Impact of milking hygiene on *salmonella* contamination in cow's milk: a study in Jember Regency

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Abstract. Milk is a liquid produced by the mammary glands of female mammals. The nutritional content of milk almost covers the entire nutritional needs required by living things. The appearance of *Salmonella* sp. in cow's milk is due to milking methods that do not meet the standards, especially regarding milking hygiene management. This study examines the relationship between milking hygiene and *Salmonella* sp. contamination in cow's milk in Jember Regency.This study used observational analytic research with a cross-sectional research design. Primary data were obtained through direct observation of milkers using a checklist related to milking. observe *Salmonella* sp. contamination using culture techniques on SCA (Salmonella Chromogenic Agar) as selective media agar for *Salmonella* sp. and Gram staining. The results of this study show that the hygiene process pre-milking (78.9%), during-milking (71.1%), and post-milking (89.5%) have been implemented. The statistical test results showed a relationship between the pre-milking hygiene process with *Salmonella* sp. contamination (p-value < 0.05), but insignificant results were seen in the during and post-milking hygiene processes (p-value> 0.05). The conclusion from this study showed that of the three results only the hygiene processes pre-milking have results associated with *Salmonella* sp. contamination in cows' milk.

1 Introduction

The need for animal protein in Indonesian society is always increasing from year to year along with the increasing population and the level of awareness of nutritional needs. As one of the products with the highest protein content, milk is chosen because it has a low price and is easily available. Milk is a liquid produced by the mammary glands of female mammals. The nutritional content of milk almost covers the entire nutritional needs required by living things [1]. Among the many milk-producing mammals, cow's milk is still the main choice for consumption. According to data from the Badan Pusat Statistik, in 2021 dairy milk production

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in Indonesia reached 107 thousand liters [2]. Along with the high rate of cow's milk consumption per year, cow's milk has the potential to cause foodborne disease. Foodborne disease is a disease caused by consuming food or drinks that have been contaminated by biological agents such as microorganisms or chemical agents. This is supported by the content of protein, glucose, lipids, mineral salts, and vitamins at a pH of around 6.80 so that it can support microorganisms to grow rapidly [3,4]. Bacteria that usually contaminate cow's milk include *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp. and *Listeria monocytogens*. Milk provided for human beings should not contain any pathogenic microorganisms. Based on the National Standardization Agency on the Maximum Limit of Microbial Contaminants and the Maximum Limit of Residues in Foodstuffs of Animal Origin, the limit of microorganism contamination in fresh milk is Total Plate Count (TPC) 1 x 10^6 CFU/mL, coliform 2 x 10^1 CFU/mL, and negative for *Salmonella* sp [5].

Salmonella sp. is a bacillus-shaped facultative aerobic and anaerobic bacterium that causes gastroenteritis, septicemia, and enteric fever [6,7]. *Salmonella* comprises two species, *Salmonella bongori* and *Salmonella enterica*. Depending on the bacterial outer membrane somatic 'O' antigen, and flagellar 'H' antigen over 2700 different serovars of *Salmonella* have been characterized. Out of these 2700 serovars, nearly 1500 belong to the *Salmonella enterica subsp*. *Salmonella* sp. bacteria enter through the oral-fecal route with a medium of transmission in the form of contaminated food and drink. Infection from *Salmonella* sp. can cause Salmonellosis with symptoms such as acute fever, nausea, vomiting, and diarrhea [8]. Salmonellosis is a medical term for a disease caused by *Salmonella* sp. infection, both by *Salmonella* typhoid and *Salmonella* non-typhoid. Specifically, *Salmonella* typhoid can cause typhoid fever which is estimated to cause around thirteen million deaths in Asia each year. Meanwhile, typhoid disease patients in Indonesia reach 810 per 100,000 population each year [9].

The appearance of *Salmonella* sp. in cow's milk is due to milking methods that do not meet the standards, especially regarding milking hygiene management [10]. The contamination of raw milk by pathogenic microorganisms including Salmonella comes from feces of infected cattle, contaminated skin, infected udder, contaminated milking equipment, air, feed, and water, and from milkers. Milking hygiene is a preventive effort carried out by cow milkers to maintain the safety of dairy products from pre-, during, and post-milking sources of contamination [11]. Many milkers have not implemented milking hygiene to prevent contamination of milk. Lack of awareness of milking hygiene can be influenced by various factors such as the level of knowledge and awareness of the milking method. East Java Province has the largest dairy cattle population when compared to other provinces in Indonesia. In 2021, the total dairy cattle population in East Java reached 301,780 heads with Jember Regency as one of the contributors to quite a lot of dairy cows [2]. The government has implemented strict rules for milkers in processing milk for both traditional and modern milkers. This study aims to also see the level of compliance of milkers. Based on the description of the background above, it is necessary to conduct research on the relationship between milking hygiene and *Salmonella* sp. contamination on cow's milk in Jember Regency.

2 Materials and Methods

This study used observational analytic research with a cross-sectional research design. The research was conducted in August 2022 - February 2023 in Jember Regency.

2.1 Participants

The population used in this study included all cow milkers in Jember Regency with a total of 38 milkers spread across 10 sub-districts. The sample used included all cow milkers and their milking products. The sampling technique used was total sampling because the population was relatively small [12]. We excluded cow milkers that used modern techniques

2.2 Data collection tools

Primary data were obtained through direct observation of cow milkers in Jember using a checklist related to milking hygiene. The inclusion criteria applied in this study include cow milkers on dairy farms in Jember Regency who are willing to become research subjects without coercion and have been given an explanation of the flow of research by signing informed consent, cow milkers who use manual milking techniques without any tools, cow's milk that is still fresh and not stored for more than 24 hours, cow's milk obtained from healthy cows. Utmost efforts were made to prevent contamination and cross-contamination of milk in the course of sample collection. Nearly 10 ml of raw milk samples were collected into sterile screw-capped bottles that had been incubated. The milk samples were then held in an icebox with ice packs and transported to the Laboratory. Examination of cow milk samples was carried out at the Microbiology Laboratory of the Faculty of Medicine, University of Jember to observe *Salmonella* sp. contamination using culture techniques on SCA (*Salmonella Chromogenic Agar*) as selective media agar for *Salmonella* sp. and Gram staining.

2.3 Data evaluation and statistical analysis

The data obtained were analyzed using the IBM SPSS Statistic 25 application with Fisher's Exact test**.**

3 Results and Discussion

Sample Characteristics

A total of 38 samples in the form of observation results regarding milking hygiene behavior and cow's milk were obtained and further examined through a series of methods. This study shows that the gender of cow milkers in Jember Regency is more male (92.1%) than female (7.9%) as shown in Table 1. On the other hand, this finding is in line with research by Ahmed in 2020 in Southwest Delhi, India where all respondents used the manual milking process (detailed description of the milking process can be seen in the Table.3). According to Ahmed, the process of milking and searching for animal feed in a manual or traditional way requires more energy so that it is dominated by male milkers [13]. The grouping of milking characteristics is divided based on productive age (15-65 years) and unproductive age (>65 years) [14]. An overview of the characteristics of milking by age shows that cow milking in Jember Regency is at most between15-65 years as much as 97.4% with an average age of 41 years. This finding is in line with research by Nyokabi in 2021 in Central Kenya which showed that the majority of milkers in the city were between 30 and 60 years old [15]. In the age range of 15-65 years, a person can still carry out daily activities well and does not have degenerative complaints, therefore more than 90% of milkers are still at a productive age.

Table 1. Sample characteristics of cow milkers

Sample characteristics		Total	
		n	%
Gender			
a.	Male	35	92.1
b.	Female	3	7.9
Age (years)			
a.	15-65 years	37	97.4
b.	>65 years	1	2.6
Last education			
a.	Did not attend school	5	13.2
b.	Elementary school	10	263
c.	Junior high school	5	13.2
d.	Senior high school/vocational school	18	47.4
Length of employment			
a.	≤ 10 years	22	57.9
b.	>10 years	16	42.1
Total		38	**100**

The last education taken by milkers in Jember was dominated by senior high school/vocational school graduates as many as 18 milkers (47.4%). Milkers who take education up to senior high school/vocational school are categorized as having a good education because the Indonesian government has established a 12-year compulsory education program or up to high school/vocational school. The results of this study are not in line with research by Maulana in 2021 which found that cow milkers in Sleman Regency have a higher proportion of milkers with lower education levels [10]. Education affects the insight of a milker regarding the hygiene aspects in the milking process, so that the higher the education taken by a milker can increase her awareness to implement it in the milking process. Milker's actions taken from hereditary knowledge are the basis for the limited knowledge of milkers in theory, especially regarding milking hygiene [16].

Most of the milkers in Jember Regency had working experience below 10 years (57.9%). These results indicate that the majority of milkers in Jember Regency do not have long enough work experience. The findings in this study are inversely proportional to research by Maulana which shows that there are more cow milkers with more than 10 years of work experience with 38 out of 40 milkers in Sleman Regency [10]. Based on interviews with several milkers in Jember Regency, the large number of new milkers is due to the increased demand for cow's milk during the pandemic, making it possible to be used as a new business opportunity to increase income.

Milking Hygiene

Milking hygiene is a series of efforts to minimize bacterial contamination from the environment into milk through milking actions which include preparation, milking process, and post-milking [17]. This study examines three milking hygiene processes which include the hygiene process pre, during, and post-milking. According to Suranindyah, improving hygiene treatment before and after milking can affect the level of acidity and the number of bacteria contaminated in milk [18]. The results obtained from this study show that cow milkers in Jember Regency have

implemented the hygiene process that is shown in the hygiene process pre-milking (78.9%), during milking (71.1%), and post-milking (89.5%) as shown in Table 2. The determination indicator in this study is using the median value of the total questions on each observation sheet which is divided into three groups: pre, during, and post-milking hygiene process. Good results in milking hygiene indicate that milkers already understand ways to prevent contamination of their milk products. The good results in this study were influenced by the fact that most milkers in Jember District have experience forming cooperatives that work with multinational dairy companies. This finding is not in line with research by Shanta et al. (2021) in Baghabarighat, Bangladesh found that the practice of milking hygiene in that location was far from reaching the standard. The poor milking hygiene in Baghabarighat is caused by the milkers lack of understanding of milking hygiene that can contaminate cow's milk so they do not apply hygiene practices to the process [19].

Although this study found that many milking hygiene processes have been carried out, there are still some actions that are rarely (10.5%) considered by milkers such as the use of PPE (masks and gloves) when milking, the use of vaseline as a lubricant when milking, and teat dipping treatment. In animal husbandry, the use of PPE such as masks and gloves can prevent cross-contamination between livestock and farmers. Milking hygiene will be closely related to the contamination of cow's milk, especially Salmonella sp. Hand hygiene, clothing, nails, hair, infectious diseases in milkers, cleanliness of equipment, milking techniques, smoking while milking, cleanliness of cows and stalls, and milk filtration methods all affect the appearance of *Salmonella* sp. in cow's milk [17].

Table 2. Frequency distribution of milking hygiene process and *Salmonella* sp. contamination**.**

Description	n	Total	%
Pre-milking hygiene process	Good	30	78.9
	Bad	8	21.1
During milking hygiene process	Good	27	71.1
	Bad	11	28.9
Post-milking hygiene process	Good	34	89.5
	Bad	4	10.5
Salmonella sp. contamination in cow's milk	Positive	23	60.5
	Negative	15	39.5

Salmonella sp. Contamination

Based on Table 2, the results of the examination of cow's milk showed that 60.5% of the samples contained *Salmonella* sp. contamination from a total of 38 samples examined. How to determine *Salmonella* sp contamination is obtained using the culture technique on SCA media which produces a light purple color [20]. After culture was carried out on SCA media, continued by microscopic confirmation examination with the characteristics of having a rod shape and reddish color.

Referring to the National Standardization Agency regarding the Maximum Limit Standard for Microbial Contamination in Food SNI 7388 : 2009, especially dairy products, Salmonella

sp. contamination must be negative or no bacteria are found [21]. These findings indicate that unprocessed fresh milk in Jember District is contaminated with *Salmonella* sp. bacteria, making

Table 3. Observation data checklist

Checklist point	Result			
	Yes		No	
	N	%	N	%
Pre-milking hygiene process				
Cleaning the cage thoroughly	32	84.2	6	15.8
Wearing clean special clothes	29	76.3	9	23.7
Hand washing with clean water and soap/disinfectant before milking	29	76.3	9	23.7
Milker's nails are not long (not exceeding the milker's skin) and dirty	27	71.1	11	28.9
Milker are free from infectious diseases Example: cough, runny nose, sneezing	33	86.8	5	13.2
No open wounds	35	92.1	3	7.9
Bathing the cows before milking	34	89.5	4	10.5
Milker wears a mask and gloves	4	10.5	34	89.5
Milker uses a head cover	24	63.2	14	36.8
Using a stainless-steel container	28	73.7	10	26.3
Wash equipment before milking with water and soap/disinfectant	32	84.2	6	15.8
During milking hygiene process				
Cleaning the cow's udder and teats	31	81.6	7	18.4
Drying the udder with a clean dry cloth	12	31.6	26	68.4
Milker does not smoke during milking	31	81.6	7	18.4
Do not use butter or oil as a lubricant during milking (use vaseline)	5	13.2	33	86.8
Tie the cow's tail during milking	12	31.6	26	68.4
Milking until the end	36	94.7	2	5.3
Discarding the first drop of cow's milk	30	78.9	8	21.1
Post-milking hygiene process				
Filtering after milking	36	94.7	2	5.3
Re-cleaning the cow's udder and teats	28	73.7	10	26.3
Performing teat dipping	14	36.8	24	63.2
Wash equipment that has been used using soap or detergent then rinsed clean and dried	36	94.7	2	5.3
The used rag is washed thoroughly	21	55.3	17	44.7
Put milking equipment in a special place free from sources of pollution	28	73.7	10	26.3

it unsafe for direct consumption. This result is not in accordance with research by Kusumaningsih et al. (2013) and Satria et al. (2021) who obtained negative results in the examination of Salmonella sp. contamination in all samples [22,23]. Milk contaminated with Salmonella sp. was found in a study by Castañeda-Salaza et al. (2021) which showed contamination in 23 samples (20.5%) of 112 samples studied at Sabana in Bogota [24]. In general, cow's milk has a very high nutritional content, making it easier for various bacteria, especially Salmonella, to grow [3]. Salmonella contamination in cow's milk can occur due to

poor milking hygiene levels in milkers [17]. Milker who have poor milking hygiene will cause *Salmonella* sp. attached to the outside of the body such as skin, nails, hair, and clothing cannot be cleaned optimally, thus facilitating the transmission of bacteria into cow's milk. The incidence of *Salmonella* sp. contamination found can also be influenced by the contact of cow's milk to the surrounding environment such as the cleanliness of the cage, the cleanliness of the cow's udder and teats, the cleanliness of the equipment (collection containers and filtering tools) [25]. Lack of environmental cleanliness can be caused by poor milking hygiene habits such as not cleaning the barn before milking, not bathing the cows, leaving dirty equipment without proper cleaning and drying, and the location of the barn mixed with equipment storage.

Relationship between Milking Hygiene Process and *Salmonella* sp. Contamination

This study examined three milking hygiene processes which include hygiene processes pre, during, and post-milking. So the three processes were each analyzed bivariately using Fisher's Exact test as shown in Table 4. The statistical test results showed a relationship between the pre-milking hygiene process with *Salmonella* sp. contamination (p-value < 0.05), but insignificant results were seen in the during milking and post-milking hygiene process (p-value > 0.05) which showed no relationship with *Salmonella* sp. contamination. The results of the study are not fully in accordance with research by Yusuf et al, (2021) and Solechah et al. (2017) which state that the better the milking hygiene behavior, the lower the level of bacterial contamination [26].

Table 4. Fisher's Exact test results between pre, during, and post-milking hygiene processes and *Salmonella* sp. contamination in cow's milk in Jember District

Milking hygiene		*Salmonella* contamination in cow				Total	Approx. Sig.
		Positive		Negative			
		N	%	N	%	N	
Pre-milking hygiene process							
a.	Good	15	50	15	50	30	.013
b.	Bad	8	100	0	0	8	
During-milking hygiene process							
a.	Good	14	51,9	13	48,1	27	.145
b.	Bad	9	81,8	2	18,1	11	
Post-milking hygiene process							
a.	Good	19	55,9	15	44,1	34	.138
b.	Bad	4	100	0	0	4	

Insignificant association between the hygiene process during and after milking with *Salmonella* sp. contamination in cow's milk can be caused by other factors that arise during the process such as cage sanitation. As in research by Permatasari et al. (2018) which says that the cleanliness of the cage, the entry of sunlight, air circulation, manure storage, the presence of ditches and drainage, to the direction of the cage can trigger bacterial growth in the cage [25]. This research only looked for the relationship between milking hygiene and Salmonella contamination, so it cannot compare other factors that may affect contamination. These factors

may include cage sanitation, cage construction, and the source of clean water used to bathe the cows. This research also did not look at how people's habits towards processing of the cow's milk until it is ready for consumption because based on the results of interviews with one of the milkers, there are still people who consume cow's milk without the pasteurization process.

4 Conclusion

The hygiene processes observed in this study are divided into three which include hygiene processes pre, during, and post-milking. The three results show that only the hygiene process pre-milking has results associated with *Salmonella* sp. contamination in cow's milk in Jember District. Therefore, cow milkers are expected to maintain and improve milking hygiene measures especially before milking to prevent contamination of cow's milk. In addition, advanced processes such as pasteurization are needed for consumers of cow's milk in order to avoid bacterial contamination. Regulators are expected to be able to provide a variety of holistic efforts including health promotion starting from making standard regulations regarding cow milking, conducting periodic socialization about the importance of hygiene processes, and providing education about the process of processing cow's milk until it is ready for consumption.

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