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Abstract. Curio is the central area for buffalo milk production in Enrekang Regency, South Sulawesi, Indonesia. Curio's farmers carried out the milking process manually, which enabled bacterial contamination to the milk. This study was aimed to identify the quality of buffalo milk in Curio by evaluating the total plate count and *Escherichia coli* count. Raw buffalo milk samples were collected from three farmers in Curio, separated as udder samples and farm samples. Total plate count was evaluated using Nutrient Agar media and total *E. coli* was evaluated using Eosin Methylene Blue Agar (EMBA) media. Isolated *E. coli* was further confirmed by Gram staining, IMViC test, TSIA Test, and Carbohydrate Fermentation Test. Results showed that 33.33% of udder milk samples met the requirements of Total Plate Count for SNI No. 01–3141–2011 of raw milk standard. Evaluation of total *E. coli* showed that 100% of milk samples exceeded the requirements of SNI No. 01–3141–2011. The Total Plate Count and *E. coli* in the farm samples were higher than the udder samples. The isolated *E. coli* were confirmed by Gram staining, IMViC test, TSIA Test, and Carbohydrate Fermentation Test.

1. Introduction

Raw milk is a liquid produced from a clean and healthy udder, which is obtained by correct milking, whose natural content is not reduced or reduced to anything and received no treatment other than cooling [1]. Milk is a good medium for the growth of bacteria which may cause milk damage due to its high nutrition [2]. The production process at the farm level is the first step to producing milk. Every dairy farmer always strives so that the milk produced can be fully utilized without any damage. The efforts made are not only focused on hygiene but also on the quality of milk [3].

Curio District is the center of buffalo milk production at Enrekang Regency. Most buffalo milk is used to make dangke on the scale of domestic business. The Curio sub-district is one of the largest areas of buffalo milk production compared to other sub-districts, but awareness of environmental hygiene and sanitation and hygiene practices is not yet considered. This will affect the microbiological quality of buffalo milk due to various microorganisms that cause contamination of buffalo milk. Milk contamination can originate from various sources, such as the skin of cattle, udders, water, soil, dust, humans, equipment and air [4]. Contamination due to pathogenic microorganisms can cause damage to milk, disease (especially digestive tract disease) and even poisoning to humans [5]. One of the pathogenic microorganisms found in milk is *Escherichia coli* [6].

Escherichia coli is pathogenic bacteria that belongs to the family Enterobacteriaceae and can cause damage to milk [7]. Infection of verotoxigenic *E. coli* in humans had caused 16,000 cases of foodborne diseases [8]. On June 2, 2009, as many as 293 elementary school students in Sindangkarta sub-district, Bandung Regency suffered from stomach and head-aches after consuming raw milk. According to the Food and Drug Examination Agency (BPOM) in 2009, the case was caused by *E. coli* [9].

The microbiological quality of a food is determined by the count of microorganisms or bacteria contained in the food. The food safety of microorganisms is determined by the number of pathogenic species. The higher number of bacteria may increase the chances of pathogenic bacteria growth [3]. The government had issued a reference to protect consumers from the presence of pathogenic bacteria contamination in milk in the form of the Indonesian National Standard (SNI) Number 01-3141-2011, which stipulates that microbial contamination in raw milk had a maximum limit of 1×10^3 CFU/ml Enterobacteriaceae and total bacteria (Total Plate Count) maximum 1×10^6 CFU/ml. Based on this description, it is necessary to test the milk quality in terms of the total plate count and of *E. coli* count in raw buffalo milk in Curio district, Enrekang Regency.

2. Materials and methods

2.1. Milk sampling

Raw buffalo milk samples were collected from three farmers in Curio, separated as udder samples and farm samples. Udder milk samples were taken from three different udders in each buffalo which were put directly into a sterile container. Farm samples were taken from milk storage on the farm. Then the milk was put in a sterile container and stored in a cool box during transportation from Enrekang to Makassar.

2.2. Total plate count and total Escherichia coli enumeration

Total plate count was evaluated using Nutrient Agar media and total *Escherichia coli* was evaluated using Eosin Methylene Blue Agar (EMBA) media. Bacteria numeration were evaluated by pour plate method with serial dilution [10]. The unit of measurement was CFU/ml. Further, colonies suspected of *E. coli* were isolated to be followed by confirmation test through Gram staining, Indol test, Methyl Red Test, Voges Proskauer Test and Citrate Test (IMViC), Triple Sugar Iron Agar Test, and Carbohydrate Fermentation Test.

2.3. Data analysis

Data collected were shown as figures and tables and analyzed descriptively. Survey method on dairy buffalo farms was conducted to support the research result.

3. Results and discussion

3.1. Total plate count

The results of the calculation of the total plate count of buffalo milk in udder milk samples and farm milk samples in Curio District, Enrekang Regency was showed in table 1. Based on SNI Number 01–141–2011, regarding the quality requirements of raw milk based on the total plate count which is not more than 1×10^6 CFU/ml, the results showed that 33.33% of udder milk samples met the requirements of Total Plate Count of raw milk standard. The sample K3 did not exceed the SNI standard with the average total number of bacteria was 2.3×10^5 CFU/ml which mean less than 1×10^6 CFU/ml. While farm milk samples showed that all samples (P1, P2, P3) (100%) exceeded the SNI standard with an average total number of bacteria as much as 6.5×10^7 CFU/ml higher than the entire average sample of udder milk. The total number of bacteria in the udder and farmer samples were converted to $\log_{10}$ and the value showed that the total number of bacteria in the udder milk sample K3 of 5.2 ($\log_{10}$ CFU/ml), met the SNI standard. Survey data showed that the K3 buffalo was cleaner than K1 and K2.

Table 1. Total plate count in raw buffalo milk collected from udder samples and farm samples in Curio District Enrekang Regency

Sample code	TPC CFU/ml	Log10 CFU/ml
A1	8.1×10^{6}	6.9
A2	8.3×10^{7}	7.9
A3	2.8×10^{5}	5.4
Average 1 (K1)	3.0×10^{7}	6.7
A4	7.8×10^{7}	7.9
A5	5.4×10^{7}	7.7
A6	3.6×10^{7}	7.5
Average 2 (K2)	5.6×10^{7}	7.7
A7	3.9×10^{5}	5.6
A8	4.6×10^{4}	4.7
A9	2.5×10^{5}	5.4
Average 3 (K3)	2.3×10^{5}	5.2
P1	4.1×10^{7}	7.6
P2	8.5×10^{7}	7.9
P3	7.0×10^{7}	7.8
Average (SP)	6.5×10^{7}	7.8
SNI	1×10^{6}	6

Description: A=Udder Samples, K=average of udder samples, P=farm samples, SP=average of farm samples

Contaminant microorganisms in milk can come from the environment, equipment, milk, and livestock. There were variations in the total number of bacteria between samples of udder milk and milk from of all farm milk samples was higher than in the udder milk samples. The farm milk samples P2 contained the highest total plate count is 7.9 ($\log_{10}$ CFU/mL), higher than the K2 udder milk sample (7.7 $\log_{10}$ CFU/mL). High and low levels of total plate count in milk were closely related to hygiene and sanitation during the production of raw milk on the farm. In the farm milk samples, the total plate count was higher than in the udder milk samples, since the farm milk samples were taken from milk containers made from used plastic bottles so that the contamination occurred due to the contact directly from milk with less hygiene equipment. According to Hempen *et al.* [11], equipment that is not properly cleaned and sterilized before and after use is one of the pathways for the entry of pathogenic bacteria. In addition, Rombaut [4] stated that the level of contamination comes from all sources and depends on the method of sanitation carried out. A very significant source of contamination is from the surface that is in direct contact with the milk.

The buffalo condition in the Curio was dirty, especially the area of the udder. Farmers did not clean the udders before milking. This farming practices caused the udder bacterium to enter the milk, and increased the total plate count. According to Cahyono *et al.* [3], the process of microbial contamination in milk begins when the milking process conducted, due to the presence of microbes around the udder. The process of cleaning the udders and nipples before and after milking is a very important factor in reducing the total plate count in raw milk by up to 70% by immersing it in

iodophor solution or antiseptic solution [12]. This was also added by Knappstein *et al.* [13] that udder cleaning decreases the total plate count approximately 10,000 CFU/ml.

Other contaminants may come from the hands of the farmers. Before milking, farmers in Curio wash their hands only with water without soap so that there was still a possibility of bacteria sticking to the hands of the milk. According to Sanjaya *et al.* [14] before milking, the hands must first wash with soap and brushed thoroughly. Furthermore, Nanu [15] mentioned that water used for cleaning equipment, reddened hands, and udder also affect the level of contamination in milk. Perkins *et al.* [16] stated that the quality of water used for cleaning equipment had a significant effect on the quality of raw milk produced. Chyel *et al.* [17] added that the presence of coliform bacteria and pathogens in milk indicates the possibility of contamination from udders, equipment or water.

3.2. Escherichia coli count

Escherichia coli is often used as a marker organism. The acquisition and calculation of *E. coli* are used as a reliable indicator of fecal contamination and indicates the possibility of the presence of enteropathogenic or toxigenic microorganisms, which are public health threats [18]. The results of the calculation of the *E. coli* count of buffalo milk in udder milk samples and farm milk samples in Curio District, Enrekang Regency showed in table 2. The limit of *E. coli* contamination according to SNI Number 01-3141-2011 is 1×10^3 CFU/ml. Table 2 showed that all samples of udder milk and farm milk (100%) exceed the SNI standard because the number of *E. coli* was more than 1×10^3 CFU/ml. The average number of *E. coli* in farm milk samples was 2.2×10^7 higher than the average sample of udder milk.

Table 2. *E. coli* count in raw buffalo milk collected from udder samples and farm samples in Curio District Enrekang Regency

Sample Code	*Escherichia coli* CFU/ml	Log_{10} CFU/ml
A1	3.6×10^6	6.5
A2	2.0×10^7	7.3
A3	1.1×10^5	5
Average 1 (K1)	7.9×10^6	6.3
A4	1.4×10^7	7.1
A5	4.6×10^7	7.6
A6	3.9×10^6	6.6
Average 2 (K2)	2.1×10^7	7.1
A7	4.6×10^4	4.7
A8	4.6×10^4	4.7
A9	1.6×10^4	4.2
Average 3 (K3)	3.6×10^4	4.5
P1	2.6×10^7	7.4
P2	2.9×10^7	7.5
P3	1.2×10^7	7.1
Average (SP)	2.2×10^7	7.3
SNI	1×10^3	3

Description: A=udder samples, K=average of udder samples, P=farm samples, SP=average of farm samples

The number of *E. coli* in udder milk samples and farm milk samples were converted in log_{10} (CFU/ml). The result showed that all udder milk samples and farm milk samples exceeded the SNI standard, with the value 3 (log_{10} CFU/ml). The count of *E. coli* in farm milk samples was higher than the udder milk samples. Farm milk samples P2 contained the highest amount of *E. coli* which was 7.5 (log_{10} CFU/ml), higher than the udder milk sample (K2) of 7.1 (log_{10} CFU/ml). Farm samples had higher *E. coli* count than udder milk samples because farm milk samples were taken from a collection

of milk that had been mixed and placed in a milk container. The container used was made of used plastic packaging bottles which enabled cross-contamination. Duguid and North [19] stated that cross-contamination could occur due to bacteria from one of the contaminated sources that were not contaminated usually through kitchen utensils or hands that were not washed properly.

The high rate of *E. coli* contamination in all udder milk samples and farm milk samples in Curio could be caused by several factors. These factors were mainly low hygiene and sanitation during raw milk production at the farm level. According to Nurwantoro and Mulyani [20], contamination by *E. coli* bacteria could be caused by poor handling of milk and less aseptic, and inadequate sanitation and environment. Suwito [9] stated that milking sanitation conditions determine the level of contamination. The presence of *E. coli* in milk showed the possibility of contamination from the soil, polluted water, fecal contamination and poor handling of milk [21]. Farmers in Curio did not separate the stool waste area with cages or milking areas, this might cause *E. coli* bacteria to enter through the dust carried by the wind. The cleaning process was not carried throughout the body of the livestock, including on the udder and nipples, before milking. According to Malaka [6], sources of contamination in milk could come from objects that fall or are carried away by the wind entering the milk during the milking process. Hadiwiyoto [22] mentioned another source of contamination that could affect the quality of milk was dirty animals which caused cross contamination to milk produced.

3.3. Confirmation test of Escherichia coli

Confirmation test of *E. coli* was conducted through Gram staining, Indol test, Methyl Red Test, Voges Proskauer Test, and Citrate Test (IMViC), Triple Sugar Iron Agar Test, and Carbohydrate Fermentation Test. The isolated *Escherichia coli* was then observed for morphology and the results were round colonies, metallic green, curved surfaces, edges and size of colonies 1-3 mm (fig. 3a). Simatupang [23] stated that macroscopic *E. coli* colonies on solid medium have a round shape, small to medium size, convex surface and smooth with flat edges. The results of Gram staining were rod-shaped bacteria and red in color (fig. 3b).

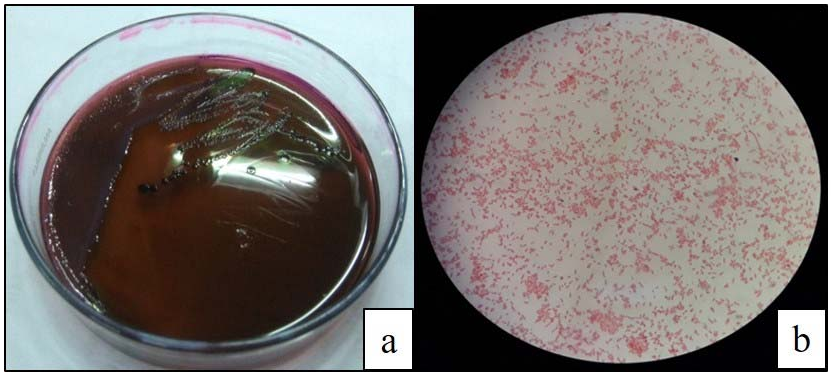

Figure 3. *E. coli* from buffalo milk in EMBA media (a), Gram staining (b)

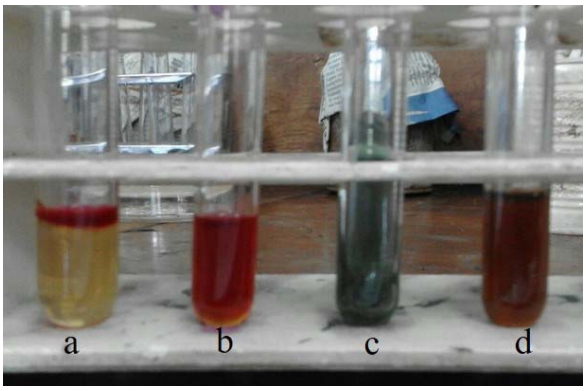

Figure 4. Indole, Methyl-Red, Voges-Proskauer, and Citrate (IMViC) Biochemical Test results on *E. coli* from raw buffalo milk. Indole test result (a), Methyl-Red test result (b), Citrate test result (c), Voges–Proskauer test Result (d)

The IMViC test results were presented in figure 4. The indole test and Methyl-Red test showed a positive result, Citrate test and Voges-Proskauer (VP) test showed a negative result. TSIA test showed that the acid/acid results were marked on the stock (bottom), as well as the slope (slope) was also yellow, indicated an acid atmosphere at the end and inclined with positive gas and H2S negative. According to Leboffe and Pierre [24], the yellow color of the whole medium was caused by *E. coli* in the bacterial media that ferment glucose, lactose, and sucrose. The gas produced comes from the fermentation of carbohydrates, which is the result of the metabolism of *E. coli*. The carbohydrates (sugars) fermentation test showed positive results for glucose, lactose, maltose, and sucrose fermentation.

4. Conclusion

Raw milk microbial quality in Curio District, Enrekang Regency showed that 33.33% of udder milk samples met the requirements of Total Plate Count for SNI No. 01–3141–2011 of raw milk standard. Evaluation of total *E. coli* showed that 100% of milk samples exceeded the requirements of SNI No. 01–3141–2011. The Total Plate Count and *E. coli* in the farm samples were higher than the udder samples. The isolated *E. coli* were confirmed by IMVIC test, indicated that raw buffalo milk containing *E. coli*.

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