MICROBIOLOGICAL PROPERTIES OF BEEF IN VARIOUS MEAT SHOPS AT SEMARANG, INDONESIA

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Received March 30, 2012; Accepted May 14, 2012

ABSTRAK

Penelitian ini bertujuan untuk mengkaji kualitas bakteriologis daging sapi yang dijual di pasar-pasar kota Semarang-Indonesia. Persyaratan kualitas daging sapi yang dijual di pasar-pasar Indonesia adalah: (1) *total plate count* (TPC) maksimal 10^6 CFU/g, (2) total bakteri *coliform* maksimal 10^2 CFU/g, (3) total bakteri *Escherichia coli* maksimal 10 CFU/g, (4) total bakteri *Staphylococcus aureus* maksimal 10^2 CFU/g dan (5) bakteri *Salmonella* negatif per 25 g (SNI 3932, 2008). Sampel-sampel daging sapi yang diteliti diambil secara acak sederhana dari pasar tradisional, depot daging dan supermarket. Hasil penelitian menunjukkan bahwa semua sampel daging sapi yang diuji tidak memenuhi syarat secara mutlak berdasarkan ketentuan pemerintah Indonesia tentang kualitas daging sapi. Semua sampel daging sapi yang diuji tidak diketemukan bakteri *Salmonella.* Kesimpulan, sampel daging sapi yang diambil dari beberapa pasar, umumnya tidak dapat memenuhi lima syarat bakteriologis secara mutlak.

Kata kunci: daging sapi, kualitas, mikrobiologis, pasar daging

ABSTRACT

The aim of the study was to assess microbiological properties of beef sold in various meat shops in Semarang. There are five Indonesian goverment standard requirements to maintain the quality of beef sold in Indonesia markets, as follows: (1) total plate count (TPC) for a maximum of 10^6 CFU/g, (2) total coliform bacteria for a maximum of 10^2 CFU/g, (3) total *Escherichia coli* up to 10 CFU/g, (4) total *Staphylococcus aureus* for a maximum of 10^2 CFU/g and (5) negative for *Salmonella* per 25 g samples (SNI 3932, 2008). Beef samples were randomly taken from several traditional markets, meat shops and supermarkets. The result showed that all samples did not contain *Salmonella* but still could not meet one or some of the Indonesian government standard regulation. In conclusion, beef samples gathered from some of the markets, generaly could not meet one or some of the five strictly requirements of the bacteriological properties.

Keywords: beef, microbiological properties, meat shops

INTRODUCTION

Beef is a food that contains lots of nutrients, composed of water 73.1%; protein 23.2%; fat 2.8%; various minerals and vitamins (Williams, 2007). The nutrients of beef is a suitable media for microorganisms such as bacteria, molds and yeasts (Aslam *et al.,* 2000). Microorganisms contamination in beef started from the slaughterhouse environment during slaughtering (Whelehan *et al.,* 1986) that was come from: the cattle itself (skin and feces), manure, water and feed as well as the transportation equipments (Berry *et al.,* 2010), rumen contents (bolus), carcass washing equipments and water (Yoder *et al.,* 2010). Another microorganisms sources contamination were come from the workers at the slaughterhouse, the process during distribution and until ready to consume (Aslam *et al.,* 2009).

Various researches in bacterial contamination in beef have been done. Aslam *et al.* (2000) reported that the number of coliform bacteria in ground beef in Pakistan varies from $1.7x10^5$ to $3.2x10^5$ CFU/g and mold from $1.0x10^4$ to $3.2x10^4$ CFU/g. Sartika *et al.* (2005) reported that beef came from Cibinong and Bogor then being measured in Bogor-Indonesia was containing *E. coli* bacteria; which 60% contamination came

from water and 41.7% came from workers. Cohen *et al*. (2008) reported that 79 out of 150 beef samples contained fecal coliform bacteria, *E.coli, S. aureus* and *Clostridium perfringens* bacteria, also had aerobic bacteria 10^7 CFU/g. Yanti *et al.* (2008) examined the bacteria content in beef at Pekanbaru markets, Indonesia, that was about $9.7x10^5$ CFU/g, while, the study in Bogor-Indonesia was reported that beef contaminated by coliform for $7.9x10^4$ CFU/g and contaminated by *E. coli* bacteria for $3.0x10^4$ CFU/g. Arifin *et al.* (2008) reported that the bacterial content in beef samples were $1.0x10^7$ CFU/g. Aslam *et al.* (2009) was successfully isolated *E. coli* bacteria from the slaughtering equipments and cow hide that have been skined. Masana *et al.* (2010) reported that 54 samples of beef that come from the slaughterhouse in Argentina (slaughtering period of November 2006 up to April 2008) were containing *E. coli* bacteria. Ingham *et al.* (2010) reported that beef has potential to get *E. coli* bacteria and non fecal coliform bacteria contamination

Indonesia government's regulation on beef and carcass quality defined five strictly requirements as follows: TPC for a maximum 10^6 CFU/g, total coliform bacteria for a maximum 10^2 CFU/g, total *E. coli* bacteria up to 10 CFU/g, total *S. aureus* bacteria for a maximum of 10^2 CFU/g and zero tolerant for *Salmonella* bacteria per 25 g samples (SNI 3932, 2008). If the bacteria content in beef samples exceeded from predetermined standard, therefore the beef is considered prohibited to be consumed. A pathogenic bacteria is also should be in zero tolerant contained in a processed beef products due to it can cause food intoxication for the consumer. The risk of potential bacteria contamination in beef is still threated the consumers. Therefore, the study was done to give some information in microbilogical properties in the meat shops at Semarang area.

MATERIALS AND METHODS

The samples collection

Beef samples were gathered from the traditional markets, meat shops and supermarkets in Semarang city, Indonesia in February 2012. Simple random method was used for gathered the beef samples (Mendenhall *et al.*, 1971). Samples was collected two times. The first time was done at 15 selected locations and gathered in the morning. The second time was done at the three selected locations and gathered in the afternoon. Afterwards, beef samples were put in the cooler box with the temperature about 5°C (Cohen *et al.*, 2008). Beef samples were then examined at the microbilogy laboratory.

Bacteriological Analysis

Bacteria determination of beef samples was done immediately after samples gathered (no longer than 1 hour after sampling). The variables for bacteria determination were included: total bacteria, total coliform bacteria, total *E. coli* bacteria, total *S. aureus* bacteria and qualitative determination for *Salmonella* bacteria in accordance with the requirements of SNI 3932 (2008). Bacterial culture was proceeded by serial dilution. Mixed solution of 225 mL Buffered Peptone Water (BPW) 0.1% and 25 g ground beef samples were then diluted in a serial dilution with dilution factor of 10 (Pao and Ettinger, 2009).

Pour plate method was used to determine total bacteria. Nutrient Agar (NA) as a culture media used for pour plate method was incubated at 35°C for 24-48 hours (Aslam *et al*., 2000). The Most Probable Number (MPN) was used for determination of total coliform bacteria. Brilliant Green Lactose Bile Broth (BGLBB) as a culture media used MPN method was incubated at 35°C for 24-48 hours. Afterwards, a confirmatory test was done using Lauryl Sulphate Tryptose Broth (LSTB) as a culture media which had incubated at 35°C for 48 hours (SNI 2897, 2008). Pour plate method also used for total *E. coli* bacteria determination. Mac Conkey Agar (MCA) as a culture media for pour plate method was incubated at 35°C for 24-48 hours (Fardiaz, 1993). Confirmatory test was done using *E. coli* broth (ECB) media which had been incubated at 45.5°C for 48 hours (SNI 2897, 2008). The casting cup method was used for total *S. aureus* determination. Vogel-Johnson Agar (VJA) as a culture media for the casting cup method was incubated at 35°C for 45 - 48 hours (Fardiaz, 1993). Confirmatory test using Gram staining and coagulate test were done afterwards (SNI 2897, 2008). There were two steps enrichment for qualitative *Salmonella* bacteria determination. Pre enrichment using Lactose Broth (LB) as a culture media had been incubated at 35°C for 24 hours, followed by enrichment step using Selenite Cystine Broth (SCB) as a culture media that had been incubated at 43°C for 24 hours. Isolation and identification method used Triple Sugar Iron Agar (TSIA) and Lysine Iron Agar (LIA) that had been incubated at 35°C for 24 hours. *Salmonella*

Table 1. Total Bacteria and *Salmonella* Bacteria in the Qualitative Determination of Beef Samples in the Morning

Source of Beef	Total Bacteria (CFU/g)	Total Coliform Bacteria (MPN/g)	Total *E. coli* Bacteria (CFU/g)	Total *S. aureus* Bacteria (CFU/g)	*Salmonella* Bacteria
Traditional Markets					
1.	$2.8x10^6$	$>2.4x10^4$	$6.4x10^4$	$1.6x10^3$	Negative
2.	$2.9x10^6$	$>2.4x10^4$	0	$1.5x10^5$	Negative
3.	$3.1x10^7$	$>2.4x10^4$	$5.0x10^6$	$9.0x10^5$	Negative
4.	$1.1x10^7$	$>2.4x10^4$	$3.0x10^5$	$7.0x10^6$	Negative
5.	$1.5x10^7$	$>2.4x10^4$	0	$2.2x10^5$	Negative
6.	$5.2x10^7$	$>2.4x10^4$	$3.0x10^5$	$1.8x10^5$	Negative
Meat Shops					
1.	$9.6x10^5$	$>2.4x10^4$	0	$8.4x10^4$	Negative
2.	$7.6x10^4$	$>2.4x10^4$	0	0	Negative
3.	$6.6x10^6$	$>2.4x10^4$	$8.0x10^4$	$3.2x10^5$	Negative
Supermarkets					
1.	$2.3x10^4$	$2.3x10^2$	0	$6.0x10^3$	Negative
2.	$2.4x10^6$	$>2.4x10^4$	$1.1x10^6$	$1.4x10^4$	Negative
3.	$2.0x10^4$	$9.3x10^2$	0	0	Negative
4.	$5.8x10^4$	$9.3x10^2$	0	$1.5x10^5$	Negative
5.	$6.1x10^6$	$>2.4x10^4$	$2.4x10^4$	$1.2x10^5$	Negative
6.	$2.3x10^5$	$2.3x10^2$	0	$1.6x10^4$	Negative

suspected colonies in a culture media were then subjected to biochemical confirmatory tests (SNI 2897, 2008).

RESULTS AND DISCUSSIONS

Results

Total viable bacteria calculated from samples of beef (morning samples) are presented in Table 1. Total bacteria in beef ranged from traditional markets $2.8x10^6$ to $5.2x10^7$ CFU/g, meat shops around $7.6x10^4$ to $6.6x10^6$ CFU/g and supermarkets around $2.0x10^4$ to $6.1x10^5$ CFU/g. Total coliform bacteria in all samples of beef were more than 10^2 MPN/g. Total *E. coli* bacteria in beef varied, from traditional markets ranged from zero to $5.0x10^6$ CFU/g, meat shops ranged from zero to $8.0x10^4$ CFU/g and supermarkets ranged from zero to $1.1x10^6$ CFU/g. Total *S. aureus* bacteria in beef ranges from traditional markets $1.6x10^3$ to $7.0x10^6$ CFU/g, meat shops ranges from zero to $3.2x10^5$ CFU/g and supermarkets ranges from zero to $1.5x10^5$ CFU/g. Qualitative test of *Salmonella* bacteria in all samples of beef results are negative, whereas some bacterial species identified are listed in Table 2. The most frequent bacterial species identified were *Klebsiella pneumoniae*. This study was also conducted on samples of beef in the afternoon, from traditional market, meat shop and supemarket each as a single location (Table 3). The results showed that the content of bacteria in ground beef samples were relatively higher in the afternoon than in the morning. Based on the results of a qualitative test, all of beef samples were not found *Salmonella* bacteria, but based on the testing it was identified several other bacterial species (Table 3). The most frequent bacterial species identified were *Klebsiella pneumoniae.*

Table 2. Bacteria Identified in Beef Samples in the Morning

Source of Beef	Kinds of Bacteria
Traditional Markets	
1.	*Staphylococos saprophyticus, Klebsiella pneumoniae, Enterobacter aglomerans*
2.	*Proteus vulgaris, Staphylococcus saprophyticus, Citrobacter diversus,Enterobacter aglomerans*
3.	*Klebsiella pneumoniae, Staphylococcus saprophyticus,Citrobacter diversus*
4.	*Klebsiella pneumoniae, Staphylococcus saprophyticus, Serratia liquefaciens*
5.	*Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus saprophyticus*
6.	*Klebsiella pneumoniae, Pseudomonas aeruginosa, Citrobacter freundii, Staphylococcus saprophyticus*
Meat Shops	
1.	*Proteus vulgaris, Staphylococcus saprophyticus, Klebsiella pneumoniae*
2.	*Klebsiella pneumoniae, Enterobacter aerogenes*
3.	*Klebsiella pneumoniae, Citrobacter diversus, Staphylococcus saprophyticus, Streptococcus alpha*
Supermarkets	
1.	*Pseudomonas aeruginosa, Staphylococcus epidermidis, Staphylococcus saprophyticus*
2.	*Klebsiella pneumoniae, Streptococcus alpha, Proteus vulgaris*
3.	*Klebsiella pneumoniae, Citrobacter diversus*
4.	*Klebsiella pneumoniae, Citrobacter diversus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Streptococcus alpha*
5.	*Klebsiella pneumoniae, Providensia stuartii, Staphylococcus epidermidis*
6.	*Klebsiella pneumoniae, Enterobacter aerogenes, Staphylococcus saprophyticus, Proteus mirabilis*

Discussions

Based on the results, it can be concluded that all of the beef samples which came from traditional markets, meat shops and supermarkets (in the morning and afternoon time gathered) could not meet the five strictly requirements of SNI 3932 (2008). Bacterial contamination in beef that being sold in the markets were occurred from the slaughterhouse. Yoder *et al.* (2010) reported that the main source of bacterial contamination in carcasses at the slaughterhouse came from the water and the slaughter equipments. Furthermore, bacterial contamination would increase during distribution of marketing. According to Aslam *et al.* (2000), the distribution methods, sales worker and sale equipments are factors that can increase the number of bacteria contamination in beef samples. The quality of beef samples in general is better from the meat shops and supermarkets than those from traditional markets. The system of meat sale in traditional markets is more open than in the supermarkets. The consumer can easily choose the meat by touching or holding it (Yanti *et al.,* 2008). The system of meat sale in the supermarkets and meat shops are more strictly, hygienic than in the traditional markets due to consumer had no opportunities to touch the meat. The display room temperature in the supermarkets also always is maintained at the temperature below 5°C. Whereas, the temperature for displaying in the meat shops and traditional markets is about 25°C. Low temperature proven can inhibit the growth of bacteria in meat. According to Birk *et al.* (2010) the majority of pathogenic bacteria in humans are sensitive to the media temperature of about 4°C. Jay *et al.* (2005) reported that most of the meat microorganisms in a refrigerator begin to grow in the temperature of

Table 3. Bacteria Determination and Kinds of Bacteria Identified in the Afternoon

Source of Beef	Total Bacteria (CFU/g)	Total Coliform Bacteria (MPN/g)	Total *E. coli* Bacteria (CFU/g)	Total *S. aureus* Bacteria (CFU/g)	Kinds of Bacteria
Traditional Market	$7.2x10^7$	$>2.4x10^4$	0	$5.0x10^6$	*Klebsiella pneumoniae, Staphylococcus epidermidis, Enterobacter aglomerans*
Meat Shop	$3.0x10^7$	$>2.4x10^4$	0	$4.6x10^6$	*Klebsiella pneumoniae, Providensia stuartii, Streptococcus alpha, Staphylococcus saprophyticus*
Supermarket	$1.2x10^6$	$2.4x10^3$	$1.0x10^3$	$1.6x10^6$	*Klebisella pneumoniae, Providensia stuartii, Staphylococcus epidermidis*

5-7°C. Therefore, the display room temperature in the markets should be always maintained at about 5°C to suppress the growth of microorganisms during displaying for sale. All of the beef samples that gathered in the afternoon had the bacterial contamination that was higher than in the morning. One of the factors that led to increase bacterial population is influenced by nutrients factors in meat (Jay *et al.*, 2005). Beef contains the main form of nutrients, included water, proteins, fats, minerals and vitamins. The nutrients are water (73.77-75.56%), protein (18.38-20.22%), fat (0.72-1.80%) and total mineral (0.97-1.2%).

CONCLUSION

Based on the study, it could be concluded that all of the beef samples gathered from some of the markets (traditional markets, meat shops and supermarkets in Semarang-Indonesia), generally could not meet one or some of the five strictly requirements of the bacteriological properties. There was no present *Salmonella* bacteria in all of the beef samples.

ACKNOWLEDGMENTS

Highly appreciated acknowledgments was for Directorate General of Higher Education, Ministry of Education and Culture, Indonesia that gave research funding for this study. The authors also thank to Elly Karlina, Happy Haryanta and Indarto who helped the bacteriological and chemical analysis of beef samples.

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