

25 coliform. The overall prevalence for *Staphylococcus* spp. in beef and
26 chicken were 19.6% and 12.9%. *Escherichia coli* were detected in 23.9%
27 of beef and 12.9% of chicken. Non-compliances for *Salmonella* were found
28 in 13% and 9.3% of beef and chicken samples. This study determined the
29 presence of foodborne pathogen in cooked meat products and indicated
30 the possibilities of cross contamination and lack of hygiene during food
31 handling.

32

33 **Keywords:** foodborne pathogen; food handlers; street-vended food

34

35 **Running title:** Microbiological Quality of Cooked Meat Products

36

37 **Introduction**

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

47

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

60 Foodborne illnesses, particularly food poisoning cases are on the rise,
61 especially during the fasting month (Soon *et al.*, 2011; Soon, 2013). This
62 may be due to the surge in consumption of take away meals and a higher
63 number of reported cases. Inappropriate food handling practices will result
64 in cross contamination and/or recontamination events. Pérez-Rodríguez *et*
65 *al.* (2008) defined cross contamination as “a general term which refers to
66 the transfer, direct or indirect, of bacteria or virus from a contaminated
67 product to a non-contaminated product” and recontamination as
68 “contamination of food after it has been submitted to an inactivation
69 process”. Lacking in personnel hygiene among food handlers is one of the
70 most commonly reported practices contributing to foodborne illnesses
71 (Lues and Van Tonder, 2007). Tirado and Schmidt (2000) also concluded

72 that this substantial proportion of foodborne diseases can be attributed to
73 food preparation practices in the domestic environment. Some of the main
74 risk factors are inappropriate storage (32%), inadequate heat treatment
75 (26%) and cross contamination from raw to cooked foods (25%) (Smerdon
76 *et al.*, 2001).

77 Street vended foods are popular among urban people as they are
78 inexpensive, convenient and attractive (WHO, 1996). Studies from
79 Bangladesh (Al Mamun *et al.*, 2013), China (Liu *et al.*, 2014), Korea (Cho *et*
80 *al.*, 2011), Philippines (Azanza, 2005; Manguiat and Fang, 2013), Senegal
81 (Cardinale *et al.*, 2005), South Africa (Mosupye *et al.*, 2002; Oguttu *et al.*,
82 2014), Taiwan (Manguiat and Fang, 2013) reported that the microbiological
83 quality of street vended food and beverages were found unsatisfactory. In
84 Malaysia, a number of studies in the safety of street vended foods (Haryani
85 *et al.*, 2007) and ready-to-eat (RTE) foods (Marian *et al.*, 2012; Jamali *et*
86 *al.*, 2013) had been conducted. This was followed by a few other studies
87 on Knowledge, Attitudes and Practices (KAP) (Toh and Birchenough, 2000;
88 Noor-Azira *et al.*, 2012; Norrakiah and Siow, 2014), food handlers' attitude
89 at school canteens (Saidatul and Hayati, 2013) hand hygiene practices
90 (Tan *et al.*, 2014) and food service hygiene factors (Ungku *et al.*, 2011). It is
91 crucial that the foods prepared and sold are handled in a clean and safe
92 manner. Thus, this research focused on evaluating the microbiological
93 quality of cooked meat products (beef and chicken) from food bazaars and
94 street-vended foods So far to our knowledge the present study represents

95 the first microbiological quality survey of cooked meat products sold in food
96 bazaars during the Ramadhan.

97

98 **Materials and Methods**

99

100 *Study sites and sampling*

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

110

111 *Laboratory procedures for meat samples*

112 Meat samples were collected in sterile bags and transported to the
113 laboratory in a carrier box containing ice packs. Analyses were performed
114 upon receipt of samples at the laboratory. However, if a laboratory analysis
115 was postponed due to delayed arrival of samples, the samples were
116 refrigerated at 0 – 4°C until examination but were not kept longer than 36
117 hours (Al Mamun *et al.*, 2013). 25 g of each sample were homogenised in
118 1% buffered peptone water in a Stomacher 400 Circulator (Seward, UK)

119 blender for 2 minutes. Following homogenization, all meat samples were
120 tested for coliform bacteria, *Escherichia coli*, *Salmonella* spp., and
121 *Staphylococcus* spp. Total coliform were enumerated using multiple tube
122 fermentation technique. MacConkey and Eosin methylene blue agar were
123 used to determine the presence of *E. coli* followed by indole tests.
124 Rappaport Vasiliadis broth was used as selective broth for enrichment of
125 *Salmonella* spp. and Xylose lysine deoxycholate agar was used as
126 selective agar for detection of *Salmonella* spp. Suspected black colonies
127 were sub-cultured to obtain pure colonies and confirmed with Triple sugar
128 iron (TSI) agar tests. Mannitol salt agar was used as selective medium for
129 *Staphylococcus* spp. Acid production as the result of fermentation of
130 mannitol results in formation of yellow colonies and zones (APHA, 2001).
131 Coliform counts of less than 10^2 per g were considered acceptable (ICMSF,
132 1986). *E. coli* should be < 3 cfu/g, coagulase positive staphylococci should
133 be $< 10^2$ per g and *Salmonella* spp. should be absent in 25 g (EC No
134 2073/2005; FSANZ, 2001).

135

136 **Results and Discussion**

137

138 Of the total meat samples (n=100), 62% were tested positive for total
139 coliforms. 42% were found to be unsatisfactory (total coliforms $\geq 10^2$ per g).
140 Results revealed that all samples from Tanah Merah were unsatisfactory.
141 On the other hand, all samples from Pasir Puteh and Machang districts
142 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.

191 However, the majority of pathogenic bacteria that can spread at slaughter
192 by cross-contamination were traced back to production on the farm rather
193 than originating from slaughter plant (Soon *et al.*, 2011). Besides applying
194 correct food handling techniques, on-farm intervention strategies to reduce
195 microbial load are crucial to reduce contamination in the food chain.

196 Meanwhile, the presence of *Salmonella* spp. in RTE foods may be a
197 result of undercooking, poor handling practices and cross contamination
198 (NSW Food Authority, 2009). Cooked foods are vulnerable if touched by
199 *Salmonella*-contaminated fingers that have been contaminated by low
200 numbers of the bacteria (Guzewich and Ross, 1999). A person may carry
201 *Salmonella* in their faeces without any signs of infection. They may then
202 contaminate food by not washing their hands after using the toilet thus
203 spreading *Salmonella* to others through contaminated food. There was no
204 reported outbreak of typhoid fever in Kelantan during the Ramadhan
205 period. It is possible that sporadic cases occurred but were unreported
206 (Soon *et al.*, 2011).

207

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

209 Cross contamination via cooking utensils, food handlers, processing
210 equipments, deficient hygiene practices, inadequate cooking and storage
211 are closely related to foodborne outbreaks (Carrasco *et al.*, 2012).
212 Inappropriate food handling practices such as using the same cutting board
213 for raw and RTE food is a potential vehicle for cross contamination. *Listeria*
214 *monocytogenes* (Goh *et al.*, 2014) and *Campylobacter jejuni* (Tang *et al.*,

215 2011) were transmitted from raw chicken meat to cooked chicken meat via
216 cutting boards. Cutting boards are commonly perceived as significant
217 fomites in cross contamination of foodstuffs with foodborne agents
218 (Carrasco *et al.*, 2012). But studies by Moore *et al.* (2007) underlined that
219 food contact surfaces that are “easy to clean” (e.g. Formica and stainless
220 steel) may be more likely to release foodborne pathogens during common
221 food preparation practices.

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

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

239 implementation of a food safety management system (Arvanitoyannis and
240 Kassaveti, 2009). To be effective, food safety training needs to target
241 changing the behaviour. Griffith (2000) argued that behavioural change (i.e.
242 the implementation of required hygiene practices) is not easily achieved
243 and that consideration must be given to motivation, constraints, barriers
244 and facilities as well as to cultural aspects. Food safety practices will only
245 be implemented given adequate resources and appropriate management
246 culture (Clayton and Griffith, 2008). Besides educating and training in
247 appropriate food handling practices, food handlers or operators can be
248 trained in simple qualitative risk assessments (risk matrix: severity x
249 probability) to determine food safety risks (Manning and Soon, 2013).

250

251 **Conclusion**

252

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

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261

262

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519 Table 1 Description of cooked meat products sold at bazaars during

520 Ramadhan

Cooked	meat	Brief description
products		
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 <i>kurma</i>		Beef or chicken cooked in <i>kurma</i> gravy made from mixed curry powder, spices, potatoes, coconut milk and coriander.
<i>Singgang</i> 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 <i>gulai</i>		<i>Gulai</i> is similar to curry except lighter in taste and colour.
Beef / chicken <i>kerutuk</i>		Beef or chicken cooked in mixture of <i>kerisik</i> (toasted grated coconut) and <i>kerutuk</i> spices (coriander powder, cardamom seeds, clove,

Cooked	meat	Brief description
products		fennel seeds, cumin, black peppercorns, turmeric, galangal, lemongrass bulbs and garlic)
<i>Beef gulai acar / dalca</i>		Beef or chicken cooked in curry powder with potatoes, carrots, eggplants, green beans, chillies, curry leaves and baby corns.
<i>Beef kawah</i>		Beef cooked in a large pot of curry. Popular during wedding ceremonies in Kelantan.
<i>Air asam perut lembu</i>		Cow intestines are sliced and boiled with vinegar, lime, chives, onion, shrimp paste, chillies and tamarind
<i>Kunyit beef</i>		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.
<i>Chicken Tom yam</i>		Chicken cooked in mixed spicy chilli paste with lime leaf and lemongrass. Tom yam

Cooked meat products	Brief description
	originates from Thailand.
<i>Percik</i> chicken	Chicken cooked in coconut milk, dried chillies, garlic and lemongrass and then roasted.
Paprika chicken	Chicken cooked with lime leaves, <i>tom yam</i> paste, lemongrass, hot pepper, fish sauce, ginger, onion, garlic, sweet soy sauce and some vegetables.
<i>Kerisik</i> chicken	Chicken cooked with <i>kerisik</i> (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.
<i>Ayam tiga rasa</i>	Chicken cooked with ginger, plum sauce, sweet soy sauce, tomato sauce, lime, spicy pepper and ginger to produce sweet, sour and spicy taste.
<i>Ayam peparu kicap</i>	Chicken lungs cooked with soy sauce, onion, garlic and capsicum.

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

Districts	Sample	<i>Staphylococcus</i> spp.	Prevalence (%)	Unsatisfactory	Prevalence (%)	Sample	<i>Staphylococcus</i> 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	<i>Isolated bacteria</i>			
	<i>E. coli</i>		<i>Salmonella</i> 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)

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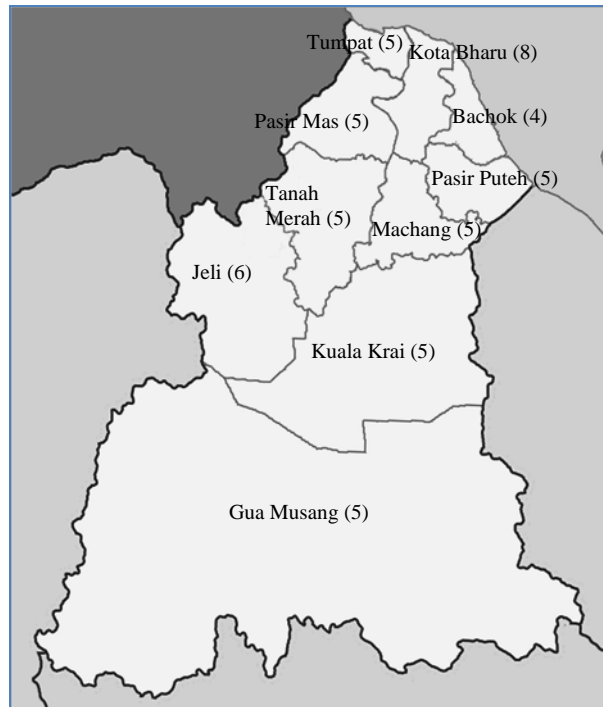
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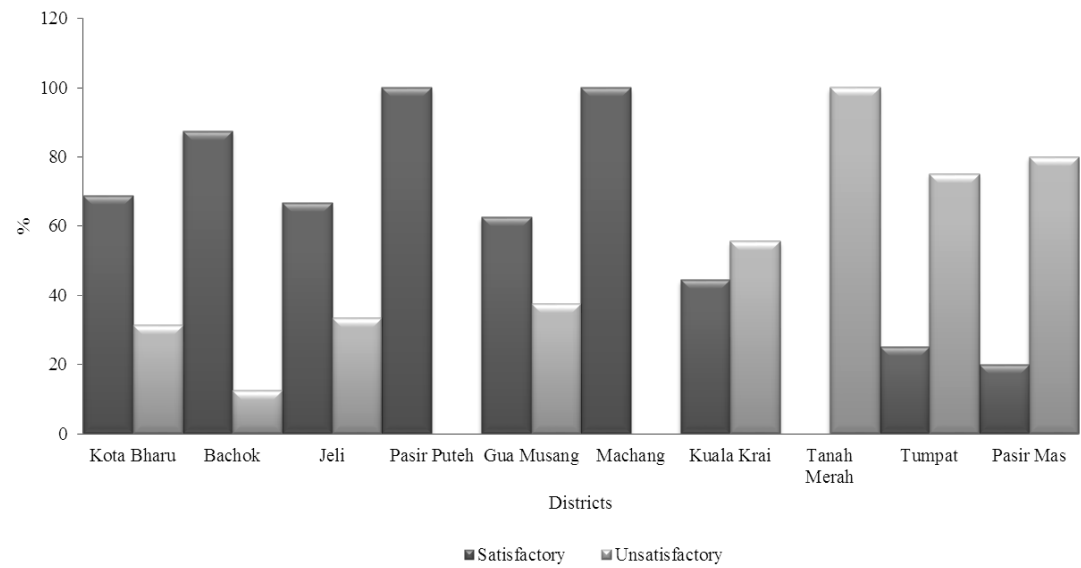
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538 Figure 1. Map showing the number of bazaars where beef and chicken
539 meats were sampled in Kelantan, Malaysia

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