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Detection of Different Enteric Protozoa Parasites with Combination of Immunological and Microscopic Methods, in Albania

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Abstract

Enteric protozoa are associated with diarrhoeal illnesses in humans, particularly in children, and represent a significant threat to public health that often was neglected. Several enteric protozoa cause severe morbidity and mortality in both humans and animals worldwide. Therefore, the study aims were to estimate the prevalence of enteric protozoa in children, comparison of the efficiency of microscopy and ELISA procedure in diagnose of protozoa, and in addition to shed light on risk behaviour for enteric protozoa.

During September 2013-August 2014 we have examined 115 hospitalized patients in "Mother Theresa" hospital center in Tirana Albania, for *Entamoeba histolytica*; *Cryptosporidium parvum* and *Giardia lamblia*. Two methods, classic microscopy and ELISA were used for examination of enteric parasites in our study.

The average year was 6.66 and the minimum age was 3 months old and maximum 15 years old. *Based to the data 53.04% were female and 46.95% male. The prevalence of* E histolytica; *C parvum* and *G lamblia* resulted 4.34%, 2.6% and 12.17% respectively by microscopy. By ELISA method the prevalence resulted 7.82%, 4.34% and 20.87% respectively. Also about 18; 44; 44 samples respectively are considered as equivocal by ELISA test. This high result of equivocal test to patients maybe were as result of the cross reaction between protozoa parasites. Depended of the methods that we have used the male were the most contaminated sex.

In our study ELISA methods resulted to be more sensitive compared to classic microscopic, but other tests like PCR-based tests need to be used for understanding the actual prevalence and epidemiology of these protozoan parasites.

Keywords: Enteric parasites; Classic microscopy test; ELISA test; Pediatric ages; Albania

Introduction

Enteric Protozoa (EP) are associated with diarrhoeal illnesses in humans, particularly in children, and represent a significant threat to public health that often was neglected [1]. Several EP cause severe morbidity and mortality in both humans and animals worldwide [2]. The World Health Organization (WHO) ranks diarrhoeal disease as the second highest cause of morbidity and mortality in children in the developing world [3-5]. In those countries the impact of protozoan pathogens represents a major cause of gastrointestinal illnesses and is becoming of growing impact [6]. EP is transmitted by the fecal-oral route and exhibit life cycles consisting of a cyst stage and a trophozoite stage. Particularly, more than 58 million cases of diarrhoeal detected per year in children are associated to EP infections with high morbidity and mortality infection rates [7]. *Giardia*, *Cryptosporidium parvum*, *Dientamoeba fragilis*, *Entamoeba* spp. (including non-pathogenic species), *Blastocystis* spp., *Cyclospora cayetanensis, Escherichia coli*, particularly enterotoxigenic and enteropathogenic, Rotavirus etc are the most important and prevalent infections reported in developed country settings [8-11].

Cryptosporidiasis as a disease is caused by *Cryptosporidium* spp. The global burden of this disease is still under ascertained. Clinical underestimations of protozoan etiology in developed countries contribute to the underestimation of the worldwide burden. In children, cryptosporidiosis encumber is even less recorded and often misidentified due to physiological reasons such as early-age unpaired immunological response [2]. Giardiasis disease is caused by *Giardia duodenalis* (syn. lamblia or intestinalis) is the most frequent cause of nonbacterial diarrhoeal throughout the world [12]. Each year 500 000 new cases are reported and about 200 million people develop symptomatic giardiasis [13]. *Entamoeba histolytica* is an invasive intestinal pathogenic parasitic protozoan that causes amebiasis. About 40-50 million people develop clinical amoebiasis each year that resulting on up to 100 000 deaths [14]. Most *Entamoeba histolytica* infections are asymptomatic and trophozoites remain in the intestinal lumen feeding on surrounding nutrients. About 10-20% of the infections develop into amoebiasis [15]. Traditionally parasites have been identified by simple microscopy and serologic methods [16-19]. The study aim was to estimate the prevalence of enteric protozoa in children and to compare the efficiency of microscopy and ELISA procedure in diagnose, so on this article we have presented the optimal diagnostic approaches of pathogenic protozoa in our country. In addition the study aims to shed light on risk behaviour for EP.

Materials and Methods

Over one year (September 2013-August 2014) 115 patients hospitalized in hospital centre "Mother Theresa" in Tirana, Albania, with problems in gastrointestinal tract have been examined for enteric protozoa like *Giardia lamblia, Entamoeba hystolitica and Criptosporidium parvum*. A single faecal sample was collected from each boy and girl aged 3 months until 15 years. With assistance of children parents and nurses, a fresh specimen was collected from each child into a plastic container (approximately 1 g of stool). The fresh stool samples were labeled with unique identification numbers (IDs), and were transferred to the laboratory of Parasitology in Institute of Public Health to assess for intestinal protozoa cysts.

Firstly, stool samples were examined macroscopically. This was done for color, consistency and to ensure that they do not have gross parasitic stages (adult worms, larvae and/or segments of tapeworms). After that they were examined microscopically by wet mount preparations, followed by the formalin-ethyl acetate 10% concentration method. Dry, stained smears were checked for protozoan cysts (at ×100, after trichome staining) and *Cryptosporidium* oocysts (at ×400, after DMSO staining).

Finally, detection of enteric protozoa antigen in all stool samples was done by using RIDASCREEN® *ELISA test*. RIDA® Quick *Giardia lamblia/Entamoeba hystolitica/Criptosporidium parvum test* (R-Biopharm AG, Darmstadt, Germany) is an enzyme immunoassay based on the detection of antigens of *parasites* cysts and trophozoites in stool specimen.

A standardized questioner was provided to the parents of each child. The questioner collected date on demographic, socioeconomic, hygiene behavior and clinical symptoms. SPSS version 19 was used for calculation of data. Selection probabilities were calculated and used to weight the data in the analysis Associations between positivity and potential risk factors for enteric protozoa were investigated via univariate logistic regression analysis. We have calculate odds ratio (OR) and 95% confidence interval and a *p*-value of <0·05 was considered indicative of a statistically significant difference or association.

Results

Overall, 115 patients (61 girls and 54 males) have been diagnose with diarrhea and gastrointestinal disorder hospitalized in pediatric hospital center "Mother Theresa" in Tirana, Albania during September 2013-August 2014. In Figure 1, the prevalence of enteric parasites diagnose with two methods (Microscopy and ELISA) is presented.

ELISA methods	Microscopy methods		Number of samples tested	Sensitivity of ELISA (%)	Specificity of ELISA (%)
	Positive	Negative			
Entamoeba spp. Positive cases	5	10	15	100% [47.82% to 100%]	90.91% [83.92% to 95.55%]
Entamoeba spp. Negative cases	0	100	100		
Entamoeba spp. Total number	5	110	115		
Cryptosporidium parvum Positive cases	3	5	8	100% [76.84% to 100%]	95.54% [66.74% to 84.14%]
Cryptosporidium parvum Negative cases	0	107	107		
Cryptosporidium parvum Total number	3	112	115		
Giardia duodenalis Positive cases	14	24	38	100% [76.84% to 100%]	76.24% [82.54% to 95.15%]
Giardia duodenalis Negative cases	0	77	77		
Giardia duodenalis Total number	14	101	115		

Table 1: Comparison of efficacy of ELISA method versus to microscopy method.

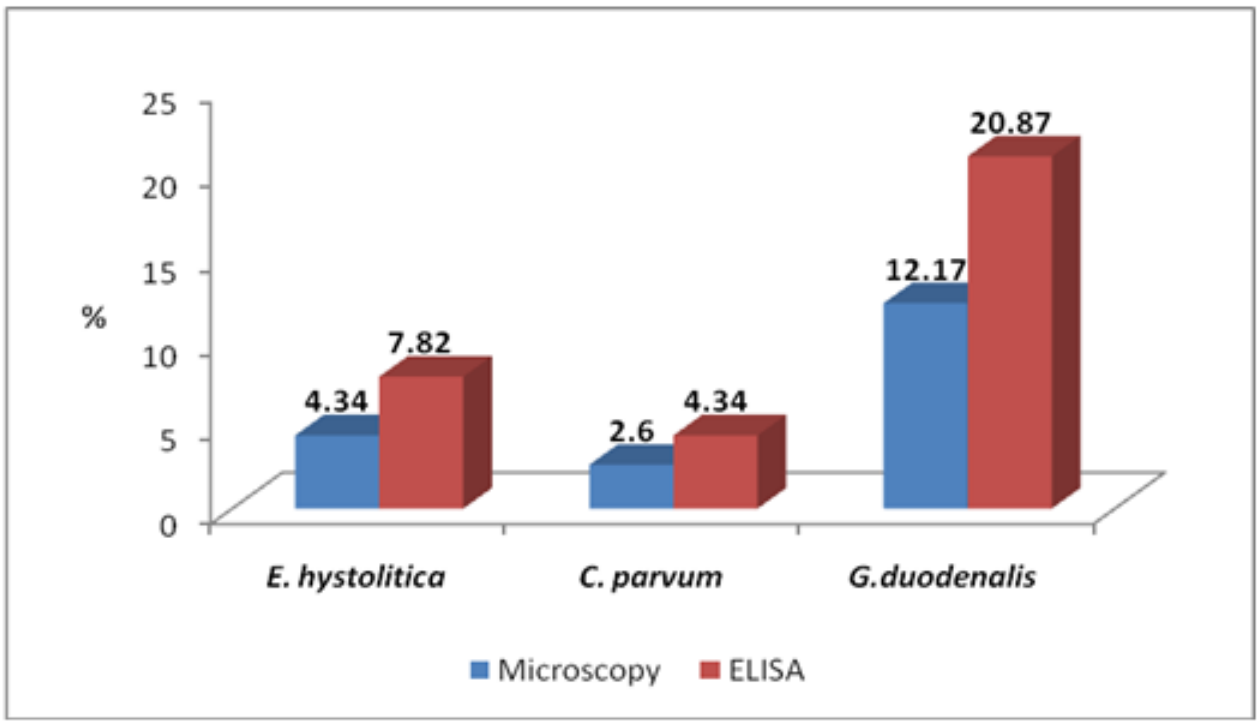

Figure 1: The prevalence of enteric protozoa by microscopy and ELISA methods.

Table 1 presented the comparison of efficacy of ELISA method versus to microscopy method. We have examined the same patients for *Entamoeba hystolitica*, *Giardia duodenalis* and also for *Cryptosporidium parvum*.

Table 2 presented the logistic regressions for risk factor and the prevalence of enteric protozoa.

Risk factor		***Entamoeba hystolitica***		***Cryptosporidium parvum***		***Giardia duodenalis***	
		Prevalence %	p-value Odds Ratio CI 95%	Prevalence %	p-value Odds Ratio CI 95%	Prevalence %	p-value Odds Ratio CI 95%
Sex							
	Female	8.2%	Reference	6.55%	Reference	19.67%	Reference
	Male	18.51%	3.3 [0.8 to 8.3] p=0.1	7.40%	1.14 [0.27 to 5] p=0.85	48.15%	3.8 [1.6 to 9] p=0.0016
Age							
	0-6 years old	11.42%	Reference	7.14%	Reference	35.71%	Reference
	>6-15 years old	15.55%	1.42 [0.48 to 5] p=0.5	6.66%	1.07 [0.24 to 4.74] p=0.92	28.8%	1.36 [0.60 to 3.07] p=0.44
Economic status							
	High	14.7%	Reference	5.88%	Reference	8.7%	Reference

	Low	12.34%	1.22 [0.38 to 3.89] p=0.7	7.4%	1.28 [0.24 to 7.14] p=0.76	24.34%	1.28 [0.53 to 3.03] p=0.59
Contact with different animals							
	No	3.37%	Reference	1.12%	Reference	6.95%	Reference
	Yes	46.15%	25 [6.25 to 100] p<0.0001	26.9%	33.3 [3.84 to 333.3] p=0.0015	26.08%	3.84 [1.66 to 9.09] p=0.0016
Washing hand before eating							
	Yes	9.52%	Reference	7.14%	Reference	35.71%	Reference
	No	15.06%	1.58 [0.47 to 5.28] p=0.45	6.84%	1.05 [0.23 to 4.76] p=0.95	45.2%	1.26 [0.61 to 2.59] p=0.5
Washing hand after defecation							
	Yes	7.17%	Reference	3.57%	Reference	30.35%	Reference
	No	18.64%	2.61 [0.78 to 8.67] p=0.11	10.16%	2.84 [0.55 to 14.7] p=0.2	52.54%	4.2 [1.71 to 10.31] p=0.0017
Living condition							
	Good	10.71%	Reference	3.57%		26.19%	
	Bad	19.35%	1.8 [0.59 to 5.5] p=0.2	16.12%	4.54 [1.02to 20.8] p=0.04	83.87%	3.3 [1.61 to 6.6] p=0.0011

Table 2: The results of logistic regression on the impact of risk factors on positivity for enteric protozoa.

Table 3 presented the logistic regressions for some of symptoms related to the enteric protozoa and the prevalence of each of them.

Symptoms		***Entamoeba hystolitica***		***Cryptosporidium parvum***		***Giardia duodenalis***	
		Prevalence % N	p-value Odds Ratio CI 95%	Prevalence %	p-value Odds Ratio CI 95%	Prevalence %	p-value Odds Ratio CI 95%
Abdominal pain	No	2.77%	Reference	0%	Reference	30.5%	Reference
	Yes	17.72%	7.69 [0.95 to 62.5] p=0.05	10.12%	9.09 [0.48 to 166.6] p=0.14	34.17%	1.19 [0.50 to 2.7] p=0.7
Diarrhea	No	13.63%	Reference	4.54%	Reference	90.90%	Reference
	Yes	12.9%	2.56 [0.30 to 25] p=0.39	7.52%	1.72 [0.19 to 16.6] p=0.62	19.35%	41.66 [8.91 to 194.73] p<0.0001
Digestive symptoms	No	6.55%	Reference	3.27%	Reference	24.6%	Reference
	Yes	20.37%	3.7	11.11%	3.7 [0.69 to 20] p=0.12	42.6%	2.32

			[1.08 to 12.5] p=0.03				[1.03 to 5.26] p=0.04
Vomiting	No	13.8%	Reference	5.74%	Reference	28.73%	Reference
	Yes	10.71%	1.33 [0.34 to 5.11] p=0.67	10.71%	2 [0.44 to 9.09] p=0.37	46.42%	2.17 [0.9 to 5.26] p=0.08
Fever	No	15.38%	Reference	6.59%	Reference	35.16%	Reference
	Yes	4.16%	4.18 [0.52 to 33.5] p=0.17	8.33%	1.29 [0.24 to 7.14] p=0.76	25%	1.62 [0.58 to 4.50] p=0.34
Other person in family with gastrointestinal symptoms	No	15.11%	Reference	9.3%	Reference	11.6%	Reference
	Yes	6.89%	2.4 [0.5 to 11.35] p=0.26	0%	6.38 [0.35 to 114.20] p=0.2	96.55%	238 [33.3 to 2000] p<0.0001

Table 3: The result of logistic regression for the relation between clinical symptoms and positivity for enteric protozoa.

Discussion

The prevalence for enteric protozoa especially the parasites that cause diarrhoeal illnesses or gastro enteric diseases is still underestimation in our country. Most of persons with diarrhoeal illnesses especially children are still ascribed to an unknown etiology.

Actually, in Albania, the detection of intestinal parasites on the large part of clinical microbiology laboratories (public and private) is still almost exclusively based on native (wet mount preparations) methods microscopic examination. The other laboratories used the concentration methods like Sulphate-Zinc for detection of parasites. In Institute of Public Health, in Tirana the examination of parasites was based to the different methods such as; native, formalin-ethyl acetate 10% concentration method, staining smear and also antigen detection by ELISA (Enzyme-linked immunosorbent *assay*). This article demonstrates the prevalence of enteric protozoa in children with gastro enteric diseases and also we have done comparisons of the efficiency of different procedures in diagnose of protozoa parasites.

Protozoan infections significantly contribute to the burden of gastrointestinal illness worldwide [1,20,21]. Nowadays *Cryptosporidium* and *Giardia* are still major cause of diarrhoeal diseases of humans worldwide and are included in the World Health Organisation's Neglected Diseases Initiative [22,23]. The most affected by those parasites are the poorest population's often living inadequate condition, in remote and rural areas. So those diseases often are indicators of poverty and disadvantage [24].

For all protozoa-related gastroenteritis, direct observation of the parasite from stools is the confirmatory diagnostic methods [25]. Microscopic examination method is the traditional method for stool parasite testing but this is labour-intensive and requires a high level of skill for optimal interpretation, this test remains the cornerstone of diagnostic testing for the intestinal protozoa.

In our study we have used the conventional microscopy and RIDASCREEN® *Giardia/Criptosporidium/Entamoeba ELISA tests.* Our results confirm that EP is a public health issue on our country. More than half (61/115) of the children were infected with at least one of the three (*G. duodenalis*, *Entamoeba hystolitica* and *C. parvum*) pathogenic intestinal protozoa.

The prevalence of *G duodenalis*, *E. hystolitica* and *C. parvum* in our study resulted 12.17%, 4.34%, 2.6% respectively by microscopy and by ELISA test the prevalence resulted 20.87%, 7.82% and 4.34 respectively. Also the vast majority of samples are considered as equivocal by ELISA test. This high result of equivocal test to children samples can be explain as result of the cross reaction between protozoa parasites.

The result taken in our study by RIDASCREEN Giardia/ Criptosporidium/Entamoeba (ELISA) test was far better than microscopy. The sensitivity and specificity of ELISA test in comparison with microscopy were presented in Table 1. The finding indicates that the sensitivity for three parasites was 100% but specificity was more 90% for CI 95%. Stool antigen enzyme-linked immunosorbent assay (ELISA) may be helpful in cases were examination with microscope concentration methods are negative but patients have consistent symptoms and parasites are still suspected.

Multiple studies have evaluated the sensitivities and specificities of the available kits and found overall [26-29].

In Table 2, the risk factors associated with EP are presented. For this analyse we have done the logistic regression for each risk factor. For demographic data we have analysed the sex and age. We found that boys were more contaminated with any of the *Entamoeba*, *Criptospiridium* and *Giardia* compared to girls. Other authors founded similar results in their study carried out to the children [30,31]. A strong significance level was been seen between male and presence of *G. lamblia* parasites. Male were 3.8 times in risk compared to female for CI 95% p value=0.0016. We have not found an association between other parasites and sex.

Regarding the age the mean age was 6.66 year with minimum age 3 months old and maximum 15 years old. All children have grouped into two major groups 0-6 years old the first and >6-15 years old the second group. Children 0-6 years old had a low rate of infection compared to other group >6-15 years old. In this group the infection rate was highest. This may be for reason that in this group the children are

independent in toilet use. Also this group are more involved in outdoor activities compared to other group [32]. About the association between age group and infection we have not found (p value resulted>0.05).

Regarding the other risk factor (economic status, contact with different animals, hand washing before eating, after defecation and living condition), we have not found an association between high and low economic status and infection, p value resulted>0.05. A strong association we have found for contact with different animals. Children, positive with *Entamoeba* and have contact with animals were 25 times in risk compared to the children without contact with animals (CI 95% [6.25 to 100] p<0.0001). Children, positive with *Cryptosporidium* and contact with animals were 33.3 times in risk compared to the children without contact with animals (CI 95% [3.84 to 333.3] p=0.0015). Children, positive with *Giardia* and contact with animals were 3.84 times in risk compared to the children without contact with animals (CI 95% [1.66 to 9.09] p=0.0016).

A strong association was found only to the children who present presence of *G. Lamblia* and them that washing hand before defecation. Those children were 4.2 times in risk compared to children that washing hand CI 95% p value =0.0017. And for living condition (good or bad) an association we have found between presence of *Cryptosporidium* and *Giardia* in children that living in bad condition p value were 0.04 for them with *Cryptosporidium* positive and 0.0011 for them with *Giardia* positive (Table 2).

About the association between symptoms and presence of parasites a significant association was been found for abdominal pain and presence of Entamoeba (odds ratio 7.69 CI 95% [0.95 to 62.5] p=0.05, for diarrhoea and presence of *Giardia* (odds ratio 41.66 CI 95% [8.91 to 194.73] p<0.0001, for digestive symptoms and presence of *Entamoeba* and *Giardia* p value <0.03 and 0.04 respectively and for other persons in family with gastrointestinal symptoms and presence of Giardia we have found a strong significance (odds ratio 238 CI 95% [33.3 to 2000] p value <0.0001) (Table 3).

This fact often was for available detection methods, such as microscopy used in many laboratories, have low sensitivity.

Conclusion

This is the first study performed in Albania reporting the prevalence and the clinical epidemiological data associated with enteric protozoan infections among children hospitalized in Tirana "Hospital Center Mother Thereza". Our study indicates that enteric protozoa infections are highly prevalent among children especially when ELISA methods are used. The most predominant protozoan found in our study with two methods was *Giardia lamblia* in comparison with *Entamoeba hystolitica* and *Cryptosporidium parvum*. But the prevalence of those protozoa are still underestimation in our country because the methods used in diagnosis are not standardized and there is a difficulty in accurately of diagnosing infections and also the lack of qualification staff (staff with experience in diagnosis of parasites in humans). Also detection methods, such as microscopy used in many laboratories, have low sensitivity. The difficulty in diagnosing enteric protozoa infections in large samples with different methods is more evident in our country. More than 50% of children reported symptoms (abdominal pain, diarrhea, vomiting, digestive symptoms etc), and some of them we found association. ELISA methods resulted to be more sensitive compared to classic microscopic, but other tests like PCR-based tests need to be used for understanding the actual prevalence and epidemiology of these protozoan parasites. Thus, it is too necessary to designs strategies in prevention and control to reduce the burden of these protozoan infections, especially in children.

References

1. Osman M, El Safadi D, Cian A, Benamrouz S, Nourrisson C, et al. (2016) Prevalence and Risk Factors for Intestinal Protozoan Infections with Cryptosporidium, Giardia, Blastocystis and Dientamoeba among Schoolchildren in Tripoli, Lebanon. PlosNegl Trop Dis 10: e0004643.
2. Fletcher SM, Stark D, Harkness J, Ellis J (2012) Enteric Protozoa in the Developed World: a Public Health Perspective. Clin Microbiol Rev 25: 420-449.
3. WHO (2005) Making every mother and child count: World Health Report, World Health Organization, Geneva, Switzerland.
4. WHO (1996) Fighting disease, fostering development: World Health Report, World Health Organization, Geneva, Switzerland.
5. Pelayo LL, Nuñez FA, Rojas LL, Wilke HH, Hansen EEF, et al. (2008) Molecular and epidemiological investigations of cryptosporidiosis in Cuban children. Ann Trop Med Parasitol 102: 659-669.
6. Ajjampur SS, Gladstone BP, Selvapandian D, Muliyil JP, Ward H, et al. (2007) Molecular and spatial epidemiology of cryptosporidiosis in children in a semiurban community in South India. J Clin Microbiol 45: 915-920.
7. World Health Organisation (1992) WHO/PAHO informal consultation on intestinal protozoal infections Tech. Rep. WHO/CDS/IPI/92.2, World Health Organisation, Geneva, Switzerland.
8. Fletcher SM, Stark D, Harkness J, Ellis J (2012) Enteric protozoa in the developed world: a public health perspective. Clin Microbiol Rev 25: 420-449.
9. Goodgame RW (1996) Understanding intestinal spore-forming protozoa: cryptosporidia, microsporidia, Isospora, and Cyclospora. Ann Intern Med 124: 429-441.
10. Eckmann L (2002) Small bowel infections. Curr Opin Gastroenterol 18: 197-202.
11. Pierce KK, Kirkpatrick BD (2009) Update on human infections caused by intestinal protozoa. Curr Opin Gastroenterol 25: 12-17.
12. Adam RD (2001) Biology of Giardia lamblia. Clin Microbiol Rev 14: 447-475.
13. Department of Health and Human Services U.S (1994) Addressing Emerging Infectious Disease Threats: A Prevention Strategy for the United States, Centers for Disease Control and Prevention, Atlanta, Ga, USA.
14. Walsh AL (1988) Prevalence in Entamoeba histolytica infection" in Amebiasis: Human Infection by Entamoeba histolytica, J. I. Ravdin, Ed., John Wiley & Sons, New York, NY, USA, pp. 93-105, .
15. http://www.parasitesinhumans.org/entamoeba-histolytica-amoebiasis.html.
16. Svenungsson B, Lagergren A, Ekwall E, Evengård B, Hedlund KO, et al. (2000) Enteropathogens in adult patients with diarrhea and healthy control subjects: a 1-year prospective study in a Swedish clinic for infectious diseases. Clin Infect Dis 30: 770-778.
17. Gascón J, Vargas M, Schellenberg D, Urassa H, Casals C, et al. (2000) Diarrhea in children under 5 years of age from Ifakara, Tanzania: a case-control study. J Clin Microbiol 38: 4459-4462.
18. Germani Y, Morillon M, Begaud E, Dubourdieu H, Costa R, et al. (1994) Two-year study of endemic enteric pathogens associated with acute diarrhea in New Caledonia. J Clin Microbiol 32: 1532-1536.
19. Youssef M, Shurman A, Bougnoux ME, Rawashdeh M, Bretagne S, et al. (2000) Bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from northern Jordan. FEMS Immunol Med Microbiol 28: 257-263.
20. Alum A, Rubino JR, Ijaz MK (2010) The global war against intestinal parasites-should we use a holistic approach? Int J Infect Dis 14: e732-e738.

21. McHardy IH, Wu M, Shimizu-Cohen R, Couturier MR, Humphries RM, et al. (2014) Detection of Intestinal Protozoa in the Clinical Laboratory. J Clin Microbiol 52: 712-720.
22. Savioli L, Smith H, Thompson A (2006) Giardia and Cryptosporidium join the 'neglected diseases initiative". Trends Parasitol 22: 203-208.
23. Neglected Diseases Initiative of the World Health Organization.
24. Putignani L, Menichella D (2010) Global Distribution, Public Health and Clinical Impact of the Protozoan Pathogen Cryptosporidium. Interdiscip Perspect Infect Dis 2010 : 753512.
25. Baron EJ, Miller JM, Weinstein MP, Richter SS, Gilligan PH, et al. (2013) A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). Clin Infect Dis 57: e22-e121.
26. Garcia LS, Shimizu RY (1997) Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of Giardia lamblia and Cryptosporidium parvum in human fecal specimens. J Clin Microbiol 35: 1526-1529.
27. Al-Saeed AT, Issa SH (2010) Detection of Giardia lamblia antigen in stool specimens using enzyme-linked immunosorbent assay. East Mediterr Health J 16: 362-64.
28. Chakarova B (2010) Comparative evaluation of the diagnostic methods for detection of Giardia intestinalis in human fecal samples. Trakia J Sci 8:174-179.
29. Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, et al. (2007) Laboratory diagnostic techniques for Entamoeba species. Clin Microbiol Rev 20: 511-532.
30. Traoré SG, Odermatt P, Bonfoh B, Utzinger J, Aka ND, et al. (2011) No Paragonimus in high-risk groups in Côte d'Ivoire, but considerable prevalence of helminths and intestinal protozoon infections. Parasit Vectors 4: 96.
31. Cifuentes E, Suarez L, Espinosa M, Juarez-Figueroa L, Martinez-Palomo A (2004) Risk of Giardia intestinalis infection in children from an artificially recharged groundwater area in Mexico City. Am J Trop Med Hyg 71: 65-70.
32. Mercado R, Otto JP, Perez M (1999) Seasonal variation of intestinal protozoa infection in outpatients of the north section of Santiago, Chile, 1995-1996. Bol Chil Parasitol 54: 41-44.