Open Access

Hasanuddin J. Anim. Sci.
Vol. 2, No. 2:76-82 November 2020
pISSN 2621-9182 eISSN 2621-9190

Evaluation of Pathogenic Contamination of The Liver and Meat From Traditional Markets In Makassar

Ratmawati Malaka[1*], Kusumandari Indah Prahesti[1], Hajrawati[1]

[1] Department of Animal Production, Faculty of Animal Science, Hasanuddin University
Jalan Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia

*Corresponding author: E-mail: malaka_ag39@yahoo.co.id

Abstract

Many factors influence the growth of microorganisms on and in meat, including temperature, moisture content, oxygen, pH, and meat nutrition. Meat is a medium that is very suitable for the growth of microorganisms because it has a high water content of 68-75%, high nutrition, complete mineral content and a pH 5.3 - 6.5. Contamination of microorganisms on the surface of meat or carcass has started since the slaughter of livestock until the meat is consumed. The purpose of this study was to evaluate the presence of microorganism contamination in meat sold in traditional markets in Makassar. Beef and beef liver were taken from traditional markets in Makassar and then tested for Total Bacteria, *Salmonella* sp. as an indicator of pathogenic bacteria contamination in meat. The total bacterial contamination of beef and beef liver was still higher than the provisions of the Indonesian National Standard and the bacteria that could be suspected were *Salmonella* sp.

Keywords : Meat, microorganism, *Salmonella*, traditional market

INTRODUCTION

Microorganisms found in meat can originate from live livestock infections, and post mortem meat contamination (Mohamed, 2007). Sources of contamination or infection can come from the slaughterhouse can come from the surrounding soil, skin, contents of the digestive tract, water, the tools used during the carcass preparation process (for example knives, saws, pulleys and hooks, and tools for viscera), floors, dirt in the air and also slaughterhouse workers (Diyantoro and Wardhana, 2019). Meat from slaughterhouses distributed to traditional markets will experience additional contamination from air, dust or hands from sellers and buyers in the market (Rustam *et al*., 2019; Martiana *et al.*, 2020).

The initial contamination of meat can come from microorganisms that enter the blood stream at the time of slaughter if the tools used for removing blood are not sterile because the blood is still circulating some time after slaughtering (Diyantoro and Wardhana, 2019; Mohamed, 2007). Subsequent contamination can occur through the surface of the meat during meat preparation operations, namely the process of cleavage of the carcass, cooling, cutting of

carcass/meat, storage, distribution, and others (Sohaib *et al.*, 2016). Anything that comes into contact with meat directly or indirectly can be a source of microbial contamination (Haruni *et al*., 2019). To overcome or reduce this contamination requires hygienic handling of meat with the best possible sanitation system (Aburi, 2012). Microbial contamination of meat will determine the quality and shelf life of meat and processed meat (Lee, 2018). This study aims to obtain an overview of the microorganisms in meat and liver circulating in traditional markets in Makassar, South Sulawesi, Indonesia. This research is very important considering that all consumers' health depends on the food they consume which is included in the realm of veterinary public health.

MATERIALS AND METHODS

Sample collection

Meat and beef liver 10 g are taken from 3 traditional markets in Makassar. Meat is put in a cold box and transported to the microbiology laboratory of the Faculty of Animal Husbandry, Hasanuddin University, Makassar.

Total Plate count

Twenty-five grams of beef and beef liver were put in a stomacher and mixed with 225 ml of physiological NaCl (1:10 dilution), then again diluted in series and each dilution was taken 1 ml and put into a sterile petri dish. Plate Count For a sterile agar with a temperature of 50°C, it is poured into the petri dish while shaking it. After the agar has solidified, it is incubated at 37°C for 20-24 hours in an inverted position. The growing colonies were then counted using a colony counter (Montville and Matthews, 2008).

Isolation and Identification of *salmonella* sp.

The meat and liver were sterilized on the outside with a spatel, then cut into 1 cm^3 width with sterile scissors. A total of 25 g was added to 225 ml of pre-enrichment media and peptone water, and incubated at 35-37^oC for 16-20 hours. After that, the sample was inoculated on the Selenite Cystein Broth and tetra thyonate enrichment medium and incubated at 35 – 37^oC for 18-24 hours. From this enrichment medium then etched on Selenite BPLS and XLD agar and incubated at 35 - 37^oC for 20-24 hours

Biochemical Identification

Colonies characterizing Salmonella were grown on TSIA and LIA and incubated at 35-37^oC for 20-24 hours (Mikoleit, 2015).

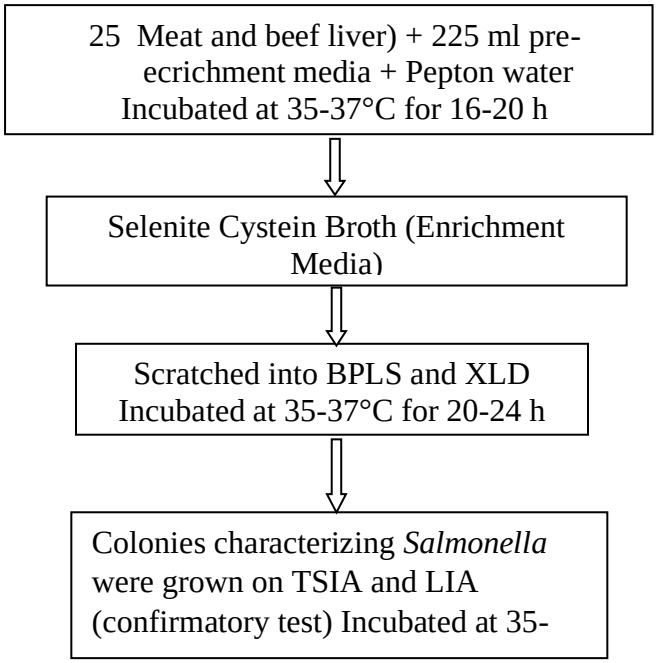

Figure 1. The flow of examination for bacteria in meat and beef liver

RESULTS AND DISCUSSION

The microflora in meat varies, where the bacteria can be contaminated from livestock with the environment and the various ways of raising animals and post-harvest processing with varied feed sources (Lulietto *et al*., 2016; Alegbeleye *et al*., 2018). These meat micro flora may be mesophilic bacteria originating from the animal itself or bacteria from the soil or floors of slaughterhouses, from workers in slaughterhouses or markets, from the air, and from equipment during processing (Diyantoro, and Wardhana, 2019; Prattis *et al*., 2015).

Table 1. Total Plate Count Agar from meat and beef liver samples from traditional markets in Makassar

Sample	10^{-4}
Meat	ICBC (>300)
Beef Liver	ICBC (>300)

Note: ICBC= It Cannot be counted

In Table 1, data on the total number of bacteria in beef and beef liver is more than 3,000,000 or 3 x 10^6 / g. This result exceeds the requirements of the Indonesian National Standard (INS) 7388-2009. This indicates that the number of microbes in beef and beef liver is more than the maximum limit in INS, indicating that the level of sanitation during meat processing is still many sources and critical points that allow contamination. The surface of freshly slaughtered meat usually contains about 10^2-10^4 bacteria / inch², consisting mainly of

mesophilic bacteria which probably originates from contamination from the digestive tract and from the skin (Harlia, e*t al*., 2017). The number of mesophilic bacteria in raw meat indicates sanitation at the time of slaughter (Jaja, *et al*., 2018). The types of bacteria that are often found in fresh meat include coliform, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, *Salmonella*, and *Enterococci* (Bintsis, 2017).

Isolation and Identification of *Salmonella* sp.

In BPLS media, 2 kinds of colonies grow, namely yellow with a yellow zone around the colony which indicates that the bacteria ferments lactose or sucrose so that it is suspected that the bacteria are *Escherichia coli*, *Enterobacter*, or maybe *Citrobacter*, *Klebsiella*, *Citrobacter* or other enteroctericeae (Chauhan *et al*., 2013). While the other colonies are red with a bright red zone around the colony which indicates that the bacteria do not ferment lactose and sucrose so that the bacteria are thought to be *Salmonella*, *Proteus*, or *Pseudomonas*.

BPLS media is a selective medium for the identification of *Salmonella* (except *S. typhosa*) in meat, meat products, and other food ingredients. This medium contains sodium deoxycholate which specifically inhibits the growth of *Proteus* whose colonies spread. The composition of this medium is peptone from meat 10.0; meat extract 5.0; yeast extract 3.0; disodium hydrogen phosphate 1,0; sodium hydrogen phosphate 0.6; lactose 10.0; sucrose 10.0; phenol red 0.09; brilliant green 0.0047 and order 12.0. If the growing colony is red surrounded by a bright red zone, the microorganisms do not ferment lactose and sucrose (*Salmonella*, *Proteus*, *Pseudomonas*). If the colony is yellow surrounded by a yellow zone, the bacteria are fermenting lactose and sucrose (*Escherichia coli*, *Enterobacter*, *Klebsiella*).

This medium contains lactose, so that if the bacteria can fertilize lactose it will be degraded into acids as indicated by the phenol red pH indicator which changes color to yellow. The indicator is generally a dark red color in the alkaline range. The growth of Gram-positive microbial flora accompanying the samples, for example, *Salmonella typhi* and *Shigella* was largely inhibited by Brilliant-green. *Salmonella* growth, however, is enhanced by a richer nutrient substrate medium. The increase in the growth of the microorganisms that accompany it is greatly prevented by increasing the concentration of Brilliant-Green. Salmonellae cannot ferment lactose or sucrose.

Table 2. The characteristics of the colonies that grew on BPLS and XLD media from meat samples from traditional markets in Makassar

Media	Coloni	Coloni Characteristics
BPLS	1	Yellow color, flat edge, convex surface, shiny, yellow zone formed around the colony
	2	Red color, flat edge, convex, shiny, a bright red zone is formed around the colony
XLD	1	The colony color is black, flat edge, convex, shiny, there is no zone around the colony.
	2	The colony color is yellow and a yellow zone is formed around the colony, opaque.
	3	Colonies are light yellow in color, opaque, and a yellow zone is formed around the colony, and precipitation is formed around the colony

BPLS : Brilliant-green Phenol-red Lactose Sucrose Agar
XLD : Xylose Lysine Deoxyclolate

On XLD media (Xylose Lysine Deoxycholate Agar) three kinds of colonies were grown. The first colony is a yellow colony with a black center and does not form a zone around the colony that characterizes *Salmonella*. The second colony is a yellow colony with a yellow zone, opaque which characterizes *Klebsiella*, *Serratia,* or *Hafnia*. Whereas in the 3rd colony the colony was cream (light yellow), opaque and there was precipitation around the colony which characterized *Escherichia coli*, *Enterobacter* or *Aeromonas*.

XLD media is a specific medium for isolating and differentiating pathogenic enterobactericeae, particularly *Shigella* spp. and *Salmonella* spp. (Park *et al*., 2012). The degradation of xylose, lactose, and sucrose into acids causes the red phenol to turn yellow. H_2S production is an indication that the thiosulfate and iron (III) salt reacts causing black precipitation of iron sulfide in the colony. Bacteria that decarboxylase lysine cause lysine to cause a purple color around the colony due to an increase in pH. A longer incubation it can cause the yellow discoloration to turn red.

Biochemical Identification

The results of the biochemical identification can be seen in Table 3. Colonies that grew on XLD and BPLS media characterized *Salmonella* sp. planted on TSIA and LIA media. In TSIA media, it turns out that the butt shows an acid reaction which indicates that the bacteria ferments glucose and the slant is red which means it does not ferment lactose and sucrose. Besides that, there is the production of H_2S (forming a black color) so that the bacteria is thought to be *S. typhosa* or *S. gallinarum.*

Table 3. The characteristics of the expected bacteria *Salmonella* sp. on TSIA and LIA media

Media		Butt	Slant	H_2S	Gas
TSIA	I	Black	Red	+	-
	II	Black	Red	+	-
LIA	I	Yellow	Red	-	-
	II	Yellow	Red	-	-

Note: TSIA (Triple Sugar Iron Agar)
LIA (Lysine Iron Agar)

The LIA medium on Butt shows a yellow color (glucose fermentation) and Slat is red and no H_2S is formed so that it is suspected that the bacteria is Proteus (Parija, 2012). For a more accurate confirmation test to the species and subspecies level, DNA extraction by PCR and sequencing can be followed to confirm the contaminant bacteria.

CONCLUSION

Evaluation of the beef and meat liver collected from traditional markets in Makassar city, in terms of microbiological quality, the meat and liver contain a number of microbes that exceed the Indonesian National standard, and are suspected to be containing pathogenic bacteria. Based on the results of colony observation and biochemical characteristics, that bacteria were identified. Therefore, to process the meat until it is ready for consumption, it must go through the correct cooking process so that the nutritional value contained in the meat reaches the consumer's table.

REFERENCES

Alegbeleye, O. O., I. Singleton, and A. S. San'Ana. 2018. Sources and contamination routes of microbial pathogen to fresh produce during field cultivation: A review. Food Microbiol. 73: 177-208.

Aburi, P. A. S. 2012. Assesment of Hygiene Practice Used by Small Butchers and Slaughter Slabs in Beef Value Chain in Juba Town-South Sudan. University of Applied Science.Van Hall Larenstein. Sudan.

Bintsis, T. 2017. Food borne pathogens. AIMS Microbiol. 3(3):529-563. Doi: 10.3934/microbiol.2017.3.529

Chaucan, S., A. Saini, D. P. Singh, U. Dhaked, and P. Gupta. 2013. Studies on microbiological quality of sprouts of mung bean (*Vigna radiate* L). J. Drug Deliv. Ther. 3(6): 44-50.

Diyantoro and D. K. Wardhana. 2019. Risk factors for bacterial contamination of bovine meat during slaughter in ten Indonesian Abattoar. Vet. Med. Int. 2019:1-6. Doi: https://doi.org/10.1155/2019/2/2707064

Harlia, E., D. Suryanto, N. Teguh, and K. N. Rahmah. 2017. Food safety on meat products based on coliform contamination. The 7th International Seminar on Tropical Animal Production Contribution of Livestock Production on Food Sovereighty in tropical Countries. September 12-14, Yokyakarta, Indonesia.

Haruni, S. R. Malaka, and H. M. Ali. 2019. Prevalence of Microbial Contamination and Antibiotic Residue in Chicken Meat, Beef, and Offal in South Sulawesi. Hasanuddin J. Anim. Sci. 1(1): 1-11. https://doi.org/10.20956/hajas.v1i1.6649

Jaja, I. F., E. Green, and V. Muchenje. 2018. Aerobic mesophilic, coliform, Escherichia coli, and Staphylococcus aureus counts of raw meat from the formal and informal meat sectors in South Africa. Int. J. Enrivon. Res. Public. Health. 15(4):819 https://doi.org/10.3390/ijerph15040819

Lee, K. T. 2018. Shelf-life Extension of Fresh and Processed Meat Products By Various Packaging Applications. The 7thInternational Seminar on Tropical Animal Production Contribution of Livestock Production on Food Sovereignty in Tropical Countries September 12-14, Yogyakarta, Indonesia.

Lulietto, M. F., P. Sechi, E. Borgogni, and B. T. Cenci-Goga. 2016. Meat Spoilage: a critical review of a neglected alteration due to ropy slime producing bacteria. Ital. J. Anim. Sci. 14(3):316-326. Doi: Https://doi.org/10.4081/ijas.2015.4011.

Martiana, A., I. I. Arief, H. Nuraini, and E. Taufik. 2020. The quality of Bali beef from East Nusa Tenggara during distribution process from slauhterhouse to consumers. JIPTHP 8(1): 8-14. https://doi.org/10.29244/jipthp.8.1.8-14

Mikoleit, M. L. 2015. Laboratory Protocol: Biochemical Identification of Salmonella and Shigella using an Abbreviated Panel of Tests. WHO Global Foodborne Infections Network. USA

Mohamed, E. A. A. 2007.The Microbiological Load of Fresh and Processed Meat in Khartoum State-Sudan. Thesis of Philosophy Doctor. University of Khartoun, Faculty of Veterinary Medicine. Sudan.

Montville, T., and K. R. Matthews. 2008. Food Microbiology. An Introduction.2nd edition. ASM Press, Wahington, DC.

Odeyemi, O. A., O. O. Alegbeleye, M. Strateva, and D. Stratev. 2019. Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. Compr. Rev. Food Sci. Food Saf. 19:311-331. Doi:https://doi.org/10.1111/1541-4337.12526

Parija, S. C. 2012. Textbook of Microbiology & Immunology. 2nd edition. Elsevier. India.

Park, S-H., S. Ryu, and D. H. Kang. 2012. Development of an improved selective and differential medium for isolation of *Salmonella* spp. J. Clin. Microbiol. 50(10):3222-3226. Doi: 10.1128/JCM.01228-12

Prattis, S. M., H. A. Shaib, R. Zgheib, A. Raad, Z. A. Hassan, and R. Rifai. 2015. Microbiome populations in Lebanese Slaughterhouse setting. SOJ Vet. Sci. 1(1):1-14. http://dx.doi.org/10.15226/2381-2907/1/1/00101

Rustam, M. A. Tamal, and J. Ariansyah. 2019. Existence of Salmonella sp. in Beef Meat in Sangatta, Kutai Timur District. Hasanuddin J. Anim. Sci. 1(2): 10-14. Doi: https://doi.org/10.20956/hajas.v1i2.7450

Sohaib, M., F. M. Anjum, M. S. Arshad, and U. U. Rahman. 2016. Postharvest intervention technologies for safety enhancement of meat and meat based products; a critical review. J. Food Sci. Technol. 53(1):19-30. Doi: https://doi.org/10.1007/s13197-015-1985-y