Microbiological quality of cooked meat products sold in Kelantan, Malaysia during Ramadhan month

1,2Ain Auzureen, M. Z., 3Erkihun Aklilu, 2Mohd Azam Khan, G. K., 2Ruhil Hayati, H., 2Al Sultan, I. I. and 3Soon, J. M.

1Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan
2Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, City Campus, 16100 Pengkalan Chepa, Kelantan
3International Institute of Nutritional Sciences and Applied Food Safety Studies, School of Sport and Wellbeing, University of Central Lancashire, Preston, UK

Abstract
The Ramadhan fast is a form of worship where Muslims spend the daylight hours in a complete fast. During the month of Ramadhan, a large number of people tend to purchase food from stalls or bazaars for breaking fast. It is crucial that the foods prepared and sold are handled in a clean and safe manner. Thus, this research focused on evaluating the microbiological quality of cooked meat products (beef and chicken) from food bazaars and street-vended foods in 10 districts throughout the state of Kelantan. A total of 100 samples were collected from all 10 districts in Kelantan and were tested for coliform, *Escherichia coli*, *Salmonella* spp., and *Staphylococcus* spp. Microbiological analysis of the meat samples showed unsatisfactory results where a total of 42% of the samples were found unsatisfactory for coliform. The overall prevalence for *Staphylococcus* spp. in beef and chicken were 19.6% and 12.9%. *Escherichia coli* were detected in 23.9% of beef and 12.9% of chicken. Non-compliances for *Salmonella* were found in 13% and 9.3% of beef and chicken samples. This study determined the presence of foodborne pathogen in cooked meat products and indicated the possibilities of cross contamination and lack of hygiene during food handling.

Keywords
Foodborne pathogen
Food handlers
Street-vended food
FRAP

Introduction
Ramadhan is the ninth month of the Islamic lunar calendar and Muslims will spend the daylight hours in a complete fast (Dodge, 2014). Fasting refers to the condition of not eating for a varying duration of time (Fasting Center International, 2002). The Ramadhan fast is a form of worship that is a part of the five pillars of Islam, and is required of every healthy adult Muslim for a complete month (between 28-30 days) for approximately 14 hours/day (Gustaviani et al., 2004). Muslims will break fast when the sun sets and it is crucial that the community observing the fasting period receives an adequate and balanced meal.

Since Ramadhan is a very special occasion, a number of street vended food stalls and bazaars will operate to sell a variety of dishes. Food bazaars in Malaysia are a congregation of food stalls in an opened area. During the fasting period, the calorie intake ranged between 1300 – 1400 cal/day (Gustaviani et al., 2004) compared to a normal (without fasting) daily intake of 1800-2000 cal/day. However, the surge in calories usually occurred during the breaking of fast. People also have less time to prepare home-cooked food and due to the fasting period, people will be more lethargic and tend to purchase take away meals. Meat products represent one of the main breaking fast dishes consumed by Muslims community. Hence it is important that the foods sold at food stalls are hygienic and safe to prevent foodborne illnesses.

Foodborne illnesses, particularly food poisoning cases are on the rise, especially during the fasting month (Soon et al., 2011; Soon, 2013). This may be due to the surge in consumption of take away meals and a higher number of reported cases. Inappropriate food handling practices will result in cross contamination and/or recontamination events. Pérez-Rodríguez et al. (2008) defined cross contamination as “a general term which refers to the transfer, direct or indirect, of bacteria or virus from a contaminated product to a non-contaminated product” and recontamination as “contamination of food after it has been submitted to an inactivation process”. Lacking in personnel hygiene among food handlers is one of the most commonly reported practices contributing to foodborne illnesses (Lues and Van Tonder, 2007).
Tirado and Schmidt (2000) also concluded that this substantial proportion of foodborne diseases can be attributed to food preparation practices in the domestic environment. Some of the main risk factors are inappropriate storage (32%), inadequate heat treatment (26%) and cross contamination from raw to cooked foods (25%) (Smerdon et al., 2001).

Street vended foods are popular among urban people as they are inexpensive, convenient and attractive (WHO, 1996). Studies from Bangladesh (Al Mamun et al., 2013), China (Liu et al., 2014), Korea (Cho et al., 2011), Philippines (Azanza, 2005; Manguiat and Fang, 2013), Senegal (Cardinale et al., 2005), South Africa (Mosupye et al., 2002; Oguttu et al., 2014), Taiwan (Manguiat and Fang, 2013) reported that the microbiological quality of street vended food and beverages were found unsatisfactory. In Malaysia, a number of studies in the safety of street vended foods (Haryani et al., 2007) and ready-to-eat (RTE) foods (Marian et al., 2012; Jamali et al., 2013) had been conducted. This was followed by a few other studies on Knowledge, Attitudes and Practices (KAP) (Toh and Birchenough, 2000; Noor-Azira et al., 2012; Norrakiah and Siow, 2014), food handlers’ attitude at school canteens (Saidatul and Hayati, 2013) hand hygiene practices (Tan et al., 2014) and food service hygiene factors (Ungku et al., 2011). It is crucial that the foods prepared and sold are handled in a clean and safe manner. Thus, this research focused on evaluating the microbiological quality of cooked meat products (beef and chicken) from food bazaars and street-vended foods. So far to our knowledge the present study represents the first microbiological quality survey of cooked meat products sold in food bazaars during the Ramadhan.

Materials and Methods

Study sites and sampling
A total of 53 bazaars from all 10 districts in the state of Kelantan were selected. Kelantan has the highest Muslim population in Malaysia. The districts include: Kota Bharu, Bachok, Pasir Puteh, Tumpat, Pasir Mas, Machang, Tanah Merah, Jeli, Gua Musang and Kuala Krai (Figure 1). A total of 46 beef and 54 chicken samples were collected in July 2014 for laboratory analysis. The 100 samples were purchased from all 53 bazaars and were selected based on availability and variability of types of cooked meat products. A description of some selected cooked meat products sold at bazaars is shown in Table 1.

Laboratory procedures for meat samples
Meat samples were collected in sterile bags and transported to the laboratory in a carrier box containing ice packs. Analyses were performed upon receipt of samples at the laboratory. However, if a laboratory analysis was postponed due to delayed arrival of samples, the samples were refrigerated at 0–4°C until examination but were not kept longer than 36 hours (Al Mamun et al., 2013). 25 g of each sample were homogenised in 1% buffered peptone water in a Stomacher 400 Circulator (Seward, UK) blender for 2 minutes. Following homogenization, all meat samples were tested for coliform bacteria, Escherichia coli, Salmonella spp., and Staphylococcus spp. Total coliform were enumerated using multiple tube fermentation technique. MacConkey and Eosin methylene blue agar were used to determine the presence of E. coli followed by indole tests. Rappaport Vassiliadis broth was used as selective broth for enrichment of Salmonella spp. and Xylose lysine deoxycholate agar was used as selective agar for detection of Salmonella spp. Suspected black colonies were sub-cultured to obtain pure colonies and confirmed with Triple sugar iron (TSI) agar tests. Mannitol salt agar was used as selective medium for Staphylococcus spp. Acid production as the result of fermentation of mannitol results in formation of yellow colonies and zones (APHA, 2001). Coliform counts of less than 10^2 per g were considered acceptable (ICMSF, 1986). E. coli should be < 3 cfu/g, coagulase positive staphylococci should be < 10^2 per g and Salmonella spp. should be absent in 25 g (EC No 2073/2005; FSANZ, 2001).
Results and Discussion

Of the total meat samples (n=100), 62% were tested positive for total coliforms. 42% were found to be unsatisfactory (total coliforms ≥ 10² per g). Results revealed that all samples from Tanah Merah were unsatisfactory. On the other hand, all samples from Pasir Puteh and Machang districts were found satisfactory (Figure 2). Table 2 shows the overall prevalence of *Staphylococcus* was 16%. There was significance difference in the prevalence among the districts (DF = 9, p < 0.05). The overall prevalence of *Staphylococcus* spp. in chicken was 12.9% while beef was 19.6%. The overall prevalence of unsatisfactory quality beef and chicken was 8.7% and 3.7%. These are meat products contaminated with *Staphylococcus* spp. at concentration greater than 10² per g. Coagulase tests were carried out and tested negative for S. aureus.

In the samples tested, no coagulase positive staphylococci were detected. Coagulase positive staphylococci such as *S. aureus* cause food poisoning and superficial skin infections (Chakraborty et al., 2011). However staphylococci can be routinely isolated from humans and associated environments. Staphylococci are ubiquitously distributed in man’s environment and strains present in the nose often contaminate the back of hands, fingers and face (Garcia et al., 1986; Lues and Van Tonder, 2007). Most food sellers did not wear gloves, masks or
aprons. Hands are the most important anatomy of food handlers and are the main culprits for cross contamination. At times, food handlers are not aware of their own movements and may rub their faces, nose and other body parts. Tan et al. (2014) isolated multidrug resistant *S. aureus* strains from food handlers' hands in Malaysia. Presence of *S. aureus* strains would facilitate the transmission of bacteria into food and staphylococcal food poisoning is one of the most common foodborne diseases that affects hundreds of thousands of people worldwide annually (Hennekinne et al., 2012; Ji-Yeon et al., 2013). Tan et al. (2013) also reported that the least practiced habits among food handlers in Malaysia were hand washing and usage of face masks during food preparation. Pérez-Rodríguez et al. (2010) observed infrequent hand washing practices after handling raw products and/or before slicing cooked meat products. Coliforms, Enterobacteriaceae and *S. aureus* were found on both food handlers' hands and their aprons (Lues and Van Tonder, 2007). There was also a lack of hand washing facilities. This is in agreement with the present study where all food stalls did not have portable hand washing sinks.

*Escherichia coli* were detected in all cooked meat products except Pasir Puteh, Gua Musang, Machang and Pasir Mas (Table 3). Results showed that the difference between cooked beef and chicken samples collected from the rest of the districts were significantly different (*p < 0.05*). This is in agreement with Saif et al. (2009) and Viswanathan and Kaur (2000) who suggested that *E. coli* were found and transmitted mainly in food derived from cattle. *Salmonella* spp. was detected in 11 meat samples from all districts. The percentage of positive samples for *Salmonella* spp. corresponded to 13% for beef and 9.3% for chicken. The incidences of potential foodborne pathogens such as *E. coli* (18% of samples) and *Salmonella* (11% of samples) are relatively high (Table 3).

The presence of coliform and *E. coli* in fully cooked RTE can be an indication of poor hygiene and sanitation or inadequate heat treatment (NSW Food Authority, 2009). Most meat-borne outbreaks were due to improper food handling practices and consumption of undercooked meat. However, the majority of pathogenic bacteria that can spread at slaughter by cross-contamination were traced back to production on the farm rather than originating from food handlers' hands.
from slaughter plant (Soon et al., 2011). Besides applying correct food handling techniques, on-farm intervention strategies to reduce microbial load are crucial to reduce contamination in the food chain. Meanwhile, the presence of Salmonella spp. in RTE foods may be a result of undercooking, poor handling practices and cross contamination (NSW Food Authority, 2009). Cooked foods are vulnerable if touched by Salmonella-contaminated fingers that have been contaminated by low numbers of the bacteria (Guzewich and Ross, 1999). A person may carry Salmonella in their faeces without any signs of infection. They may then contaminate food by not washing their hands after using the toilet thus spreading Salmonella to others through contaminated food. There was no reported outbreak of typhoid fever in Kelantan during the Ramadhan period. It is possible that sporadic cases occurred but were unreported (Soon et al., 2011).

**Cross contamination from food contact surfaces to cooked meat products**

Cross contamination via cooking utensils, food handlers, processing equipments, deficient hygiene practices, inadequate cooking and storage are closely related to foodborne outbreaks (Carrasco et al., 2012). Inappropriate food handling practices such as using the same cutting board for raw and RTE food is a potential vehicle for cross contamination. *Listeria monocytogenes* (Goh et al., 2014) and *Campylobacter jejuni* (Tang et al., 2011) were transmitted from raw chicken meat to cooked chicken meat via cutting boards. Cutting boards are commonly perceived as significant fomites in cross contamination of foodstuffs with foodborne agents (Carrasco et al., 2012). But studies by Moore et al. (2007) underlined that food contact surfaces that are “easy to clean” (e.g. Formica and stainless steel) may be more likely to release foodborne pathogens during common food preparation practices.

Additionally, foodborne pathogens readily transmit from wet kitchen sponges to stainless steel surfaces to food (Kusumaningrum et al., 2003) and from poultry meats to stainless steel surfaces (Malheiros et al., 2010). In fact, Kusumaningrum et al. (2003) and Takahashi et al. (2011) found that pathogens remain viable on dry stainless steel surfaces and present a contamination hazard for considerable periods of time. Pests particularly flies are potential vectors for pathogens. Pest control practices observed at most bazaars include usage of candles, hand-made fly swat or adhesive paper to trap flies.

Hand washing is easy to do and it’s one of the most effective ways to prevent the spread of many types of infection and illness in all setting (CDC, 2013). This is because the hands of food handlers can be vector to spread harmful microorganism through cross contamination. Food handlers can also spread microorganisms during and after they experience gastrointestinal infections (Baş et al., 2006). Training is crucial to any food safety systems. Poor staff training in food hygiene is a real threat to food safety; hence effective training is an important prerequisite to successful implementation of a food safety management system (Arvanitoyannis and Kassaveti, 2009). To be effective, food safety training

<table>
<thead>
<tr>
<th>Districts</th>
<th>Isolated bacteria</th>
<th>E. coli</th>
<th>Salmonella spp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beef</td>
<td>Chicken</td>
<td>Beef</td>
</tr>
<tr>
<td>Kota Bharu</td>
<td>62.5%</td>
<td>25%</td>
<td>12.5%</td>
</tr>
<tr>
<td>Bachok</td>
<td>50%</td>
<td>0%</td>
<td>25%</td>
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<tr>
<td>Jeli</td>
<td>50%</td>
<td>25%</td>
<td>0%</td>
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<tr>
<td>Pasir Pateh</td>
<td>0%</td>
<td>5%</td>
<td>0%</td>
</tr>
<tr>
<td>Gua Musang</td>
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<td>0%</td>
</tr>
<tr>
<td>Machang</td>
<td>0%</td>
<td>0%</td>
<td>50%</td>
</tr>
<tr>
<td>Kuala Krai</td>
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<td>33.3%</td>
</tr>
<tr>
<td>Tanah Merah</td>
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<td>0%</td>
</tr>
<tr>
<td>Tunap</td>
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<td>25%</td>
<td>0%</td>
</tr>
<tr>
<td>Pasir Mas</td>
<td>0%</td>
<td>0%</td>
<td>20%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>23.9%</td>
<td>12.9%</td>
<td>13%</td>
</tr>
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</table>

**Table 3. E. coli and Salmonella spp. in cooked meat samples**
needs to target changing the behaviour. Griffith (2000) argued that behavioural change (i.e. the implementation of required hygiene practices) is not easily achieved and that consideration must be given to motivation, constraints, barriers and facilities as well as to cultural aspects. Food safety practices will only be implemented given adequate resources and appropriate management culture (Clayton and Griffith, 2008). Besides educating and training in appropriate food handling practices, food handlers or operators can be trained in simple qualitative risk assessments (risk matrix: severity x probability) to determine food safety risks (Manning and Soon, 2013).

Conclusion

Food stalls and bazaars fulfil the demands of consumers and assist in socio-economic growth of food vendors. However, the safety of food sold may be compromised due to unhygienic handling and inappropriate storage temperature. Hence, priority should be placed in assisting food vendors in understanding the importance and requirements of food safety. All food sellers and handlers should be registered and trained under the Food Handlers’ Training Programme and foodstalls inspected for hygiene.

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