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Isolation and Identification of Shigella Sp. in Cattle Feces in Tamangapa Final Disposal Place, Makassar City

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Abstract

Beef cattle are one of the most widely developed types of fauna by the people of Indonesia because they are supported by Indonesia's land area which reaches 1,922,570 km² and has a favorable climate, namely a tropical climate. However, this does not apply to urban areas where limited grazing land is one of the reasons urban breeders graze cattle in final disposal site (TPA) as is done by the people around the Tamangapa TPA, Makassar City. One of the impacts of grazing cattle in the final disposal site is contamination of the cow feed with various microorganisms such as *Shigella* sp bacteria which can cause *shigellosis*. *Shigellosis* is a zoonotic disease and can be transmitted through contaminated food such as raw eggs, raw meat, vegetables or from contaminated water. Diarrhea in toddlers 26.7% is caused by *Shigella sp*. This study aims to isolate and identify *Shigella sp.* in cow feces at the Tamangapa final disposal site (TPA) in Makassar City. The samples taken were 28 samples from 17 farms around the TPA Tamangapa Makassar City. Samples that have been taken are then stored in a transport medium and then the isolation and identification process is carried out using *Nutrient Agar* (NA) media, gram staining, *Salmonella Shigella Agar* (SSA) and biochemical tests which include TSIA, SIM, MR-VP, *urease* and *citrate*. The results showed the level of infection with *Shigella sp*. in the feces of cows grazed around the TPA Tamangapa Makassar City was low where of the 28 stool samples tested, only 2 samples (7%) were positively contaminated with *Shigella sp*

Keywords: Shigella sp, Cattle Feces, Shigellosis, TPA Tamangapa

Introduction

Beef cattle are one of the most widely developed types of fauna by the people of Indonesia. Indonesia's land area of around 1,922,570 km² really supports the community to develop beef cattle farming business. Indonesia's climate is also very supportive of livestock business development because in the tropical climate it experiences rainy and summer seasons every year, making it very easy to fulfill forage and water for beef cattle farming. One of the provinces with the largest number of beef cattle in Indonesia is South Sulawesi Province with around 1,434,999 beef cattle in 2017 (Martini and Wandi, 2019).

The breeding methods carried out by most people's farms in Indonesia are still very traditional because most of the livestock business carried out is a sideline business with

limited funds so that breeders cannot make appropriate cages, provide proper feed and control animal health properly (Martini and Wandi, 2019). One of the raising methods of concern is the grazing of beef cattle in landfills (TPA) as was done at TPA Tamangapa, Makassar City. The limited grazing land in urban areas is one of the reasons urban farmers graze their cows in landfills (Nangkiawa et al., 2015).

Feeding that is not suitable for cattle can affect the productivity of the cow itself. Cattle can experience malnutrition as a result of the low quality of feed given so that cattle will be more susceptible to disease. One example of a disease that is often experienced by cattle due to inappropriate feeding is diarrhea. One of the causes of diarrheal disease in cattle is infection with the bacterium *Shigella sp.* which is characterized by the occurrence of acute watery diarrhea which is commonly called *Shigellosis*. *Shigellosis* is an acute disease and is endemic in the world, especially in developing countries because it is one of the causes of high morbidity and mortality in children (Zakwan *et al*., 2018).

Seeing the high morbidity and mortality rates caused by *Shigella sp*. This makes the authors feel the need to conduct research to detect the presence of *Shigella sp*. on the faeces of cows kept by the community around the TPA Tamangapa Makassar City. This is due to the close relationship between the community around the TPA Tamangapa Makassar City and the cows they raise because most of the cows are raised around the residents' yards. Therefore it is necessary to do research on the isolation and identification of *Shigella sp* in cow feces at TPA Tamangapa, Makassar City.

Meterials and Methods

Sample

The sample in this study were all cows that were grazed at the Tamangapa landfill in Makassar city and used landfill waste as the main food source. The number of cattle herders at TPA Tamangapa Makassar City is 17 people with a total of 971 cows. Calculation of the number of samples based on the 95% confidence level, the assumption of the prevalence of the disease with the consideration that there is no data related to cases of *Shigella sp*. infection in cattle in Makassar City and obtained a total sample of 28 samples.

Sample Collecting Method

To make sampling easier, the cows were first restrained using clamps. Stool samples are then taken by reaching directly using a sterile hand glove. The faeces were then put into the transport medium (MT) and given a tag (label) and then brought to the Integrated Laboratory of the Hasanuddin University Teaching Animal Hospital to be isolated and identified.

Tools and Materials

Tools: ose, object glass, bunsen, microscope, test tube, pipette, incubator, test tube rack, petri dish, oven, coolbox, erlenmeyer, clamp, rope, boots.
Materials: bovine faecal samples, transport Media, nutrient agar (NA), salmonella shigella agar (SSA), TSI media, SIM media, α-naphtol, KOH, urease media, Simmon's citrate media, MRVP media, immersion oil, NaCL solution, alcohol 95 %, methyl red reagent, voges proskauer reagent, kovacs reagent, crystal violet, aquadest, Lugol's solution, safranin, match, tissue, cotton, label paper, sterile swab, ice cubes, hand gloves, mask.

Isolation

The isolation process was carried out using Nutrient Agar media. Samples on the transport medium were taken using sterile loops and then scratched on the NA medium using 3 quadrant strokes. The scratched samples were then incubated for 24-48 hours at 34-37°C.

Identification

Gram Staining: The first step is to sterilize the glass object with 95% alcohol and pass it over a bunsen flame. Bacterial isolates were taken using a sterile ose and smeared as thinly as possible on an object glass. Do the fixation by passing the object glass over the bunsen flame. The first staining was carried out by dripping crystal violet until all the bacterial isolates that had been smeared were completely covered and let stand for 1 minute then washed using distilled water for 5 seconds. The second coloring is done using lugol, let stand for 1 minute.

The third staining is done using 95% alcohol. Fourth stain using safranin and let stand for 1 minute then rinse with water slowly for 5 seconds. The results of gram staining were observed by looking at the color that appeared on observation with a microscope. Bacteria that appear purple in color indicate gram-positive bacteria while those that appear pink indicate gram-negative bacteria.

***Salmonella Shigella Agar* (SSA):** Planting bacteria on SSA media was carried out by taking bacterial colonies that had grown on the rearing medium using sterile loops and scratching them on SSA media. The media was then incubated for 1x24 hours at 37^{0}C in the incubator.

Biochemical Test: In this study, 4 biochemical tests were carried out, namely Triple Sugar Iron Agar (TSIA), Sulfida Indol Motility (SIM), Methyl Red-Voges Proskauer (MR-VP), Urease and Citrate.

Data Presentation

Data were analyzed descriptively by observing the parameter in the form of the number of positive samples of *Shigella sp.*

Results and Discussion

The results showed that of the 28 stool samples tested, 2 of them were positive for *Shigella sp.* Besides *Shigella sp.*, there are several other bacteria that have been identified. Four samples were positively identified as containing *Salmonella sp.*, *Proteus vulgaris* and *E.Coli.*

Table 1. Isolation Results, Gram Staining and Embedding on SSA Media

No Sample	NA	Gram Staining Gram Characteristic	Shape	SSA
1	+	+	Basil	
2	+	+	Basil	
3	+	+	Basil	
4	+	+	Basil	
5	+	+	Basil	
6	+	-	Basil	White colony
7	+	-	Basil	White colony
8	+	+	Basil	
9	+	-	Basil	-
10	+	+	Basil	
11	+	-	Basil	-
12	+	+	Basil	
13	+	+	Basil	
14	+	-	Basil	Black colony
15	+	-	Basil	White colony
16	+	-	Basil	Black colony
17	+	+	Basil	
18	+	-	Basil	-
19	+	-	Basil	-

20	+	-	Basil	-
21	+	-	Basil	Black colony
22	+	-	Basil	-
23	+	-	Basil	-
24	+	+	Basil	
25	+	-	Basil	-
26	+	-	Basil	White colony
27	+	-	Basil	-
28	+	-	Basil	Pink colony

Samples that have been grown on SSA media and show the growth of clear or white colonies are followed by biochemical tests. The results of the biochemical test can be seen in the following table.

Table 2. Biochemical Test Results

No Sample	TSIA	H_2S	SIM	MR	VP	Urease	Citrate
6	K/A	-	-	+	-	-	-
7	A/A	+	+	+	-	+	+
15	K/A	-	-	+	-	-	-

Description: K/A: Glucose fermentation only, peptone catabolized; A/A: Glucose and lactose and/or sucrose fermentation.

Nutrient Agar is one of the most commonly used media for bacterial growth (Sakinah *et al.*, 2018). The NA media content can grow most types of bacteria because it is composed of 0.8% protein, 1.2% agar and the rest is water (Sinaga *et al.*, 2022). *Shigella sp.* It is characterized by convex, circular, transparent colonies with intact margins reaching a diameter of about 2 mm after 24 h incubation (Carroll *et al.*, 2016). Of the 28 samples grown on NA media, all showed bacterial growth with characteristics consistent with the colony morphology of *Shigella sp.*

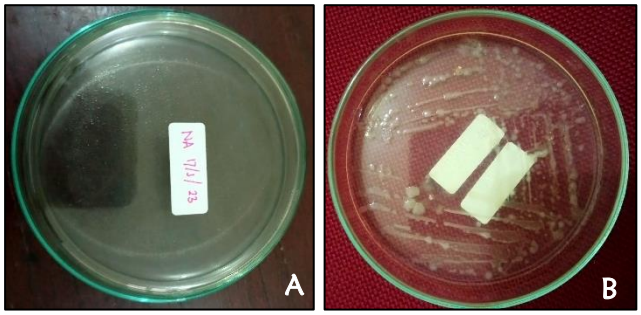

Fig. 1 NA medium. (A) before incubation, (B) after incubation

Bacterial colonies that had grown on NA media were followed by gram staining. *Shigella sp.* are gram-negative bacteria in the form of slender rods (Carroll *et al.*, 2016). Gram negative bacteria are characterized by the appearance of bacteria in the form of bacilli and are pink in color. Of the 28 samples that had been stained, 17 of them showed the characteristics of gram-negative bacteria and 11 of them showed gram-positive bacteria.

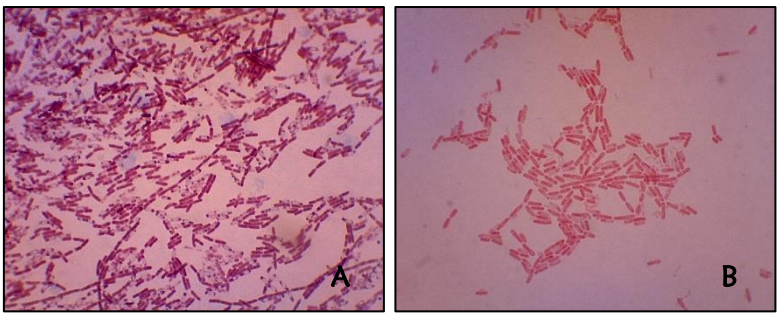

Fig. 2 Gram Staining. (A) Gram Positive Bacteria, (B) Gram Negative Bacteria

Gram staining aims to identify bacteria based on gram properties related to the physical and chemical properties of the cell wall of a bacterium. The difference between gram positive and negative bacteria lies in the nature of the cell wall where gram positive bacteria have a thick *peptidoglycan* content while gram negative bacteria have a cell wall composed of thick lipids. Gram-negative bacteria have a low affinity so that the crystal violet dye does not absorb into the cells and only leaves the safranin dye (Purwaningsih and Wulandari, 2021).

Salmonella shigella agar (SSA) is a selective medium used to isolate *Salmonella sp.* and *Shigella sp.* originating from various samples such as faecal, urine and food samples (Fatiqin *et al.*, 2019). SSA media consists of various components that play a role in selectivity such as lactose, peptone, bile salts, iron (III) citrate and red retusal indicator. The selectivity of bacteria on SSA media is based on their metabolic abilities (Aini, 2018). The growth of *Shigella sp.* on SSA media characterized by smooth and translucent white colonies. Of the 17 samples that had been planted on SSA media, 3 of them showed the growth of Shigella sp.

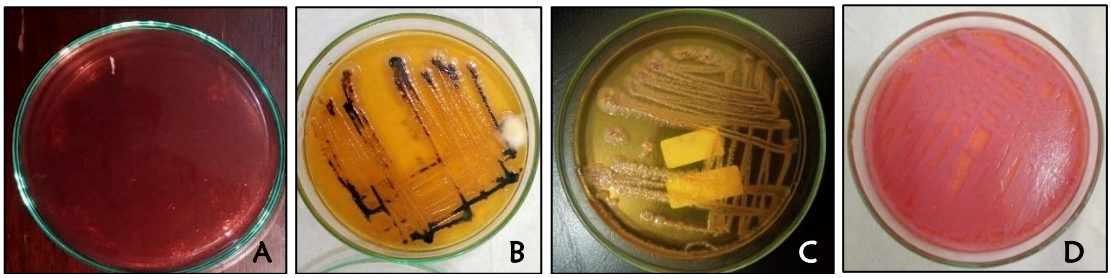

Fig. 3 SSA Media. (A) before incubation, (B) Salmonella sp., (B) Shigella sp., (B) E-Coli

Bacteria from the genus *Salmonella* can produce H_2S and *thiosulfate reductase* so that on SSA media they will form clear colonies with black spots as shown in Figure 3 (B). Bacteria from members of the genus *Shigella sp.* does not ferment lactose and does not produce H_2S gas so that clear colonies without black spots form on SSA media as shown in figure 3 (C). These results are in accordance with the theory of Aini (2018) which states that if the colonies formed are white or colorless, it indicates that the bacteria are unable to ferment lactose and do not produce H_2S gas or *thiosulfate reductase* enzymes which are characteristic of *Shigella sp.* Figure 3(D) shows the formation of pink colonies. The colony is suspected as a colony of *E-coli* bacteria. Although SSA media is a selective medium for growing only *salmonella* and *shigella* bacteria, other bacteria that have the same properties as the bacterial media can also grow. On SSA media, *coliform Escherichia coli* can also grow with pink colonies because they have the ability to ferment lactose without producing H_2S gas so that black precipitate will not form in the media (Aini, 2018).

Table 3. Biochemical Characteristic of Shigella sp.

Biochemical Characteristic	*Shigella sp.*
TSIA	
Glukosa	+
Sukrosa	-
Laktosa	-
H_2S	-
Gas	-
SIM	-
MR	+
VP	-
Urease	-
Citrate	-

Samples that have been grown on SSA media and have similar characteristics to *Shigella sp.* then proceed with biochemical tests. The bacterial biochemical test is a method or treatment carried out to identify and determine a pure culture of isolated bacteria through its physiological properties (Rahayu and Gumilar, 2017). Of the three samples on SSA media which were suspected of being colonies of *Shigella sp.* followed by biochemical tests and 2 of them had biochemical properties that match those of *Shigella sp.* (samples 6 and 15) while one other bacterium has biochemical properties that are in accordance with the bacterium *Proteus vulgaris* (sample 7).

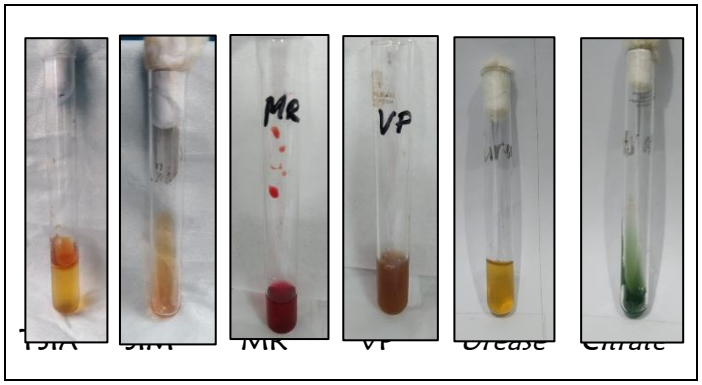

Fig. 4 Positive Sample Biochemical Test Results Shigella sp.

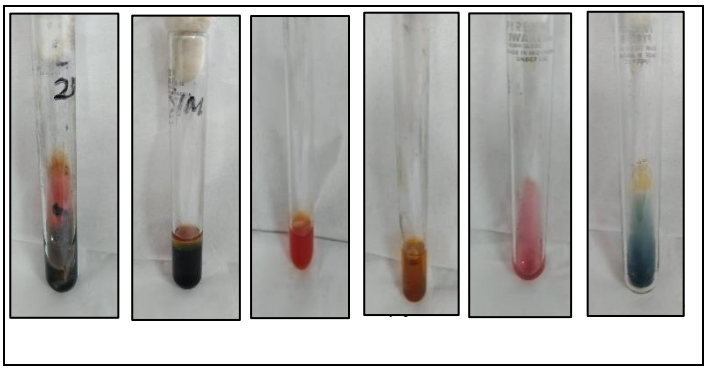

Fig. 5 Positive Sample Biochemical Test Results Proteus vulgaris

TSIA test helps in the identification of microorganisms belonging to *Enterobacteriacea*. This test medium consisted of 3 sugars, 0.1% glucose, 1% lactose and sucrose each. Phenol red and sulfate are used as indicators (Shoaib *et al.*, 2020). *Shigella sp.* has biochemical properties capable of fermenting glucose without producing gas so that it will appear on the

red media on the slant and yellow on the butt. *Shigella sp*. unable to produce H_2S gas so no black precipitate will form. This is consistent with the biochemical properties of the bacterial colonies in samples number 6 and 15. In sample number 7, the media appeared to turn yellow in the slant and butt portions indicating the occurrence of glucose, sucrose and lactose fermentation. On the surface of the tube also appears black precipitate which indicates the ability of the bacteria to produce H_2S gas. This is consistent with the biochemical properties of *Proteus vulgaris*.

SIM media is used to determine the ability of bacteria to produce H_2S gas, *indole* production and motility or movement of a bacterium (Suarjana *et al*., 2017). *Shigella sp*. cannot produce H_2S, *indole* and is not motile so that in the SIM test there will be no change in the media after dropping the reagent as seen in samples number 6 and 15. In sample number 7, a pink precipitate appears to form at the top of the tube indicating the result positive *indole* test and there is a black precipitate which indicates the ability to produce H_2S gas. This is consistent with the biochemical properties of *Proteus vulgaris*.

The MR test aims to determine the ability of bacteria to ferment mixed acids which causes an increase in the pH of the media to become acidic. The addition of the methyl red indicator to an acidic medium causes a change in the color of the media to a red color (Stephanandra, 2011). *Shigella sp*. and *Proteus vulgaris* are positive on the MR test so that there will be a change in the color of the media to red as found in samples number 6, 7 and 15. VP test aims to determine the formation of 2,3 butanediol (Stephanandra, 2011). *Shigella sp*. and *Proteus vulgaris* are negative in the VP test so that there will not be a change in the color of the media to red as seen in samples 6, 7 and 15.

The urease test aims to determine the ability of bacteria to hydrolyze urea to ammonia which results in a change in the pH of the media to become alkaline (Stephanandra, 2011). *Shigella* had negative urease test results so that the media would not experience a discoloration as was seen in samples number 6 and 15. In sample number 7, the media color changed to pink which indicated a positive result of the urease test. This is consistent with the biochemical properties of Proteus *vulgaris bacteria*.

The citrate test aims to determine the ability of bacteria to use sodium citrate as a carbon source and ammonium phosphate as a nitrogen source (Stephanandra, 2011). *Shigella* is negative in the citrate test so that there will be no change in the color of the media as found in samples number 6 and 15. In sample number 7, a change in the color of the media appears to be blue which indicates a positive result of the citrate test. This is consistent with the biochemical properties of *Proteus vulgaris*.

Proteus sp. is a genus of pathogenic bacteria that are harmful to humans and other animals. The main habitat of Proteus sp. is the intestinal tract of animals. *Proteus sp*. is a straight rod, gram negative, does not form spores, lives facultatively anaerobic, moves with a flagellum. *Proteus sp.* can grow on Salmonella Shigella Agar media (Lempang, 2014). *Proteus sp*. has white-gray colonies, with a flat colony surface (Kundera et al., 2020). Based on the results of the biochemical test of sample number 7, in the TSIA test there was a change in the color of the media, namely A/A yellow on the butt (base) and yellow on the slant (slanted surface).

Table 4. Number of Cases of Bacterial Infection Shigella sp.

No	Variabel		Number of Samples	Infection of *Shigella sp*	
				+	-
1	Stool	Liquid	15	2	13
	Consistency	Normal	8	0	8

		Congested	5	0	15
2	Age	< 2 years	10	2	8
		≥ 2 years	18	0	18

The samples in this study all came from cattle that were grazed around the TPA Tamangapa Makassar City. The cows are given food in the form of waste in the landfill without any other feed mixture. Two samples contaminated with *Shigella sp.* came from the age group under 2 years, namely 1 year and 1.5 years with the consistency of liquid feces.

The results showed that of the 28 stool samples tested, 2 of them were positively contaminated with *Shigella sp.* Several risk factors for *Shigella sp.* in cattle can be affected by feeding and water contaminated with *Shigella sp.*, poor sanitation of the stables and environment and age. Cattle that are grazed at the landfill site are carried out using an extensive method where the cows are just released and done every day. Cattle that are grazed at this TPA will eat food that comes from piles of waste consisting of vegetables, fruits, grass waste, leaf waste and food scraps that have been mixed with various decomposing waste and will drink water mixed with existing feces. around the landfill. According to Qu *et al.*, (2012) animals kept in environments with poor sanitation, difficulty accessing clean drinking water and long-term exposure to contaminated food are susceptible to *Shigella sp* infection.

Fig. 5 The condition of the cow pens around the Tamangapa TPA

The cage used is a simple cage with a base in the form of soil with feces that accumulate and mix with urine. This is due to the condition of the base of the cage which is not possible and the farmers do not routinely clean the cage. According to Mahagamage *et al.* (2020), *Shigella sp.* is a bacterial pathogen that is commonly found in environments with poor sanitation, because it is excreted by human feces, livestock and wild animals.

Positive samples came from the young age group, namely under 2 years of age with good stool consistency. The high-risk group infected with *Shigella sp.* namely very young and old age animals. The poor immune ability of young calves makes them susceptible to infection. 15 samples that had the consistency of liquid feces which is a symptom of *Shigella sp.* infection. not all of them have a positive test result. This is due to the factors that cause watery diarrhea in cattle, not only due to infection with *Shigella sp.* just. Other bacterial infections such as *Salmonella sp.* and *E-coli* also give almost the same symptoms, namely watery diarrhea. In addition to infectious factors, non-infectious factors can also cause diarrhea such as stress or poor environmental hygiene.

Conclusion

From the results of this study it can be concluded that the infection rate of *Shigella sp.* in cattle that were grazed at TPA Tamangapa Makassar City was relatively low where only 2 out of 28 samples (7%) samples were identified as containing *Shigella sp.*

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