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Original Article

Performance profile of coproscopic techniques Coproplus® and Hoffman, Pons and Janner in the diagnosis of giardiasis

Perfil de desempenho de técnicas coproscópicas Coproplus® e Hoffman, Pons e Janner no diagnóstico de giardíase

Perfil de rendimiento de las técnicas coproscópicas Coproplus® y Hoffman, Pons y Janner en el diagnóstico de giardiasis

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ABSTRACT

Background and Objectives: Intestinal parasites are a public health problem in Brazil. The identification of parasites in feces is routinely performed by several diagnostic techniques; many of these methods are still criticized for their limitations such as the Hoffman, Pons, and Janner one. We thus considered valid to evaluate the degree of diagnostic sensitivity of this technique in Coproplus® coproscopic collecting and filtering method, since this methodology is also based on the concentration of parasitic structures, and this is a practical adaptation to the usual methods, since there are diagnosis documents of protozoa. **Methods:** The graphic analysis by the Bland-Altman method showed that there is agreement between the two methods of identification of cysts evaluated when plotting the differences between the number of cysts against the means of both values. **Results:** For protozoa, the use of a single parasitological method – Hoffman, Pons and Janner – is not sufficient to identify all samples. **Conclusion:** The analyzed methods were effective in identifying intestinal parasites, but not all agents were identified simultaneously in both techniques and numbers of cysts, which leads to the conclusion that the two techniques are complementary.

Keywords: Giardiase. Diagnosis. Sensitivity.

RESUMO
Justificativa e Objetivos: Os parasitas intestinais representam um problema de saúde pública no Brasil, e sua identificação é feita rotineiramente, por meio de várias técnicas diagnósticas. Muitas dessas técnicas são criticadas por suas limitações, como a de Hoffman, Pons e Janner. Considerou-se avaliar o grau de sensibilidade diagnóstica dessa técnica em comparação ao método coproscópico de coleta e filtragem Coproplus®, uma vez que esta metodologia também é baseada na concentração de estruturas parasíticas e é uma adaptação prática aos métodos usuais, pois não há documentos diagnósticos de protozoários. **Métodos:** A análise gráfica pelo método de Bland-Altman mostrou que há concordância entre os dois métodos de identificação dos cistos avaliados, ao traçar as diferenças entre o número de cistos contra as médias de ambos os valores. **Resultados:** Verificou-se que, para os protozoários, o uso de apenas um método parasitológico de Hoffman, Pons e Janner não é suficiente para identificar todas as amostras. **Conclusão:** Os métodos têm se mostrado eficazes na identificação de parasitas intestinais, mas nem todos os agentes foram identificados simultaneamente em ambas as técnicas e números de cistos, o que leva à conclusão de que uma técnica pode complementar a outra.
Descritores: Giardíase. Diagnóstico. Sensibilidade.

RESUMEN
Justificación y Objetivos: Los parásitos intestinales son un problema de salud pública en Brasil, y la identificación de parásitos se realiza de forma rutinaria mediante diversas técnicas de diagnóstico. Incluso con la existencia de numerosos métodos de diagnóstico, muchos aún son criticados por sus limitaciones, como el de Hoffman, Pons y Janner. Se consideró oportuno evaluar el grado de sensibilidad diagnóstica de esta técnica en el método de coprofia de recolección y filtro Coproplus®, ya que esta metodología también se basa en la concentración de estructuras parásitas y es una adaptación práctica a los métodos habituales, y no hay documentos de diagnóstico de protozoos. **Métodos:** El análisis gráfico por el método de Bland-Altman mostró que existe una concordancia entre los dos métodos de identificación de los quistes evaluados al rastrear las diferencias entre el número de quistes y los promedios de ambos valores. **Resultados:** Se ha encontrado que, para los protozoos, el uso de un solo método parasitológico (Hoffman, Pons y Janner) no es suficiente para identificar todas las muestras. **Conclusión:** Se ha demostrado que los métodos son eficaces para identificar parásitos intestinales, pero no todos los agentes se han identificado simultáneamente en las técnicas y en el número de quistes, lo que lleva a la conclusión de que una técnica puede complementar a la otra.
Palabras Clave: Giardiasis. Diagnóstico. Sensibilidad.

INTRODUCTION

Intestinal parasites remain a collective health problem in Brazil and can be considered indicators of low socioeconomic, environmental and sanitary conditions in a certain region. They affect large portions of the population, especially children, and are directly related to the lack of basic sanitation. These diseases are directly inserted in the determinant complex associated with poverty that evolves in a vicious cycle, always involving social ills.[1,2]

Giardiasis is one of the most prevalent parasitic diseases and part of this condition. This pathology is caused by the protozoan *Giardia lamblia* and is very common due to the ease of

transmission.[3] Cases of giardiasis during childhood may compromise physical and mental performance, impairing school development. At a stage of their evolutionary cycle, giardia are located in the individual's digestive tract, and before being eliminated in feces they become cysts, allowing them to survive outside the intestine for months.[2-4]

Cysts are the most common route of contamination, via the accidental ingestion of water without proper treatment and unsanitized food.[4] Once inside the host, the cysts hatch and the parasites are released, restarting the disease cycle. The most usual symptoms of giardiasis are watery diarrhea followed by abdominal cramps, nausea, vomiting, malnutrition caused by intestinal malabsorption and significant weight loss.[3,4] However, some carriers of this disease are asymptomatic but still spread the parasite via their feces, and they may expand the distribution of cysts to the environment and increase the incidence of this parasitosis; these situations show the power of dissemination of this protozoan.[2-4]

The identification of parasites (usually cysts) in feces is routinely performed by several diagnostic techniques, which should have high sensitivity (correct diagnosis and positive predictive), since specific treatment depends on these conditions.[4,5]

Although numerous quantitative and qualitative methods of parasitological diagnosis exist, many are still criticized for their limitations, technical complexity, low sensitivity, and high cost, restricting their use in the routine of some laboratories.[6] In laboratory practice, more than one method should be used to detect immature forms of helminths or protozoa to reduce inconclusive results, since important variations in the positivity of feces examination occurs, which significantly influence the detection of infection, such as parasitic load, experience of the technician performing the analysis, and infection time.[6] Some authors corroborate these ideas and have addressed that no test is considered 100% sensitive for diagnosis. A single feces sample examined for parasitological investigation leads to the detection of about 30% of infections. The sensitivity of the diagnosis increases to about 50% if three fecal samples are used, and sensitivity may almost reach 100% if seven samples are used, which often becomes a time-consuming and costly process.[7]

In practice, few adaptations are made in relation to fecal examinations nowadays, with the Hoffman, Pons and Janner spontaneous sedimentation technique (HPJ) as one of the most common.[8,9] Therefore, we considered appropriate to evaluate the degree of diagnostic sensitivity of this technique compared to the croposcopy method of collecting and filtering Coproplus®, since this methodology is based on the concentration of parasitic structures and a practical adaptation to the usual methods, based on the modification of Ritchie's method.[9-11]

METHODOLOGY

Two samples donated by the manufacturer (NL diagnóstica) served as an analytical parameter (a positive and a negative fecal sample, both previously confirmed by analysts of the supplier company and used as a quality control parameter).

After the pre-analytical phase, the samples were directly examined using 5 g of feces per technique. In total, 50 slides were prepared for each technique, added with 200 mg of feces diluted according to the methodologies applied, assuming a specific mass equal to 1 g/mL, stained with lugol. Any slides whose liquid surplus exceeded the determined volume and observed under optical microscope were discarded. Parasitic load evaluation was based on the classification described in the literature, which considers mild infection from 1-100 cysts or oocysts/slide; moderate from 101-300; and severe with more than 301 cysts or oocysts/slide.[9-12]

The slides were analyzed in duplicate by two technicians for three minutes and compared by a third rater for the verdict of positivity.[13]

Based on the cyst count results, the prevalence and parasitic density were analyzed, adding a methodology of graphic analysis proposed by Bland and Altman (Bland-Altmangraphical analysis) to evaluate the agreement between coproscopy methods used, plotting the differences between parasite counts obtained with the types of tests, against the means of both values. The limits of this agreement are in the interval between the mean difference observed in both methods, added or subtracted from 1.96 standard deviations (mean of differences ± 1.96 SD). The analysis of diagnostic sensitivity between techniques and in combination followed the accuracy parameters performed in other studies.[12,13] All evaluations used 0.05 alpha as the basis for accepting statistical hypotheses.[14] The donated samples did not have any type of clinical or documentary data from donors and were used only for quality assurance tests.

RESULTS

Positive samples were found by the Coproplus technique® 47 slides considered as true positive (TP), 3 false negative slides (FN), 4 false positive slides (FP) and 46 true negative slides (TN), indicating sensitivity of 94% (confidence interval: 89-96%).

Hoffman's method presented: 49 TP slides, 1 FN slide, 3 FP slides and 47 TN slides, indicating 98% sensitivity (confidence interval: 95-99%).

The graphic analysis by the Bland-Altman method showed agreement between the two methods of identification of cysts evaluated, when the differences between the amount of cysts against the means of both values are plotted, since most of the plotted values remained within the agreement limit of ± 1.96 SD (Figures 1 and 2). Comparing the amount of cysts in the uncontaminated samples was impossible (Figure 3), so the estimation of diagnostic specificity is not feasible.

Figure 1. Bland-Altman graphic analysis of the amount of cysts observed in contaminated samples (percentage values), identified with the Coproplus kit® and the HPJ method.

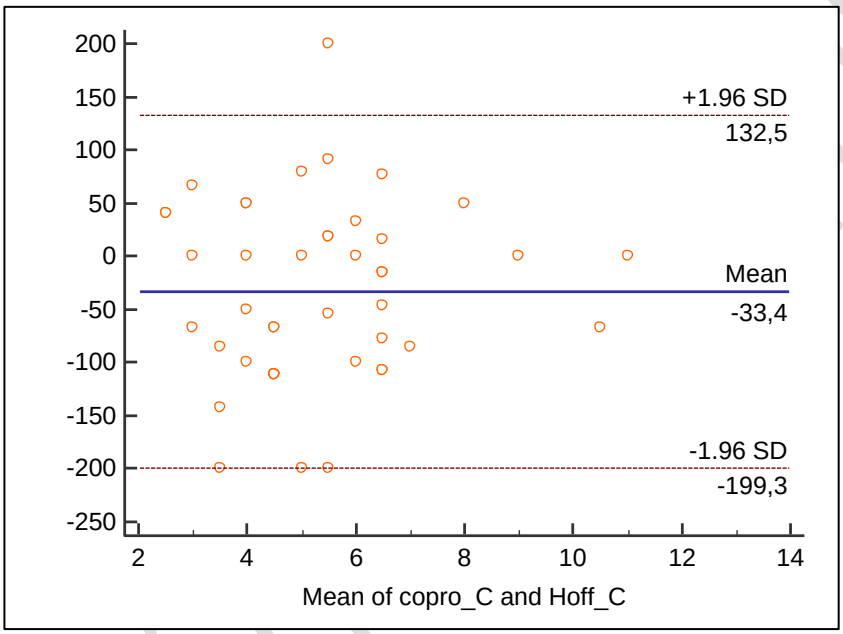


Figure 2. Bland-Altman graphic analysis of the amount of cysts observed in contaminated samples, identified with the Coproplus kit® and the HPJ method.

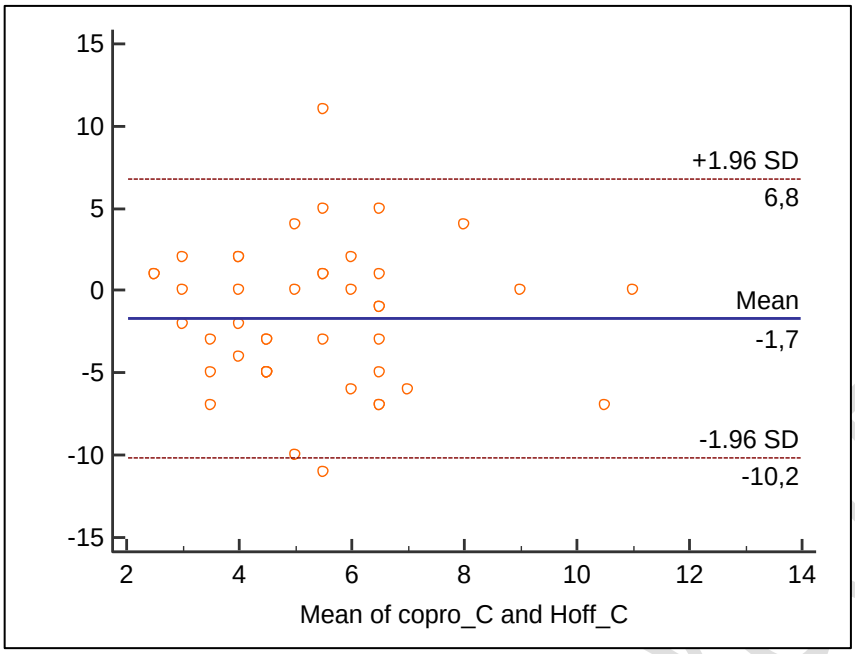
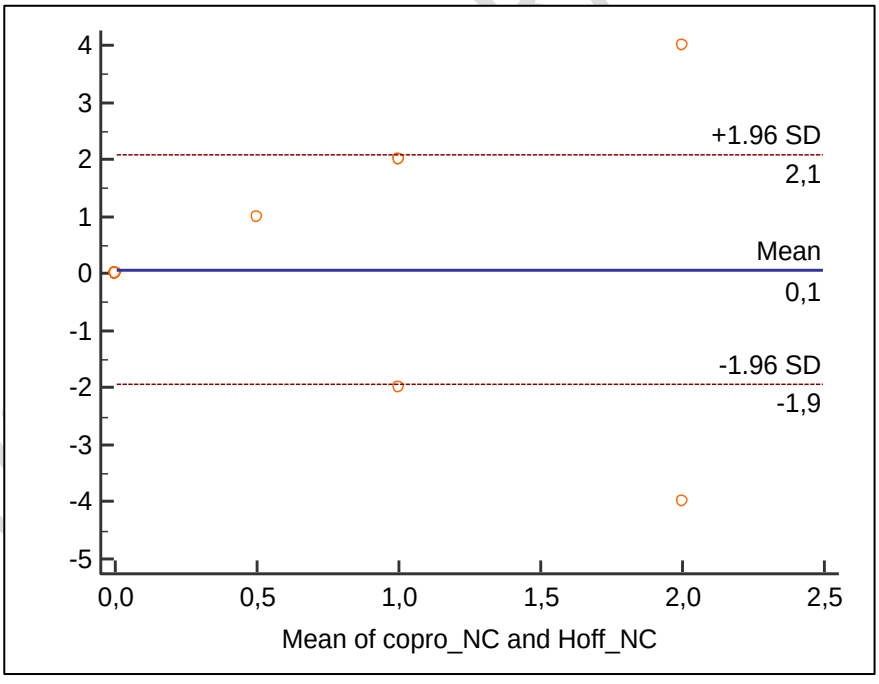


Figure 3. Data could not be interpreted from Bland-Altman graphic analysis due to the small amount of cysts observed in non-contaminated samples, identified with the Coproplus kit® and the HPJ method.



DISCUSSION

This study has an important aspect about dichotomous diagnostic accuracy techniques: the method's ability to present a correct diagnosis. The purpose of this study is to establish a

link between the answers since these interpretations contribute to the achievement of desirable results.

The Coproplus® technique is presented as a recent application with parasitic practices, and the statistical analysis of its performance can expand both its use and epidemiological surveys, given its practicality and efficiency, both in pre- and analytical phases.[8]

When comparing the Coproplus® and HPJ methods, we found that the sensitivity difference is 4%; to us, such value shows how these methods are functional, so that the methods are reasonable within laboratory applications.

Efficacy analyses of these parasitological techniques have already been standardized for a possible quantification for helminth eggs.[9] The results of this research are corroborated, since we observed a similar performance profile between methods; however, with the data obtained in our study, it was found that, for protozoa, the use of only one parasitological method is not sufficient to identify all samples. The methods showed efficiency to identify intestinal parasites, but not all agents were identified simultaneously in both techniques and in numbers of cysts; one can thus conclude that the techniques are complementary, suggesting the use of both based on increased diagnostic sensitivity. According to Mendes et al.,[10] in routine laboratories it would be important to perform more than one diagnostic method to detect the parasitic forms of protozoa and helminths, especially when there is low parasitic load. The methods used proved to be fast and inexpensive means for the study of cysts as well, being noninvasive tests and useful for diagnostic and epidemiological profile surveys.

Further studies should be conducted based on population samples, collected in conventional situations involving living public in areas of high endemicity, so this test can be challenged.

REFERENCES

1. Pacheco SJ. Estigmatização social pela leishmaniose cutânea no estado do Rio de Janeiro, Brasil. Reciis – Rev Eletron Comun Inf Inov Saúde. 2017;11(3). https://doi.org/10.29397/reciis.v11i3.1091

2. Grott SC, Hartmann B, Silva Filho HH, Franco RMB, Goulart JAG. Detecção de cistos de *Giardia spp*. e oocistos de *Cryptosporidium spp*. na água bruta das estações de tratamento no município de Blumenau, SC, Brasil. Rev Ambient Água. 2016;11(3):689-701. http://dx.doi.org/10.4136/ambi-agua.1853

3. Dias LR, Pequeno IFP, Cavalcante UMB, Silva CR, Lima MBL, Freitas FIS. Estudo coproparasitológico e epidemiológico de crianças e manipuladores de alimentos durante 3 anos em uma creche da Paraíba. Rev Epidemiol Control Infec. 2017;7(2):90-5. http://dx.doi.org/10.17058/reci.v7i2.7981

4. Pina ILT. Deteção de Cryptosporidium, Giardia e Escherichia coli em vegetais consumidos crus e possíveis implicações em saúde pública [tese] [Internet]. Lisboa: Faculdade de Medicina Veterinária, Universidade de Lisboa; 2017 [citado 2019 jul 14]. Disponível em: https://www.repository.utl.pt/handle/10400.5/13220

5. Brandão ML, Chame M, Cordeiro JLP, Chaves SAM. Diversidade de helmintos intestinais em mamíferos silvestres e domésticos na Caatinga do Parque Nacional Serra da Capivara, Sudeste do Piauí, Brasil. Rev Bras Parasitol Vet. 2009;18 Supl. 1:19-28. http://dx.doi.org/10.4322/rbpv.018e1004

6. Sudré AP, Macedo HW, Peralta RHS, Peralta JM. Diagnóstico da estrongiloidíase humana: importância e técnicas. Rev Patol Trop. 2006;35(3):173-84. https://doi.org/10.5216/rpt.v35i3.1876

7. Hoffman WA, Pons JA, Janer JL. The sedimentation-concentration method in schistosomiasis mansoni. J Public Health Trop Med. 1934;9:238-98.

8. Araújo AJUS, Kanamura HY, Dias LCS, Gomes JF, Araújo SM. Coprotest® quantitativo: quantificação de ovos de helmintos em amostras fecais utilizando-se sistema de diagnóstico comercial. J Bras Patol Med Lab. 2003;39(2):115-24. https://doi.org/10.1590/S1676-24442003000200005

9. De Carli GA. Parasitologia clínica*:* seleção de métodos e técnicas de laboratório para o diagnóstico das parasitoses humanas [Internet]. 2a ed. São Paulo: Atheneu; 2007 [citado 2020 jun 29]. Disponível em: https://www.academia.edu/28431021/Parasitologia_Cl%C3%ADnica-Geraldo_Attilio_de_Carli_biomedlivros

10. Mendes CR, Teixeira ATLS, Pereira RAT, Dias LCS. Estudo comparativo de técnicas parasitológicas: Kato-Katz e coprotest®. Rev Soc Bras Med Trop. 2005;38(2):178-80. https://doi.org/10.1590/S0037-86822005000200010

11. Pinto RM, Vicente JJ, Noronha D, Gonçalves L, Gomes DC. Helminth parasites of conventionally maintained laboratory mice. Mem Inst Oswaldo Cruz. 1994;89(1):22-40. https://doi.org/10.1590/S0074-02761994000100007

12. Ferraz RRN, Namba TK, Nigro CA, Rodrigues FSM, Fornari JV, Barnabé AS. Comparação entre os métodos de extração de metacercárias de *Ascocotyle sp* (Trematoda: Digenea) dos tecidos de *Mugil liza* Valenciennes, 1836 (Teleostei: Mugilidae). Ciênc Anim Bras. 2010;15(3):354-61. http://dx.doi.org/10.1590/1809-6891v15i328163

13. Altman DG, Bland JM. assessing agreement between methods of measurement. Clin Chem. 2017;63(10):1653-4. https://doi.org/10.1373/clinchem.2016.268870

14. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):10-6. https://doi.org/10.1186/2046-4053-4-1

Authors' contribution:

Carolina Praeiro da Silva, Anderson Sena Barnabe, and Renato N. Ferraz contributed to the conception, design of the article, reading of samples, analysis and writing of the article, in addition to statistical analyses. Katia R. Aranda and Tatiana de Campos Mello contributed to the planning, review of the English and Spanish versions of the abstract, contribution to text analysis and revision, and final approval of the article.