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Prevalence and potential risk factors for gastrointestinal parasitic infections in children in urban Bissau, Guinea-Bissau

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Background: Gastrointestinal (GI) parasitic infections cause significant morbidity and mortality in tropical and subtropical countries. We aimed to investigate the prevalence of GI parasitic infections in children from Bissau, Guinea-Bissau and to identify the possible risk factors for these infections.

Methods: We performed an observational study on two comparable cohorts of children 2–15 y of age: one study covering health care–seeking children (n=748) and one study covering children from the background population (n=851). A total of 1274 faecal samples from the two cohorts were investigated for parasites by microscopy and the risk factors for infection were identified by logistic regression.

Results: Intestinal parasitic infections were found in 54.8% of health care–seeking children and 55.5% of children from the background population. Helminth infections were more common among health care–seeking children and were mainly due to hookworms. Pathogenic protozoa included *Entamoeba histolytica/dispar* and *Giardia lamblia*. The risk factors for infections included increasing age, household crowding and poor sanitation and water supply.

Conclusions: We found a high prevalence of intestinal parasites among both health care–seeking children and children from the background population. Compared with previous studies, we found a decreasing prevalence of intestinal helminths. This study further identifies potential risk factors for infections, including inadequate sanitation and water supply.

Keywords: Guinea-Bissau, helminthiasis, neglected diseases, parasitic diseases, parasitology, protozoan infections

Introduction

Intestinal parasitic infections caused by helminths and protozoa are among the most common infections in humans and contribute significantly to global morbidity and mortality.[1] Intestinal parasites are distributed worldwide, with the highest prevalence in poor and developing countries. Soil-transmitted helminths (STHs), including *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms, are estimated to affect >25% of the world's population.[2] *Giardia lamblia* is the most prevalent intestinal parasite worldwide, with prevalence ratios of 10–50% reported in children in tropical regions and >250 million people infected worldwide.[3] Recent reports estimate the global burden of amoebiasis (caused by *Entamoeba histolytica*) to be approximately 400 000 cases annually, accounting for 40 000–110 000 deaths.[3,4] The symptomology of intestinal parasitic infections varies widely between different species and further depends on the infection intensity and the patient's age, nutritional state, immunocompetence and genetic factors. Although intestinal parasite infections may be asymptomatic, severe symptoms and complications include diarrhoea and dysentery, anaemia, stunted growth and impaired cognitive development.[2,5]

Guinea-Bissau, a small country located in western Africa, is one of the poorest countries in the world and has one of the highest infant mortality rates.[6] Over the past 3 decades, several studies have found that gastrointestinal (GI) infections and associated diarrhoea has been and still is among the leading causes of morbidity and mortality in children in Guinea-Bissau.[7,8] The main focus in previous studies has been on children <5 y of age, where the most prevalent causes were reported to be rotavirus, *Cryptosporidium* spp., *G. lamblia* and pathogenic strains of *Entamoeba coli*.[9,10]

The aim of the present study was to estimate the prevalence of intestinal parasitic infections and potential risk factors among both health care–seeking children and children from the background population in urban Bissau, Guinea-Bissau.

Methods

Study area

The study was conducted in Bissau, the capital in Guinea-Bissau, in two neighbouring suburbs, Bandim I and Bandim II, which are part of the Bandim Health Project (BHP) study area.[11] The map is available online on the BHP homepage.[11]

The particular study area was chosen due to logistics and because the socio-economic status, including income and standard of living, does not vary significantly between the two suburbs. The climate of Guinea-Bissau is tropical, with a rainy season from June to November, when the monthly precipitation can be >500 mm.

Study design, population and period

We performed an observational study of two comparable cohorts: one cohort covered children 2–15 y of age whose parent or guardian sought medical attention for their child at a local health centre due to fever, diarrhoea and vomiting (referred to as cohort I) and the other cohort consisted of children who were identified in the BHP Health and Demographic Surveillance Site database (referred to as cohort II). These children were matched on age, sex and residency with children from cohort I and they were visited at their private address and invited to participate. The two-cohort design was chosen to investigate prevalence among children seeking medical attention and the general population of children with no apparent signs of disease.

The study was conducted from August 2015 to April 2017, providing data from both the dry and rainy seasons. Questionnaires and stool samples were collected. Questionnaires included baseline information, such as age and residency, and information regarding possible risk factors for intestinal parasite infections, such as household construction, animal husbandry and sanitary standards.

All information in the questionnaire was obtained by a local health professional based on a structured interview with the participant's parent or guardian. All participants were assigned a unique study identifier on inclusion.

Intestinal parasite examination

Upon inclusion in the study, the participant's parent or guardian was instructed to use a collection tube to collect a stool sample from the participant on the morning of the investigation. Fresh stool samples were kept in a refrigerator until examination, which was performed within 18 h of sample delivery. Microscopy examination was performed by independent, trained laboratory technicians at the Bandim Health Centre.

Faecal samples were investigated by three distinct light microscopy–based techniques to allow detection of the most common GI parasites, including those reported in previous studies. Direct light microscopy of a wet mount was used to detect and quantify trophozoites of *G. lamblia* and *Entamoeba* spp. and to detect *Strongyloides stercoralis* larvae.[12] For the detection and quantification of helminth eggs, the flotation method of Willis was performed.[13] The formol-ether technique was used to detect and quantify protozoan cysts.[12] *Entamoeba* cysts were characterized as either *E. coli* or *E. histolytica/dispar*, the latter two species being indistinguishable from one another by microscopic examination.[14] Each sample was investigated by one technician and the three applied techniques were in adherence with the local routine for parasitological investigation.

Data handling and management

Questionnaires and laboratory forms containing results from the microscopic examination of each faecal sample were entered manually using EpiData Entry Client version 4.0.2 (EpiData Association, Odense, Denmark; www.epidata.dk). The 250 matched questionnaires and 250 laboratory forms (19.6% of all paper forms) were double-entered by another investigator to verify data and minimize errors due to manual entry. No major differences appeared between the two entries, with errors in written names only and not in the numerical data.

Statistical methods

Absolute numbers and percentages were used to describe the two cohorts and the overall prevalence of intestinal parasites was identified in two age groups: 2–7 and 8–15 y old. The three most prevalent pathogenic intestinal parasites in both cohorts—hookworm, *E. histolytica/dispar* and *G. lamblia*—were used as outcomes in logistic regression models to identify possible risk factors for infection with these species. Risk factors were calculated for both cohorts as odds ratios (ORs) with corresponding 95% confidence intervals (CIs) and p-values. We performed univariate and multivariate logistic regressions, with the latter adjusted for age, sex and residency. Due to a low prevalence and thus a lack of statistical power, we did not perform logistic regression analyses for other intestinal parasites.

Between-cohort differences were calculated using the Pearson χ^2 test or Fisher's exact test, when appropriate. P-values <0.05 were considered significant. All statistical analyses were performed using Stata 15.1 (StataCorp, College Station, TX, USA).

Ethical statement

This project was approved by the Ethical Committee of Guinea-Bissau (reference no. 0029/CNES/INASA/2015). Participants were enrolled after oral and written consent was obtained from the parent or guardian. Any positive parasitological finding was treated according to local standard protocols, free of charge. The present study was performed in accordance with the Helsinki Declaration.

Results

Study size and baseline characteristics

A total of 1599 individuals (748 from cohort I and 851 from cohort II) gave consent to participate and provided complete

questionnaire data. Of these, 304 completed the questionnaire but failed to return a stool sample for parasitological investigation. Another 21 participants delivered a stool sample but did not complete the questionnaire. In total, 566 children from cohort I and 708 children from cohort II provided the completed questionnaire and stool sample, yielding a total study size of 1274 participants. The characteristics of the two cohorts are shown in Table 1.

The median age for both sexes was 6 y, ranging from 2 to 14 y in cohort I and 2 to 15 y in cohort II. Both cohorts were divided into two age groups: 2–7 y (377 in cohort I and 471 in cohort II; N=848 [66.6%]) and 8–15 y (189 in cohort I and 237 in cohort II; N=426 [33.4%]). The sex distribution did not vary between the two cohorts (54.1% and 53.4% were boys in cohort I and II, respectively; p=0.811) or between the age groups (53.2% and 54.7% were boys in the younger and older age group, respectively; p=0.975).

Household size, husbandry and hygiene indicators

Information on household size, animal husbandry and hygiene indicators (source of drinking water and toilet source) is shown in Table 1. The household size did not differ between the two cohorts and sharing a bed with other children was equally common between the two. However, we found that it was more common for children in cohort I to share a bed with one or more adults (age >15 y) compared with children from cohort II (p<0.005).

Animal husbandry was equally common in the two cohorts, as 43.5% (246/566) in cohort I and 44.6% (316/708) in cohort II reported at least one animal in the household (p=0.668) and approximately 20% (110/566 and 149/708 for cohort I and II, respectively) reported more than one animal. The most common animals were chickens (17.5% and 23.5% for cohort I and II, respectively), pigs (22.6% and 18.4%) and dogs (19.8% and 18.8%). In cohort I, it was equally or more common to keep animals inside the house (including chickens and pigs) vs outside, whereas this was not the case for the homes of the children in cohort II.

No apparent differences existed between the two cohorts regarding toilet facilities, as in both cohorts it was more common to have access to poor toilet facilities rather than good (79.2% and 76.6%, respectively; p=0.279), However, the source of drinking water was different between the two cohorts. In general, a poor water source was more common in cohort I than in cohort II (59.5% of children in cohort I used a well or public water station vs 25.0% in cohort II; p<0.005)

Household construction, animal husbandry and hygiene indicators were regarded as proxy measurements of socio-economic status among the participants and their families.

Helminth infections

Helminths were more common in cohort I than in cohort II (78 [13.8%] samples versus 68 [9.6%], p=0.021) and seemed to be more common in older children than younger, although that difference was not statistically significant (Table 2). We found no differences between the two different suburbs. For both cohorts, positive helminth samples were mainly due to hookworm (73.1% of all samples were positive for helminths in cohort I and 61.8% of all samples were positive for helminths in cohort II). We found a very low prevalence of other helminths, with no major differences between the cohorts.

Protozoan infections

The overall prevalence of samples positive for intestinal protozoans was 41.5% (235/566) in cohort I and 46.0% (326/708) in cohort II, with no significant difference between either the cohorts or the age groups (Table 2). *E. histolytica/dispar* was found in 17.3% (98/566) of the children in cohort I and 17.2% (122/708) of the children in cohort II, with the highest prevalence in older children for both cohorts. *G. lamblia* was significantly more common among children in cohort II, as it was found in 20.5% (116/566) in cohort I and in 26.6% (188/708) in cohort II. The prevalence of *G. lamblia* was lower in younger children, an effect only observed in cohort I. No differences were observed in the prevalence of any of the protozoans between suburbs.

Multiple intestinal parasites

More than one intestinal parasite was detected by microscopy in 16.2% (92/566) of the children in cohort I and 15.8% (112/788) of the children in cohort II (Table 3). Multiple infections were equally common between the two cohorts (p=0.844) and equally distributed across age groups (p=0.174). The most common combinations were *E. coli* and *E. histolytica/dispar* or a combination of *E. histolytica/dispar* and *G. lamblia*. Co-infection with either of the non-pathogenic protozoa (*E. coli* and *Endolimax nana*) significantly increased the risk of also having an infection with *E. histolytica/dispar* in both cohorts (OR 3.40, p<0.001 and OR 4.27, p<0.001, respectively) and further increased the risk of having an infection with *G. lamblia* in cohort I (OR 1.72, p=0.041).

Associations between intestinal parasite infections and potential risk factors

Overall, some risk factors were shared across the two cohorts, whereas others were observed in only one (Table 4 and Supplementary Tables 1–3). Female children in cohort II were found to have marginally higher odds of infection with hookworm (OR 1.90, p=0.049), but sex did not seem to affect the odds for other parasites in any of the cohorts. In both cohorts, the odds of hookworm infection increased in the older age group (OR 3.11, p<0.001 in cohort I; OR 4.56, p<0.001 in cohort II) and children in the older age group in cohort II also had higher odds of infection with *E. histolytica/dispar* (OR 1.56, p=0.030). Children in the older age group in cohort II had lower odds of *G. lamblia* infection (OR 0.41, p<0.001).

Household construction had a varying impact as a risk factor. In cohort I, sharing a house with four or more children increased the odds of hookworm infection (adjusted OR [OR_{adj}] 2.48, p=0.032), an observation that was also seen in cohort II with respect to infection with *G. lamblia* (OR_{adj} 1.64, p=0.045).

Table 1. Characteristics of the residence, age and sex distributions, household composition, season of inclusion, animal husbandry and sanitation standards in two cohorts: 566 health care–seeking children (cohort I) and 708 children from the background population (cohort II), all of whom were 2–15 y of age and from urban Bissau, Guinea-Bissau

Characteristics	Cohort I (N=566), n (%)	Cohort II (N=708), n (%)	Total (N=1274), n (%)	p-Value
Subdivision				0.105
Bandim I	410 (72.4)	541 (76.4)	951 (74.6)	
Bandim II	156 (27.6)	167 (23.6)	323 (25.4)	
Sex				0.811
Male	306 (54.1)	378 (53.4)	684 (53.7)	
Female	260 (45.9)	330 (46.6)	590 (46.3)	
Age group (years)				0.975
2–7	377 (66.6)	471 (66.5)	848 (66.6)	
8–15	189 (33.4)	237 (33.5)	426 (33.4)	
Household				
Adults in house				0.108
1–2	213 (37.6)	299 (42.2)	512 (40.2)	
3–4	232 (41.0)	287 (40.5)	519 (40.7)	
≥5	121 (21.4)	122 (17.2)	243 (19.1)	
Children in house				0.296
0–1	173 (30.6)	191 (27.0)	364 (28.6)	
2–3	260 (45.9)	331 (46.8)	591 (46.4)	
≥4	133 (23.5)	186 (26.3)	319 (25.0)	
Adults sharing bed with participant				**<0.005**
0	110 (19.4)	207 (29.2)	317 (24.9)	
1	329 (58.1)	380 (53.7)	709 (55.7)	
≥2	127 (22.4)	121 (17.1)	248 (19.5)	
Children sharing bed with participant				0.054
0	221 (39.0)	262 (37.0)	483 (37.9)	
1	208 (36.7)	304 (42.9)	512 (40.2)	
≥2	137 (24.2)	142 (20.1)	279 (21.9)	
Season				**0.014**
Dry season	270 (47.7)	387 (54.7)	657 (51.6)	
Rainy season	296 (52.3)	321 (45.3)	617 (48.4)	
Husbandry				
Any animal	246 (43.7)	316 (44.6)	562 (44.1)	0.668
Pigs				0.158
None	438 (77.4)	578 (81.6)	1016 (79.7)	
Outside	30 (5.3)	28 (4.0)	58 (4.6)	
Inside	98 (17.3)	102 (14.4)	200 (15.7)	
Chicken				**<0.005**
None	467 (82.5)	542 (76.6)	1009 (79.2)	
Outside	40 (7.1)	93 (13.1)	133 (10.4)	
Inside	59 (10.4)	73 (10.3)	132 (10.4)	
Sanitation				
Water source				**<0.005**
Well/public water station	337 (59.5)	177 (25.0)	514 (40.3)	
Private post/running water	229 (40.5)	531 (75.0)	760 (59.7)	
Toilet source				0.279
No toilet/public latrine	448 (79.2)	542 (76.6)	990 (77.7)	
Private latrine/toilet	118 (20.8)	166 (23.4)	284 (22.3)	

p-Values indicate statistical differences between the two cohorts by the Pearson χ^2 test or Fisher's exact test, when appropriate. p-Values <0.05 are considered statistically significant and are marked with **bold**.

Table 2. Prevalence of intestinal parasites in two cohorts: 566 health care–seeking children (cohort I) and 708 children from the background population (cohort II), all of whom were 2–15 y of age and from urban Bissau, Guinea-Bissau. For multi-infected participants, each positive finding is included in the table

Characteristics	2–7 years		8–15 years		Total		p-Value
	Cohort I, n (%)	Cohort II, n (%)	Cohort I, n (%)	Cohort II, n (%)	Cohort I, n (%)	Cohort II, n (%)	
No. of children	377 (66.6)	471 (66.5)	189 (33.4)	237 (33.5)	566 (100)	708 (100)	
Uninfected	200 (53.1)	233 (49.5)	84 (44.4)	118 (49.8)	284 (50.2)	351 (49.6)	0.844
Infected with ≥1 parasite	177 (46.9)	238 (51.5)	105 (55.6)	119 (51.2)	282 (49.8)	357 (50.4)	
Infected with 1 parasite	122 (32.4)	165 (35)	68 (36)	80 (33.8)	190 (33.6)	245 (34.6)	
Infected with 2 parasites	44 (11.7)	64 (13.6)	32 (16.9)	33 (13.9)	76 (13.4)	97 (13.7)	
Infected with ≥3 parasites	11 (2.9)	9 (1.9)	5 (2.6)	6 (2.5)	16 (2.8)	15 (2.1)	
Helminths							
Any helminth	38 (10.1)	37 (7.9)	40 (10.6)	31 (6.6)	78 (13.8)	68 (9.6)	0.021
Hookworm	24 (6.4)	14 (3.0)	33 (8.8)	28 (5.9)	57 (10.1)	42 (5.9)	0.008
Hymenolepis diminuta	3 (0.8)	3 (0.6)	1 (0.3)	1 (0.2)	4 (0.7)	4 (0.6)	0.739
Hymenolepis nana	11 (2.9)	19 (4.0)	5 (1.3)	3 (0.6)	16 (2.8)	22 (3.1)	0.869
Enterobius vermicularis	0 (0.0)	1(0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	NA
Ascaris lumbricoides	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	NA
Strongyloides stercoralis	2 (0.5)	0 (0.0)	2 (0.5)	0 (0.0)	4 (0.7)	0 (0.0)	0.039
Trichuris trichiura	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	NA
Taenia saginata	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	n/a
Protozoa							
Any protozoa	152 (40.3)	223 (47.3)	83 (22.0)	103 (21.9)	235 (41.5)	326 (46.0)	0.112
Entamoeba coli	34 (9.0)	27 (5.7)	16 (4.2)	25 (5.3)	50 (8.8)	52 (7.3)	0.351
Entamoeba histolytica/dispar	58 (15.4)	71 (15.1)	40 (10.6)	51 (10.8)	98 (17.3)	122 (17.2)	0.999
Giardia lamblia	84 (22.3)	150 (31.8)	32 (8.5)	38 (8.1)	116 (20.5)	188 (26.6)	0.012
Endolimax nana	28 (7.4)	33 (7.0)	17 (4.5)	18 (3.8)	45 (8.0)	51 (7.2)	0.669
Chilomastix mesnili	2 (0.5)	0 (0.0)	1 (0.3)	0 (0.0)	3 (0.5)	0 (0.0)	0.087
Iodamoeaba butschlii	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)	0.444

p-Values were calculated between the two cohorts by Fischer's exact test. p-Values <0.05 are considered statistically significant and are marked with bold. NA: not applicable due to low prevalence.

Sharing a house with four or more children marginally decreased the odds of infection with *G. lamblia* in cohort I (OR_{adj} 0.54, p=0.046). Sharing a bed with one other child decreased the odds of infection with *E. histolytica/dispar* in cohort I (OR_{adj} 0.52, p=0.014), and a similar observation was seen in living with three to four adults in cohort II (OR_{adj} 0.61, p=0.039).

Animal husbandry had a limited effect on the risk of intestinal parasite infections and having the animal inside the house did not seem to be a greater risk than outside.

In both cohorts, using a private latrine or toilet decreased the odds of hookworm infection (OR_{adj} 0.32, p=0.019 and OR_{adj} 0.21, p=0.011, respectively). In cohort II, obtaining drinking water from a private water post or from running water in the house significantly decreased the odds of infection with hookworm (OR_{adj} 0.48, p=0.026) and *E. histolytica/dispar* (OR_{adj} 0.58, p=0.013). In cohort I, using a private latrine or toilet decreased the odds of *G. lamblia* infection (OR_{adj} 0.48, p=0.016), whereas the source of drinking water did not seem to influence the odds of infection.

We did not observe any significant association between the prevalence of the intestinal parasites and season.

Discussion

This two-cohort study found a high prevalence of GI parasites among children 2–15 y of age in urban Bissau, Guinea-Bissau. The high prevalence was found in both health care–seeking children (cohort I) and children from the background population (cohort II), suggesting that intestinal parasitic infections are not the main reason to seek medical attention for children in Bissau-Guinean and further suggesting that intestinal parasite infections are widely distributed among the background population. Approximately 50% of all participants in both cohorts were infected with at least one intestinal parasite (Table 2). The major intestinal parasite species were pathogenic protozoa, including *E. histolytica/dispar* and *G. lamblia*. Since *E. histolytica* and *E. dispar* are indistinguishable by microscopy, the high prevalence in

Table 3. Distribution of multiple intestinal parasite infections in two cohorts: 566 health care–seeking children (cohort I) and 708 children from the background population (cohort II), all of whom were 2–15 y of age and from urban Bissau, Guinea-Bissau

Characteristics	2–7 y		8–15 y		Total	
	Cohort I, n (%)	Cohort II, n (%)	Cohort I, n (%)	Cohort II, n (%)	Cohort I, n (%)	Cohort II, n (%)
No. of children	377 (66.6)	471 (66.5)	189 (33.4)	237 (33.5)	566 (100%)	708 (100%)
Infected with 2 parasites	44 (11.7)	64 (13.6)	32 (16.9)	33 (13.9)	76 (13.4)	97 (13.7)
Entamoeba histolytica/dispar and *Giardia lamblia*	9 (2.4)	15 (3.2)	1 (0.5)	5 (2.1)	10 (1.8)	20 (2.8)
Entamoeba histolytica/dispar and *Entamoeba coli*	6 (1.6)	12 (2.5)	5 (2.6)	8 (3.4)	11 (1.9)	20 (2.8)
Hymenolepis nana and *Giardia lamblia*	1 (0.3)	8 (1.7)	2 (1.1)	0 (0)	3 (0.5)	8 (1.1)
Giardia lamblia and *Endolimax nana*	5 (1.3)	7 (1.5)	2 (1.1)	2 (0.8)	7 (1.2)	9 (1.3)
Entamoeba histolytica/dispar and *Endolimax nana*	2 (0.5)	6 (1.3)	4 (2.1)	4 (1.7)	6 (1.1)	10 (1.4)
Giardia lamblia and hookworm	2 (0.5)	6 (1.3)	5 (2.6)	4 (1.7)	7 (1.2)	10 (1.4)
Entamoeba histolytica/dispar and hookworm	2 (0.5)	1 (0.2)	5 (2.6)	3 (1.3)	7 (1.2)	4 (0.6)
Entamoeba coli and *Endolimax nana*	5 (1.3)	1 (0.2)	1 (0.5)	3 (1.3)	6 (1.1)	4 (0.6)
Entamoeba coli and *Giardia lamblia*	4 (1.1)	1 (0.2)	2 (1.1)	1 (0.4)	6 (1.1)	2 (0.3)
Other combinations	8 (2.1)	7 (1.5)	5 (2.6)	3 (1.3)	13 (2.3)	10 (1.4)
Infected with ≥3 parasites	11 (2.9)	9 (1.9)	5 (2.6)	6 (2.5)	16 (2.8)	15 (2.1)
Entamoeba histolytica/dispar, Entamoeba coli and *Giardia lamblia*	4 (1.1)	0 (0)	0 (0)	0 (0)	4 (0.7)	0 (0)
Entamoeba histolytica/dispar, Endolimax nana and *Giardia lamblia*	1 (0.3)	3 (0.6)	2 (1.1)	0 (0)	3 (0.5)	3 (0.4)
Entamoeba coli, Endolimax nana and *Giardia lamblia*	1 (0.3)	2 (0.4)	0 (0)	0 (0)	1 (0.2)	2 (0.3)
Other combinations	5 (1.3)	4 (0.8)	3 (1.6)	6 (2.5)	8 (1.4)	10 (1.4)

both cohorts may reflect the non-pathogenic *E. dispar*. Amoebiasis from *E. histolytica* is a serious and sometimes fatal disease, and molecular characterization by polymerase chain reaction (PCR) may, in the future, shed light on the actual distribution of the two species.

Although overall intestinal parasite prevalence was equal between the two cohorts, some significant differences were found, which should be considered when interpreting the results. The overall prevalence of the helminths was higher in cohort I than in cohort II, with the prevalence of hookworm being especially different (Table 2). Furthermore, *G. lamblia* prevalence was statistically higher in cohort II than in cohort I (Table 2), although this may not have any clinical relevance. In addition, some discrepancies exist between the two cohorts with regard to baseline characteristics. A poor drinking water source was more common among children in cohort I, which could explain, at least partly, the higher prevalence of hookworm, as infection follows the faecal–oral route. However, a poor source of drinking water also would be expected to impact the prevalence of *G. lamblia*, as discussed below, suggesting that sanitation is not the only driving factor.

A previous study of 706 children, 4–12 y of age, conducted in the dry season of 2001 in the same urban area of Bissau as we investigated, reported a high prevalence of helminths (44.2% of all included), including hookworm (31.9%) and pathogenic protozoa (51.1%), including *G. lamblia* (34.7%) and *E. histolytica/dispar* (26.6%).[15] Whereas the prevalence of protozoa was comparable with our findings, and thus seems to be relatively stable over time, the prevalence of helminths seems to have declined. One possible explanation for this may be the introduction of biannual mass administration of mebendazole alongside vitamin A supplementation in children 6 months–5 y of age, which was introduced in 2006. Mass drug treatment is known to be an efficient strategy to reduce the prevalence of STHs, although concerns regarding limited efficacy due to potential reinfection and drug resistance should be considered.[16] Despite the supposed decline in helminth prevalence, distribution among different species remained more or less intact. Mass drug administration of mebendazole may induce a certain cohort effect on overall helminth prevalence, as treated children are less likely to pass the infection to other family members. Our results support this, as we found that the prevalence of STHs was higher in the older age group (8–15 y) compared with the young group, regardless of cohort. This is consistent with previous publications from Guinea-Bissau and worldwide.[15,17] Other possible explanations for the decline in helminth prevalence include improvements in living conditions. This is more speculative, however, as no data support this, and furthermore, we would expect that this would also impact the prevalence of intestinal protozoa.

In another more recent but smaller study of 52 children, 0–5 y of age, with diarrhoea and living in urban Bissau, the prevalence of *G. lamblia* and *E. histolytica* was reported to be 44.0% and 0.0%, respectively, as determined by PCR.[18] The prevalence determined by microscopy was quite different from the highly sensitive PCR method (6% for *G. lamblia* and 2% for *E.*

Table 4. Logistic regression analysis of the potential risk factors for intestinal parasite infections in two cohorts of children from urban Bissau, Guinea-Bissau.

Risk factor	Hookworm						*Entamoeba histolytica/dispar*						*Giardia lamblia*					
	Cohort I (n positive=57)			Cohort II (n positive=42)			Cohort I (n positive=98)			Cohort II (n positive=122)			Cohort I (n positive=116)			Cohort II (n positive=188)		
	OR	OR_{adj}	p-Value	OR	OR_{adj}	p-Value	OR	OR_{adj}	p-Value	OR	OR_{adj}	p-Value	OR	OR_{adj}	p-Value	OR	OR_{adj}	p-Value
Sex																		
Male	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
Female	1.35	–	0.279	1.90	–	**0.049**	0.74	–	0.186	0.92	–	0.693	1.41	–	0.100	1.03	–	0.858
Age group (years)																		
2–7	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
8–15	3.11	–	**<0.001**	4.56	–	**<0.001**	1.47	–	0.095	1.56	–	**0.030**	0.69	–	0.116	0.41	–	**<0.001**
Adults in house																		
1–2	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
3–4	0.75	0.69	0.261	1.12	1.06	0.865	1.32	1.26	0.370	0.63	0.61	**0.039**	1.08	1.14	0.596	0.90	0.93	0.713
≥5	0.96	0.84	0.651	0.68	0.59	0.313	1.09	1.04	0.892	1.69	1.60	0.068	1.46	1.53	0.126	0.84	0.93	0.777
Children in house																		
0–1	Ref.						Ref.			Ref.			Ref.			Ref.		
2–3	1.15	1.19	0.641	2.00	2.06	0.109	1.34	1.39	0.234	1.06	1.04	0.872	0.89	0.86	0.534	1.49	1.55	**0.044**
≥4	2.48	2.25	**0.032**	1.80	1.48	0.434	1.68	1.68	0.095	1.03	0.95	0.863	0.55	0.54	**0.046**	1.41	1.64	**0.045**
Adults sharing bed with patient																		
0	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
1	0.61	0.79	0.498	0.80	1.15	0.707	1.06	1.17	0.588	0.85	0.94	0.789	1.18	1.09	0.775	1.37	1.13	0.551
≥2	0.72	1.20	0.680	0.55	1.19	0.760	0.84	0.99	0.977	0.73	0.86	0.650	1.20	1.04	0.908	1.48	1.08	0.772
Children sharing bed with patient																		
0	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
1	0.60	0.60	0.165	1.12	1.13	0.761	0.51	0.52	**0.014**	0.94	0.93	0.761	0.72	0.70	0.144	0.87	0.88	0.517
≥2	2.00	1.62	0.145	1.76	1.54	0.310	0.87	0.84	0.524	1.00	0.94	0.813	0.87	0.90	0.707	0.94	1.06	0.818
Season																		
Dry season	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
Rainy season	0.80	0.71	0.241	1.11	1.12	0.725	1.04	1.04	0.852	0.99	0.99	0.950	1.15	1.15	0.515	0.81	0.81	0.229
Animal husbandry																		
Pigs																		
None	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
Outside	1.41	1.27	0.681	–	–	–	1.18	1.22	0.683	0.79	0.73	0.566	1.46	1.41	0.428	0.91	1.04	0.924
Inside	1.04	0.96	0.908	0.77	0.64	0.375	0.86	0.80	0.478	1.03	0.96	0.892	1.04	1.10	0.723	1.06	1.19	0.481
Chickens																		
None	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
Outside	0.69	0.55	0.344	0.50	0.53	0.314	1.17	1.14	0.759	0.66	0.66	0.203	0.91	0.93	0.853	0.59	0.57	0.052
Inside	0.79	0.69	0.453	1.11	0.84	0.739	0.73	0.71	0.394	0.70	0.63	0.204	0.57	0.58	0.176	1.06	1.28	0.390
Sanitation																		
Water source																		

Continued

Table 4. *Continued*

Risk factor	Hookworm						*Entamoeba histolytica/dispar*						*Giardia lamblia*					
	Cohort I (n positive=57)			Cohort II (n positive=42)			Cohort I (n positive=98)			Cohort II (n positive=122)			Cohort I (n positive=116)			Cohort II (n positive=188)		
	OR	OR_{adj}	p-Value	OR	OR_{adj}	p-Value	OR	OR_{adj}	p-Value	OR	OR_{adj}	p-Value	OR	OR_{adj}	p-Value	OR	OR_{adj}	p-Value
Well or public water station	Ref.			Ref.			Ref.			Ref.			Ref.			Ref.		
Private water post or running water	0.85	0.85	0.591	0.43	0.48	**0.026**	0.79	0.77	0.263	0.57	0.58	**0.013**	0.88	0.90	0.626	0.78	0.73	0.116
Toilet source																		
No toilet access or public latrine	Ref.						Ref.			Ref.			Ref.			Ref.		
Private latrine or toilet	0.34	0.32	**0.019**	0.24	0.21	**0.011**	1.58	1.52	0.102	1.20	1.18	0.480	0.46	0.48	**0.016**	0.84	0.87	0.581

Crude ORs are unadjusted. Multivariate odds ratios (OR_{adj}) are adjusted for age, sex and residence of the participant).
95% CIs are found in Supplementary Tables 1–3. P-values <0.05 are considered statistically significant and marked with **bold**.

histolytica). Diarrhoea may influence the sensitivity of microscopy as a detection method for intestinal protozoa, as the density of both trophozoites and cysts in the faecal output is dependent on stool consistency.[19] PCR has several advantages, including improved sensitivity, and microscopy may underestimate the actual prevalence.[19] However, in a developing country with no access to molecular laboratory facilities, microscopy remains a cheap, reproducible and feasible method for the identification of intestinal parasites, with acceptable sensitivity and specificity.[20]

Several explanations are possible for the high prevalence of intestinal parasites both among health care–seeking children and children from the background population. Among the most important risk factors are poor hygienic standards, as demonstrated by our analyses. Our results suggest that the drinking water source and toilet facilities are major contributors to the risk of infection (Table 4). The relatively high prevalence of the non-pathogenic protozoa *E. coli* and *E. nana* could indeed support this, as these are often regarded as indicators or surrogate measures of faecal contamination in the local environment due to the route of infection.[21] Our results support a relationship between these non-pathogenic protozoans and infection with *E. histolytica/dispar* and further demonstrate a relationship with *G. lamblia* infection in cohort I.

With respect to *G. lamblia* infections, drinking water may easily be contaminated with cysts from infected humans and domestic animals.[22] Although no recent studies have investigated the water quality in urban and suburban Bissau, previous studies have reported the presence of both amoeba and pathogenic coliform bacteria in water sources.[23,24]

We found that having pigs and chickens inside the house was common. Despite this, and the fact that both chickens and pigs are able to passively spread infective helminth eggs, our analysis did not find any association between pathogenic intestinal parasites and animal husbandry.[25] The overall low prevalence of helminths and the introduction of mass drug administration to children may explain why we do not find any association between animal husbandry and helminth infection. A possible explanation for the high prevalence of co-infections with multiple intestinal parasites is that most intestinal parasites follow the faecal–oral route of infection and are transmitted in the same habitats.[17,26]

The present study has some limitations to consider. Primarily, our prevalence estimates are based on microscopy. We aimed to describe the prevalence using available standard methods in Guinea-Bissau. Although molecular diagnosis by PCR is superior to microscopy in terms of sensitivity and specificity, as discussed previously, it is not available in Guinea-Bissau at the present time. Furthermore, PCR would be able to discriminate between pathogenic *E. histolytica* and non-pathogenic *E. dispar*, which would provide better insight into the intestinal parasitic disease burden and enhance the importance of the identified possible risk factors.

Furthermore, the findings of this study represent an overview of the prevalence of parasitic infections at a specific point in time. Although our study was conducted over a period of 20 months, covering both rainy and dry seasons, we did not detect any seasonal variation in prevalence. Previous studies from sub-Saharan Africa have found that the transmission of STHs was

significantly lower in the dry season.[27] The overall low prevalence of helminths in our study may explain why we did not see the same effects. Furthermore, the prevalence of intestinal parasites is highly dynamic within the population, and reinfection is likely to occur. In addition, the faecal output of intestinal parasites may vary between samples from the same individual and from day to day.[28] We only investigated one faecal sample per participant and our results may thus underestimate the actual prevalence. The sensitivity and specificity of microscopy may be increased by examining two or three samples on alternating days, although some might argue that one sample is sufficient.[29]

Conclusions

This study updates the current knowledge on the prevalence, distribution and risk factors for intestinal parasites in children from Bissau, Guinea-Bissau. We find an overall high prevalence of intestinal protozoa, including *E. histolytica/dispar* and *G. lamblia*, both among children seeking medical attention and among children from the background population. The high prevalence among children from the background population suggests that intestinal parasitic infections are not necessarily symptomatic. We found that risk factors for protozoan infections are poor hygienic standards, including the source of drinking water and toilet access. We also found a pronounced decline in the prevalence of helminths compared with previous studies, which may be explained by the introduction of mass administration of anthelmintic drugs.

Future work should focus on the identification of infection sources, including examination of wells and sewage systems for the presence of both helminths and protozoa. By identifying the source of infections, the improvement of WASH factors (water, sanitation, and hygiene) may help lower the prevalence, as these have been demonstrated to be at least as effective as mass treatment campaigns.[30]

Supplementary data

Supplementary data are available at Transactions online (http://trstmh.oxfordjournals.org/).

Authors' contributions: PK and UH designed the study. SH and PK performed the data collection, entry, handling and statistical analyses. SH and UH acquired funding. SH drafted the manuscript. SH, PK and UH critically revised the manuscript and read and approved the final manuscript. SH, PK and UH are guarantors of the paper.

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Ethical approval: The present study was performed in accordance with the Helsinki Declaration. Oral and written consent to participate was obtained from either a parent or guardian prior to inclusion of their child in the study. The study was approved by the local ethical committee of Guinea-Bissau.

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