ABSTRACT

The aim of the study was to assess microbiological properties of beef sold in various meat shops in Semarang. There are five Indonesian government 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, generally 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 (Whelahan 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 $1.7 \times 10^3$ to $3.2 \times 10^4$ CFU/g and mold from $1.0 \times 10^4$ to $3.2 \times 10^4$ 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 in 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 7.9x10^6 CFU/g and contaminated by *E. coli* for 3.0x10^6 CFU/g. Arifin et al. (2008) reported that the bacterial content in beef samples were 1.0x10^5 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 10^5 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 threatened the consumers. Therefore, the study was done to give some information in microbiological 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 microbiology 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*
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 ranging from 7.6x10^5 to 6.6x10^6 CFU/g and supermarkets ranging from 2.0x10^5 to 6.1x10^6 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 supermarket 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.

| 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          |
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

| Source of Beef  | Kinds of Bacteria                                      |
|-----------------|-------------------------------------------------------|
| Traditional Markets |                                                       |
| 1.              | *Staphylococcus saprophyticus, Klebsiella pneumoniae, Enterobacter agglomerans* |
| 2.              | *Proteus vulgaris, Staphylococcus saprophyticus, Citrobacter diversus, Enterobacter agglomerans* |
| 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* |
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%).

| 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 agglomerans |
| Meat Shop           | 3.0x10^7               | >2.4x10^4                       | 0                             | 4.6x10^6                        | Klebsiella pneumoniae, Providencia stuartii, Streptococcus alpha, Staphylococcus saprophyticus |
| Supermarket         | 1.2x10^6               | 2.4x10^3                        | 1.0x10^3                      | 1.6x10^6                        | Klebsiella pneumoniae, Providencia stuartii, Staphylococcus epidermidis |

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.

REFERENCES

Arifin, M., B. Dwiloka and D. E. Patriani. 2008. Penurunan kualitas daging sapi yang terjadi selama proses pemotongan dan distribusi di kota Semarang. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner, Bogor. Pp. 99-104

Aslam, A., I. Mariam, I. Haq and S. Ali. 2000. Microbiology of raw minced beef. Pakistan J. Biological Sci. 3(8):1341-1342

Aslam, M., M. S. Diarra, C. Service and H. Rempel. 2009. Antimicrobial resistance genes in Escherichia coli isolates recovered from a commercial beef processing plant. J. Food Protect. 72(5):1089-1093

Berry, E. D., J. E. Wells, T. T. Arthur, B. L. Woodbury, J. A. Nienaber, T. M. Brown-Brandi and R. A. Eigenberg. 2010. Soil versus pond ash surfacing of feedlot pens: occurrence of Escherichia coli O157:H7 in cattle and persistence in manure. J. Food Protect. 73(7):1269-1277

Birk, T., A. C. Grönlund, B. B. Christensen, S. Knöchel, K. Lohse and H. Rosenquist. 2008. Effect of organic acids and marination ingredients on the survival of Campylobacter jejuni on meat. J. Food Protect. 73(2):258-265

Cohen, N., I. Filliol, B. Karraouan, S. Badri, I.
Carle, H.; Ennaji, B.; Bouchrif, M.; Hassar and H. Karib. 2008. Microbial quality control of raw ground beef and fresh sausage in Casablanca (Morocco). J. Environmental Health. 71(4):51-55
Fardiaz, S. 1993. Analisis Mikrobiologi Pangan. PT Raja Grafindo Persada, Jakarta.
Ingham, S. C., R. Y. Algino, B. H. Ingham and R. F. Schell. 2010. Identification of Escherichia coli O157:H7 surrogate organisms to evaluate beef carcass intervention treatment efficacy. J. Food Protect. 73(10):1864-1874
Jay, J. M., M. J. Loessner and D. A. Golden. 2005. Modern Food Microbiology. Springer Science & Business Media, Inc., New York.
Masana, M. O., G. A. Leotta, L. L. Del-Castillo, B. A. D’Astek, P. M. Palladino, I. Galli, E. Vilacoba, C. Carbonari, H. R. Rodriguez and M. Rivas. 2010. Prevalence, characterization, and genotype analysis of Escherichia coli O157:H7/NM from selected beef exporting abattoirs of Argentina. J. Food Protect. 73(4):649-656
Mendenhall, M., L. Ott and R. L. Scheaffer. 1971. Survey Sampling. Wadsworth Publishing Company, Inc. Belmont, California.
Pao, S. and M. R. Ettinger. 2009. Comparison of the microbial quality of ground beef and ground beef patties from internet and local retail markets (dagger). J. Food Protect. 72(8):1722-1727
Sartika, R. A. D., Y. M. Indrawani and T. Sudiarti. 2005. Analisis mikrobiologi Escherichia coli O157:H7 pada olahan hewan sapi dalam proses produksinya. Makara Kesehatan. 9(1):23-28
Standard Nasional Indonesia (SNI) 2897. 2008. Metode Pengujian Cemaran Mikroba dalam Daging, Telur dan Susu, serta Hasil Olahannya. Badan Standardisasi Nasional (BSN), Jakarta.
Standard Nasional Indonesia (SNI) 3932. 2008. Mutu Karkas dan Daging Sapi. Badan Standardisasi Nasional (BSN), Jakarta.
Whelehan, O. P., W. R. Hudson and T. A. Roberts. 1986. Microbiology of beef carcasses before and after slaughterline automation. J. Hyg. Camb. 96:205-216
Williams, P. G. 2007. Nutritional composition of red meat. Research Online. University of Wollongsong, Australia.
Yanti, H., Hidayati and Elfawati. 2008. Kualitas daging sapi dengan kemasan plastik PE (polyethylene) dan plastik PP (polypropylen) di pasar Arengka Kota Pekanbaru. J. Peternakan. 5(1):22-27
Yoder, S. F., W. B. Henning, E. W. Mills, S. Doores, N. Ostiguy and C. N. Cutter. 2010. Investigation of water washes suitable for very small meat plants to reduce pathogens on beef surfaces. J. Food Protect. 73(5):907-915