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Comparison of the performance of two spontaneous sedimentation techniques for the diagnosis of human intestinal parasites in the absence of a gold standard

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ABSTRACT

Performance evaluation of diagnostic tests is critical in the search for accurate diagnoses. A gold standard test is usually absent in parasitology, thus rendering satisfactory assessment of diagnostic accuracy difficult. Moreover, reliability (assessed by the study of repeatability) is a rarely studied characteristic of diagnostic tests. This study compared and evaluated the performance (repeatability, concordance and accuracy) of the spontaneous sedimentation technique (SST) and the Paratest for the diagnosis of *Giardia lamblia*, *Entamoeba histolytica* complex, *Blastocystis* spp., *Ascaris lumbricoides*, hookworm, *Trichuris trichiura* and *Calodium hepaticum*. Fecal samples of 143 individuals were separated into three replicates for each test. Concordance and homogeneity of the results between replicates of each test and between tests were evaluated. Proportions of positives, sensitivity and specificity were estimated using a Bayesian Latent Class Model. High repeatability of both tests was found for the detection of intestinal parasites, except for *Blastocystis* spp. and hookworm. Concordance between tests was generally high (concordance correlation coefficient, 0.72–0.88), except for *Blastocystis* spp., hookworm and *T. trichiura*. The Paratest detected more cases of *Blastocystis* spp. and fewer of hookworm than the SST. The tests were quite discordant in the detection of *T. trichiura*. A low sensitivity (39.4–49.2% for SST, 35.8–53.8% for Paratest) and a high specificity (93.2–97.2%) were found for both tests. The Paratest presented a slightly higher sensitivity for the diagnosis of *Blastocystis* spp. (53.8%), and SST did so for hookworm (49.2%). This is the first study on repeatability and accuracy (using a Bayesian approach) of two spontaneous sedimentation techniques. These results suggest underdiagnosis of little dense parasitic forms due to technical limitations in both tests. We conclude that the combined study of repeatability, concordance and accuracy is a key strategy for better evaluation of the performance of tests and is also useful for the identification of technical limitations.

1. Introduction

Intestinal parasites are, even today, major contributors to the global burden of disease, affecting especially the population living in regions in the developing world (Alum et al., 2010). Of particular importance worldwide are the soil-transmitted helminths *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* and the protozoans *Entamoeba histolytica* and *Giardia lamblia* (Bethony et al., 2006; Fenwick, 2012; Harhay et al., 2010). In the Amazon region, *Calodium hepaticum* is a zoonotic helminth that has been increasingly reported as a cause of spurious infection in humans (Gonçalves et al., 2012), and *Blastocystis* spp. is a highly prevalent suspected pathogenic protozoan (Borges et al., 2009). *C. hepaticum* is also the causative agent of a rarely reported liver disease (hepatic calodiasis) found worldwide. This helminth infects the hepatic parenchyma of various mammals (rodents being the principle hosts). Hepatic infection occurs following the ingestion of embryonated eggs present in the ground or contaminated food.

Abbreviations: SST, spontaneous sedimentation technique; Kappa IRM, kappa index for repeatability measure; Kappa IBT, kappa index between tests; CCC, concordance correlation coefficient; CI, confidence interval; CrI, credible interval.

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In spurious infection, non-embryonated eggs are ingested (from the ground, contaminated food or liver of mammals) and directly exit with the stools without causing liver disease (Fuehrer et al., 2011; Gonçalves et al., 2012). Transmission of intestinal parasites depends on the availability of clean water, socio-economic conditions, education, personal and public hygiene practices, temperature, humidity and the survival of the environmental stages of the parasites (Alum et al., 2010).

Evaluation of the performance of diagnostics tests is critical in the search for accurate diagnostic techniques to provide adequate patient care, assess drug efficacy, monitor the effectiveness of control programs and obtain better understanding of the epidemiology of intestinal parasites (Harhay et al., 2011; Tarafder et al., 2010). In order to evaluate diagnostic tests it is important to take into account that few, if any, gold standard tests (i.e. a diagnostic test with 100% accuracy against which the sensitivity and specificity of other tests are estimated) are available in parasitology and, in particular, do not exist for the detection of intestinal parasite infection (Basso et al., 2013; Tarafder et al., 2010). Nevertheless, most studies estimating the sensitivity and specificity of tests for the diagnosis of intestinal parasites 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; Carvalho et al., 2012; Devera et al., 2008a; Dogruman-Al et al., 2010; Glinz et al., 2010; Inês et al., 2011; Knopp et al., 2011; Levecke et al., 2011; Steinmann et al., 2012). These practices have led to biased estimations of accuracy. The use of statistical models that consider the assumption of absence of a gold standard test can overcome this problem, generating more reliable information as to the accuracy of diagnostic tests. Up to now, only three articles in the area of human intestinal parasites have presented estimations of the sensitivity and specificity of diagnostic tests using the concept of absence of a gold standard test (Booth et al., 2003; Tarafder et al., 2010; Traub et al., 2009).

Another important aspect during the evaluation of the performance of diagnostic tests is the repeatability of the results (Sanchez et al., 2002). Repeatability refers to the extent of agreement among repeat assessments of the same sample using the same technique in the same laboratory by the same operator (Braun-Munzinger and Southgate, 1992; White and van den Broek, 2004). Although the repeatability of a test refers to its reliability (White and van den Broek, 2004), this important characteristic has been little evaluated in studies of the performance of diagnostic tests (Charlier et al., 2005; Sanchez et al., 2002; Thomas et al., 1981).

Among the diagnostic tests based on optical microscopy those based on spontaneous sedimentation are among the least expensive and easiest to perform and enable the simultaneous detection of helminth and protozoan intestinal infections (Brandelli et al., 2011; Camacho et al., 2013; Carvalho et al., 2012; Ribeiro and Furst, 2012; Tello et al., 2012). For these reasons, in some economically underdeveloped settings their use is preferred over the tests based on centrifuge-sedimentation or centrifuge-flotation. Nevertheless, techniques based on centrifugation have demonstrated to be better in relation to those based only on spontaneous sedimentation (Carvalho et al., 2012; Gomes et al., 2004), although exceptions have been reported (Devera et al., 2008a; Tello et al., 2012).

The spontaneous sedimentation technique (SST) (also known as the Lutz technique or the Hoffman, Pons and Janer technique) is a traditional test widely used for clinical diagnosis and epidemiological surveys in Brazil and, also, Venezuela (Brandelli et al., 2011; Carvalho et al., 2012; de Souza et al., 2007; Devera et al., 2008a,b; Pinheiro et al., 2011; Santos et al., 2013; Velásquez et al., 2005). In this test, stool samples (previously preserved or diluted in water) are filtered through a gauze strip into a conical cup and subsequently submitted to sedimentation in tap water for 1 or 2 h (De Carli, 2007a). On the other hand, the Paratest (DK Diagnostics, São Paulo, Brazil) is a commercial kit for spontaneous sedimentation of preserved stool, developed with the aim of expanding new methods based on the simplification of laboratory procedures thereby improving biosecurity (Brandelli et al., 2011). The kit provides a stool container that has a cap equipped with a filter of 266 μm. This characteristic synthesizes the manipulation and examination of stool samples by performing the steps of conservation, filtration and concentration in the container itself. The amount of feces is standardized (2 g), whereas variable quantities can be used (1–5 g) in the SST (Brandelli et al., 2011; De Carli, 2007a; Hoffman et al., 1934). The Paratest is faster (15 or 30 min of sedimentation) than the SST and its compact structure allows the performance of the test in remote places. Despite the widespread use of the spontaneous sedimentation techniques, no study has evaluated their repeatability and accuracy taking into account the absence of a gold standard in the latter case.

The aim of this study was to evaluate and compare the performance (repeatability, concordance and accuracy) of two spontaneous sedimentation techniques (SST and Paratest) in the detection of infection by several pathogenic (or suspected pathogenic) intestinal parasites (*G. lamblia*, *E. histolytica* complex, *Blastocystis* spp., *A. lumbricoides*, hookworm, *T. trichiura* and *C. hepaticum*), using a Bayesian approach for the estimation of the proportion of positives, sensitivity and specificity.

2. Materials and methods

2.1. Study area and population

This study was carried out in 2009 with the collection of stool samples from children and adults from the agricultural community of Rio Pardo of the municipality of Presidente Figueiredo, located ~160 km to the north of the city of Manaus (~1°48′ S; 60°19′ W), Amazonas State, Brazil.

2.2. Field and laboratory procedures

Participants were asked to submit one fresh stool sample. The collection of samples was conducted in the households with two daily visits of the staff of the project, once in the morning and another in the afternoon. The samples were initially processed 1–3 h after collection in a local laboratory unit located in the community, as follows: firstly, thorough homogenization of each specimen was performed by stirring with a wooden spatula for at least 1 min. After homogenization, each sample was separated into three equal replicates of feces for each test. For the Paratest the replicates were deposited into three different plastic stool containers provided by the Paratest kit using a device that enables the collection of 1 g of feces. Each replicate was composed of 2 g of feces diluted in 7 ml of the preservative (5% buffered formalin pH 7.0) contained in each container. For the SST the replicates were deposited into three different containers with sodium acetate–acetic acid–formaldehyde (SAF). For each replicate of the SST, 2 g of feces (measured with the device provided by the Paratest kit) were diluted in 7 ml of SAF. In the case of a diarrheal sample, three measurements of the device provided by the Paratest kit were applied for the two tests. Samples that could not achieve the total of six replicates due to the lack of a sufficient quantity of feces were not included in the study.

The two sedimentation techniques were processed and examined in the Leonidas e Maria Deane Institute (Fiocruz, Manaus) by an experienced laboratory technician. The delay in time from stool sample processing to microscopic reading ranged from 3 to 17 days. The Paratest was carried out according to the manufacturer's instructions. In brief, each container with the diluted feces was