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Risk Factors for Cryptosporidiosis among Children in a Semi Urban Slum in Southern India: A Nested Case-Control Study

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Abstract. The risk factors for acquisition of cryptosporidial infection in resource-poor settings are poorly understood. A nested case-control study was conducted to assess factors associated with childhood cryptosporidiosis (detected by stool polymerase chain reaction) in an endemic, Indian slum community using data from two community-based studies with 580 children followed prospectively until their second birthday. Factors were assessed for overall cryptosporidiosis ($N = 406$), and for multiple ($N = 208$), asymptomatic ($N = 243$), and symptomatic ($N = 163$) infections, respectively. Presence of older siblings (odds ratio [OR] = 1.88, $P = 0.002$) and stunting at 6 months of age (OR = 1.74, $P = 0.019$) were important risk factors for childhood cryptosporidiosis. Always boiling drinking water before consumption, the use of a toilet by all members of the family, and maternal age ≥ 23 years were protective. These results provide insights into acquisition of childhood cryptosporidiosis in settings with poor environmental sanitation, contaminated public water supply systems, and close human–animal contact. Disease control strategies will require a multifaceted approach.

INTRODUCTION

Cryptosporidium spp. is an obligate, intracellular, protozoan parasite that infects the gastrointestinal tract of humans and animals.[1] It is a highly infectious parasite with a minimal infectious dose as low as nine oocysts.[2] Transmission is predominantly fecal–oral and occurs by multiple routes including direct person-to-person spread, ingestion of contaminated food or water, or contact with infected animals.[3] The parasite attaches itself to the intestinal epithelium of the host, resulting in varying degrees of villous atrophy and inflammatory infiltration of the lamina propria.[4] The clinical presentation is highly variable, but is frequently characterized by watery diarrhea, sometimes accompanied by abdominal pain, low-grade fever, malaise, nausea, vomiting, and loss of appetite. Symptoms usually appear 2–10 days after infection and can last for a few weeks.[5,6]

Cryptosporidiosis is often asymptomatic and almost always self-limiting in immunocompetent hosts, but may be severe and life threatening in immunocompromised patients such as those with acquired immunodeficiency syndrome (AIDS) or severe malnutrition.[6] Children, especially those living in resource poor settings, are the worst affected.[7,8] Early childhood cryptosporidiosis has been associated with growth retardation, cognitive deficits, and a higher overall risk of mortality.[9–11] There is no consistently effective treatment available for cryptosporidiosis in vulnerable populations.[12] Hence, the identification of factors associated with cryptosporidial infection, particularly in childhood, is essential in designing strategies to prevent or control disease caused by this protozoan parasite.

Previous studies assessing the factors that affect acquisition of infection in children have identified low socioeconomic status, crowded living conditions, age < 2 years, male gender, presence of animals (pigs, cats, and dogs) in the household, storage of cooked food, diarrhea in the family, drinking non-potable water, rainy season, low birth weight, stunting, and lack of breastfeeding as important risk factors for cryptosporidiosis in children (Table 1). However, risk differs with settings (Table 1); hence, a comprehensive understanding of the transmission dynamics of *Cryptosporidium* spp. requires well-defined populations and a wide range of social and environmental conditions.

This study investigated risk of acquisition and protection from cryptosporidial infection in children living in a slum in southern India. Previous studies in the same community have shown a high burden of childhood cryptosporidiosis.[31,32]

MATERIALS AND METHODS

Study design and subjects. A nested case-control study was conducted among children residing in a slum community in the western outskirts of Vellore, Tamil Nadu, India, using data from two community-based studies on childhood cryptosporidiosis between 2008 and 2013. The first study was a quasi-experimental study on the effect of protected drinking water supply (bottled drinking water) in preventing childhood cryptosporidiosis, where 176 children were recruited at birth or during exclusive breastfeeding and followed weekly until 2 years of age[33]; drinking bottled water did not confer additional protection against cryptosporidial infections.[34] The second study was a birth-cohort study on immune responses to *Cryptosporidium* spp. that investigated symptomatic and asymptomatic cryptosporidial infections in 497 children during the first 3 years of their life through biweekly follow-up visits.[35]

Ethical approvals for both studies were obtained from the Institutional Review Boards of Christian Medical College, Vellore and Tufts University Health Sciences Campus, Boston. Written informed consent was provided by parents or legal guardians of all participating children, before enrollment. A total of 580 children (160 from the quasi-experimental study and 420 from the birth cohort study) completed 2 years of follow-up.

Follow-up and sample collection. In both studies, socio-demographic and birth details, information on water usage and storage, toilet use, and presence of cows and other animals

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Table 1
Studies on the risk and protective factors of cryptosporidial infections in developed and developing countries

Location	Year(s)	Study design	Study setting	Sample size	Target population	*Cryptosporidium* detection method	Risk and protective factors	Reference
Guatemala	1985–1986	Cohort	Community	130	< 1 year	Microscopy	*Risk:* Liquid or solid foods in the diet; presence of domestic animals (dogs, cats, or poultry); absence of toilet facilities	13
Guinea-Bissau	1988–1990	Case-control	Community	250	0–37 months	Microscopy	*Risk:* Presence of pigs and dogs in the household; storage of cooked food for later consumption; male gender; *Protection:* Breastfeeding	14
Mexico	1988–1989	Cross-sectional	Community	403	< 5 years	Indirect immunofluorescent assay	*Risk:* Malnutrition; non-breastfed children	15
Brazil	1989–1993	Cohort	Community	189	≤ 4 years	Microscopy	*Risk:* Low birth weight; crowded living conditions	16
Bangladesh	1991–1994	Case-control	Hospital	272	< 5 years	Microscopy	*Risk:* Age below 2 years; non-breastfed children; stunting	17
Indonesia	1992–1993	Cross-sectional	Community	4,368	All age groups	Microscopy	*Risk:* contact with cats; rainy season; flooding; crowded living conditions	18
Peru	1995–1998	Cohort	Community	368	< 12 years	Microscopy	*Risk:* Houses without a latrine or toilet	19
Zambia	1995–1996	Cross-sectional	Hospital	222	< 12 years	Microscopy	*Risk:* Rainy season; breastfeeding; living in households that owned their house	20
USA	1996–1997	Cross-sectional	Hospital	285	6 months–13 years	Serum ELISA	*Risk:* Consumption of municipal water; increased age of the child; lower annual household income	21
Brazil	1998–1999	Cross-sectional	Hospital	445	≤ 10 years	Direct immunofluorescent assay	*Risk:* Age < 2 yrs; male gender; day care attendance; having children with diarrhea in the household	22
USA	1999–2001	Case-control	Community*	282	All age groups	Fluorescent microscopy	*Risk:* International travel; contact with cattle; contact with persons >2 to 11 years of age with diarrhea; freshwater swimming; *Protection:* Eating raw vegetables	23
Mexico	–	Cross-sectional	Community	132	1–15 years	Microscopy	*Risk:* Diarrhea in the family; crowded living conditions; drinking non-potable water	24
UK	2000–2003	Case-control	Community*	6,736	All age groups	PCR	*Risk:* High socioeconomic status; age less than four years; residing in areas with poor water treatment	25
UK	2001–2002	Case-control	Community*	854	All age groups	PCR	*Risk:* Travel outside of the country; contact with another person with diarrhea; touching cattle; *Protection:* Eating ice cream and raw vegetables	26
Malaysia	2004	Cross-sectional	Community	276	2–15 years	Microscopy	*Risk:* Low birth weight; large family size; breastfeeding	27
Iran	2005–2006	Cross-sectional	Hospital	171	< 5 years	Stool ELISA	*Risk:* Low birth weight; breastfeeding for less than one month	28
Nigeria	2006–2007	Cross-sectional	Community	692	19.5–72 months	PCR	*Risk:* Stunting; younger age	29

(continued)

TABLE 1
Continued

Location	Year(s)	Study design	Study setting	Sample size	Target population	*Cryptosporidium* detection method	Risk and protective factors	Reference
Venezuela	2008	Cross-sectional	Community	536	All age groups	Microscopy	*Risk:* Living in a hut or small residence; extreme poverty; open air defecation; crowded living condition	30

*Laboratory-confirmed cases of cryptosporidiosis detected through an ongoing surveillance program.
ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction.

in close proximity to the house were collected at the time of recruitment. Information on anthropometry (height/weight), household hygiene, and breastfeeding practices were collected at multiple times during the follow-up.

During their at least weekly visits, the field workers enquired about diarrheal and other morbidities experienced by children during the preceding week. Surveillance stool samples were collected every month and diarrheal stool samples collected every time the child had an episode of diarrhea (defined as three or more loose watery stools over a 24-hour period[36]). Stool samples were tested for the presence of *Cryptosporidium* spp. by polymerase chain reaction (PCR) using previously described protocols.[31,37]

Definition of cases and controls. Children who developed one or more episodes of cryptosporidiosis at any time during the follow-up were considered cases. Controls were children with no evidence of cryptosporidial infection (as detected by stool PCR). Analysis was restricted to the 580 children who completed 2 years of follow-up.

Assessment of malnutrition. Nutritional deficiency in children was assessed by computing the height-for-age (HAZ), weight-for-height (WHZ), and weight-for-age (WAZ) *z*-scores, using the 2006 WHO child growth standards as the reference.[38] Children were then classified as stunted (HAZ < –2 SD), wasted (WHZ < –2 SD), underweight (WAZ < –2 SD), or normal based on their *z*-scores. Children who were stunted (HAZ < –2 SD) at 6 months, and remained so at 12, 18, and 24 months of age were classified as persistently stunted. Similarly, children who showed evidence of wasting (WHZ < –2 SD) or were underweight (WAZ < –2 SD) at 6, 12, 18, and 24 months of age were considered to be persistently wasted or underweight, respectively. Children with one or more growth deficiencies (stunted, wasted, and/or underweight) at any time point were classified as malnourished.

Assessment of household hygiene. Household hygiene was assessed using an 18-point scale, which covered aspects of water, food, and personal hygiene. The questionnaire has previously been validated and used in the same community.[39,40] The hygiene measurement of children closest to the time of weaning was used for this analysis, and families with a score of ≥ 12 (upper tertile of the hygiene score) were considered to have good household hygiene.

Environmental risk assessment. The presence of potential environmental contaminants such as cow or other animal sheds, garbage dumping sites, sewage channels, and open-air defecation fields, within a specified perimeter of the study house was assessed using geographic information system (GIS) data collected through Garmin GPS V receivers (Garmin International Inc., Olathe, KS) and mapped using ArcGIS 10 software (Environmental Systems Research Institute Inc., Redlands, CA). Nearest distances between study households and the potential environmental contaminant was calculated using the "distance between points (between layers)" feature in the Hawth's Analysis Tools 3.26 (http://www.spatialecology.com/htools), an

TABLE 2
Comparison of baseline characteristics between children who completed 2 years of follow-up (N = 580) and those who were lost to follow-up (N = 93)

	Completed follow-up	Lost to follow-up	*P* value
Male child	312 (54%)	46 (49%)	0.437†
Nuclear family	320 (55%)	56 (60%)	0.363†
Median (IQR) birth weight (in kg)*	2.9 (2.6–3.2)	2.8 (2.5–3.1)	0.203‡
Normal vaginal delivery	469 (81%)	72 (77%)	0.438†
Birth in a healthcare facility	568 (98%)	93 (100%)	0.444§
Median (IQR) family size	5 (4–7)	6 (4–7)	0.836¶
Crowding (≥ 5 per room)	183 (32%)	29 (31%)	0.943†
Presence of older siblings	346 (60%)	51 (55%)	0.381†
Median (IQR) age of the mother (in years)	23 (21–26)	23 (21–25)	0.192‡
Median (IQR) years of completed maternal education	5.5 (0–10)	5 (0–9)	0.400¶
Median (IQR) years of completed education of the head of the household	5 (0–8)	5 (0–8)	0.612¶
Living in a "kutcha" house‖	81 (14%)	16 (17%)	0.409†
Low socio-economic status	379 (65%)	66 (71%)	0.288†
Firewood as the primary cooking mode	257 (44%)	32 (34%)	0.073†
Good household hygiene*	262 (45%)	52 (57%)	0.054†

*Data on birth weight and household hygiene missing for 16 and 2 children, respectively.
Tests of significance: †χ^2 test; ‡Two-tailed *t* test; §Fisher's exact test; ¶Mann-Whitney *U* test.
‖"Kutcha" house: a house with wall and roof of mud/tin/asbestos/thatch.

TABLE 3
Results of the univariate logistic regression analysis of factors associated with childhood cryptosporidiosis

		Any infection			Multiple infections			Asymptomatic infection(s)			Symptomatic infection(s)		
	Controls (N = 174)	Cases (N = 406)	Odds ratio (95% CI)	P value	Cases (N = 208)	Odds ratio (95% CI)	P value	Cases (N = 243)	Odds ratio (95% CI)	P value	Cases (N = 163)	Odds ratio (95% CI)	P value
Male child	96 (55%)	216 (53%)	0.92 (0.65–1.32)	0.663	118 (57%)	1.06 (0.71–1.60)	0.760	122 (50%)	0.82 (0.55–1.21)	0.317	94 (58%)	1.11 (0.72–1.70)	0.644
Maternal age: ≥ 23 years	110 (63%)	230 (56%)	0.76 (0.53–1.10)	0.142	114 (55%)	0.71 (0.47–1.07)	0.097	136 (56%)	0.74 (0.50–1.10)	0.138	94 (58%)	0.79 (0.51–1.23)	0.298
Maternal education													
High school and above (> 8 years)	63 (36%)	122 (30%)	0.81 (0.52–1.25)	0.336	61 (29%)	0.87 (0.53–1.45)	0.599	76 (31%)	0.84 (0.52–1.36)	0.476	46 (28%)	0.76 (0.44–1.29)	0.310
Middle school (6–8 years)	30 (17%)	75 (19%)	1.04 (0.61–1.77)	0.879	44 (21%)	1.32 (0.73–2.39)	0.353	43 (18%)	1.00 (0.56–1.78)	0.994	32 (20%)	1.11 (0.59–2.07)	0.750
Primary school (1–5 years)	26 (15%)	77 (19%)	1.23 (0.72–2.13)	0.449	42 (20%)	1.46 (0.79–2.68)	0.227	45 (19%)	1.20 (0.67–2.18)	0.538	32 (20%)	1.28 (0.67–2.42)	0.454
No formal education*	55 (32%)	132 (33%)	1	–	61 (29%)	1	–	79 (33%)	1	–	53 (33%)	1	–
Education of the head of the household													
High school and above (> 8 years)	48 (28%)	85 (21%)	0.80 (0.51–1.26)	0.332	44 (21%)	0.72 (0.43–1.20)	0.210	47 (19%)	0.75 (0.45–1.25)	0.270	38 (23%)	0.87 (0.50–1.50)	0.608
Middle school (6–8 years)	27 (16%)	80 (20%)	1.34 (0.79–2.25)	0.275	33 (16%)	0.96 (0.53–1.74)	0.889	50 (21%)	1.42 (0.81–2.49)	0.223	30 (18%)	1.22 (0.65–2.27)	0.536
Primary school (1–5 years)	30 (17%)	88 (22%)	1.32 (0.80–2.19)	0.275	43 (21%)	1.12 (0.64–1.97)	0.684	56 (23%)	1.43 (0.83–2.46)	0.196	32 (20%)	1.17 (0.64–2.14)	0.614
No formal education*	69 (40%)	153 (38%)	1	–	88 (42%)	1	–	90 (37%)	1	–	63 (39%)	1	–
House ownership: Own house	116 (67%)	246 (61%)	0.77 (0.53–1.12)	0.167	125 (60%)	0.75 (0.49–1.15)	0.186	147 (60%)	0.77 (0.51–1.15)	0.198	99 (61%)	0.77 (0.50–1.21)	0.258
Living in a “kutcha” house§	21 (12%)	60 (15%)	1.26 (0.74–2.15)	0.389	35 (17%)	1.47 (0.82–2.64)	0.192	41 (17%)	1.48 (0.84–2.60)	0.176	19 (12%)	0.96 (0.50–1.86)	0.907
Firewood as the primary cooking mode	82 (47%)	175 (43%)	0.85 (0.60–1.21)	0.372	93 (45%)	0.91 (0.61–1.36)	0.637	106 (44%)	0.87 (0.59–1.28)	0.478	69 (42%)	0.82 (0.54–1.27)	0.377
Socio-economic status													
Low	110 (63%)	269 (66%)	1.53 (0.49–4.77)	0.465	141 (68%)	3.20 (0.61–16.83)	0.169	164 (68%)	1.24 (0.37–4.17)	0.725	105 (64%)	2.39 (0.45–12.57)	0.305
Middle	59 (34%)	129 (32%)	1.37 (0.43–4.35)	0.597	65 (31%)	2.75 (0.5–14.74)	0.236	73 (30%)	1.03 (0.30–3.55)	0.961	56 (34%)	2.37 (0.44–12.73)	0.313
High*	5 (3%)	8 (2%)	1	–	2 (1%)	1	–	6 (3%)	1	–	2 (1%)	1	–
Low birth weightΔ	25 (15%)	63 (16%)	1.08 (0.65–1.79)	0.761	34 (17%)	1.15 (0.66–2.02)	0.619	37 (15%)	1.06 (0.61–1.83)	0.843	26 (16%)	1.12 (0.62–2.03)	0.716
Exclusive breastfeeding for ≥ 6 months	24 (14%)	69 (17%)	1.28 (0.77–2.12)	0.336	35 (17%)	1.26 (0.72–2.22)	0.415	43 (18%)	1.34 (0.78–2.31)	0.286	26 (16%)	1.19 (0.65–2.16)	0.578
Stunting (HAZ < −2 SD)													
Stunted at 6 months of age	28 (16%)	105 (26%)	1.82 (1.15–2.89)	0.011	60 (29%)	2.11 (1.28–3.50)	0.004	59 (24%)	1.67 (1.01–2.76)	0.044	46 (28%)	2.05 (1.21–3.48)	0.008
Persistently stunted	8 (5%)	54 (13%)	3.18 (1.48–6.84)	0.003	32 (15%)	3.77 (1.69–8.42)	0.001	30 (12%)	2.92 (1.31–6.54)	0.009	24 (15%)	3.58 (1.56–8.23)	0.003
Wasting (WHZ < −2 SD)													
Wasted at 6 months of age	32 (18%)	54 (13%)	0.68 (0.42–1.10)	0.115	26 (13%)	0.63 (0.36–1.11)	0.112	26 (11%)	0.53 (0.30–0.93)	0.027	28 (17%)	0.92 (0.53–1.61)	0.771
Persistently wasted	7 (4%)	11 (3%)	0.66 (0.25–1.74)	0.406	3 (1%)	0.35 (0.09–1.37)	0.132	7 (3%)	0.71 (0.24–2.06)	0.525	4 (3%)	0.60 (0.17–2.09)	0.423
Underweight (WAZ < −2 SD)													
Underweight at 6 months of age	38 (22%)	114 (28%)	1.40 (0.92–2.13)	0.118	55 (26%)	1.29 (0.80–2.07)	0.297	68 (28%)	1.39 (0.88–2.19)	0.156	46 (28%)	1.41 (0.86–2.31)	0.177

(continued)

TABLE 3
Continued

	Controls (N = 174)	Any infection: Cases (N = 406)	Any infection: Odds ratio (95% CI)	Any infection: P value	Multiple infections: Cases (N = 208)	Multiple infections: Odds ratio (95% CI)	Multiple infections: P value	Asymptomatic infection(s): Cases (N = 243)	Asymptomatic infection(s): Odds ratio (95% CI)	Asymptomatic infection(s): P value	Symptomatic infection(s): Cases (N = 163)	Symptomatic infection(s): Odds ratio (95% CI)	Symptomatic infection(s): P value
Persistently underweight	16 (9%)	63 (15%)	1.81 (1.02–3.24)	0.044	35 (17%)	2.00 (1.06–3.75)	0.031	39 (16%)	1.89 (1.02–3.50)	0.044	24 (15%)	1.71 (0.87–3.34)	0.120
Malnourished (stunted, wasted and/or underweight)													
Malnourished at 6 months of age	66 (38%)	171 (42%)	1.19 (0.83–1.71)	0.347	89 (43%)	1.22 (0.81–1.85)	0.336	94 (39%)	1.03 (0.69–1.54)	0.876	77 (47%)	1.47 (0.95–2.26)	0.085
Persistently malnourished	29 (17%)	93 (23%)	1.49 (0.94–2.36)	0.092	50 (24%)	1.58 (0.95–2.63)	0.078	55 (23%)	1.47 (0.89–2.41)	0.135	38 (23%)	1.52 (0.89–2.61)	0.128
Presence of older sibling(s)	91 (52%)	255 (63%)	1.54 (1.07–2.21)	0.018	126 (61%)	1.40 (0.93–2.11)	0.104	158 (65%)	1.70 (1.14–2.52)	0.009	97 (60%)	1.34 (0.87–2.06)	0.183
Overcrowding (persons per room ≥ 5)	45 (26%)	138 (34%)	1.48 (0.99–2.19)	0.050	70 (34%)	1.45 (0.93–2.27)	0.099	89 (37%)	1.66 (1.08–2.54)	0.021	49 (30%)	1.23 (0.76–1.98)	0.391
Good hygienic practices (hygiene score ≥ 12)	83 (48%)	179 (44%)	0.86 (0.61–1.23)	0.423	86 (41%)	0.77 (0.52–1.16)	0.213	108 (44%)	0.88 (0.59–1.30)	0.510	71 (44%)	0.85 (0.55–1.30)	0.446
Boiling drinking water before consumption													
Always	26 (15%)	44 (11%)	0.71 (0.42–1.21)	0.205	17 (8%)	0.53 (0.28–1.03)	0.060	34 (14%)	0.94 (0.54–1.65)	0.831	10 (6%)	0.39 (0.18–0.84)	0.016
Occasionally	25 (14%)	69 (17%)	1.16 (0.70–1.92)	0.567	40 (19%)	1.30 (0.75–2.27)	0.348	38 (16%)	1.09 (0.63–1.91)	0.753	31 (19%)	1.25 (0.70–2.24)	0.453
Never*	123 (71%)	293 (72%)	1	–	151 (73%)	1	–	171 (70%)	1	–	122 (75%)	1	–
Mud flooring‡	5 (3%)	21 (5%)	1.88 (0.70–5.08)	0.212	12 (6%)	2.08 (0.72–6.04)	0.177	16 (7%)	2.42 (0.87–6.74)	0.091	5 (3%)	1.10 (0.31–3.88)	0.881
Presence of toilet‡	122 (72%)	260 (67%)	0.79 (0.53–1.17)	0.242	129 (64%)	0.69 (0.44–1.08)	0.101	150 (64%)	0.70 (0.45–1.07)	0.099	110 (71%)	0.96 (0.59–1.56)	0.879
Toilet used by all members of the family‡	111 (66%)	228 (59%)	0.75 (0.51–1.09)	0.133	112 (56%)	0.66 (0.43–1.00)	0.052	127 (55%)	0.63 (0.42–0.94)	0.025	101 (66%)	0.99 (0.63–1.58)	0.986
Presence of animals in the house¶	43 (25%)	110 (28%)	1.16 (0.77–1.75)	0.480	62 (31%)	1.31 (0.83–2.07)	0.251	68 (29%)	1.20 (0.77–1.88)	0.423	42 (27%)	1.10 (0.67–1.80)	0.709
Direct contact with animals‡	44 (26%)	115 (30%)	1.20 (0.80–1.80)	0.377	56 (28%)	1.10 (0.69–1.74)	0.694	74 (32%)	1.32 (0.85–2.05)	0.214	41 (27%)	1.03 (0.63–1.69)	0.905
Presence of cow in the house/handling of cow dung by the primary caregiver¶	43 (25%)	119 (31%)	1.30 (0.86–1.95)	0.212	67 (33%)	1.47 (0.93–2.31)	0.099	70 (30%)	1.25 (0.80–1.95)	0.325	49 (32%)	1.37 (0.84–2.22)	0.206
Cow or other animal shed within 10 m of the house	29 (17%)	67 (17%)	0.99 (0.61–1.59)	0.961	31 (15%)	0.88 (0.50–1.52)	0.637	43 (18%)	1.08 (0.64–1.80)	0.784	24 (15%)	0.86 (0.48–1.56)	0.625
Garbage dump within 10 m of the house	75 (43%)	186 (46%)	1.12 (0.78–1.60)	0.548	93 (45%)	1.07 (0.71–1.60)	0.753	106 (44%)	1.02 (0.69–1.51)	0.916	80 (49%)	1.27 (0.83–1.95)	0.272
Open sewage channel within 10 m of the house	50 (29%)	115 (28%)	0.98 (0.66–1.45)	0.920	58 (29%)	0.96 (0.61–1.50)	0.854	71 (29%)	1.02 (0.67–1.57)	0.915	44 (27%)	0.92 (0.57–1.48)	0.722
Open-air defecation area within 50 m of the house	18 (10%)	39 (10%)	0.92 (0.51–1.66)	0.784	21 (10%)	0.97 (0.50–1.89)	0.936	26 (11%)	1.04 (0.55–1.96)	0.907	13 (8%)	0.75 (0.36–1.59)	0.453

*Reference category.
Data missing for Δ9, ¶23, and ‡24 children respectively.
§"Kutcha" house: A house with wall and roof of mud / tin / asbestos / thatched leaves.

extension of the ArcGIS software. Occupants of houses within a specified distance (50 m for open-air defecation fields, 10 m for other attributes) of a potential environmental contaminant were considered to have an increased risk of acquiring cryptosporidial infection.

Statistical analysis. Data were analyzed using STATA 10.1 for Windows (StataCorp, College Station, TX). The socio-demographic and other baseline characteristics of children who completed the study and those who dropped out was compared using χ^2 test or Fisher's exact test for categorical variables and two-tailed *t* test or Mann-Whitney *U* test for continuous variables, depending on the distribution of data.

The risk factors for cryptosporidiosis were ascertained using logistic regression analysis. Univariate analysis was performed at first for all exposure variables, and crude odds ratios (ORs) and 95% confidence interval (95% CI) calculated. The variables significant at $P \leq 0.2$ level and/or those that were known risk factors for childhood cryptosporidiosis were then included in the multivariate analysis and a final model built using the backward stepwise method. Analysis was performed for all cases at first, and then children with multiple cryptosporidial infections (defined as two or more asymptomatic or symptomatic infections) were compared separately with the controls. Similarly, the factors associated with asymptomatic and symptomatic (associated with diarrhea) cryptosporidiosis were assessed by separately comparing the control children with those having asymptomatic (defined as cryptosporidial infection detected by stool PCR on biweekly or monthly surveillance samples) and symptomatic (defined as cryptosporidial infection detected by stool PCR within ± 7 days of a diarrheal episode) infections, respectively. Population attributable fraction (PAF) was calculated for selected variables that were significant in the final multivariate model using the maximum likelihood method.[41] The PAF quantifies the proportion of disease burden that can theoretically be reduced if a particular risk factor was eliminated from the entire population.[42]

RESULTS

Baseline comparison. A greater proportion of children who completed the study (N = 580) were males (312, 54%), belonged to nuclear families (320, 55%), and had poor household hygiene (318, 55%). Almost all children were born in a healthcare facility (568, 98%) and had normal birth weight (483, 85%). The median (interquartile range [IQR]) family size was 5 (4 to 7). The majority of children had an older sibling (346, 60%). Approximately two-thirds (379, 65%) of the participating families were classified as being of low socio-economic status, with 499 (86%) children living in either a "pucca" (a house with walls of brick/cement and roof of concrete/tiles) or a "mixed" (a house with walls of brick/cement and roof of tin/asbestos/thatch) house; 81 (14%) children lived in a "kutcha" house (a house with walls and roof of mud/tin/asbestos/thatch). Comparison of the baseline socio-demographic characteristics of children who completed 2 years of follow-up (N = 580) with those who were lost to follow-up (N = 93) revealed no statistically significant differences (Table 2).

Of the 580 children who completed 2 years of follow-up, 406 (70%) developed one or more episodes of cryptosporidiosis detected by stool PCR. One hundred and ninety-eight (49%) of the 406 children with cryptosporidiosis had only one infection, whereas 208 (51%) had multiple infections, ranging from 2 to 6 episodes. The majority of children with cryptosporidiosis (243, 60%) had only asymptomatic infections; 163 (40%) children reported one or more episodes of cryptosporidial diarrhea (range 1 to 4 episodes). The median (IQR) age at first cryptosporidial infection was 10.5 (6 to 17) months. For children with only asymptomatic infections (N = 243), the first infection occurred at a median (IQR) age of 12 (6 to 18) months, whereas those reporting cryptosporidial diarrhea anytime during follow-up (N = 163) had their first infection at an earlier age of 9 (5 to 15) months (P = 0.002).

Factors associated with cryptosporidiosis. The factors associated with acquisition of cryptosporidial infections in the study children were assessed over a wide range of demographic, socio-economic, nutritional, hygiene, and environmental variables (Table 3).

Any infection. In the univariate analysis, children who were stunted at 6 months of age were found to have a higher risk of cryptosporidial infections (OR = 1.81, P = 0.011). Children who were persistently stunted (OR = 3.18, P = 0.003) or underweight (OR = 1.81, P = 0.044) also had a significantly higher likelihood of having cryptosporidial infection during the first 2 years of their life. The presence of an older sibling (OR = 1.54, P = 0.018) and crowded living conditions (ratio of ≥ 5 people per room) (OR = 1.48, P = 0.050) were also associated with an increased risk of childhood cryptosporidiosis in the univariate analysis. On the other hand, always boiling drinking water before consumption (OR = 0.71, P = 0.205), maternal age of ≥ 23 years (OR = 0.76, P = 0.142), owning their home (OR = 0.77, P = 0.167), and the use of a toilet by all members of the family (OR = 0.75, P = 0.133) showed some degree of protection against cryptosporidial infections, although these associations were not statistically significant. No associations between childhood cryptosporidiosis and socio-economic status, household hygiene, presence of cows and other domestic animals in the house, and presence of potential environmental contaminants such as sewage channels, garbage dumps, animal sheds, or open-air defecation areas near the house were observed in the univariate analysis (Table 3).

In the multivariate analysis, presence of one or more older siblings in the house (OR = 1.88, P = 0.002) and stunting at 6 months of age (OR = 1.74, P = 0.019) were associated with an increased risk of cryptosporidial infections, whereas maternal age ≥ 23 years was found to be protective (OR = 0.60, P = 0.016). The results of the multivariate analysis are presented in Table 4.

Calculating the PAFs for the various risk factors in the multivariate model, the largest proportion of childhood cryptosporidiosis was found to be attributable to the presence of older siblings in the house (PAF = 29%, 95% CI = 13–43%), followed by maternal age < 23 years (PAF = 17%, 95% CI = 5–28%). Stunting at 6 months of age had a PAF of 11% (95% CI = 3–19%).

Multiple infections. When children with multiple episodes of cryptosporidial infection (N = 208) were compared with those without any infection (N = 174), children stunted at 6 months of age (OR = 2.11, P = 0.004) had a significantly higher risk of multiple infections in the univariate analysis.

TABLE 4
Results of the multivariate logistic regression analysis of the factors associated with childhood cryptosporidiosis

	Any infection		Multiple infections		Asymptomatic infection(s)		Symptomatic infection(s)	
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Presence of sibling(s)	1.88 (1.26–2.80)	0.002	–	–	2.05 (1.28–3.26)	0.003	1.71 (1.06–2.76)	0.029
Maternal age: ≥ 23 years	0.60 (0.40–0.91)	0.016	–	–	0.53 (0.33–0.85)	0.008	0.70 (0.43–1.14)	0.151
Stunted (HAZ < −2 SD) at 6 months of age	1.74 (1.09–2.78)	0.019	2.01 (1.20–3.36)	0.008	–	–	1.99 (1.16–3.42)	0.013
Boiling drinking water before consumption								
Always	–	–	0.49 (0.25–0.97)	0.041	–	–	0.35 (0.16–0.76)	0.009
Occasionally	–	–	1.29 (0.72–2.31)	0.385	–	–	1.22 (0.67–2.21)	0.515
Never*	–	–	1	–	–	–	1	–
Toilet used by all members of the family†	–	–	0.63 (0.41–0.97)	0.036	0.66 (0.43–0.99)	0.047	–	–

*Reference category.
†Data missing for 24 children.

Living in a joint family (OR = 1.60, *P* = 0.092) and in houses with a person-to-room ratio of ≥ 5 (OR = 1.45, *P* = 0.099) also resulted in an increased risk of multiple infections, although it was not statistically significant (Table 3). Similarly, the presence of a cow in the house or handling of cow dung by the primary caregiver was associated with a slightly elevated risk of multiple infections in the univariate analysis (OR = 1.47, *P* = 0.099). On the other hand, always boiling drinking water before consumption (OR = 0.53, *P* = 0.060), having a toilet in the house (OR = 0.69, *P* = 0.101), or the use of a toilet by all members of the family (OR = 0.66, *P* = 0.052) conferred some degree of protection against multiple infections, as did maternal age ≥ 23 years (OR = 0.71, *P* = 0.097). Children belonging to low socio-economic status families were three times more likely to have multiple cryptosporidial infections in the univariate analysis, but this too was not statistically significant (*P* = 0.169).

In the multivariate analysis, children who were stunted at 6 months of age were found to have an increased risk of multiple cryptosporidial infections (OR = 2.02, *P* = 0.007), with a PAF (95% CI) of 15% (5–24%). The use of a toilet by all members of the family (OR = 0.64, *P* = 0.044) and always boiling drinking water before consumption (OR = 0.47, *P* = 0.028) were found to be protective against multiple infections (Table 4). None of the other factors were significant in the multivariate model.

Asymptomatic infection(s) and *Cryptosporidium*-associated diarrhea. Similar to what was observed for cryptosporidiosis overall and for multiple infections, stunting at 6 months of age was associated with a higher risk of both asymptomatic (OR = 1.67, *P* = 0.044) and symptomatic (OR = 2.05, *P* = 0.008) cryptosporidial infections in the univariate analysis (Table 3). Other significant risk factors for asymptomatic cryptosporidial infections in the univariate analysis included presence of one or more older siblings in the house (OR = 1.70, *P* = 0.021) and overcrowded living conditions (person-to-room ratio ≥ 5) (OR = 1.66, *P* = 0.021). The use of a toilet by all members of the family conferred significant protection against asymptomatic infections in the univariate analysis (OR = 0.63, *P* = 0.025). On the other hand, always boiling drinking water before consumption conferred significant protection against *Cryptosporidium*-associated diarrhea (OR = 0.39, *P* = 0.016). The presence of one or more older siblings in the house was also associated with an increased risk of *Cryptosporidium*-associated diarrhea, but it was not statistically significant in the univariate analysis (OR = 1.34, *P* = 0.183). Similarly, living in a "kutcha" house (a house with wall and roof of mud/tin/asbestos/thatched leaves) (OR = 1.48, *P* = 0.176) or one with a mud flooring (OR = 2.42, *P* = 0.091) was associated with an increased, albeit non-significant, risk of asymptomatic infections, whereas presence of a toilet in the house (OR = 0.70, *P* = 0.099) and maternal age ≥ 23 years (OR = 0.74, *P* = 0.138), conferred some degree of protection.

In the multivariate analysis, presence of one or more older siblings in the house was significantly associated with a higher risk of both asymptomatic (OR = 2.05, *P* = 0.003; PAF = 18%, 95% CI = 6–28%) and symptomatic (OR = 1.71, *P* = 0.029; PAF = 14%, 95% CI = 1–26%) cryptosporidial infections. Children who were stunted at 6 months of age had an increased risk of *Cryptosporidium*-associated diarrhea (OR = 1.99, *P* = 0.013; PAF = 7%, 95% CI = 2–13%), whereas those who always drank boiled water were protected (OR = 0.35, *P* = 0.009). On the other hand, use of a toilet by all members of the family (OR = 0.66, *P* = 0.047) and maternal age ≥ 23 years (OR = 0.53, *P* = 0.008) were protective against asymptomatic infections in the multivariate model (Table 4).

DISCUSSION

The factors that affect the acquisition of cryptosporidial infection in developing countries are poorly understood. Our previous study had indicated that in this setting, a protected drinking water source did not decrease the risk of cryptosporidial infection and disease[34]; hence, in this study, a wide range of potential risk and protective factors were systematically investigated to evaluate association with cryptosporidiosis among children in an Indian slum community.

Presence of an older sibling in the house was a significant risk factor for cryptosporidiosis among the study children. In a post-outbreak case-control study among residents of Milwaukee, Wisconsin, people living in households with children < 5 years of age were found to have a higher risk of endemic cryptosporidiosis.[43] In another study in England and Wales, living in areas with a larger proportion of children in the 0–4 year age group was associated with an increased risk of infection.[25] Contact with children with diarrhea > 2–11 years of age,[23] changing diapers of children < 5 years of age, or helping them use the toilet[26] have also been reported as risk factors for cryptosporidiosis. Taken together, these data highlight the importance of close person-to-person contact, especially with younger children, in the transmission of *Cryptosporidium* spp.

In this study, stunting at 6 months of age was associated with a higher risk of cryptosporidial infection in children, with stunted children almost twice as likely to have multiple infections or *Cryptosporidium*-associated diarrhea. Furthermore, children who were persistently malnourished (stunted and/or underweight) had a higher probability of being infected with *Cryptosporidium* spp. The association between malnutrition and childhood cryptosporidiosis has earlier been reported from cross-sectional studies in Mexico[15] and Nigeria.[29] Longitudinal studies from Brazil,[44] Peru,[9] and Guinea-Bissau[45] have shown that both symptomatic and asymptomatic cryptosporidiosis have an adverse and sustained impact on child growth, the effect being more protracted in younger and malnourished children.[9] Cryptosporidiosis in malnourished children has also been associated with more severe and prolonged illness than in normally nourished children.[46,47] It has been hypothesized that malnutrition impairs cell-mediated immunity, predisposing children to infection which, in turn, impairs nutrient absorption and results in further growth impairment.[48]

An interesting, but unexpected observation in this study was the inverse association between maternal age and the risk of cryptosporidiosis, both asymptomatic and symptomatic. Although previous studies on cryptosporidiosis have not found any association between maternal age and infection,[21,28] the protective effect of increasing maternal age on diarrhea in general,[49] and on persistent diarrhea in particular,[50] has previously been documented. A possible reason for this observed protection could be a result of differences in child-rearing practices between the younger and older mothers. Adjusting for psycho-social factors such as low self-esteem, depression, social support, and cognitive abilities, older mothers were found to have a more positive child-rearing attitude.[51]

In a meta-analysis study, drinking boiled water was associated with a 38% reduction in the risk of endemic cryptosporidiosis.[52] In this study too, children belonging to families who always boiled their drinking water had a significantly lower risk of multiple cryptosporidial infections and cryptosporidial diarrhea than those drinking unboiled water, although no protection against cryptosporidiosis overall or asymptomatic infections was observed. Additionally, providing a protected drinking water source (bottled drinking water) did not prevent or delay cryptosporidial infections (both symptomatic and asymptomatic) in children in the same community.[34] These findings suggest that, although provision of safe drinking water may not be sufficient in preventing the acquisition of cryptosporidiosis in endemic communities with poor environmental sanitation and opportunities for recontamination, point-of-use decontamination before consumption can help reduce the frequency and severity of infection.

In this study, reported usage of a toilet by all members of the family was found to be protective against asymptomatic and multiple cryptosporidial infections in children, even though the presence of a toilet in the house or its usage by some members of the family did not confer additional protection. Previous studies have reported a lower risk of cryptosporidiosis among children residing in houses with a functional toilet.[13,19] Together, these findings highlight the beneficial role of appropriate sanitation in reducing the risk of cryptosporidial infections, even in communities living in highly unsanitary conditions.

Even though the role of animals in the transmission of cryptosporidiosis has been well documented,[53,54] the presence of cows or other animals in and around the house were not associated with a significantly elevated risk of childhood cryptosporidiosis in this study. A possible reason for this observed lack of association could be the preponderance of anthroponotic species of *Cryptosporidium* in urban Vellore.[31,34] Another reason could be the increased likelihood of animal–human contact among the residents of the study area, as both domesticated (cows, goats, and poultry) and stray (cats and dogs) animals are allowed to roam freely on the streets and near children's playgrounds.

Unlike some earlier studies where low socio-economic status has been reported as either a risk[21] or a protective factor,[25] no association between socio-economic status and the risk of cryptosporidiosis was noticed in this study. This apparent lack of association could be caused by the relative homogeneity of the study population in terms of their socio-economic status. No association between wealth indices and burden of cryptosporidiosis was observed in an impoverished community in Lima, Peru.[55]

In conclusion, this study comprehensively examined the factors associated with childhood cryptosporidiosis in an endemic Indian slum community. The nested case-control analysis used here is a powerful epidemiological design that overcomes many of the biases inherent to case-control studies.[42] The results of this study provide insights into the factors associated with acquisition of childhood cryptosporidiosis in an area with poor sanitary conditions, a grossly contaminated public water supply system,[39] and close human–animal contact. Effective disease control strategies in such settings will require a multifaceted approach that takes into account the complex nature of the host–parasite interaction.

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