doi: 10.5216/rpt.v53i1.77216

ORIGINAL ARTICLE

PREVALENCE OF ENTEROPARASITES IN RURAL AND URBAN CHILDREN IN THE MUNICIPALITIES OF BOCAIÚVA DO SUL AND COLOMBO, PARANÁ, BRAZIL

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ABSTRACT

The aim of this study was to diagnose enteroparasites and to relate them to socioeconomic factors in schoolchildren from the public system in the municipalities of Colombo and Bocaiúva do Sul, located in the Metropolitan Region from the city of Curitiba, Brazil. Fecal samples were processed using the modified Ritchie and Ziehl-Neelsen staining methods. Analysis of the risk factors, using Tukey test and Odds ratio (OR), was obtained by connecting questionnaires with parasitological results. Of the 414 schoolchildren analyzed, 32.4% (134) were positive for at least one enteroparasite species. The most prevalent were *Blastocystis* spp. (21.7%), *Entamoeba coli* (5.5%), *Endolimax nana* (5.5%), *Giardia duodenalis* (3.9%), *Trichuris trichiura* (3.7%) and *Ascaris lumbricoides* (2.9%). Schoolchildren from rural areas had a higher occurrence of enteric parasites ($p<0.05$), showing that is necessary to continue with health education, being careful with the food, basic sanitation and parasitological examination of feces before treatment.

KEY WORDS: Enteroparasites; monoparasitism; multiparasitism; children; socio-echonomic factors.

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Received for publication: 20/10/2023. Reviewed: 8/1/2024. Accepted: 9/2/2024.

INTRODUCTION

Infections caused by enteroparasites are one of the most prevalent parasitic diseases in tropical and subtropical countries, being an important public health problem and a global socioeconomic concern (WHO, 2017). It is estimated that 3.5 billion people are affected by enteroparasites and, of these, 450 million have clinical manifestations (WHO, 2013). Approximately 5.18 million deaths annually are caused by infections attributed to intestinal parasites, where helminths (*Ascaris lumbricoides, Trichuris trichiura, Necator americanus* and *Ancylostoma duodenale*) and protozoa (*Cryptosporidium* sp. and *Entamoeba histolytica/ dispar/ moshkovskii* complex) are considered to cause deaths (Lozano et al., 2012; PAHO, 2018).

This group of infectious agents are part of the neglected tropical diseases. There is currently a list of priority diseases (human African trypanosomiasis, onchocerciasis, scabies, schistosomiasis and visceral leishmaniasis) for the WHO, directing research for "Paediatric Drug Optimization" (PADO), aiming to meet the specific needs for babies and children (WHO, 2023).

Among the main risk factors associated with parasitic infections are the lack of basic sanitation, deficient socioeconomic and educational characteristics, crowding, consumption of untreated water and food contamination (Nobre et al., 2013; Rodrigues et al., 2020). The enteroparasites prevalence in the Brazilian nation is underestimated due to the lack of studies in many regions of the country also because it is not compulsory to report them. In the State of Paraná, previous studies have shown parasites infection ranging from 2 to 24.8% (Freckleton et al., 2019; Oishi et al., 2019). Although the Metropolitan Region of Curitiba (MRC) presents a Human Development Index (HDI) of 0.809, greater than other regions of Brazil such as the Metropolitan Region of Porto Alegre (HDI of 0.795), the presence of enteroparasites in the Metropolitan Region of Curitiba (MRC) schoolchildren (Ogliari & Passos 2002; Oishi et al., 2019) have the prevalence ranging from 24.8 to 89.7%, highlighting the presence of *A. lumbricoides, T. trichiura, Entamoeba coli* and *Blastocystis* sp. Thus, the purpose of this study was to diagnose enteroparasites in schoolchildren from public schools in the city of Colombo and Bocaiúva do Sul, located in the MRC, and to relate them to their socioeconomic and hygienic characteristics.

MATERIAL AND METHODS

Study area

The study was carried out in two municipalities in the MRC in the State of Paraná. The city of Colombo is 17 km away from Curitiba, the State´s capital, with an estimated population of 243,726 inhabitants and the city of Bocaiúva do Sul is 40,6 km away from Curitiba, with an estimated population of 12,944 inhabitants (IBGE, 2017ab) (Figure 1).

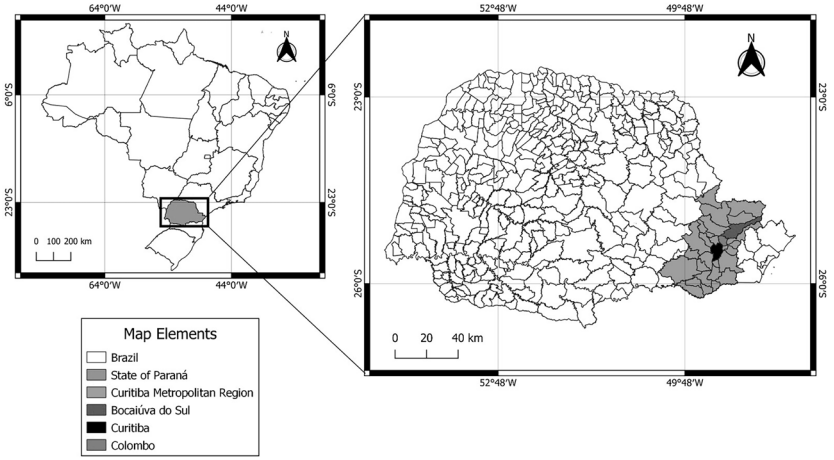

Figure 1. Map of Brazil, the location of the State of Paraná and the municipalities of Colombo and Bocaiúva do Sul.

Two schools from Colombo, one urban (School I) and one rural (School II), and other two schools from Bocaiúva do Sul (School III and IV), have some children from urban, rural and from unknown origin, were included in our study. A total of 414 students were enrolled, 207 from Colombo and 207 from Bocaiúva do Sul. Only students between 1 and 10 years of age participated in our study, with an informed consent signed by their guardians. In addition, guardians answered a closed structured questionnaire with socio-economic and hygienic aspects to assess risk factors for enteroparasites.

Approval of the ethics committee

The research was approved by the Research Ethics Committee from the Federal University of Paraná (CAAE 91542618.0.0000.0102).

Sample collection and processing methods

Fresh morning fecal samples were transported in a refrigerated Styrofoam box to the Laboratory of Parasitology at the Federal University of Paraná and classified according to the Bristol scale. For the fecal analysis the modified Ritchie technique (Knight et al., 1976) was performed, changing the use of ether to ethyl acetate. The observation was carried out in triplicate. To detect *Cryptosporidium* spp. and other coccidian protozoa, the modified Ziehl-Neelsen method was applied. A Leitz Dialux 22 optical microscope was used. Measurements were carried out in micrometers (μm) and were taken with a Zeiss ocular micrometer calibrated using a standard Zeiss scale. The conversion of the micrometers measurements was as follows: objective (6.3×)= 18.5 μm, (16×)= 7.3 μm, (25×)= 4.65 μm and (40×)= 2.9 μm to access the structure's size. All samples were analyzed in triplicate.

Statistical analysis

The data obtained were tabulated in the Excel® 2016 program and analyzed both by descriptive statistics and by inferential statistics using the R. Studio® version 3.6.2. The information provided through prevalence calculations made it possible to establish population risk statistics (Odds-Ratio) between children who tested positive for enteroparasites and children who tested negative, describing the relationships between a set of variables making it possible to characterize a group based on its positive or negative sample, using the chi-square test or Fisher's test when necessary. All inferences were accompanied by a 95% confidence interval. The tabulated data were transformed into logistic regression and a multivariate analysis (ANOVA) was performed, to verify multifactorial relationships.

RESULTS

Overall prevalence of infection with at least one parasite species reached 32.4% (134/414). Three species of helminths were detected in 2.4% (10/414) and protozoa (species and genera) were present in 30% (124/414) of the samples analyzed. In addition to the two municipalities, the helminth species found were *T. trichiura* (1.2%) and *A. lumbricoides* (1%). For protozoa, the results demonstrated that the most prevalent were *Blastocystis* spp. (21.7%), followed by *E. coli* (5.5%), *E. nana* (5.5%), *G. duodenalis* (3.9%), *E. hartmanni* (2.6%), (Table 1, Figure 2). Infection was detected with a frequency of 68% (91/134) in formed feces, 24.6% (33/134) in pasty stools and 7.5% (10/134) in diarrheal stools.

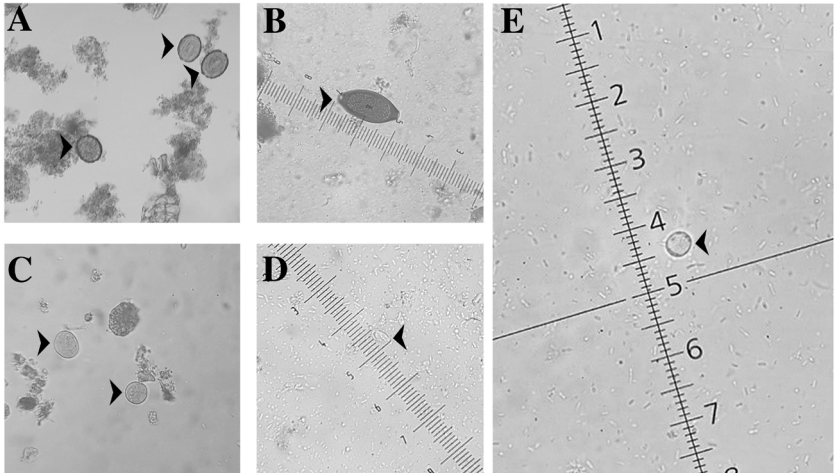

Figure 2. A: *Ascaris lumbricoides* eggs; B: *Trichuris trichiura* egg; C: *Entamoeba coli* cysts; D: *Giardia duodenalis* cyst; E: *Blastocystis* spp. vacuolar (indicated by the arrow).

Higher overall prevalence of infection appears in Colombo (18.4%) than in Bocaiúva do Sul (14%), with no statistically significant differences. Approximately 74.6% (100/134) of the children presented monoparasitism and 25.4% (34/134) multiparasitism with two or more species. *Blastocystis* spp. was the parasitic form with the greatest association (Table 2).

In the Table 3 results from urban and rural environments infection is shown. In general, enteroparasites infection resulted more frequent between children from rural environments (50.7%) (68/134) rather than those from urban ones (40.3%) (54/134) with statistically significant differences (Tukey's test, $p< 0.05$). *Blastocystis* spp. was most observed in children from rural environments (38.1%) rather than in those from urban ones (23.1%) with significant statistical differences (Tukey's test, $p=0.002$).

Table 4 shows the relationships between infection results and different variables recorded, allowing the Odds-Ratio calculations. Although no statistically significant differences were observed in the overall infection among both municipalities studied, schoolchildren from rural environments result statistically 2.6 times more likely to became infected. Moreover, given the different schools studied, schoolchildren from school II (Rural-Colombo) present statistically 12.6 times more likely to present infection. The most affected age group was from 6 to 10 years old, with a statistical difference, showing a 1.8% chance of being affected by at least one parasite (Odds-Ratio and Tukey Test, $p< 0.05$).

Table 1. Prevalence of enteroparasites in schoolchildren from Colombo and Bocaiúva do Sul municipalities (August 2017 to July 2019).

	N (%)	Colombo (n= 207)		Bocaiúva do Sul (n= 207)		
		n	%	n	%	p-value
Helminths	10 (2.4)	5		5		
Trichuris trichiura	5 (1.2)	3	0.7	2	0.5	1.000
Ascaris lumbricoides	4 (1)	1	0.2	3	0.7	0.623
Enterobius vermicularis	1 (0.2)	1	0.2	0	0.0	1.000
Protozoa	124 (30)	71		53		
Blastocystis spp.	90 (21.7)	53	12.8	37	8.9	0.074
Entamoeba coli	23 (5.5)	12	2.9	11	2.7	1.000
Endolimax nana	23 (5.5)	13	3.1	10	2.4	0.668
Giardia duodenalis	16 (3.9)	9	2.2	7	1.7	0.798
Entamoeba hartmanni	11 (2.6)	6	1.4	5	1.2	1.000
Iodamoeba bütschlii	6 (1.4)	3	0.7	3	0.7	1.000
Entamoeba histolytica/dispar/ moshkovskii complex	3 (0.7)	2	0.5	1	0.2	1.000
Cryptosporidium spp.	1 (0.2)	0	0.0	1	0.2	1.000
Total	134 (32.4)	76 (18.4)		58 (14)		

[N: total positive samples; n: observation; %; prevalence= (n/Nx100)].

Table 2. Frequency of monoparasitism and multiparasitism detected in Colombo and Bocaiúva do Sul municipalities (August 2017 to July 2019).

	N	Colombo		Bocaiúva do Sul		p-value
		n	%	n	%	
Monoparasitism						
Helminths	5					
Ascaris lumbricoides	2	0	0	2	1.5	
Trichuris trichiura	2	2	1.5	0	0	
Enterobius vermicularis	1	1	0.7	0	0	
Protozoa	124					
Giardia duodenalis	8	3	2.2	5	3.7	
Entamoeba histolytica/ dispar/ moshkovskii complex	2	2	1.5	0	0	
Entamoeba hartmanni	6	2	1.5	4	3	
Entamoeba coli	10	6	4.5	4	3	
Endolimax nana	7	3	2.2	4	3	
Iodamoeba bütschilii	2	2	1.5	0	0	
Blastocystis spp.	60	36	27.0	22	16.4	
Cryptosporidium spp.	1	1	0.7	0	0	0.104
2 parasites						
Giardia duodenalis + Entamoeba hartmanni	1	1	0.7	0	0	
Giardia duodenalis + *Blastocystis* spp.	4	2	1.5	2	1.5	
Entamoeba coli+*Blastocystis* spp.	8	3	2.2	5	3.7	
Blastocystis spp.+*Endolimax nana*	7	5	3.7	2	1.5	
Blastocystis spp.+*Trichuris trichiura*	2	0	0	2	1.5	
Endolimax nana+ *Entamoeba histolytica/dispar/moshkovskii* complex	2	2	1.5	0	0	0.557

3 or more parasites						
Entamoeba histolytica/dispar/ moshkovskii complex + *Iodamoeba bütschilii* + *Blastocystis* spp.	1	1	0.7	0	0	
Ascaris lumbricoides + *Blastocystis* spp. + *Endolimax nana*	1	1	0.7	0	0	
Giardia duodenalis + *Endolimax nana* + *Trichuris trichiura*	1	1	0.7	0	0	
Ascaris lumbricoides + *Blastocystis* spp. +*Entamoeba coli*	1	1	0.7	0	0	
Entamoeba coli+*Blastocystis* spp. + *Entamoeba histolytica/dispar/ moshkovskii* complex	1	0	0	1	0.7	
Blastocystis spp. + *Endolimax nana*+*Entamoeba Entamoeba histolytica/dispar/moshkovskii* complex	1	0	0	1	0.7	
Blastocystis spp. + *Endolimax nana*+*Entamoeba coli*	1	0	0	1	0.7	
Entamoeba coli+*Blastocystis* spp. +*Giardia duodenalis* +*Enterobius vermicularis*	1	1	0.7	0	0	
Blastocystis spp. + *Endolimax nana*+*Entamoeba coli*+*Giardia duodenalis*	1	1	0.7	0	0	1
Total Infected	134	76		58		

[N: total of positive samples; n: observations; %= (n/NX100)].

Table 3. Frequencies of enteroparasites infection, in rural and urban environments from Colombo and Bocaiúva do Sul municipalities (August 2017 to July 2019).

	N	Urban		Rural		Uninformed		p-value
		n	%	n	%	n	%	
Helminths	10	4		6	4.5	0	0	
Trichuris trichiura	5	1	0.7	4	3.0	0	0	0.255
Ascaris lumbricoides	4	3	2.2	1	0.7	0	0	0.756
Enterobius vermicularis	1	0	0.0	1	0.7	0	0	1.000
Protozoa	124	50 (40.3)		62 (50)		12 (9.7)		
Blastocystis spp.*	90	31	23.1	51	38.1	8	6.0	0.002
Entamoeba coli	23	10	7.5	11	8.2	2	1.5	0.482
Endolimax nana	23	6	4.5	15	11.2	2	0.0	0.669
Giardia duodenalis	16	7	5.2	8	6.0	1	0.7	0.543
Entamoeba hartmanni	11	4	3.0	5	3.7	2	0	0.230
Iodamoeba bütschlii	6	5	3.7	1	0.7	0	0.0	0.538
Entamoeba histolytica/ dispar/ moshkovskii complex	3	2	1.5	1	0.7	0	0.0	1.000
Cryptosporidium spp.	1	0	0.0	1	0.7	0	0	0.452
Total Infected	134	54 (40.3)		68 (50.7)		12		

[N: total of positive samples; n: observations; % = (n/NX100); *: p< 0.05 calculated between rural and urban areas].

Table 4. Infection results and characteristics of schoolchildren from Colombo and Bocaiúva do Sul municipalities, in relation to the frequencies of enteroparasites (August 2017 to July 2019).

Variables	N	N	%	OR	p-value
Municipality					
Colombo	207	76	36.7	1.5	p> 0.05
Bocaiúva do Sul	207	58	28.0	1.0	
Environment					
Urban	227	54	40.3	1.0	
Rural*	152	68	50.7	2.6	p< 0.05
Not Informed	35	12	9.0	1.6	
Schools					
School I (Urban - Colombo)	91	23	17.2	5.1	
School II (Rural - Colombo)*	116	53	39.6	12.6	
School III (Urban - Bocaiúva do Sul)	120	30	22.4	5.0	
School III (Rural - Bocaiúva do Sul)*	21	9	6.7	11.3	
School III (Uninformed- Bocaiúva do Sul)	26	7	5.2	5.5	p< 0.05
School IV (Urban - Bocaiúva do Sul)	16	1	0.7	1.0	
School IV (Rural - Bocaiúva do Sul)*	15	6	4.5	10.0	
School IV (Uninformed - Bocaiúva do Sul)	9	5	3.7	18.8	
Gender					
Feminine	233	75	32.2	1.0	p> 0.05
Masculine	181	59	32.6	1.0	
Age group					
1-5	158	40	9.7	1.0	
6-10*	232	87	21.0	1.8	p< 0.05
Not Informed	24	7	1.7	0.8	
Total	414	134	32.4		-

[N: total samples; n: observations; %: prevalence rate= (n/NX100); *: p<0.05; OR: Odds Ratio].

Amidst all the variables analyzed (medication, water source, place of evacuation, waste destination, sewage treatment and presence of animals) for those children who were positive for some form of parasite, no significant statistical differences were observed (Odds-Ratio and Tukey test, $p< 0.05$) (Table 5). When observing the socioeconomic data of both municipalities, children who had already taken some medication (Albendazole or Nitazoxanide) and/or natural treatment (mint tea) were 30% less likely to have a parasite (Tukey, $p<0.05$). It was noted that in Colombo 25.0% of the samples (5/20) that had a positive result had already taken Albendazole, 50.0% (1/2) had taken Nitazoxanide and 22.2% (4/18) mint tea. For Bocaiúva do Sul, mint tea was most used by the individuals sampled and parasitized, 8.7% (14/67), followed by Albendazole with 8.2 (13/48) and Nitazoxanide with 5.1 (8/ 31).

Table 5. Odds-Ratio calculation between infection results and socio-economic and hygienic characteristics of schoolchildren from Colombo and Bocaiúva do Sul municipalities, Paraná (August 2017 to July 2019).

	Colombo					Bocaiúva do Sul				
Variables	(N=66)	n	%	OR	p value	(N=164)	n	%	OR	p value
Medicine or natural treatment for worm										
albendazole	20	5	25.0	0.3	0.106	48	13	8.2	1.02	1.000
nitazoxanide	2	1	50.0	1.4	1.000	31	8	5.1	0.94	1.000
mint	18	4	22.2	0.3	0.079	67	14	8.7	0.59	0.213
Water source										
sanepar	53	22	41.5	1.5	0.792	111	29	26.1	0.90	0.916
source	12	7	58.3	2.2	0.363	59	19	32.2	1.52	0.327
filtered	1	0	0.0	—	1.000	90	26	28.9	1.26	0.632
boiled	—	—	—	—	—	6	2	33.3	1.38	1.000
Place of evacuation of feces										
bathroom	65	27	41.5	—	0.877	80	17	21.2	0.80	0.648
latrine	—	—	—	—	—	4	2	50	2.81	0.626
land around	—	—	—	—	—	4	1	25	0.91	1.000
Waste destination										
city hall	66	28	42.4	—	1.000	102	24	23.5	0.65	0.298
land around	—	—	—	—	—	29	11	7	1.89	0.209
lurned	—	—	—	—	—	4	1	0.6	0.91	1.000

Sewage treatment										
sanepar	11	4	36.4	—	0.911	153	39	24.7	0.41	0.275
septic tank	—	—	—	—	—	8	2	1.3	0.90	1.000
not informed	55	24	43.6	—	0.911	2	2	100	—	0.071
Presence of animals at home										
puppy	54	23	42.6	0.46	0.363	45	12	7.6	0.99	1.000
cat	23	12	52.2	1.84	0.362	18	4	2.5	0.76	0.853
hen	22	12	54.5	2.1	0.252	9	1	0.6	0.33	0.447
pork	4	3	75.0	4.44	0.402	84	21	13.3	0.83	0.715
rabbit	4	0	0.0	—	0.212	22	8	5.1	1.68	0.409

[N: number of responses per municipality; n: frequency of positive results for some parasitic form; %: prevalence rate= (n/NX100); OR: Odds Ratio].

DISCUSSION

Overall enteroparasites prevalence detected (32.4%) was lower than that found in the coast of the State of Paraná (46.1%) (Seguí et al., 2018). However, results from Colombo (18.4%) are close to those from Campo do Tenente (24.8%) (Oishi et al., 2019). Protozoa prevalence resulted higher than that of helminths, similar to previous Brazilian study (Oishi et al., 2019). *Blastocystis*, considered as the most observed intestinal protozoan in humans (Coco, 2017), was the most prevalent species in both municipalities.

However, although the decrease in helminths prevalence detected may be due to health education and mass treatment programs (Guilherme et al., 2004; Oishi et al., 2019). some protozoa considered commensals for humans were detected in this study, such as *E. coli, E. nana, E. hartmanni* and *I. bütschlii* and they are indicators of failure in personal hygiene. Most infection was detected in those not symptomatic schoolchildren, with 68% having formed feces, according to Belloto et al., 2011.

In the State of Amazonas, with indigenous populations, a parasitic association of 14 species was observed, with emphasis on *A. lumbricoides, G. duodenalis* and *E. coli* (Oliveira et al., 2016). However, we have only found single counts of four different parasite species associations, while the most prevalent way of infection was monoparasitism in both municipalities of MRC. Different enteroparasites species detected resulted similar in both municipalities studied. However, children from rural environments were more frequently infected than those from urban ones. A contradictory fact to highlight is the higher *A. lumbricoides* infection in urban rather than in rural environments.

No differences of infection between males and females were observed, other authors result detected a higher prevalence in females (Coco et al., 2017; Oishi et al., 2019). Children aged 6 to10 years, presented the highest prevalence and higher risk of infection, which corroborates previous research in the State of Minas Gerais, Brazil, where they observed protozoa, such as *E. histolytica* complex and *E. coli*, in children of that age-group (Belo et al., 2012). Previous studies showed that mass treatment with anthelmintic (Albendazole) reduced the prevalence of parasites (Machado et al., 1996) and the use of mint has a positive effect in the treatment of the protozoan *G. duodenalis* (Vidal et al., 2007). In that sense, the use of antiparasitic treatment is a habitual consumption, drugs mainly in Colombo and natural treatment mainly in Bocaiúva do Sul. However, many people take medicine without undergoing a previous laboratory diagnosis test (Cavagnolli et al., 2015).

Water consumption from the public network is responsible for the decline in the prevalence of parasitic species (Cavagnolli et al., 2015; Viana et al., 2017). The largest water supplier for the children's homes in both municipalities is the "Companhia de Saneamento do Paraná" (Sanepar) who supplies treated drinking water. Although the presence of animals such as pigs, chickens and cows in children's homes is considered as a risk factor for infection or reinfection of zoonoses (Zanetti et al., 2021), in the present study, it cannot be considered as a risk factor of infection in neither of the two municipalities studied.

In conclusion, this research is the first parasite prevalence study in schoolchildren from the municipalities of Colombo and Bocaiúva do Sul, where transmission of parasitic species was exposed, with emphasis on *Blastocystis* spp. Knowing the importance of the fecal-oral route of transmission, there is an urgent need to improve the public health policies for parasite control, prevention methods and improvement of social awareness.

ACKNOWLEDGMENTS

We deeply thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for financing the project, and the Municipal Schools of Bocaiúva and Colombo for accepting and collaborating in the implementation of the project.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to disclose.

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