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Salmonella sp. and Escherichia coli Contamination on Beef from Slaughterhouse and Traditional Markets in Medan City

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Abstract. *Salmonella sp* and *Escherichia coli are* zoonotic pathogenic bacteria that can be found on beef. The presence of bacterial contamination on meat will impact the meat quality physical and in microbiological. The environment of the slaughter area is one of the factors causing *Salmonella sp* and *Escherichia coli* contamination in beef. The study aimed to determine the presence of *Salmonella sp.* and *Escherichia coli* contamination on meat from Slaughterhouses (RPH) and Medan Traditional Markets. Sampling was taken in 4 locations; RPH, and three traditional markets: Brayan, MMTC, and Central Market. Meat samples researched from October to December 2021 at Laboratorium Balai Penguji Penyidik Penyakit Balai Veteriner Regional Wilayah I. Data analysis used Paired Sample T-Test. The positive result of Salmonella spp contamination was found one a meat samples from Brayan Market. Meanwhile, Negative results of *Salmonella spp* contamination were found on meat samples from Slaughterhouse, MMTC Market, and Central Market. Furthermore, *Escherichia coli is* also found on almost all meat samples from RPH, and traditional markets.

Keywords : contamination, *escherichia coli*, *salmonella sp,* slaughterhouse, traditional market

1. Introduction

Beef, which is part of the muscles of the cow's body after slaughter, includes meat that is often consumed. Meat is a suitable container for the growth of microbes. These things happen due to the high content of fat, protein, and water in it. Those high content compounds made the bacteria present in the meat, causing it to spoil quickly [1]. Good processing meat comes from meat with good physical quality so that the meat is suitable for consumption. The decline in the quality of meat caused by bacterial contamination negatively impacts health. At the time, slaughtering animals until the meat was consumed is one of the causes of bacterial contamination in meat. Carcass quality is badly influenced by the conditions of the environment and infrastructure at the time of slaughtering beef at the Slaughterhouse (RPH) [2]. The meat most likely can be contaminated by bacteria starting from Slaughterhouses (RPH) through traditional markets.

Directly contact with bacterial contamination by workers, ambient air, and unhygienic surfaces can occur in Slaughterhouses (RPH) [3]. The market is divided into two types of markets, namely modern markets and traditional markets, which are a means of transactions between sellers and buyers. Cleanliness, sanitation, buyer's economy, and Standard Operating Procedure (SOP) are things that are observed in the differences between the two types of markets [4]. Animal food products that contain bacteria are not safe for consumption, this can be referred to as *Food Borne Disease*, namely consuming food of animal origin that is dangerous that can cause disease [5]. Several bacteria often contaminate meat, one of which is *Salmonella sp* . and *Escherichia coli*. The causative etiologic agent *Salmonellosi*s in humans and animals is also found in *Salmonella sp*. The common enteric disease that can spread widely worldwide can be called *Salmonellosis* [6]. The most common symptoms are nausea, fever, chills, headache, throw up. This will appear after 8 to 72 hours of eating contaminated meat [7]. *Escherichia coli,* commonly abbreviated as *E.coli,* includes a significant species of gram-negative bacteria. *E. coli,* including facultative anaerobes. In general, *Escherichia coli bacteria* result in humans health problems, such as vomiting, diarrhoea, and other digestive issues [8]. The magnitude of the risk caused by the bacteria *Salmonella sp* and *Escherichia coli* made researchers interested in researching bacterial contamination of beef at Slaughterhouses (RPH) and Medan City Traditional Markets to maintain the quality of the meat and to ensure that it reaches consumers safely.

2. Materials and Methods

This research was carried out at the Slaughterhouse and 3 Traditional Markets in Medan City, which consisted of Pasar Brayan, Central Market Center, and MMTC Market and carried out bacterial testing at the Laboratory of Disease Investigation Research Center Regional Veterinary Center Region I Jl. Gen. Gatot Subroto No.255-A, Lalang, Medan Sunggal District, Medan City, North Sumatra. The implementation of this research took place in October – December 2021.

The tools used in this study were rubber gloves, sterile knives, paper labels, stationery, sterile white plastic (*bag stomacher*) for packing samples, *cool boxes*, *ice gel*, thermometers, *freezers*, spatulas, test tubes, Erlenmeyer, tube racks, Bunsen, lighter, petri dish, *incubator*, autoclave, sterile cabinet, analytical balance, cutting board, *biosafety*, mask, tip, micropipette, *cling wrap*, *aluminium foil, hot plate*, *vortex*, measuring cup, needle loop, *magnetic silver*, duster, *tissue, hockey stick*, tweezers, *mortar and pestle*.

Ingredients used are fresh beef on The quadriceps (*Shank*) were 16 samples obtained from the abattoir and three traditional markets in Medan City. The media for testing were a solution of

Buffered Pepton Water (BPW), *Chromocult Agar* (CA), *Lactose Broth (LB), Tetrathionate Broth (TTB), Xylose Lysine Deoxycholate Agar (XLD), Rambach Agar*, Aquadest, and 70% Alcohol.

2.1. Research Design

The research was conducted using an experimental method using contamination from the bacteria *Salmonella sp.* and *Escherichia coli* in beef at the Slaughterhouse and Traditional Market in Medan City. The p*urposive sampling method was* used to study research samples totaling 16 samples. Information about the researcher's meat condition was obtained with direct observation at the sampling time. The data was observed and observed directly based on the place's cleanliness, the general condition of the workers involved, the environment around the cutting site, and the equipment used [9].

2.2. Research Implementation

the research was carried out with two activities; the first was activities that occurred directly in the field. Then the second activity was a procedure in the laboratory when the researchers tested the contamination of *Salmonella sp.* and *Escherichia coli.*

2.3. Procedures in the Field

Prepare the tools used, such as rubber gloves, sterile knives, white plastic, label paper, and pens. Samples that have been researched will then be put into sterile white plastic that, given the date and observation time labeled of the sample and then stored in the *coolbox*. Beef sampling was carried out at the Medan City Slaughterhouse and 3 Traditional Markets (Brayan Market, Central Market Center, and MMTC Market). Fresh beef samples at the Medan City RPH were carried out at 03.00 WIB, totaling four samples, namely two samples for the *Salmonella sp*. and two pieces for the *Escherichia coli bacteria test.* Fresh beef from 3 Traditional Markets was taken simultaneously at 07.00 WIB, totaling 12 samples consisting of 4 pieces at Brayan Market, four samples at MMTC Market, four samples at the Central Market Center, namely six samples for the test of *Salmonella sp* and six samples for *Escherichia coli bacteria test* . The part of the sample taken is the quadriceps (*Shank*).

2.4. Procedures in the Laboratory

Testing *for Salmonella sp.* (SNI 2897:2008)

- Pre-enrichment (pre-enrinchment)
- Enrichment

- Isolation and Identification

***Escherichia coli* testing (SNI 2897:2008)**

- Making a solution of *Buffered Peptone Water* (*BPW*).
- Preparation of *Chromocult Agar* (*CA*) Media
- Dilution
- Colony number analysis

2.5. Data analysis

Observational data is in the form of tables and uses descriptive analysis. Then the results obtained were compared with the standards for microbiological quality requirements of the Indonesian National Beef (SNI) 3932:2008, namely *Salmonella sp* negative 25 g/sample, to see the difference in the number of *Escherichia coli* found in fresh beef at the Medan City Hospital and Medan City Traditional Market, followed by a paired T-Test Differential Test. Different test Paired t-test is the object of the study, which is subjected to two other treatments which will produce two samples of the primary treatment and also the second or also called the data contained in the second sample is the result of changes from the first sample data [10]

With a linear model [11]:

$$t : \frac{\bar{\bar{d}}}{S\bar{d}}$$

Information :

t : Value t count

$\bar{d}$: Average difference of 2 mean/mean

$S\bar{d}$: Standard deviation difference difference

Decision Rules :

- If t-count > t-table: Significantly different (Ho is rejected)
- If t-count < t-table: Not significantly different (Ho is accepted)

3. Results and Discussion

3.1. Hygiene and Sanitation Information and Conditions

Table 3. Results of observation of information and conditions of RPH and Traditional Markets

	Criteria	Beef Pick Up Location			
		RPH	Brayan Market	Central Market Center	MMTC Market
Sanitation	Water for cleaning right meat	The running water is clean	Water in the basin	Water in the basin	Water in the basin
	Water for cleaning right equipment	Flowing and clean water	Water in the basin	Water in the basin	Water in the basin
	Worker Cleanliness/ Beef seller	Some use aprons and wear boots but do not use gloves	Do not use Apron and gloves	Using an apron but not wearing gloves	Do not use Apron and gloves
Equipment	Cutting Mat	Porcelain/ ceramic	Wooden Cutting Board	Wooden Cutting Board	Wooden Cutting Board
	Cleanliness	Clean but not sterilized	Not clean and not sterilized	Clean and not sterilized	Clean and not sterilized
keeper-Masan	Packaging used to wrap	Use sack or plastic and transported in open trucks when taken from the RPH to the Traditional Market	Using a plastic bag	Using a plastic bag	Using a plastic bag

	Criteria	Beef Pick Up Location			
		RPH	Brayan Market	Central Market Center	MMTC Market
	The environment around the RPH or Traditional Market	Clean	Not clean enough	Pretty Clean	Pretty Clean

	Floor Cleaning	Clean floor	The table is not clean	Table clean	Table clean
Floor/ Table	Floor used for selling	Porcelain/ ceramic	Porcelain/ Cracked ceramic	Porcelain/ ceramic	Porcelain/ ceramic
Condition Location	setting	Separate place for slaughtering and cleaning of carcass and place for removing innards cow	In place- right next door to other essential grocery sellers	Placed in a special place with beef sellers only	In place- right next door to other basic grocery sellers

Based on “Table 3”, the conditions in the RPH in terms of sanitation and equipment are good, with the floor used being porcelain/ceramic, which makes the floor easy to clean. So that it can reduce bacterial contamination that sticks to the bottom, cleaning the floor in the RPH can be done by flushing the floor using a clean water hose that has heavy pressure. Clean water has many functions for the sanitation process in the slaughter room, and clean water is also used for washing carcasses and offal. [12] If the water is intended for washing carcasses, it must use water that is drinkable, clean, and has heavy pressure because if the water is not clean, it can cause bacterial contamination in processed slaughterhouse meat so that the meat becomes of poor quality and hygienic.

Figure 1. Medan City Slaughterhouse

The tools used in the RPH are already good and clean, because the equipment is one of the factors causing bacterial contamination in beef. [13] Bacterial contamination, which is found in meat and

slaughtering animals processing, comes from tools such as blades or tools used in splitting and hooks.

Due to skinning process, removal of viscera, carcass cleavage, storage of contamination is unavoidable [14]. In addition when the viscera are removed and the carcass is split open, the rumen and intestinal fluids spills can make meat contaminated with bacteria [15]. The condition of beef traders in Brayan Market and MMTC Market is placed in an open location. The arrangement of its merchandise are mixed and side by side with other sellers of essential commodities. The Central Market Center is where the sellers are placed in a secure location, and the conditions for the arrangement of the traders tend to be more neatly arranged in a particular area for meat sellers. The cleanliness and sanitation of the sellers in the Brayan Market are not clean because the area around the market still has garbage piled up around the cattle traders. At the same time, the MMTC Market is also not completely clean due to puddles of water around the beef selling area.

3.2. Detection of *Salmonella sp Bacteria Contamination* in Beef

Table 4. Results of Testing the Amount of *Salmonella sp bacteria contamination* in beef

Name	Sample Code	Test Type	Results	SNI standard	Information
			Slaughterhouse		
	RPH 1	.	Negative	< BMCM	
	RPH 2		Negative	< BMCM	
			Brayan Market		
	PB 1		**Positive**	**>BMCM**	
	PB 2		**Positive**	**>BMCM**	
			Central Market Center *Salmonella sp.* Negative/ 25 gr		
	PS 1		Negative	< BMCM	
	PS 2		Negative	< BMCM	
			MMTC Market		
	PM 1		Negative	< BMCM	
	PM 2		Negative	< BMCM	

Source: Medan City Veterinary Center Laboratory
Note: BMCM = Minimum Limit of Microbial Contamination

Sample results were positive for *Salmonella sp.* The results obtained from microbiological testing on *Xylose Lysine Deoxycholate Agar* and *Rambach Agar media* were the emergence of new pink colonies and had a shiny black core. [16] If Bacteria *Salmonella sp. was suspected to* be positive from *XLD agar* media, made pink large or there was no shiny black core found. The bacteria *Salmonella sp.* can ferment *xylose,* decarboxylate *lysine* and produce *hydrogen sulfide* from sodium thiosulfate.

Figure 2. Positive results for *Salmonella sp* . on *Xylose Lysine Deoxycholate Agar* and *Rambach Agar media*

Based on “Table 3” above, it can be concluded that *hygiene* and sanitation in Brayan Market are not entirely following the standards that have been set regarding healthy markets compared to other observed markets, such as the MMTC Market and Central Market Center, and RPH. Due to the lack of sanitation equipment used in cutting meat, such as cutting boards used are still made of wood, traders who have not used aprons and equipment that is not clean. According to Decree of the Ministry of Health of the Republic of Indonesia Number 519/MENKES/SK/VI/2008, which states that: requirements for the use of equipment such as cutting tools (cutting boards) are not made of wood, the existence of a special place for washing food and utensils, the availability of handwashing places, trash cans, sellers who are free of *vectors of* dangerous disease transmission, and lastly, sellers must wear head coverings, wear gloves and also an apron.

Figure 3. Equipment for beef slaughter at Brayan Market

Salmonella sp contamination in traditional markets is caused by poor *hygiene* and environmental sanitation around the market [17]. That is why the contamination of *Salmonella sp* in Brayan Market is high. There is *Salmonella sp.* existence on meat from Brayan Market, which is weirdly closer to RPH than other traditional markets. The positive ones in Brayan Market are also affected by poor butcher's *hygiene* and poor quality of cleanliness around the seller's table. The butcher should be concern on personal *hygiene* and sanitation. It will improve sanitation around butchers like cleanliness of cutting tools and the availability of clean water, which is the most important thing for washing hands before cutting and after cutting meat [18].

3.3. Detection of *Escherichia coli Bacterial Contamination* in Beef

Table 5. Results of Testing for the Amount of *Escherichia coli Bacterial Contamination* in Beef

Name	Sample Code	Test Type	Results	SNI standard (CFU/g)	Information
			Slaughterhouse		
	RPH 1		$0.7x10^1$ cfu/g	< BMCM	
	RPH 2		$2.275x10^3$ cfu/g	**>BMCM**	
			Brayan Market		
	PB 1		$2.93x10^3$ cfu/g	**>BMCM**	
	PB 2		$5.56x10^3$ cfu/g	**>BMCM**	
			Escherichia coli $1x10^1$		
			Central Market Center		
	PS 1		$3.1 x10^3$ cfu/g	**>BMCM**	
	PS 2		$5.6 x10^3$ cfu/g	**>BMCM**	
			MMTC Market		
	PM 1		$3.173x10^4$ cfu/g	**>BMCM**	
	PM 2		$2.973x10^4$ cfu/g	**>BMCM**	

Source: Medan City Veterinary Center Laboratory

Note: BMCM = Minimum Limit of Microbial Contamination

Based on "Table 5" the examination result of eight meat from the Slaughterhouse and Traditional Market in Medan City had been High exposed to microbial contamination showed the presence of *Escherichia coli* of 7 samples from RPH 2, Brayan Market, Central Market Center, and MMTC Market, while those that did not exceed were one sample from RPH 1. Positive result of *Escherichia coli* from microbiological tested using *Chromocult Agar (CA) media* which was marked by the presence of purple spots. [19] the principle of the test using *Chromocult Agar (CA) media* is to detect glucuronidase's activity, which is commonly used to differentiate *E.coli* and *Coliform* bacteria. *E. coli* has a uidA *gene* that can be *encoded* in *the* -glucuronidase *enzyme*, while *Coliform* bacteria have *lacZ gene*, which causes it to be *encoded in the* -glucuronidase enzyme. As a result, *Escherichia coli bacteria* can produce a purple color because it can bind all chromogens.

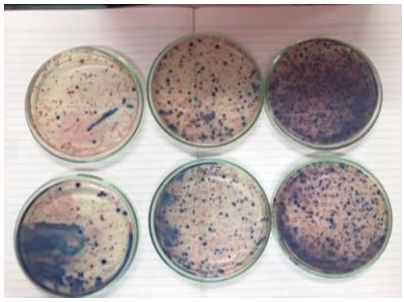

Figure 4. *Chromocult Agar (CA)* media with positive bacteria *Escherichia coli*

The test results using *Chromocult* agar medium are the presence of isolates and can calculate *Enterobacteriaceae* from the sample. This medium has been shown to be effective in identifying *E.coli* and *Coliforms* in the environment without the need for further biochemical tests to confirm [20].

Table 6. Calculation results of *Escherichia coli* t-test at RPH and Brayan Traditional Market

Sample No	Sample Origin		mean	Paired t-test T-count result	t-table (0.05), db 1
	RPH	Brayan Market			
1	$0.7x10^1$ cfu/g	$2.93x10^3$ cfu/g	2923	13.93363	12.71
2	$2.275x10^3$ cfu/g	$5.56x10^3$ cfu/g	3375		

Source: Processed data

We can see from "Table 6" that the results of the t-count will be greater than the t-table. It can be concluded that if there is a real or significant difference in contamination from bacteria in beef samples at the RPH with Brayan Market (close distance) with a t-count value (13.93363) > t-table value (12.71).

Table 7. Calculation results of *Escherichia coli* t-test at RPH and Central Market Traditional Market

Sample No	Sample Origin		mean	Paired t-test T-count result	t-table (0.05), db 1
	RPH	P.Central Market		27.66379	12.71
1	$0.7x10^1$ cfu/g	$3.1x10^3$ cfu/g	3039		
2	$2.275x10^3$ cfu/g	$5.6x10^3$ cfu/g	3325		

Source: Processed dat

Based on "Table 7" it could be seen that the t-count will be greater in number compared to the t-table. It is concluded that if there is a real or significant difference in beef bacterial contamination at the RPH with the Central Market Center (medium distance. The t-count value (27.66379) > the t-table value (12.71).

Table 8. Calculation Results of *Escherichia coli t-test* at RPH and MMTC Traditional Market

Sample No	Origin of Sanpel		mean	Paired t-test T-count result	t-table (0.05), db 1
	RPH	MMTC Market			
1	$0.7x10^1$ cfu/g	$3.173x10^4$ cfu/g	31723	13.86551	12.71
2	$2.275x10^3$ cfu/g	$2.973x10^4$ cfu/g	27455		

Source: Processed data

The results from "Table 8" above show that t-count results will be greater than the t-table. The conclusion is found if there is a real or significant difference in bacterial contamination in beef samples at the RPH with the MMTC market (far away) with an at-count value (13.86551) > t-table value (12.71). From Tables 6, 7, and 8 it can be concluded that between the RPH and markets that are close, medium, and far from the RPH, namely Brayan Market, Central Market Center, and MMTC Market, bacterial contamination does not only come from the abattoir but environmental conditions in the area. Traditional markets significantly affect the amount of bacterial contamination in beef. [21] If the selling environment of beef affects the intensity of *Escherichia coli* contamination and really need to pay attention to the condition of the meat market. When on the market, the low probability of contamination from Slaughterhouses provides many opportunities for *Escherichia coli* contamination in beef to breed.

One of the factors that influence the contamination of *Escherichia coli bacteria* is the means of transportation and environmental conditions. [22] If there are stages transporting meat from the slaughterhouse directly to the traditional market can also result in contamination of the meat , a lot of meat being transported uses an open car and is not given a base or only wrapped in jute/plastic and not given an additional cover so that the meat will be more susceptible to contamination from bacteria.

Figure 5. Transport vehicles

How long is the cutting time? And the time of purchase by consumers can affect the amount of bacterial spread. Meat contaminated with bacteria from abattoirs to traditional markets can grow

bacteria that will split cells until the meat is stored [23]. The level of *Escherichia coli* contamination in seven samples from eight beef samples at RPH and Traditional Markets in Medan City. This shows that the development of *Escherichia coli* contamination in beef can be said to exceed the standard so that it can interfere with the digestive system in humans and can cause disease. [24] *Escherichia coli* is a cause of diarrheal disease that spreads very quickly.

3.4. Prevention of Microorganism Contamination

[25] In maintaining the quality of food , there is much to be seen in order to reduce the impact of microbial contamination on foodstuffs are :

1) Increase knowledge about natural ecology and epidemiology used in determining reliable diagnostic methods ,
2) Selecting critical points of contamination that enter the food chain of animal origin,
3) Increase knowledge , care and awareness in the community sensitive to diseases caused by microbial contamination, and
4) Create networks and improve coordination with related agencies/agencies.

To prevent things that make infection can be done by :

- cooks meat, as well as ground beef.
- Stay away from unpasteurized milk.
- Before and after cutting beef, hands are washed first
- use clean tools and surfaces to prepare ingredients
- Wash every utensil that has been used, be it boards or cutting tools thoroughly using hot soapy water and rinse before using it again.

Good sanitation to prevent the development of microorganism contamination is by processing beef with a temperature of around 65°C or more, and reheating the dish; then, the part of the beef that has not been consumed would be better stored the beef in the refrigerator at a temperature less 4°C. These things are a way to control the growth of microorganisms [26].

4. Conclusion

From the research results that have been carried out by researchers, it is concluded that beef in the RPH and Traditional Markets in Medan City has positive results of *Salmonella sp*

contamination. Namely, those from the Brayan Market, while the negative results of *Salmonella sp contamination* were those from the RPH, Central Market Center and MMTC Market.

Beef that has *Escherichia coli contamination* above the Maximum Microorganism Contamination Limit (BMCM) is from RPH 2, Brayan Market, Central Market Center and MMTC Market, while those with *Escherichia coli contamination* are below the Maximum Microorganism Contamination Limit (BMCM) which comes from RPH 1.

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