2012 Thesis

An estimation of thermophilic *Campylobacter* population in ready-to-eat roast beef and chicken and the hygiene practices of sellers in beer bars in Arusha, Tanzania

Veterinary Medicine Unit, Department of Veterinary Medicine,
School of Veterinary Medicine, Rakuno Gakuen University

Supervisor: Dr. Makita Kohei

20703042 Toyomaki Haruya

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Ⅰ. Introduction

Campylobacteriosis is a zoonosis, a disease transmitted to humans from animals or animal products［44］, caused by *Campylobacter*. *Campylobacter jejuni* is one of the most commonly identified bacterial causes of acute gastroenteritis worldwide［2］and a typical case is characterized by diarrhea, fever and abdominal cramps［15, 38, 41］. *Campylobacter* infections are generally mild, but can be fatal among very young children, elderly and immunosuppressed individuals［44］, and often occur more frequently per year than *Salmonella* species, *Shigella* species or *Esherichia coli* O157:H7 infections［2, 36, 39］. In addition to diarrheal symptoms, *Campylobacter* infections have been identified as the most common antecedent to an acute neurological disease, the Guillain-Barré syndrome［30, 35］.

Campylobacter species are gram-negative bacilli that have a curved or spiral shape, microaerophilic, non-fermenting, motile rods with a single polar flagellum; they are oxidase-positive and grow optimally at 37° or 42°C［35］. Some *Campylobacter* species grow best at 42°C, called thermophilic *Campylobacter* and particularly, *C. jejuni* and *C. coli* are the clinically most important thermophilic *Campylobacters* to humans. *C. jejuni* and *C. coli* are common components of the gut flora of all warm-blooded animals including livestock (cattle, sheep and pigs), domestic pets and wild animals, and especially prevalent in avian species［8, 38］. Therefore, the most frequent source of contamination of carcasses or meat with *Campylobacter* is feces during slaughtering［44］. *Campylobacter* are particularly sensitive to drying and reduced pH［16］. *C. jejuni* is relatively sensitive to the lethal effects of heat, D_{55} values ranging from 0.6 to 2.3 min［16］.

In developed countries, *Campylobacter* infections are largely sporadic and observed during the warmer months of the summer and autumn, suggesting a seasonal pattern associated with ambient temperature［9, 17, 33］. On the other hand in developing countries, *Campylobacter* infections are hyper-endemic among young children, especially those aged less than two years, and asymptomatic infections occur commonly in both children and adults. The illness lacks the marked seasonal patterns observed in industrialized nations［2］.

Every year, 2 billions of diarrhea cases occur for all age groups and 1.5 million children under five die each year due to this illness worldwide［45］. The large proportion of the cases occurs in developing world because of lack of sanitation and unregulated food distribution

system; more than 80% of child deaths due to diarrhea occur in South Asia and Africa ［42］. Diarrhea is the second cause of child deaths following pneumonia ［42］. A great proportion of these cases can be attributed to contamination of food and drinking water and *Campylobacter* can be one of the important causal pathogens.

The Safe Food Fair Food (SFFF) project of the International Livestock Research Institute (ILRI), funded by BMZ, aimed to build a capacity to conduct participatory risk analysis in resource-poor sub Saharan African countries in order to improve food safety of animal source foods in informal markets while enhancing market access of poor farmers [12, 24]. One of the project activities in Tanzania focused on popular ready-to-eat foods served in beer bars called 'nyama-choma' (roast beef) and 'mishikaki' (skewer beef) which are seasoned with salt and black pepper and served with relish. The risk assessment for thermophilic *Campylobacter* from consumption of ready-to-eat roast beef in Arusha showed that the incidence rate of campylobacteriosis was 6.4 people (90% CI: 3.4-10.4) per 1000 people per day but the sensitivity analysis showed that the concentration of *Campylobacter* in beef, which was not studied, was the most influencing factor to the risk assessed ［23］. Therefore, the present study was conducted to understand the concentration of *Campylobacter* on ready-to-eat meat in Arusha, under the SFFF project, focusing on the most important thermophilic *Campylobacter*, *C. jejuni* and *C. coli*.

The concentration of *Campylobacter* on meat has been studied in the world ［1, 19, 21］ but in Tanzania, such study has not been published yet, although *C. jejuni* is known to be the predominant *Campylobacter* species among intestines of cattle, pigs, poultry and ducks, and *Campylobacter* diarrheal disease of human ［27-28, 31-32］. The concentration of *Campylobacter* on roast meat has not been studied in the world and the present study in Tanzania would be the first report.

The Most Probable Number (MPN) is a dilution method to estimate the density of organisms in a liquid without any directing count. This method is used principally for estimation of bacterial densities in water and milk ［10］. The present study uses the MPN method to estimate the concentration of thermophilic *Campylobacter* on roast beef and chicken surfaces as well as on raw beef sold in Arusha, Tanzania and at the same time describes the practices related with food hygiene in the butchers and the beer bars studied.

Ⅱ．Materials and Methods

1．Study areas

The study areas were the urban and peri-urban areas of Arusha Municipality in Tanzania. Arusha is the largest city in northern Tanzania located at latitude 3°22' to 3°37'S and longitude 36°41' to 36°68'E with an elevation of 1265 meters above sea level ［23］．

2．Sampling

Each one sample of raw beef was collected from 30 butchers, and each one sample of roast beef from 30 beer bars and each one sample of roast chicken from 10 beer bars were collected in September and October 2010. Sample size was determined based on the availability of fund. Purpose of this study was not estimating prevalence but concentration of *Campylobacter* in beef and chicken, and the sample size was not calculated. The estimated numbers of butchers and beer bars in the North, Central and South zones were provided by the meat inspector at the Arusha Abattoir and the numbers of samples were proportionally allocated to the zones. As there was no complete list available for the locations of butchers and beer bars, these sellers were visited based on the residents' information.

3．Interviews

The butchers and bar owners were interviewed using a structured questionnaire during the visits for sampling. The questionnaire included quantity of sales per day, business days per week, type of meat for sale, possession of refrigerator, source of water, attendance to a hygienic training and the use of same knives for both beef and chicken, and raw and roast beef. Pilot study was conducted in a butcher and a beer bar prior to the study. The level of urbanization was classified and recorded during sampling based on the rapid classification method ［25］．

4．Isolation of *Campylobacter*

Isolation of *Campylobacter* was conducted at the Veterinary Investigation Centre, Arusha, Tanzania. Fifty grams of samples were rinsed with 25 ml of Phosphate Buffered Saline (PBS) and 1 ml of each three replicates of this solution and their 10 and 100 times diluted solutions

were inoculated to Bolton selective enrichment broth (OXOID co.) in airtight test tubes and incubated at 42ºC for 24 hours.　The enrichment cultures were then inoculated to CCDA agar (OXOID co.) and incubated at 42ºC for 48-72 hours again in a microaerobic jar with AneroPack MicroAero (MITSUBISHI GAS CHEMICAL co., Inc.). The colonies on CCDA agar were selected and sub-cultured on blood agar at 42 °C for 48-72 hours. Conventional microbiological tests (Gram stain, Oxidase and Catalase tests) were performed for the isolates sub-cultured and the DNA of all the isolates was extracted using InstaGene Matrix (BIO RAD). The DNA was sent to Japan for the molecular analysis.

5 ． Identification of *Campylobacter*

Polymerase chain reaction (PCR) ［20］ was performed on the extracted DNA as the definitive identification for *C. jejuni* and *C. coli* in Rakuno Gakuen University, Japan. . At first, PCR based on 16S rRNA (*rrs*) gene was performed to co-identify *C. jejuni* and *C. coli* for all DNA samples. The *rrs* gene-positive samples were tested for *hip* gene (specific to *C. jejuni*) and CCCH (specific to *C. coli*). All PCR amplifications were performed in a solution containing Go Taq Green (Promega) 12.5µl, 1µM primer and 2µl DNA sample. Reaction mixes were subjected to 25 cycles of amplification in a DNA thermal cycler. The cycling was as follows: for *C. jejuni - C. coli*, denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute and extension at 72°C for 1minute; for *C. jejuni*, denaturation at 94°C for 1 minute, annealing at 66°C for 1 minute and extension at 72°C for 1 minute; and for *C. coli*, denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute and extension at 72°C for 1 minute. PCR amplicons were electrophoresed in 1% agarose gels, stained with ethidium bromide and photographed under UV light.

6 ． Estimation of the Most Probable Number

The mean of the MPN was estimated based on the MPN table. The standard error of MPN was estimated by using $0.55\sqrt{\frac{\log_{10}\alpha}{n}}$ where n is the number of samples per dilutions and a is dilution ratio ［6］ . The 90% confidence interval was estimated using the mean and the standard deviation calculated using @Risk (Palisade), under the assumption that the bacteria

concentration follows Log-Normal distribution.

Ⅲ．Results

1．Descriptive summary of business of butchers and beer bars

Although rigorous random sampling was not achieved in the present study, samples were proportionally allocated to three zones (North, Central and South) and the summary of the data obtained can show a fair representation of butchers and beer bars serving roast meats in Arusha. Seventeen percent (5/30) of butchers and 37.5% (15/40) of beer bars sampled were located in urban areas and the other sellers were located in peri-urban areas. These proportions were not significantly different (x^2=2.7, df=1, p=0.10).

Table 1 shows the meat sales business of butchers and beer bars in Arusha. Most of the butchers (93.1%) and all the beer bars operated seven days a week. Most of the butchers sold only beef (93.3%) and a few butchers sold the other types of meat. It suggested that chicken are slaughtered at either home or eating places such as restaurants and beer bars. All the beer bars sold roast meat sold beef and roast chicken was served at 19 of 40 beer bars studied (47.5%). Roast mutton was sold at 15 of 40 beer bars (37.5%). Median beef sale per day was 42.5kg in butchers and 13kg in beer bars. Median sale of roast chicken at beer bars was 5 birds a day. Butchers in urban areas sold more beef (110.8kg/day) than in peri-urban areas (39.1kg/day, t=4.34, p=0.005), and beer bars in urban areas sold more roast beef (19.9kg/day) than in peri-urban areas (9.0kg, t=3.4, p=0.002, data not shown in a table).

2．Prevalence of *Campylobacter* in meats

Table 2 shows the prevalence of *C. jejuni* and *C. coli* for the different types of meat. Only one isolate from a sample of roast chicken was identified as *C. coli* by PCR. *C. jejuni* was not detected from any of the samples. Therefore, the prevalence of *C. coli* was 0% (0/30) for raw beef at butchers, 0% (0/30) for roast beef and 10% (1/10) for roast chicken. The MPN of the *C. coli* was estimated to be 0.37/g of meat (90% CI: 0.03 – 1.2). The standard error of MPN was calculated as 0.335.

Table 1. Meat sales business of butchers and beer bars participated in the study

Items	Butchers (n=30)	Beer bars (n=40)
Business operation per week[*1, *2]		
Five days	1 (3.4%)	0 (0%)
Six days	1 (3.4%)	0 (0%)
Seven days	27 (93.1%)	39 (100%)
Types of meat for sale		
Only beef	28 (93.3%)	14 (35%)
Beef and chicken	0 (0%)	11 (27.5%)
Beef and mutton	1 (3.3%)	7 (17.5%)
Beef, chicken and mutton	1 (3.3%)	8 (20%)
Median and range of beef sale/ day[*2]	42.5kg (5-200)	13kg (2-80)
Median and range of chicken sale/ day[*2]	5 birds (n=1)	5birds (1-20, n=18)

*1: Data include one missing data among butchers

*2: Data include one missing data among beer bars

Table 2. The prevalence of *C. jejuni* and *C. coli* in raw and roast meat

Type of meat	Number of samples	*C. jejuni* (%)	*C. coli* (%)
Raw beef	30	0 (0%)	0 (0%)
Roast beef	30	0 (0%)	0 (0%)
Roast chicken	10	0 (0%)	1 (10%)
Total	70	0 (0%)	1 (1.4%)

3．Hygiene practice of meat sales

Table 3 shows the hygienic practice related with the sales of meat in butchers and beer bars. Large proportions of butchers (23/30, 76.7%) and beer bars (32/40, 80.0%) did not have a refrigerator. Water was provided in the studied areas of Arusha and all the butchers and beer bars were using tap water for their business. About half of the butchers (16/30, 53.3%) and

beer bar owners received hygienic training from the public health authority (20/40, 50%). Out of 2 butchers and 26 beer bars selling different types of animal meats (Table 1), 2 butchers (100%) and 18 beer bars (69.2%) used same utensils for these different types of meats. Out of 39 beer bars responded, 18 (46.2%) used same utensils for both raw and roasted meats.

By observations during the fieldwork, after meats were ordered by customers, meats were roasted well with fire of woods, then were either cut immediately on a cutting board or placed on the iron grill slightly far from fire a while and were cut. Roast meat cut into pieces were placed on a plate and were served to customers.

According to the beer bar owner who sold the roast chicken from which *C. coli* was recovered, he used same utensils for beef, chicken and mutton but used separate utensils for raw and roast meat; the owner did not use the same utensils for raw and roasted meat but a contamination had occurred. This beer bar was located in urban area and did not have a refrigerator. The owner had received a hygiene training by the public health authority in Arusha.

In order to assess the efficacy of a hygiene training, a Chi-squared test was performed. There was no association between an experience of a hygiene training and the practice of using separate utensils for raw and roast meat (Chi-squared=0.22, df=1, p=0.64).

Table 3. Hygiene practice among butchers and beer bars participated in the study

Items	Butchers (n=30)	Beer bars (n=40)
Possession of a refrigerator	7 (23.3%)	8 (20%)
Use of tap water	30 (100%)	40 (100%)
Experience of a hygiene training	16 (53.3%)	20 (50%)
Use same utensils for meat of different types of animals	2/2 (100%)	18/26 (69.2%)
Use same utensils for raw and roasted meat	NA	18 (46.2%)*

*One beer bar owner did not respond to the question

IV. Discussion

The purpose of the present study was to estimate the bacteria concentration of thermophilic *Campylobacter* in roast beef. This literal aim was not achieved because thermophilic *Campylobacter* was not detected from any of roast beef samples. However *C. coli* was isolated from a roast chicken sample and the MPN was 0.37/g (90%CI: 0.03-1.2). Surprisingly only *C. coli* was detected in the present study, although *C. jejuni* is the predominant species in Tanzania[27-28, 31-32]. Considering the observed roasting process on fire and the weakness of *Campylobacter* against dryness and heat [16] , *Campylobacter* on roast meat should have been killed completely. The *C. coli* isolated in the present study may be contaminated after the chicken was roasted and cooled. The beer bar owner where *C. coli* was isolated stated that he used separate utensils between raw and roast meats; however it is questionable whether a cutting board was included in 'utensils' in his reply, according to the fieldwork team; post-roast contamination might be occurred on a cutting board or during improper handling. In case such contamination occurs on roast beef, the bacteria concentration can be similar with which we found from roast chicken; thus the MPN obtained can be applied to that of roast beef.

In retail raw meat, bacteria concentration of thermophilic *Campylobacter* on chicken meat tends to be higher than the other types of meat. In New Zealand, among a total of 48 samples of beef, lamb, mutton and pork contaminated with thermophilic *Campylobacter*, the bacteria concentrations were less than 0.3MPN/g, and one unweaned veal sample had more than 10.9MPN/g [46] . In USA, the concentration in ground beef was 1.1cfu/g [1] . Whereas in retail chicken meat, although 40.2% had less than 0.3MPN/g, 50.5% had 0.3-10.0MPN/g, 8.8% had 10.1-50.0MPN/g and 0.5% had 110MPN/g in New Zealand [46] . In England, the bacteria concentrations on retail chicken meat were even higher; $\log_{10}$ geometric means were 4.9 (SD=1.0) in chicken carcass-rinse samples [18] . An integrated report from 25 countries in EU presented the concentration on broiler carcasses at slaughter houses; 47% had less than 10cfu/g, 7.5% had 10-39cfu/g, 4.7% had 40-99cfu/g, 19.3% had 100-999cfu/g, 15.8% had 1,000-10,000cfu/g and 5.8% had over 10,000cfu/g [7] . The MPN on roast chicken in the present study was equivalent with the bacteria concentration on raw meat which contaminated with *Campylobacter* at a low level.

The prevalence of thermophilic *Campylobacter* in raw and roast beef in Arusha cannot be estimated in the present study, as the sample size was small and probabilistic sampling was not used. However there is a significant gap in the prevalence of thermophilic *Campylobacter* in raw and roast beef between the present study and the previous study by Mahundi (2012): 12.3% (9/73) in raw beef and 17.8% (8/45) in roast beef. The difference of the results may be attributable to the identification methods. The discriminatory power of conventional biochemical tests is lower than that of DNA-based techniques［28］. Mahundi (2012) used conventional biochemical tests for identification, and it might overestimate the contamination rate. In the present study, extracted DNAs were shipped to Japan and initially the condition during shipment was hypothesized to have affected the quality of DNAs. However non-specific bands of DNAs were detected from the negative samples (data not shown in the texts) and DNAs were proved not to have been damaged. The low prevalence of thermophilic *Campylobacter* in roast meats in the present study was similar with the other studies in poultry dishes; 0% in poultry related cooked products in Northern Ireland［29］, 0% in roast chicken in Mexico［5］, 0.7% in ready-to-eat street-vended poultry dishes in Senegal［4］and 1.2% in ready-to-eat poultry products in Poland［22］. Quiñones-Ramírez *et al.* (2000) detected Campylobacter from 27% of roasted chicken tacos samples, however all positive samples were collected from one location where poor hygiene in handling practices suggested a cross-contamination of the cooked product.

The low prevalence (0%) in raw beef in the present study was also similar with the other studies; 2% in retail raw beef in Kenya［34］, 3% in retail raw beef in Tanzania［32］, 0.1% in retail raw beef in USA［47］and 1.5% in provincially inspected cattle slaughter facilities in Canada［3］. Furthermore, most butchers in Tanzania do not have a refrigerator as shown in the present study and they hang raw meats for sale in shops in the dry environment which is critical for the survival of *Campylobacter*.

The risk of cross-contamination for ready-to-eat beef with thermophilic *Campylobacter* can be higher at the beer bars dealing with chicken meat as well. Regardless of developed or developing countries, the contamination rate of *Campylobacter* in chicken is high at the farm level［7, 17, 19, 46］. In a cooking process, there is non-negligible probability of contamination. *Campylobacter* spp. survived on wooden and plastic cutting boards after 3h of exposure in

food preparation areas ［43］ and on sponges, dishcloths or scourers and hands or tea towels after washing-up and cleaning ［26］ . The most important food-specific risk factor of *Campylobacter* infections was consumption of chicken in USA ［11, 14］ .

　The results of interviews suggested that hygienic training was not effective in preventing use of same utensils for raw and roast meat. The hygiene practice could have been elucidated clearer if questions were asked about handling of meat, cutting board and washing hands. Careful food preparation and cooking practices prevent foodborne illnesses ［13］ and future study should focus on the incentives for the compliance of recommended good hygiene practice and education of food safety.

　Although the present study showed low prevalence and concentration of *Campylobacter* in roast beef, quantitative risk assessment for campylobacteriosis through consumption of ready-to-eat beef needs to be carried out using the data shown in this study in order to understand the risks in population in Arusha, Tanzania.

V．Abstract

An estimation of thermophilic *Campylobacter* population in ready-to-eat roast beef, chicken and raw beef was conducted in Arusha, Tanzania in order to generate the data necessary for a reliable food safety risk assessment.

Thirty samples of beef sold at 30 butchers, 30 samples of roast beef and 10 samples of roast chicken sold at 40 beer bars were collected in September and October in 2010. These 70 samples were tested for thermophilic *Campylobacter* to estimate the MPN using triplicate method. The isolates cultured on CCDA agar were analyzed for *C. jejuni* and *C. coli* by PCR as the definitive identification. The MPN and the standard deviation were calculated based on a published method. The confidence interval of the MPN estimated was obtained using @Risk.

Out of 70 samples, only one *C. coli* isolate was detected from a roast chicken sample. The MPN of *Campylobacter* was 0.37/g (90% CI: 0.03-1.2). The fact that *Campylobacter* was detected from roast meat suggested post-roast cross contamination although the sample was taken from a beer bar whose owner uses separate utensils for raw and roast meat. According to the interviews with beer bar owners, 46.2% (18/26) used same utensils for raw and roast meat even though 50% (20/40) received hygienic training and there was no association between an experience of the training and the practice (Chi-squared=0.22, df=1, p=0.64).

This suggested the necessity of improving quality of food hygiene training in beer bars in Arusha in order to prevent the post-roast cross contamination.

VI. Acknowledgement

VII. Reference

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