Research Article

Risk Factors of *Cryptosporidium* spp. Infection Through Drinking Water Sources in Farming Communities in Sukowono District Jember Regency

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Abstract

Cryptosporidiosis is a zoonotic, emerging waterborne and protozoan disease. This parasite infects the digestive and respiratory tracts of humans and various animals. This study aims to determine the risk factors of *Cryptosporidium* spp. infection through drinking water sources in farming communities in Sukowono District, Jember Regency and using an observational analytic study with a cross-sectional approach. The research sample was reviewed using observational sheets and stool samples with concentration method and modified Ziehl-Neelsen staining on research subjects' samples based on inclusion and exclusion criteria totalling 52 people. The results of this study indicate the characteristics of the research subjects in Sukowono District, Jember Regency, dominated by female gender (57.7%), adult age category (42.3%), elementary school graduate (53.8%), and identified as a farmer or rancher (34.6%). The examination of stool samples showed *Cryptosporidium* spp. an infestation in 1 (one) person. The results of the bivariate analysis using the Fisher's Exact alternative test obtained p of 0.601 ($p>0.05$). The conclusion is that most research subjects use unhealthy healthy water as a drinking water source; one person is infected. Also, all factors from drinking water sources are not at risk of this parasitic infection in Sukowono District, Jember Regency.

Keywords : *Cryptosporidium*, drinking water, the farming community
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INTRODUCTION

Cryptosporidiosis is an emerging waterborne disease that is important in public health worldwide (Dhaliwal & Juyal, 2015). These organisms live under the cell membranes of the small intestine and the respiratory epithelium of some mammals, including humans (Bogitsh, Carter, & Oeltmann, 2013; İnci et al., 2018). *Cryptosporidium* spp. the infection has been recognized as a major cause of diarrhoea worldwide, especially in mild to severe diarrhoea (Bamaiyi & Redhuan, 2016). About 60% of species of the genus *Cryptosporidium* are the most common diarrhoea-causing protozoa and are transmitted by water worldwide from 2004 to 2010 (Baldursson & Karanis, 2011). The incidence and prevalence of cryptosporidiosis are higher in developing countries due to inadequate infrastructure or basic amenities that allow oocysts to be transmitted through faeces to contaminate food and drinking water. Humans and animals become infected when they consume food and beverages that contain these oocysts (Burnet et al., 2014).

Cryptosporidium spp. oocyst found as much as 39% in pure water sources contaminated with oocysts, although it appears clear. Additionally, these oocysts resist disinfectants like chlorine, which is frequently used to sanitize drinking water, water parks, and swimming pools (Bennett, J. E., Dolin, R., Blaser, 2015; Wilkes et al., 2013). Research by Gharpure et al. (2019) also Hardiyanti & Umniyati (2017) found a relationship between this parasite contamination in drinking water sources and the incidence of diarrhoea. Factors that facilitate contamination are micron size so that it escapes the filtration process, high infectiousness, population density, livestock pens, excystation in large quantities around 10^5-10^7 per gram of faeces, and oocysts do not have a maturation period (Ahmed & Karanis, 2020; Ramsay et al., 2014; Sarkar et al., 2013). Water can be contaminated through sewage overflows, improperly functioning sewage systems, polluted rainwater runoff, and agricultural runoff (Centers for Disease Control and Prevention et al., 2015).

Water is the optimal transmission medium in the transmission of *Cryptosporidium* spp. oocysts, both drinking water and water used for recreational purposes, such as swimming pools, rivers, lakes, and others (Ramsay et al., 2014; Wandrivel, Suharti, & Lestari, 2012). A total of 4.5% of cases of *Cryptosporidium* spp. globally related to the availability of drinking water (Gharpure et al., 2019). Although this parasite infection has been linked in numerous studies to the accessibility of drinking water, Jember Regency has never addressed this issue. The availability of clean drinking water in the Jember Regency is still at 29.467%, and Sukowono District reaches 24.95% (Dinas Kesehatan Kabupaten Jember, 2019). The community's main livelihood in the Sukowono District is in the agricultural sector, which has 2,845 people in the workforce, including farmers (Badan Pusat Statistik Kabupaten Jember, 2019). Until now, there have been no reports of *Cryptosporidium* spp. infection, and there has not been much research done on risk factors related to this parasitic infection in agricultural communities, especially farming communities

METHODS

This research has a cross-sectional design and is an observational analytic study. The study was conducted in Sukokerto Village, Sukowono District, and the Parasitology Laboratory of the Faculty of Medicine, the University of Jember, from July 2022 to August 2022. Ethical clearance of this study has been released by the Faculty of Medicine Ethics Committee of the Faculty of Medicine, Jember University, with the number 1610/H25.1.11/KE/2022 on June 27, 2022. The samples of this study were those who resided in a single house and owned livestock that met the inclusion and exclusion criteria in Sukokerto Village, Sukowono District, Jember Regency. The sample size of this study was 52 samples from Sukokerto Village, Sukowono District. Non-probability sampling combined with the purposive sampling approach is the sampling methodology employed in this study.

Primary data collection in this study was carried out after obtaining permission from the Ethics Commission of the Faculty of Medicine, University of Jember. The drinking water sources factors were identified through questionnaire interviews. Each respondent's faecal sample was put into a small bottle (pot) with 2.5% potassium dichromate solution, then labelled with the identity code of each respondent and wrapped in a different sterile plastic bag for each sample. Respondents are advised to collect faeces in the morning, faeces should not be mixed with toilet water or urine, and the amount of faeces collected should be around 50 mg (or the size of a thumb). These samples were examined using the concentration method with diethyl-ether sedimentation and modified Ziehl-Neelsen (MZN) staining technique at the Parasitology Laboratory, Faculty of Medicine, University of Jember. Faecal sample preparations were observed using a microscope with a magnification of 1000x to identify the oocysts. Data on drinking water sources factors that have been obtained are then processed using Fisher's Exact alternative test and relative risk.

RESULTS

Table 4.1 shows the distribution of research themes by gender, age, educational level, and occupation. From Table 4.1, it is known that there are more female subjects than male subjects. Most study participants were 18-45 years old or adults, up to 22 (42.3%). The education level of those surveyed mainly was elementary school, with a total of 28 (53.8%). Most respondents were 20 farmers (34.6%) and 14 housewives (26.9%).

Cryptosporidium spp. infection was identified through stool sample examination using the concentration method with diethyl-ether sedimentation. Oocyst had been found in one subject, as shown in Figure 1. The results of the distribution of *Cryptosporidium* spp. infection can be seen in Table 1 and Table 2.

Risk factors' association between drinking water sources factors and *Cryptosporidium* spp. infection was determined using Fisher's Exact alternative test. The researcher found p-values greater than 0.05 ($p>0.05$) for all the risk factors studied. This shows no significant relationship exists between groups of risk factors for drinking water sources (location of drinking water sources, processing, and storage of drinking water) and *Cryptosporidium* spp. infection on the research subjects. The risk factors from the place of drinking water sources, processing, and storage of drinking water cannot be defined as definitive risk factors for this parasite infection. These findings can be seen in Table 3.

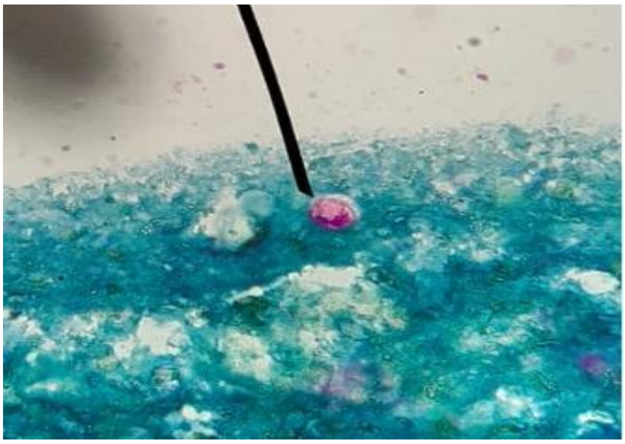

Figure 1. The results of observations on stool examination after the concentration method and stained with modified Ziehl-Neelsen (MZN) were carried out using a 1000x magnification microscope to figure *Cryptosporidium* spp. oocyst.

Table 1. Research subjects characteristics

Characteristic	Freq. (n)	Per cent (%)	Infected with *Cryptosporidium* spp.	
			Positive	Negative
Gender				
Female	30	57.7	0	31
Male	22	42.3	1	20
Age				
0–1 year old	2	3.8	0	2
1–6 years old	6	11.5	1	5
6–18 years old	7	13.5	0	7
18–45 years old	22	42.3	0	22
45–59 years old	11	21.2	0	11

≥60 years old	4	7.7	0	4
Education level				
No education	7	13,5	1	6
Elementary school	28	53,8	0	28
Junior high school	15	28,8	0	15
Senior high school	2	3,8	0	2
College				
Occupation				
Farmer	20	34.6	0	20
Housewife	14	26.9	0	14
Student/Pre-schooler	10	19.2	1	9
Other	8	15.4	0	8

Table 2. Distribution of stool samples examination

Faecal Sample Examination	Frequency (n)	Per cent (%)
Positive	1	1.9
Negative	51	98.1
Total	52	100%

Table 3. Relation of factors for drinking water sources with *Cryptosporidium* spp. infection

Drinking Water Sources Factors		Negative STH contamination		Positive STH contamination		*p*-value	RR (95% CI)
		n	%	n	%		
The source of water is used for cooking, drinking, and daily needs	Well water	47	97.9	1	2.1	0.923	-
	Chlorinated well water	4	100	0	0		
The well meets the following requirements. a. Well wall measuring > 3-meter waterproof b. Well, lip measuring > 1 meter c. Well, floor width >	Yes	25	100	0	0	0.519	-
	No	26	96.3	1	3.7		

1.5 meters d. The well has a cover							
The source of drinking water comes from clean water sources	Yes No	42 9	97.7 100	1 0	2.3 0	0.827	-
The source of drinking water is the same as the source of drinking water for livestock	Yes No	39 12	97.5 100	1 0	2.5 0	0.769	-
Sources of drinking water are also used for daily needs	Yes No	23 28	95.8 100	1 0	4.2 0	0.462	-
Distance from the septic tank to the water source ≥ 10 m	Yes No	31 20	96.9 100	1 0	3.1 0	0.615	-
The drinking water used is cooked or processed	Yes No	47 4	97.9 100	1 0	2.1 0	0.923	-
Drinking water used for washing foodstuffs is the water used for drinking	Yes No	49 2	98 100	1 0	2 0	0.962	-
Ever drunk uncooked water	Yes No	18 33	94.7 100	1 0	5.3 0	0.365	-
Drinking water is stored in a closed container	Yes No	47 1	97.9 100	1 0	2.1 0	0.923	-

DISCUSSION

The results of this study indicate the number of female research subjects is as many as 30 (57.7%) people, while the male sex is as many as 22 (42.3%) people. One person among the male research subjects was infected with *Cryptosporidium* spp. oocysts. This study has resulted similar to a study in Baghdad, India, which reported the number of cases infected with *Cryptosporidium* spp. oocysts higher in males than females (Whaeeb et al., 2020). Similar research was conducted by Bamaiyi & Redhuan (2016) in Southeast Asian countries, including Indonesia, which stated that cases of infection with *Cryptosporidium* spp. found more in males than females. According to Pane & Putignani (2022), this can occur because men tend to activity and are in contact with the environment more often than women, but the correlation between the incidence of *Cryptosporidium* spp. oocyst infection and gender have not been proven (Sayal, 2019; Whaeeb et al., 2020).

The highest number of research subjects was found in the age category of 18-45 years (adults) numbered 22 (42.3%) people, but 1 (one) person in the 1–6 years category was infected with *Cryptosporidium* spp. The results of this study followed studies in South Asia and Sub-Saharan Africa which reported that ages 1-6 years or under one year of age were highly susceptible to *Cryptosporidium* spp. infection (King, Tyler, & Hunter, 2019; Sow et al., 2016). This is due to malnutrition, a developing immune system, consumption of unclean drinking water, and the habit of playing with soil contaminated with oocysts and supported by a poor environmental sanitation system, such as the absence of proper waste disposal facilities and water. insufficient net income (Latif & Rossle, 2015; Tamomh et al., 2021).

The subjects of this study generally had a low level of education (no school or elementary school graduates), as many as 28 people (53.8%). One research subject in the no-education category was infected with *Cryptosporidium* spp. oocysts. The same thing was found in the research conducted by Saputra et al. (2016) at SDN Papanggo 01 Jakarta. Two out of 100 students (2%) were reportedly infected with *Cryptosporidium* spp. . Lack of education about personal hygiene from kindergarten to higher education causes difficulties in handling the transmission of *Cryptosporidium* spp. in an area, such as the habit of washing hands before eating and after defecating (Zheng et al., 2018).

The research subjects who worked as farmers or ranchers were 20 people (34.6%). One person in the student or pre-schooler category was infected with *Cryptosporidium* spp. . This study's results align with the research of Drinkard et al. (2015) reported that an outbreak occurred among veterinary students in Philadelphia. This is supported by Siddique et al. (2021), who state that transmission often occurs in children of pre-primary school age to students such as veterinary students. Transmission can occur due to a lack of habit of washing hands before eating, living areas that are too shabby and crowded, and poor water sanitation (Deichsel et al., 2020).

This study demonstrates no causal link between drinking water source risk factors and *Cryptosporidium* spp. infection. The possibility that causes this to happen is the presence of other more dominant risk factors, such as different body immunity, travel history, and low socioeconomic status. Research conducted by Hardiyanti & Umniyati (2017a) in Batanghari River, Jambi, supports this study, which reported no connection between the risk factors of drinking water sources, including its location, and *Cryptosporidium* spp. infection. This is supported by the CDC, which states that the incidence of *Cryptosporidium* spp. in the San Francisco Bay Area does not have a significant relationship with drinking water sources (Putignani & Menichella, 2010). The location of drinking water sources is also not significantly related and has been proven through research conducted by Kifleyohannes & Robertson (2020) in Ethiopia. The same finding occurred in the study of Maryanti et al. (2019) in Pekanbaru City, which found no connection between risk factors for drinking water treatment and infection with *Cryptosporidium* spp. .

Most of the water sources used by the research subjects came from well water which did not comply with the requirements of healthy well water, so it was required to treat well water to make it fit for consumption. Treating drinking water before consumption can reduce the risk of infection with *Cryptosporidium* spp. In addition, there is no relationship between risk factors from drinking water sources and infection with *Cryptosporidium* spp. in this study can be caused by the drinking water storage habits of most of the research subjects. Research subjects have a habit of storing drinking water in closed containers so that drinking water is protected from contamination with *Cryptosporidium* spp. oocysts. Therefore, oocysts were not found in the faeces of the study subjects. A similar finding was reached in the study of Saputra et al. (2016) in North Jakarta stated that there was no relationship between beverage processing using PDAM water or well water and infection with *Cryptosporidium* spp. It was also not found that consuming bottled water can reduce the risk of infection with *Cryptosporidium* spp. (Sarkar et al., 2013).

The limitation of this study is the faecal storage period (more than six months) could interfere with the integrity of *Cryptosporidium* spp. oocysts. Another limitation is the rinsing process using distilled water in the concentration method with diethyl-ether sedimentation, which can reduce the concentration of oocysts deposited in the subject's faeces. No sequencing method was carried out in this study to identify *Cryptosporidium* species.

CONCLUSION

Based on the research results carried out in Sukowono District, Jember Regency, with many research subjects, 52 people, one person was found to be infected with Cryptosporidium spp. Factors of origin of drinking water sources, processing, and storage were not found to be significant or not at risk factors of infection with Cryptosporidium spp. Most farming communities in Sukowono District use well water that does not meet the requirements of healthy wells as a source of drinking water, daily needs, and livestock needs. Water is boiled before consumption and stored in clean water containers.

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