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ORIGINAL ARTICLE

Low sensitivity of the ImmunocardSTAT® Crypto/Giardia Rapid Assay test for the detection of *Giardia* and *Cryptosporidium* in fecal samples from children living in Libreville, Central Africa

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Abstract Giardiasis and cryptosporidiosis are now recognized as neglected tropical parasitic diseases. The risk of their dissemination in developing countries, such as Gabon, is increasing, due to urban crowding and poor sanitation. Accurate, simple and rapid diagnosis tools are thus necessary for the estimation of their real burden. The aim of this study was to evaluate the performances of the ImmunocardSTAT® *Crypto/Giardia* Rapid Assay test for the detection of *Cryptosporidium* (*C.*) spp. and *Giardia* (*G.*) *duodenalis* in children living in Libreville, Gabon. Stool samples of 173 healthy children were screened by routine microscopic using the merthiolate iodine formol concentration technique for *Giardia*, the modified Ziehl Neelsen (ZN) staining for *Cryptosporidium* and the ImmunocardSTAT® Crypto/Giardia RDT for the detection of *Giardia* and *Cryptosporidium* parasite forms and antigens respectively. *G. duodenalis* was detected with microscopy and the ImmunocardSTAT® Crypto/Giardia in 27 (15.6 %) and 22 (13.3 %) fecal samples respectively. *C.* spp. oocysts were found in 18 (10.4 %) ones, whereas only one sample was positive with the immunochromatographic assay. When microscopic examination was considered as the reference method, sensitivity and specificity of the ImmunocardSTAT® Crypto/Giardia Rapid Assay were found to be 63.0 %, 96.6 and 5.5 %, 99.3 % for *G. duodenalis* and *C.* spp. respectively. The prevalence of *G. duodenalis* and *C.* spp. carriage is high in children from Libreville. A low sensitivity of the ImmunocardSTAT® Crypto/Giardia for the detection of both parasites is observed. It is thus inappropriate as a diagnostic tool for detecting asymptomatic carriers.

Keywords *Cryptosporidium* spp. · *Giardia duodenalis* · RDTs · Children · Gabon

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Introduction

Diarrhea remains one of the leading causes of death among children under five years. It accounts for 9 % of all under-five deaths (Walker et al. 2013). Children from low and middle income sub-Saharan African countries remain at higher risk of frequent diarrhea episodes and their long term complications (Fischer Walker et al. 2012). Rotavirus, *Cryptosporidium (C.)* spp., enterotoxigenic *Escherichia coli* and *Shigella* are the most attributable causes of moderate to severe diarrhea and deaths (Kotloff et al. 2013; Lamberti et al. 2014). Most of these pathogens are now considered public health problems and specific diagnostic tools (Rapid diagnostic tests, RDTs, molecular techniques), preventive strategies including vaccine developments and mass treatments are thus being implemented. Intestinal protozoan parasites including, *C.* spp. and *Giardia* (*G.*) *duodenalis,* are the most frequent cause of chronic diarrhea beside bacterial and viral etiologies (Kotloff et al. 2013). The prevalence of both protozoan in diarrheic individuals vary from 2 to 45 % for *G. duodenalis* and 0.5 to 26 % for *C.* spp. (Muhsen and Levine 2012; Desai et al. 2012; Kotloff et al. 2013). The outcome could be worsened in case of acute diarrhea with dehydration in malnourished or in case of HIV-infection. Furthermore, the risk of transmission of both parasites in developing countries, such as Gabon, is increasing, due to urban crowding and poor

sanitation (Dillingham et al. 2002). Previous data from Libreville have shown the prevalence of *G. duodenalis* (2.2–8.6 %) and *C.* spp. (3.0–5.0 %) to be non-negligible in an asymptomatic population, as well as in pediatric patients with acute diarrhea (24.0 %) (Mabika Mamfoumbi et al. 2009; Duong et al. 1995). The clinical spectrum of these two parasitic infections ranges from asymptomatic carrier status to severe diarrhea, depending on host nutritional and immune status. *Giardia (G.) duodenalis* is found in stool samples from immunocompetent patients, whereas *C.* spp. mostly causes acute or chronic diarrhea in HIV-infected patients (Muhsen and Levine 2012; Stark et al. 2009). However, it has been shown that *C.* spp. is not restricted to immunocompromised hosts, it is also found in immunocompetent individuals (Nchito et al. 1998; Gatei et al. 2006; Salyer et al. 2012). This parasite frequently infects children, in which it may have adverse effects on nutritional outcomes, affecting intestinal absorption, nutrition, and childhood development (Checkley et al. 1997; Mondal et al. 2009; Mølbak et al. 1997). Data on asymptomatic individual carriage are scarce although *C.* spp. is now recognized to contribute to the global diseases burden in children (Kotloff et al. 2013; Natchu and Bhatnagar 2013). Indeed, giardiasis and cryptosporidiosis are now recognized as neglected tropical parasitic diseases (Savioli et al. 2006). It is thus essential to identify the reservoirs of these parasites (i.e. individuals with asymptomatic infections), to facilitate the establishment of effective control strategies and to protect the population at greatest risk: children, malnourished and immunocompromised hosts. In case of symptomatic infection, urgent medical action is required, because acute diarrhea can be life-threatening. Moreover, biological diagnosis of both pathogens is routinely performed at first level public health centers or reference laboratories in urban cities from Sub-Saharan Africa. This would enable caregivers to provide an immediate diagnosis and clinical interventions, when necessary. Antigens specific to *G. duodenalis* and *C.* spp. can be detected in fecal samples by immunochromatographic tests (ICTs) or rapid diagnostic tests (RDTs) (Garcia et al. 2003; Minak et al. 2012; Agnamey et al. 2011; Stark et al. 2014). In resource-limited settings, RDTs are highly suitable for point of care testing, with many advantages. They are simple to perform, and they provide results very rapidly, making possible to diagnose the infection and initiate specific treatment during a single visit (Minak et al. 2012; Agnamey et al. 2011). Providing simple, accurate and rapid new diagnostic tool will help for the project on the estimation of *C.* spp. and *G. duodenalis* global prevalence throughout the country.

The aim of this study was to determine the sensitivity and specificity of the ImmunocardSTAT® Crypto/Giardia for their detection in human stool samples, by comparing this method with the gold standard, microscopy. The positive and negative predictive values were also determined.

Methods

In a community-based study performed for the detection of a *Schistosoma mansoni* infection focus within the Plaine-Orety district of Libreville, fresh human fecal specimens were collected in 10 % formalin, from February to May 2012. Plaine-Orety is traversed by a large channel of almost 2 m wide and 1.75 m high which is a landscaped development of the Langoune river. It was built to prevent the recurrent floods observed in this district. It is also widely used for various household activities, fishing and swimming for children. Among the 848 inhabitants of the neighborhood, 420 patients who were aged more than 2 years old and who have not changed their place of residence for more than six months, agreed to participate in the study. All the samples of the 173 included children were used as biological support for the comparison of techniques. Agreement and informed consent for sample testing were obtained before processing. All the samples collected were analyzed prospectively by the gold standard methods, merthiolate-iodine-formaldehyde staining of cysts and vegetative forms (MIFc) for *G. duodenalis* and Ziel–Neelsen (ZN) staining for *Cryptosporidium*. The non-enzymatic rapid immunoassay test ImmunocardSTAT® ® *Crypto/Giardia* Rapid Assay (Meridian Bioscience, Inc.) was performed according to the manufacturer's instructions. This test uses specific antibodies to capture antigens specific to *Giardia* and *Cryptosporidium* and immobilize them on a membrane. Two drops of buffer and 60 µl of stool specimen were added into a tube. Two additional drops of a *Giardia* capture antibody conjugate and colloidal carbon-conjugated detection reagent for *Giardia* and *Cryptosporidium* were then added to the tube, mixed with the sample and immediately poured into the test device. Assay results were read after 10 min and remained valid until 15 min. Grey-black lines (regardless of their intensity) in the appropriate position in the results window indicate a positive result.

Data analysis

All data were entered using Epi-info version 3.3.2 (2005 CDC Atlanta) to resolve discordances and delete double entries. Analysis was performed with Statview 5.0 (SAS Institute, Cary, NC, USA). Microscopy was considered as the gold standard method. Sensitivity (Se), specificity (Sp), negative predictive value (NPV), and positive predictive value (PPV) were calculated with 95 % confidence intervals (CIs). A p value less than 0.05 was significant.

Results

Most of the samples (90.7 %, $n = 157/173$) consisted of solid, formed stools, six were pasty and 10 (5.8 %) were liquid.

G. duodenalis was detected by MIFc in 15.6 % ($n = 27$) of the samples and parasite antigens were detected in 13.3 % ($n = 22$) of the samples with the RDT (Table 1a). Vegetative forms of *G. duodenalis* were identified in seven of the 10 diarrheal stool samples and were associated with cysts in six of these samples. The sensitivity and the specificity of the ImmunocardSTAT ® test for the detection of *G. duodenalis* were 63.0 % and 96.6 % respectively (Table 2). A relationship was found between positive results for the RDT, *G. duodenalis* parasite and stool consistency. Indeed, the ImmunocardSTAT® ® test detected this species in four (40.0 %) of the 10 diarrheal stools, 17 (10.8 %) of the formed stools and one pasty stool sample ($p = 0.03$). However, this test had a sensitivity of 60.0 % when used on formed stools and 50.0 % when used on diarrheal stools. Twenty-one (21) of the 27 tests were positive before 10 min, three between 10 and 15 min, and three between 15 and 20 min with a clear positive line. These last three tests were performed with one diarrheal stool sample containing vegetative forms and two formed stools containing cysts, they were considered positive. By combining the results of microscopic examination, ImmunocardSTAT® tests, the overall prevalence of *G. duodenalis* was 18.5 % ($n = 32/173$).

C. spp. was identified on ZN-stained slides from 18 (10.4 %) stool samples, whereas two (1.2 %) samples tested positive for this parasite with the RDT (Table 1b). No *Cryptosporidium* oocysts were found in any of the diarrheal and pasty stool samples. Parasites of this genus were detected by both techniques in one of the samples, which contained 14 oocysts/gram of feces. For the 17 samples giving false-negative results for *C.* spp., parasite density was between 4 and 164 oocysts/gram of feces. The sensitivity of the ImmunocardSTAT ® test was 5.5 % for the detection of *C.* spp. Its specificity exceeded 94.0 % (Table 2). The overall prevalence of *C.* spp. was 11.0 % ($n = 19/173$).

Table 1 Comparison of microscopy and ImmunoCardStat® RDTs results

	ImmunocardSTAT® test results		
	Positive	Negative	Total (N)
(a) Detection of *Giardia duodenalis*			
MIF staining			
Presence	17	9	26
Absence	6	141	147
(b) Detection of *Cryptosporidium* spp.			
Ziel–Neelsen staining			
Presence	1	17	18
Absence	1	154	155

MIF Merthiolate-iodine-formaldehyde

Discussion

Implementation of point of care diagnosis of infections endemic in sub-Saharan Africa will improve the patient care management. Indeed, tests enabling fast and multiparametric identification of a spectrum of microbial pathogens associated with a syndromic infection will guide the first few critical hours of patient management and will lead to important socio-economic benefits for populations in low and middle income areas were these life-threatening pathogens predominate.

Few studies in Central Africa have evaluated the performance of multitest RDTs, such as ImmunocardSTAT®, for the diagnosis of *G. duodenalis* and *C.* spp. infections in humans, despite the high frequency of these two parasites (Wumba et al. 2012; Sarfati et al. 2006). Indeed, *G. duodenalis* is considered to be one of the most common protozoa responsible for diarrhea in children living in developing countries (Kotloff et al. 2013). This is the first evaluation of a RDT performance in fecal samples of Gabonese children. Moreover, the Immunocardstat Crypto/Giardia was not already evaluated in non diarrheic patients. Cysts and vegetative forms were detected in 18.5 % of children, confirming the existence of a non-negligible human reservoir of *G. duodenalis* among adults and children, as described elsewhere (Ouattara et al. 2008; Wegayehu et al. 2013). Moreover, at the Plaine-Orety district of Gabon, the proportion of children with *C.* spp. oocysts in their stools was found to be 11.0 %, a surprisingly high rate given the lack of symptoms in these individuals. This rate is comparable to the prevalence observed in diarrheic individuals which vary from 3 to 10 % according to HIV prevalence (Wumba et al. 2012; Gatei et al. 2006; Morse et al. 2007). The existence of this reservoir therefore constitutes a potential risk for the infection of malnourished and immunocompromised individuals.

Table 2 ImmunoCardSTAT® RDT performances

	Giardia duodenalis	*Cryptosporidium* spp.
Sensitivity	63.0 [42.4–80.6]	5.5 [0.1–27.3]
Specificity	96.6 [92.2–98.9]	99.3 [96.5–100.0]
FP	22.7 [7.8–45.4]	50.0 [1.3–98.7]
FN	6.0 [2.8–11.1]	9.9 [5.9–15.4]
PPV	77.3 [54.6–92.2]	50.0 [1.2–98.7]
NPV	93.4 [88.1–96.8]	90.1 [84.9–93.9]

The overall sensitivity of the ImmunoCardSTAT ® test was 65.3 % for the detection of *G. duodenalis*, greater than that observed in Germany (58.0 %), Turkey (33.3 %) and in a study on gorillas in Gabon (57.9 %) (Oster et al. 2006; Bayramoglu et al. 2013; VanZijllLanghout et al. 2010). A higher value (68.0 %) was reported by one study in USA (Agnamey et al. 2011). Using diarrheic samples of immunocompromised patients, higher values of this RDTs sensitivity have been noted, ranging from 70.0 to 94.0 %, in France, Saudi Arabia, California and Bangladesh (Garcia et al. 2003. Agnamey et al. 2011; Minak et al. 2012). Higher sensitivity values (>70 %) of ImmunoCard®STAT have been notified for the diagnosis of *C.* spp. infection compared to the 5.5 % found here (Minak et al. 2012; Garcia et al. 2003; Zaglool et al. 2013). A high threshold for the detection of specific antigens is presumably in cause, accounting for the low sensitivity observed here, as sensitivities of 62 % to 81 % have been obtained, depending on parasite load in previous studies (Chartier et al. 2013). Indeed, the sensitivity of the Immunocard-STAT® test is at least 80 % for parasite density above 100,000 oocysts per gram of feces, concentration that is often found in patients with diarrhea (Llorente et al. 2002; Chartier et al. 2013). In the study population, parasite densities for *C.* spp. microscopy-positive samples were frequently below 200 oocysts per gram of feces.

Alternatively, the low sensitivity of this RDT could also be associated to the parasite species. The RDT target is *C. parvum,* the main species identified in diarrheal samples (Llorente et al. 2002; Abd el kader et al. 2012; Agnamey et al. 2011). Infections due to species other than *C. parvum* may generate false-negative results (Llorente et al. 2002). A high frequency of infections due to *C. hominis* could thus partly explain the observed low sensitivity (Agnamey et al. 2011). This species is a strictly human parasite and Plaine-Orety is a district of Libreville in which the human population rarely comes into contact with the *C. parvum* parasite non human reservoir. Moreover, in tropical areas, most surveys on the general population, and even on patients with diarrhea, have shown that *C. hominis* predominates (Wumba et al. 2012; Gatei et al. 2006; Morse et al. 2007; Walker al. 2010). Molecular identification of the *Cryptosporidium* species circulating in Libreville would allow improving the choice of RDT for the diagnosis of infections with these parasites. Overall specificity was good, at more than 95.0 %, a value similar to that found for surveys performed in the general population and for patients with diarrhea (Minak et al. 2012).

This study has some limitations. It was nested, not previously designed to assess the RDT performances and conducted in a population that was presumed to be asymptomatic, and therefore who have a low probability of excreting large numbers of parasites. Another specific study should be performed in others areas and in populations with diarrhea. Moreover, the parasite load of *G. duodenalis* was not estimated, PCR genotyping of *Cryptosporidium* species was not performed.

Conclusion

Asymptomatic carriage of *G. duodenalis* and *C.* spp. is important among children living in a slum of Libreville. The sensitivity of ImmunoCard®STAT Crypto/Giardia RDTs is low, especially for the detection of *C.* spp. The performance of this RDT is therefore too poor to provide added value for diagnosis and it cannot be considered as a reliable alternative to the microscopy method for individuals with a low parasite density or for mass screening of the entire population. More sensitive diagnostic tools are required for the detection of *C.* spp. and *G. duodenalis* in our settings.

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Conflict of interest The authors declare that they have no competing interests.

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