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Intestinal parasitism in Moroccan children: comparative quantitative study of the Faust's and Ritchie's coprologic methods

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ABSTRACT: A study of intestinal parasites in school children in urban and rural areas of Tetouan (Morocco) was conducted. Before it was performed a comparative quantitative study of Faust's and Ritchie's techniques in order to optimize intestinal parasites diagnosis and to determine the techniques effectiveness. The Ritchie's technique resulted the most effective for the detection of both protozoan and helminthes, especially under conditions of low parasite burdens. The prevalence of intestinal parasites was 65% and 71% in rural and urban areas respectively. Overall, the prevalence of protozoa that was found was higher than the one detected for helminths. The most frequent of the intestinal parasites was the protozoa *Blastocystis hominis* and the most frequent pathogenic protozoa were *Giardia lamblia* followed by *Cyclospora cayetanensis*. Among the helminths *Trichuris trichiura*, *Hymenolepis nana*, *H. diminuta, Enterobius vermicularis*, *Taenia saginata*, *Ascaris lumbricoides* and *Fasciola hepatica.*

Giardia lamblia showed notable differences between boys and girls in urban areas. To compare the prevalence of parasites in children with the same sex in different areas the differences were only found in boys infected by *B. hominis*, *G. lamblia* and *E. nana*.

Multiple parasitism appeared in 29% of the samples presenting two, three or four parasites.

KEYWORDS: Faust, Ritchie, quantitative, enteroparasite, prevalence, control

1 INTRODUCTION

Intestinal parasitism can be considered indicative of the socioeconomic status and sanitation levels of a population. The death triangle of poor hygiene, inadequate sanitation and lack of access to safe drinking water claims children's lives each day, mostly in developing countries [1]. Inadequate water supply and sanitation are largely responsible for millions of estimated cases of diarrheal disease and deaths in developing countries every year [2].

Children constitute the sector most affected by intestinal parasites. Concerning the morbidity that this causes, the World Health Assembly, in Resolution 54.19 of 2001 [3], indicated that at least 75% of school-age children living in high-risk areas for geohelminths and *Schistosoma* would need access to regular chemotherapy during the year 2010. WHO have therefore, for some years, been providing incentives to improve sanitation such as the Projects of Healthy Schools [4].

Intestinal parasites can cause a wide range of symptoms including diarrhea, loss of appetite, joint pain, mucus in stools, fever, listlessness, foul-smelling stools, stomach cramps, coughing and vomiting.

The diagnosis of intestinal parasitic infections is based primarily on microscopic analysis of fecal samples, including direct observation, concentration and permanent staining smears. Concentration techniques are essential since the amount of parasitic forms in fecal samples is often very low and can give as negative positive samples, which consequence would be the maintenance of the transmission of these parasites. There are many concentration techniques described, however it these techniques have limitations, particularly regarding to their sensitivity. Two of the most common techniques for its low cost and effectiveness to recover both cysts as eggs and larvae are Ritchie's and Faust's [5], [6], [7]. The choice of the concentration technique is important for rigorous epidemiological studies that allow to establish appropriate control programs.

Although intestinal parasites are common in the Moroccan population, there are few references in this regard. The first published study specifically on children dates back to 1955 and, as in most studies, showed a higher prevalence of the protozoan *Giardia lamblia* of 10% [8].

Parasites such as *Chilomastix mesnilii*, *Pentatrichomonas hominis* and *Enteromonas* are also frequent within the adult and child population in the province of Fez [9], [8]. Further studies in this province indicated a simple parasitization index (SPI) of intestinal parasites of 0.46 in 1974 [10] and 0.0938 in 1989 [11].

In all studies undertaken in Fez, protozoa are more frequent than intestinal helminths as in Tangier and Rabat [12], [13]. In Rabat, which studies show has a lower SPI than that of Fez [10], studies of the intestinal parasites more frequently found in hospitalized children showed *Entamoeba histolytica*, *Giardia intestinalis* and *Entamoeba coli* among the protozoa, and *Enterobius* and *Hymenolepis nana* among the helminths [14], [15].

Furthermore in children, Garcia-Fernández et al. conducted a study in 1984 [16] in Ceuta on intestinal parasites of school children, predominantly Muslim in low socio-economic areas in which *Trichuris trichiura*, *Entamoeba coli*, *Giardia intestinalis*, *Iodamoeba butschlii*, *Endolimax nana*, *Hymenolepis nana*, *Ascaris lumbricoides* and *Enterobius vermicularis* were prevalent.

These are also the predominant intestinal parasites in Tetouan, Larache and Tangier [17]. *G. lamblia* is prominent among protozoans, which in all studies predominate over the helminths, while *E. vermicularis* is prominent among helminths.

The intestinal parasitization of urban and rural populations has been compared in the provinces of Taounate, Beni Mellal and Tizinit. Two-thirds of the rural population and 50% of the urban population were affected. The amoebae were the most common parasites, followed by flagellates and helminths [18].

The analysis in 2001 in Tetouan, Rabat and Marrakech, of 1003 fecal samples of people from all ages in both rural and urban areas, showed that the parasitization was greater in rural areas, in males, and in children aged 6-15 years. The most common parasite was *Trichuris trichiura* with a prevalence of 21% (Hoummad El Fatni, unpublished results, Doctoral thesis, University of Granada).

More recent studies in children in Tiflet (Morocco) demonstrate that intestinal parasites have a very high incidence with a prevalence of pathogenic protozoa of 25.8%, highlighting *B. hominis*, *E. vermicularis* and *H. nana* [19]. These children were aged 7-15 years old and of them those between 10 and 12 years old were more parasitized (84.1%).

A retrospective study of enteroparasites published in 2009 [20] reviewed the diagnosed intestinal parasitism in the Provincial Hospital Center (Kenitra, Morocco) for the years 1996-2005, and showed an overall prevalence of 14.15%. Amoeba were frequently observed (47.04%) followed by flagellates such as *G. lamblia* (22.71%). Helminths were less common, with a predominance of *Ascaris lumbricoides* (11.87%), *T. trichiura* (5.64%), *H. nana* (2.68%) and *E. vermicularis* (2.08%).

It is clear that one way of infection with of children intestinal parasites is via food, vegetables and fruits irrigated with wastewater. Health risks of raw sewage have been extensively studied which enabled the presence, of abundant eggs in water to be demonstrated of *A. lumbricoides*, *T. trichiura*, *E. vermicularis*, *H. nana* and *Taenia saginata* as well as cysts of *E. histolytica* and *G. intestinalis.* It has also been shown that 50.8% of children living in areas where wastewater is used in irrigation are parasitized, compared to 8.2% in areas where this practice is not utilized [21], [22], [23], [24], [25].

The most recent work on intestinal parasites in children in Morocco has been in the city of Sale, finding a prevalence of 61.7% in children aged 12-14. The protozoa were found more frequently than the helminths with 57.7% and 26% respectively, and 36.6 % of children were multi-parasitized [26].

The Spanish Agency for Intemational Cooperation (Agencia Española de Cooperación Internacional) have been financing since 2009 a project of prevention, control, and treatment of intestinal parasites in Tetouan, to lower the prevalence of parasitic infections among children.

With this aim, in the present study the techniques of Ritchie [27] and Faust [28] were quantitatively compared to select the more sensitive coprologic diagnostic technique. After, an epidemiological study of the intestinal parasitisms in children, of rural and urban areas of the province of Tetouan, was performed in order to treat sick children and develop monitoring programs against the most frequent pathogen enteroparasites.

2 Materials And Methods

This study was carried out in four schools in the province of Tetouan, which is located in the North of Morocco. It has an area of 3242 km^2 and has a population of approximately 700,000 inhabitants. The province has a mountainous landscape and is crossed by numerous rivers. It has a Mediterranean climate with variable temperatures, with a maximum of 32°C in summer and 5°C in winter.

In this work, we studied the frequency of intestinal parasites in children from four schools in both rural and urban areas, in order to treat sick children and establish prevention and control programs against intestinal parasites.

Samples, 546, were collected from the schools 18 Noviembre and Ahmed El Bakal, situated in urban areas, and Jamaate Ben Karich and Azla, situated in rural areas. Samples were collected from November 2009 until the beginning of the year 2011. In all cases these were public schools attended by children of different social classes.

In urban areas, 276 samples were collected from the two schools located in the mountainous area from various socio-economic levels. There were large differences in the dwellings of the population; on the one hand some lived in houses made from adobe with no access to sewerage and drinking water from wells, while on the other hand others lived in large modern buildings. Pets and other domestic animals coexist with people.

In the rural areas, 270 samples were collected from the two schools. In this case the location of schools is different; the college of Jamaate Ben Karich is located in the mountainous area and the college of Azla is located on the coast. Both schools were attended by children with various socio-economic levels; some live in houses of adobe and may or may not have potable water and sewage. Pets and domestic animals live outside the houses in the immediate surroundings.

Data of age, sex and state of health of the family were obtained from a questionnaire interview. Following the interview a small container for a stool sample was given to each child. After the fecal samples were collected, the children were weighed and measured to complete the questionnaire.

Samples were preserved in 10 ml of potassium dichromate at 2.5% and kept at 4ºC until macroscopic and microscopic observation.

Macroscopic inspection determined the consistency and mucosity, as well as the blood and fat contents of the samples. After examination under a binocular microscope, and under a light microscope using lugol in some cases, samples were stained with Zielh-Neelsen and Giemsa.

Since some samples had low number of parasites it was decided to compare quantitatively the Faust's [28] and Ritchie's [27] techniques and see which one offers better results for coproparasitological diagnosis, in order to select the best of them for further epidemiological studies.

A hundred of samples were subjected to Faust and Ritchie concentration techniques. For both concentration techniques, fecal suspensions were filtered through gauze and the material on the filter was diluted with 10 ml of sterile water. This suspension was centrifuged at 500 x *g* for 2 min in conical propylene tubes, followed by supernatant decantation. Sterile water was then added to reach 10 ml of total volume and the process was repeated until a clear supernatant was obtained. Each sample was examined at 40x magnification for the presence of eggs, larvae, trophozoites or cysts and some samples were stained with Ziehl-Neelsen and Giemsa. For the Faust's technique the final pellet was resuspended in 10 ml of $ZnSO_4 \cdot 7 H_2O$ (703 g/l, 33%, specific gravity 1.118) for flotation. The tube was inverted six times and the fluid removed with a pipette to fill both chambers of Neubauer and to quantify the cysts, oocysts, and eggs 10 min after loading the slide [29]. Using the McMaster slide identified only helminth eggs.

For Ritchie's technique the final pellet was resuspended in 10 ml of 10% formaldehyde solution, mixed and finally stand for 10 min. After, 5 ml of ether were added, the tubes were capped with rubber stoppers and shaken vigorously for 30 seconds. Then the mix was spinned down for 2 minutes at 1500 rpm and an aliquot of the pellet were placed in both chambers of Newbaur and McMaster for examination at 40x and 100x in order to quantify the number of parasites per ml and/or per gram of fecal sample.

Although the results showed clearly the greater effectiveness of the Ritchie technique, the statistical test of Mann-Whitney (Wilcoxon) was applied to compare means.

As Ritchie came out to be the most effective technique, the rest of the samples (446) were analized by this technique.

A comparative study of enteroparasitosis has been made for zone (urban/rural) and sex. Statistical analysis was done to study the prevalence of each parasite. It has been realized a study to compare proportions between the rates of infestation in urban and rural areas and between sexes through a hypothesis test. Multiparasitism was studies too.

3 RESULTS

We analyzed 546 fecal samples of school children from the province of Tetouan. In urban areas, 276 samples were collected; in the college of 18 Noviembre, 108 samples were analyzed, (56 from girls and 52 from boys), and from the college of Ahmed Bakal, 168 samples were analyzed, (93 from girls and 75 from boys). From rural areas, 270 samples were collected; 118 samples, (66 from girls and 52 from boys), from the college of Jamaate Ben Karich, and 152 samples, (70 from girls and 82 from boys), from the college of Azla.

A hundred of these samples were used for a comparative quantitative study of the Ritchie's and Faust's techniques. Results showed that Ritchie's technique was more sensitive than the Faust's. Ritchie's allowed to recover more parasitic forms, almost the double, 2062 x 10^4/ml by Neubauer and 2082 eggs/g by McMaster and by Faust's 1014 x 10^4/ml by Neubauer and 1025 eggs/g by McMaster (Table I). This fact was also demonstrated by analyzing individually the 2 techniques for each parasite, which is especially important in pathogens such as *Giardia lamblia* (where 1027 cysts were recovered by Ritchie's and and 503 cysts by Faust's technique), *Hymenolepis spp*. (35/47 by Ritchie versus 12/20 by Faust) and *Cyclospora cayetanensis* (10 by Ritchie versus 7 by Faust) as is showed in Table 1. By the contrast test of Mann-Whitney (W) the midrange of the results was 93.4167 for the Ritchie technique and 63.5833 for Faust technique, where W = 1878.5 with a p-value = 0.0000185924. These statistical results confirmed with confidence level of 95% that the method of Ritchie was significantly better than the Faust technique in the recovery of enteroparasites.

As Ritchie came out to be the most effective technique, the rest of the samples (446) were analized by this technique.

The general prevalence of intestinal parasitism was found to be 68%, slightly higher in the urban areas (71%) than in the rural areas (65%). Although the prevalence of intestinal parasites was similar between boys and girls, 68% and 69% respectively, in urban areas the prevalence was 66.1% in boys and 76.5% in girls, while in rural areas it was 70% in boys and 61.5% in girls.

The protozoa detected were *Giardia lamblia, Entamoeba coli/histolytica, Cyclospora cayetanensis, Isospora belli, Iodamoeba buschlii, Endolimax nana, Chilomastix mesnili,* and *Cryptosporidium spp*. *Blastocystis hominis* was the most frequent. The helminths were *Trichuris trichiura, Hymenolepis nana, H. diminuta, Enterobius vermicularis, Taenia saginata, Ascaris lumbricoides* and *Fasciola hepatica.* Table II shows the general prevalence of each parasite and the prevalence for both urban and rural zones. *E. nana* only was observed in rural areas. Similar prevalences of the parasites were observed between both areas. Table IIIa shows the prevalence of each parasite in boys and girls of urban and rural zones and the study to compare proportions between the rates of infestation of boys and girls in these areas through a hypothesis test. Only *Giardia lamblia* showed notable differences between boys and girls in urban areas. To compare the prevalence of parasites in children with the same sex in different areas the result were shown in Table IIIb. In this case, the differences were only found in boys when they were infected by *B. hominis*, *G. lamblia* and *E. nana*.

Multiple parasitic infections were common (Table IV), with 276 children presenting intestinal parasitosis, and 80 (29%) of these with two, three, or four intestinal parasites. The most frequent cases of multiple parasitism were *B. hominis* and *G. lamblia*, and *B. hominis* and *C. cayetanensis*. Notably, pathogenic enteroparasites as *G. lamblia* and *C. cayetanensis* were associated in samples with three or four.

4 DISCUSSION

The prevalence of enteroparasitoses is linked to both poor hygiene and inadequate sources of drinking water [2]. Also, intestinal parasites have become a public health concern in developed countries because of the increase in the number of inmunocompromised subjects. Physicians in developed and developing countries are now requesting frequent stool examinations for intestinal parasites, or they are recommending at least one stool examination per year, especially for immunocompromised patients [30]. Parasitologic techniques provide true diagnoses since the causative agent is demonstrated directly and have low cost differing from other more sophisticated techniques. The epidemiological studies that lead to the establishment of prevention and control programs against intestinal parasites in developing countries requires economic and sensitive techniques. Several studies have demonstrated that Ritchie and Faust are the better techniques for recuperation of parasites of fecal samples and their low cost are the most useful consideration in developing countries even comparing both techniques with the TF-Test, which have a higher sensitivity than these traditional techniques [6], [7].

In this study, Ritchie's and Faust's techniques were compared quantitatively in order to select the one that allows greater recovery of parasites. Our global results showed a marked difference between the two techniques. Recovery of parasites (Table I) was more than double in the Ritchie technique than in Faust (2062 x 10^4/ml or 2082/gr versus 1014 x 10^4/ml or 1025/gr), this fact let that some enteroparasites were identified by the technique of Ritchie in negative samples by the technique of Faust. This happened with 20% of the samples. Also we analyzed each parasite by both techniques (Table I) and never were recovered more parasitic forms by Faust technique that by Ritchie, and in the majority of cases the parasites recovered were double or more by Ritchie than by Faust. This is especially important in the case of enteropathogens as *G. lamblia* (1027 x 10^4/ml versus 503 x 10^4/ml), *C. cayetanensis* (10 x 10^4/ml versus 6 x 10^4/ml), *Hymenolepis spp*. (35 x 10^4/ml or 4735 x 10^4 versus 12 x 10^4/ml or 20/gr) or *Taenia spp*. (4 x 10^4 / ml or 635 x 10^4 or 6/gr versus 2 x 10^4/ ml or 3/gr). In the case of *B. hominis,* that actually is considered as well as a pathogen, [31], [32] occurred equally (956 x 10^4/ml versus 478 x 10^4/ml). This confirms the results of previous work [6] where it was refuted that *B. hominis* could not be identified by Ritchie. Other parasites like *E. coli* or *D. dentriticum* not even diagnosed by Faust technique.

Many laboratories have developed modifications in the traditional Ritchie's method [33], [34], [35] in order to avoid toxicological effects but none is more efficient than the original.

For all this, an epidemiological study of esteroparasites in Moroccan children was done using Ritchie's technique. This study shows that intestinal parasitic infections are a public health problem in Morocco, because the general prevalence of enteroparasites was found to be 68%. This is somewhat higher than the prevalence found in previous years ranging from 14 - 57%, with the higher values corresponding to children [17], [18], [26], [20]. This indicates that the socioeconomic and sanitary conditions have not changed significantly during these years or have perhaps worsened, as practically the same parasitic diseases occur in urban and rural areas.

Table 2 shows that the most prevalent parasite was *Blastocystis hominis* (56.04%). *B. hominis* has long been described as a nonpathogenic protozoan parasite until recently when claims were made that it may be a cause of intestinal disorders [31], [32]. Its frequency in children and adults in developing countries has been widely proven [31], [36], [37]. The presence of five or more *B. hominis* forms per high-powered field is reported by most laboratories, leaving the clinical significance and the decision to treat to the consulting physician based on clinical evidence [38]. In these cases we recommended treating with co-trimoxazole or metronidazole.

Among pathogenic protozoa, *G. lamblia* was the most prevalent (16.6%). This prevalence is somewhat lower than that found in the most recent published works [24], [39] in which it appears in 22.71% of fecal samples analyzed. This difference may be due to the fact that these samples were from hospital (from symptomatic persons), while in our study we examined samples of theoretically healthy children. *G. lamblia* (Table II) appeared almost at the same percentage in children from urban areas (17%) as from rural areas (16.2%). This may be due to the zoonotic nature of this parasite, as living with pets is common in the neighborhoods studied, both in the urban and in the rural areas. For *G. lamblia*, zoonotic transmission has been described [40], [30]. By adopting appropriate descriptive and molecular epidemiological studies, particularly in defined endemic foci and where close coexistence between susceptible humans, livestock and companion animals exists, the zoonotic potential of this parasite should be able to be elucidated [41], [42], [43], [44]. Within the *G. lamblia* species complex, there are currently eight described assemblages [45]. The majority of these assemblages are host-specific. However, two, assemblages A and B, are considered zoonotic and are the only assemblages commonly accepted as being infectious to humans [43]. Therefore, one, or both, will be responsible for the infection of children, and are also transmitted between them. The prevalence in the urban area was higher in boys (22.1%) than in girls (11.02%) while there were no major differences between the two sexes in the rural areas (Table IIIa). This also happened with helminths such as *H. nana* and *H. diminuta*, and may be due to better personal hygiene in girls. When prevalence of parasites was compared in boys of different areas, (Table IIIb), significant differences appeared for *B. hominis, G. lamblia and E. nana*, with higher prevalence in boys of urban areas than in boys in rural. This may be because rural and urban schools hygienic conditions were similar and some parasites are endemic in Tetuoan.

Cyclospora cayetanensis presented a prevalence of 6.6%, lower than that of *G. lamblia* (Table II). The cyclosporiosis is an antroponosis whereby no animal transmission occurs, with the main transmission routes instead being food and water. In our study there were few differences between prevalences values of urban and rural areas, as is the case with the majority of diagnosed parasites. This may be because the standard of living in studied urban areas is not homogeneous and buildings with drinking water alternate with houses where water is supplied from wells, in the majority of cases, left open or not properly closed. Similar results were therefore obtained in other studies carried out also in Morocco [18]. It should also be noted that the parental educational pattern was similar in both groups. Both mother and father of rural and urban group were poorly educated, which may be a factor in the prevalence of human intestinal parasitic infection in developing countries [46].

The prevalence of helminths was significantly lower than that of protozoa (Table II), as in other studies in Morocco [18], [19], [20], [26]. Thus the most common were *Hymenolepis nana* and *H. diminuta* with a prevalence of only 1.28%.

The few differences in the prevalence of intestinal parasites in rural and urban areas (Table II) could also be indicative of the sensitivity of the Ritchie's technique chosen. But it could also been seen that children in urban areas were mostly asymptomatic and they presented quantitatively lower number of parasites in their feces while in rural areas 60% the children had diarrheal stools and other symptoms associated with parasitic infections; their feces also carried a higher number of cysts, eggs and trofozoites.

Multiple parasitic infections were common 29% of these with two, three, or four intestinal parasites (Table 4). The most frequent association was *B. hominis + G. lamblia* (43 samples) *and B. hominis and C. cayetanesis* (10 samples). These children had severe diarrhea as well as those who had *B. hominis*, *G. lamblia* and *C. cayetanensis* (4 samples), and were treated immediately. This also indicates the extent of the problem of intestinal parasites in Morocco and its severity in children. This type of study therefore highlights the importance of prevention and control programs as intestinal infection is one of the major causes of childhood malnutrition, anemia, stunted physical and mental growth, psycho-social problems with high morbidity in children, and remains a major cause of high infant and child mortality [46].

Table I. Comparison of enteroparasites recovered by Ritchie's and Faust's technique in faecal samples of Moroccan children.

Parasites	Ritchie	Faust
	N/Mc	N/Mc
Giardia lamblia	1027	503
Blastocystis hominis	956	478
Hymenolepis spp.	35/47	12/20
Cyclospora cayetanensis	10	7
Chilomastix mesnili	9	2
Entamoeba coli	8	0
Ascaris lumbricoides	5/8	5/6
Taenia spp.	4/6	2/3
Endolimax nana	3	3
Iodamoeba bustchlii	3	1
Enterobius vermicularis	1/3	1/2
Dicrocoelium dendriticum	1/2	0/0
	2062/2082	**1014/1025**

N: *number of parasites counted with Neubauer chamber x* 10^4 */ml*
Mc: *number of parasites counted with McMaster chamber / g*

By the contrast test of Mann-Whitney (W) the midrange of the results was 93.4167 for the Ritchie technique and 63.5833 for Faust technique.
W = 1878.5
p-value = 0.0000185924.

Table II. Prevalence (%) of intestinal parasites in children of urban and rural zones of Tetouan.

Parasites	Urban (nº=276)		Rural (nº=270)		Total (nº=546)		p-value *
	Nº	%	Nº	%	Nº	%	
Blastocystis hominis	160	58	146	54.07	306	56.04	0,3549
Giardia lamblia	47	17.02	44	16.2	91	16.6	0,7969
Chilomastix mesnili	3	1.08	5	1.85	8	1.46	0,4534
Isospora belli	0	0	1	0.37	1	0.18	0,3118
Cyclospora	20	7.2	16	5.9	36	6.6	0,5395
Cryptosporidium	1	0.36	0	0	1	0.18	0,3237
Entamoeba coli	8	2.9	4	1.48	12	2.1	0,2578
Entamoeba histolytica	2	0.72	0	0	2	0.36	0,1625
Endolimax nana	0	0	4	1.48	4	0.73	0,0425**
Iodamoeba bustchlii	1	0.36	1	0.37	2	0.36	0,9845
Ascaris lumbricoides	1	0.36	0	0	1	0.18	0,3237
Trichuris trichiura	4	1.45	2	0.74	6	1.1	0,4246
Enterobius vermicularis	2	0.72	0	0	2	0.36	0,1625
Taenia saginata	0	0	1	0.37	1	0.18	0,3118
Hymenolepis nana	4	1.45	3	1.11	7	1.28	0,7236
Hymenolepis diminuta	4	1.45	3	1.11	7	1.28	0,7236
Fasciola hepatica	1	0.36	0	0	1	0.18	0,3237

Nº: *number of samples*

** Study to compare proportions between the rates of infestation in urban and rural areas through a hypothesis test. The level of significance was set at p<0.05.*

*** The data shows significant evidences to not accept the proportions equality between the rural and urban areas.*

Table IIIa: Prevalence of intestinal parasites in children of urban and rural zones on Tetouan by sexes

Parasites	Urban					Rural					Total	
	Girls (Nº=127)		Boys (Nº=149)		p-value*	Girls (Nº=140)		Boys (Nº=130)		p-value*	Total (Nº=546)	
	Nº	%	Nº	%		Nº	%	Nº	%		Nº	%
Blastocystis hominis	69	53.3	91	61.07	0,1931	79	56.4	67	51.5	0,4195	306	56.04
Giardia lamblia	14	11.02	33	22.1	0,0146**	24	17.1	20	15.3	0,6886	91	16.6
Chilomastix mesnili	1	0.7	2	1.3	0,6216	2	1.4	3	2.3	0,5818	8	1.46
Isospora belli	0	0	0	0	-	0	0	1	0.76	0,3014	1	0.18
Cyclospora	11	8.6	9	6.1	0,4247	9	6.4	7	5.3	0,7008	36	6.6
Cryptosporidium	0	0	1	0.67	0,3554	0	0	0	0	-	1	0.18
Entamoeba coli	4	3.1	4	2.03	0,5721	2	1.4	2	1.5	0,9452	12	2.1
Entamoeba histolytica	0	0	2	1.3	0,1972	0	0	0	0	-	2	0.36
Endolimax nana	0	0	0	0	-	2	1.4	2	1.5	0,9452	4	0.73
Iodamoeba butschlii	1	0.78	0	0	0,2801	1	0.71	0	0	0,3358	2	0.36
Ascaris lumbricoides	0	0	1	0.67	0,3554	0	0	0	0	-	1	0.18
Trichuris trichiura	2	1.57	2	1.34	0,8732	1	0.71	1	0.76	0,9616	6	1.1
Enterobius vermicularis	1	0.78	1	0.67	0,9142	0	0	0	0	-	2	0.36
Taenia saginata	0	0	0	0	-	1	0.71	0	0	0,3358	1	0.18
Hymenolepis nana	1	0.78	3	2.01	0,3933	2	1.4	1	0.76	0,6131	7	1.28
Hymenolepis diminuta	1	0.78	3	2.01	0,3933	2	1.4	1	0.76	0,6131	7	1.28
Fasciola hepatica	0	0	1	0.67	0,3554	0	0	0	0	-	1	0.18

Nº:*number of samples*

** Study to compare proportions between the rates of infestation of men and women in urban and rural areas through a hypothesis test. The level of significance was set at p<0.05.*

***The data shows significant evidences to not accept the proportions equality between two sexes in urban areas.*

Table IIIb: Prevalence of intestinal parasites in children of urban and rural zones on Tetouan by sexes

Parasites	Girls					Boys						
	Urban (Nº=127)		Rural (Nº=140)		p-value*	Urban (Nº=149)		Rural (Nº=130)		p-value*	Total (Nº=546)	
	Nº	%	Nº	%		Nº	%	Nº	%		Nº	%
Blastocystis hominis	69	53.3	79	56.4	0.6112	91	61.07	67	51.5	0.1076**	306	56.04
Giardia lamblia	14	11.02	24	17.1	0.1553	33	22.1	20	15.3	0.1481**	91	16.6
Chilomastix mesnili	1	0.7	2	1.4	0.5782	2	1.3	3	2.3	0.5270	8	1.46
Isospora belli	0	0	0	0	-	0	0	1	0.76	0.2864	1	0.18
Cyclospora	11	8.6	9	6.4	0.4941	9	6.1	7	5.3	0.7742	36	6.6
Cryptosporidium	0	0	0	0	-	1	0.67	0	0	0.3498	1	0.18
Entamoeba coli	4	3.1	2	1.4	0.3452	4	2.03	2	1.5	0.7386	12	2.1
Entamoeba histolytica	0	0	0	0	-	2	1.3	0	0	0.1921	2	0.36
Endolimax nana	0	0	2	1.4	0.1808	0	0	2	1.5	0.1335**	4	0.73
Iodamoeba butschlii	1	0.78	1	0.71	0.9470	0	0	0	0	-	2	0.36
Ascaris lumbricoides	0	0	0	0	-	1	0.67	0	0	0.3498	1	0.18
Trichuris trichiura	2	1.57	1	0.71	0.5047	2	1.34	1	0.76	0.6385	6	1.1
Enterobius vermicularis	1	0.78	0	0	0.2951	1	0.67	0	0	0.3498	2	0.36
Taenia saginata	0	0	1	0.71	0.3414	0	0	0	0	-	1	0.18
Hymenolepis nana	1	0.78	2	1.4	0.6284	3	2.01	1	0.76	0.3799	7	1.28
Hymenolepis diminuta	1	0.78	2	1.4	0.6284	3	2.01	1	0.76	0.3799	7	1.28
Fasciola hepatica	0	0	0	0	-	1	0.67	0	0	0.3498	1	0.18

Nº:*number of samples*

** Study to compare proportions between the rates of infestation in urban and rural areas for boys and girls through a hypothesis test. The level of significance was set at p<0.05.*

***The data shows significant evidences to not accept the proportions equality between boys of different areas.*

Table IV:The most frequent multiple infections by intestinal parasites in children of Tetouan

	Number of cases
2 parasites	
Blastocystis hominis + Giardia lamblia	43
Blastocystis hominis + Cyclospora cayetanensis	10
Blastocystis hominis + Chilomastix mesnili	3
Blastocystis hominis + Endolimax nana	1
Blastocystis hominis + Entamoeba coli	1
Giardia lamblia + Entamoeba coli	1
Chilomastix mesnilii + Endolimax nana	1
Iodamoeba butschlii + Cyclospora cayetanensis	1
Isospora belli + Cyclospora cayetanensis	1
Blastocytis hominis + Enterobius vermicularis	1
Giardia lamblia + Enterobius vermicularis	1
Blastocystis homines + Tania saginata	1
Blastocystis hominis+ Trichiuris trichiura	1
Cyclospora cayetanesis + Trichuris trichura	1
Giardia lambia + Hymenolepis nana	1
Blastocystis hominis + Hymenolepis dimunita	1
Hymenolepis nana + Hymenolepis dimunita	1
3 parasites	
B. hominis + G. lamblia + C. cayetanensis	4
B. hominis + H. nana + H. diminuta	4
B. hominis + G. lamblia + E. coli	2
B. hominis + E. coli + C. cayetanensis	1
B. hominis + G. lamblia + E. nana	1
B. hominis + E. nana + C. mesnili	1
B. hominis + E. coli + E. histolytica	1
B. hominis + E. coli + C. mesnili	1
B. hominis + C. cayetanensis + Iodamoeba bustchlii	1
C. mesnili+C. cayetanensis +B. hominis	1
4 parasites	
B. hominis + G. lamblia + C. cayetanensis + E. coli	1
B. hominis+ G. lamblia + C. cayetanensis + T. trichura	1

5 CONCLUSION

This study shows that intestinal parasitic infections are actually a public health problem in Morocco, because the general prevalence of enteroparasites was found to be 68%. The most frequent of the intestinal parasites was *Blastocystis hominis* (56%) and the most frequent pathogenic protozoa were *Giardia lamblia* (16.6%) followed by *Cyclospora cayetanensis* (6.6%).

We recommended the Ritchie's technique for enteroparasites diagnostic, of both protozoan and helminthes, especially under conditions of low parasite burdens, which is important in pathogens.

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