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ORIGINAL ARTICLE

Comparison of parasitological techniques for the diagnosis of intestinal parasitic infections in patients with presumptive malabsorption

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Abstract Intestinal parasitic infections still remain a public health problem, overall in tropical and subtropical regions. Frequently, patients with malabsorption syndrome can be infected with intestinal parasites, independent that they could be the etiological agents. To compare three coproparasitological techniques Paratest®, Ritchie and direct wet mount for the diagnosis of intestinal parasitic infections in patients with suspected malabsorption syndrome. A descriptive cross sectional survey was carried out in 82 patients with presumptive symptoms of intestinal malabsorption. Three consecutive stool samples were collected from every patient and they were analyzed by three coproparasitological techniques. The degree of agreement was almost perfect when all parasitological techniques were compared for all protozoan infections. Nevertheless, the agreement between Paratest® and Ritchie's methods was slightly lower because this last method was superior for intestinal infections with commensals. The technique of Ritchie showed 100% of sensitivity for protozoa infections in general. However, the direct wet mount and the Paratest®, showed lower sensitivity. When all techniques were compared only for infections with no pathogenic protozoa, the Paratest® had the lowest sensitivity, and less predictive value for negatives. Ritchie's method showed a higher superiority than Paratest® for the diagnosis of intestinal protozoa infections in this group of patients. We would recommend the evaluation of new techniques in local conditions before to decide the introduction in the public health network of laboratories.

Keywords Parasitology · Feces · Diagnostic techniques

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Introduction

Intestinal parasitic infections still remain a public health problem, overall in tropical and subtropical regions, mainly in countries where sanitary and socioeconomic conditions are less developed (Carvalho et al. 2012).

Intestinal parasitoses are classified by the World Health Organization (WHO) among Neglected Tropical Diseases (NTD); they affect thousands of millions people worldwide (Morris 2010). In Mexico, intestinal parasitic infections are distributed all over the country and they are among the 20th causes of diseases; with rates ranging from 1000 to 1500 (Aquino et al. 2012). In Cameroon, like most developing countries in Sub-Saharan Africa, intestinal parasites are widely distributed partly due to the low level of environmental and personal hygiene, fecal contamination of food and drinking water and poor housing (Nsagha et al. 2015).

In our country two National surveys were carried out in 1984 and 2009; however, the prevalence of intestinal parasitic infections was found lower in the second study despite of Kato–Katz thick smear was added in the last one (Rojas et al. 2012).

Coproparasitological techniques have suffered few variations in the last 50 years, but they still are the choice methods for the diagnosis of intestinal parasitic infections because they are relatively cheap and simple to perform (Núñez et al. 1991; Núñez and Cordoví 2003). Among parasitological techniques, the direct wet mount and the Ritchie technique are good methods to identify both, pathogenic and commensal protozoa However; these techniques have limitations to identify helminthic eggs. In addition, some authors have indicated limitations of formalin ether method to concentrate trophozoites of protozoa (Núñez et al. 1991; Núñez and Cordoví 2003; Speich et al. 2014). Over the past few years, a Brazilian industry called Diagnostek (Itu, SP, Brazil) created the Paratest® System, a new concentration method by sedimentation, with the objective to increase new methods to facility these diagnosis (Brandelli et al. 2011).

Frequently, patients with malabsorption syndrome can be infected with intestinal parasites (Behera et al. 2008), that they could be the causal agents of this pathology.

In this study was used Paratest's technique for the diagnosis of intestinal parasitoses in patients with presumptive diagnosis of intestinal malabsorption and it was compared with the direct wet mount and Ritchie's method to determine its efficacy, accuracy and the degree of agreement of the different methods for diagnosis of intestinal parasitic infections. The main objective of this study was to compare these croproparasitological techniques.

Materials and methods

Study population

A descriptive cross sectional survey was carried out in 82 adult's patients with suggested symptoms of intestinal malabsorption attended at "Hermanos Ameijeiras" Clinical Surgery Hospital, from March 2012 to March 2013. Every patient signed an informed consent and they filled a standardized questionnaire with clinical and epidemiological data.

Stool samples

Three consecutive stool samples were collected from every patient and they were analyzed by three coproparasitological techniques: the direct wet mount (Núñez and Cordoví 2003), the Paratest® method (Brandelli et al. 2011), and Ritchie's formalin ether procedure (Núñez and Cordoví 2003). The samples were preserved on formalin 5% and were examined at the National Reference Laboratory of Intestinal Parasitic Infections at "Pedro Kouri" Institute.

Inclusion and exclusion criteria

Inclusion criteria

Only patients who fulfilled all major eligibility criteria: (1) clinical symptoms of malabsorption syndrome (chronic diarrhea, anemia or weight loss), with or without the result of the biopsy (2) written informed consent, and (3) voluntariness to participate in this study.

Exclusion criteria

Individuals who did not comply with inclusions criteria, denial to participate, that they were taking any antiparasitic drug since last three months, patients submitted to radio or chemotherapy, or patients exhibiting psychological symptoms.

Statistical analysis

All data were double-entered into an Excel file (Microsoft 2010) and cross-checked. Statistical analysis was performed using EPIINFO version 6.04 (Dean et al. 1994) and EPIDAT 3.1 (Santiago Pérez et al. 2010).

As the diagnostic "gold" standard, was considered the combined results from all parasitological tests. Each sample found positive with either method was considered as "true positive". Prevalence, sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and Kappa index (KI) for each method were calculated with 95% confidence intervals (95% CI). Cohen's kappa measure was used to assess agreement between two methods as follows: $\kappa < 0$, no agreement; $\kappa = 0$–0.20, poor agreement; $\kappa = 0.21$–0.40, fair agreement; $\kappa = 0.41$–0.60, moderate agreement; $\kappa = 0.61$–0.80, substantial agreement; and $\kappa = 0.81$–1.00, almost perfect agreement. All P value less than 0.05 were considered statistically significant.

Ethical considerations

The study was reviewed and approved by the Ethics Committee of Pedro Kourí Institute and "Hermanos Ameijeiras" Clinical and Surgical Hospital. Samples were collected after informed consent were obtained from patients, following the principles expressed in the Declaration of Helsinki (WMA 2000) and the exposed on the Operational Guidelines for Ethics Committees that review Biomedical Research (WHO 2000). Both committees evaluated and certificated its approval from the ethical, scientific and methodological point of views.

Results

A total of 82 outpatients were referred to malabsorption consulting room at "Hermanos Ameijeiras" hospital were studied, from March 2012 to March 2013.

Out of 82 included cases, 32 (39.02%) were infected in general, with pathogenic or non-pathogenic parasites. Of these, 10 (12.19%) were infected with pathogenic protozoa and 26 (31.71%) with commensals (Table 1).

On Table 2, we analyzed the concordance (kappa index) between the coproparasitological techniques employed in the group of patients. The degree of agreement was almost perfect (kappa between 0.81 and 1) when all parasitological techniques were compared for protozoan infections. Nevertheless, the agreement between Paratest® and Ritchie's methods was slightly lower (substantial or good: between 0.61 and 0.80), because this last method was superior for the diagnosis of intestinal infections with commensals.

On Table 3, it was compared the frequency of positives for protozoa in general, it was observed that Ritchie's concentration technique was superior to Paratest® ($P < 0.05$); the last one was similar to the direct wet mount ($P > 0.05$), and in the group of commensals Ritchie's method was superior to Paratest® ($P < 0.05$).

Table 1 Type of intestinal infection in a group of patients with presumptive diagnosis of malabsorption

Type of intestinal infection	No.	(%)
Infected in general	32	(39.02)
Infected with pathogenic protozoa	10	(12.19)
Infected with commensals	26	(31.71)

(n = 82)

Table 2 Concordance between coproparasitological techniques employed

Techniques	Kappa index (95% CI)
Infections with protozoa in general	
Paratest® and direct wet mount	0.84 (0.69–0.99)
Paratest® and Ritchie	0.81 (0.68–0.94)
Direct wet mount and Ritchie	0.81 (0.68–0.94)
Infections with pathogenic protozoa	
Paratest® and direct wet mount	0.75 (0.52–0.98)[a]
Paratest® and Ritchie	0.94 (0.82–1.00)
Direct wet mount and Ritchie	0.82 (0.62–1.00)
Infections with commensals	
Paratest® and direct wet mount	0.87 (0.74–0.99)
Paratest® and Ritchie	0.76 (0.60–0.91)[a]
Direct wet mount and Ritchie	0.79 (0.65–0.94)[a]

[a] Degree of agreement substantial or good, according to the classification by Landis and Koch

To analyze the sensitivity and specificity of coproparasitological techniques employed in this study, as well as, the positive and negative predictive values, we found the 100% of specificity and positive predictive values of 100% too, in all techniques and in the three groups of infections. Ritchie's method showed a 100% of sensitivity for protozoa infections in general; however, the direct wet mount and the Paratest®, showed a lower sensitivity (68.13%) and less predictive value for negatives (87.72%). In addition, in the group of commensals, Paratest® had less sensitivity (76.92%) and less predictive values for negatives (90.32%).

Discussion

The analysis of the accuracy of one diagnosis test, count the computation of its sensitivity and specificity, indicators that allow to compare directly the efficacy of one diagnosis test with other, and to expect similar results when they are applied in different countries, regions or fields (Jaime-Cerda and Lorena-Cifuentes 2010).

Most studies estimating the sensitivity and specificity of tests for the diagnosis of intestinal parasitic infections consider the results of one of two tests compared (usually the traditional test) or the combination of the results of several diagnostic tests as the gold standard (Brandelli et al. 2011; Devera et al. 2008).

The world of diagnostic tests is highly dynamic. New tests are developed at a fast rate, and the technology of existing tests is continuously being improved (Bossuyt et al. 2003). However, the majority of parasitological methods used for the diagnosis of intestinal helminths and protozoan infections in humans have underwent few modifications during the last years, and they are still routinely used (Carvalho et al. 2012).

Some Brazilian authors have used the Paratest® method (Diagnostek, Brazil) to preserve or to process stool samples in parasitological tests (Escobar-Pardo et al. 2010; Ponciano et al. 2012). In fact, a group of researchers of this country, on 2011, completed a comparative study between the spontaneous sedimentation technique and Paratest® in 140 stool samples, and they found with the first method a prevalence of 12.7% while with the Paratest ® was only 5.7%. The formalin—ether technique showed a high proportion of negative false results, a lower sensitivity and lower negative predictive values than the spontaneous sedimentation technique (Brandelli et al. 2011).

Gonçalves and collaborators completed a comparative study between the Paratest® method and the spontaneous sedimentation technique, it presented similar results to our study and Brandelli's in 2011; this research showed low values of sensitivity and high specificity of this new technique (Gonçalves et al. 2014).

Table 3 Sensitivity, specificity, negative and positive predictive values obtained with coproparasitological techniques employed in the group of infected patients

Group of infections/techniques	No. (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Infections with protozoa in general (n = 32)					
Direct wet mount	25 (78.12)	68.13 (62.24–94.01)	100 (99.0–100)	100 (98.0–100)	87.72 (78.32–97.12)
Paratest®	25 (78.12)	68.13 (62.24–94.01)	100 (99.0–100)	100 (98.0–100)	87.72 (78.32–97.12)
Ritchie	32 (100)	100 (98.44–100)	100 (99.0–100)	100 (98.4–100)	100 (99.0–100)
	$P = 0.02$				
Infections with pathogenic protozoa (n = 10)					
Direct wet mount	8 (80.0)	80.00 (50.21–100)	100 (99.31–100)	100 (93.7–100)	97.30 (92.23–100)
Paratest®	9 (90.0)	90.00 (66.41–100)	100 (99.31–100)	100 (94.4–100)	98.63 (95.28–100)
Ritchie	10 (100)	100 (95–100)	100 (99.31–100)	100 (95–100)	100 (99.31–100)
	$P = 0.33$				
Infections with commensals (n = 26)					
Direct wet mount	21 (80.77)	80.77 (63.70–97.84)	100 (99.11–100)	100 (97.6–100)	91.80 (84.10–99.51)
Paratest®	20 (76.92)	76.92 (58.81–95.04)	100 (99.11–100)	100 (97.5–100)	90.32 (82.16–98.49)
Ritchie	26 (100)	100 (98.08–100)	100 (99.11–100)	100 (98.1–100)	100 (99.11–100)
	$P = 0.04$				

CI confidence intervals, *PPV* positive predictive value, *NPV* negative predictive value

In our study, Ritchie's method showed a higher superiority than Paratest® and direct wet mount. In fact, the formalin-ether diagnosed a high frequency of protozoan infections in general, and commensals in particular, as well as, a high sensitivity and negative predictive values in all group of protozoa infections. The conventional formalin-ether method is considered as a gold standard for the diagnosis of parasite infections (Won et al. 2015). Some authors have considered this method as a valuable alternative to the Kato–Katz method for the diagnosis of soil-transmitted helminthes infections (Speich et al. 2014); others showed the superiority of Ritchie's method over the direct wet mount for the diagnosis of protozoa infections in day care children (Mendoza et al. 2003).

Among possible limitations of this study it is necessary to highlight that the lack of helminthic infections in the group of adult patients avoided evaluate the accuracy of these diagnostic methods for this group of intestinal parasitic infections. In addition, the majority of 82 patients were from La Habana province; they probably harboring similar pathogenic or commensal organisms and this fact could reduce the broadness of this comparison. Despite of this, these results recommend that in the future new evaluations of parasitological techniques should be always carried out in local conditions before to decide the final introduction in the public health network of laboratories.

Compliance with ethical standards

Conflict of interest The author(s) declare that they have no conflict of interests.

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