Risk Factor Analysis of *Cryptosporidium* sp. Contamination in Dairy Cow Milk in Jember, Indonesia

Begawan Sa'bani[1], Bagus Hermansyah[2]*, Kristianningrum Dian Sofiana[3], Yunita Armiyanti[2], Wiwien Sugih Utami[2]

[1]Medical Education, Faculty of Medicine, University of Jember, Jember, Indonesia, [2]Department of Parasitology, Faculty of Medicine, University of Jember, Jember, Indonesia, [3]Department of Physiology, Faculty of Medicine, University of Jember, Jember, Indonesia.
*Corresponding author: bagus_hermansyah@unej.ac.id

Abstract

This study aimed to determine the factors influencing *Cryptosporidium* sp. contamination in dairy cow milk in Jember. This study used an observational analytic design with a cross-sectional approach. The number of samples in this study was 30 cow farmers, using a purposive sampling technique. Data sources were obtained from questionnaire interviews, observation sheets, and laboratory examination results of milk samples. Data were then analyzed using univariate, bivariate, and multivariate analysis. Bivariate analysis used the Kruskal-Wallis comparison test, and multivariate analysis used the logistic regression test. The results showed *Cryptosporidium* sp. contamination in the milk of dairy cows by 6.7%. Most of the cow farmers in Jember had sufficient knowledge (40%), milking hygiene was categorized as sufficient (70%), and equipment sanitation was sufficient (66.7%). The statistical analysis showed no significant relationship between farmers' knowledge and equipment sanitation and *Cryptosporidium* sp. contamination ($p > 0.05$). There was a significant relationship between milk hygiene and *Cryptosporidium* sp. contamination in dairy cow milk in Jember ($p < 0.05$). In conclusion, milking hygiene was a risk factor affecting *Cryptosporidium* sp. contamination in dairy cow milk in Jember.

Keywords: *Cryptosporidium* sp., dairy cow milk, equipment sanitation, farmers' knowledge, milking hygiene

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INTRODUCTION

Milk-borne cryptosporidiosis is a disease caused by consuming milk contaminated with *Cryptosporidium* sp. Globally, milk-borne cryptosporidiosis outbreaks were reported between 1984 and 2017 (Chalmers *et al.*, 2020). Other reports mention that milk-borne cryptosporidiosis outbreaks have occurred in Australia (1983), Mexico (1985), England (1995), Australia (2001), and Germany (2005) (Ahlinder *et al.*, 2022). The main factor causing the outbreak of milk-borne cryptosporidiosis is the consumption of unpasteurized milk or milk that is not standardized pasteurized (Ursini *et al.*, 2020).

Milk is an essential source of nutrients for all age categories (Christi *et al.*, 2019). The largest milk producer in the world comes from dairy cows, which account for 70%. Because of this, the term milk often refers to dairy cows' milk (Susilawati *et al.*, 2021). The high calcium and vitamin D content in cow's milk is essential for growth from toddlerhood to adolescence. Several studies have shown that cow's milk stimulates Insulin-like Growth Factor-1 (IGF-1), which mediates growth hormone (Grenov *et al.*, 2021; Purnama *et al.*, 2021). These crucial benefits are to be considered because toddlers are vulnerable to infection with *Cryptosporidium* sp. (Costa *et al.*, 2020). Symptoms of cryptosporidiosis in infants and young children can progress to persistent chronic diarrhea and increase the risk of growth disorders (Adem *et al.*, 2021; Wijayanti, 2018). Cow's milk can become a growth inhibitor if it is contaminated with *Cryptosporidium* sp.

Some risk factors for milk contamination include farmers' knowledge, milking hygiene, and equipment sanitation. Milking hygiene is a clean milking activity to prevent contamination (Syamsi *et al.*, 2020). Investigation by Yusuf *et al.* (2021) showed that milking hygiene significantly influences milk contamination.

Other factors that can cause milk contamination are poor sanitation and dirty and unsterilized equipment (Ursini *et al*., 2020). Equipment sanitation is the cleanliness of equipment, including the washing process, until the tool is used. Investigation by Rahadyan *et al*. (2023) showed that poor equipment sanitation was positively correlated with milk contamination. A study by Yenew *et al*. (2022) showed that farmers' knowledge is another factor affecting milk contamination. Farmers' knowledge is everything the farmer knows regarding good husbandry management and milking techniques. Good knowledge can encourage the implementation of good behaviors, such as milking hygiene practices and equipment sanitation (Arifin *et al*., 2019; Maulana *et al*., 2021).

Jember is one of the regencies in East Java Province with high dairy milk production. Dairy milk production in Jember, amounting to 2,992.59 tons, is dominated by dairy cow milk, which is 99.64% (BPS Provinsi Jawa Timur, 2019). A preliminary survey reported that some small-scale farms in Jember sell cow's milk products directly to the public without going through pasteurization. There have been no reported cases of milk-borne cryptosporidiosis in Indonesia so far. However, the risk of contamination still exists, so efforts to prevent contamination of cow's milk need to be considered. Study on risk factors for *Cryptosporidium* sp. contamination in dairy cow milk, especially in Jember, is still limited. Based on this description, this study was conducted to investigate the prevalence of *Cryptosporidium* sp. contamination, evaluate the risk factors for dairy cow milk contamination, and know the factors that influence *Cryptosporidium* sp. contamination in dairy cow milk in Jember.

MATERIALS AND METHODS

Ethical Approval

This study has received ethical approval from the Ethics Commission of the Faculty of Medicine, University of Jember, with number 1.752/H25.1.11/KE/2023.

Study Period and Location

This study used an observational analytic design with a cross-sectional approach. This study was conducted from October 2022 until March 2023 at the Parasitology Laboratory of the Faculty of Medicine, University of Jember, and dairy farms in Jember.

Samples

The sample consisted of 30 cow farmers. This study's sampling technique used purposive sampling with inclusion and exclusion criteria. The inclusion criteria consisted of farmers ready to have their data collected by filling out an informed consent form as a sign of agreement to become respondents and farmers willing to have their dairy milk samples taken for examination. The milk sampled was fresh milk from healthy dairy cows. Exclusion criteria consisted of farmers who used milking equipment in milking and farmers who had cows in the dry period or not in the lactation period.

Risk Factor Evaluation

The risk factors studied included farmers' knowledge, milking hygiene, and equipment sanitation. Farmers' knowledge data was measured using a Guttman scale questionnaire, while milking hygiene and equipment sanitation data were measured using a Guttman scale observational sheet. The questionnaire and observational sheet consisted of 10 assessments. Each correct evaluation will be given one point, while the wrong assessment will be zero points.

Data on farmers' knowledge, milking hygiene, and equipment sanitation were then classified into three categories with an ordinal data scale, consisting of good if the score > mean + standard deviation, sufficient if the mean - standard deviation ≥ score ≤ mean + standard deviation, poor if the score < mean - standard deviation. The questionnaire used in this study is a questionnaire that has been tested for validity and reliability. At the same time, the observation sheet is taken from guidelines by the Food and Agriculture Organization (FAO) and previous study (FAO, 2011; Prabandari, 2021).

The milk used as examination samples is fresh milk that has not received any treatment other than the chilling process. Samples were taken from the milk container and put into sterile glass bottles by the cow farmers to avoid contamination by the researcher's hands. Sterile glass bottles were labeled and put into clean and closed containers during transportation. Laboratory examination of dairy cow milk samples used the sedimentation method with Modified Ziehl-Neelsen (MZN) staining. Observation of *Cryptosporidium* sp. oocyst morphology using a light microscope with 1000× magnification. Data from the examination of milk contamination were classified into two categories, positive and negative, with a nominal data scale.

Data Analysis

The data were analyzed using the SPSS version 24 program and presented as tables and narratives. Data were analyzed using univariate, bivariate, and multivariate analysis. Univariate analysis was conducted on each variable to describe the characteristics and evaluated the distribution of each study variable. Bivariate analysis used Cramer's V correlation test if the test conditions were met or the Kruskal-Wallis comparison test if the test conditions were not. Data were then analyzed multivariate using a logistic regression test if there were at least two independent variables with a calculation value of $p < 0.25$ in bivariate analysis (Dahlan, 2020).

RESULTS AND DISCUSSION

Distribution of Respondent Characteristics

Respondent characteristics included gender, age, education level, and working period. The distribution of cow farmers' features in Jember can be seen in Table 1. The most cow farmers in the Jember are male, with 27 farmers (90%). The age distribution varies, with most aged 31–40 years (30%) and 41–50 years (30%). The youngest cow farmer is 19 years old, while the oldest is 62 years old. All cow farmers are in the productive age range of 15–65 years (Kementerian Kesehatan Republik Indonesia, 2019). Most of the farmers had an education level between elementary school (36.7%) and high school (36.7%). Arikunto (2021) categorizes education at the primary to junior high school level as low education, while education at the senior high school to university level is high education. Based on this category, 63.3% of cow farmers in this study still have an education up to junior high school level, or the majority are still classified as low. Based on the working period, most farmers had worked for 1–10 years (46.7%).

Distribution of Farmers' Knowledge

The results showed that most cow farmers had sufficient knowledge (40%), followed by the good (30%) and poor (30%) categories (Table 2). This result aligns with investigation by Pratiwi *et al*. (2016), who reported that most cow farmers in Cipageran Village, North Cimahi District, Cimahi City, had sufficient knowledge (66.67%). Learning can be influenced by a person's level of education and age (Kevin *et al*., 2021). Education encourages a person to obtain more information to understand something better. A higher level of education is expected to increase someone's knowledge. Investigation by Suolaniemi *et al*. (2022) showed that cow farmers aged < 40 years had a better understanding than farmers aged ≥ 40 years. This result may be because older farmers are more closed to new information and slower to implement innovations, so they tend to milk in the traditional way they have received from generation to generation (Komala *et al*., 2022). In this study, most farmers had a low level of education and were ≥ 40 years old, which caused most farmers to lack sufficient knowledge.

Distribution of Milking Hygiene

The data reported that 70% of farmers had sufficient milking hygiene, 16.7% had good hygiene, and 13.3% had poor hygiene (Table 2). These findings align with study by Ahmed *et al*. (2020), which reported that 60% of cow farmers had sufficient milking hygiene, 21.6% were good, and 18.3% were poor. Different results were demonstrated by Yenew *et al*. (2022), who found that 56.7% of farmers had poor milking hygiene, 32% were sufficient, and 11.3% were good. Working periods and education levels can influence milking hygiene practices. The work experience of cow farmers can increase efficiency in milking. Those are important because efficient milking can reduce contact with the farm environment and reduce the risk of milk contamination (Yenew *et al*., 2022). Education can influence milking hygiene practices through the knowledge gained. Good knowledge can encourage the implementation of good behavior, such as milking hygiene practices (Septiyani *et al*., 2021). Most of the cow farmers in this study had a relatively brief working period of 1–10 years and a low level of education, which caused the milking hygiene applied to be mostly classified as sufficient.

Distribution of Equipment Sanitation

This study showed that 66.7% of cow farmers had sufficient sanitation equipment, 20% were good, and 13.3% were poor (Table 2). This result aligns with study by Komala *et al*. (2022), which reported that most cow farmers in Cijeruk Bogor had sufficient sanitation equipment. The level of education and working period can influence the application of equipment sanitation. Similar to hygiene, good knowledge can encourage the application of good sanitation, and the working period can improve the application of sanitation through the experience gained over time (Nurfikrizd and Rustiawan, 2020; Septiyani *et al*., 2021). Through interviews by the researcher, farmers knew that the cow's udder should be dried with a clean cloth immediately after bathing the cow so that no water drips on the milk. This was because some farmers received feedback from buyers regarding the change in milk flavor from usual. After analysis, the farmers learned that the remaining water when bathing the cows should not drip on the milk as it can affect the taste of the milk. This indicated that work experience could improve farmers' hygiene and sanitation. Most of the cow farmers in this study had a low level of education and a relatively brief working period of 1–10 years, which causes the equipment sanitation applied by the majority to be classified as sufficient.

Correlation Between Farmers' Knowledge and *Cryptosporidium* sp. Contamination

There was one cell with an actual count of 0, namely in the category of poor farmers' knowledge with positive contamination, and there were three cells (50%) with an expected count value < 5. The analysis yielded a significance value of 0.622 ($p > 0.05$) (Table 3).

The bivariate analysis showed no significant ($p > 0.05$) relationship between farmers' knowledge and *Cryptosporidium* sp. contamination in dairy cows' milk. This result aligns with study by Aliyo and Teklemariam (2022), which mentioned no significant relationship between knowledge and milk contamination. The data in this study showed two positive *Cryptosporidium* sp. contaminations came from cow farmers with sufficient knowledge. The absence of a relationship between knowledge and milking hygiene practices can cause this. Investigation by Maulana *et al*. (2021) showed no association between cow farmers' knowledge and hygiene practices. Another study by Hartini (2022) also showed no significant relationship between knowledge and sanitary hygiene practices, but there was a relationship between the attitudes and hygienic hygiene practices of food handlers. This could be because there are two kinds of knowledge.

Knowledge is divided into implicit knowledge and explicit knowledge. Tacit knowledge is subjective and usually only based on experience. Implicit knowledge forms someone's principles, beliefs, and attitudes toward something. In this study, all farmers knew milk collection containers should be washed before milking, but only 25 farmers practiced this. This shows that five farmers knew the importance of cleaning the equipment before use, but due to different attitudes, they did not implement their knowledge. Although the results showed an insignificant relationship between farmers' knowledge and milk contamination, farmers are expected to have good knowledge because the basis of practicing good hygiene and sanitation is having knowledge related to hygiene and sanitation.

Table 1. Distribution of respondent characteristics

Respondent Characteristics	Number (n)	Percentage (%)
Gender		
a. Male	27	90
b. Female	3	10
Total	30	100
Age		
a. 16–20 years old	1	3.3
b. 21–30 years old	4	13.3
c. 31–40 years old	9	30
d. 41–50 years old	9	30
e. 51–60 years old	6	20
f. 61–65 years old	1	3.3
Total	30	100
Education Level		
a. Not attending school	5	16.7
b. Elementary School / Equivalent	11	36.7
c. Junior High School / Equivalent	3	10
d. High School / Equivalent	11	36.7
Total	30	100
Working Period		
a. <1 year	1	3.3
b. 1–10 years	14	46.7
c. 11–20 years	6	20
d. 21–30 years	4	13.3
e. 31–40 years	4	13.3
f. 41–50 years	1	3.3
Total	30	100

Table 2. Distribution of farmers' knowledge, milking hygiene, equipment sanitation, and *Cryptosporidium sp.* contamination

Variables	Number (n)	Percentage (%)
Farmers' Knowledge		
Good	9	30
Sufficient	12	40
Poor	9	30
Total	30	100
Milking Hygiene		
Good	5	16.7
Sufficient	21	70
Poor	4	13.3
Total	30	100
Equipment Sanitation		
Good	6	20
Sufficient	20	66.7
Poor	4	13.3
Total	30	100
***Cryptosporidium sp.* Contamination**		
Positive	2	6.7
Negative	28	93.3
Total	30	100

Table 3. Analysis of the correlation between farmers' knowledge, milking hygiene, equipment sanitation, and *Cryptosporidium sp.* contamination

	***Cryptosporidium sp.* Contamination**				**Total**	***p-value***
	Negative		**Positive**			
	Number (n)	**Percentage (%)**	**Number (n)**	**Percentage (%)**		
Farmers' Knowledge						
Good	8	28.6	1	50	9	
Sufficient	11	39.3	1	50	12	0.622
Poor	9	32.1	0	0	9	
Total	28	100	2	100	30	
Milking Hygiene						
Good	4	14.3	0	0	4	
Sufficient	21	75	0	0	21	0.006*
Poor	3	10.7	2	100	5	
Total	28	100	2	100	30	
Equipment Sanitation						
Good	4	14.3	0	0	4	
Sufficient	19	67.9	1	50	20	0.523
Poor	5	17.9	1	50	6	
Total	28	100	2	100	30	

Correlation Between Equipment Sanitation, Milking Hygiene, and *Cryptosporidium* sp. Contamination

There were two cells with an actual count of 0, namely in the categories of good milking hygiene with positive contamination and sufficient milking hygiene with positive contamination. In addition, there were five cells (83.3%) with an expected count value < 5. The significance value obtained was 0.006 ($p < 0.05$) (Table 3).

There was one cell with an actual count of 0, namely in the category of good equipment sanitation with positive contamination, and there were four cells (66.7%) with an expected count value < 5. The resulting significance value was 0.523 ($p > 0.05$) (Table 3).

The analysis results in Table 3 show no significant ($p > 0.05$) relationship between equipment sanitation and *Cryptosporidium* sp. contamination in dairy cow milk. These results align with study by Kurniawan *et al*. (2018), which showed no relationship between equipment sanitation and milk contamination. Different results were demonstrated by Suwito *et al*. (2018), mentioning the relationship between equipment sanitation and milk contamination with an odds ratio value of 3. This data reported two *Cryptosporidium* sp. contaminations in milk came from farmers with sufficient and poor sanitation equipment. The data suggest that good equipment sanitation can prevent milk contamination. The non-significant results may be due to milk contamination also being influenced by other factors, such as water source, environmental humidity, and milk temperature and pH.

The water source, environmental humidity, milk temperature, and pH were not measured or investigated in this study. Liquid cow feces containing *Cryptosporidium* sp. oocysts can seep through the soil and contaminate water sources (Ramadhani *et al*., 2022). Water is used on cattle farms to clean cows' udders, teats, and wash equipment. Clean and uncontaminated water can reduce the risk of milk contamination (Pradika *et al*., 2019). Other factors that may lead to insignificant results are the shape of the container surface and the number of oocysts attached to the milk container before it is used for milking. A small number of oocysts may cause a false negative because the average volume of milk collection containers used by cow farmers ranges from 20–40 liters, while the milk sample taken is about 50 mL. The surface shape can also affect oocyst attachment to the milk container. Most milk containers used by cow farmers are made from stainless steel. Stainless steel containers are easier to clean and non-porous, minimizing oocyst attachment (Aritonang, 2017). Although the results showed no association between equipment sanitation and milk contamination, farmers are expected to continue implementing good equipment sanitation because the risk of contamination from milk containers still exists.

CONCLUSION

The results found that the prevalence of *Cryptosporidium* sp. contamination in milk from dairy cows in Jember was 6.7%. Most of the farmers in Jember had sufficient knowledge (40%), sufficient milking hygiene (70%), and sufficient equipment sanitation (66.7%). Of the three risk factors for contamination studied, milk hygiene is a risk factor that affects *Cryptosporidium* sp. contamination in dairy cow milk in Jember. People who buy fresh, unpasteurized cow's milk are suggested to heat it at a minimum temperature of 71.5°C for 15 seconds to eliminate *Cryptosporidium* sp. oocysts.

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AUTHORS' CONTRIBUTIONS

BH: Conceptualization and drafted the manuscript. BH, YA, and WSU: Validation, supervision, and formal analysis. BS, BH, KDS, YA, and WSU: Performed sample evaluation. BH, YA, and WSU: Performed the statistical analysis and the preparation of tables. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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