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Preliminary Investigation of Association Between Sanitation and Water Contamination by *Cryptosporidium* at Livestock Area in Jember

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ABSTRACT

Cryptosporidium sp. is one of the waterborne protozoa involving water as the main medium of transmission causing cryptosporidiosis in animals and humans and its presence is influenced by various things including hygiene and sanitation of the community. This cross-sectional study aims to determine the association between the implementation of the community-led total sanitation and the presence of *Cryptosporidium* oocysts in clean water sources. The research was conducted from October 2020 to June 2021 in Sukokerto Village, Sukowono, Jember Regency. The data were obtained from interview with the respondents using structured questionnaires. The presence of oocysts was examined by microscopic observations with sedimentation methods and Modified Ziehl=Neelsen staining, and the data were analyzed using Spearman rho statistical test. The results showed that there were 12 (20.3%) household with good category at the implementation of community-led total sanitation, 28 (47.5%) household with moderate category, and 19 (32.2%) household with poor category. The presence of *Cryptosporidium* oocysts was found in 6 (10.2%) samples of clean water sources. The results of statistical tests with Spearman rho showed a p-value=0.098, so that there was no significant association between the implementation of community-led total sanitation and the presence of *Cryptosporidium* oocysts in clean water sources in study location.

Keywords: *Cryptosporidium* sp; waterborne protozoa; Community-led total sanitation

INTRODUCTION

The availability of clean water is still a problem in several countries, especially developing countries. The World Health Organization stated that in 2017, there were still around two billion people who used air contaminated with feces as a source of drinking water, and this caused 485,000 deaths from diarrhea every year (WHO, 2019). It is known that protozoa are the main etiology of waterborne diarrhea outbreaks (Lim & Nissapatorn, 2017). There have been 905 reported diarrheal outbreaks from the 1900s to December 2016 caused by protozoa. In addition, it is known that more than 60% of cases are caused by *Cryptosporidium* sp. (Baldursson & Karanis, 2011; Efstratiou et al., 2017). Cryptosporidium sp. is a waterborne protozoon that causes a water-borne disease, the main symptom of which is diarrhea in animals and humans. It is called cryptosporidiosis (Cho et al., 2013). *Cryptosporidium hominis* and *C. parvum* are the main species that cause cryptosporidiosis (Checkley et al., 2014; Kooh et al., 2020). Cryptosporidiosis can occur at various levels of age and gender, depending on the patient's immune status (Maryanti, 2011). Weak immune conditions, such as HIV/AIDS patients, are at greater risk of suffering from cryptosporidiosis (Ahmadpour et al., 2020). The spread of this protozoa can occur from animals to humans or from humans to humans via the fecal-oral route and is related to drinking water supply systems, wastewater disposal, and environmental sanitation (Nugraha et al., 2011). *Cryptosporidium* sp infection is more common in developing countries, especially in rural areas with minimal access to adequate drinking water, poor sanitary conditions, and exposure to livestock (Checkley et al., 2014; Bouzid et al., 2018). In addition, areas with warm environmental temperatures and high rainfall, such as tropical areas, are associated with an increased risk of cryptosporidiosis (Checkley et al., 2014).

Water has an important role in the spread of *Cryptosporidium* sp. These protozoa can contaminate various types of water sources and processed products. Several previous studies succeeded in detecting the presence of *Cryptosporidium* sp. oocysts in various types of water sources in several countries spread across the continents of Asia, America, Africa, Europe and Southeast Asia (Kumar et al., 2014, 2016; Vermeulen et al., 2019). In Indonesia, *Cryptosporidium* sp oocysts were found in the water of the Citarum River in Bandung and the Ciliwung River in Jakarta (Nufutomo & Muntalif, 2017; Mahardianti et al., 2020). One study in Jember stated that around 31-55% of local community sanitation behavior was still classified as moderate to poor. Even 99% of residents throw rubbish carelessly. More than 60% of people do not have bathrooms and toilets, and 50% of residents who do not have toilets dispose of their wastewater in rivers and gardens. Apart from that, 44% of residents who have livestock throw away livestock waste without processing it. This is enough to put water sources in Jember at risk of being contaminated with parasites, in this case, especially at risk of being contaminated with *Cryptosporidium* sp. oocysts (Khoiron and Rokhmah, 2015).

As an effort to improve community hygiene and sanitation, the government then implemented the Community-Led Total Sanitation (CLTS) program, which has five pillars: stopping open defecation, washing hands with soap, managing household drinking water and food, and securing household waste. Household and safeguarding household liquid waste (Ministry of Health of the Republic of Indonesia, 2014). The CLTS program aims to improve community hygiene and sanitary behavior through triggering programs to change unsanitary behavior into clean and healthy living behavior. Since it was first implemented, community hygiene and sanitary behavior have shown improvement. The national achievement of CLTS implementation in Indonesia in 2019 was 69.43%. East Java is one of the three provinces with the highest realization of CLTS implementation. However, only around 51% of villages in East Java have ODF (Open Defecation Free) status (Indonesian Ministry of Health, 2020). This is because most people who have toilets still defecate in the open. Access to toilets and defecation behavior are the main indicators for assessing the achievements of CLTS implementation. Access to toilets is still low, and defecation behavior is poor, indicating that in this area, the hygiene and sanitation conditions of the community are also still low. Based on data accessed from the CLTS website as of November 15, 2020, the percentage of latrine access in Jember has reached 75%. However, 166,988 families in Jember still defecate in the open. There are 10 sub-districts with the lowest percentage of latrine access in Jember, one of which is Sukowono District (Kementerian Kesehatan RI, 2019).

Not much research has studied the relationship between hygiene and sanitation and water contamination by parasites, especially in rural areas. There is no previous research in Jember. Therefore, it is deemed necessary to conduct research on the relationship between hygiene behavior and sanitation, in this case regarding the implementation of CLTS with *Cryptosporidium* sp. contamination as one of the parasites that causes the most contamination in water sources and is the main etiology in several outbreaks of diarrhea. Apart from that, in rural areas where hygiene conditions and community sanitation behavior are still poor, almost all people use river water, well water, groundwater, and other surface water as the main water source. This causes people in these areas to be at high risk of infection if there is Cryptosporidium sp. contamination in their water sources.

METHOD

This research is a type of observational analytical research with a cross-sectional design which was carried out from October 2020 to June 2021 in Sukokerto Village, Sukowono District, Jember Regency. The research sample size was calculated using the following formula, and 59 samples were obtained (Lemeshow et al., 1990).

$$n = \frac{Z\alpha^2 \, P \, (1\text{-}P) \, N}{d^2 (N\text{-}1) + Z\alpha^2 \, P \, (1\text{-}P)}$$

Information:
n = Number of samples
α = Type I error, which is 5%.
Zα = Standard value of alpha (α). The standard value of alpha is 5% or 1.96.
P = Proportion of certain characteristics or variables found in the population; the value is obtained from previous research, namely 20% (Lim and Nissapatorn, 2017).
N = Total research population is 1,186 families (Jember Regency Central Statistics Agency, 2020).
d = Research precision is 10%.

Data was obtained from family or family representatives willing to become research respondents. Data regarding the implementation of CLTS was obtained through interviews with a CLTS questionnaire guide. Meanwhile, data on the presence of *Cryptosporidium* sp. oocysts in clean water samples originating from respondents' homes was obtained through a microscopic examination using the modified Ziehl-Neelsen (MZN) sedimentation and staining method (Nufutomo

& Muntalif, 2017; Sakran et al., 2017; Mahardianti et al., 2020). The results of observations regarding the presence of *Cryptosporidium* sp. oocysts were measured in diameter using the Image Raster 3.0 application and confirmed with at least 2 competent lecturers in the field of Parasitology. Both data were then analyzed using the Spearman rho statistical test with a significance level of 0.05 via the SPSS application (Dahlan, 2014).

RESULT

Based on the respondents' characteristics, as shown in Table 1 below, most research respondents were female. This is because when the research was carried out, most of the men who were heads of families were out for work so family members who were at home were filling in the questionnaires. The research respondents ranged from 18 to over 50 years old, and most respondents were in the 25–50-year-old range. The education level of most respondents is still in the low category or elementary school level, or no schooling. Most respondents do not work or are only housewives and farmers. More than 50% of respondents have livestock (55.9%), and the type of clean water source used by respondents is mostly dug well water.

Table 1. Characteristics of Respondents (n=59)

Characteristics of Respondents	Frequency	Percentage
Gender		
Male	8	13.6
Female	51	86.4
Age (years)		
18-24	3	5.1
25-50	46	78.0
>50	10	16.9
Education level		
Low	38	64.4
Moderate	16	27.1
High	5	8.5
Occupation		
None/ Housewife	30	50.8
Farmer / farm labor	15	25.4
Others	14	23.8
Cattle Ownership		
Yes	33	55.9
No	26	44.1
Type of water source used		
Dug well water	36	61.0
Drilled well water	1	1.7
Dug well water and river water	20	33.9
Dug well water and local water company's water	1	1.7
Dug well water and refill drinking water	1	1.7

The implementation of CLTS from the families of research respondents is shown in Table 2 below. Based on this table, it is known that most respondents (47.5%) are in the moderate CLTS implementation category.

Table 2. Implementation of CLTS

Implementation of CLTS	Frequency	Percentage
Good	12	20.3
Moderate	28	47.5
Poor	19	32.2
Total	59	100

Table 3 below shows that of the 59 clean water samples obtained from respondents' homes, 6 (10.2%) were positive for *Cryptosporidium* sp. oocysts. The distribution of *Cryptosporidium* sp. oocyst contamination is shown by the map in Figure 1. Meanwhile, Figure 2 is one of the forms of *Cryptosporidium* sp. oocysts that have been identified.

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Table 3. Presence of *Cryptosporidium* sp oocysts

The presence of *Cryptosporidium* sp. oocysts	Frequency	Percentage
Positive	6	10.2
Negative	53	89.8
Total	59	100

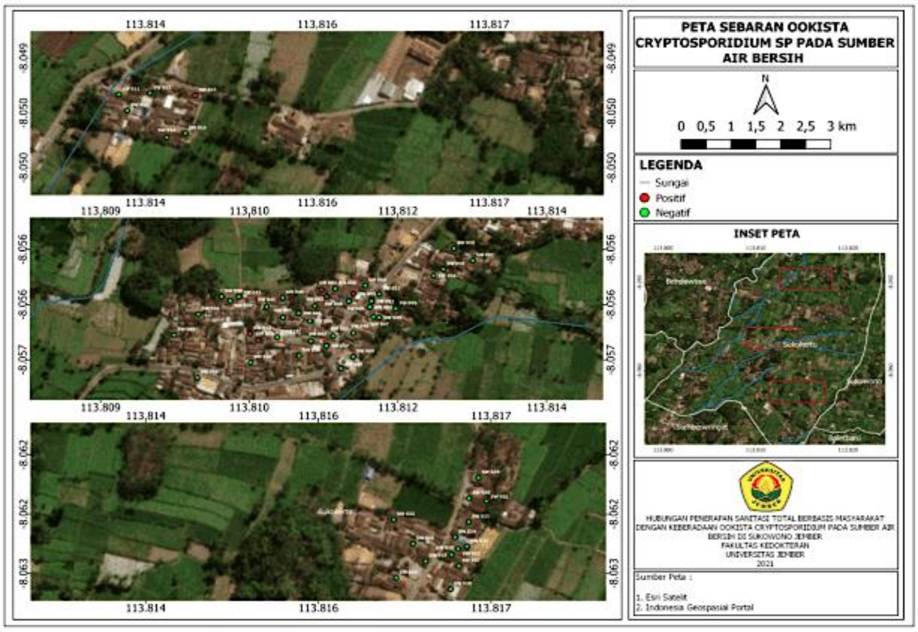

Figure 1. Distribution Map of *Cryptosporidium* sp oocysts in Clean Water Sources

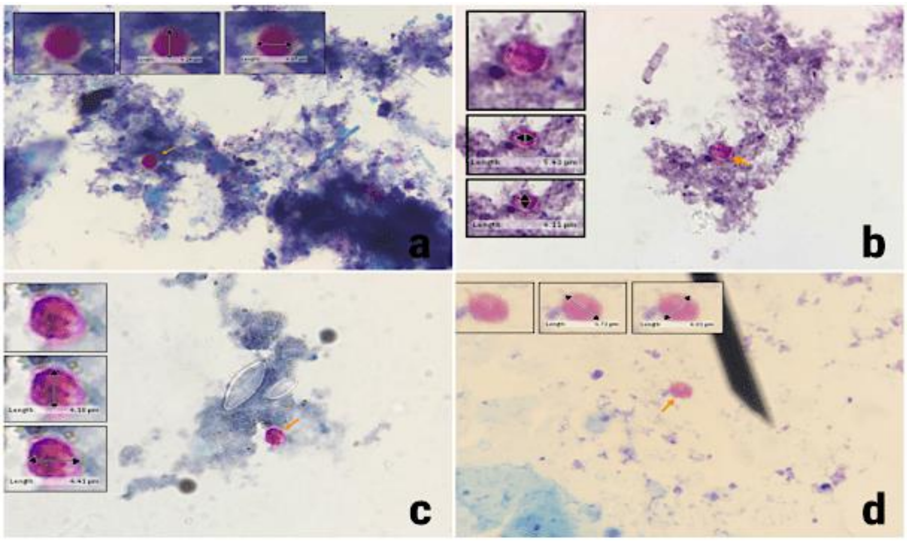

Figure 2. *Cryptosporidium* sp oocysts in Water Samples with MZN Staining and 100x Objective Lens Magnification

The limitation of this study is that it is only limited to rural areas in Jember, especially Sukowono Village. These results cannot be generalized in other areas. The potential bias of this study may be related to the limited area of the research.

DISCUSSION

Implementation of CLTS

In this research, CLTS implementation is grouped into three categories, namely good, moderate, and poor as described in Table 2. CLTS implementation is related to understanding or knowledge, motivation, and availability of facilities. CLTS is a new approach or paradigm for developing sanitation hygiene in Indonesia by triggering. The triggering aims to provide understanding and motivation to the community to raise awareness to implement the five pillars of CLTS independently (Kementerian Kesehatan RI, 2014). Community understanding of the triggering carried out is influenced by the level of education, while the availability of facilities to support the implementation of CLTS is related to funding. Previous research states that education and funding influence the sustainability of the implementation of the CLTS program (Priatno et al., 2014; Lahudin, 2017).

In this research, it was discovered that most respondents' education was still in the low category (not attending or elementary school). The level of education influences a person's mindset, way of receiving, understanding, and knowledge of information (Rudolfo et al., 2022; Kautsar et al., 2023). Low education makes it difficult for people to understand the importance of sanitation and hygiene, such as implementing CLTS well (Hidayatullah, 2015). This is in line with previous research conducted by Marwanto (2019). This research shows a relationship between the level of knowledge and the behavior of implementing the first pillar of CLTS and someone with a higher level of education tends to have better concern for health.

The presence of *Cryptosporidium* sp. oocysts in clean water sources

The results of the observations have been briefly described in Table 3. Based on this table, it is known that of the 59 water samples taken from respondents' homes, 6 (10.2%) of the samples were positive for *Cryptosporidium* sp. oocysts. Figure 2 shows several images of Cryptosporidium sp. oocyst formations were identified from respondents' water samples. The image of *Cryptosporidium* sp. oocysts that do not appear uniform can be caused by several factors, one of which is the technical painting process. Splashing too much with water or paint that has been stored for a long time so that the quality becomes poor can affect the formation of found oocysts. *Cryptosporidium* sp. oocysts are identified based on size, morphology, and type of staining used. Size and morphology are very important for determination because, with the MZN staining technique, *Cryptosporidium* sp. oocysts are not the only parasites colored red. Parasites also colored red with MZN staining are *Cyclospora cayetanensis* and *Cystoisospora belli* (WHO, 1994). Apart from that, other parasites such as Entamoeba sp. and Giardia sp. in the form of cysts or trophozoites can also be identified using MZN staining, but these parasites are colored blue or greenish blue. In this study, researchers also identified parasites as mentioned above, namely *Cyclospora cayetanensis* and *Entamoeba* sp. as shown in Figure 3 and Figure 4.

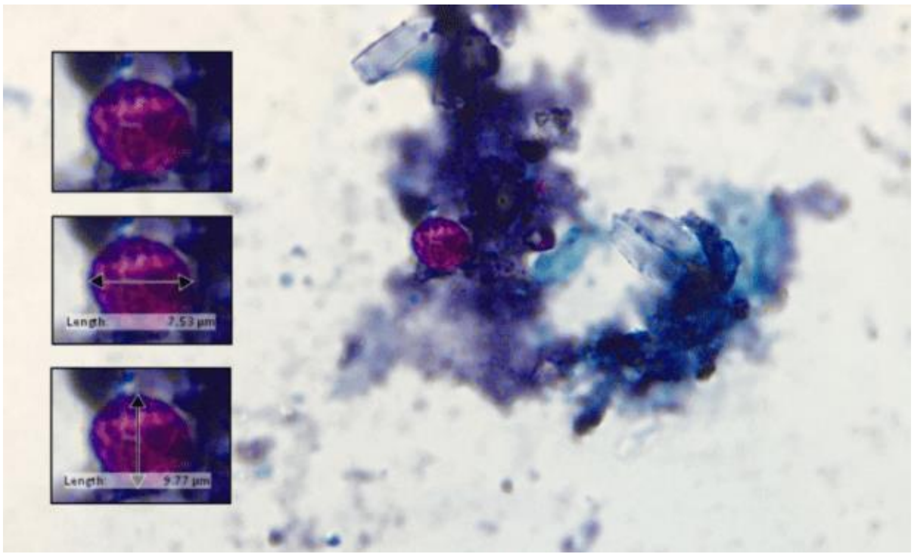

Figure 3. *Cyclospora cayetanensis* oocysts in Water Samples with MZN Staining and 100x Objective Lens Magnification

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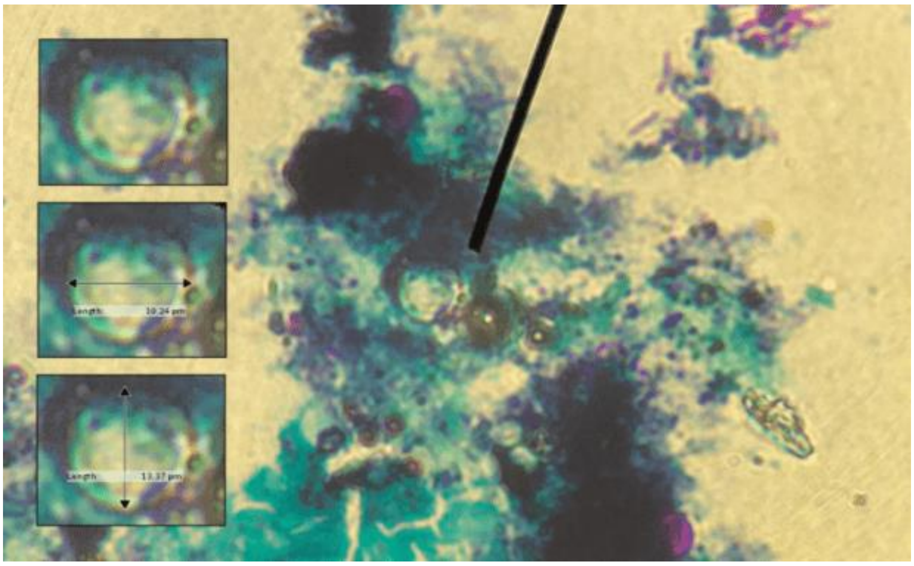

Figure 4. *Entamoeba* sp. cysts in Water Samples with MZN Staining and 100x Objective Lens Magnification

If we look at it at a glance and compare Figure 2 and Figure 3, morphologically there is not much difference. Therefore, distinguishing between the two formations is based on their size. The difference in the size of the oocysts of *Cryptosporidium* sp., *Cyclospora cayetanensis,* and *Cystoisospora belli* is actually not very significant, so we need to be careful in estimating their size. The size of *Cryptosporidium* sp. oocysts ranges from 4-6 µm. Meanwhile, the size of *Cyclospora cayetanensis* ranges from 7.5-10 µm or 8-10 µm, and the size of *Cystoisospora belli* ranges from 20-33 µm x 10-19 µm with an oval shape (WHO, 1994; CDC, 2016, 2017, 2019).

The distribution of *Cryptosporidium* sp. oocyst contamination in clean water sources identified in this study is shown by the map in Figure 1. In the map image, six sample points were positive for *Cryptosporidium* sp. oocysts and are marked in red. If we look again, five sample points are in close or neighboring locations. This shows that there is a possibility that if there is contamination in a water source, the surrounding water sources will also be at risk of becoming contaminated. Contamination mechanisms can occur directly or indirectly. Direct spread of contamination can occur through the possibility of runoff or seepage between adjacent water sources.

Meanwhile, the spread of contamination indirectly can occur involving the role of humans and animals. This mechanism can form a continuous cycle. Using water sources contaminated by *Cryptosporidium* sp. oocysts can put humans or animals who consume them at risk of infection. Suppose this is followed by poor sanitation and hygiene behavior on the part of the individual, such as throwing livestock waste carelessly, defecating carelessly, and not washing hands properly. In that case, the surrounding water sources will also be at risk of being contaminated by *Cryptosporidium* sp. oocysts.

Furthermore, it can be seen again on the map that the source of clean water that was positive for *Cryptosporidium* sp. oocysts was a source of clean water taken from a settlement near a river. Surface water, such as rivers, is more likely to be contaminated by parasites, in this case by *Cryptosporidium* sp. This is because many people still channel household waste or throw animal and human waste along river flows. Apart from that, agricultural irrigation also uses river water and flows it back into the river. Based on observations, the dug well, the main source of clean water the community uses at the research location, has a relatively low depth. The distance between the ground surface and the water surface of the dug well is only around 1-2 meters. The low depth of the dug well allows seepage to occur from the flow around the dug well, both from waste disposal and flow formed during rain or river water. So, there is a possibility that if *Cryptosporidium* sp. oocyst contamination is found in groundwater or dug well water close to a river flow, then it is necessary to suspect that the source of contamination could be from the river flow.

Relationship between the Implementation of CLTS and the Presence of *Cryptosporidium* sp. Oocysts in Clean Water Sources

The results of the Spearman rho statistical test to determine the relationship between the independent variable implementation of CLTS and the dependent variable of the presence of *Cryptosporidium* sp. oocysts in clean water sources in Sukokerto Village, Sukowono District, Jember Regency show a p-value=0.098, there is no association between the implementation of CLTS in a family and the presence of *Cryptosporidium* sp. oocysts in the clean water source used by the family.

Not many studies directly link the application of CLTS to the presence of parasites, especially *Cryptosporidium* sp. oocysts, in clean water sources. Several factors, including natural and human factors, influence the presence of *Cryptosporidium* sp. oocysts in a clean water source. The natural factors include climate, weather, and geographic conditions (Lake et al., 2005; Sterk et al., 2016). Meanwhile, human factors include contamination from agricultural waste, animal waste, human waste, and household waste disposal that is not managed properly (Branco, Leal, & Franco, 2012; Toledo et al., 2017). Agricultural waste, such as from irrigation flows, can affect the quality of surrounding water sources. This relates to the possibility of using manure as plant fertilizer. So, suppose the manure used contains *Cryptosporidium* sp oocysts and is carried by the flow from the irrigation process or rain to nearby water sources. In that case, it can cause the possibility of *Cryptosporidium* sp oocyst contamination in the water source.

Indirectly, this is also related to livestock manure management. In this study, no respondents managed livestock manure for plant fertilizer. However, almost all respondents who have livestock dispose of their livestock waste by dumping it in an area, especially plantation areas or rice fields. Even though it does not directly contaminate the dug wells, which are the main source of water used by respondents in this study, the disposal of livestock waste can risk becoming a source of contamination for surrounding water sources, such as river water. Considering that in this research, quite a lot of people in Sukokerto Village, Sukowono District, Jember Regency still use river water, namely 20 (33.9%) respondents.

In this study, none of the families in the category of poor CLTS implementation were found to have *Cryptosporidium* sp. oocysts in the clean water sources used. This indicates that there may be other factors that influence these results. The water samples taken from the dug well were drawn directly, flowing through a tap. However, none of the water samples were positive for *Cryptosporidium* sp. oocysts. If described based on respondents' answers in the category of poor implementation of CLTS, all these families do not have latrines and defecate in rivers or ditches. This means that the dug well water has no risk of contamination from the septic tank. However, the possibility of contamination still exists because most of these families use dug well water and river water for their daily needs. River water and other surface water have a higher risk of *Cryptosporidium* sp. oocyst contamination than groundwater, so if after doing activities or defecating in the river, they don't wash their hands properly and correctly, the risk of being infected with *Cryptosporidium* sp. or other parasites will be greater and will also be at risk. become a source of contamination for water sources in the house. However, in this study, all families in the category of poor implementation of CLTS answered that the respondents had hand-washing facilities and washed their hands with soap. Respondents also know when it is important to wash their hands, especially after defecating in the river. Respondents added that they had washed their hands again when they got home. This shows that washing hands can break the chain of disease transmission, especially diseases caused by protozoan parasites.

Furthermore, 6 clean water samples positive for *Cryptosporidium* sp. oocysts were from homes with moderate and good CLTS implementation categories. A total of 4 positive water samples were obtained from families with moderate CLTS implementation and 2 others were obtained from families with good CLTS implementation. If described based on the sampling location, conditions at the time of sampling, and the respondent's answer to each questionnaire question, several factors are likely to influence these results, namely the sampling location and possible contamination due to poor hand washing techniques from the respondent, contamination from the septic tank, and possible contamination from livestock.

The clean water samples that were positive for *Cryptosporidium* sp. oocysts all came from dug well water, but the collection locations differed. One positive sample was obtained from taking dug well water directly, while the other five positive samples were obtained from dug well water stored in the bathroom tub. The discovery of *Cryptosporidium* sp. oocysts in bathroom tub water may have occurred due to poor technical hand washing. Of the five positive samples taken from dug well water, which was stored in the bathroom tub, all respondents had a toilet or latrine, and all family members used the toilet or latrine for defecation. So there is a possibility that if one or all of the family members are positively infected with *Cryptosporidium* sp. and after defecating, they do not wash their hands properly and correctly, then accidentally dip their hands into the bathroom tub, then this can contaminate the water in the bathroom tub. Even though the water in the bathroom tub is not used directly for drinking, it is used for gargling, washing their face, and bathing, which can be swallowed. So it is important to drain and clean the bathroom tub regularly and periodically.

Furthermore, one positive water sample was obtained from direct dug well water less than 10 meters from the feces dump. The distance between feces disposal and dug wells is important because feces disposal is a source of groundwater pollution. Dug well water is groundwater that can come from rainwater, which seeps into the soil layers, enters the unsaturated zone, and then seeps deeper into the saturated zone to become groundwater. Apart from the possibility of contamination from fecal disposal, such as septic tank leaks, the use of culverts, which are basically in direct contact with the ground, water pollution from dug wells can also come from seepage or runoff from household liquid waste disposal (drainage) channels that do not meet the requirements, as well as water flow. livestock and agricultural waste (Chibuogwu and Eze, 2015; Deby et al., 2016). So, to minimize the risk of seepage from these sources of pollution, the recommended minimum distance between dug wells and sources of pollution such as cesspools, septic tanks, rubbish dumps, etc., must be more than 10 meters (Kementerian PUPR, 2016)

In addition, four positive water samples for *Cryptosporidium* sp. oocysts came from families with livestock, such as chickens and cows. Meanwhile, the other two samples did not have livestock. It is necessary to suspect that the possibility of contamination comes from cage sanitation or poor sanitation hygiene behavior of livestock keepers. Remember previous research by Rinanto (2017), which stated a relationship between cage sanitation and the incidence of intestinal protozoan infections, in this case *Cryptosporidium* sp., in livestock animals. Suppose the sanitation of the pen is poor, so livestock are susceptible to *Cryptosporidium* sp. infection, and the sanitary hygiene behavior of the livestock owner is also poor. In that case, this can cause the livestock owner to be susceptible to infection, and water sources around the pen or places where livestock waste is disposed of are also susceptible to being contaminated with *Cryptosporidium* sp. oocysts.

CONCLUSION

Based on preliminary investigation, it can be concluded that the implementation of sanitation in livestock areas, Sukowono District, Jember Regency is mostly still in the moderate and poor categories. There is contamination of *Cryptosporidium* sp. oocysts in clean water sources. The main source of contaminated clean water came from dug well water. There is no association between implementing sanitation and contaminating *Cryptosporidium* sp. oocysts in clean water sources in Sukowono District, Jember Regency.

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