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Prevalence of intestinal opportunistic parasites infections in the University hospital of Bobo-Dioulasso, Burkina Faso

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Abstract

Background: Gastrointestinal parasites infections are widespread in Africa and their prevalence infections vary from country to country. This study aimed at assessing the prevalence of opportunistic intestinal parasites infection and other gastrointestinal parasites infection among patients attending the laboratory of Parasitology and Mycology of the University Hospital Souro Sanou of Bobo-Dioulasso.

Methods: A hospital cross-sectional based study was conducted from April to August, 2012. Participants were persons whom parasitological examination of stools has been prescribed by a clinician. The stools examination methods included direct wet saline examination, lugol's iodine staining technique, formol-ether concentration and modified Ziehl-Neelsen staining. We recorded age and sex information for each patient.

Results: The overall prevalence of intestinal parasite infections was 65.3 % (190/291). Majority of the parasitic infections was waterborne (64.3 %) consisting of high prevalence of *Cryptosporidium sp.* (26.5 %) and *Entamoeba histolytica/dispar* (23.4 %). The prevalence of opportunistic parasites was 28.9 % and *Cryptosporidium sp.* was the most prevalent species followed by *Blastocystis sp.* (1.0 %), *Cyclospora sp.* (0.7 %) and *Isospora belli* (0.7 %). The prevalence of intestinal helminthes was 1.7 %.

Conclusions: The prevalence of intestinal parasitism in general remains high in Bobo-Dioulasso requiring the establishment of adequate diagnostic techniques, treatment and prevention.

Keywords: Intestinal opportunistic parasite, Prevalence, Bobo-Dioulasso, Burkina Faso

Background

Intestinal parasitic infections caused by helminthes and protozoans are among the most widespread and remain an important cause of morbidity and mortality in developing countries [1]. The prevalence of infection is remarkably high in sub-Saharan Africa, where the largest burden of human immunodeficiency virus/ acquired immunodeficiency syndrome (HIV/AIDS) cases is concentrated [2, 3]. HIV infections, tropical and subtropical climate, high population density, poverty, very low

hygienic conditions and health education are the major factors for the transmission of intestinal parasites [1].

Indeed in the previous studies, the prevalence of certain intestinal parasite known as opportunistic is significantly higher among HIV infected individuals with chronic diarrhea and CD4 lymphocytes counts of 200 cells/mm³ [4–8]. The presence of the opportunistic intestinal parasites, *Cryptosporidium sp.*, *Cyclospora cayetanensis*, *Isospora belli*, *Blastocystis hominis* and *Microsporidia* is well documented in patients with HIV/AIDS [4, 5, 9–12]. However, the incidence and the prevalence of the opportunistic intestinal parasites in HIV/AIDS patients are likely to depend upon the endemicity in the community.

Infections by the major human gastrointestinal parasites are widespread in Africa and the prevalence of these infections varies from country to country. Intestinal parasites

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are a major public health problem in Burkina Faso [13]. Their prevalence was estimated at 54.7 % throughout the country [14]. In addition, the epidemiology of intestinal parasitism is very wide and is significantly different between sahelian area (38.9 % in the Sahel) and humid area (65.8 % in the East) [13]. In the capital city of Ouagadougou, a prevalence of 60.82 and 52.47 % have been previously reported respectively by Ouermi *et al.*, [15] and Karou *et al.*, [16]. In Bobo-Dioulasso, economic capital of the country, the prevalence of intestinal parasites was 23.8 % [17]. Published data about opportunistic intestinal parasites infection in Burkina Faso are extremely rare. Only one study performed in 1998 at the Pediatric Department of Bobo-Dioulasso Hospital showed a prevalence of 5.2 % of cryptosporidiosis [18].

This study aimed at assessing the prevalence of opportunistic intestinal parasites among gastrointestinal parasites in patients attending the Laboratory of Parasitology and Mycology of the University Hospital Sourou Sanou of Bobo-Dioulasso for diagnosis.

Methods

Study design

A descriptive cross-sectional hospital based study was carried out from April to August, 2012 in the University Hospital Sourou Sanou of Bobo-Dioulasso, Burkina Faso.

Bobo-Dioulasso (11°10'42"N; 4°17'35"W) is located in the western part of the country at 360 km from Ouagadougou, the capital city of Burkina Faso. The city population is around 1.5 million inhabitants. The climate is Sudan climatic types and is characterized by a rainy season from June to October with relatively abundant rainfalls (the annual rainfall ranges from 1000 to 1200 mm) and a dry season from November to May. The annual average temperature is about 28 °C. Sanitary conditions are insufficient in large sectors of the city.

The University Hospital Sourou Sanou is the only referral and teaching hospital in the region. The hospital provides various health services.

Participants were persons whom parasitological examination of stools has been prescribed by a clinician. The sample size was determined using the single proportion population formula. It was calculated assuming a prevalence of 50 % with a margin of error of 0.05 and a confidence level of 95 %, that a minimum size of 291 participants.

Fecal sample collection

A written informed consent form has been administered to each participant. Then, a single fresh stool sample was collected from each consenting study participant in a sterile fecal container early morning. The specimens were soon transported to the laboratory of Parasitology-

Mycology of the University Hospital Sourou Sanou of Bobo-Dioulasso.

Sociodemographic characteristics of the study participants including age and sex were registered for each patient on the data collection form.

Parasitological methods

Freshly stool specimens were collected, processed and examined microscopically in saline wet mount to detect larva, eggs, trophozoites and cysts of various parasites. In addition, formol-ether concentration was performed and modified Ziehl-Neelsen (ZN) method was used to detect coccidian species.

Direct examination

A portion of stool was examined by direct wet saline mount preparation (0.90 % sodium chloride solution) to observe motile intestinal parasites and trophozoites under light microscope at 10× and 40× magnifications. Lugol's iodine staining method was also performed to observe cysts of the intestinal protozoan parasites.

The remaining part of stool was processed by the following methods.

Formalin ether concentration

About 7 ml of 10 % formalin was added to approximately 1 g of feces and mixed using an applicator stick. The stool sample was sieved with cotton gauze and transferred to 15 ml centrifuge tube Falcon®. After adding 3 ml of diethyl ether to the mixture and hand shaking, the content was centrifuged at 2000 rpm for 3 min. The supernatant was poured and a drop of sediment was transferred to slide. Finally, the entire zone under the cover slip was systematically examined using 10X and 40X objective lenses to observe ova, cyst and larvae of different intestinal parasites according to the protocol of Ritchie [19, 20].

Modified Ziehl-Neelsen method

In this method, thin smears were prepared from preserved as well as sediments of concentrated stool samples, air-dried, and fixed with absolute methanol for 5 min. The smears were stained with carbol-fuchsin for 10 min and thereafter, washed with tap water. The slides were decolorized in chlorhydric acid-ethanol 1 % for 2 min and were counter stained with methylene blue 0.25 % for 1 min [21, 22]. Finally the stained smears were examined using oil immersion objective to detect oocysts of *Cryptosporidium* sp., of *Isospora belli*, of *Cyclospora* sp. and cyst of *Blastocystis* sp.

Quality control

Each stool sample was examined by two different laboratory technicians. In case of discordant results, the stool

sample slide was read by a third technician, and his report was considered as the final result.

Randomly selected samples were also sent to the laboratory of Parasitology of Centre MURAZ, Bobo-Dioulasso to check the reproducibility of the results.

Data analysis

Data were double-entered based on EpiData 3.1. Statistical analysis was performed with SPSS Statistics 17.0 (SPSS Inc., Chicago, IL). Chi-square test was used to compare the categorical variables. Fisher's exact test was used when the expected value in any cell was less than 5.

Ethics statement

The study protocol was approved by the institutional review boards of the University Hospital of Bobo-Dioulasso for routine analyses, Burkina Faso. Participants were contacted through the hospital practitioners and the objectives, procedures, and potential risks were carefully explained to all potential participants. Interested individuals were asked to sign a written informed consent or their parents in the case of minors before being involved in the study. Personal data from participant and all diagnostic results were kept strictly confidential. Results of participants with parasitic infections were sent, as soon as possible to clinicians for their case management.

Results

Participants' characteristics

Among the 291 participants, 146 (50.2 %) were male, 145 (49.8 %) were female (Table 1) with a 1.1 sex ratio. The mean age of the participants was 29.2 ± 20.8 years (ranged, 0–89 years).

Prevalence of gastrointestinal parasite

The overall prevalence of intestinal parasite infections was 65.3 % (190/291). Majority of the parasitic infections were waterborne protozoa, prevalence of 64.3 % (187/291) with few helminthes, 1.7 % (5/291). *Cryptosporidium sp.*

Table 1 Distribution of sociodemographic characteristics

Age group (years)	Total number of individual		Total
	Gender		
	Male	Female	
0–5	22	18	40 (13.7 %)
6–9	14	16	30 (10.3 %)
10–14	12	7	19 (6.5 %)
15–29	33	37	70 (24.1 %)
30–59	61	63	124 (42.6 %)
≥60	4	4	8 (2.7 %)
Total	146	145	291

and *Entamoeba histolytica/dispar* were found at 26.5 and 23.4 %, respectively, constituting the majority of parasitic infections, followed by others protozoa *Entamoeba coli* (19.6 %), *Giardia lamblia* (4.8 %), *Trichomonas intestinalis* (1.7 %), *Blastocystis hominis* (1.0 %), *Cyclospora sp.* (0.7 %) and *Isoospora belli* (0.7 %) were also observed (Table 2). Among the helminthic parasites, one case of eggs of *Ascaris lumbricoides*, *Trichuris trichiura*, *Ankylostomidae*, *Hymenolepis nana*, *Dicrocoelium sp* and larvae of *Strongyloides stercoralis* were observed at a rate of 0.3 % respectively.

Prevalence of intestinal opportunistic parasites

Regarding the intestinal opportunistic parasites infection, the results revealed a total of 84 out the 291 patients, thus the prevalence was 28.9 %. *Cryptosporidium sp.* infection was the most diagnosed (26.5 %, 77/291) followed by infections with *Blastocystis sp.* (1.0 %; 3/291), *Cyclospora* (0.7 %, 2/291) and *Isoospora belli* (0.7 %, 2/291) (Table 3).

A break-up of the 291 persons (146 males and 145 females) revealed no difference as both the sexes were equally affected with the opportunistic intestinal parasites. Both age ($P = 0.6$) was not significantly associated the opportunistic intestinal parasites (Table 3).

Global co-infection of gastrointestinal parasite and prevalence co-infection of opportunistic parasites species

The overall prevalence of multiple infection of gastrointestinal parasite was 21.0 % (61/291) and co-infection of opportunistic parasites was 2.1 (6/291).

Table 2 Prevalence of different species intestinal parasites

Parasite species	Number of positive samples (Prevalence %)
Protozoa	187 (64.3)
<i>Entamoeba histolytica</i>	68 (23.4)
<i>Entamoeba coli</i>	57 (19.6)
<i>Giardia lamblia</i>	14 (4.8)
<i>Trichomonas intestinalis</i>	5 (1.7)
<i>Cryptosporidium sp.</i>	77 (26.5)
<i>Blastocystis sp</i>	3 (1.0)
<i>Cyclospora</i>	2 (0.7)
<i>Isoospora belli</i>	1 (0.3)
Helminth	5 (1.7)
<i>Ankylostomidae</i>	1 (0.3)
<i>Ascaris lumbricoides</i>	1 (0.3)
<i>Dicrocoelium sp</i>	1 (0.3)
<i>Hymenolepis nana</i>	1 (0.3)
<i>Trichuris trichiura</i>	1 (0.3)

Table 3 Prevalence of intestinal opportunistic intestinal parasites according age and sex

Risk factor	Opportunistic parasite			<i>Cryptosporidium</i>	
	No. samples examined	No. positive (Prevalence %)	P value	No. positive (Prevalence %)	P value
Age group (years)					
0–5	40	18 (45)		15 (37.5)	
6–9	30	10 (33.3)		10 (33.3)	
10–14	19	5 (26.3)	0.2	5 (26.3)	0.5
15–29	70	16 (22.9)		16 (22.9)	
30–59	124	32 (25.8)		29 (23.4)	
>60	8	3 (37.5)		2 (25.0)	
Gender					
Male	146	44 (30.1)		41 (28.1)	
Female	145	40 (30.1)	0.6	36 (24.8)	0.5

Discussion

Overall 190 out of 291 (65.3 %) participants harbored intestinal parasites in the the laboratory of Parasitology and Mycology laboratory of University hospital of Bobo-Dioulasso. The national prevalence for intestinal parasite infection was 54.7 % [23].

This prevalence of intestinal parasitism is high compared with previous findings in Burkina Faso. Other authors reported 23.8 % in Bobo-Dioulasso [17, 23] 52.4 and 60.8 % in Ouagadougou [15, 16]. The difference could be due mainly to coproparasitological techniques used. In our study, modified Ziehl-Neelsen method was systematically performed. This technique increased the sensitivity of study by diagnosing 78 cases of parasitism compared to direct examination and formol ether concentration.

The high prevalence of protozoa parasitism, (64.3 %) compared to that of helminth infections (1.7 %) in this study is in agreement with previous findings in Burkina Faso [23] and in other developing countries in Africa [6, 24–26]. The drug mass administration with albendazole could explain the low rate of helminthes infection as previously reported [23, 27, 28].

The prevalence of intestinal opportunistic parasites was 28.9 % and the most prevalent species was *Cryptosporidium sp.* (26.5 %). This prevalence of *Cryptosporidium sp.* is higher than those reported in Burkina Faso (5.2 %) [18], and as well as in west Africa (ranging from 7.7 to 25.71 %) [29–31]. The difference could be due to sample size as our study presented a larger sample size than that reported by other authors.

The presence of opportunistic intestinal parasites such as *Blastocystis* (1.10 %), *Cyclospora* (0.7 %) and *Isospora belli* (0.7 %) should not be neglected. The pathogenicity of certain species such as *Blastocystis sp.* among immunocompetent persons is not excluded [32, 33].

Gender and age did not significantly affect the prevalence of intestinal parasitic infections while it was not a risk factor for acquiring these infections. The distribution

of the parasitic infections in both sexes as well as, among the various age groups suggested that sex and age were not predetermining factors for parasitic infections in our study.

Our study had some limitations. First, host immunity such as HIV status was not investigated in the present study. Second, we did not use the Weber trichome staining or PCR which would have improved the diagnosis of microsporidiosis [34]. The present study is a pilot study and we are planning a large-scale longitudinal study.

One perspective of our staff is to use the molecular tool to study the epidemiology of different opportunistic species. In fact, for example, the identification and characterization of *Cryptosporidium* species and population variants (genotypes and subgenotypes) is fundamental to study the epidemiology of cryptosporidiosis, being a valid support for prevention and control strategies [35]. The large scale of human cases of cryptosporidiosis worldwide are caused by two species, *Cryptosporidium parvum* and *C. hominis* [36]. However, other species, including *C. felis*, *C. meleagridis*, *C. canis*, *C. suis*, *C. muris* and, more rarely, *C. cuniculus*, *C. ubiquitum* and *C. andersoni* can also infect humans, especially children under the age of 5 years and immunocompromised individuals [37, 38]. Oocyst morphology, host specificity or preferences in infection sites do not provide sufficient information for the identification of *Cryptosporidium* species, genotypes or subgenotypes. The confirmation of their species status and determination of virulence and pathogenic profiles might explain why some patients are asymptomatic while others present clinical symptoms [38].

Conclusions

The overall prevalence of intestinal parasites was therefore 65.3 % in our study. The prevalence of intestinal opportunistic parasites was 28.9 % and *Cryptosporidium sp.* was the most prevalent species.

Prompt diagnosis of parasitic infections in HIV-negative and positive patients, especially intestinal parasitic infections using staining and molecular diagnostic tools is advocated in order to improve the management and quality of life of HIV-infected individuals.

Abbreviations

CI: Confident interval; °C: Degree celsius; CD4: Lymphocyte cluster differentiation 4; HIV/AIDS: Human immunodeficiency virus/ acquired immunodeficiency syndrom; %: Percent; mm: Millimeter; ZN: Ziehl-Neelsen.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

IS and GTR conceived and designed the experiments. IS, BR, YKB, SR performed the experiments. IS and CM analyzed the data. IS, BS, DKR and GTR contributed reagents/materials/analysis tools. IS wrote the paper. All authors read and approved the final version of the manuscript.

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