

**Artikel Asli/Original Articles****Presence of *Salmonella* spp. on Beef Carcasses and Meat Contact Surfaces at Local Abattoirs in Selangor, Malaysia****(Kehadiran *Salmonella* spp. pada Karkas Daging Lembu dan Permukaan Sentuhan Daging di Abatoir Tempatan di Selangor, Malaysia)**

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## ABSTRACT

*Salmonella* spp. is a pathogenic microbial contaminant in beef of worldwide importance. It has the ability to colonize the gastrointestinal tract of animals without producing any clinical sign. It may lead to infections in human when the contaminated meat was consumed. The main objective of this study was to evaluate the contamination of *Salmonella* spp. on beef carcasses and meat contact surfaces at selected abattoirs in Selangor, Malaysia. A total of 152 swabs from beef carcasses ( $n = 104$ ) and meat contact surfaces ( $n = 48$ ) were collected from the selected abattoirs in October 2015 to June 2016. The collected samples were examined for total viable count and prevalence of *Salmonella* spp. *Salmonella*-positive samples were confirmed by routine biochemical tests and Gram staining. The results showed that all samples contained an average viable count of  $4.56 \pm 1.23$  Log CFU/cm<sup>2</sup>. The overall prevalence of *Salmonella* spp. was 21.05% which beef carcasses and meat contact surfaces contributed 11.18% and 9.87%, respectively to the overall prevalence. The prevalence of *Salmonella* spp. on meat contact surfaces was higher than that on beef carcasses could be attributed to poor hygienic practices at the abattoirs. However, despite a lower prevalence of *Salmonella* spp. on the beef carcasses, beef could still be a potential vehicle for foodborne infections. This study suggests implementation of preventive measures and good hygienic practices at abattoirs in order to avoid cross-contamination on beef prepared for retail markets.

**Keywords:** *Salmonella*; beef carcass; meat contact surfaces; abattoir

## ABSTRAK

*Salmonella* spp. merupakan kontaminan mikrob patogenik yang penting pada daging lembu di seluruh dunia. Ia mempunyai keupayaan untuk mengkolonisasi saluran gastrousus haiwan tanpa menunjukkan sebarang tanda klinikal. Perkara ini boleh menyebabkan jangkitan kepada manusia sekiranya termakan daging yang telah terkontaminasi. Objektif utama kajian ini adalah untuk menilai kontaminasi *Salmonella* spp. pada karkas daging lembu dan permukaan sentuhan daging di abatoir terpilih yang terletak di Selangor, Malaysia. Sebanyak 152 swab daripada karkas daging lembu ( $n = 104$ ) dan permukaan sentuhan daging ( $n = 48$ ) telah diambil dari abatoir terpilih daripada Oktober 2015 sehingga Jun 2016. Sampel diuji bagi menentukan bacaan jumlah koloni hidup dan prevalens *Salmonella* spp. Sampel positif *Salmonella* spp. disahkan dengan ujian rutin biokimia dan juga perwarnaan Gram. Hasil menunjukkan bahawa semua sampel mempunyai purata bacaan jumlah koloni hidup sebanyak  $4.56 \pm 1.23$  Log CFU/cm<sup>2</sup>. Prevalens keseluruhan *Salmonella* spp. adalah 21.05% di mana atribut karkas daging lembu dan permukaan sentuhan daging adalah masing-masing sebanyak 11.18% dan 9.87%. Prevalens *Salmonella* spp. didapati adalah lebih tinggi pada permukaan sentuhan daging berbanding dengan karkas daging lembu, ini mungkin disebabkan oleh amalan kebersihan yang rendah di abatoir. Walaupun prevalens *Salmonella* spp. pada karkas daging lembu adalah lebih rendah, tetapi daging lembu masih berpotensi untuk menjadi sebagai perantara jangkitan bawaan makanan. Kajian ini mencadangkan pelaksanaan langkah kawalan dan juga pengamalan kebersihan yang baik perlu dilakukan di abatoir bagi mengelakkan kontaminasi silang pada daging lembu yang disediakan untuk pasaran.

**Kata kunci:** *Salmonella*; karkas daging lembu; permukaan sentuhan daging; abatoir

## INTRODUCTION

*Salmonella* spp. causes Salmonellosis which has long been recognized as a major human public health threat in both developed and developing countries (European Food Safety Authority 2010; FAO/WHO 2002). The Centre for Diseases Control and Prevention (CDC) estimates that approximately

1.2 million cases occur annually which results in about 450 deaths in the United States (Scallan et al. 2011). Hamdan et al. (2006) reported that the incidence rate for typhoid and paratyphoid fever is approximately 0.77 per 100,000 populations in Malaysia. However, the actual incidence rate could be higher as many cases often go unreported in many Asian countries including Malaysia (Pui et al. 2011).

Generally, poultry, eggs and meat are acknowledged as constant vehicles for *Salmonella* spp. and often involved in the infectious disease (Wilson 2002). Although findings on prevalence of *Salmonella* spp. in foods in Malaysia have been reported, yet little is known on the prevalence of *Salmonella* spp. in meat and meat products as well as at abattoirs (Arumugaswamy et al. 1995; Rusul et al. 1996; Salleh et al. 2003; Tunung et al. 2007). With increasing meat demand among Malaysians (Department of Veterinary Services 2015), biosafety of the ruminant industry deserves extra deliberation. The main aim of this study was to evaluate the prevalence of *Salmonella* spp. on beef carcasses and meat contact surfaces at local abattoirs in Selangor, Malaysia. The total viable count (TVC) was determined as to represent the overall hygienic status of the selected abattoirs. Besides, the data acquired from this study could also serve as a reference on the prevalence of *Salmonella* spp. at local abattoirs.

## MATERIALS AND METHODS

### DESIGN OF THE STUDY

Samples were collected in October 2015 to June 2016 from two abattoirs in Selangor, hence named abattoir A and abattoir B. Abattoir A was a big scale abattoir that slaughters approximately 200 cattle per day while abattoir B was a relatively small scale abattoir that slaughters approximately 30 cattle per day.

### SAMPLING OF BEEF CARCASSES

Sampling was done according to the Australian *E. coli* and *Salmonella* spp. Monitoring Programme guidelines (2003) with slight modifications. Carcass swabs were taken from three sites: brisket, flank and rump. A polyurethane sponge (3M, United States) was first moistened with 15 ml of buffered peptone water (BPW; Merck, Germany) and a template delineating 100 cm<sup>2</sup> areas was pressed on the carcass site to be sampled. In total, 104 carcasses were sampled on the slaughter line (immediately after evisceration). All samples were placed in an insulated container with ice packs and transported back to the laboratory.

### SAMPLING OF MEAT CONTACT SURFACES

Sampling on meat contact surfaces was done according to the procedure by Midura and Bryant (2001). The meat contact surfaces were knife (used for dehiding) and splitting tool (used for evisceration). Samples from meat contact surfaces were collected before any slaughter process starts on the day of sampling and after the slaughtering process (after one batch of cattle were slaughtered). The whole knife blade was swabbed using a sterile cotton tip that was moistened with BPW and was then re-swabbed using a dry sterile cotton tip. The cotton tips were placed

in a universal bottle containing 20 ml of BPW. The same sampling procedure was repeated for splitting tool. In total, 48 meat contact surfaces (before and after slaughter process) were collected. All samples were placed in an insulated container with ice packs and transported back to the laboratory.

### SAMPLE PREPARATION

At the laboratory, 10 ml of BPW was added to carcass swabs and then homogenised using a stomacher for 3 mins whereas meat contact surfaces samples were homogenised using vortex for 30 s. All the samples were analysed within 24 hrs.

### DETECTION OF *SALMONELLA* SPP.

For *Salmonella* spp. detection, samples were pre-enriched by incubation for 20 h at 37°C to allow resuscitation of injured cells. Aliquots of 0.1 ml pre-enriched samples were transferred to 10 ml of Rappaport Vassiliadis *Salmonella* enrichment broth (RVS, Merck, Germany) and then incubated at 42°C for 24 h. The cultures were then plated onto xylose lysine deoxycholate agar (XLD; Merck, Germany) and incubated at 37°C for 24 h. Black colonies were identified as presumptive *Salmonella* spp. The presumptive *Salmonella* spp. isolates was then confirmed via a series of biochemical (indole, methyl red-voges prokauer and citrate agar) tests and Gram staining.

### DETERMINATION OF TOTAL VIABLE COUNT (TVC)

For determination of TVC, serial dilutions were prepared in 0.1% peptone water (Merck, Germany) using 0.1 ml aliquots. An aliquot (0.1 ml) from appropriate dilutions were plated onto plate count agar (Merck, Germany) and subsequently incubated at 37°C for 24 hrs. The colonies were counted and the results were recorded as Log CFU/cm<sup>2</sup>.

### STATISTICAL ANALYSIS

One sample *t*-test was performed to compare the TVC results with the international standards (Australian ESAM 2003; European standards 2001) (at  $\alpha = 0.05$ ). All statistical tests were performed using the statistical package IBM SPSS Statistics 22.0.

### RESULTS

The results obtained for both TVC and the prevalence of *Salmonella* spp. on beef carcass samples and meat contact surfaces samples are presented in Table 1. The prevalence of *Salmonella* spp. found in this study was 21.05% with 32 positive samples. The presence of *Salmonella* spp. was higher in meat contact surfaces (31.25%) than in beef carcasses (16.35%). *Salmonella* spp. was detected on 2/12 (16.67%) knives (sampled before slaughter), 4/12

(33.33%) splitting tools (sampled before slaughter), 17/104 (16.35%) beef carcasses, 3/12 (30.00%) knives (sampled after slaughter) and 6/12 (50.00%) splitting tools (sampled after slaughter). Of the 4 types of meat contact surfaces, splitting tools (sampled after slaughter) were the most contaminated. The TVC recovered from splitting tools was the highest among all the sample surfaces ( $5.83 \pm 2.70$  Log CFU/cm<sup>2</sup>). TVC on meat contact surfaces and beef carcasses were also compared to European Union (EU) standards and Australian standards, respectively. The TVC recovered from beef carcasses was  $4.35 \pm 1.17$  Log CFU/cm<sup>2</sup> and the value exceeded the acceptable range recommended by the Australian standards ( $p < 0.05$ ). TVC recovered from meat contact surfaces, on the other hand, achieved  $5.01 \pm 1.24$  Log CFU/cm<sup>2</sup> and too, exceeded the acceptable range recommended by the EU standard ( $p < 0.05$ ).

TABLE 1. Total viable count and the prevalence of *Salmonella* spp.

| Type of sample             | No. of samples | No. of <i>Salmonella</i> - positive samples (%) | TVC Log CFU/cm <sup>2</sup> (Mean $\pm$ SD) |
|----------------------------|----------------|---|---|
| Beef Carcasses             | 104            | 16.35(17/104)                                   | $4.35 \pm 1.17$                             |
| Knives Before <sup>1</sup> | 12             | 16.67 (2/12)                                    | $3.64 \pm 1.53$                             |
| Splitting tools Before     | 12             | 33.33 (4/12)                                    | $5.57 \pm 2.56$                             |
| Knives After <sup>2</sup>  | 12             | 25.00(3/12)                                     | $4.99 \pm 2.23$                             |
| Splitting tools After      | 12             | 50.00 (6/12)                                    | $5.83 \pm 2.70$                             |

1: 'Before' denotes samples collected before slaughter process, 2: 'After' denotes samples collected after slaughter process

## DISCUSSION

Evaluation of the prevalence of *Salmonella* spp. in beef carcasses and the processing environment is crucial as meat is often associated with the increasing incidence of food borne illnesses worldwide (Wilson 2002). Most of the studies in Malaysia were focused on the prevalence of *Salmonella* spp. in foods other than beef and beef products, such as raw vegetable (Salleh et al. 2003; Najwa et al. 2015), fruits and fruit juices (Pui et al. 2011; Diane et al. 2013) and retail poultry (Arumugaswamy et al. 1995; Rusul et al. 1996). Hence, there is a need to look into the prevalence of *Salmonella* spp. in beef in Malaysia.

Our findings reported a higher prevalence of *Salmonella* spp. on beef carcasses (16.35%) than that reported previously. For instance, *Salmonella* spp. was isolated from 14/355 (3.94%) samples by Li et al. (2004) whereas 5/290 (1.724%) by Dong et al. (2014). The relatively higher prevalence of *Salmonella* spp. found on beef carcasses and processing tools suggests potential health threat by locally processed beef to consumers. According to the Australian ESAM (2003), there should be a total absence of *Salmonella* spp. on beef carcasses. In addition, the TVC recovered from the samples also exceeded the levels recommended by the Australian ESAM (2003) and was therefore considered

as unsatisfactory and non-conformance. The Australian ESAM also suggests continuous monitoring programme and further training for abattoir operators in order to avoid the bacterial contamination in beef and beef products.

There are several steps in the slaughtering process that render possibilities for *Salmonella* spp. contamination such as hide removal and evisceration (Gill et al. 2003). Cattle are normally asymptomatic carrier for *Salmonella* spp. and shed bacteria in their feces (Pinto & Tenreiro 2012). If the hide of the slaughtered cattle was highly contaminated with feces, these microorganisms could contaminate the carcass surfaces via airborne transfer, direct contact of the contaminated hide to the carcass surfaces (due to lack of skills of the meat handler) and also due to the exertion of contaminated equipment (Doyle & Erikson 2006). Carcasses would then also become contaminated during evisceration due to intestinal breakage and fecal leakage (Haeghebaert et al. 2003). Since none of the visited abattoirs in this study implement pathogen reduction intervention, therefore it is important that additional intervention strategies should be implemented at these identified critical control points. Maintaining the cleanliness of lairage and abattoir environment is another key effort to control *Salmonella* spp. contamination as Hurd et al. (2001) and Swanenburg et al. (2001) suggested that lairage is probably the major source for *Salmonella* infection in cattle.

Narváez et al. (2013) has reported that final product of the carcasses is 5.96 times likely positive with *Salmonella* spp. if the carcass was found *Salmonella*-positive at the pre evisceration phase. Ideally, slaughtering process in the abattoirs aims to reduce cross-contamination of animal products by foodborne pathogens that are of animal origin. The production process should prevent an increase of pathogenic loads in the final products. A similar study done by Brichta-Harhay et al. (2008) has reported that the prevalence of *Salmonella* spp. on post intervention carcasses was significantly decreased by the processing interventions employed. This showed that control and monitoring at each critical point are important for pathogen loads reduction. The World Health Organization, WHO (1999), reported that most of foodborne outbreaks are closely associated with cross-contamination events involving deficient hygienic practices, contaminated equipment and contamination via food handlers, processing or inadequate storage. As cattle frequently carry *Salmonella* spp. in their gastrointestinal tract, during slaughtering process, the pathogen could cross contaminate equipment and tool surfaces during dehiding or evisceration (Fegan et al. 2005). Once it is introduced on meat contact surfaces, *Salmonella* spp. persists over time in the processing environment as it is highly tolerant and readily resistant in various environments (Sallam et al. 2014). The findings of this study revealed that cleaning procedures at the abattoirs might be ineffective at time being, as *Salmonella* spp. was detected on meat contact surfaces (both knives and splitting tools) even before the slaughter process began. This is especially risky as many



reports have shown that *Salmonella* spp. is capable of colonizing different inert food contact surfaces and forms biofilm which in turn become a persistent source of food contamination (Joseph et al. 2011).

To date, there are no microbiological guidelines available at national level, hence, TVC for both beef carcasses and meat contact surfaces were compared to the international guidelines in this study. According to the Australian ESAM (2003), the upper limit of TVC for beef carcasses is 5.00 Log CFU/cm<sup>2</sup> while the EU legislation (2001) stated that TVC for food contact surfaces should not exceed 1.00 Log CFU/cm<sup>2</sup>. Based on these guidelines, the overall abattoir hygiene in this study can be deemed as poor. It is a pivotal step for abattoir management to work on minimizing bacterial contamination on both beef carcasses and meat contact surfaces. Good and sufficient hygienic practice is one of the crucial factors to improve the hygiene of abattoirs and quality of animal products.

## CONCLUSION

Studies on the prevalence of *Salmonella* spp. in beef and beef processing environments need to be emphasized as it poses a risk for cross-contamination in beef products which is then possibly transferred to consumers. This study also highlighted the need for improving cleaning and hygienic procedures at local abattoirs. Controls such as hot water rinses of the knives in between slaughtering a carcass to prevent cross-contamination can be implemented as to mitigate *Salmonella* spp. contamination at abattoirs. Data acquired from this study will be able to serve as a baseline study in order to propose a national standard and enhances meat safety awareness among the processors and consumers thus reducing the risk of foodborne illnesses.

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