

Prevalence and Sources of Contamination of *Escherichia coli* and *Salmonella* spp. In Cow Milk Dangke, Indonesian Fresh Soft Cheese

¹Wahniyathi Hatta, ²Mirnowati Sudarwanto,
²Idwan Sudirman and ¹Ratmawati Malaka

¹Faculty of Animal Husbandry, Hasanuddin University,
Perintis Kemerdekaan Street Km. 10 Tamalanrea Makassar, 90245, Indonesia

²Faculty of Veterinary Medicine, Bogor Agriculture University,
Agatis Street Darmaga Bogor, 16680, Indonesia

Abstract: The presence of pathogenic bacteria in foods potentially causes health problems to consumers, such as infectious diseases and food poisoning. This research was aimed to determine the level of contamination of *Escherichia coli* (*E. coli*) in cow milk dangke and its relationship with workers and equipment of processing (molds and packaging materials); in addition, the presence of *Salmonella* spp. was also detected. Dangke samples, swabs of the workers' right and left hands, swabs of molds and packaging materials were collected from the 30 cow dairy processing businesses of dangke randomly selected in Enrekang regency. The standard laboratory method was used to detect the presence of *E.coli* and *Salmonella* spp. Contamination levels of *E. coli* were 73% (22/30), 40% (12/30), 63% (19/30) and 40% (12/30) in dangke, workers' hands, molds and packaging materials respectively. The presence of *Salmonella* spp. was found in 7% (2/30) of dangke and 3% (1/30) of molds. The workers and equipment of dangke processing might become risk factors for dangke to be contaminated by *E. coli*. Dangke cheese produced by the farmers potentially causes health problems for consumers if it was eaten raw. Efforts on extension, coaching and supervision on hygiene sanitation practices must be provided to workers dangke consistently and continuously.

Key words: Food Safety • Pathogenic Bacteria • Workers' Hands • Coconut Shells • Banana Leaf

INTRODUCTION

The presence of pathogenic bacteria in foods potentially causes health problems to consumers, such as infectious diseases and food poisoning. Food borne bacteria that often contaminate dairy products in developing countries are *Escherichia coli* (*E. coli*) and *Salmonella* spp. The presence of bacteria in dairy products is linked with poor hygiene practices in handling raw milk and end products [1, 2]. *E. coli* is a normal micro-flora in the digestive tract of human and warm-blooded animals, but some of its strains are pathogens causing diseases in humans [3]. Several serotypes of *E. coli* are associated with mild and severe cases of diarrhea in children and adults, especially in developing countries [4]. *Salmonella* spp. genus is one of

the pathogens that was reportedly involved in several outbreaks of diseases through food in France and other countries due to the consumptions of contaminated milk and its products [5].

Dangke is an Indonesian traditional dairy product, a type of fresh soft cheese that is usually made from fresh cow milk or buffalo milk by the farmers' households in Enrekang regency, South Sulawesi province. Cow milk dangke has a high nutrient content (water content of 55%, protein of 23.8%, fat of 14.8% and ash of 2.1%) and its near-normal pH value of 6.4 [6]. Its shelf life is generally two days at room temperature, while at the refrigerator temperature, it can reach five to seven days. Dangke is consumed as a side dish accompanying rice or as a snack.

Corresponding Author: Wahniyathi Hatta, Faculty of Animal Husbandry, Hasanuddin University,
Perintis Kemerdekaan Street Km. 10 Tamalanrea Makassar, 90245, Indonesia.

Dangke manufacturing principles include coagulating milk proteins through heating and adding a solution of papaya latex as the coagulant enzyme. Heating process is generally carried out until the temperature reaches 80 to 100°C. The curd straining process from the whey is the shaping stage of making dangke using coconut shells with a hole at the bottom where the whey passes through. Dangke is packed with a banana leaf after the shaping process and ready to be consumed or stored.

Dangke is made through a simple process without salting, the use of starter cultures and ripening period as in the manufacture of cheese in general. Dangke is a ready-to-eat food product in which its subsequent processing before consumption does not guarantee its product safety. Based on this, the unhygienic process of making dangke can cause contamination with pathogenic bacteria that affect the health of consumers. Contamination of pathogenic bacteria in dangke can occur in various stages of production so that prevention and supervising require information on the major sources of contamination in dangke production chain.

A small number of studies on the contamination of pathogenic bacteria in dangke in Enrekang Regency have been carried; therefore, the availability of scientific information on the topic is limited. This research aimed to determine the contamination level of *E. coli* and *Salmonella* spp. bacteria in dangke, as well as to determine whether workers, molds (coconut shells) and packaging materials (banana leaves) were the sources of bacterial contamination in dangke or not.

MATERIALS AND METHODS

Sample Collection: The samples were collected from 30 cow dairy processing businesses of dangke randomly selected in Cendana district, Enrekang regency. A total of 150 samples comprising the dangke (30), right hand swabs (30) and left hands swabs (30), swabs of surfaces of coconut shells (30) and swabs of the surfaces of banana leaves (30) were collected from March 2012 to June 2012.

Swab samples were taken before the process, while the dangke samples packed in banana leaves were collected two hours after making the products. Sample collection procedures were performed based on Harrigan [7]. Both dangke samples in sterile plastic containers and swab samples in a glass jar with a lid were immediately placed in a cool box with ice packs to maintain their temperature below 5°C until the samples were reached the laboratory.

Microbiological Testing: Testing of bacterial contaminations in all samples was carried out qualitatively including pre-enrichment stage, enrichment, isolation and identification and biochemical testing to confirm the results [8].

For the detection of *E. coli*, buffer pepton water medium (Difco 1221951) was used as a pre-enrichment medium, *E. coli* broth (Oxoid 1199624) as an enrichment medium and levine's eosin-methylene blue agar (Difco 2254461) as a selective medium for identification-isolation. Biochemical testing was performed with IMViC test using media of tryptone broth, methyl red-voges proskauer broth and koser's citrate broth.

For the detection of *Salmonella* spp., buffer pepton water medium (Difco 1221951) was used as a pre-enrichment medium, rappaport vasiliadis broth (Difco 8105415) was used as the enrichment medium, hektoen enteric agar (Difco 0341710), bismuth sulfite agar (Difco 1153880) and xylose lysine deoxycholate agar (Oxoid 1162584) were used as a selective medium for isolation-identification. The biochemical test used were the media of a triple sugar iron agar (Difco 8137638), lysine indole agar (Difco 0298030), urea broth, simmon citrate agar and tryptone broth.

Statistical Analysis: Table of frequency distribution was used to compare the percentage of samples contaminated and uncontaminated by *E. coli* and *Salmonella* spp. Statistical analysis were performed using SPSS version 19.0 program.

RESULTS

The contamination levels of pathogen bacteria in dangke found in this research were *E. coli* by 73% and *Salmonella* spp. by 7%. The percentage of dangke workers' hands contaminated with *E. coli* reached 40%, while the *Salmonella* spp. was not found in all samples. In addition, the results of this research showed that 63% from the mold samples and 40% from the packaging samples were contaminated by *E. coli*. Contamination of *Salmonella* spp. was not found in all packaging samples, but it was found in one mold sample (Table 1).

Table 1: Contamination levels of *Escherichia coli* and *Salmonella* spp. in dangke, workers' hands and dangke processing equipment (n =30)

Sample	<i>E. coli</i> n (%)	<i>Salmonella</i> spp. n (%)
Dangke	22 (73)	2 (7)
Workers' hands	12 (40)	0 (0)
Molds (coconut shells)	19 (63)	1 (3)
Packaging materials (banana leaf)	12 (40)	0 (0)

n = Number of samples

DISCUSSION

The presence of *E. coli* and *Salmonella* spp. showed that dangke made by the farmers in Enrekang regency was unhygienic and could threaten public health if it was eaten raw. The regulation of the Republic of Indonesia requires that *Salmonella* spp. should be negative by 25^{-1} g and the number of *E. coli* maximally reaches 10 MPN g^{-1} in all kinds of cheese (SNI 7388:2009).

Contamination of *E. coli* and *Salmonella* spp. in soft cheeses was also reported by the researchers from other countries. Najand and Ghanbarpour [9] isolated *E. coli* from 98.7% of domestic soft cheese samples obtained from various wholesales and markets in Kerman, Iran. *E. coli* was found in all specimens (60) and *Salmonella* spp. was found in two specimens of fresh white cheese presented for sale in bazaars Canakkale, Turkey [10]. The contamination level of *E. coli* in this research was lower than that in the findings of the two studies. This can be attributed to the temperature of the milk heating in the making of dangke reached by 80 to 100°C, thus suppressing bacterial contamination, while domestic soft cheese from Iran and white cheese from Turkey were made by using used raw milk without pasteurization. Effective pasteurization of raw milk is essential to minimize microbial load in the manufacture of the cheese [11]. Antimicrobial activity in papaya latex was used as a milk clotting enzyme likely contributed to the reduction in the level of bacterial contamination in dangke.

The contamination levels of bacteria in this research were in accordance with the report by Carvalho *et al.* [12] who detected the presence of *E. coli* at 78.26% in Minas Frescal cheese production by the household businesses in Gerais Minas, Brazil, but contamination with *Salmonella* spp. was not detected in the cheese. Similar results were also reported by Godbole *et al.* [13] stating that 72% of Paneer cheese in Nagpur city, India were contaminated by *E. coli*; however, the level of contamination of *Salmonella* spp. was larger i.e. 34%. Although the milk heating temperature in the manufacture of both types of cheese and dangke achieved the pasteurization standards, the level of contamination of *E. coli* detected remained high. This indicates that bacterial contamination in cheese might occur after processing. The microbiological contamination during production of white cheese can be originated from the workers, equipment and factory environment [14].

The presence of *Salmonella* spp. implies the needs of other treatments besides heating in the processing to prevent contaminations of pathogenic bacteria in dangke.

Mennane *et al.* [15] stating that *E. coli* and *Salmonella* spp. were not found in klila samples, traditional Moroccan cheese, correlated with low water activity product as a result of drying treatment in the sun. Hamid and El Owni [16] suggested that *Salmonella* spp. undetected in Gibna Bayda cheese samples in Sudan, because the cheese had a high salt content. The ripening process [17] and the use of starter cultures [18] in cheese making could also reduce the level of contamination of pathogenic bacteria. Tesfaye *et al.* [19] showed that the use of mixed lactate acid bacteria culture in the making of Ayib cheese, traditional Ethiopian cheese, could reduce the number of *E. coli* and *Salmonella Typhimurium* in the cheese when stored both at a refrigerated condition and at an ambient temperature.

Almost all the dangke workers are the wives of the farmers who make dangke at home while looking after the house and family. This situation might cause their hands to be contaminated with bacteria from various sources during household activities. The presence of *E. coli* on the hands of workers has significance for food safety because of its potential to cause health problems if its isolates include pathogenic strains, such as enterohaemorrhagic *E. coli* O157:H7 [20]. In addition, contamination of *E. coli* implies the presence of other enteric pathogens on the hands of workers. Shojaei *et al.* [21] reported the presence of the bacteria of *Bacillus* spp., *E. coli*, *Enterobacter* spp., *Klebsiella* spp. and *Staphylococcus aureus* in food hand processing in Iran. The presence of bacterial pathogens can cause serious health problems for consumers because it can move into the food through the hands of workers who process it, especially after the heating process [22]. The results of this research indicated that the hygiene practices of the dangke workers were inadequate. Direct observation in the research field showed that most of the dangke workers did not use to wash hands with soap and water after doing any activity associated with dirt. Hand washing based on the hygiene standards is a simple way to reduce the level of bacterial contamination on the hands [23].

Dangke has a unique shape because it is inserted into a coconut shell mold and the dangke is then wrapped in a banana leaf. The use of the two materials has been preserved until now and the shape becomes the characteristic of the dangke. The presence of pathogenic bacteria on the molds can be associated with poor hygiene practices of the workers, such as delaying mold washing after use, washing molds without soap and washing kitchen utensils and dangke equipment in the same basin. In addition, the coconut shells after being washed and dried are not stored in a sealed container so

that they have the risk of bacterial contamination from the air of the processing area and the vector [24]. Levels of microbial contamination on the surfaces of the materials used in the processing of dairy products might indicate the effectiveness of the equipment cleaning and air quality within the factory environment [25]. The use of a dirty cloth and the cloth which is not reserved only for packaging were suspected to cause a high level of contamination of *E. coli* on a banana leaf. Contamination of *E. coli* on food equipments could also be sourced from the contaminated hands of workers [26]. The unhygienic molds and packaging potentially contaminate the products because the equipment has a direct contact with dangke after the heating process. The processes of molding and packaging are the final stage of processing before dangke is sold or consumed; therefore, the hygiene of the molds and packaging indirectly plays a role in the incidence of disease outbreaks through food.

CONCLUSION

Dangke cheese which was produced by the farmers potentially causes health problems for consumers if it was eaten raw. The worker's and processing equipment are the main sources of contaminations of bacterial pathogens in dangke. Efforts on extension, coaching and supervision on the hygiene sanitation practices must be given to the dangke workers consistently and continuously.

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