

BACTERIAL CONTAMINATION ON BEEF CARCASS AT SELECTED ABATTOIRS LOCATED IN SELANGOR, MALAYSIA

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ABSTRACT

The ruminant industry is one of the most burgeoning sectors in Malaysia. With increasing beef productions in Malaysia, the issue of bacterial contamination on beef carcass deserves extra attention as to ensure public food safety. The main objective of this study was to determine the level of bacterial contamination of beef carcasses by determining the presence of selected microorganisms (total plate count, Enterobacteriaceae, *Escherichia coli* and *Salmonella* spp.). Swab samples were taken from the two abattoirs located in Selangor, Malaysia from October 2015 to February 2016. The results obtained showed that the samples ($n = 40$) of the two abattoirs has recorded an average reading of 4.00 ± 0.934 log CFU/cm² for total plate count. Enterobacteriaceae was detected from 82.5% of total samples with an average reading of 2.728 ± 0.936 log CFU/cm². While for *E. coli* was isolated from 55% of total samples with an average reading of 1.87 log CFU/cm². A total of 4 samples (10%) were tested positive for the presence of *Salmonella* spp. The result reflect on the level of contamination of locally produced beef in Malaysian abattoirs. Thus, this study will allow better interventions from related authorities in order to improve the safety and the quality of locally produced beef.

Key words: Bacterial contamination, beef carcass, abattoir, Enterobacteriaceae, *E. coli*

INTRODUCTION

In this new era, food borne illnesses associated with consumption of beef remain as a significant public health problem worldwide. The annual incidence rate of food borne illnesses in developed countries range from 2,600 cases per 100,000 inhabitants in the United Kingdom and more than 25,000 cases per 100,000 inhabitants in Australia and the United States (Teisl & Roe, 2010). In Malaysia, the recorded incidence rate was 47.79 per inhabitant with 0.04 mortality rate in 2013 (Ministry of Health Malaysia, 2013). However, the actual incidence is likely higher as food borne illnesses events often goes unreported in Malaysia (Soon *et al.*, 2011) which is a tremendous cause of concern.

Contamination of beef carcasses is inevitable and may happen during dressing, primarily during

skinning and evisceration phase (Nastasijevic *et al.*, 2009). Pathogens such as *Escherichia coli* and *Salmonella* spp. are often the major cause of food borne illnesses (Sofos & Smith, 2014). Moreover, these pathogens could enter the food chain through faecal contamination in the abattoir and through contact with dirty equipment and surface without any visible contamination (Gill, 2004; Abdallah *et al.*, 2009). Without strict adherence to hygienic slaughter practices, beef could be contaminated and pose potential public health risk.

Beef consumption in Malaysia has increased from 5.75 kg per capita in 2003 to 6.29 kg per capita in 2013 as reported by the Department of Veterinary (DVS, 2014). With the increasing demand of beef in Malaysia, bacterial contamination of beef carcasses in the local abattoirs should be evaluated as an initiative to safeguard the public from possible beef consumption related food borne illnesses. The main aim of this study is to determine

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the level of bacterial contamination of beef carcasses in local abattoir. The data acquired from this study will be useful for implementation of better interventions as to reduce the bacterial contamination and improve the Malaysian beef quality.

MATERIALS AND METHODS

Sample Collection

A total of 40 samples were collected from two local abattoirs (referred as A and B) located in Selangor in October 2015 until February 2016. Carcass sampling was performed after evisceration. By swabbing a total area of 300 cm² on three different sites of the beef carcasses as stipulated by Australian monitoring standard, Australian *E. coli* and *Salmonella* Monitoring Program (ESAM) as shown in Figure 1 (Australian ESAM, 2003). Samples were obtained using pre-moistened sterile sponges (3M, USA) with 10 ml of Buffered Peptone Water, BPW (Merck, Germany). With a 100 cm² template, the sponge was wiped over approximately ten times vertically and horizontally direction, on the flank area of the carcass, followed with the brisket area using the same side of the sponge. The step was repeated on the rump area of the carcass using the other side of the sponge. The sponge was then placed into the sample bag and transported back to the laboratory in a sanitized and insulated cool box containing frozen ice packs.

Microbial Analysis

Total Viable Counts (TVCs), Enterobacteriaceae and *E. coli*

Upon arrival in the laboratory, 15 mL of BPW were added to each sample bags and homogenized for 3 minutes using a stomacher (MiniMix 100PCC Lab Blender, Interscience, France). Detection and enumeration of TVCs, Enterobacteriaceae and *E. coli* were conducted according to the methods of Bacteriological Analytical Manual, BAM (2001; 2002) with some modifications. Suspensions (15 mL) were drawn out from the homogenised sample and subjected to ten-fold serial dilution. The homogenate aliquots (0.1 mL) from each dilutions were spread on plate count agar (PCA) (Merck, Germany) for total viable count, violet red bile dextrose agar (VRBD) (Merck, Germany) for Enterobacteriaceae and eosin methylene blue agar (EMB) (Merck, Germany) for *E. coli* in duplicate. Plates were incubated aerobically at 37°C for 24 hours. Counts were calculated as log CFU/cm².

Detection of *Salmonella* spp.

Salmonella spp. was detected according to the methods of BAM (2004) with some modifications. Bacterial suspension (10 mL) was incubated at 37°C for 20 hours as pre-enrichment for *Salmonella* spp. detection. Aliquot of 0.1 mL of the incubated suspension were then transferred to 10 mL of Rappaport-Vassiliadis soy broth (RVS broth) (Merck, Germany) and vortexed thoroughly before

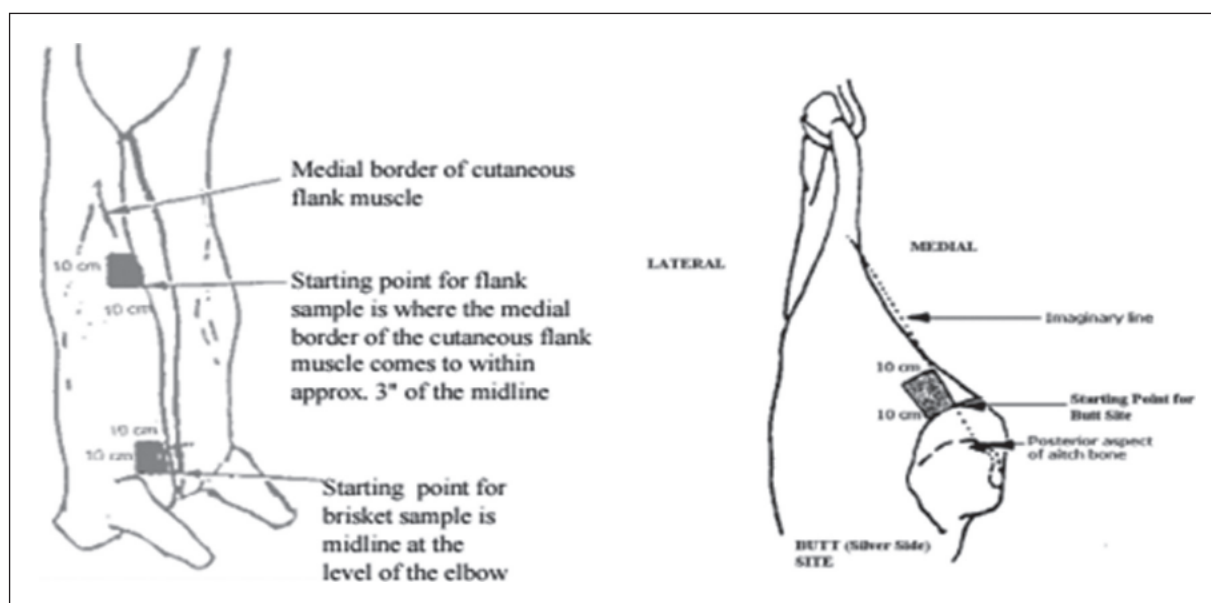


Fig. 1. Beef Carcass Sampling Site (Flank, Brisket, Rump). Adapted from Australian ESAM (2003).

incubated for another 20 hours at 40°C. A loopful of the enriched suspension was then streaked onto xylose lysine deoxycholate agar (XLD) (Merck, Germany) and incubated at 37°C for 24 hours.

Statistical Analysis

In order to evaluate significant differences for TVCs, Enterobacteriaceae counts and *E. coli* counts between abattoir, paired t-test were used, and a significant level of $\alpha = 0.05$ was chosen. All the statistical analysis was performed using IBM SPSS Statistic Version 21.0.

RESULTS

Total Viable Counts (TVCs), Enterobacteriaceae, *E. coli* and *Salmonella* spp.

Total viable counts (TVCs), Enterobacteriaceae *E. coli* and *Salmonella* spp. are summarised in Table 1. From the total samples collected (n=40), the occurrence and mean \pm standard deviation of TVCs were 100% and 4.005 ± 0.934 , 75% and 2.728 ± 0.936 for Enterobacteriaceae and 55% and 1.867 ± 0.508 for *E. coli*. While the occurrence for *Salmonella* spp. was 10% in overall.

The average TVCs were 4.041 log CFU/cm² for abattoir A and 3.968 log CFU/cm² for abattoir B (Table 1). No significant difference of TVCs was observed between the two abattoirs. The

Enterobacteriaceae counts was significantly higher in abattoir A (3.285 ± 0.999 log CFU/cm²) than in abattoir B (2.318 ± 0.646 log CFU/cm²) where the *p* value is less than 0.05. Abattoir B (65%) have higher occurrence of *E. coli* than in abattoir A (45%) (Table 1). However, there was no significant difference of the *E. coli* counts between the two abattoirs ($p > 0.05$). Both abattoirs showed similar occurrence of *Salmonella* spp. (10% samples).

Comparison of TVCs and *E. coli* counts to Australian standard

To date, there are no microbiological standards for carcasses available in Malaysia. Therefore, the results for TVCs, *E. coli* counts and *Salmonella* spp. in this study were compared to the Australian red meat standard (Australian ESAM, 2003). The Australian ESAM has classified the microbiological results to excellent, good, acceptable and marginal (Table 2). In the present study, the TVCs of the samples collected are generally within the range of "Good" and "Excellent", despite 5% of the samples collected from abattoir A were in the marginal range as shown in Figure 2. For *E. coli* counts, samples collected in abattoir A showed a very high percentage of counts in the range of "Excellent". Only 2 (10%) samples exceed 3.0 Log CFU/cm². Similar situation was observed in abattoir B, where only 20% of the samples collected are in the marginal range (Figure 3.). Only 10% of the samples

Table 1. Total Viable Counts (TVCs), Enterobacteriaceae, *E. coli* and *Salmonella* spp. for abattoir A and abattoir B

Abattoir	TVCs*			Enterobacteriaceae*			<i>E. coli</i> *			<i>Salmonella</i> spp.**
	n (%)	Mean	SD	n (%)	Mean	SD	n (%)	Mean	SD	n (%)
A	20(100)	4.041	1.044	14(70)	3.285	0.999	9(45)	1.962	0.529	2(10)
B	20(100)	3.968	0.836	19(9)	2.318	0.646	13(65)	1.801	0.504	2(10)
Total	40(100)	4.005	0.934	33(82.5)	2.728	0.936	22(55)	1.867	0.508	4(10)

*Counts are expressed in log CFU/cm² of positive samples only.

**Expressed in absence/presence detection.

Table 2. Classification of counts based on Australian *E. coli* and *Salmonella* Monitoring Program (Australian ESAM, 2003)

Category	TVCs*	<i>E. coli</i> *	<i>Salmonella</i> spp.
Excellent	<3.00	Not detected	Absent in the area tested per carcass
Good	3.00–4.00	0.00–1.00	
Acceptable	4.00–5.00	1.00–2.00	
Marginal (action required)	>5.00	2.00–3.00	Present in the area tested per carcass

*Counts are expressed in log CFU/cm²

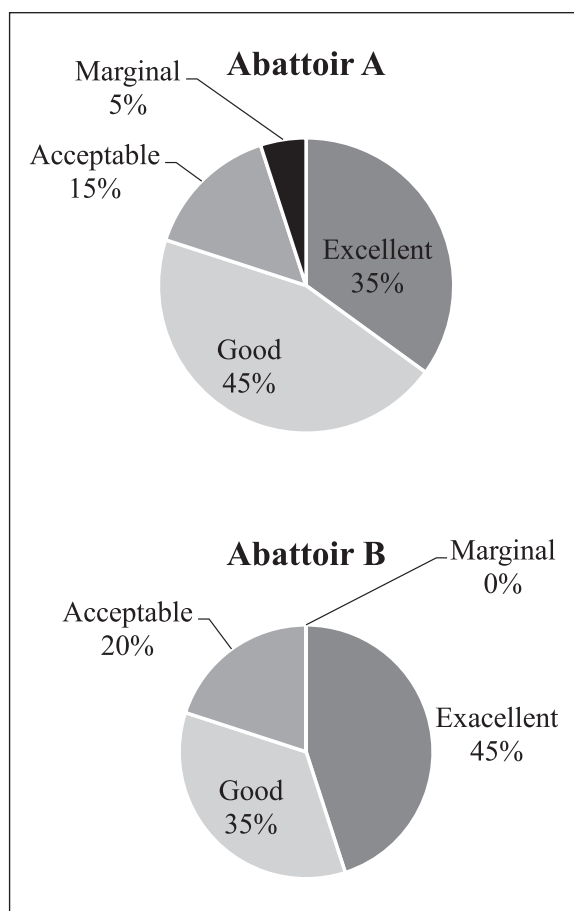


Fig. 2. Distribution of samples based on ESAM classifications for Total viable counts.

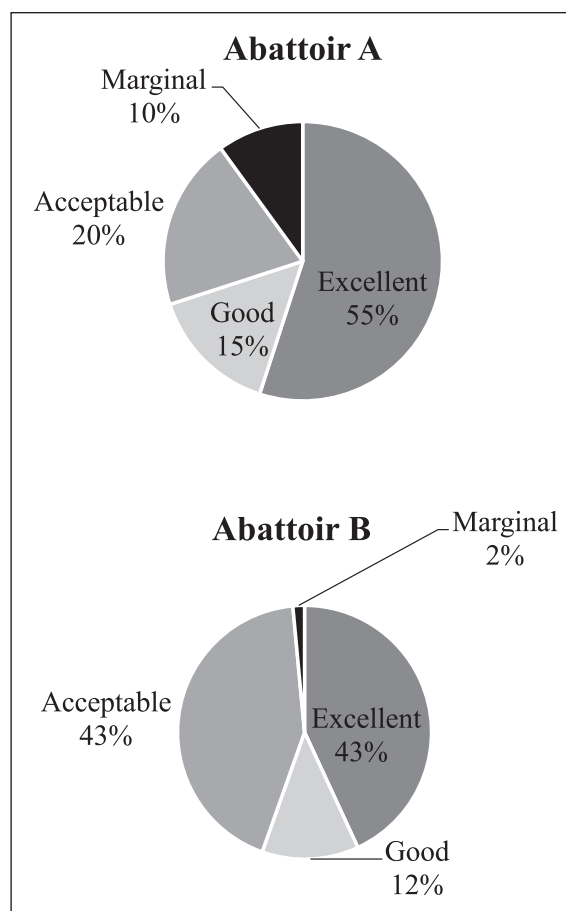


Fig. 3. Distribution of samples based on ESAM classification for *E. coli* counts.

were found positive with *Salmonella* spp that can be considered as unacceptable according to ESAM.

DISCUSSION

Indicator microorganisms can be used to evaluate the general hygiene and contamination of faecal origin (EFSA, 2010). The indicator microorganisms used in this study are: total viable count, Enterobacteriaceae and *E. coli*. Even though there was no proven correlation between the level of indicator microorganisms and the prevalence of pathogens, pathogens might be a positive fraction of the indicator microorganisms (Brown *et al.*, 2000).

For both abattoirs, the TVCs and *E. coli* counts showed average contamination levels and most of the samples were within the satisfactory range based on the Australian standards. A previous study in Malaysia on retail beef showed lower occurrence (36%) of *E. coli* (Radu *et al.*, 1998). No previous reports on beef carcass in the abattoirs were available but it can be deemed to be the first control point of

beef contamination. Retail beef can be contaminated during sale preparation and by poor hygiene of the environment (Eyi & Arslan, 2012). Another study by Radu *et al* (1997) indicated that beef may serve as a reservoir for multi-antibiotic resistant and plasmid containing *E. coli*. As such, more studies are needed to assess the prevalence of pathogenic bacteria on beef, especially *E. coli* as its presence on the beef carcasses could be a fraction of pathogenic *E. coli*. It should be a great cause of concern as it has been established that the infectious dose for pathogenic *E. coli* such as O157:H7 are as low as 10 to 100 cells (Desmarchelier & Fegan, 2003).

From this study, it can be concluded that only a small fraction of locally produced beef is contaminated with *Salmonella* spp. It is possible that the low prevalence of *Salmonella* spp. in this study could be the result of healthy cattle as cattle were found positive with *Salmonella* spp. (a carrier) has a likelihood of 3 times higher of being tested positive at the pre evisceration stage (Narváez-Bravo *et al.*, 2013). The occurrence of *Salmonella* spp. in this study, however, was considered as unacceptable

according to Australian ESAM and should be treated as non-conformances as the presence of *Salmonella* spp. in the beef carcass could pose a risk of food born illnesses if consumed. This is dangerous as *Salmonella* spp. is readily resistant to various conditions even to harmful influence and has high multiplication rate (Sallam *et al.*, 2014). Further sampling should be carried out and in case of repetition of unsatisfactory result, the abattoir system need to be re-assessed and further trainings of the abattoir operators are needed (Australian ESAM, 2003).

By comparing the microbiological profiles between abattoirs, evaluation of effectiveness of the hygiene practices applied in the abattoir system could be conducted (Zweifel *et al.*, 2005). In this study, there were no significant differences found for TVCs and *E. coli* counts between the two abattoirs, and the occurrence of *Salmonella* spp. for both abattoirs is the same. Considering that both of the abattoirs are regulated by the Malaysian Department of Veterinary Services, the abattoir systems (i.e. slaughtering process, and technique used) are generally the same, which explains the lack of differences of microbiological examination results (TVCs, *E. coli* counts and *Salmonella* spp.) between these two abattoirs.

On the other hand, abattoir A was found to have higher Enterobacteriaceae count compared to abattoir B. Barco *et al* (2014) has suggested that Enterobacteriaceae counts are most likely influenced by the design of the abattoir plant. It is assumed that when circular rail design was not made available in the abattoir, the carcass is likely to be in contact with the floor and thus resulting in higher contamination rate (FAO, 1991). However, this suggestion is not consistent with this study; Abattoir A is a large scale abattoir equipped with circular rails while abattoir B is a smaller scale abattoir with limited hanging devices. The higher count of Enterobacteriaceae are most likely influenced by other factor as contamination could arise from the workers, dirty equipment or the animal (Schegelova *et al.*, 2004; FAO, 2014). The source of Enterobacteriaceae contamination could be from leakage of intestinal contents or cross contamination with dirty cattle hides (Koohmaraie *et al.*, 2005; Small *et al.*, 2005).

The TVCs and *E. coli* counts in this study were considered to be within satisfactory range. Such results indicate the effectiveness of the hygienic practice in the local abattoir system in Malaysia. Regardless of satisfactory bacterial counts, significant difference of Enterobacteriaceae counts

were observed in between the abattoirs and 10% of samples collected were positive with *Salmonella* spp. Such findings indicate ample room for improvements in the local abattoir to achieve excellent meat quality. Hence, more interventions are needed in order to further improve the quality of locally produced beef in Malaysia.

Yalcin *et al* (2001) has suggested that an additional step of washing the carcass should be implemented for reduction of microbial loads. Other than washing, chilling is also deemed as another important procedure in reducing the microbial loads on carcasses (Yalcin *et al.*, 2004). It has been reported that *E. coli* and coliforms counts were reduced during chilling (Bacon *et al.*, 2000), which prove that chilling the carcasses could help in inhibiting the bacterial growth thus extending the shelf life of the beef products. It is advisable that carcasses should be placed in the chillers immediately after dressing or weighing process until the surface of the carcasses are dry and cold when touch (FAO, 1991). Chillers in the local abattoir require additional costs; which leads to most of the carcasses were transported for sale directly after the weighing process without being chilled. The regulatory authority plays an important role to make sure that the chilling procedure is being carried out as part of the effort to enhance beef quality and safety.

CONCLUSION

In conclusion, the microbiological data presented in this study highlights that the occurrence and bacterial counts (TVCs, Enterobacteriaceae, and *E. coli*.) are within satisfactory range except for *Salmonella* spp. These findings indicated that there is a need to achieve better microbiological quality in locally produced beef in Malaysia. Hence, regular monitoring programme is essential as to further improve quality and maximize the productivity.

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REFERENCE

- Abdallah, F.B., Chaieb, K., Zmantar, T., Kallel, H. & Bakhrouf, A. 2009. Adherence assays and slime production of *Vibrio alginolyticus* and *Vibrio parahaemolyticus*. *Brazilian Journal of Microbiology*, **40**: 394-398.
- Australian ESAM. *E. coli* and *Salmonella* Monitoring Program. 2003. Australian Quarantine and Inspection Services (AQIS). Australia.
- Bacon, R.T., Belk, K.E., Sofos, J.N., Clayton, R.P., Reagan, J.O. & Smith, G.C. 2000. Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. *Journal of Food Protection*, **63**: 1080-1086.
- Bacteriological Analytical Manual (BAM). 2001. *BAM: Aerobic Plate Count*. USFDA. United States of Food and Drug Administration. <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.htm> (accessed on 12 June 2016).
- Bacteriological Analytical Manual (BAM). 2002. *BAM: Enumeration of Escherichia coli and the Coliform Bacteria*. USFDA. United States of Food and Drug Administration. <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm064948.htm> (accessed on 12 June 2016).
- Bacteriological Analytical Manual (BAM). 2004. *BAM: Salmonella*. USFDA. United States of Food and Drug Administration. <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm> (accessed on 12 June 2016).
- Barco, L., Bellico, S., Roccato, A. & Ricci, A. 2014. *Escherichia coli* and Enterobacteriaceae counts on pig and ruminant carcasses along the slaughter line, factors influencing the counts and relationship between visual faecal contamination of carcasses and counts: A review. *European Food Safety Authority Supporting Publication: External Scientific Report*, **31**:45-49.
- Brown, M.H., Gill, C.O., Hollingsworth, J., Nickelson, R., Seward, S. & Sheridan, J.J. 2000. The role of microbiological testing in systems for assuring the safety of beef. *International Journal of Food Microbiology*, **62**: 7-16.
- Desmarchelier, P. & Fegan, N. 2003. Enteropathogenic *Escherichia coli*. In: Hocking, A.D. (eds.). *Foodborne Microorganisms of Public Health Significance*. AIFST Inc, NSW, Australia. pp.128-134.
- DVS. Department of Veterinary Services. 2014. Malaysia: Output of Livestock Products, 2005-2014. Department of Veterinary Services Malaysia. Malaysia.
- EFSA, 2010. The assessment of the comparison of the Australian monitoring programme for carcasses to requirements in Regulation (EC) No. 2073/2005 on microbiological criteria on foodstuffs. *European Food Safety Authority Journal*, **8**: 1452-1503.
- Eyi, A. & Arslan, S. 2012. Prevalence of *Escherichia coli* in retail poultry meat, ground beef and beef. *Medycyna Weterynaryjna*, **68**: 237-240.
- FAO. Food and Agricultural Organization. 1991. Guidelines for slaughtering, meat cutting and further processing. *FAO Animal Production and Health Paper* 91. <http://www.fao.org/docrep/004/t0279e/T0279E00.htm> (accessed on 14 January 2014).
- FAO. Food and Agricultural Organization. Slaughter house cleaning and sanitation. <http://www.fao.org/docrep/003/x6557e/X6557E01.htm#ch1> (accessed on 14 January 2014).
- Gill, C.O. 2004. Visible contamination on animals and carcasses and the microbiological condition of meat. *Journal of Food Protection*, **67**: 413-419.
- Koohmaraie, M., Arthur, T.M., Bosilevac, J.M., Guerini, M.N., Shackelford, S.D. & Wheeler, T.L. 2005. Post-harvest interventions to reduce/eliminate pathogens in beef. *Meat Science*, **71**: 79-91.
- Ministry of Health Malaysia. 2013. Incidence rate and mortality rate of communicable diseases. <http://www.moh.gov.my/index.php/pages/view/324> (accessed on 10 June 2016).
- Narváez-Bravo, C., Miller, M., Jackson, T., Jackson, S., Rodas-González, A., Pond, K., Echeverry, A. & Brashears, M.M. 2013. *Salmonella* and *E. coli* O157:H7 prevalence in cattle and on carcasses in a vertically integrated feedlot and harvest plant in Mexico. *Journal of Food Protection*, **76**: 786-795.
- Nastasijevic, I., Mitrovic, R. & Buncic, S. 2009. The occurrence of *Escherichia coli* O157 in/on faeces, carcasses and fresh meats from cattle. *Meat Science*, **82**: 101-105.
- Radu, S., Mutalib, S.A., Rusul, G., Ahmad, Z., Morigaki, T., Asai, N., Kim, Y.B., Okuda, J. & Nishibuchi, M. 1998. Detection of *Escherichia coli* O157:H7 in the beef marketed in Malaysia. *Applied and Environmental Microbiology*, **64**: 1153-1156.

- Radu, S., Rozila, A.A., Rusul, G., Zainuri, A., Raha, A.R. & Sahilah, A.M. 1997. Antibiotic resistance and plasmid profiles of *Escherichia coli* in raw milk and beef. *Malaysian Applied Biology*, **26**: 77-81.
- Sallam, K.I., Mohammed, M.A., Hassan, M.A. & Tamura Tomohiro. 2014. Prevalence, molecular identification and antimicrobial resistance profile of *Salmonella* serovars isolated from retail beef products in Mansoura, Egypt. *Food Control*, **38**: 209-214.
- Schegelova, J.E., Napravnikova, M., Dendis, R., Horvath, J., Benedik, V., Babak, E., Klimova, P., Navratilova, A. & Sustackova. 2004. Beef carcass contamination in a slaughterhouse and prevalence of resistance to antimicrobial drugs in isolates of selected microbial species. *Meat Science*, **66**: 557-565.
- Small, A., Wells-Burr, B. & Buncic, S. 2005. An evaluation of selected methods for the decontamination of cattle hides prior to skinning. *Meat Science*, **69**: 263-268.
- Sofos, J.N. & Smith, G.C. 2009. *The role of slaughter hygiene in food safety. Agricultural Mechanization and Automation*. 2nd ed. Oxford, United Kingdom. pp. 393-412.
- Soon, J.M., Singh, H. & Baines, R. 2011. Foodborne diseases in Malaysia: a review. *Food Control*, **22**: 823-830.
- Teisl, M.F. & Roe, B.E. 2010. Consumer willingness to-pay to reduce the probability of retail foodborne pathogen contamination. *Food Policy*, **35**: 521-530.
- Yalcin, S., Nizamlioglu, M. & Gurbuz, U. 2001. Faecal coliform contamination of beef carcasses during the slaughtering process. *Journal of Food Safety*, **21**: 225-231.
- Yalcin, S., Nizamlioglu, M. & Gurbuz, U. 2004. Microbiological conditions of sheep carcasses during the slaughtering process. *Journal of Food Safety*, **24**: 87-93.
- Zweifel C., Baltzer, D. & Stephan R. 2005. Microbiological contamination of cattle and pig carcasses at five abattoirs determined by swab sampling in accordance with EU decision 2001/471/EC. *Meat Science*, **69**: 559-566.

