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Research article

Prevalence of *Campylobacter* spp. on retail fresh chicken carcasses in Hanoi, Vietnam

Vu Dung Minh[1], Tongkorn Meeyam[1,2,*], Fred Unger[3], Greta Gölz[4], Pham Thi Ngoc[5], Truong Thi Huong Giang[5], Thomas Alter[4] and Duangporn Pichpol[2]

[1] *Veterinary Public Health and Food Safety Centre for Asia Pacific, Chiang Mai University, Chiang Mai, 50100, Thailand*
[2] *Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, 50100, Thailand*
[3] *International Livestock Research Institute (ILRI), Hanoi,10000, Vietnam*
[4] *Institute of Food Safety and Food Hygiene, School of Veterinary Medicine, Freie Universität Berlin, Berlin, 14163, Germany*
[5] *National Institute of Veterinary Research (NIVR), Hanoi 10000, Vietnam*

Abstract

This study was conducted to determine the prevalence of *Campylobacter* spp. on fresh chicken carcasses at retail markets in four selected urban districts in Hanoi, Vietnam. A total of 107 chicken carcass samples were collected. *Campylobacter* spp. was found on 44 chicken carcasses and accounted for a prevalence of 41.1%. Among the *Campylobacter* species detected, *C. coli* was the predominant *Campylobacter* spp., being detected in 24 of the 44 samples, followed by C. *jejuni*, detected in 19 of 44 samples. One carcass was positive for both C. *jejuni* and *C. coli*. The high prevalence of zoonotic C. *jejuni* and *C. coli* on chicken carcasses emphasizes the need to enhance awareness of the public and relevant stakeholders on food safety and foodborne diseases in Vietnam.

Keywords: *Campylobacter,* Chicken carcass, Retail market, Vietnam

Corresponding author: : Tongkorn Meeyam, Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, 50100, Thailand, E-mail: tongkorn.meeyam@cmu.ac.th

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INTRODUCTION

The chicken production chain is a potential harbor of the foodborne pathogen (Lampang et al., 2014; Chotinan, 2015; Zhu et al., 2017; Vidayanti et al., 2020; Wangroongsarb et al., 2021; Akter, 2022). *Campylobacter* is one of the most common bacterial agents causing gastroenteritis in humans worldwide. In humans, infection with *C. jejuni* is predominant and generally associated with consuming contaminated food (Altekruse et al., 1999; Nyati and Nyati, 2013; Kaakoush et al., 2015), especially poultry meat (Mughini Gras et al., 2012). Nevertheless, *C. coli* accounts also for a significant share of human infections (Gürtler et al., 2005; Awadallah et al., 2014).

Epidemiological data on *Campylobacter* infections in South-East Asia are still scarce (WHO, 2012). A recent review summarized the available information on *Campylobacter* infections in South-East Asia, including Cambodia, Laos, and Vietnam (Nguyen et al., 2017).

In Vietnam, chicken ranks second in meat consumption (the average per head per year is 14.5 kg of chicken meat), following pork consumption. Due to increasing income and rising meat consumption, meat consumption in urban areas is considered significantly higher than in rural areas in Vietnam (Ton et al., 2011).

In recent years, Vietnam has experienced rapid economic development and urbanization. In parallel, modern retail formats, such as supermarkets and boutique food shops, claim high quality and safety of foods and are expanding and replacing traditional markets to a certain degree, especially in urban areas (Maruyama and Trung, 2007; Figuié and Moustier, 2009). Typically, products at traditional markets are displayed uncovered at ambient temperature. In contrast to traditional markets, animal products in supermarkets and boutique shops are kept in cool cabinets. The expansion of these modern retail formats is expected to increase food safety (Wertheim-Heck et al., 2015). Nevertheless, limited information is available on food safety issues related to the consumption of chicken meat or the status of different food outlets regarding food safety and food hygiene aspects.

So far, only single study investigated *Campylobacter* prevalence in chicken meat at retail level in Vietnam. In an older study, Luu et al. (2006) observed a prevalence of approx. 31% on chicken meat collected at retail markets in Vietnam.

Since information is still scarce, this study aimed to determine the prevalence and quantitative load of *Campylobacter* spp. on chicken carcasses at different retail outlet types.

MATERIALS AND METHODS

Whole eviscerated chicken carcasses without feet and heads (n=107) were collected from retail markets in four urban districts in Hanoi, Vietnam, including Ba Dinh, Hoan Kiem, Dong Da, Cau Giay. These districts are high population densities, economic centers, and tourist destinations (Vo, 2017). In each district, five traditional markets (n=60), two supermarkets (n=23), and three boutique food shops (n=24) were selected for sampling. All samples were handled separately, kept in cooling boxes, and performed laboratory analysis

within 12 hours after sample collection. The sample collection was done from July to October 2017. The expected prevalence of 50% calculated the sample size due to a lack of data, a confidence level of 90%, and an 8% accepted error. Isolation and species identification of *Campylobacter* spp. was performed according to ISO 10272-1: 2006 (ISO, 2006) with slight modifications. Briefly, a whole carcass was rinsed with 200 ml of Phosphate buffer saline (PBS). The rinsing fluid of 25 ml was enriched in 225 ml Bolton broth (OXOID, UK) containing modified Bolton broth selective supplement (OXOID, UK) and 5% Laked Horse Blood. The suspensions were incubated at 37°C for 4-6 hours under microaerobic conditions generated by CampyGen packs (OXOID, UK). Then, they were continuously incubated at 41.5°C for 40-48 hours under microaerobic conditions. After enrichment, a loop of Bolton broth was streaked on Modified Charcoal Cefoperazone Deoxycholate agar - mCCDA (OXOID, UK) containing selective supplements (OXOID, UK) and incubated at 41.5°C for 40-48 hours under microaerobic conditions. Typical *Campylobacter* colonies on mCCDA were selected and sub-cultured on Columbia Blood Agar (OXOID, UK) containing 5% Defibrinated Sheep Blood. And they were incubated at 41.5°C for 40-48 hours under microaerobic conditions.

A multiplex PCR assay was performed to simultaneously identify the two most common *Campylobacter* spp., *C. jejuni*, and *C. coli*. Colonies presumed to be *Campylobacter* were selected for subsequent confirmation by multiplex PCR. DNA was extracted by heating the cells at 100°C for 10 minutes. PCR conditions and primers were used according to Denis et al. (1999). A Chi-square test was carried out to determine the significance of prevalence differences of *Campylobacter* among the different outlet types and sampling districts (R Version 3.2.2) with $p < 0.05$.

Enumeration of *Campylobacter* spp. was carried out according to ISO 10272-2:2017 (ISO, 2017), with slight modifications. Briefly, serial ten-fold dilutions were prepared with PBS from the rinsing solution. Aliquots of 0.5 ml of each dilution step were spread directly onto mCCDA in duplicate, then incubated for 40-48 hours at 41.5°C under microaerobic conditions. After incubation, all colonies with typical *Campylobacter* morphology were counted. Presumptive *Campylobacter* colonies from each sample are proportionally representative of all colony types. They were selected, and sub-cultured on Columbia Blood Agar containing 5% defibrinated sheep blood and incubated for 40-48 hours at 41.5°C under microaerobic conditions. The quantitative load of *Campylobacter* spp. was expressed as $\log_{10}$ CFU/carcass (the lower detection limit of *Campylobacter* spp. by direct enumeration was 3.3 $\log_{10}$ CFU/carcass).

RESULTS

Prevalence of *Campylobacter* spp. on chicken carcasses

Overall, *Campylobacter* spp. was detected in 41.1% (44/107) of the samples. When splitting into individual outlet types, *Campylobacter* spp. was detectable in 46.7% (28/60) of the samples from traditional markets, in 56.5% (13/23) of the samples from supermarkets, and 12.5% (3/24) of the samples taken from boutique food shops (Table 1). Prevalence differs significantly between boutique shops, supermarkets, and traditional markets ($p < 0.05$).

The prevalences were found in Hoan Kiem and Cau Giay districts (each 44.4%), followed by Dong Da (42.3%) and Ba Dinh (33.3%) (data not shown). However, there are no statistically significant differences in the prevalence of *Campylobacter* spp. among these four districts.

Table 1 Prevalence of *Campylobacter* spp. on fresh chicken carcasses collected at different outlet types in Hanoi, Vietnam

Outlet type	Number of samples	*Campylobacter*-positive	*Campylobacter* prevalence (%)
Traditional markets	60	28	46.7
Supermarkets	23	13	56.5
Boutique shops	24	3	12.5*
Overall	107	44	41.1

*$p < 0.05$

Campylobacter species distribution

In the 44 samples that were *Campylobacter*-positive, *C. coli* was found on 24 chicken carcasses; *C. jejuni* was found on 19 samples, and one sample was positive for both *C. coli* and *C. jejuni*.

Quantitative load of *Campylobacter*

We were able to quantify *Campylobacter* from 33 chicken carcass samples. Forty percent (24/60) of the samples from traditional markets and 39.1% (9/23) of the samples from supermarkets were above the detection limit for quantitative analysis. None of the samples from boutique food shops (0/24) demonstrated *Campylobacter* loads above the detection limit.

For all quantifiable samples, the median *Campylobacter* spp. load was 5.58 $\log_{10}$ CFU/carcass. When comparing samples from traditional markets, supermarkets, and boutique food shops, chicken carcass samples from traditional markets showed significantly higher *Campylobacter* spp. Loads (with a median of 5.81 $\log_{10}$ CFU/carcass; 95% CI 5.15-6.2), compared to samples from supermarkets (with a median of 5.0 $\log_{10}$ CFU/carcass; 95% CI 3.9-5.64) (data not shown). The Mann – Whitney test was applied to determine significant differences ($p<0.05$) in the median loads of *Campylobacter* spp.

DISCUSSION

In this study, we estimated the prevalence of *Campylobacter* on fresh chicken carcasses from different outlet types in four urban districts of Hanoi, Vietnam. This finding corresponds well with previous observations from South East Asia, showing a prevalence of chicken meat at retail markets in Hanoi, Vietnam (Luu et al., 2006) and in Nueve Ecija, Philippines (Sison et al., 2014). Significantly higher prevalence was detected in carcasses from traditional markets and supermarkets compared to boutique food shops. However, no significant difference between *Campylobacter* prevalence in traditional markets and supermarkets was detected. Garin et al. (2012) already demonstrated that semi-industrial automated slaughter processing lines (as applied for a large share of supermarket chicken) did not result in a lower prevalence of *Campylobacter* compared to manual slaughter (slaughter by the seller), often carried out in traditional markets.

For boutique shops, chickens originated from small-scale contracted farms and were slaughtered in small batches under controlled conditions. After slaughter, chicken carcasses are packed individually into plastic bags and immediately stored at 4-8°C up to retail. Such improved hygienic conditions can lead to lower contamination and cross-contamination, which might explain the lower detection rate in these samples.

The high quantitative loads were obtained from traditional markets and supermarkets compared to boutique food shops. This finding is higher than the previous study by Garin et al. (2012) included quantitative data that presented the enumeration of chicken neck skin samples from Ho-Chi-Minh City, Vietnam. However, variations in the types of samples and different enumeration methods complicate the comparison of our data to these results (Jorgensen et al., 2002). Moreover, two predominant species found in this study are *C. coli* and *C. jejuni,* similar to the previous studies in Asia (Meeyam et al., 2004; Sison et al., 2014; Zhu et al., 2017).

The rapid development of economic conditions in Vietnam led to the introduction of integrated food chains and the expansion of modern retail formats. It expects to provide safer food, especially in populous urban areas.

Our findings highlight the need to implement integrated intervention measures to enhance food safety associated with chicken consumption and improve hygienic situations at all steps in poultry production. Raising the awareness of vendors, especially at traditional markets) Furthermore, consumers' towards food safety and safe handling of raw chicken meat at the market and the consumer level are also needed to mitigate the effects of foodborne infections associated with *Campylobacter* in Vietnam.

CONCLUSIONS

Our study detected a high prevalence of *Campylobacter* in chicken meat at the retail level in Hanoi, Vietnam. To establish intervention measures, improve hygienic conditions, and reduce the number and prevalence of *Campylobacter* in fresh chicken meat in Vietnam. Further studies should focus on identifying risk factors throughout the poultry processing chain, including poultry farms, slaughterhouses, and retail shops.

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AUTHOR CONTRIBUTIONS

Vu Dung Minh Conception or design of the work, Sample collection, Perform experiment, Data analysis and interpretation, Drafting the manuscript.
Tongkorn Meeyam Conception or design of the work, Perform experiment, Data analysis and interpretation, Final approval of the version to be published.
Fred Unger Conception or design of the work.
Greta Gölz Perform experiment.
Pham Thi Ngoc Conception or design of the work, Perform experiment.
Truong Thi Huong Giang Perform experiment.
Thomas Alter Conception or design of the work, Perform experiment, Data analysis and interpretation, Final approval of the version to be published.
Duangporn Pichpol Conception or design of the work, Perform experiment, Data analysis and interpretation, Critical revision of the manuscript, Final approval of the version to be published.

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