

## **Enterobius vermicularis (Nematoda: Rhabditida) at a rural school in the Córdoba province, Argentina: diagnosis and perception of parasitism**

## **Enterobius vermicularis (Nematoda: Rhabditida) en una escuela rural de la provincia de Córdoba, Argentina: diagnóstico y percepción de parasitismo**

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**ABSTRACT:** *Enterobius vermicularis* is the causal agent of enterobiasis, a parasitic infection that primarily affects school-age children. This study aimed to achieve the following objectives due to the limited knowledge about prevalence of parasitic infections in the Córdoba region: to determine the prevalence of *E. vermicularis* in male and female students attending a rural primary school located in Sierras Chicas, Córdoba province, analyzing perianal and subungual samples; to investigate the correlation between the parasite presence and factors such as sex, age, and classroom; and, to assess the perception of parasitism based on symptoms, signs, and hygienic habits. Perianal samples were collected using the Graham method from a total of 37 students aged between 4 and 11 years old. Additionally, samples of the subungual region contents from both hands were also collected. A survey was conducted to gather information on both hygiene habits and symptoms from each school participant. The prevalence of *E. vermicularis* on perianal samples was 48.6%. However, no helminth eggs were detected in subungual samples. A correlation was observed between both the parasitic infection and children age. On the other hand, no significant association was observed between hygiene habits, symptoms reported by the students, and the parasitosis presence at the school population.

**Keywords:** Córdoba, elementary school, enterobiasis, pinworm, preschool.

**RESUMEN:** *Enterobius vermicularis* causa enterobiasis, una parasitosis que afecta a la población infantil en edad escolar. Ante el escaso conocimiento sobre la prevalencia de parasitismo en Córdoba, se plantearon los siguientes objetivos: determinar la prevalencia de *E. vermicularis* en alumnos y alumnas de una escuela rural de nivel primario de Sierras Chicas (Córdoba) mediante el análisis de muestras de la región perianal y subungueal; relacionar la presencia del parásito con el sexo, la edad y el aula, así como la percepción del parasitismo por síntomas y/o signos y hábitos higiénicos manifestados. Las muestras de la región perianal fueron tomadas siguiendo el método de Graham a alumnos y alumnas de 4 a 11 años de edad (n= 37). Además, se tomaron muestras del contenido de la región subungueal de ambas manos. Se realizó una encuesta para relevar datos sobre hábitos de higiene y síntomas de cada escolar. La prevalencia de *E. vermicularis* en las muestras perianales fue del 48,6%. No se hallaron huevos del helminto en las muestras de la región subungueal. Se encontró relación entre el parasitismo y la edad de los niños, sin embargo, no se observó relación entre los hábitos de higiene y los síntomas de cada escolar con la parasitosis.

**Palabras clave:** Córdoba, enterobiasis, oxiuros, preescolar, primaria.

Intestinal parasitic diseases are an increasing globally public health concern, particularly affecting impoverished and rural communities in developing countries. In Argentina, efforts have been made to develop and to implement prevention and control programs targeting parasitic diseases. Despite these advancements, parasitic diseases continue to be a significant concern for the public health system (Pezzani *et al.*, 2009; Cociancic *et al.*, 2020). Enterobiasis

or oxyuriasis produced by the pinworm nematode *Enterobius vermicularis* have been mentioned among the most frequent intestinal parasitosis in the world (Merad *et al.*, 2018).

The highest *E. vermicularis* prevalence has been observed in communities of a great population density, as well as institutional settings such as mental health facilities, hospitals, orphanages, and boarding schools. Preschool children rank as second as a vulnerable

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group of infection risk followed by mothers who care for parasitized children (Requena *et al.*, 2007). An extensive research has revealed that the infection rate of *E. vermicularis* is influenced by numerous factors. These include the dietary habits of individuals under study, personal hygiene practices, and environmental conditions. These variables play a significant role in determining the variation of infection rates among different populations (Muliawati *et al.*, 2020). The primary mode of *E. vermicularis* transmission is a direct contact between both infected and uninfected individuals typically through transferring pinworm eggs from anus to fingers leading to the ingestion of eggs. Moreover, the disease can easily spread among family members through contaminated hands, objects (i.e. fomites), and even inhalation of eggs (Merad *et al.*, 2018). Children attending school regularly engage on collective activities which create favorable conditions for this endoparasite transmission. Furthermore, their inadequate hygiene practices and behavioral patterns contribute to the infection spread (Merad *et al.*, 2018; Muliawati *et al.*, 2020).

Pinworms typically attach themselves to the mucosal layer and primarily reside in the cecal region, vermiform appendix, and distal intestine. While many cases of pinworm infection are asymptomatic, children with heavy infestations may experience symptoms such as irritability, appetite lack, bed-wetting, nightmares, nausea, insomnia, teeth grinding, diarrhea, anal itching (pruritus ani), inflammation of mucous membranes (catarrhal inflammation), vulva itching (pruritus vulvae), recurrent cellulitis, and endometritis (Khadka and Maharjan, 2018).

The prevalence of enteroparasites has been taken as an indicator of the population health status (Pezzani *et al.*, 2009), such as the prevalence of pinworms in developed countries is relatively low at about 10%. The pinworm prevalence in both kindergartens and primary schools was observed to be below 20% in Taiwan, Korea, China, Turkey, and Venezuela. However, the prevalence in countries such as Sri Lanka, Thailand, Malaysia, and Argentina was observed to be around 40% (Guignard *et al.*, 2000; Rivero *et al.*, 2022). The climatic and socioeconomic variability in Argentina influences the parasitosis frequency showing a decreasing trend from north to south, and from east to west (Navone *et al.*, 2017). The Córdoba province in Argentina lacks comprehensive statistical information regarding to the parasitism prevalence within its population despite of being an endemic region for several parasitic infections (Bracciaforte *et al.*, 2010). Furthermore, few studies that have been conducted in the Córdoba province regarding to parasitic infections often lack a strict standardization for identification methods of parasites. Additionally, some studies have even neglected to

results further hindering our understanding to the parasitism extent in the region (Guignard *et al.*, 2000).

It is crucial to establish monitoring systems and reassess data regarding to the status of *E. vermicularis* in communities due to the aforementioned symptoms and lack of information on pinworm prevalences. Therefore, the objective of this study was to determine the prevalence of pinworm infection among students attending both kindergarten and primary school from the Río Ceballos town located in the Córdoba province, Argentina. Additionally, we aimed to investigate potential associations between both the infection prevalence in these school-age children and factors such as age, sex, exhibited symptoms and/or signs, and hygienic habits.

The study was conducted on both sex children attending a rural kindergarten and elementary school from the Río Ceballos town located at the foot of Sierras Chicas Mountains. The school follows a rural modality and implements a multi-grade system. Either preschool or kindergarten students are situated in one classroom comprising 4 and 5 year old students. There are first, second, and third-grade students at two other classrooms whereas the fourth, fifth, and sixth-grade students are accommodated in another classroom. For simplicity, these classrooms will be referred as classrooms 1, 2, and 3, respectively. This research includes more than 45% from the total student population who voluntarily participated in the study (n= 37).

Informative talks were conducted to inform the population about *E. vermicularis* and to emphasize the significance of routine analyses for its detection before sampling. These talks were organized for both students and parents, and illustrative brochures were distributed throughout the school staff. In addition, educational games, hands-on observation under a stereoscopic magnifying glass, and several activities promoting prevention and hygiene were carried out at the school. The objective of these initiatives was to enhance awareness and encourage proactive measures against pinworm infections.

Questionnaires were given to the students' parents to assess both risk factors and clinical manifestations associated with enterobiosis. Questionnaires collected personal data of students such as name, age, and sex, as well as information about their families. Additionally, forms included questions regarding to symptoms and/or signs commonly associated with enterobiosis such as anal itching, nervousness, irritability, insomnia, bruxism (teeth grinding), and school distraction.

Furthermore, questionnaires aimed to investigate children hygienic behaviors which might contribute to the transmission of pinworm eggs. These included factors such as onychophagia (nail-biting), finger sucking, and handwashing frequency. These inquiries were conducted to gather comprehensive data of potential risk factors and clinical manifestations associated with

enterobiosis in the study population.

Information about the use of antiparasitic drugs was collected excluding students who had received such medication within one month either prior to or during the study period to ensure accurate results. Parent-teacher meetings were held at the school to inform the participants about the study objectives and sample collection methods which were involved using the Graham method. Those who chose to participate were provided with three slides equipped with transparent adhesive tapes for sampling the perianal region. These samples were collected over three consecutive days, preferably on the early morning before defecation and prior to washing the perianal area. Subsequently, anal adhesive slides were transported to the Parasitology Laboratory of the National University of Córdoba where they were examined within 48 hours from collection. The examination was conducted using a light microscope with magnifications ranging from 10X to 40X to identify the presence of *E. vermicularis* eggs.

Samples were collected from the subungual area in order to investigate the potential transmission of parasites through nails. Each morning the students refrained from washing their hands before going to school. The collection method involved firmly pressing the end of each finger, particularly the nearest area to the inner side of nails onto an adhesive tape with a sticky surface facing upwards. The tape was then attached to a slide allowing the material from the subungual area from all fingers to be collected on the same slide. This process allowed to examine the presence of parasites in the subungual region and explore the possibility of parasite transport through nails.

The statistical analysis was performed using the SPSS 20 software for Windows (SPSS Inc, Chicago, Illinois, USA). The Fisher's Exact Test was used to assess the association between each studied variable (i.e. sex, symptoms, hygiene habits, and classrooms), and the frequency of students infected with *E. vermicularis*. A p-value lower than 0.05 was considered statistically significant. The relationship between both parasitism and the age of individuals was evaluated using a Student's t-test.

The study was conducted with a maximum respect to

the physical, mental, and moral integrity of participants. The authorization was obtained from the principal school and students' parents who voluntarily agreed to participate. Numerical codes were used to identify the participants on databases to protect confidentiality. Laboratory studies were conducted free of charge, and results were delivered to parents in sealed envelopes.

The prevalence of *E. vermicularis* eggs in analyzed samples from students at the rural school was 48.65%. This prevalence is higher compared to findings from other studies conducted in Argentina, and numerous Latin American countries. For instance, the oxyuriasis prevalence (pinworm infection) in children was reported to be 19.4% in Venezuela (Moosazadeh et al., 2017). In Argentina, different studies provided prevalences of *Enterobius* in some Argentinian provinces: e.g. Salta (13.6%), Formosa (20.4%), Corrientes (20.0%), Chubut (21.7%), Mendoza (24.8%), Buenos Aires (34.7% y 37.7%), Entre Ríos (39.2%), Misiones (42.9%), and La Pampa (50.9%) (Navone et al., 2017; Cociancic et al., 2020; Rivero et al., 2022). Two studies were carried out in a residence for orphaned and homeless children in the Unquillo town (P= 43.4%) (Guignard et al., 2000), and a peri-urban town from the Santa María department (P=54.0%), Córdoba province (Bracciaforte et al., 2010). The oxyuriasis prevalence rates were also variable although they were generally high (Requena et al., 2007).

Sample means of parasitized and non-parasitized students suggest that infected children with *E. vermicularis* have a higher average age than non-infected ones with significant differences observed between ages of both parasitized (8.65 ± 0.45) and non-parasitized children (6.84 ± 0.46) (t = -2.72, df = 36, p = 0.01). The highest prevalence rate was found in ages between 10-14, followed by those of 6-9, and 4-5 years old. It was observed that boys presented a higher percentage of positive samples than girls when the analysis was carried out by sex (Table 1), but differences were not significant (p = 1). Table 1 also shows that the highest percentage of parasitized children was found in classroom 3 - a group of fourth, fifth, and sixth-grade students- although no significant differences were found between the groups of students at the three analyzed

**Table 1. Prevalence of *Enterobius vermicularis* according to both classroom number and sex of Córdoba province's students.**

Classroom	Females			Males			Total		
	N	Parasitized		N	Parasitized		N	Parasitized	
		n	%		n	%		n	%
1	3	1	33.33	7	3	42.86	10	4	40.00
2	10	5	50.00	7	3	42.86	17	8	47.06
3	4	2	50.00	6	4	66.67	10	6	60.00
Total	17	8	47.06	20	10	50.00	37	18	48.65

classrooms ( $p = 0.77$ ). Our findings are similar to those reported by Stoyanova *et al.* (2020), who found a higher prevalence in ages between 7-13 years old related to younger children. This phenomenon could be attributed to the fact that kindergartens or preschools typically have more effective prevention systems in place. Younger children in these settings are often closely supervised and guided in their hygiene practices which helps reducing the enterobiosis risk. On the other hand, older children begin to develop more independence on their hygiene routines, and may not have fully formed habits yet, particularly those in a school-age range. This may contribute to a higher enterobiosis prevalence in school-age children compared to preschoolers. Furthermore, other studies conducted by various authors have also reported a higher prevalence of intestinal parasites including enterobiosis in school-age children between 6 and 10 years old compared to those in the preschool-age group. This age range may be more susceptible to infection due to several factors such as increased social interactions and exposure to contaminated environments (Feroli *et al.*, 2020). Other studies have demonstrated no significant inter-sex differences on infection prevalences in agreement with the present results (Moosazadeh *et al.*, 2017; Navone *et al.*, 2017; Stoyanova *et al.*, 2020). It has been pointed out that in the early childhood there is no differences between sexes. But, after the pubertal age infection rates remain in boys, and decrease in girls because the latter usually follow hygiene rules earlier than boys (Atias, 1999).

The parasitosis perception by families who agreed to the study was also analyzed in this work. From 37 students' parents who completed the questionnaire, 89.19% indicated that their children had symptoms. About a half of them (51.52%) were parasitized. Almost the remaining half (48.48%) said they had symptoms but they were not parasitized. Another remarkable fact is that the 25% of students, whose parents stated that they had no symptoms, were parasitized. Anal itching, nervousness, sleep talking, and bruxism were the most mentioned symptoms by parents of parasitized students (Table 2). However, the test has not showed significant differences between symptoms and/or signs reported by children with parasitosis ( $p > 0.05$ ). No significant differences were found between parasitized and non-parasitized students regarding to hygienic habits and/or behaviors manifested by parents (handwashing frequency before eating food), and onychophagia. Significant differences were found between both (parasitized or not) groups of students on the finger-sucking habit ( $p = 0.01$ ) (Table 2).

Regarding to hygienic habits reported by the analyzed students' parents, they were not related to parasitoses, and significant differences were only found on the habit of toys or finger- sucking. However, this habit has not

*et al.* (2020) observed a similar phenomenon. By contrast, other studies showed that finger-sucking habits, fingernail-biting, toy or pencil sucking could be determinant factors of *E. vermicularis* infections especially in younger age children (Merad *et al.*, 2018).

No *E. vermicularis* eggs were found in any of 37 analyzed samples from the subungual region. Despite

**Table 2. Symptoms and hygienic habits and/or behaviors manifested by parents of the parasitized students with *Enterobius vermicularis* in the Córdoba province, Argentina.**

Variable	No. Examined	No. Positive	Prevalence (%)	p-value
<b>Anal itching</b>				
Yes	21	12	57.14	0.33
No	16	6	37.50	
<b>Nervousness / Irritability</b>				
Yes	21	9	42.86	0.52
No	16	9	56.25	
<b>School distraction</b>				
Yes	5	4	80.00	0.18
No	32	14	43.75	
<b>Insomnia</b>				
Yes	4	1	25.00	0.60
No	33	17	51.52	
<b>Skin spots</b>				
Yes	3	2	66.67	0.60
No	34	16	47.06	
<b>Asleep talk</b>				
Yes	17	7	41.18	0.52
No	20	11	55.00	
<b>Bruxism</b>				
Yes	17	7	41.18	0.52
No	20	11	55.00	
<b>Handwashing before eating</b>				
Yes	12	5	41.67	0.73
No	0	0	0	
Sometimes	25	13	52.00	
<b>Onychophagia</b>				
Yes	7	3	42.86	1.00
No	30	15	50.00	
<b>Finger or toy sucking</b>				
Yes	10	1	10.00	0.01
No	27	17	62.96	

the high prevalence of *E. vermicularis* on the Graham method, this finding is consistent with those who showed no cases of helminths diagnosed in the subungual deposit. Usually, the main contamination source is the transfer of eggs from hands to mouth after scratching the anal canal and perianal area by self-inoculation/ autoinfection (Rawla and Sharma, 2020; Ahmed, 2023). The observation of parasites in the subungual bed is not always possible since it does not depend exclusively on the parasite load but it does depend on hygienic habits of children and their parents (Requena et al., 2007). The absence of parasites in nail samples can be attributed to the action of parents and teachers who, fearing the study, washed children's hands while denying having to do that.

Signs and/or symptoms of parasitism are usually confusing and although the irritability, school distraction, nervousness, skin spots, even anal itching are manifestations of *E. vermicularis* parasitism, and they can also have other multiple causes.

In conclusion, the *E. vermicularis* prevalence in both kindergarten and elementary school students from the Córdoba province was high. However, no relationship could be established between the presence of these helminthes in anal samples and subungual area because no parasite eggs were found in student nails. Therefore, schools would not be a source of spillover although they are the place to carry out informative/preventive actions.

Current data highlight the importance of deploying educational programs to reduce the infection incidence and the attention to the personal hygiene in rural kindergarten and elementary schools. Intestinal parasites can be prevented by avoiding symptoms of parasitized children. In Argentina, both the enteroparasitosis prevalence and the spectrum of predominant species vary considerably from one locality to another. In addition, large-scale studies are required to establish the infection extent in other rural regions from the country.

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