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Salmonella sp. and *Escherichia coli* Contamination on Cattle Beef from Binjai City Slaughterhouse and Tavip Market

S. M. Capah[1], Hasnudi[1], P. Patriani[*1], and M.I. Aja[2]

[1]Animal Production Program Study, Faculty of Agriculture, Universitas Sumatra Utara, Padang Bulan, Medan 20155

[2]IPB University
**correspondent author :peni@usu.ac.id*

Abstract. *Salmonella sp.* and *Escherichia coli* became contaminator bacteria that influenced the microbiological quality of beef. Environmental conditions, facilities, and slaughterhouse infrastructure cause contamination. This study aimed to determine the presence of *Salmonella sp*. and *Escherichia coli* contamination on slaughtered beef from Binjai City slaughterhouse and Tavip Market. This study used a descriptive and purposive sampling technique and 12 samples (6 from the slaughterhouse and six from Tavip Market). This study was conducted at Binjai City Slaughterhouse and Tavip Market. It was analyzed at the Cell and Tissue Cultures Laboratory in the University of North Sumatera Faculty of Medicine in November 2021. Data analysis used Paired Sample T-Test. The result showed the absence of *Salmonella sp.* contamination on the Binjai City slaughterhouse and Tavip Market sample. Furthermore, all the samples were contaminated by *Escherichia coli*, and 5 out of 6 samples surpassed the SNI 3932:2008. This study concludes that the average of *Escherichia coli* in slaughterhouse samples differs significantly from the sample from Tavip Market.

Keywords: beef, *escherichia coli*, contamination, *salmonella sp*, slaughterhouse.

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1. Introduction

Beef is a product of the animal husbandry industry. An increase in slaughter intensity follows the high demand for beef in the community. That is why there is more attention to the existence of Slaughterhouses as a place for slaughtering beef [1]. Beef-contaminating bacteria have been causing various problems such as food poisoning, nausea, vomiting, stomach cramps, and digestive tract irritation.

Contamination by microorganisms in food is often referred to as foodborne disease [2]. Contaminants usually found in beef come from the bacteria *Salmonella sp.* and *Escherichia coli*

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which generally can cause disturbances to human digestive health [3]. Based on observations, the location around the Binjai City slaughterhouse is an area of hot weather and dusty air with an average temperature of 32^{o}C. The hygiene aspect of the place and the equipment used in every stage of slaughtering cattle at the Binjai City slaughterhouse is still lacking because the slaughterhouse building has a lot of cobwebs and blood stains on the walls, slippery and perforated floors, and unpleasant odors. Microbiological test on beef regarding contamination of *Salmonella sp.* and *Escherichia coli* as indicator of pollution in Binjai City slaughterhouse is needed to determine the presence of contamination so that it can be a reference for the level of hygiene for public consumption and food safety. Based on the background explanation, the authors are interested in conducting research related to *Salmonella sp.* and *Escherichia coli* at the Slaughterhouse and Tavip Market in Binjai City.

2. Materials and Methods

The materials and tools used in this study were six cattle beef samples from Binjai slaughterhouse and six cattle beef samples from Binjai Tavip Market (Shank), distilled water, 70% alcohol, Lactose Broth (LB), Salmonella Shigella Agar (SSA), and Eosin Methylene Blue Agar (EMBA), rubber gloves, sterile knives, cool boxes, ice gel, test tubes, Erlenmeyer, autoclave, analytical scales, biosafety cabinet, hot plates, magnetic stirrer, shaker, micro pipe, prop pipe, hockey stick, ose needle, vortex, cling wrap, aluminum foil, petri dish.

Bacterial testing occurs by conducting laboratory tests. This study used a purposive sampling technique. 6 samples were taken from each location, namely 3 for the Salmonella sp. test and 3 for the Escherichia coli test. Observational data collected from sampling time can be seen directly, namely the cleanliness of the slaughter location and all equipment used, the condition of the people who take part in general, and the environment around the slaughter place.

2.1 Research Procedure

a. Preparation of Lactose Broth (LB) solution

3.25 grams of Lactose Broth powder was weighed, put into an Erlenmeyer, and dissolved (homogenized) with 250 ml of sterile distilled water. 1.3 grams of Lactose Broth powder was weighed and put into an Erlenmeyer and dissolved with 100 ml of sterile distilled water. A sample code tag is affixed to each Erlenmeyer. Then heated on a hot plate until boiling, covered with aluminum foil and cling wrap for sterilization using an autoclave at a temperature of 121^{o}C, 1 atm for 15 minutes. Do as many as the number of samples.

b. Preparation of Salmonella Shigella Agar (SSA) and Eosin Methylene Blue Agar (EMBA)

The SSA powder weighed 24 grams and dissolved into 400 ml of sterile distilled water. EMBA powder weighed 15 grams and dissolved into 400 ml of sterile distilled water. Then stir until completely dissolved and heat on a hot plate while stirring using a magnetic stirrer. After boiling, it was covered with aluminum foil and cling wrap for sterilization using an autoclave at a temperature of 121ºC, 1 atm for 15 minutes.

c. Dilution

After the LB solution is made, weigh the beef sample as much as 25 grams for testing *Salmonella sp.* and 10 grams for testing *Escherichia coli* bacteria. Put the sample into each Erlenmeyer containing the LB solution according to the sample tag, then homogenize with a shaker for up to 24 hours. Then do the sample dilution into a 10^{-1} dilution and a 10^{-2} dilution. Furthermore, vigorous shaking (vortex) for a few seconds so that the sample dissolves homogeneously.

d. Isolation of bacteria with the Steak Plate Method

After the SSA and EMBA media have been made, it poured evenly into a petri dish and allow to solidify. Samples from the slaughterhouse were coded BS1, BS2, and BS3 for *Salmonella sp.* and BE1, BE2, and BE3 for *Escherichia coli*. Samples from the Tavip market were coded TS1, TS2, TS3 for *Salmonella sp.* and TE1, TE2, and TE3 for *Escherichia coli*. Samples incubated at 35ºC for 24-48 hours.

e. Counting the number of colonies

Observations were made on the colonies of *Salmonella sp.* and *Escherichia coli* bacteria growing on SSA and EMBA media. Then the number of colonies of *Salmonella sp.* and *Escherichia coli* bacteria was calculated. Colonies of *Salmonella sp.* (+) are transparent, with a black spot in the middle. Colonies of *Escherichia coli* (+) are metallic green with a black spot in the middle.

2.2. Analysis of Data

Data obtained from direct observation and analyzed with a descriptive method, then compared with SNI 3932:2008. Furthermore, to find out the differences in the number of *Escherichia coli* in cattle beef at the Binjai City slaughterhouse and Tavip Market was followed by a paired t-test with statistical software.

The linear model of paired t-test [5]:

$$t_{count} = \frac{\overline{D}}{S_D/\sqrt{n}}$$

Description: t: t count value
$\overline{D}$: Average difference between slaughterhouse and tavip market E. coli

S_D: Standard deviation of the difference between slaughterhouse and tavip market E. coli

N: Number of samples

3. Results and Discussion

3.1. Overview of Binjai City Slaughterhouse

Sanitation in the equipment and buildings of the Binjai City slaughterhouse is not carried out using the sterilization and disinfection method before and after the slaughtering activity. The equipment used in slaughter is a modern tool. Furthermore, the cow to be slaughtered can be stunned by using a stunning gun first and then slaughtered, or it can be slaughtered directly with the help of a restraining box.

The cattle slaughtered at the Binjai City slaughterhouse are then transported to Tavip Market at a distance of 4.8 km using an open pickup truck without any cover to be marketed immediately starting at 06.00 WIB. Improper transportation methods, such as those used by the Tavip Market cattle traders, can increase the risk of beef contamination by microorganisms and reduce the quality of beef because the beef is left exposed and exposed to the air during the journey to the market.

3.2. Overview of Binjai City Tavip Market

The condition of lighting and air circulation in the Tavip market is quite good because traders use the help of lights and selling locations located on the side of the road so that they are exposed to sunlight, and the air is free to enter and leave. However, the condition of the hallway selling is muddy and dirty. Flies flew and landed on the beef because it was displayed on a porcelain table and hung with a Steel Beef Hook. The risk of direct contamination from the air, flies, and human hands during the buying and selling process cannot be avoided.

Table 1. Descriptive observations of the location of data collection in the field

Variable	Sampling Location	
	Slaughterhouse	Tavip Market
Location conditions	The floor is dirty and wet. There are many flies on the floor and beef	Dirty and soggy, but the beef was neatly arranged. There are many flies on the beef
Beef cleaning	Remove the contents of the innards. Carcasses and non-carcasses are cleaned with running water from the water tower	Carcass and non-carcass were cleaned with water that has been accommodated. The beef is neatly arranged on a cleaned porcelain table
Slaughter tool	Clean and sharp, not sterilized	Clean and sharp, not sterilized
Slaughter mat	Cement floor	Wooden cutting board
Storage		
- Temperature	28-32^{o}C	28-32^{o}C
- Beef conditions	Fresh	Fresh
- Packaging	Not use packaging, mat, or covers when transported from the slaughterhouse to Tavip Market	Plastic bag

Workers conditions	Do not wear an apron and gloves. Some don't wear clothes and boots	Not wearing an apron and gloves

The average temperature of Binjai City is 32°C which can be one of the factors supporting the rapid growth of *Salmonella sp.* and *Escherichia coli*. According to [6], *Salmonella sp.* lives in an environment of 5-45°C and grows optimally in an environment of 35-37°C. The statement [7] is that *Escherichia coli* can grow well in an environment of 8-46°C with an optimum temperature of 37°C.

According to [8], the Sanitation Standard Operating Procedure (SSOP) is a standard for sanitation requirements in operating production facilities or company areas. In addition, [9] states that several requirements in the SSOP include the condition and cleanliness of all surfaces that come into direct contact with food, preventing the risk of cross-contamination, and monitoring personal health conditions that can increase the risk of contamination.

3.3. Detection of *Salmonella sp*.

Table 2. Test results for *Salmonella sp.* on beef at the Binjai City slaughterhouse and Tavip Market

Sample Code*	SNI Standard (CFU/g)	Test result	
		Slaughterhouse (B)	Tavip Market (T)
S1	Negative /25g	Negative	Negative
S2		Negative	Negative
S3		Negative	Negative

Description: B = slaughterhouse location code

T = tavip market location code

* = sampling from slaughterhouses and tavip markets is done at different times

Based on the test results in "Table 2", it can be seen that in the examination of 3 beef samples at the Binjai City slaughterhouse and three beef samples at the Binjai City Tavip Market, all samples were negative or there was no *Salmonella sp.* According to [4], the presence or absence of *Salmonella sp.* in beef is due to several factors, namely the existence of conditions that do not support the growth of *Salmonella sp.* in beef. There is competition with other bacterial contamination, such as spoilage bacteria and lactic acid bacteria, which can inhibit the growth of *Salmonella sp* bacteria. This is because *Salmonella sp.* cannot compete against common bacteria in food products. [6] stated that one of the extrinsic factors that affect the growth ability of microorganisms in beef is the presence or absence of microorganisms inhibiting substances. According to [10], This can happen because beef contains a lot of nitrogenous substances of different complexity and several carbohydrates that bacteria can ferment.

The application of sanitation and hygiene at the slaughterhouse and Tavip Market is categorized as not good enough, starting from the conditions of workers, beef storage, and transportation. Still, based on the test results, 100% of the samples are clean (negative) from contamination by *Salmonella sp*., which means that although the sanitation and hygiene at the slaughterhouse and Tavip Market are not good, beef samples can be free from *Salmonella sp*. contamination.

The observations at Binjai City Slaughterhouse show that livestock that will be slaughtered are always rested for approximately 6 hours. This follows the statement [11] that treating livestock before slaughter will affect the number of microbes in the beef. This can also occur from healthy and well-treated individual cattle before slaughter.

3.4. Detection of *Escherichia coli*

Table 3. Test results of *Escherichia coli* bacteria on beef at slaughterhouse and Tavip Market, Binjai City

Sample code*	SNI Standard (CFU/g)	Total *Escherichia coli* (CFU/g)			
		Slaughterhouse (B)	Desc.	Tavip Market (T)	Desc.
E1	1×10^1	0.5×10^1	Under	5.5×10^3	Over
E2		1.6×10^3	Over	4.68×10^3	Over
E3		3.46×10^3	Over	6.37×10^3	Over
Average**		1.68×10^3		5.5×10^3	

Description: B = slaughterhouse location code
T = tavip market location code
* = sampling from slaughterhouses and tavip markets is done at different times
** = calculated value > T table. Significantly different (Ho rejected) or significant at 0.05 probability (P=0.05)

The results from "Table 3" show the number of *Escherichia coli*. 83.3% of beef samples slaughtered at the Binjai City Slaughterhouse exceed the BMCM of *Escherichia coli* bacteria. This means that from the six beef samples tested, there is only 1 sample that meets the established SNI requirements, namely the sample originating from the slaughterhouse with the sample code BE1, which has *Escherichia coli* bacteria of 0.5 x 101 CFU/g. The other two samples from the slaughterhouse did not meet the requirements because sample BE2 had *Escherichia coli* bacteria of 1.6 x 103 CFU/g, and sample BE3 had *Escherichia coli* bacteria of 3.46 x 103 CFU/g.

Based on the results of the t-test, it was found that the value of the number of *Escherichia coli* bacteria in the Tavip market was higher than the value of the number of *Escherichia coli* bacteria in the slaughterhouse. The statistical test results showed a significant difference between the average number of *Escherichia coli* bacteria in the beef samples slaughtered at the slaughterhouses and those sold at the Tavip Market, Binjai City ($p<0.05$). The average colony of *Escherichia coli* bacteria in the slaughterhouse was 1.68 x 103 CFU/g, and the average bacterial colony in the Tavip market was 5.5 x 103 CFU/g.

Figure 1. Condition of the slaughterhouse (left) and Tavip Market (right)

The high level of contamination and differences in the number of *Escherichia coli* bacteria in beef at the slaughterhouse and the Tavip market is caused by several factors, such as the level of the slaughter process to sales, place hygiene, and personal hygiene of workers. It can be seen in Figure 3 that the place for skinning and removing offal and carcass is carried out in the same building, so microbial contamination from waste offal and the outer skin of the carcass is hazardous. The slaughter place and all tools that have been used are not sterilized and not disinfected but only cleaned using running water.

Based on the observation's results, the livestock will be rested for ± 6 hours before slaughtering. Then the slaughtering process is carried out at the Binjai City slaughterhouse, which begins with a stunning gun containing empty bullets without electrical stimulation to the livestock and immediately slaughtering. But all the slaughter tools used by workers are not sterile. Before the knife is used, the butcher washes it first without sterilizing it and then sharpening it. According to [12], one of the factors after slaughter that can affect the quality and quality of beef is electrical stimulation.

After the slaughter, beef transport to tavip market is carried out without mats and covers, so the beef is exposed to the open air. According to [13], the correct beef transport vehicle uses a closed boxcar covered with a heat insulator. And other people or objects are not allowed to enter or be inside the interior of this transport vehicle.

This research is relevant to research on the Pekanbaru slaughterhouse [14]. The things that caused the high level of microbial contamination are the condition of the slaughter place and equipment not being cleaned immediately after use, unavoidable cross-contamination due to clean and dirty rooms that were not separated, and personal hygiene workers not appropriately applied. The workers are not provided or wear complete clothing such as covered clothing, boots, gloves, masks, and head coverings. Also, the supporting equipment is rarely cleaned or disinfected, the level of supervision and awareness of employees at the slaughterhouse on the importance of implementing sanitation is minimal, and beef transport facilities are inadequate.

Beef sold in the Tavip market is not packaged in advance but is immediately displayed openly and arranged in stacks so that consumers can choose their beef independently. Some are hung using a steel beef hook. According to [15], beef that is sold openly causes consumers to be free to choose beef and often touches the desired part of the beef so that there is direct contact between the microbes on the hands of consumers and the beef that can contaminate the beef.

Contamination of *Escherichia coli* bacteria in beef at the slaughterhouse and Tavip Market can pose a risk of digestive disorders and infections to the community as consumers and the surrounding environment. This follows the opinion [16] that *Escherichia coli* can develop naturally in the human intestine but is an opportunistic pathogen. Therefore, cases of diarrhea caused by *Escherichia coli* only occur when a person's body defenses are weak or because the number of *Escherichia coli* bacteria in the body exceeds the standard limit.

3.5. Prevention of Microbial Contamination

[17] stated that the main concerns to controlling the impact of microbial contamination on livestock products are:

- Knowledge in natural ecology and epidemiology that is useful as a parameter to establish an accurate method of diagnosis;

- Identification of critical points in the event of contamination of disease agents in the middle of the livestock food chain;

- The level of knowledge as a form of public awareness and care for several diseases that can be caused by microbial contamination; and

- Stakeholder relations with agencies/agencies in coordinating microbial pollution problems.

Applying good sanitation and hygiene during the beef cooking process can reduce the risk of infection by consuming beef contaminated with microbes. SNI 7388:2009 states that good personal hygiene can be implemented with the application of good sanitation, including:

- Wash hands before and after cooking fresh beef;

- Use utensils with clean surfaces when preparing pre-cooking beef; and

- Wash all utensils used with hot soapy water and rinse before using them to prepare other foods.

4. Conclusion

Salmonella sp. contamination. the beef samples from the Slaughterhouse and Tavip Market in Binjai City have met the standard of SNI 3932:2008. *Escherichia coli* bacterial contamination has exceeded the Maximum Microbial Contamination Limit in beef samples from the Binjai City

Slaughterhouse and 100% from the Tavip Market in Binjai City. A total of 5 out of 6 samples did not meet the standard of SNI 3932:2008. The average number of *Escherichia coli* bacteria in beef samples from the Binjai City slaughterhouse significantly differed from samples from Binjai City Tavip Market.

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