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RESEARCH ARTICLE

Seasonal variation of diarrhoeal pathogens among Guinea-Bissauan children under five years of age

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Abstract

Background

Diarrhoea remains a major cause of childhood morbidity and mortality in low-income countries (LICs). The frequency of diarrhoeal episodes may vary by season, yet few prospective cohort studies have examined seasonal variation among various diarrhoeal pathogens using multiplex qPCR to analyse bacterial, viral and parasitic pathogens.

Methods

We combined our recent qPCR data of diarrhoeal pathogens (nine bacterial, five viral and four parasitic) among Guinea-Bissauan children under five years old with individual background data, dividing by season. The associations of season (dry winter and rainy summer) and the various pathogens were explored among infants (0–11 months) and young children (12–59 months) and those with and without diarrhoea.

Results

Many bacterial pathogens, especially EAEC, ETEC and *Campylobacter*, and parasitic *Cryptosporidium*, prevailed in the rainy season, whereas many viruses, particularly the adenovirus, astrovirus and rotavirus proved common in the dry season. Noroviruses were found constantly throughout the year. Seasonal variation was observed in both age groups.

Conclusion

In childhood diarrhoea in a West African LIC, seasonal variation appears to favour EAEC, ETEC, and *Cryptosporidium* in the rainy and viral pathogens in the dry season.

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Author summary

Childhood diarrhoea, a major cause of mortality in low-income countries (LICs), is known to be influenced by seasonal variations. Studies on the seasonality of diarrhoeal pathogens have mostly been conducted in the temperate zone where the seasons differ by temperature. In Guinea-Bissau and many other LICs, seasonality is characterized by precipitation changes with negligible temperature variations. The monsoon-type rainy season lasts from June to October and the dry season from November to May. We expect this seasonality to influence stool pathogen distribution.

To investigate the associations between various diarrhoeal pathogens and seasonality in childhood diarrhoea in Guinea-Bissau, we explored a large variety of bacterial, viral and parasitic agents among children with and without diarrhoea during the rainy versus dry seasons.

We found diarrhoeal pathogens not to be equally present over the year but show seasonal variation with respect to age and precipitation. The waterborne *Cryptosporidium* showed highest prevalence during the wetter months, while viral pathogens (adenovirus, astrovirus and rotavirus) were found most frequently during the arid months. Of bacterial pathogens, EAEC, ETEC, and *Campylobacter* prevailed in the rainy season. Such data add to our understanding of childhood diarrhoea in LICs and serve as a tool for devising preventive measures.

Introduction

The seasonality of diarrhoeal pathogens has mostly been examined in temperate regions [1], where seasons are mainly characterized by variations in temperature. Fewer studies have been conducted in tropical low-income countries (LICs), where the seasons are generally defined by rainfall [2–3]. Data on seasonal variations are quite scarce, but deeper insight into the impact of seasonality on each pathogen's epidemiology may offer tools for prevention and even guide treatment.

In Guinea-Bissau, like in all equatorial tropical countries, the temperature differences over the year are minimal, mostly with averages ranging between 28 and 32 degrees. There is, however, a monsoonal-like rainy season from June to October and a dry season from November to May, with monthly rainfall averages of 70–600mm and 0–20mm, respectively [4]. The data remain limited, but weather conditions may have a considerable effect on the types of prevailing pathogens. For example, in the Global Enteric Multicenter Study (GEMS) conducted in four sub-Saharan African countries (Kenya, Mali, Mozambique, and Gambia) and three South Asian (Bangladesh, India, and Pakistan) [5], Chao et al. report rotavirus prevailing in the drier and bacterial pathogens in the wetter months [6]. Only a few studies have explored seasonality of diarrhoeagenic pathogens among children in Guinea-Bissau [7–9], mainly focusing on rotavirus and *Cryptosporidium*. In the absence of national surveillance programmes, academic research provides despite its limitations, for example, with respect to continuity over time, an important source of data on the seasonality of the agents causing diarrhoea in LICs.

To investigate the associations between various diarrhoeal pathogens and seasonality in childhood diarrhoea, we explored a large variety of bacterial, viral and parasitic agents among children with and without diarrhoea during both rainy and dry seasons. Moreover, while we previously observed differences in the occurrence of various pathogens between infants and young children [10], we now scrutinized whether these differences were seasonally impacted. Characterizing such epidemiology of diarrhoeal pathogens can enhance clinical approaches

for diagnostics and treatment by season. In addition, defining the weather conditions for each pathogen should enable identification of pathways of pathogen spread and prediction of large outbreaks.

Materials and methods

Ethics statement

The study protocol was approved by the Comité Nacional de Ética na Saúde, Instituto Nacional de Saúde Pública, Guinea-Bissau (No: 031/CNES/2010). As described in our previous report in the same study setting [10], the children's parents or caregivers were informed about the aim of the study and they signed a written informed consent form.

Study population and sample material

This study was a secondary analysis of data from an unmatched, health facility-based case-control study, the procedures and primary findings of which have been reported elsewhere [10]. The study was conducted at the Bandim Health and Demographic Surveillance Site (HDSS) serving an area of 16 km^2 in suburban Bissau, the capital of Guinea-Bissau (www.bandim.org). A total of 561 children were recruited between November 2010 and October 2012 from among consecutive patients (excluding weekends and night-time) seeking medical care at the Bandim Health Centre, which is one of the three health centres in the Bandim HDSS (Fig 1). The study population was selected to cover children with and without diarrhoea in two age groups: infants (0–11 months) and young children (12–59 months). The inclusion criteria comprised age less than five years and information available on the study nurse's interview form concerning presence/lack of diarrhoea at the time of sampling; those with ongoing diarrhoea were included in the diarrhoea group and those with no diarrhoeal symptoms over the past seven days in the control group. Children requiring hospital care were not eligible.

Definition of children's diarrhoea

Diarrhoea was defined by WHO criteria [11] as passage of three or more loose or liquid stools per day or, more frequently than is normal for the individual. The children were defined as not having diarrhoea if they had not had diarrhoea during the last seven days prior to attending the health centre.

Weather data, definition of dry and rainy season and age stratification

Monthly averages of daily temperature and precipitation for Bissau were extracted from WorldWeatherOnline.com [4] and visualized by plotting them in time series graphs alongside taxon- and species-specific enteropathogen detection rates. The rainy season was defined as the months in which the average rainfall exceeded 50 mm (June to October) and the dry season as months it did not exceed this limit (November to May). The data were also analysed by age group, allowing comparisons between infants (0–11 months) and small children (12–59 months).

PCR for the detection of diarrhoeal pathogens

PCRs for the detection of diarrhoeal pathogens were described in our previous study [10]. Briefly, we used qPCR assays with a large coverage of pathogens: nine enteric bacteria, *Campylobacter*, enteroaggregative (EAEC), enterohaemorrhagic (EHEC), enteroinvasive (EIEC)/*Shigella*, enteropathogenic (EPEC), enterotoxigenic *E. coli* (ETEC), *Salmonella*, *Yersinia* and *Vibrio cholerae* O1 [12]; five viruses: adenovirus 40 and 41, astrovirus, norovirus GI and GII,

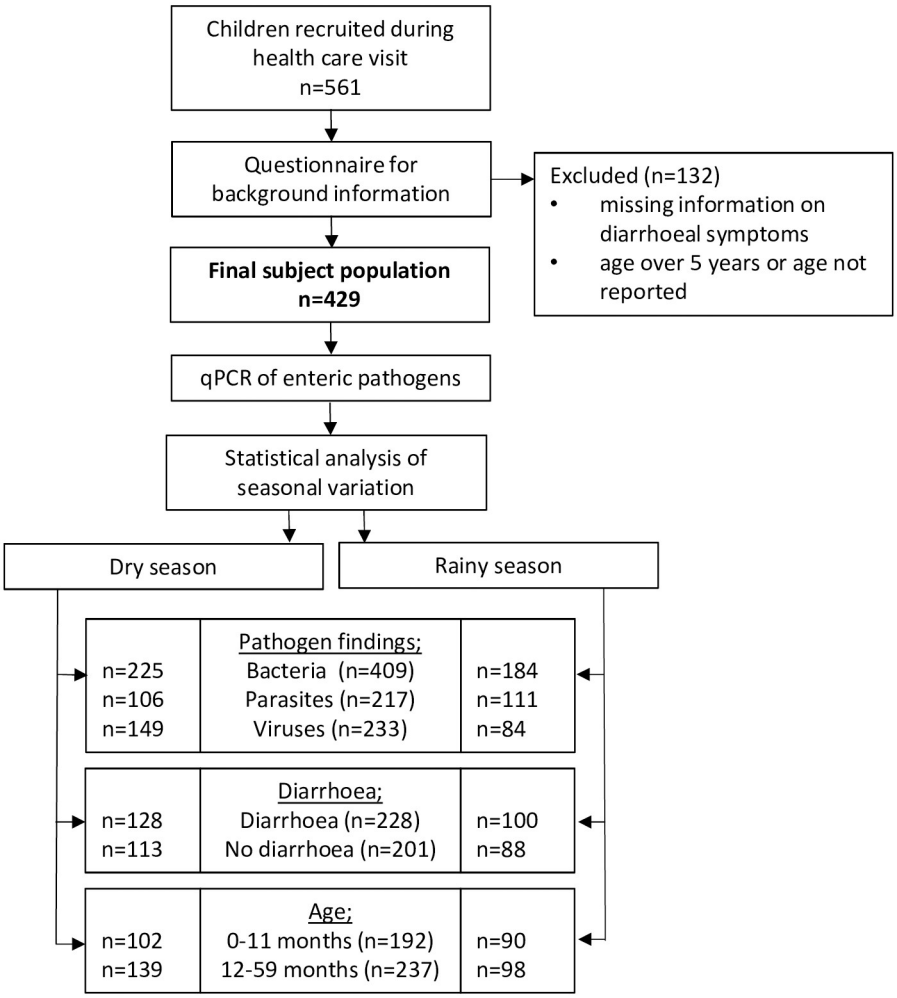

Fig 1. Flow chart of the study. The final study population comprised 429 children; 424 of these were tested for bacterial, 397 for viral, and 426 for parasitic pathogens.

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rotavirus A and sapovirus (Amplidiag Viral GE; Mobidiag Ltd, Helsinki, Finland); and four parasites: *Cryptosporidium*, *Dientamoeba fragilis*, *Entamoeba histolytica* and *Giardia duodenalis* (Amplidiag Stool Parasites; Mobidiag Ltd, Helsinki, Finland).

Statistical analysis

Pearson's chi-square test or Fisher's exact test were used to compare categorical variables when applicable. Statistical significance was defined as $p<0.05$ or ORs with 95% CIs ranging either above or below 1. Exact binomial 95% CIs for proportions were calculated. The increase/decrease of pathogen findings by 100 mm of rainfall was analysed by logistic regression. The unadjusted population attributable fraction (AF) was defined as $AF = \frac{Pr\ (disease)\ - Pr(disease|not\ exposed)}{Pr\ (disease)}$ which by the Bayesian formula equals to $Pr\ (exposed \mid disease)\left(1 - \frac{1}{RR}\right)$, in which RR is the relative risk of the disease. We used logistic regression with robust standard error estimators to evaluate attributable fractions by predicting the number of cases needed for calculating the estimate of AF and its uncertainty (standard error). The unadjusted AF is estimated by

$\frac{observed\ no.\ ill - expected\ no.\ ill\ on\ removal\ of\ the\ exposure}{observed\ no.\ ill}$ [13]. The statistical analyses were carried out using SPSS 22 software (IBM Corp., Armonk, NY) and Stata 17.0 (StataCorp, College Station, TX).

Results

Study population

Of the 561 children recruited, 429 met the inclusion criteria, 228 of them having diarrhoea and 201 not having the disease. Of all children, 211 (50.8%) were females, 193 (45.0%) infants (0–11 months), and 236 (55.0%) young children (12–59 months). Flow chart of study conduct is shown in Fig 1.

Seasonality of diarrhoeal pathogens

As indicated in our previous report, coinfections with multiple pathogen species types (bacteria, viruses, parasites) were common [10]. Overall, findings of viral pathogens in the total study population were most common in the dry season (45.4% in the rainy versus 70.3% in dry season, $p<0.001$) and parasitic pathogens in the rainy season (59.4% versus 44.4%, $p = 0.002$). With bacterial pathogens, the rates were very high regardless of season (98.4% versus 94.9%) (Table 1 and Fig 2). These differences were mostly explained by six individual pathogens showing seasonal differences in prevalence (Table 1): EAEC, ETEC, *Cryptosporidium*, adenovirus, astrovirus and rotavirus (Fig 3); a seasonal variation was also observed with *Campylobacter*, yet it did not reach statistical significance (58.3% versus 48.9%, $p = 0.056$). EAEC (69.5% versus 59.9%, $p = 0.041$), ETEC (56.7% versus 45.1%, $p = 0.018$) and *Cryptosporidium* (22.5% versus 7.1%, $p<0.001$) were found more frequently during the rainy season, and rotavirus (12.4% versus 33.0%, $p<0.001$), astrovirus (4.9% versus 14.6%, $p = 0.001$), and adenovirus (11.4% versus 24.5%, $p = 0.001$) during the dry season. For the other pathogens, no clear differences were seen between seasons. A separate analysis by the amount of rainfall showed that 100 mm per month significantly increased the number of *Campylobacter* ($p = 0.026$) and *Cryptosporidium* ($p<0.001$) findings, and correspondingly decreased the number of virus findings ($p<0.001$), mainly adenovirus, astrovirus and rotavirus (Table 1).

The temperature In Guinea-Bissau does not vary much during the year and, thus, the variation in the rates of the various pathogen findings appears not to depend on changes in temperature (Fig 2b).

Seasonality of diarrhoeal pathogens by age group

In both age groups, an association was found between the findings of *Cryptosporidium* and rainy season (Table 2). As for the viral pathogens, an association with the dry season was observed for rotavirus among infants (dry versus rainy season: 40.0% versus 11.2%, $p<0.0019$, and young children (27.9% versus 13.5%, $p = 0.011$), for astrovirus among infants (16.7% versus 5.6%, $p = 0.019$) and young children (13.1% versus 4.2%, $p = 0.023$) and for adenovirus among infants (31.1% versus 10.1%, $p = 0.001$). For bacterial pathogens, we observed an association with the rainy season in the following age groups: for *Campylobacter* among infants (rainy versus dry season: 58.4% versus 43.6%, $p = 0.041$), for ETEC among infants (62.9% versus 47.5%, $p = 0.033$) and for EAEC among young children (66.3% versus 50.0%, $p = 0.013$).

Impact of season on pathogen findings among those with and without diarrhoea

We compared those with symptomatic diarrhoea to those without diarrhoea separately during each of the two seasons. Statistically significant differences were seen for viruses; they were more

Table 1. Microbial findings among 429 Guinea-Bissauan children under five years of age during dry (November to May) and rainy (June to October) seasons*.

	Total	Dry season		Rainy season		Rainy versus dry season		Impact of 100 mm monthly rainfall on pathogen findings	
	n (%)	n (%)	95% CI (%)	n (%)	95% CI (%)	OR (95% CI)	p-value	OR (95% CI)	p-value
All subjects	429 (100)	241 (56.2)		188 (43.8)					
Children with diarrhoea	228 (53.1)	128 (53.1)		100 (53.2)		1.0 (0.7–1.5)	0.987	1.0 (0.9–1.1)	0.856
Any pathogen	422 (98.4)	236 (97.9)		186 (98.9)		2.0 (0.4–10.3)	0.474		
Any bacteria [a]	409 (96.5)	225 (94.9)	91.3–97.3	184 (98.4)	95.4–99.7	3.3 (0.9–11.8)	0.056	1.1 (0.8–1.5)	0.536
Campylobacter	225 (53.1)	116 (48.9)	42.8–55.9	109 (58.3)	50.3–64.8	1.5 (1.0–2.1)	0.056	1.1 (1.0–1.2)	**0.026**
EAEC	272 (64.2)	142 (59.9)	53.4–66.3	130 (69.5)	62.8–76.3	1.5 (1.0–2.3)	**0.041**	1.0 (0.9–1.1)	0.704
EHEC	6 (1.4)	3 (1.3)	NA	3 (1.6)	NA	1.3 (0.3–6.4)	1.000	NA	NA
EIEC/*Shigella*	98 (23.1)	48 (20.3)	15.1–25.7	50 (26.7)	20.8–33.9	1.4 (0.9–2.3)	0.116	1.0 (0.9–1.2)	0.435
EPEC	266 (62.7)	143 (60.3)	53.4–66.3	123 (65.8)	58.4–72.4	1.3 (0.8–1.9)	0.250	1.0 (0.9–1.1)	0.441
ETEC	213 (50.2)	107 (45.1)	39.0–52.1	106 (56.7)	49.8–64.3	1.6 (1.1–2.3)	**0.018**	1.0 (0.9–1.1)	0.565
Salmonella	11 (2.6)	5 (2.1)	NA	6 (3.2)	NA	1.5 (0.5–5.1)	0.342	NA	NA
V. cholerae	2 (0.5)	1 (0.4)	NA	1 (0.5)	NA	1.3 (0.1–20.4)	1.000	NA	NA
Yersinia	3 (0.7)	2 (0.8)	NA	1 (0.5)	NA	0.6 (0.1–7.0)	1.000	NA	NA
Any parasites [b]	217 (50.9)	106 (44.4)	37.5–50.5	111 (59.4)	52.4–66.8	1.8 (1.2–2.7)	**0.002**	1.2 (1.1–1.3)	**0.001**
Cryptosporidium	59 (13.8)	17 (7.1)	4.2–11.2	42 (22.5)	17.9–30.5	3.8 (2.1–6.9)	<**0.001**	1.4 (1.2–1.5)	<**0.001**
D. fragilis	43 (10.7)	26 (11.9)	8.0–17.1	17 (9.2)	5.4–14.2	0.8 (0.4–1.4)	0.385	1.1 (0.9–1.2)	0.438
E. histolytica	3 (0.7)	0 (0.0)	NA	3 (1.6)	NA	NA	0.084	NA	NA
Giardia	159 (37.3)	88 (36.8)	30.2–42.8	71 (38.0)	30.1–44.4	1.1 (0.7–1.6)	0.808	1.0 (0.9–1.1)	0.474
Any virus [c]	233 (58.7)	149 (70.3)	64.3–77.0	84 (45.4)	37.1–51.8	0.4 (0.2–0.5)	<**0.001**	0.8 (0.7–0.9)	<**0.001**
Adenovirus 40, 42	73 (18.4)	52 (24.5)	19.1–31.2	21 (11.4)	7.1–16.7	0.4 (0.2–0.7)	**0.001**	0.8 (0.6–0.9)	**0.005**
Astrovirus	40 (10.1)	31 (14.6)	10.3–20.3	9 (4.9)	2.2–8.9	0.3 (0.1–0.6)	**0.001**	0.7 (0.6–1.0)	**0.034**
Norovirus GI/GII	92 (23.2)	48 (22.6)	17.4–29.1	44 (23.8)	17.2–29.7	1.1 (0.7–1.7)	0.788	1.0 (0.9–1.1)	0.871
Rotavirus A	93 (23.4)	70 (33.0)	27.0–40.2	23 (12.4)	8.0–17.9	0.3 (0.2–0.5)	<**0.001**	0.8 (0.6–0.9)	**0.001**
Sapovirus	29 (7.3)	20 (9.4)	5.9–14.3	9 (4.9)	2.2–8.9	0.5 (0.2–1.1)	0.081	0.9 (0.7–1.1)	0.426

Data is missing, n (%):

[a] 5 (1.2),

[b] 3 (0.7),

[c] 32 (7.5)

CI = confidence interval; NA = not applicable; OR = odds ratio; **bolding** indicates statistically significant at $p<0.05$ (Pearson χ^2 test or Fisher´s exact test)

*Coinfections with multiple pathogen species were observed in 97.4% of samples collected in the rainy and 92.1% in the dry season (p = 0.017; OR 3.2 95% CI 1.2–8.7). Viruses and bacteria were found in 19.5% versus 41.2% (p<0.001; OR 0.3 95% CI 0.2–0.5), bacteria and parasites in 34.1% versus 13.7% (0<0.001; OR 3.2 95% CI 2.0–5.4), viruses and parasites in 0% versus 1.0% (p = 0.500; OR NA), and all three in 24.9% versus 27.5% (p = 0.562; OR 0.9 95% CI 0.6–1.4), respectively.

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prevalent among those with diarrhoea than those without the disease during both seasons (Table 3). A tendency was seen for practically all viruses, but statistical significance was found for norovirus (rainy season: diarrhoea 29.6% versus no diarrhoea 17.2%; dry season: diarrhoea 28.9% with no diarrhoea 15.5%) and astrovirus (rainy season: diarrhoea 8.2% versus no diarrhoea 1.1%; dry season: diarrhoea 19.3% versus no diarrhoea 9.2%) in both seasons. Of the parasites, the higher rates of cases among those with diarrhoea were seen with *Cryptosporidium* during both seasons. As for bacteria, the only significant findings were those for ETEC, which showed in the dry season higher rates among those with diarrhoea than those without it (rainy season: diarrhoea 57.0% versus no diarrhoea 56.3%; dry season: diarrhoea 52.8% versus no diarrhoea 36.4%). In the rainy season, most of the diarrhoea cases were attributable to norovirus (8%) and *Cryptosporidium* (6%) and in the dry season to ETEC (13%) and norovirus (8%) (Table 3).

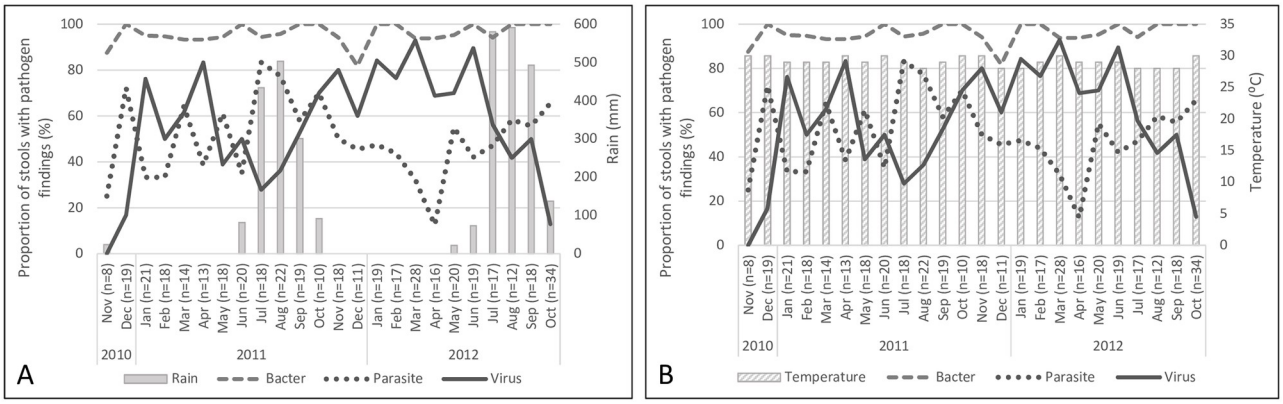

Fig 2. Seasonal variation of diarrhoeal pathogens identified over the 24-month study period with respect to rainfall [4] (A) and temperature (B). Monthly proportions given for those with samples positive for bacterial, parasitic, or viral diarrhoeal pathogens.

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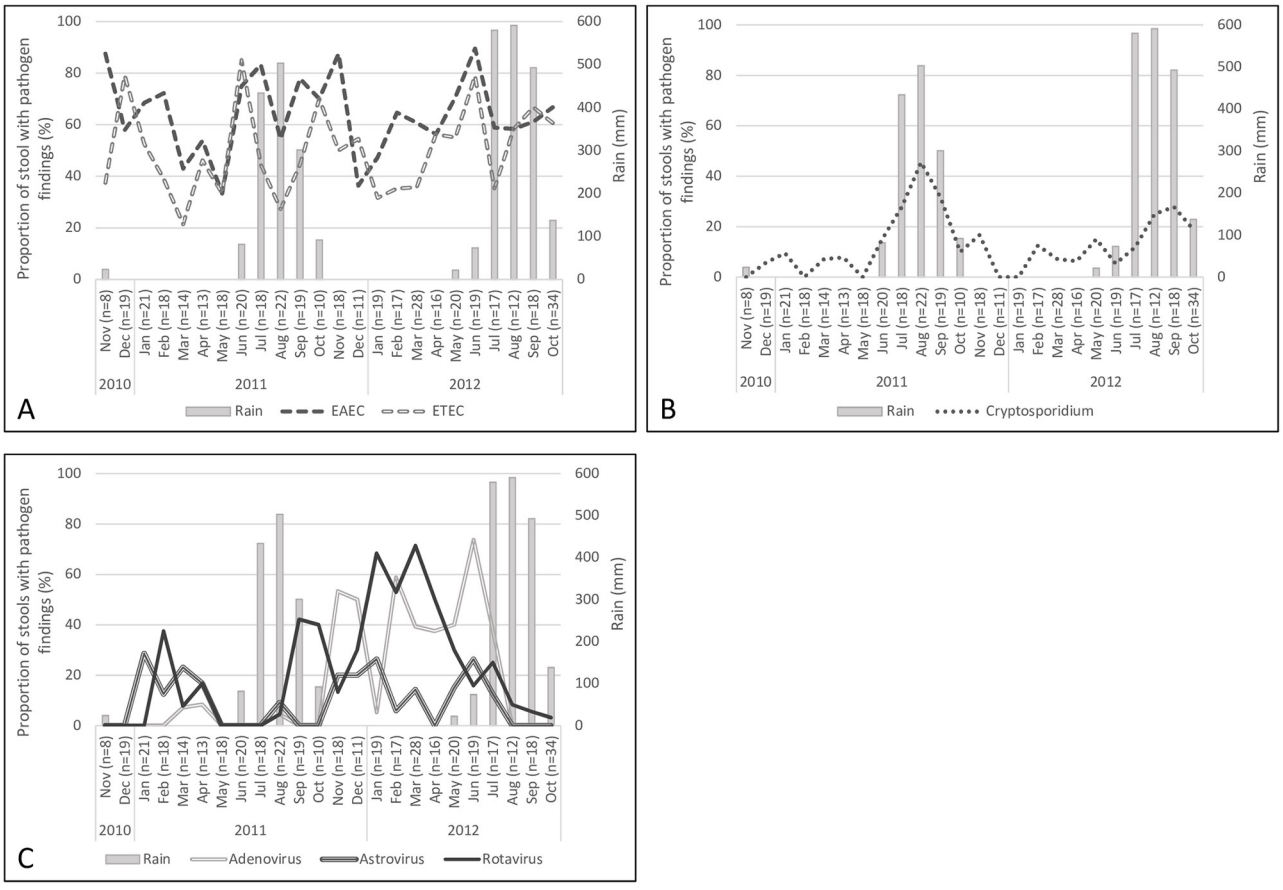

Fig 3. Seasonal variation of diarrhoeal pathogens with respect to rainfall [4]. For the six pathogens showing seasonal variation in incidence, data are shown as monthly proportions of samples positive for A) bacterial (EAEC, ETEC), B) parasitic (*Cryptosporidium)*, and C) viral pathogens (adeno-, astro-, and rotavirus). Details of all pathogen findings are shown in S1 Fig. Numbers of children are indicated in parentheses for each month.

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Table 2. Effect of seasonality on microbial findings by age group.

	Total	0–11 months				12–59 months			
	n (%)	Rainy season n (%)	Dry season n (%)	Rainy versus dry season OR (95% CI) p-value		Rainy season n (%)	Dry season n (%)	Rainy versus dry season OR (95% CI) p-value	
All	429 (100)	90 (46.9)	102 (52.1)			98 (41.4)	139 (58.6)		
Children with diarrhoea	228 (53.1)	42 (46.7)	57 (55.9)	0.7 (0.4–1.2)	0.202	58 (59.2)	71 (51.1)	1.4 (0.8–2.3)	0.217
Any pathogen	422 (98.4)	88 (97.8)	101 (99.0)	0.4 (0.0–4.9)	0.601	98 (100)	135 (97.1)	NA	0.144
Any bacteria [a]	409 (96.5)	87 (97.8)	97 (96.0)	1.8 (0.3–10.0)	0.686	97 (99.0)	128 (94.1)	6.1 (0.7–49.3)	0.083
Campylobacter	225 (53.1)	52 (58.4)	44 (43.6)	1.8 (1.0–3.2)	**0.041**	57 (58.2)	72 (52.9)	1.2 (0.7–2.1)	0.428
EAEC	272 (64.2)	65 (73.0)	74 (73.3)	1.0 (0.5–1.9)	0.971	65 (66.3)	68 (50.0)	2.0 (1.2–3.4)	**0.013**
EHEC	6 (1.4)	1 (1.1)	0 (0.0)	NA	0.468	2 (2.0)	3 (2.2)	0.9 (0.2–5.6)	1.000
EIEC/*Shigella*	98 (23.1)	14 (15.7)	14 (13.9)	1.2 (0.5–2.6)	0.717	36 (36.7)	34 (25.0)	1.7 (1.0–3.1)	0.053
EPEC	266 (62.7)	61 (68.5)	62 (61.4)	1.4 (0.8–2.5)	0.303	62 (63.3)	81 (59.6)	1.2 (0.7–2.0)	0.566
ETEC	213 (50.2)	56 (62.9)	48 (47.5)	1.9 (1.0–3.4)	**0.033**	50 (51.0)	59 (43.4)	1.4 (0.8–2.3)	0.248
Salmonella	11 (2.6)	4 (4.5)	4 (4.0)	1.1 (0.3–4.7)	1.000	2 (2.0)	1 (0.7)	2.8 (0.3–4.7)	0.573
V. cholerae	2 (0.5)	0 (0.0)	1 (1.0)	NA	1.000	1 (1.0)	0 (0.0)	NA	0.419
Yersinia	3 (0.7)	1 (1.1)	1 (1.0)	1.1 (0.1–18.4)	1.000	0 (0.0)	1 (0.7)	NA	1.000
Any parasites [b]	217 (50.9)	46 (51.1)	29 (28.7)	2.6 (1.4–4.7)	**0.002**	65 (67.0)	77 (55.8)	1.6 (0.9–2.8)	0.084
Cryptosporidium	59 (13.8)	23 (25.6)	8 (7.9)	4.0 (1.7–9.5)	**0.001**	19 (19.6)	9 (6.5)	3.5 (1.5–8.1)	**0.002**
D. fragilis	43 (10.7)	2 (2.3)	7 (7.5)	0.3 (0.1–1.4)	0.170	15 (15.6)	19 (15.2)	1.0 (0.5–2.2)	0.931
E. histolytica	3 (0.7)	1 (1.1)	0 (0.0)	NA	0.471	2 (2.1)	0 (0.0)	NA	0.169
Giardia	159 (37.3)	24 (26.7)	21 (20.8)	1.4 (0.7–2.7)	0.340	47 (48.5)	67 (48.6)	1.0 (0.6–1.7)	0.988
Any viruses [c]	233 (58.7)	44 (49.4)	72 (80.0)	0.2 (0.1–0.5)	**<0.001**	40 (41.7)	77 (63.1)	0.4 (0.2–0.7)	**0.002**
Adenovirus 40, 42	73 (18.4)	9 (10.1)	28 (31.1)	0.3 (0.1–0.6)	**0.001**	12 (12.5)	24 (19.7)	0.6 (0.3–1.2)	0.157
Astrovirus	40 (10.1)	5 (5.6)	15 (16.7)	0.3 (0.1–0.9)	**0.019**	4 (4.2)	16 (13.1)	0.3 (0.1–0.9)	**0.023**
Norovirus GI/GII	92 (23.2)	26 (29.2)	26 (28.9)	1.0 (0.5–1.9)	0.962	18 (18.8)	22 (18.0)	1.0 (0.5–2.1)	0.892
Rotavirus A	93 (23.4)	10 (11.2)	36 (40.0)	0.2 (0.1–0.4)	**<0.001**	13 (13.5)	34 (27.9)	0.4 (0.2–0.8)	**0.011**
Sapovirus	29 (7.3)	4 (4.5)	10 (11.1)	0.4 (0.1–1.2)	0.099	5 (5.2)	10 (8.2)	0.6 (0.2–1.9)	0.387

Data is missing, n (%):

[a] 5 (1.2),

[b] 3 (0.7),

[c] 32 (7.5)

CI = confidence interval; NA = not applicable; OR = odds ratio; **bolding** indicates statistically significant at $p<0.05$ (Pearson χ^2 test or Fisher´s exact test)

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Discussion

The prevalence of individual pathogens differed by season and age group, and according to whether or not the children had diarrhoea. *Cryptosporidium* was most prevalent during the rainy season, whereas viral pathogens (adenovirus, astrovirus and rotavirus) were most frequently found during the dry season. Bacterial pathogens showed a tendency towards slightly higher rates in the rainy (98.4%) than the dry (94.9%) season, but overall, EAEC, EPEC, ETEC and *Campylobacter* were the most prevalent pathogens in both seasons.

Impact of temperature on occurrence of diarrhoea and diarrhoeal pathogens

Many research findings from high-income countries connect diarrhoeal pathogens with temperature rather than rainfall [1,14–17]. In their systematic literature review covering 59 studies

Table 3. Diarrhoeal pathogens in children with and without diarrhoea during the dry and rainy seasons.

	Total	Dry season					Rainy season				
		No diarrhoea	Diarrhoea	Diarrhoea versus no diarrhoea			No diarrhoea	Diarrhoea	Diarrhoea versus no diarrhoea		
	n (%)	n (%)	n (%)	OR (95% CI)	p-value	AF (95% CI)	n (%)	n (%)	OR (95% CI)	p-value	AF (95% CI)
Total	429 (100)	113 (46.9)	128 (53.1)				88 (46.8)	100 (53.2)			
Any pathogen	422 (98.4)	109 (96.5)	127 (99.2)	4.7 (0.5–42.3)	0.189		87 (98.9)	99 (99.0)	1.1 (0.1–18.5)	1.000	
Any bacteria [a]	409 (96.5)	103 (93.6)	122 (96.1)	1.7 (0.5–5.4)	0.395	-0.2 (-0.5–0.6)	85 (97.7)	99 (99.0)	2.3 (0.2–26.1)	0.598	0.4 (-2.1–0.9)
Campylobacter	225 (53.1)	56 (50.9)	60 (47.2)	0.9 (0.5–1.4)	0.574	-0.0 (-0.2–0.1)	51 (58.6)	58 (58.0)	1.0 (0.5–1.4)	0.932	0.1 (-0.2–0.2)
EAEC	272 (64.2)	71 (64.5)	71 (55.9)	0.7 (0.4–1.2)	0.176	-0.1 (-0.3–0.0)	63 (72.4)	67 (67.0)	0.8 (0.4–1.5)	0.422	-0.1 (-0.3–0.1)
EHEC	6 (1.4)	3 (2.7)	0 (0.0)	NA	0.099	NA	2 (2.3)	1 (1.0)	0.4 (0.0–4.8)	0.598	NA
EIEC/*Shigella*	98 (23.1)	17 (15.5)	31 (24.4)	1.8 (0.9–3.4)	0.087	0.1 (-0.0–0.1)	19 (21.8)	31 (31.0)	1.6 (0.8–3.1)	0.158	0.1 (-0.0–0.1)
EPEC	266 (62.7)	66 (60.0)	77 (60.6)	1.0 (0.6–1.7)	0.921	0.0 (-0.1–0.2)	60 (69.0)	63 (63.0)	0.8 (0.4–1.4)	0.391	-0.1 (-0.3–0.1)
ETEC	213 (50.2)	40 (36.4)	67 (52.8)	2.0 (1.2–3.3)	**0.011**	0.1 (0.0–0.2)	49 (56.3)	57 (57.0)	1.0 (0.6–1.8)	0.926	-0.0 (-0.2–0.1)
Salmonella	11 (2.6)	3 (2.7)	2 (1.6)	0.6 (0.1–3.5)	0.665	NA	3 (3.4)	3 (3.0)	0.9 (0.2–4.4)	1.000	NA
V. cholerae	2 (0.5)	0 (0.0)	1 (0.8)	NA	1.000	NA	0 (0.0)	1 (1.0)	NA	1.000	NA
Yersinia	3 (0.7)	1 (0.9)	1 (0.8)	0.9 (0.1–14.0)	1.000	NA	0 (0.0)	1 (1.0)	NA	1.000	NA
Any parasites [b]	217 (50.9)	49 (43.8)	57 (44.9)	1.0 (0.6–1.7)	0.860	0.0 (-0.1–0.1)	48 (55.2)	63 (63.0)	1.4 (0.8–2.5)	0.277	0.1 (-0.1–0.2)
Cryptosporidium	59 (13.8)	3 (2.7)	14 (11.0)	4.5 (1.3–16.0)	**0.012**	0.0 (0.0–0.1)	14 (16.1)	28 (28.0)	2.0 (1.0–4.2)	0.052	0.1 (-0.0–0.1)
D. fragilis	43 (10.7)	13 (12.9)	13 (11.1)	0.8 (0.4–1.9)	0.689	-0.0 (-0.1–0.1)	7 (8.0)	10 (10.3)	1.3 (0.5–3.6)	0.597	0.0 (-0.0–0.1)
E. histolytica	3 (0.7)	0 (0.0)	0 (0.0)	NA	NA	NA	3 (3.4)	0 (0.0)	NA	0.099	NA
Giardia	159 (37.3)	43 (38.4)	45 (35.4)	0.9 (0.5–1.5)	0.636	-0.0 (-0.1–0.1)	31 (35.6)	40 (40.0)	1.2 (0.7–2.2)	0.539	0.0 (-0.1–0.1)
Any viruses [c]	233 (58.7)	59 (60.2)	90 (78.9)	2.5 (1.4–4.5)	**0.003**	0.3 (0.1–0.4)	31 (35.6)	53 (54.1)	2.1 (1.2–3.8)	**0.012**	0.2 (0.0–0.3)
Adenovirus 40, 42	73 (18.4)	27 (27.6)	25 (21.9)	0.7 (0.4–1.4)	0.343	-0.0 (-0.1–0.0)	6 (6.9)	15 (15.3)	2.4 (0.9–6.6)	0.072	0.0 (-0.0–0.1)
Astrovirus	40 (10.1)	9 (9.2)	22 (19.3)	2.4 (1.0–5.4)	**0.038**	0.1 (0.0–0.1)	1 (1.1)	8 (8.2)	7.6 (0.9–62.4)	**0.037**	0.0 (0.0–0.1)
Norovirus GI/GII	92 (23.2)	15 (15.3)	33 (28.9)	2.3 (1.1–4.5)	**0.018**	0.1 (0.0–0.1)	15 (17.2)	29 (29.6)	2.0 (1.0–4.1)	**0.049**	0.1 (0.0–0.2)
Rotavirus A	93 (23.4)	30 (30.6)	40 (35.1)	1.2 (0.7–2.2)	0.490	0.0 (-0.1–0.1)	10 (11.5)	13 (13.3)	1.2 (0.5–2.8)	0.716	0.0 (-0.0–0.1)
Sapovirus	29 (7.3)	8 (8.2)	12 (10.5)	1.3 (0.5–3.4)	0.557	0.0 (-0.0–0.1)	2 (2.3)	7 (7.1)	3.3 (0.7–16.2)	0.176	0.0 (-0.0–0.0)

Data missing, n (%):

[a)] 5 (1.2),

[b)] 3 (0.7),

[c)] 32 (7.5)

AF = attributable fraction; CI = confidence interval; NA = not applicable; OR = odds ratio; **bolding** indicates statistically significant at $p<0.05$ (Pearson χ^2 test or Fisher´s exact test)

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published 1974–2010, Philipsborn et al. report an association between high temperature and findings of diarrhoeagenic *E. coli* (DEC). They depict a growth of 8% in DEC incidence for each 1˚C increase in monthly temperature; however, nontropical industrialized countries were also included in the review [14]. The impact of temperature may be related to various factors, such as enhanced pathogen survival, increased pathogen load in (animal) reservoirs, and prolonged transmission seasons [14] or differences in expression of virulence genes [1]. Since the temperature in Guinea-Bissau does not vary much by season [4], no major temperature-related impact appeared likely.

Impact of rainfall on occurrence of diarrhoea

As there are substantial seasonal variations in rainfall in Guinea-Bissau, we also expected to see such variation in diarrhoea cases. Indeed, Colombatti et al. report severe faecal

contamination of drinking water from taps and wells during the rainy season in the country [18]. In the literature, however, the impact of rainfall on the occurrence of diarrhoea remains somewhat controversial [19–20]. In many African countries, such as Botswana [19] Mozambique [20] and Niger [21], rainfall has been associated with diarrhoea. A Ghanaian investigation found diarrhoea risk to grow 5.1% for every 10mm increase in rainfall [21]. Flooding and heavy precipitation may flush faecal and other waste into areas where humans easily become exposed [22]. The seasons may also affect water quality and access to water as well as various wildlife and agricultural activities [20,23–24]. Some studies suggest that in households with poor-quality water sources, the risk of diarrhoea grows with heavy rainfall, while dry conditions decrease it [20–22,25]. On the other hand, during the dry season, drought and smaller rainfall can increase the proportion of wastewater in surface water, leading to greater pathogen concentration in both drinking and irrigation water [26]. In dry conditions water sources are also more likely to be shared by human and animal populations [1]. Some studies [19,27–28] relate the higher rate of pathogen findings in the dry season to decreased access to handwashing [22]. Indeed, handwashing with soap can bring about a reduction of up to 27% of diarrhoeal cases [29].

Importantly, only about 15% of Guinea-Bissauan inhabitants have been estimated to have access to adequate sanitation, and only 2% to basic waste management services (waste separation, treatment and safe disposal) [30]. Although we did not see a change in the rates of diarrhoea per se, the prevalence of various pathogen findings changed by season, as discussed below.

Impact of season on occurrence of diarrhoeal pathogens

***Cryptosporidium* common during rainy season.** According with previous studies [2,14,21], the pathogen most strongly associated with the rainy season was *Cryptosporidium*, a major cause of waterborne gastrointestinal disease with potential to large outbreaks [31]. An earlier study carried out in Guinea-Bissau 1991–1997 also connects the peak in the prevalence of *Cryptosporidium* with wetter times of the year; very few cases having this pathogen have been reported for dry seasons [7]. Indeed, cryptosporidiosis incidence has been suggested to be related to contamination of water supplies through heavy rainfall [1–2,6,32]. This tallies with our number of detected cases increasing sharply in July, peaking in August when the rains are heaviest, and decreasing again with the sparse precipitation in October (Fig 3).

As for the other parasites in our study, we saw no signs of seasonality for *Giardia* and *Dientamoeba*. This difference between the various parasites seems logical, since *Giardia* and *Dientamoeba* are often transmitted directly via the faecal-oral route rather than water like *Cryptosporidium*. Their spread can be largely controlled by improving hygiene and sanitation conditions, according with their non-seasonal occurrence [33–34].

Bacterial pathogens prevalent all year. Bacterial pathogens proved very common throughout the year (rainy season 98.4% versus dry season 94.9%; p = 0.056) (Table 1). Findings of *Campylobacter* increased by the amount of rainfall. Moreover, ETEC and EAEC were associated with the rainy season for all children (Table 1). The extensive cohort study of ETEC infections conducted by Steinsland et al. in Guinea-Bissau 1996 and 1998 monitored children from birth to two years of age with weekly faecal sampling. Observing an increase in ETEC during the 1997 rainy season, they concluded that rainy season epidemics may be annual [35]. This accords with our ETEC data for infants: they had more ETEC findings during the rainy season, whereas no seasonal difference was seen in the older age group, a possible consequence of gradually developing intestinal immunity to this highly common pathogen. A similar seasonal trend was also seen for *Campylobacter* infections. In

industrialized countries, *Campylobacter* infections peak over warmer summer months, but many African studies show no strong seasonal trends [36–37]. However, an earlier investigation carried out in Guinea-Bissau 1987–88 [38] accords with our findings. The seasonal difference for EAEC was only seen among young children, but not infants, though (Table 2). The reason for this remains unknown.

Viral pathogens common during dry season. According with earlier research [2,14,21,39–40], decrease in the amount of rainfall and the dry season were strongly associated with findings of adenovirus, astrovirus, and rotavirus (Fig 2). In fact, their incidences dropped dramatically already at the beginning of the rainy season (Fig 3). These findings agree with a study exploring rotavirus diarrhoea in Guinea-Bissau between 1996 and 1998 [9]. The virus's high prevalence in the dry season has been suggested to be attributable to aridity of soil increasing aerial transport of dried faecal material in the form of droplet nuclei. In addition, dust may serve as a vehicle for virus particles [26,41].

Unlike the case of the other viruses in our study, norovirus findings were evenly distributed throughout the year. This difference may be ascribed to noroviruses being transmitted not only by the faecal-oral route but also aerosols from vomiting. Noroviruses' seasonality has barely been studied in LICs, but among Malawian children aged 18 months or younger, noroviruses have been reported to prevail in the rainy season [42]. In cooler regions, norovirus infections have been found mainly to be related to colder temperatures ("winter vomiting disease") [43]. Indeed, the literature suggests low temperature (-5–20˚C) and low relative humidity (10–60%) to be associated with the occurrence of norovirus epidemics worldwide [44].

Impact of participants' ages on seasonal differences. The associations between age, season and diarrhoea are complicated. For example, highly prevalent pathogens encountered early in life may no longer cause diarrhoea for older children due to pre-existing intestinal immunity elicited by previous exposures [5,36]. The frequency and load of pathogens to which children become exposed may vary by age and season, resulting in differences between pathogen findings for infants and young children. Indeed, when scrutinizing the seasonality of the pathogens in the two age groups, the association appeared stronger among infants than young children. Among infants, seasonal differences were observed for *Campylobacter*, ETEC, *Cryptosporidium*, adeno-, astro- and rotavirus, while among young children such differences were seen for EAEC, *Cryptosporidium*, astro-, and rotavirus. Our data encourage further research, particularly since we found no recent studies that would compare pathogens' seasonality and children's ages.

Diarrhoeal pathogens found in dry and rainy seasons among children with and without diarrhoea. Some of the previous studies from West Africa report DEC rates (1–15%) lower than ours (23–64%) [45–47]. Apart from differences in study sites and PCR cut-off values, the explanation may be merely methodological: we detected DEC directly from stools by PCR, while they used culture-based methods an approach known to be substantially less sensitive [48]. Our rates concur with those reported among local children in LICs [49–50] and traveller studies conducted in West Africa [51–52], all assessing the pathogens directly from stools by PCR.

Our prevalence analysis of various pathogens and diarrhoeal symptoms during the dry or rainy season scrutinizes a subject barely covered in scientific literature. Our data does not show many significant differences apart from greater prevalence of astrovirus, norovirus and *Cryptosporidium* among those with diarrhoea than those without in both seasons. It is noteworthy that these pathogens all have low infectious doses. As for bacterial pathogens, the only statistically significant finding was that of ETEC during the dry season; it proved more prevalent among those with diarrhoeal symptoms than those without any—despite ETEC's greater occurrence in the rainy season. This phenomenon may be ascribed to ETEC's high infectious

doses: as the inoculum may be diluted during the rainy season, many of the exposed individuals do not catch infectious doses. The finding may also be explained by the presence of ETEC in biofilms in water tanks used during the dry season for irrigation or washing fresh vegetables and fruits [53]. Biofilms may serve as reservoirs for this group of *E. coli* [54]. Bacterial recovery from dry-surface biofilm has been shown to amount to 100% [55]. The relatively low attributable fractions for practically all single pathogens reveal the polymicrobial character of childhood diarrhoea in West Africa, highlighting the preventive value of hygiene measures.

Limitations

The main limitation in our study was the restricted number of cases, which prevented some of the potential analyses used in other studies [21,26,40]: with higher numbers of patients and longer follow-up period, more flexible approaches have been used for characterizing the timing, amplitude and number of annual seasonal peaks in enteropathogen detection [40,56]. We now opted, instead, to use attributable fractions to express the proportions of the observed pathogens attributable to 11 mm increase in rainfall.

Furthermore, we did not analyse the LT (heat-labile) and ST (heat-stabile) toxin expressions of the ETEC strains or various ETEC serotypes. Such analyses could have shed light on the seasonal variation found for ETEC, since diarrhoea is associated particularly strongly with ST-producing strains [36], and in the dry and rainy seasons different serotypes may predominate, some of them more diarrhoeagenic than others.

We did not have the opportunity to identify *Cryptosporidium* species at the genus level. It would have been particularly interesting to explore which species predominate during the rainy and dry seasons, as *C. hominis* has been reported to mostly account for waterborne outbreaks and *C. parvum* for foodborne outbreaks [57]. The seasonal patterns may have been affected by the fact that some pathogens, such as noroviruses, EAEC, *Salmonella* or *Campylobacter* may be detected in stools for some weeks after the resolution of clinical symptoms [10]. This should, however, mostly impact the cases found during the first weeks of the season.

Although the data does not provide information on actual incidences, the numbers of patients attending the health centre and agreeing to participate are given. It is possible that those with diarrhoea were more willing to participate, but this should not vary by season.

Conclusion

Diarrhoeal pathogens are not equally presented over the year, but show seasonal variation with respect to precipitation in Guinea-Bissau. Waterborne pathogens such as *Cryptosporidium* can be expected during the rainy season, whereas viral pathogens appear more common during the dry season. Knowledge of pathogens' seasonal variations could not only guide empiric treatment but also provide tools for devising interventions and preventive measures.

Supporting information

S1 Fig. The monthly proportion of diarrhoea and pathogens in respect to rainfall with a confidence interval; A) diarrhoea, B) any bacter, C) *Campylobacter*, D) EAEC, E) EIEC/*Shigella*, F) EPEC, G) ETEC, H) any parasite, I) *Cryptosporidium*, J) *D. fragilis*, K) *Giardia*, L) any virus, M) adenovirus 40/41, N) astrovirus, O) norovirus GI/GII, P) rotavirus A, Q) sapovirus. Data are not presented for EHEC (n = 6), *Salmonella* (n = 11), *V. cholerae* (n = 2), *Yersinia* (n = 3) and *E. histolytica* (n = 2).
(TIF)

S1 Data. All relevant data for the study.
(XLSX)

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