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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

Running title: Microbiological Quality of Cooked Meat Products

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 Vasiliadis 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 ≥ 10^2 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).

143 Table 2 shows the overall prevalence of *Staphylococcus* spp. was
144 16%. There was significance difference in the prevalence among the
145 districts (DF = 9, $p < 0.05$). The overall prevalence of *Staphylococcus* spp.
146 in chicken was 12.9% while beef was 19.6%. The overall prevalence of
147 unsatisfactory quality beef and chicken was 8.7% and 3.7%. These are
148 meat products contaminated with *Staphylococcus* spp. at concentration
149 greater than 10^2 per g. Coagulase tests were carried out and tested
150 negative for *S. aureus*.

151 In the samples tested, no coagulase positive staphylococci were
152 detected. Coagulase positive staphylococci such as *S. aureus* cause food
153 poisoning and superficial skin infections (Chakraborty *et al*., 2011).
154 However staphylococci can be routinely isolated from humans and
155 associated environments. Staphylococci are ubiquitously distributed in
156 man's environment and strains present in the nose often contaminate the
157 back of hands, fingers and face (Garcia *et al*., 1986; Lues and Van Tonder,
158 2007). Most food sellers did not wear gloves, masks or aprons. Hands are
159 the most important anatomy of food handlers and are the main culprits for
160 cross contamination. At times, food handlers are not aware of their own
161 movements and may rub their faces, nose and other body parts. Tan *et al*.
162 (2014) isolated multidrug resistant *S. aureus* strains from food handlers'
163 hands in Malaysia. Presence of *S. aureus* strains would facilitate the
164 transmission of bacteria into food and staphylococcal food poisoning is one
165 of the most common foodborne diseases that affects hundreds of
166 thousands of people worldwide annually (Hennekinne *et al*., 2012; Ji-Yeon

167 *et al.*, 2013). Tan *et al*. (2013) also reported that the least practiced habits
168 among food handlers in Malaysia were hand washing and usage of face
169 masks during food preparation. Pérez-Rodríguez *et al*. (2010) observed
170 infrequent hand washing practices after handling raw products and/or
171 before slicing cooked meat products. Coliforms, Enterobacteriaceae and *S.*
172 *aureus* were found on both food handlers' hands and their aprons (Lues
173 and Van Tonder, 2007). There was also a lack of hand washing facilities.
174 This is in agreement with the present study where all food stalls did not
175 have portable hand washing sinks.

176 *Escherichia coli* were detected in all cooked meat products except
177 Pasir Puteh, Gua Musang, Machang and Pasir Mas (Table 3). Results
178 showed that the difference between cooked beef and chicken samples
179 collected from the rest of the districts were significantly different ($p < 0.05$).
180 This is in agreement with Saif *et al*. (2009) and Viswanathan and Kaur
181 (2000) who suggested that *E. coli* were found and transmitted mainly in
182 food derived from cattle. *Salmonella* spp. was detected in 11 meat samples
183 from all districts. The percentage of positive samples for *Salmonella* spp.
184 corresponded to 13% for beef and 9.3% for chicken. The incidences of
185 potential foodborne pathogens such as *E. coli* (18% of samples) and
186 *Salmonella* (11% of samples) are relatively high (Table 3).

187 The presence of coliform and *E. coli* in fully cooked RTE can be an
188 indication of poor hygiene and sanitation or inadequate heat treatment
189 (NSW Food Authority, 2009). Most meat-borne outbreaks were due to
190 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 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 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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519 Table 1 Description of cooked meat products sold at bazaars during

520 Ramadhan

Cooked meat products	**Brief description**
Black pepper beef	Beef marinated in soy sauce and black pepper
Soy sauce beef	Beef fried with soy sauce, onion and red pepper
Fried beef	Beef marinated with salt, onions and pepper and then fried
Beef / chicken *kurma*	Beef or chicken cooked in *kurma* gravy made from mixed curry powder, spices, potatoes, coconut milk and coriander.
Singgang beef	Beef cooked in sauce with plenty of herbs such as galangal, chillies, garlic, onion and black pepper
Spicy red beef / chicken	Beef or chicken cooked in concentrated sauce of dried chillies
Beef /chicken *gulai*	*Gulai* is similar to curry except lighter in taste and colour.
Beef / chicken *kerutuk*	Beef or chicken cooked in mixture of *kerisik* (toasted grated coconut) and *kerutuk* spices (coriander powder, cardamom seeds, clove,

Cooked meat products	Brief description
	fennel seeds, cumin, black peppercorns, turmeric, galangal, lemongrass bulbs and garlic)
Beef *gulai acar / dalca*	Beef or chicken cooked in curry powder with potatoes, carrots, eggplants, green beans, chillies, curry leaves and baby corns.
Beef *kawah*	Beef cooked in a large pot of curry. Popular during wedding ceremonies in Kelantan.
Air asam perut lembu	Cow intestines are sliced and boiled with vinegar, lime, chives, onion, shrimp paste, chillies and tamarind
Kunyit beef	Beef marinated with salt and turmeric powder and fried.
Gearbox soup	Made from bull's joints and boiled in richly flavoured soup.
Ginger chicken	Chicken marinated and cooked with salt and sliced ginger.
Roasted chicken	Chicken marinated with honey, black pepper, aniseed, soy sauce, oyster sauce and ginger before roasted.
Chicken *Tom yam*	Chicken cooked in mixed spicy chilli paste with lime leaf and lemongrass. Tom yam

Cooked meat products	Brief description
	originates from Thailand.
Percik chicken	Chicken cooked in coconut milk, dried chillies, garlic and lemongrass and then roasted.
Paprika chicken	Chicken cooked with lime leaves, *tom yam* paste, lemongrass, hot pepper, fish sauce, ginger, onion, garlic, sweet soy sauce and some vegetables.
Kerisik chicken	Chicken cooked with *kerisik* (toasted, grated coconut), galangal, chillies, ginger and brown sugar
Honey chicken butts / wings	Chicken butts / wings marinated with honey, ginger, oyster sauce, soy sauce, black pepper, and garlic and roasted.
Ayam *tiga rasa*	Chicken cooked with ginger, plum sauce, sweet soy sauce, tomato sauce, lime, spicy pepper and ginger to produce sweet, sour and spicy taste.
Ayam *peparu kicap*	Chicken lungs cooked with soy sauce, onion, garlic and capsicum.

521

522

523 Table 2. Prevalence of *Staphylococcus* spp. in cooked meat products

Districts	Sample	*Staphylococcus* spp.	Prevalence (%)	Unsatisfactory	Prevalence (%)	Sample	*Staphylococcus* spp.	Prevalence (%)	Unsatisfactory	Prevalence (%)
			Beef					**Chicken**		
Kota Bharu	8	ND	ND	-	-	8	ND	-	-	-
Bachok	4	1	25	-	-	4	1	25	-	-
Jeli	4	ND	-	-	-	8	1	25	-	-
Pasir Puteh	5	ND	-	-	-	5	ND	-	-	-
Gua Musang	4	1	25	-	-	4	ND	-	-	-
Machang	4	2	50	1	25	5	1	20	1	25
Kuala Krai	3	ND	-	-	-	6	ND	-		
Tanah Merah	5	1	20	-	-	5	2	20	1	25
Tumpat	4	2	50	2	50	4	1	25	-	-
Pasir	5	2	40	1	20	5	1	20	-	-

Mas										
Total	46	9	19.6	4	8.7	54	7	12.9	2	3.7

524 ND: Not detected

525 Table 3. *E. coli* and *Salmonella* spp. in cooked meat samples

Districts	***Isolated bacteria***			
	E. coli		*Salmonella* spp.	
	Beef	Chicken	Beef	Chicken
Kota Bharu	62.5% (5/8)	25% (2/8)	12.5% (1/8)	37.5% (3/8)
Bachok	50% (2/4)	0% (0/4)	25% (1/4)	0% (0/4)
Jeli	50% (2/4)	25% (2/8)	0% (0/4)	12.5% (1/8)
Pasir Puteh	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/5)
Gua Musang	0% (0/4)	25% (1/4)	0% (0/4)	0% (0/4)
Machang	0% (0/4)	0% (0/5)	50% (2/4)	20% (1/5)
Kuala Krai	33.3% (1/3)	0% (0/6)	33.3% (1/3)	0% (0/6)
Tanah Merah	0% (0/5)	20% (1/5)	0% (0/5)	0% (0/5)
Tumpat	25% (1/4)	25% (1/4)	0% (0/5)	0% (0/5)
Pasir Mas	0% (0/5)	0% (0/5)	20% (1/5)	0% (0/5)
Total	23.9% (11/46)	12.9% (7/54)	13% (6/46)	9.3% (5/54)

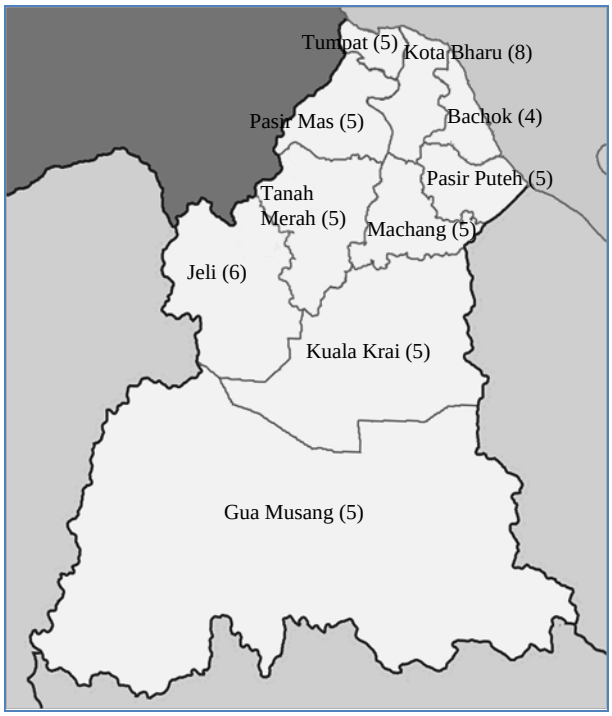
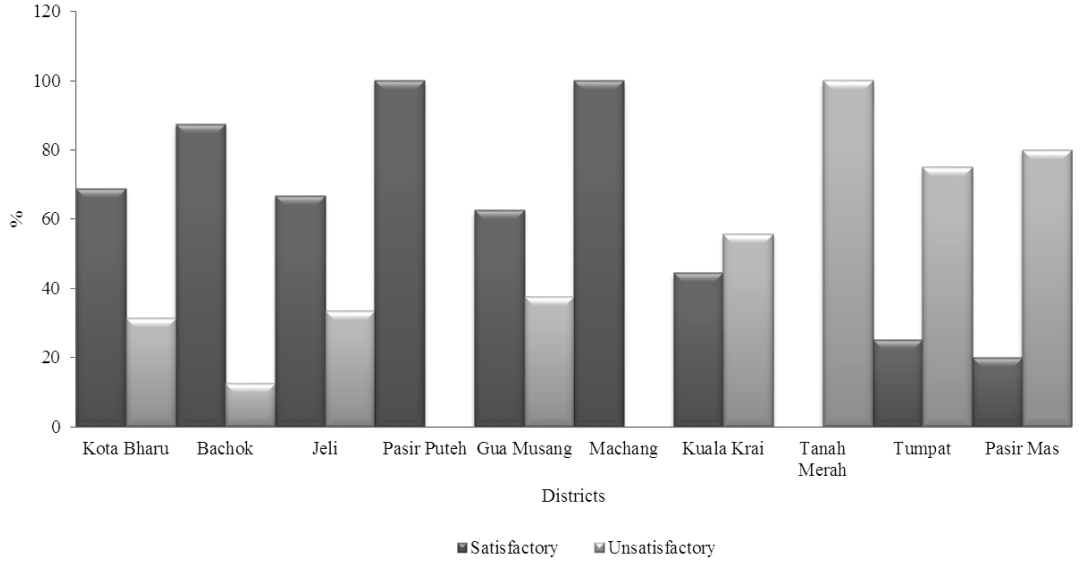


Figure 1. Map showing the number of bazaars where beef and chicken meats were sampled in Kelantan, Malaysia



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542 Figure 2. Total coliform counts in cooked meat samples (n=100) collected from different districts (unsatisfactory: total coliforms
543 ≥ 10^2 per g) (No unsatisfactory counts detected for Pasir Puteh and Machang and no satisfactory counts detected for Tanah
544 Merah)