Detection of *Campylobacter jejuni* among Commercial Broiler Chickens in East-Coast Malaysia

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ABSTRACT

Human campylobacteriosis is associated with contaminated foods and drinks or direct contact with the source of organisms. Broilers are the main reservoir of *Campylobacter* as well as the primary source of meat in many countries. The microorganism load in broilers is converted into the rate of food-related campylobacteriosis. The present study aimed to determine the *Campylobacter jejuni* colonization load in commercial broiler chickens in east coast Malaysia. Cloaca swabs were taken after consent from the owners of selected broiler farms in Kelantan, Malaysia. Swabs were kept in Amie's transport media before being placed in enrichment broth. The DNA was extracted directly from the broth and specific Polymerase Chain Reaction (PCR) was performed, which targeted hippurate hydrolase (hipO) gene of *C. jejuni*. Three broiler farms from different districts in Kelantan were identified. A total of 120 cloacal swabs were analyzed, 20% (24/120) confirmed by PCR-positive for hipO gene when the amplified product with the band size of approximately 344 bases per was visualized on agarose gel. It is concluded that the prevalence of *C. jejuni* colonization among living broiler flocks in Kelantan was at a low level. However, further studies with bigger sample size and involvement of more farms are needed.

Key words: Broiler chicken, *Campylobacter jejuni*, Cloaca swab, Polymerase Chain Reaction, Hippurate hydrolase

INTRODUCTION

More than 30 *Campylobacter* species has been identified to date (Pitkanen and Hanninen, 2017). However, *Campylobacter jejuni* and *Campylobacter coli* are the two predominant species that cause gastrointestinal infections, based on analysis of 1020 inflammatory stool samples (Ranjbar et al., 2017). The person acquired the infection after consuming contaminated food or water or after having direct contact with the source. Since the 1970s, the broiler has been the main cause of campylobacteriosis, which has been identified in connection with human nutrition. The presence of high *Campylobacter* rate in broiler chickens and their carcasses is one of the major risk factors for human acquisition (Marotta et al., 2015; Skarp et al., 2016).

Broiler chickens, specifically *Gallus gallus domesticus*, have a significant contribution to meat production worldwide (Skarp et al., 2016). It was estimated that around 20 to 30% of campylobacteriosis in the European Union was related to chicken meat consumption, as reported by European Food Safety Authority. Another report indicated 50–80% infections might be attributed to the entire chicken reservoir. It is also emphasized that consumption of broiler meat production contributed to campylobacteriosis in different regions (Skarp et al., 2016; Ranjbar and Babazadeh, 2017).

There are limited reports on *Campylobacter* species in living broiler chickens. Furthermore, no data are available for the Kelantan District, where the study is being conducted. To reduce the incidence of campylobacteriosis in human, understanding the colonization status or contamination of poultry by *Campylobacter* species is very necessary for intervention strategies and monitoring programs in the primary broiler production chain. Thus, the aim of this study was to

determine the colonization load of *C. jejuni,* which is the predominant species in local broiler farms.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Institutional Animal Care and Use Committee of the Universiti Sains Malaysia (USM) (USM/IACUC/2018/ 113, 928).

Sample collection

Three commercial broiler chicken farms were identified in the three locations in Kelantan state on the east-coast Malaysia. The farms had about 2000 to 6000 broiler each. One hundred cloacal samples were taken from the first two farms (50 samples from each farm) and another 20 samples were taken from the last farm. The samples were taken according to the availability of the broiler during sampling and with consent of owners. The samples were taken using a moistened sterile cotton swabs and immediately kept in Amie's transport medium. Within 1-2 hours, the swabs were placed in 3 mL of Blood Free *Campylobacter* broth (Oxoid, France) and incubated at 37°C for 48 hours to enhance *Campylobacter* growth.
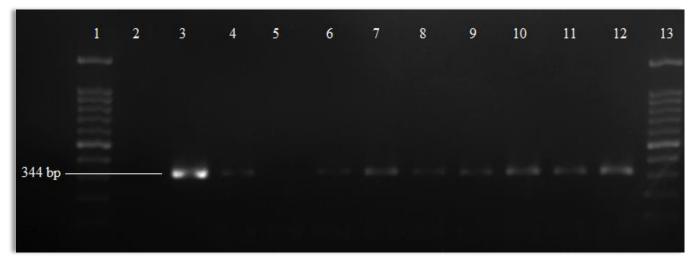
PCR

The DNA extraction was performed using a boiling lysis method directly from the broth according to the published protocols with modifications (Singh et al., 2011). The boiling method is a classic alternative procedure of DNA extraction using TE buffer to lyse the cells, followed by rapid boiling at 95°C to obtain the DNA. About 100 µL of broth was mixed with 100 µL of sterile distilled water and centrifuged at 10,000 rounds per minute (rpm) for three minutes. The supernatant was removed, and the remained pellets were suspended again with 100 µL TE buffer. The samples were vortexed to mix them up and boiled at 95°C for 10 minutes to lyse the bacteria cells. The samples were centrifuged again at 10,000 rpm for three minutes. The supernatant was used directly as a template in a PCR reaction. A set of specific forward (5'-GAC TTC GTG CAG ATA TGG ATG CTT-3') and reverse (5'-GCT ATA ACT ATC CGA AGA AGC CAT CA-3') primers were used, targeting hippurate hydrolase (hipO) gene characteristic of *C. jejuni*. The primers were proved to be specific for *C. jejuni* (Persson and Olsen, 2005). The conventional PCR was performed in a total reaction volume of 25 µL containing 12.5 µL of 2 x PCR buffers, 4.3 µL of PCR water, 1.6 µL of 5 µM of forward and reverse primers respectively and 5 µL of

extracted DNA, which served as template DNA. The reactions prepared were run in the thermocycler with an initial denaturation of 6 minutes at 94°C followed by 35 cycles of 94°C (50 seconds), 57°C (40 seconds) of annealing, and 72°C (50 seconds) extension with a final extension at 72°C for 3 minutes. The Polymerase Chain Reaction (PCR) products were analyzed on 1.5% agarose gel and a fragment with a size of 344 base per (bp) was identified as hipO gene.

RESULTS AND DISCUSSION

The PCR was considered positive for hipO gene when the amplified product with the band size of approximately 344 bp was visualized on agarose gel. A total of 120 cloaca swabs were taken and analyzed. Twenty-four (20%) samples were positive. A representative of the PCR analysis as shown in figure 1. *C. jejuni* was regarded as an intestinal flora of chickens but was recognized to cause campylobacteriosis in humans. Many researches were conducted to correlate the occurrence of the organism with the contamination and infection of human food (Skarp et al., 2016). The contamination was linked with the various stages of chicken meat production and processing chain until the consumption of chicken meat in the community. The production chain of chicken meat varied between regions, industrialized and developing countries. This would determine the contamination rate during the processes. The *Campylobacter* contamination in poultry farms subsequently reflected the contamination rate of carcasses and meat (Kaakoush et al., 2015; Skarp et al., 2016). In the recent study, the PCR was able to detect 24 (20%) positive samples among the living broilers compared to the previous data from Thailand, in which the prevalence of *Campylobacter* species in broilers ceca was reported 11.2% and 11 out of 98 ceca samples taken in the slaughterhouse were positive by culture method (Chokboonmongkol et al., 2013). Researchers postulated that the prevalence after avian influenza outbreaks declined drastically in 2004, with strict biosecurity measures in the broiler farms and the administration of antimicrobials were implemented to chickens less than 3 days old. Another study in 2017 showed a high prevalence and isolation rate around 40-60%, depend on the region and climate during the sampling (Prachantasena et al., 2017). A higher isolation rate was noted in Ecuador when the study was conducted from 2013 to 2014. About 64 to 70% *Campylobacter* species were isolated from ceca in the slaughterhouse. The chickens usually came from one identified breeding farm, but from different batches

(Vinueza-Burgos et al., 2017). Another high isolation rate was reported from Austria in 2015 and during the study of 15 broiler flocks, 80% (n = 12/15) were positive for *Campylobacter* species by some detection methods. At least samples were positive by one of the detection methods (lateral flow, direct plating, Bolton broth enrichment and real-time PCR). *C. jejuni* was detected as

predominant species in poultry flocks (Schallegger et al., 2015). A study in the United Kingdom noted the *Campylobacter* species being colonized at the slaughterhouse and the prevalence was almost 95.9%. However, the methods of detection influenced the outcome of the study (Rodgers et al., 2017).

Figure 1. Conventional PCR analysis of hipO gene from broilers in east-coast region of Malaysia. Lanes 1 and 13: DNA Ladder (100 bp); Lane 2: Negative control; Lane 3: Positive control (344bp); Lanes 4–12: Cloaca samples (Lane 4 and Lanes 6-12: Positive; Lane 5: Negative)

CONCLUSION

It is concluded that the colonization load of *C. jejuni* in local broiler farms is still low in study locations compared to other reports. However, further studies with larger sample sizes and involvement of more farms in different locations are very necessary. There is a lot of routs for improvement in order to prevent human campylobacteriosis. The control and prevention program requires a multidisciplinary approach that includes both food-chain production and the consumer as the end-users. Regular monitoring of *Campylobacter* species load in commercial broiler chickens is necessary for successful control of human campylobacteriosis in connection with meat production.

DECLARATIONS

Competing interests

The authors have declared that no competing interest exists.

Authors' contributions

Nur-Aziera-Aina CMN had performed the field-work, laboratory analysis and drafting the manuscript. Nur-Syafiqah MN had also performed the field-work and laboratory analysis, and Zaidah AR supervised the field-work, monitored the laboratory analysis, drafted the manuscript. All authors approved the final revised manuscript.

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