to these previous results. This may be due to anthropic changes in the urban environment, such that there may have been a reduction in the extent of areas of bare soil with the humidity and shading necessary for geohelminths; and also improvement in basic sanitation measures with adequate sewage disposal and treatment of drinking water, possibly in association with antiparasite treatment. According to information from Instituto Trata Brasil17, the city of Niterói occupies the 23rd position in the 2022 Sanitation Ranking of the 100 largest cities in the country. Water supply is provided to 100% of the population; 95.6% of the population are connected to a sewage disposal system; and 100% of sewage collected is treated.

In studies that have used the Graham technique for diagnosing enterobiasis among children at daycare centers in Brazil, the frequencies of parasitism have ranged from 1.9% in Presidente Prudente, SP9, to 15% in Santa Isabel do Rio Negro, AM18. The frequency of positivity found among preschool children in Uberlândia, MG (13.87%), was similar to that found in Niterói19. Among the children at community daycare centers in Niterói, *E. vermicularis* was the most common helminth, as also reported in other studies9,10,18, and in Niterói it was also the parasite, between protozoa and helminths, with greatest frequency.

Among all the parasitological techniques used in the present study, only the Graham technique resulted in positivity for enterobiasis. This can be explained by the fact that females of *E. vermicularis* deposit their eggs in the anal and perianal region4. This result may also have been due to low parasite load. Findings of eggs in feces were identified by Fantinatti & Da-Cruz5 as responsible for the majority of records referring to parasitosis in Brazil, thus resulting in underreporting of cases of parasitosis. Although the fine-mesh screening technique was considered to be adequate and easily feasible for diagnosing enterobiasis, it was not effective in the present study.

Age was significantly associated with *E. vermicularis* infection in the present study, such that children aged four to six years were more parasitized. Similarly, Bunchu *et al*.20 in Thailand and Huang *et al*.21 in China also showed that the positivity rate was higher in the four to six-year-old age group, but only Bunchu *et al*.20 showed that this difference was statistically significant. Carvalho *et al*.10 observed a significant association between enterobiasis and the age group of three and four years and correlated this with the stage of consolidation of hygiene habits. Huang *et al*.21 suggested that the greater positivity in the age group of four to six years, in China, was related to children's entry into preschool, which increases the risk of infection, since children aged three years or younger are generally at home or only attend daycare centers intermittently. From the present study, it can be suggested that in addition to consolidation of hygiene habits, the greater frequency of positivity among children aged four or over, may be related to the complete removal of the diaper, which according to Meneses22 occurs from the age of four onwards. Without wearing a diaper, at night, children would have easier access to their perianal region during their sleep, coinciding with female parasite oviposition.

In the present study, it was seen that use of two slides in combination, compared with use of three slides, for diagnosing parasitosis, was efficient and showed substantial to excellent agreement (K = 0.748-0.926). Silva *et al*.19 also correlated repeated sample collection for Graham's technique with increased sensitivity. The results obtained in the present study suggest that use of two slides is indicated for diagnosing enterobiasis, with lower cost and less embarrassment for the child and their guardian.

**CONCLUSION**

To the best of our knowledge, the present study presents the first results on the frequency of enterobiasis among children in Niterói, RJ, using the Graham technique. It can be suggested that in order to solidly expand information on this parasitic disease, the Graham technique will need to be included in prevalence studies on intestinal parasitic diseases. Furthermore, the results of the article are a starting point to direct future public policies to specifically combat this parasitic disease in order to better direct control measures, mainly in the community daycare centers included in this study.

**ACKNOWLEDGMENTS**

We gratefully acknowledge the Municipal Education Foundation of Niterói for authorize and indicate the participant community daycare centers. We are also grateful to the pedagogical teams, parents and children of the community daycare centers for their participation and interest.

**FINANCIAL SUPPORT**

The work was carried out with the authors' own resources, without formal financial support from any development agency.

**CONFLICT OF INTEREST**

None.

**REFERENCES**

1. Cook GC. *Enterobius vermicularis* infection. Gut. 1994; 35(9):1159-62. doi: https://doi.org/10.1136/gut.35.9.1159
2. Kucik CJ, Martin GL, Sortor BV. Common intestinal parasites. Am Fam Physician. 2004; 69(5):1161-1168. Disponível em: https://www.aafp.org/pubs/afp/issues/2004/0301/p1161.html
3. Georgiev VS. Chemotherapy of enterobiasis (oxyuriasis). Expert Opin Pharmacother. 2001; 2(2):267-275. doi: https://doi.org/10.1517/14656566.2.2.267
4. Wendt S, Trawinski H, Schubert S, Rodloff AC, Mössner J, Lübbert C. The diagnosis and treatment of pinworm infection. Dtsch Arztebl Int Online. 2019; 116(13):213-219. doi: https://doi.org/10.3238/arztebl.2019.0213
5. Fantinatti M, Da‐Cruz AM. *Enterobius vermicularis* in Brazil: An integrative review. Rev Soc Bras Med Trop. 2023;56(e0073). doi: https://doi.org/10.1590/0037-8682-0073-2023
6. Graham CF. A Device for the Diagnosis of Enterobius Infection. The American Am J Trop Med Hyg. 1941; 21:159-161. doi: https://doi.org/10.4269/ajtmh.1941.s1-21.159
7. Moore TA, McCarthy JS. Enterobiasis. In: Guerrant RL, Walker DH, Weller PF, editors. Tropical infectious diseases: principles, pathogens and practice 3nd ed. USA: W.B. Saunders; 2011. p. 788-790.
8. Cazorla-Perfetti D. Aspectos relevantes de la enterobiosis humana: revisión crítica. Saber. 2014; 26(3):221-242. Disponível em: https://ve.scielo.org/scielo.php?pid=S1315-01622014000300002&script=sci\_arttext
9. Tashima NT, Simões MJ. Enteroparasitic occurrence in fecal samples analyzed at the University of Western São Paulo-UNOESTE clinical laboratory, Presidente Prudente, São Paulo State, Brazil. Rev Inst Med Trop Sao Paulo. 2004; 46:243-248. doi: https://doi.org/10.1590/S0036-46652004000500002
10. Carvalho TB, Carvalho LR, Mascarini LM. Occurrence of enteroparasites in day care centers in Botucatu (São Paulo State, Brazil) with emphasis on *Cryptosporidium* sp., *Giardia duodenalis* and *Enterobius vermicularis*. Rev Inst Med Trop Sao Paulo. 2006; 48:269-273. doi: https://doi.org/10.1590/S0036-46652006000500006
11. Faust EC, D’Antoni JS, Odon V, Miller MJ, Perez C, Sawitz W, *et al*. A critical study of clinical laboratory technics of the diagnosis of protozoan cysts and helminth eggs in feces. I - Preliminary communication. Am J Trop Med Hyg. 1938; 18:169-183. doi: https://doi.org/10.4269/ajtmh.1938.s1-18.169
12. Ritchie LS. An ether sedimentation technique for routine stool examinations. Bull U S Army Med Dep. 1948; 8:326.
13. Young KH, Bullock SL, Melvin DM, Spruill CL. Ethylacetate as a substitute for diethyletherformalin-ether sedimentation technique. J Clin Microbiol. 1979; 10(6):852-853. doi: https://doi.org/10.1128/jcm.10.6.852-853.1979
14. Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. Biometrics. 1977; 33(2):363-374. doi: https://doi.org/10.2307/2529786
15. Uchôa CMA, Lobo AGB, Bastos OMP, Matos AD. Parasitoses intestinais: prevalência em creches comunitárias da cidade de Niterói, Rio de Janeiro – Brasil. Rev Inst Adolfo Lutz. 2001; 60(2):97-101. doi: https://doi.org/10.53393/rial.2001.60.35525
16. Uchôa CMA, Albuquerque MC, Carvalho FM, Falcão AO, Silva P, Bastos OMP. Parasitismo intestinal em crianças e funcionários de creches comunitárias na cidade de Niterói-RJ, Brasil. Rev Patol Trop. 2009; 38(4):267-278. doi: https://doi.org/10.5216/rpt.v38i4.8590
17. Instituto Trata Brasil [homepage on the Internet]. Niterói. Organização da Sociedade Civil de Interesse Público, formado por empresas com interesse nos avanços do saneamento básico e na proteção dos recursos hídricos do país. Brazil: Instituto Trata Brasil; 2022 [Accessed on Nov. 22, 2023] Available from: https://tratabrasil.org.br/niteroi/.
18. Valverde JG, Gomes-Silva A, De Carvalho Moreira CJ, Leles De Souza D, Jaeger LH, Martins PP, *et al*. Prevalence and epidemiology of intestinal parasitism, as revealed by three distinct techniques in an endemic area in the Brazilian Amazon. Ann Trop Med Parasitol. 2011; 105(6):413-424. doi: https://doi.org/10.1179/1364859411Y.0000000034
19. Silva JJ, Borges R, Silveira AC, Silva LP, Mendes J. Enterobiasis and other intestinal parasitoses in children attending educational institutions in Uberlândia, state of Minas Gerais, Brazil. Rev Patol Trop. 2003; 32(1):87-94. doi: https://doi.org/10.5216/rpt.v32i1.4354
20. Bunchu N, Vitta A, Thongwat D, Lamlertthon S, Pimolsri U, Waree P, et al. *Enterobius vermicularis* infection among children in lower northern Thailand. J. Trop. Med. Parasitol. 2011; 34(1):36-40. Disponível em: https://li01.tci-thaijo.org/index.php/JTMP/article/view/14330
21. Huang J, Zhu H, Zhou C, Zhu T, Zhang M, Chen Y, *et al*. Epidemiological Profile and Spatial Patterns of Enterobiasis in Children Aged 3–9 Years in China from 2016 to 2020. Trop Med Infect Dis. 2023; 8(1):25. doi: https://doi.org/10.3390/tropicalmed8010025
22. Meneses RP. Nefrologia Pediátrica: Distúrbios funcionais da micção na infância. J. Bras. Nefrol. 2000; 22(2):95-102. Disponível em: https://www.bjnephrology.org/en/article/nefrologia-pediatrica-disturbios-funcionais-da-miccao-na-infancia/

**Table 1.** Positive results for intestinal parasites using parasitological techniques on fecal and perianal samples from children at three community daycare centers in Niterói, RJ, Brazil, 2023

|  |
| --- |
|  |
| Parasite | Parasitological Technique |
|  | Graham n=92 | Faust *et al.* n=88 | Ritchie Mod. n=88 | Fine-mesh screening n=86 |
| *Enterobius vermicularis* | 11 (12%) | 0 (0%) | 0 (0%) | 0 |
| *Giardia duodenalis* | 0 | 2 (2.3%) | 2 (2.3%) | 0 |
| *Entamoeba coli* | 0 | 5 (5.7%) | 4 (4.6%) | 0 |
| *Trichuris trichiura* | 0 | 1 (1.1%) | 1 (1.1%) | 0 |

 Ritchie Mod.– Ritchie modified by Young *et al.*

**Tabel 2.** Positive results for *Enterobius vermicularis* using the Graham Technique of three different days of 74 children at three community daycare centers in Niterói, RJ, 2023

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Graham | S 1 | S 2 | S 3 | S1/ S2 | S1/ S3 | S2/ S3 | S1/S2/S3 |
| Positive | 4 | 4 | 5 | 5 | 7 | 7 | 8 |
| Negative | 70 | 70 | 69 | 69 | 67 | 67 | 66 |
| McNemar Test | 0,125 | 0,125 | 0,250 | 0,250 | 1,000 | 1,000 |  |
| Kappa | 0,641 | 0,641 | 0,748 | 0,748 | 0,926 | 0,926 |  |

S1/S2, S1/S3, S2/S3, S1/S2/S3 – result based on associated slides of the same child. McNemar Test P<0.05. Kappa value < 0,00 – without concordance, 0,00 – 0,21 – weak, 0,21 – 0,41 - Slightly Weak, 0,41 – 0,61 – Moderate, 0,61 – 0.81 – Substantial, 0,81 – 1,00 - Almost perfect (excellent)

**Tabel 3.** Positive results for *Enterobius vermicularis* and socioeconomic information and symptoms of 92 children at three community daycare centers in Niterói, RJ, Brazil, 2023

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Question | Response | N | Positive | % | p-value |
|  | Respondents (n=92) | n=11 | 12 |  |
| Sex | Female | 45 | 4 | 4.4 | 0.524 |
| Male | 47 | 7 | 7.6 |
| Age | 2-3 | 38 | 0 | 70 | 0.002\* |
| 4-6 | 54 | 11  | 12 |
| Number of residents in the house | Up to 3 | 42 | 4 | 4.4 | 0.656 |
| 4-5 | 43 | 7 | 7.6 |
| 6 or more | 5 | 0 | 0 |  |
| Not answer | 2 | 0 | 0 |  |
| Number of children in the house | 1-2 | 79 | 8 | 8.7 | 0.157 |
| 3-4 | 12 | 3 | 3.3 |
| Not answered | 1 | 0 | 0 |  |
| Father's education level | Illiterate | 5 | 0 | 0 | 0.846 |
| Fundamental (incomplete/complete) | 33 | 4 | 4.4 |
| High school (incomplete/complete) | 38 | 6 | 6,5 |  |
| Higher education (incomplete/complete) | 16 | 1 | 1.1 |  |
| Mother's education level | Illiterate | 3 | 0 | 0 | 0.789 |
| Fundamental (incomplete/complete) | 24 | 4 | 4.4 |
| High school (incomplete/complete) | 48 | 6 | 6.5 |
|  | Higher education (incomplete/complete) | 16 | 1 | 1.1 |  |
|  | Not answered | 1 | 0 | 0 |  |
| Monthly family income (R$) | < 300 | 2 | 0 | 0 | 0.437 |
| 300-600 | 5 | 1 | 1.1 |
| 600-1000 | 23 | 5 | 5,4 |
| 1000-2000 | 26 | 1 | 1.1 |
| > 2000 | 19 | 3 | 3.3 |
|  | Not answered | 17 | 1 | 1.1 |
| Itchy perianal area | Yes | 24 | 0 | 0 | 0.061 |
| No | 57 | 11 | 12 |  |
| Not answered | 11 | 0 | 0 |  |

Positive = number of children positive for enterobiasis. Fisher's exact test: \*statistical significance when p ≤ 0.05.