

## Analysis Total Plate Count (TPC) *Escherichia coli* and *Salmonella* sp. on Frozen Beef Imported through Tanjung Priok Port

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### INTRODUCTION

Beef is the third most consumed protein in Indonesia after chicken and fish. Beef is the most important source of protein used widely in a variety of traditional dishes, such as "bakso" (meatball) and "rendang" (meat dish cooked with coconut milk). Fresh meat products are been marketed either as freshly cooled or frozen.



Figure 1. Importation frozen beef (Source: [www.facebook.com/humaskarantinapriok](http://www.facebook.com/humaskarantinapriok))

Foodborne pathogens such as bacteria or toxins, viruses or parasites may lead to human disease when contaminated food is eaten. The source of contamination may vary but harmful bacteria are mostly responsible for causing gastrointestinal infections. The sources could be the animal, the environment or contamination during food processing [1].

This study was carried out to evaluate the microbiological condition and status of frozen beef samples imported through Tanjung Priok port (Figure 1). The studied microbiological parameters were Total Plate Count (TPC), *Escherichia coli*, and *Salmonella*, to determine it's risk for public health. The amount of coliform bacteria in meat ex. *E. coli* is important. This microorganisms are very good indicators concerning whether the food has been processed in hygienic conditions. *Salmonella* bacteria is not exist in food. The *Salmonella* on meat can be emphasized to be spread by the animals whose intestine, skin are cut and because of the equipment used in cutting and the unhygienic and careless working [4].

### MATERIALS AND METHODS

#### Samples Collection

During January – May 2018, 93 samples of frozen beef in this study were collected from different countries and analyzed in the same day with Total plate count for fecal coliforms bacteria, *E. coli*, and pathogen bacteria *Salmonella* in Agriculture Quarantine Tanjung Priok Laboratory.

#### Microbiological Analysis

Total 25 g frozen beef sample has been weighed placed in sterile container. A total of 225 ml of sterile 0.1% Buffered Peptone Water (BPW) solution were added to the sterile container containing the sample, homogenize with stomachers for 1 minute to 2 minutes. This is a solution with  $10^{-1}$  dilution. One ml of suspension with  $10^{-1}$  dilution is transferred with a sterile pipette to solution of 9 ml BPW to obtain  $10^{-2}$  dilution. Dilution is carried out to  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  as needed. A total of 1 ml of suspension of each dilution is introduced into the petri dish in duplo. Plate Count Agar (PCA) that has been cooled to temperature  $45\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  is added 15 ml up to 20 ml on each cup petri dish containing suspension. Screening of the cup is done with forward and backward movement or form eight figures for solution samples and PCA media are completely mixed and let until it becomes solid. The media were incubated at a temperature of  $34\text{ }^{\circ}\text{C}$  to  $36\text{ }^{\circ}\text{C}$  for 24 hours up to 48 hours by placing the plate in reverse position [2].

#### *Salmonella* Analysis

The sample was weighed as much as 25 g aseptically then inserted in a sterile container. As much as 225 ml of Lactose Broth (LB) solution was added to the sterile bag containing the sample, then homogenized with stomacher for 1 min to 2 min. the suspension is transferred into an Erlenmeyer or sterile container and incubated at  $35\text{ }^{\circ}\text{C}$ . for 24 hours  $\pm$  2 hours. the pre-enrichment culture is stirred slowly then removed and transferred each 1 ml into 10 ml Tetra Thionate Broth (TTB) medium, while for the: Rappaport Vassiliadis (RV) medium is transferred 0.1 ml into 10 ml RV

[2].

Samples with alleged contamination of *Salmonella* spp. high microbial load is incubated on an RV medium at a temperature of 42 °C ± 0.2 °C for 24 hours ± 2 hours. As for TTB media incubated at a temperature of 43 °C ± 0.2 °C for 24 hours ± 2 hours. Samples with alleged contamination of *Salmonella* spp. low microbial load were incubated on RV medium at 42 °C ± 0.2 °C for 24 hours ± 2 hours. As for TTB media incubated at a temperature of 35 °C ± 2 °C for 24 hours ± 2 hours [2].

### Isolation and Identification

Two or more colonies were taken with ose needle from each of the incubated enrichment media, and inoculated on Hektoen Enteric Agar (HE), Xylose Lysine Deoxycholate Agar (XLD) and Bismuth Sulfite Agar (BSA) media, incubated at 35° C for 24 hours ± 2 hours. For BSA if unclear can be incubated for 24 hours ± 2 hours. Salmonella colonies on HE medium appear bluish-green with or without black dots (H<sub>2</sub>S). In XLD colonies the media looks pink with or without a shiny point or visible almost all black colonies. In BSA colonies media look grayish or blackish, sometimes metallic, the media around the colony is brown and the longer the incubation time will turn black. Identification was carried out by taking alleged colonies from the three media and inoculated to TSIA and LIA by piercing to the bottom of the agar medium, subsequently scratched on the media to be inclined and incubated at 35 °C for 24 hours ± 2 hours [2].

### *Escherichia coli* Analysis

Weighed sample as much as 25 g aseptically then placed in a sterile container. 225 ml of 0.1% BPW solution pour into the sterile bag containing the sample was homogenized with stomachers for 1 min to 2 min. This was a solution with 10<sup>-1</sup> dilution. Test used 3 tube series, isolation-identification test, and biochemical test.

The 10<sup>-1</sup> dilution solution was transferred by 1 ml with a sterile pipette into a 0.1% BPM 0.1% solution to obtain 10<sup>-2</sup> dilution. In the same way as above made 10<sup>-3</sup> dilution. Each 1 ml sample of each dilution was piped into 3 series of Lauryl Sulfate Tryptose Broth (LSTB) tubes containing the Durhan tube, then incubated at 35 ° C for 24 hours up to 48 hours. Notice the existence of gas formed in the Durham tube. The test results are positive if formed gas.

### Confirmation Test (Affirmation)

The positive culture is removed by using the inoculation needle from each LSTB tube into the *Escherichia coli* Broth (ECB) tube containing the Durham tube. ECB is incubated at 45.5 °C for 24 hours ± 2 hours, if negative results are incubated

for 48 hours ± 2 hours. Note the presence of gas formed in the Durham tube. The test results are positive if formed gas.

### Isolation-Identification

Scratches made on Levine Eosin Methylene Blue Agar (L-EMBA) or VRBA medium from a positive ECB tube, incubated at 35 °C for 18 hours up to 24 hours. The alleged colony of *E. coli* is 2 mm to 3 mm in diameter, black or dark in the center of the colony, with or without glossy greenish metallic on L-EMBA media. The suspected colonies of each L-EMBA medium were taken using ose, and transferred to PCA tilt. Inclined PCA was incubated at 35 °C for 18 hours up to 24 hours for biochemical tests.

### Biochemical Test with IMViC Test [2]

Interpretation of biochemical test results the classification of *E. coli* is IMViC reaction with the + + - - or - + - - pattern (Table 1).

Table 1. Reaction Indole, Methyl Red, Voges-Proskauer, Citrate (IMViC)

<i>E. coli</i> Type	Indol	MR	VP	Citrate
<i>E. coli</i> specific	+	+	-	-
<i>E. coli</i> non spesifik	-	+	-	-

### RESULTS AND DISCUSSION

The total plate count is the enumeration of aerobic, mesophilic organisms that grow in aerobic conditions under moderate temperatures of 20-45 °C. This count includes all pathogens and nonpathogens and is used to determine the hygienic status of food produced. Total plate count can be made using plate count agar. This microbiological growth medium is not a selective medium.

The total bacteria counting results obtained from 93 sample collected from some frozen beef were given in Table 2 and 3. The 100% of frozen beef investigated according to the Figure 2. The microbiological analysis results of import frozen beefs were shown in Table 2.



Figure 2. Total plate count (Source: Private collection from Agricultural Quarantine of Tanjung Priok Laboratory)

TPC results showed the number of microbes contained in imported frozen beef ranged

from  $5.9 \times 10^2$  to  $5.35 \times 10^3$ , well below the standard SNI is  $1 \times 10^6$  [3]. It's means that frozen beef produce in good hygienic status.

## REFERENCES

Table 2. Total Plate Count

Month	Sample	Number of sample	TPC (cfu/gram)
January 2018	Frozen beef	26	589,6154 ( $5.9 \times 10^2$ )
February 2018	Frozen beef	22	5352,636 ( $5.35 \times 10^3$ )
March 2018	Frozen beef	22	2512,045 ( $2.5 \times 10^3$ )
April 2018	Frozen beef	16	2381,047 ( $2.4 \times 10^3$ )
	Frozen Buffalo Meat	3	2030 ( $2.03 \times 10^3$ )
May 2018	Frozen beef	18	1629,722 ( $1.63 \times 10^3$ )

Table 3 Total Plate Count Result for *Escherichia coli* and *Salmonella* sp.

Month	Sample	Number of Sample	<i>Salmonella</i> sp.	<i>E. coli</i>	Number of colonies
January 2018	Frozen Beef	26	Negative	Negative	
February 2018	Frozen Beef	22	Negative	20 Negative	
				2 Positive	<1cfu/g
March 2018	Frozen Beef	22	Negative	20 Negative	<1cfu/g
				2 Positive	<1cfu/g
April 2018	Frozen Beef	16	Negative	14 Negative	<1cfu/g
				2 Positive	<1cfu/g
May 2018	Frozen Beef	18	Negative	Negative	
	Frozen Buffalo Meat	7	Negative	Negative	

*Escherichia coli* are used as indicator microorganisms for evaluating the microbiological quality of food and controlling whether sanitation has been done properly [6]. *E. coli* can colonize in the intestines of animals, which could contaminate muscle meat at slaughter. *E. Coli* O157:H7 is a rare strain that produces large quantities of a potent toxin that forms and causes severe damage to the intestine. The disease is called Hemorrhagic Colitis and is characterized by bloody diarrhea [4].

*Salmonella* belonging to the pathogen bacteria group and causing diseases such as typhoid, paratyphoid, and food poisoning not be found in frozen beef [4]. *Salmonella* may be found in the intestinal tracts of livestock, poultry, dogs, cats, and other warm-blooded animals. There are about 2,000 *Salmonella* bacterial species. Freezing doesn't kill this microorganism, but it is destroyed by thorough cooking. *Salmonella* must be eaten to cause illness. They cannot enter the body through a skin cut. Cross-contamination can occur if raw meat or its juices contact cooked food or foods that will be eaten raw, such as salad [4].

Table 3 shows there was no *Salmonella* in the sample, and *E coli* found in the sample <1cfu/g is very far below the Indonesian national standard  $1 \times 10^1$ cfu/g [3]. This shows no fecal contamination on frozen beef.

## CONCLUSIONS

The current study suggests that microbiological quality of food and controlling sanitation has been done properly.

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