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ORIGINAL ARTICLE

Concordance between the zinc sulphate flotation and centrifugal sedimentation methods for the diagnosis of intestinal parasites

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Introduction: The diagnosis of intestinal parasitic infections depends on the parasite load, the specific gravity density of the parasite eggs, oocysts or cysts, and the density and viscosity of flotation or sedimentation medium where faeces are processed.
Objective: To evaluate the concordance between zinc sulphate flotation and centrifugal sedimentation in the recovery of parasites in faecal samples of children.
Materials and methods: Faecal samples of 330 children from day care centers were evaluated by zinc sulphate flotation and centrifugal sedimentation techniques. The frequencies of detection of parasites by each method were determined and the agreement between the diagnostic techniques was evaluated using the kappa index, with 95% confidence intervals.
Results: The faecal flotation in zinc sulphate diagnosed significantly more cases of *Trichuris trichiura* infection when compared to centrifugal sedimentation (39/330; 11.8% vs. 13/330; 3.9%, $p<0.001$), with low diagnostic concordance between methods (kappa=0.264; 95% CI: 0.102-0.427). Moreover, all positive samples for *Enterobius vermicularis* eggs (n=5) and *Strongyloides stercoralis* larvae (n=3) were diagnosed only by zinc sulphate. No statistical differences were observed between methods for protozoa identification.
Conclusions: The results showed that centrifugal flotation in zinc sulphate solution was significantly more likely to detect light helminths eggs such as those of *T. trichiura* and *E. vermicularis* in faeces than the centrifugal sedimentation process.

Key words: Intestinal parasites/diagnosis; child; flotation; sedimentation.

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Concordancia entre los métodos de flotación con sulfato de zinc y sedimentación centrífuga para el diagnóstico de parásitos intestinales

Introducción. El diagnóstico de infecciones parasitarias intestinales depende de la carga de parásitos, la densidad de la gravedad específica de los huevos, ooquistes o quistes de parásitos, y de la densidad y viscosidad de los reactivos de flotación o sedimentación usados para procesar las heces.
Objetivo. Evaluar la concordancia entre el método de flotación de sulfato de zinc y la sedimentación por centrifugación en la recuperación de parásitos en muestras fecales de niños.
Materiales y métodos. Se evaluaron las muestras fecales de 330 niños de guarderías mediante las técnicas de flotación con sulfato de zinc y de sedimentación por centrifugación. Se determinó la frecuencia de detección de parásitos con cada método y se evaluó la concordancia entre las técnicas de diagnóstico mediante el índice kappa, con intervalos de confianza del 95 %.
Resultados. Mediante la flotación fecal con sulfato de zinc, se diagnosticó un número significativamente mayor de casos de infección por *Trichuris trichiura* que con la sedimentación por centrifugación (39/330; 11,8 % *Vs*. 13/330; 3,9 %) ($p<0,001$), con poco acuerdo entre los métodos (kappa=0,264; $IC_{95\%}$ 0,102-0,427). Además, todas las muestras positivas para huevos de *Enterobius vermicularis* (n=5) y larvas de *Strongyloides stercoralis* (n=3) se diagnosticaron solamente por sulfato de zinc. No se observaron diferencias estadísticamente significativas entre los métodos para la identificación de protozoos.

Author's contributions:
Márcia Cristina Aquino Teixeira and Patrícia Silva de Almeida Mendes designed the study and collected the samples.
Milena Carneiro Pinto, Elizabete de Jesus Inês and Flávia Thamires Figueiredo Pacheco performed the laboratory work.
Elizabete de Jesus Inês and Milena Carneiro Pinto analyzed the results.
Márcia Cristina Aquino Teixeira, Elizabete de Jesus Inês, Hugo Costa-Ribeiro Jr, Neci Matos Soares wrote the paper.

Conclusiones. La flotación centrífuga en una solución de sulfato de zinc presentó una probabilidad significativamente mayor de detectar los huevos livianos de helmintos como *T. trichiura* y *E. vermicularis* en heces, que el proceso de sedimentación por centrifugación.

Palabras clave: parásitos intestinales/diagnóstico; niño; flotación;, sedimentación.

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The most commonly used parasitological diagnostic methods for detection of intestinal helminthic and protozoan infections in humans are inexpensive and simple to perform; however, they have important limitations, particularly regarding their sensitivity. Therefore, the use of more than one diagnostic method is necessary to detect different parasitic evolving forms, such as eggs, larvae, cysts, oocysts and trophozoites, due to the differences in size, morphology, density, and mobility among them. Moreover, the use of different parasitological methods is essential to improve sensitivity for helminth and protozoa diagnosis in patients with low parasite burdens (1).

Laboratory diagnosis of intestinal protozoa is performed by identifying the parasite cysts in faecal smears stained with iodine or by detecting the typical trophozoites in a wet or a permanent stained preparation (2). On the other hand, as the adult forms of most worms are rarely seen in faeces, the parasitological diagnosis relies on the identification of helminth eggs or larvae after concentrating the stools, which is usually realized by sedimentation or flotation techniques (3,4).

Spontaneous sedimentation, centrifugal sedimentation, or formalin-ethyl acetate are widely used for the diagnosis of intestinal protozoa and helminths in human and animal stool samples (5-8). The faecal concentration methods based on sedimentation of parasites are useful for recovering heavy parasite eggs due to the concentration of the organisms in the sediment, whereas the centrifugal-flotation in zinc sulphate (9) is used preferentially to recover protozoa cysts because of the low density of parasitic forms as compared to the salt solution. The result is a clean preparation for microscopic examination with a minimal amount of faecal debris. For instance, if the density of a helminth egg is lower or close to the density of the flotation solution, it will float along with cysts of faecal homogenate.

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The purpose of this study was to evaluate the agreement between the zinc sulphate flotation and the centrifugal sedimentation for diagnosing protozoa and helminths in children's faecal samples.

Materials and methods

Faecal samples from 330 children attending day care centers in Salvador, Bahia, Brazil, whose ages ranged between 0 and 8 years, were examined by zinc sulphate flotation (ZS) (9) and centrifugal sedimentation (CS) (7) methods. Briefly, two grams of faeces were homogenized in 10 ml of water and filtered through gauze for each diagnostic method. Subsequently, faecal homogenate was centrifuged in a 15 ml tube for 2 minutes at 400 g twice and the sediment was examined by preparing wet mounts with iodine (CS) or resuspended in zinc sulphate solution (1.18 g/mL) for the flotation process (ZS). Faecal smears from the sedimentation technique were also stained by modified Ziehl-Neelsen (mZN) for coccidian diagnosis. Three slides per sample were examined with each technique.

We determined and compared the proportion of specific parasites identified by each diagnostic method in the faecal samples analysed. We evaluated the concordance between diagnostic methods by calculating kappa indices using the Graph Pad (San Diego, USA) statistical software. The reference kappa values were inferred as follows: (<0) no agreement, (0-0.19) poor, (0.20-0.39) low, (0.40-0.59) moderate, (0.60-0.79) substantial and (0.80-1.00) excellent agreement (10). We performed the statistical analysis of the frequencies of intestinal parasites and the concordance of diagnostic results between the two parasitological methods using the chi-square test; values of $p<0.05$ were considered statistically significant.

Ethical aspects

This study was approved by the Committee of Ethics in Research of the Department of Health of Bahia. Stools were collected from children only after obtaining a formal signed consent from their parents or legal guardians, whom also received the parasitological test results. All children were attended by a pediatrician and treated for enteroparasite infections when necessary.

Results

Of the 330 children analyzed, 217 (65.8%) were infected with at least one intestinal parasite regardless of the diagnostic method employed. According to the combined results of ZS and CS, the most frequent pathogenic protozoa identified were *Giardia duodenalis* (83/330; 25.1%) and *Blastocystis* spp. (46/330; 13.9%). *Cryptosporidium* spp. and *Isospora belli* infections were found in five (1.5%) and in one child (0.3%), respectively. Regarding helminths, *Ascaris lumbricoides* and *Trichuris trichiura* had the highest occurrence in faecal samples (table 1).

Concerning the frequency of specific parasites detected by each diagnostic method, ZS presented a similar proportion of *A. lumbricoides* and *Hymenolepis nana* cases compared to CS, or identified significantly more helminths such as *T. trichiura* ($p<0.001$) (table 1), with a low concordance between methods (kappa=0.264; 95% CI, 0.102-0.427) (table 2). Moreover, all positive samples for *Enterobius vermicularis* eggs (n=5) and *Strongyloides stercoralis* larvae (n=3) were diagnosed only by ZS (table 1). As expected, *Schistosoma mansoni* egg samples were found solely in CS faecal sediments. The ZS method also detected most of the *G. duodenalis* cases (74/83; 89.1%), compared to the CS technique (67/83; 80%). Conversely, the latter identified more frequently the protozoan *Blastocystis* spp. in infected children (38/46; 82.6%) vs. the ZS (33/46; 71.7%). However, no statistical differences were observed, and there was substantial agreement between methods for both parasites: kappa=0.775; 95% CI: 0.691-0.859, and kappa=0.669; 95% CI: 0.537-0.801, respectively (table 2).

Discussion

The comparison of parasitological methods often requires study populations with high frequency of parasitic infections. In the present study, 217 out of 330 stools examined were positive for one or more intestinal parasites, allowing a correct assessment of the diagnostic concordance between the CS and ZS flotation parasitological techniques.

Usually, the goal in using the ZS flotation method is to improve the detection of protozoan cysts in stools because their densities are lower than the salt solution and they rise to the surface. Therefore, the increased quantity of *G. duodenalis* and amebae samples diagnosed in this study by ZS was expected, and it was in concordance with reports from other authors (11). Conversely, other studies, including this one, have shown that CS is a more effective technique for identification of *Blastocystis* spp. in faecal samples (12). The better performance of CS in *Blastocystis* spp. identification can be explained by the polymorphism of this protozoan, with different evolutionary forms (vacuolar, granular, amoeboid and cystic), and a wide range of sizes, varying from 2 to 200 micrometers (13-15). Moreover, the hypertonic zinc sulphate solution may not be appropriate for the detection of *Blastocystis* spp., due to the possibility of parasite membrane lysis (16,17).

In this work, we did not compare CS and ZS methods for *Cryptosporidium* spp. diagnosis. In a previous report, we showed that the concentration

Table 1. Frequency of enteroparasites in faecal samples of 330 children from day care centers, analysed by zinc sulphate (ZS) flotation and centrifugal sedimentation (CS) methods

Protozoa	Positivity					Helminths	Positivity				
	ZS		CS		ZS X CS		ZS		CS		ZS X CS
	n	(%)	n	(%)	p		n	(%)	n	(%)	p
*Giardia duodenalis**	74	(22.4)	67	(20.3)	0.506	*Ascaris lumbricoides**	67	(20.3)	68	(20.6)	0.923
*Endolimax nana**	71	(21.5)	64	(19.4)	0.499	*Trichuris trichiura**	39	(11.8)	13	(3.9)	<0.001**
*Entamoeba coli**	51	(15.4)	47	(14.2)	0.661	*Hymenolepis nana**	21	(6.4)	17	(5.1)	0.504
Blastocystis spp.*	33	(10.0)	38	(11.5)	0.530	*Enterobius vermicularis*	5	(1.5)	0	(0.0)	0.025**
*Iodamoeba butschlii**	25	(7.6)	20	(6.1)	0.440	*Strongyloides stercoralis*	3	(0,9)	0	(0.0)	0.083
*E. histolytica/dispar/ moshkovskii**	14	(4.2)	14	(4.2)	1.000	*Hookworm*	3	(0.9)	1	(0.3)	0.316
Chilomastix mesnilli	3	(0.9)	2	(0.6)	0.653	*Schistosoma mansoni*	0	(0.0)	2	(0.6)	0.157

*$p<0.05$: Parasites with statistically significant frequencies compared to total of parasitized children (217/330; 68.5%), considering results of both parasitological methods. **$p<0.05$: Statistically significant difference between ZS and CS methods for the diagnosis of *Trichuris trichiura* and *Enterobius vermicularis*; chi-square test.

Table 2. Concordance between zinc sulphate flotation and centrifugal sedimentation for diagnosing protozoan cysts and helminth eggs in faeces

	Zinc sulphate flotation			*Kappa (95% CI)	Concordance level
	Positive	**Negative**	**Total**		
Centrifugal sedimentation					
Giardia duodenalis					
Positive	58	9	67	0.775 (0.691-0.859)	Substantial
Negative	16	247	263		
Total	74	256	330		
Blastocystis spp.					
Positive	25	13	38	0.669 (0.537-0.801)	Substantial
Negative	8	284	292		
Total	33	297	330		
Entamoeba histolytica/dispar/moshkovskii					
Positive	14	0	14	1.000 (1.000 -1.000)	Excellent
Negative	0	316	316		
Total	14	316	330		
Iodamoeba butschlii					
Positive	17	3	20	0.738 (0.590-0.886)	Substantial
Negative	8	302	310		
Total	25	310	330		
Endolimax nana					
Positive	60	4	64	0.860 (0.792-0.929)	Excellent
Negative	11	255	266		
Total	71	259	330		
Entamoeba coli					
Positive	44	3	47	0.880 (0.807-0.953)	Excellent
Negative	7	276	283		
Total	51	279	330		
Ascaris lumbricoides					
Positive	55	13	68	0.767 (0.680-0.854)	Substantial
Negative	12	250	262		
Total	67	263	330		
Trichuris trichiura					
Positive	8	5	13	0.264 (0.102-0.427)	Low
Negative	31	286	317		
Total	39	291	330		
Hymenolepis nana					
Positive	17	0	17	0.888 (0.780-0.996)	Excellent
Negative	4	309	313		
Total	21	309	330		

* Only parasites with significant frequencies and detected by both parasitological methods were included for evaluation of concordance by kappa index.

of oocysts by CS, followed by mZn staining, proved to be a suitable protocol for *Cryptosporidium* detection in human faeces (7). Moreover, the ZS method showed lower sensitivity and specificity compared to saturated sugar solution flotation technique for *Cryptosporidium* spp. diagnosis (18).

The effective recovery of parasite eggs and cysts depends on their own densities and on the density and viscosity of the flotation medium. Considering these issues, the choice of a flotation solution must be matched with the characteristics of particular parasite species for optimal results. In this study, the relatively heavy helminth eggs, such as those of *S. mansoni*, were only detected by CS due to their high density and subsequent tendency to settle along with various faecal debris (19). In addition, faecal components such as food debris, fat, bacteria, fungi, etc., may affect the performance of a specific parasitological method, which may interfere with the faecal concentration process as well as the microscopic visualization of the parasitic forms (7).

The specific gravities of helminth eggs have been determined previously using density gradients (20-22). In those studies, some eggs of animal or human helminths presented the following densities (g/cm^3): *Ancylostoma duodenale* and *A. caninum,* 1.05; *A. lumbricoides,* 1.11-1.13 (fertile) and 1.20

(infertile) and *A. suum,* 1.13; *E. vermicularis,* 1.11; *Trichuris suis,* 1.13, *T. vulpis,* 1.14 and *T. trichiura,* 1.15. Considering the relatively lower density of helminth eggs compared to the zinc sulphate solution (1.18 g/cm^3), their tendency to float to the surface is expected. However, average dimensions of eggs may influence their flotation in salt or sugar solutions, as well as their sedimentation velocity in water (22). As we observed in our study, despite the closer specific gravity of *T. trichiura* eggs (size range 50 x 22 µm) to *A. lumbricoides* eggs (size range 60 x 45 µm – fertile and 90 x 40 µm - infertile), there was no significant difference in the diagnosis of the latter between ZS or CS. It is also important to note that an *A. lumbricoides* female can eliminate 10 times more eggs than a *T. trichuris* female, favouring the identification by different diagnostic techniques.

When we analyzed the diversity of helminths identified by the method employed in this work, we noticed a higher frequency of *T. trichiura* eggs when diagnosed by ZS. Previous studies have shown that ZS is very effective in detecting *T. vulpis* infections in dogs (11). In our study, CS failed to detect 31 of 44 cases of trichiuriasis, which were diagnosed exclusively by ZS. Despite the lower detection of *T. trichiura* infected children, the CS method identified five positive cases that were not diagnosed by ZS. This emphasizes the need to combine two or more methods, always including a flotation technique, for diagnosing light helminth eggs in faecal samples. As discussed above, it is feasible that the variation on the egg size range of a specific parasite population and, consequently, its density, or the low parasite load in faeces, may have accounted for the five *T. trichiura* samples diagnosed only by CS.

It is also notable that the faecal analysis identified four additional *H. nana,* and exclusively five *E. vermicularis* and three *S. stercoralis* cases by the ZS flotation method. Diagnosis of *E. vermicularis* and *S. stercoralis* infections is usually based on the recovery of typical eggs on perianal skin using a transparent tape (23) and by the Baermann-Moraes method (24), respectively, and rarely by sedimentation techniques. Thus, although it is a nonspecific laboratory tool for both parasites, the ZS may identify some enterobiasis and strongyloidiasis cases during coproparasitological analysis.

In conclusion, this study demonstrated that it is significantly more likely that the centrifugal flotation in zinc sulphate solution detect a faecal sample positive for *T. trichiura* or *E. vermicularis* than the centrifugal sedimentation process, whereas there were no significant differences between methods in the identification of other intestinal parasites, including *G. duodenalis*. Therefore, the use of both methods in the clinical laboratory will maximize the precision and improve the diagnosis of parasitic infections in children.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

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