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Are species-specific antigen detection tests needed in the diagnosis of *Giardia duodenalis* infection?

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ABSTRACT

Objective: To assess the diagnostic performance in human stool samples of a rapid, qualitative, solid-phase immunochromatographic test (Alere®) originally developed to detect *Giardia duodenalis* antigens in fecal samples of dogs.
Methods: Samples from 54 patients with a previous diagnosis of giardiasis were tested by the microscopic examination to assess the performance of an immunochromatographic kit developed to detect *Giardia duodenalis* coproantigen in dog feces.
Results: The agreement between the microscopic and the immunological methods was 83.3%. These findings are consistent with those of other studies using human specific kits.
Conclusions: It is suggested that the same immunochromatographic test could be used for *Giardia* diagnosis in both species.

1. Introduction

The flagellate protozoan *Giardia duodenalis* parasitizes a wide range of vertebrate hosts. According to the World Health Organization (WHO), 250 million people suffer from *Giardia* infection worldwide, and approximately 500 000 new cases of the disease are reported annually[1,2]. The increasing interest in the epidemic and zoonotic potential of this disease has fuelled a search for highly sensitive, specific, accurate, fast and low-cost diagnostic tests[3]. Immunochromatographic assays have been designed to detect *Giardia duodenalis* antigens excreted in the feces from various animal hosts[4,5] and have widespread use in the diagnosis of giardiasis[6]. These tests provide fast results and, as opposed to the coprological examination, do not require specialised laboratory equipment or trained personnel to examine stool samples under the microscope[7]. Its sensitivity may be higher than 97% and the specificity close to 100%[8]. Therefore, these assays would be useful for field tests and as valuable diagnostic tools in minimally equipped laboratories[7]. The aim of this study was to assess the diagnostic performance in human stool samples of a rapid, qualitative, solid-phase immunochromatographic test (Alere®) originally developed to detect *Giardia duodenalis* antigens in fecal samples of dogs.

2. Materials and methods

Fifty-four patients living in the city of Niterói, State of Rio de Janeiro (RJ), Southeast Brazil, with a previous diagnosis of giardiasis by the microscopic examination of stool samples (coprological test) were enrolled in this study. All patients were requested to submit fresh stool samples as part of this survey. Participants were provided with stool collection containers and

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This study was approved by the Research Ethics Committee of the School of Medicine/Antônio Pedro University Hospital, Fluminense Federal University (UFF), Niterói, RJ, Brazil (CAAE 44055615.0.0000.5243). All volunteers who participated in this study signed a participant consent form.

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instructions on how to sample and store the fecal specimens. No preservative was added to the stool samples. Fecal specimens were refrigerated for up to 24 h before being examined by light microscopy according to the method of Faust *et al*.[9]. The sediment recovered by washing the samples prior to the coproparasitological examination was aliquoted and then frozen at –20 ℃ for subsequent testing with an immunological technique. A commercially available immunoassay (Alere®) was used to test the stool samples according to the instructions provided in the package insert with minor modifications. According to the manufacturer's instructions, fresh fecal samples should be collected with the swab that comes with the kit and then diluted in solution and homogenized. However, to allow the use of frozen fecal samples in the present study, 50 μL of the frozen sediment from the stool samples was added to a microtube with dilution buffer provided by the manufacturer. The remaining steps of this diagnostic process were performed according to the manufacturer's instructions. The modification of the standard methodology was previously tested in fecal samples of dogs for which this kit was originally developed, and the results obtained were consistent with those provided by the original method (Costa, 2016; unpublished data). This study was approved by the Research Ethics Committee of the School of Medicine/Antônio Pedro University Hospital, Fluminense Federal University (UFF), Niterói, RJ, Brazil (CAAE 44055615.0.0000.5243). All volunteers who participated in this study signed a participant consent form.

3. Results and discussion

Stool samples from humans were examined by light microscopy and by an immunological assay, and there was an agreement of 83.3% (45/54) between the two sets of test results as seen in Table 1. Of the 54 fecal samples tested, 53 yielded positive results in at least one of the techniques; 1 of the samples yielded negative results in both assays. Samples from 3 patients (5.5%) were negative in the coprological examination and positive in the immunochromatographic technique. *Giardia duodenalis* cysts were found by light microscopy in 6 samples (11.1%), but *G. duodenalis* coproantigen was not detected in these specimens. In 44 patients (81.5%), samples were positive for *G. duodenalis* by both techniques.

Table 1

Results of the coprological examination and immunological test (Alere® immunochromatographic assay) in a survey of *Giardia duodenalis* in stool samples from individuals living in Niteroi, RJ, Brazil.

Immunochromatography	Faust technique		
	Negative	Positive	Total
Negative	1/54 (1.9%)	6/54 (11.1%)	7
Positive	3/54 (5.5%)	44/54 (81.5%)	47
Total	4	50	54

Our findings agree with those reported by other authors in previous similar surveys in which both the coprological and the immunological tests were used for the diagnosis of giardiasis in human beings[6,10-13]. In the present study, the high agreement between the two diagnostic tests shows that neither the use of a species-specific immunochromatographic test kit originally developed for the diagnosis of giardiasis in canine fecal specimens nor the modification on the standard methodology affected the survey results when this kit was used in human fecal specimens. In the present study, there was high agreement (83.3%) between test results. In surveys on the prevalence of giardiasis in human beings published elsewhere in which similar diagnostic tools were used, the comparison of the diagnostic performance between the coprological examination and species-specific immunochromatographic assays by using kits from various manufacturers yielded agreement levels ranging from 65.5% to 100%[8,10,14]. Our findings corroborate those of previous studies, and show that an immunological assay designed for the diagnosis of *G. duodenalis* infection in dogs is a rapid, reliable diagnostic test for humans. In our study, serial sampling was not the sampling method adopted. Therefore, a number of false negatives are expected in the coprological examination. Intermittent cyst shedding would account for those false negative results as reported by other authors[15]. The antigen detection assay could still yield positive results in those samples in which no intact protozoan cysts were found by light microscopy. In our survey, samples from 3 patients were negative in the coprological examination but positive in the immunochromatographic assay. Similar findings have been reported elsewhere in studies on *Giardia* in which species-specific immunological kits were used[16,17]. Protozoan cysts were absent in these three samples with negative results by coprological examination possibly due to the fact that *Giardia* cysts are not permanently excreted in the feces (intermittent cyst shedding), or because no identifiable cysts or trophozoites were present in these stool samples examined by light microscopy. Immunological assays may not be very sensitive and therefore may not detect very small amounts of antigen in a sample, and that would account for the false negative results. Previous studies have shown a correlation between the signal intensity of the ELISA and the parasite load of the fecal specimens examined[18]. Many studies reported that human stool samples tested with the ImmunoCardSTAT!®, RIDAQUICK®Combi and Duo-Strip® kits yielded false negatives, and this was due to the presence of low numbers of *Giardia* cysts in the fecal specimens examined[8,16,17,19,20].

The host specificity of *G. duodenalis* isolates from a number of animal species is a topic of debate in recent years. With regard to the diagnosis of giardiasis, our study shows that a commercially available immunochromatographic test originally designed for the

diagnosis of giardiasis in dogs which utilizes antibodies specific to *G. duodenalis* also has affinity for *Giardia* isolates found in human fecal samples. Manufacturing of multispecies diagnostic tests would allow companies to simplify production lines and reduce manufacturing costs as well. Multispecies diagnostic tests can be particularly useful to diagnose giardiasis affecting multiple mammalian species in endemic areas and in outbreaks in which the source of contamination is unknown. Immunochromatographic assays are simple, low-cost and versatile diagnostic tests that can be used for the identification of infected individuals in field surveys.

Conflict of interest statement

We declare that we have no conflict of interest.

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