Prevalence and Determinants of *Cryptosporidium* Infection in an Underdeveloped Rural Region of Southwestern China

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Abstract. Few studies have focused on the epidemiology of *Cryptosporidium* in resource-challenged settings in China. We report a community-based cross-sectional study to investigate the prevalence of *Cryptosporidium* infection and its risk factors and associations with hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) infections. The prevalence of *Cryptosporidium* infection was 12.6% (95% confidence interval = 11.0–14.3). Individuals living in households with ≥ 5 family members and raising domestic pigs tended to have a greater risk of *Cryptosporidium* infection. In addition, *Cryptosporidium* infection was significantly associated with HBV infection. There were no significant associations of *Cryptosporidium* infection with HIV viral load and HBV viral load. Further studies are needed to determine the association of *Cryptosporidium* infection with HBV infection.

BACKGROUND

Cryptosporidium is one of the most widespread diarrhea-causing parasites in humans globally and is the third most common causes of moderate-to-severe diarrhea in infants (aged 0–11 months) and the fifth most common diarrhea-causing pathogen in toddlers (aged 12–23 months), especially in low-resource settings.^{1,2} Although infections are manifested as self-limiting diarrhea in immunocompetent individuals, they can become severe and chronic in young children, elderly people, and immunocompromised patients.³ *Cryptosporidium* is a major source of opportunistic coinfection in acquired immune deficiency syndrome (AIDS) patients.⁴ Hepatitis B virus (HBV) is frequently present in individuals seropositive for human immunodeficiency virus (HIV) because of shared transmission routes. China has the world's largest burden of HBV infection with an estimated overall prevalence of 7–8% and nearly 300,000 deaths from HBV-related liver diseases every year.^{5,6} Chronic HBV infection may increase the susceptibility to human parasite infection due to damaged T-cell function and subsequent depressed production of cytokines.^{6,7} It has been indicated that HBV infection was associated with *Ascaris lumbricoides*.⁸ Yet so far, few studies have attempted to examine the relationship between HBV infection and *Cryptosporidium* infection.

In China, since the first report of human *Cryptosporidium* infection in 1987, growing attention has been paid to *Cryptosporidium* and its existence has been confirmed in at least 14 out of 32 provinces in China.^{9,10} Although there is a considerable amount of information on cryptosporidiosis in children and diarrheal patients or HIV-infected individuals, few studies have focused on the overall prevalence of *Cryptosporidium* infection and its determinants in general population in China.^{9,11} Little is known about the ecology of *Cryptosporidium* in areas with frequent human–animal contacts, which also bear a heavy burden of both HBV and HIV endemics.

The Yi Autonomous Prefecture is an underprivileged region with a high prevalence of HIV, holding more than

half (53%) of the HIV/AIDS patients in Sichuan Province, China.¹² This prefecture also bears a high HBV burden.¹³ This study investigates *Cryptosporidium* infection in residents in three selected towns. Local residents are engaged in grain cultivation and animal husbandry with high human–livestock interactions. The current study examined the occurrence of *Cryptosporidium* infection and its determinants in a population of mainly Yi people. We also examined the coexistence of HBV and HIV infections with *Cryptosporidium* infection and the possibility for HBV and HIV viral loads as predictors for *Cryptosporidium* infection.

METHODS

Study site. The study took place in a Yi autonomous prefecture of southwestern China. It has a population of approximately 4.9 million people, of which almost half belong to the Yi ethnic group.¹⁴ This region is noted for its HIV/AIDS endemic. Rugged mountainous terrain and the sparsely scattered rural populations have hindered local economic development. The present study was conducted in three towns (A, B, and C) from two counties (A from Mg County, B and C from Pg County). Pg County and Mg County were state-level poverty-stricken counties. These towns were selected because they shared similar social demographic characters (such as age structure, education level, and proportion of Yi people), meanwhile these towns were presumed to have high prevalence rate of intestinal parasites, including *Cryptosporidium* spp. According to the 2010 Chinese Census, the population of A, B, and C towns were approximately 7,000, 3,000, and 10,000, respectively.¹⁵ Local residents are engaged in mixed agriculture, have frequent human contacts with livestock, and are at a great risk for zoonotic diseases.

Survey data and sample collections. The study was carried out during the period from October 2014 to August 2015. To improve participant enrollment, village chiefs were first contacted and invited to the preparation meetings for the field work. The village chiefs then explained the purpose, procedures, potential risk and discomforts, and benefits of the study to the villagers, invited them to a scheduled survey, and distributed clean plastic containers for stool sample collection. The participants were residents of the village, who

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provided informed consent by themselves or the guardians and were willing to provide a single stool specimen. Participants were instructed to provide their fecal samples of at least 30 g collected in the morning at home and to avoid contamination. Concurrent with human fecal sample collection, a standardized questionnaire was administered to each participant by trained health workers. The survey focused on sociodemographic characteristics (including age, sex, ethnicity, marital status, education, occupation, annual family income, and number of persons/houses), personal hygiene (including drinking unboiled water, washing hands before meals and after defecation, and washing before eating raw fruits and raw vegetables), raising domestic animals, and access to safe water and sanitation facilities.

Stool samples were collected from each participant. All the participants had a finger prick to obtain about 1 mL of blood and were screened for HBV surface antigen (HBsAg), anti-hepatitis C virus (HCV), and anti-HIV antibodies by using the Diagnostic Kit for HBV (Colloidal Gold) (Livzon Pharmaceutical Group Inc., Zhuhai, China), the Diagnostic Kit for HCV (Colloidal Gold) (Livzon Pharmaceutical Group Inc.), and the Diagnostic Kit for HIV (Colloidal Gold) (Livzon Pharmaceutical Group Inc.). Colloidal gold kits are convenient and rapid tools for detecting HBsAg.¹⁶ Those with positive screening results were asked to provide a 5 mL blood sample for confirmation tests. Both stool and blood samples were transported to the local Centers for Disease Control and Prevention laboratory as soon as possible.

Laboratorial procedures. All the stool specimens were processed and labeled with a unique identification number at the same day that they were collected. Modified acid-fast staining was used to detect the existence of *Cryptosporidium* oocysts. Two thin smears were prepared from each freshly collected stool sample. Slides were air-dried and heat fixed using a candle flame for 5 seconds. The smears were stained with carbol fuchsin for 10 minutes, thoroughly washed with tap water, and then decolorized with 3% acid alcohol for 2 minutes. Slides were rinsed in tap water and then counter-stained with methylene blue for another 30 seconds. Finally, these slides were rinsed thoroughly and air-dried. In this method, *Cryptosporidium* oocysts appear as pink to red, spherical to ovoid bodies ranged between 4 and 6 μ m against blue background of debris. A stool was labeled as positive if the size of the oocysts ranged between 4 and 6 μ m. Every smear was examined by two separate technicians and a third examiner was called in, if there was a disagreement.

CD4 cell counts were measured in ethylenediaminetetraacetic acid blood using Becton Dickinson (BD) FACScout (version 1.5, Franklin Lakes, NJ; BD FACScout™ controls, Cat No.: 340166; BD FACScout™ reagents, Cat No.: 340167). Plasma aliquots were stored at -20°C and were then transported in ice to Shanghai and tested for viral load by real-time polymerase chain reaction (PCR). Plasma viral loads of HBV, HCV, and HIV were measured by using the diagnostic kit for quantification of HBV DNA, HCV RNA, and HIV-1 RNA (PCR-Fluorescence Probing) (DaAn Gene Group Inc., Zhongshan, China) in the Center for Tropical Disease Research at Fudan University. Standard procedures for sample preparation, storage, and testing were strictly followed. Not all patients had the test results of HIV or HBV viral load due to inadequate plasma aliquots. CD4 cell count was unavailable for some HIV-infected individuals because

we had no access to the equipment needed at some of the local hospitals.

Ethical considerations. The study was approved by the Ethics Review Committee of the Ethical Institute of the School of Public Health, Fudan University. All participants provided consent and were informed of their right to refuse to participate or withdraw at any point during the study.

Statistical analysis. Data were input and cross-checked by using the EpiData software (version 3.1; The EpiData Association, Odense, Denmark). Statistical analyses were conducted with the SPSS software (version 20.0; IBM SPSS Institute, Inc., Chicago, IL). Demographic characteristics and risk factors were coded as categorical variables. We used Pearson χ^2 tests to compare differences between different demographic and risk groups and crude odds ratios (ORs) and their 95% confidence intervals (CIs) were computed. Variables with a P value ≤ 0.2 from univariable results were included in the initial multivariable logistic regression model. We then used a backward selection approach to produce a final multivariable model with all variable P value ≤ 0.05 .^{17,18} We accounted for familial clustering with generalized estimating equations.^{18,19} We also developed separate multivariable models of risk factors for *Cryptosporidium* in each town. In addition, Pearson χ^2 test was used to examine the associations of *Cryptosporidium* sp. infection with HBV infection, HIV infection, HBV and HIV viral loads, and CD4 cell count. Fisher's exact test was used when indicated. P value ≤ 0.05 was considered significant in all the analyses.

RESULTS

A total of 1,635 individuals were investigated with mean age of 29 years, ranging from 1 to 82 years. Supplemental Table 1 summarizes the sociodemographic characteristics and personal behaviors of the study population. Participants were predominantly of Yi people (96.6%). Of these, 1,003 (61.3%) were female, and nearly half were unmarried (48.4%).

The prevalences of HIV, HBV, HCV, and *Cryptosporidium* infections were 2.8% (95% CI = 2.1–3.7), 8.0% (95% CI = 6.7–9.4), 1.7% (95% CI = 1.1–2.4), and 12.6% (95% CI = 11.0–14.3), respectively (Table 1). The prevalences of HIV/*Cryptosporidium*, HBV/*Cryptosporidium*, and HCV/*Cryptosporidium* coinfections were 0.1% (95% CI = 0–0.5), 1.6% (95% CI = 1.0–2.3), and 0.2% (95% CI = 0–0.3), respectively. Figure 1 shows the distribution of mixed infections in this study.

Table 2 shows potential risk factors associated with *Cryptosporidium* infection. Significant variations were observed in the risk of *Cryptosporidium* infection including county of

TABLE 1
Prevalence of infections and coinfections of HBV, HIV, HCV, and *Cryptosporidium*

	No. of participants	Cases	Prevalence (% [95% CI])
HIV	1,635	46	2.8 (2.1–3.7)
HBV	1,635	131	8.0 (6.7–9.4)
HCV	1,635	27	1.7 (1.1–2.4)
<i>Cryptosporidium</i>	1,635	206	12.6 (11.0–14.3)
Coinfection			
HIV and <i>Cryptosporidium</i>	1,635	3	0.2 (0–0.5)
HBV and <i>Cryptosporidium</i>	1,635	26	1.6 (1.0–2.3)
HCV and <i>Cryptosporidium</i>	1,635	1	0.1 (0–0.3)

CI = confidence interval; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus.

TABLE 2

Univariable and multivariable binary logistic generalized estimating equation regression analyses of risk factors for *Cryptosporidium* infection

Variable	Participants with <i>Cryptosporidium</i> infection N (%)	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Town					
A	12/260 (4.6)	1.00	< 0.01	1.00	
B	73/615 (11.9)	2.68 (1.43–5.04)	< 0.01	3.01 (1.58–5.76)	0.001
C	121/760 (15.9)	3.85 (2.08–7.12)	< 0.01	4.06 (2.20–7.52)	< 0.001
Sex					
Male	84/632 (13.3)	1.16 (0.85–1.57)	0.35		
Female	122/1,003 (12.2)	1.00			
Age (years)					
0–9	44/331 (13.3)	1.00			
10–19	49/340 (14.4)	1.08 (0.68–1.70)	0.76		
20–29	17/158 (10.8)	0.71 (0.38–1.31)	0.28		
30–39	27/272 (9.9)	0.68 (0.41–1.15)	0.15		
40–49	33/291 (11.3)	0.76 (0.46–1.25)	0.29		
50–59	24/166 (14.5)	1.01 (0.58–1.75)	0.99		
≥ 60	12/76 (15.8)	1.04 (0.51–2.15)	0.91		
Ethnicity					
Yi	200/1,580 (12.7)	1.00			
Other	6/55 (10.9)	0.72 (0.28–1.84)	0.50		
Marital status					
Not married	107/791 (13.5)	1.00			
First marriage	74/648 (11.4)	0.78 (0.57–1.08)	0.14		
Others	25/196 (12.8)	0.82 (0.51–1.34)	0.43		
No. of persons in household					
< 5	41/427 (9.6)	1.00		1.00	
≥ 5	159/1,167 (13.6)	1.52 (1.05–2.20)	0.03	1.44 (0.99–2.09)	0.05
Education					
Illiterate	88/721 (12.2)	1.00			
Primary school	101/786 (12.8)	1.11 (0.81–1.52)	0.51		
Middle school or above	17/128 (13.3)	1.09 (0.61–1.96)	0.78		
Annual family income (yuan)					
< 3,000	83/575 (14.4)	1.00			
3,000–4,999	50/441 (11.3)	0.75 (0.51–1.10)	0.14		
5,000–9,999	33/257 (12.8)	0.95 (0.61–1.48)	0.83		
≥ 10,000	39/345 (11.3)	0.77 (0.51–1.17)	0.22		
Raising cattle					
No	139/1,075 (12.9)	1.00			
Yes	67/560 (12.0)	0.92 (0.67–1.27)	0.61		
Raising pigs					
No	38,399 (9.5)	1.00		1.00	
Yes	168/1,236 (13.6)	1.54 (1.05–2.26)	0.03	1.70 (1.11–2.61)	0.01
Raising horses					
No	186/1,462 (12.7)	1.00			
Yes	20/173 (11.6)	0.88 (0.53–1.46)	0.62		
Raising poultry					
No	56/506 (11.1)	1.00			
Yes	150/1,129 (13.3)	1.26 (0.90–1.76)	0.18		
Raising cats and/or dogs					
No	124/1,010 (12.3)	1.00			
Yes	82/625 (13.1)	1.06 (0.78–1.44)	0.73		
Source of drinking water					
Spring	156/1,183 (13.2)	1.00			
Wells	28/265 (10.6)	0.79 (0.52–1.22)	0.29		
River or pond	20/170 (11.8)	0.92 (0.55–1.54)	0.76		
Drinking unboiled water					
Never	5 (10.9)	1.00			
Occasionally	26 (11.9)	1.44 (0.48–4.37)	0.46		
Always	175 (12.9)	1.48 (0.52–4.18)	0.52		
Washing hands before meals					
Always	22/368 (12.3)	1.00			
Occasionally	128/985 (13.0)	1.16 (0.74–1.80)	0.52		
Never	45/371 (12.1)	1.07 (0.64–1.79)	0.79		
Washing hands after defecation					
Always	32/282 (11.3)	1.00			
Occasionally	122/924 (13.2)	1.31 (0.84–2.06)	0.24		
Never	52/419 (12.4)	1.25 (0.76–2.06)	0.39		
Washing fruits and raw vegetables before eating					
No fruits and raw vegetables	25/196 (12.1)	1.00			
Always	70/436 (16.1)	1.17 (0.70–1.94)	0.27		
Occasionally or never	111/1,003 (11.1)	0.77 (0.48–1.23)	0.54		

(continued)

TABLE 2
Continued

Variable	Participants with <i>Cryptosporidium</i> infection N (%)	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
HIV infection					
No	203/1,589 (12.8)	1.00			
Yes	3/46 (6.5)	0.51 (0.16–1.66)	0.26		
HBV infection					
No	180/1,504 (12.0)	1.00		1.00	
Yes	26/131 (19.8)	1.82 (1.14–2.90)	0.01	1.90 (1.18–3.05)	< 0.01
HCV infection					
No	205/1,608 (12.7)	1.00			
Yes	1/27 (3.7)	0.26 (0.04–1.93)	0.19		

CI = confidence interval; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; OR = odds ratio.

residence, number of family members, raising domestic pigs, and HBV infection. The prevalence of *Cryptosporidium* was markedly lower in town A (4.6%) compared with town B (11.9%) or town C (15.9%) (Table 2). Having ≥ 5 family members was significantly associated with an increased risk of *Cryptosporidium* infection (adjusted OR [aOR] = 1.44, 95% CI = 0.99–2.09, $P = 0.05$). Individuals living with domestic pigs tended to have a greater odds of *Cryptosporidium* infection (aOR = 1.70, 95% CI = 1.11–2.61, $P = 0.01$). In addition, HBV was a predictor for increased risk of *Cryptosporidium* infection (aOR = 1.90, 95% CI = 1.18–3.05, $P < 0.01$). We found no significant associations of *Cryptosporidium* infection with HIV or HCV infection and drinking unboiled water, eating raw food, and not washing hands before meals or after defecation (Table 2). Further analysis by each town showed that only HBV infection was positively associated with *Cryptosporidium* infection (aOR = 4.07, 95% CI = 1.01–16.46, $P < 0.05$) in town A. HBV infection (aOR = 2.36, 95% CI = 1.15–4.85, $P = 0.02$) and raising domestic pigs (aOR = 3.61, 95% CI = 1.72–7.56, $P < 0.01$) were associated with *Cryptosporidium* infection in town B. No risk factor was found in town C (Supplemental Table 2).

The number of diagnosed cases with *Cryptosporidium* infection was the highest for young adolescents aged 10–19 years, followed by children aged 0–9 years (Table 1). Table 3 shows the age and sex distributions of *Cryptosporidium* prevalence.

Supplemental Table 3 shows that *Cryptosporidium* infection was not significantly associated with HIV infection (Fisher's exact $P = 0.26$), which was not modified by the presence of

HBV or HCV infection. The number of cases with HCV infection was small in the current study.

HBV infection was significantly associated with *Cryptosporidium* infection overall (Fisher's exact $P < 0.01$; Table 4) or in those free from HIV and HCV infections (Fisher's exact $P = 0.01$). For HIV-infected persons, there was no significant association between HBV and *Cryptosporidium* infections (Fisher's exact $P = 1.00$). For patients coinfecting with HIV and HCV, HBV increased the risk of *Cryptosporidium* infection although this association was not significant (Fisher's exact $P = 0.08$). For HCV-positive individuals, it was not feasible to access the association of HBV and *Cryptosporidium* infections because of small number.

Table 5 shows the association between *Cryptosporidium* infection and HIV viral load, HBV viral load, and CD4⁺ T lymphocyte count. Results of HIV and HBV viral loads were available for 26 and 98 participants, respectively. There were no significant associations of *Cryptosporidium* infection with HIV and HBV viral loads (Fisher's exact $P = 1.00$ and 0.66, respectively). The result of CD4⁺ T lymphocyte count was available for 23 patients. HIV-positive individuals with lower CD4 cell count (defined as $< 200/\mu\text{L}$) tended to have a greater risk of *Cryptosporidium* infection, although it did not reach statistical significance (Fisher's exact $P = 0.06$).

DISCUSSION

High prevalence of *Cryptosporidium* and geographical differences. Our results demonstrated that the prevalence

TABLE 3
Age and sex distributions of *Cryptosporidium* infection prevalence

Age group (years)	Prevalence of <i>Cryptosporidium</i> N (%) [95% CI]			P value
	All	Male	Female	
0–4	7/26 (26.9 [11.6–47.8])	7/21 (33.3 [14.6–57.0])	0/5 (0)	0.28
5–9	37/305 (12.1 [8.7–16.3])	23/149 (15.4 [10.0–22.3])	14/156 (9.0 [5.0–14.6])	0.11
10–14	41/259 (15.8 [11.6–20.9])	25/121 (20.7 [13.8–29.0])	16/138 (11.6 [6.8–18.1])	0.06
15–19	8/81 (9.9 [4.4–18.5])	4/33 (12.1 [3.4–28.2])	4/48 (8.3 [2.3–20.0])	0.71
20–24	6/67 (9.1 [3.3–18.5])	3/25 (12.5 [2.5–31.2])	3/42 (7.1 [1.5–19.5])	0.66
25–29	11/92 (12.0 [6.1–20.4])	4/30 (13.3 [3.8–30.7])	7/62 (11.3 [4.7–21.9])	0.75
30–34	13/120 (10.8 [5.9–17.8])	2/28 (7.1 [0.9–23.5])	11/92 (12.0 [6.1–20.4])	0.73
35–39	14/152 (9.2 [5.1–15.0])	2/48 (4.2 [0.1–14.3])	12/104 (11.5 [6.1–19.3])	0.23
40–44	15/148 (10.1 [5.8–16.2])	4/44 (9.1 [2.5–21.7])	11/100 (11.0 [5.6–18.8])	0.77
45–49	18/143 (12.6 [7.6–19.2])	2/43 (4.7 [0.1–15.8])	16/100 (16.0 [9.4–24.7])	0.06
50–54	14/90 (15.6 [8.8–24.7])	1/25 (4.0 [0.1–20.4])	13/65 (20.0 [11.1–31.8])	0.10
55–59	10/76 (13.2 [6.5–22.9])	3/26 (11.5 [2.4–30.2])	7/50 (14.0 [5.8–26.7])	1.00
≥ 60	12/76 (15.8 [8.4–26.0])	4/35 (11.4 [3.2–26.7])	8/41 (19.5 [8.8–34.9])	0.38
Total	206/1,635 (12.6 [11.0–14.3])	84/632 (13.3 [10.7–16.2])	122/1,003 (12.2 [10.2–14.3])	0.54

CI = confidence interval. P value was calculated by Fisher's exact test.

TABLE 4
Crude association between HBV infection and *Cryptosporidium* infection stratified by status of HIV and/or HCV infections

HIV (n)	HBV infection (n)	No	Yes	P value
<i>Cryptosporidium</i> infection (n)	Total	No 1,324	105	0.01
		Yes 180	26	
HIV(–) and HCV (–)	No	1,274	98	0.01
	Yes	178	25	
HIV (+) and HCV (–)	No	25	6	1
	Yes	2	0	
HIV (–) and HCV (+)	No	13	1	NA
	Yes	0	0	
HIV (+) and HCV (+)	No	12	0	0.08
	Yes	0	1	

HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; NA = not applicable. P value was calculated by Fisher's exact test.

of *Cryptosporidium* infection in rural southwestern China was 12.6% (95% CI = 11.0–14.3). Our prevalence estimates were considerably higher than the prevalences detected by modified acid-fast staining of fecal smears in similar populations from Yuxi, southern areas of Guizhou and Shiyang, China.^{11,20,21} The high prevalence of *Cryptosporidium* infection documented in our study may be due to poor sanitation, poverty, and lack of diagnostic tools.^{3,22} Since our study was performed in late summer and early autumn, these results might also reflect the seasonality of *Cryptosporidium* transmission.^{23–25} We noted that the prevalence of *Cryptosporidium* was different among the towns and therefore carried out a stratified analysis. Risk factors varied from town to town. Such geographical variations of risk factors might be responsible for the differences of *Cryptosporidium* prevalence among towns.

Age and sex distribution of *Cryptosporidium* infection.

Although *Cryptosporidium* can infect people of all ages, the prevalence of *Cryptosporidium* infection was most common among children aged 10–14 years and elderly adults aged ≥ 60 years, followed by those aged 50–54 years and young children aged 0–9 years. Similar findings have been noted in the other studies, which reflected the fact that children and the elderly are more susceptible to *Cryptosporidium* infection.²⁴ Overall, the prevalence of *Cryptosporidium* infection was similar for males and females.

Assessment of risk factors for *Cryptosporidium* infection. Participants from town A were at much lower risk of *Cryptosporidium* infection than participants from towns B

TABLE 5
Association between *Cryptosporidium* infection and HIV viral load, HBV viral load, and CD4⁺ T lymphocyte count

N (%)	No. of participants (%) with <i>Cryptosporidium</i> infection	P value
HIV viral load, IU/mL		0.53
< 10 ⁵	9/26 (34.6)	0
≥ 10 ⁵	17/26 (65.4)	2 (11.8)
HBV viral load, IU/mL		0.66
< 30 (undetectable)	15/98 (15.3)	2 (13.3)
30–99,999	40/98 (40.8)	10 (25.0)
≥ 100,000	43/98 (43.9)	8 (18.6)
CD4 ⁺ T lymphocyte (no./μL)		0.06
< 200	6/23 (26.1)	2 (33.3)
≥ 200	17/23 (73.9)	0

HBV = hepatitis B virus; HIV = human immunodeficiency virus. P value was calculated by Fisher's exact test.

FIGURE 1. Venn diagram showing the distribution of mixed infections in this study.

and C. The difference remained after adjustment for covariates, which might underscore the importance of geographical distribution of *Cryptosporidium*. Overcrowding has been associated with *Cryptosporidium* infection, specifically with *Cryptosporidium hominis*.²⁶ A high secondary infection rate (39%) was reported in households with children diagnosed with *Cryptosporidium* infection.²⁷ In the present study, we found a significant association between having more than five family members and *Cryptosporidium* infection. This might be related to the crowded conditions in a household, which may translate into more frequent human-to-human contact, especially with children diarrhea, thus increasing the risk of *Cryptosporidium* transmission within families.^{28–30}

It was previously found that residents who raised livestock or poultry were more likely to be infected with *Cryptosporidium* in this area, supporting the hypothesis of zoonotic transmission of domestic animals. Domestic pigs in this region were mostly free ranging and tended to be around the residences. Humans can be easily infected through direct contact with them or by indirect contact with their infective feces on the ground or ingestion of contaminated food or water. However, we identified no significant association of *Cryptosporidium* infection with other agricultural/domestic animals including cattle, horses, poultry, cats, and dogs. Previous studies also found that raising cattle, especially calves, was a risk factor for human *Cryptosporidium* infection.^{3,31} In this area, cattle usually graze on the nearby hills away from human settlements, thus human–cattle contacting is less frequent. Horses, cats, and dogs are primarily infected with host-specific *Cryptosporidium* species or genotypes, and therefore, the actual risk of zoonotic transmission of these animals may be low.^{32,33} Nevertheless, genotyping and subtyping studies of *Cryptosporidium* from domestic animals will help to clarify zoonotic transmission of *Cryptosporidium*. Surprisingly, unsafe drinking water sources were not found to increase the risk of *Cryptosporidium* infection, which could be the result of degradation or improper tapping of the water distribution system, exposing water to environmental contamination.³⁴ In addition, drinking unboiled water was found to be associated with *Cryptosporidium* infection in this study, for various reasons.

First, other transmission pathways, such as animal contact, or anthroponotic transmission might play a relatively important role as we found raising pigs and living in a household with ≥ 5 members were associated with greater odds of *Cryptosporidium* infection. Routinely drinking boiled water at home does not necessarily prevent drinking untreated water, for instance, during farm work far away on the hills or during a visit to a neighbor's house.

Associations of *Cryptosporidium* infection with HBV, HIV, and HCV infections and with viral loads and CD4 cell counts as indicators of immunity changes. Intriguingly, we found that *Cryptosporidium* infection was significantly associated with HBV infection, but not with HCV infection. To determine whether the immunity changes caused by HBV lead to a greater risk of *Cryptosporidium* infection, we examined the association between the levels of HBV DNA and prevalence of *Cryptosporidium*. HBV viral load was used as a surrogate marker for host immunity status and was only divided into three categories due to small sample size: < 30 IU/mL (undetectable), 30–99,999 IU/mL, and $\geq 100,000$ IU/mL. Although the prevalence of *Cryptosporidium* infection increased with HBV DNA levels, this difference was not statistically significant suggesting that HBV viral load is not likely a suitable predictor of risk of *Cryptosporidium* infection in HBV-infected individuals. Further study is needed to determine the interactions between HBV and *Cryptosporidium* infections.

Previous studies have identified HIV infection as a risk factor for *Cryptosporidium* infection.^{35,36} Unexpectedly, HIV did not appear significantly related to *Cryptosporidium* infection in our study. One possible reason for this is that most of HIV-infected individuals were less immunocompromised based on their HIV RNA levels and CD4 cell counts. Our study showed that quantitative HIV RNA levels and CD4 cell counts did not correlate with the risk of *Cryptosporidium* infection, but the two cases of *Cryptosporidium*/HIV coinfection with available test results occurred in patients with HIV viral loads $\geq 10^5$ IU/mL and CD4 cell counts $< 200/\mu\text{L}$. It should be noted that our results were preliminary and based on a small sample size.

Broadly speaking, the immunosuppression imposed by HIV results in reduced rates of HBV and HCV clearance and increases the risk of chronic liver diseases in the setting of coinfection.³⁷ Conversely, existing evidence points out that HBV and HCV coinfections are associated with worse outcomes for HIV-positive patients.^{37–39} In addition, HBV/HCV coinfection may accelerate liver disease progression and increase the risk of hepatocellular carcinoma.⁴⁰ Thus the question of whether the interplay among HIV, HBV, and HCV infections influences *Cryptosporidium* infection is important. When stratified by the status of HBV infection, we found no significant association between *Cryptosporidium* and HIV infections. For HIV-negative–HCV-negative individuals, HBV was associated with an increased risk of *Cryptosporidium* infection, but not for HIV-positive–HCV-negative persons. For patients coinfecting with HIV and HCV, HBV increased the risk of *Cryptosporidium* infection although this association was not significant. Overall, the risk of *Cryptosporidium* infection was strongly associated with HBV infection, but not with HIV infection and HIV/HBV coinfection. Coinfection with HIV might neutralize the impact of HBV infection on the risk of *Cryptosporidium* infection. However, since the

number of participants coinfecting with *Cryptosporidium* and viruses was small, our data only help to generating hypothesis concerning the interactions of these viruses and changes in the host immune system and subsequent *Cryptosporidium* infection.

Our study has several limitations. First, there were fewer males than females because many young males migrated to the cities to make a living. This underrepresentation of young males may lead to an underestimate of the prevalence of HIV, HBV, and HCV infections, as they are at greater risk than other age groups. Second, we collected only one stool sample from each participant, which may result in an underestimate of the true prevalence of *Cryptosporidium* infection. Also, misclassification may have occurred because colloidal gold kits are slightly less sensitive compared with enzyme-linked immunosorbent assay, and the sensitivity of modified acid-fast staining technique is 70% compared with that of immunofluorescent antibody stains.^{16,23} Because of limited research funds, data on *Cryptosporidium* genotypes were not available. Further research should include molecular analysis to determine the roles of anthroponotic and zoonotic transmission of *Cryptosporidium* in rural China. In view of these limitations, the findings should be interpreted discreetly.

CONCLUSIONS

Our results reveal a high prevalence of *Cryptosporidium* infection in rural areas of southwest China and its close associations with HBV infection and raising domestic pigs. Further studies are needed to clarify the association of *Cryptosporidium* infection with HBV infection.

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