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Effects of knowledge, attitudes, and practices of poultry handlers on the prevalence of *Campylobacter* along the poultry production chain in Peninsular Malaysia

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Abstract

The present work aimed to identify the effects of knowledge, attitudes, and practices (KAP) of poultry handlers on the prevalence of *Campylobacter* along the poultry production chain in Peninsular Malaysia. A total of 1230 microbiological samples were collected from five companies in different Malaysian states. The sampling points involved the entire poultry supply chain from the farm to the retailer. The collected samples were subjected to bacteriological isolation and morphological identification for microbiological analyses. All *Campylobacter*-positive samples were further confirmed with molecular identification by using a polymerase chain reaction. *Campylobacter* prevalence in poultry farms, processing plants, and retailers were identified. For the KAP survey, 300 respondents answered a questionnaire evaluating their levels of KAP regarding food safety while handling live birds and subsequently poultry products after slaughtering. Overall, the mean KAP scores of the workers at farms, processing plants, and retail outlets were assessed. The survey data indicated that even though the overall KAP levels of the poultry workers were excellent, the bacterial prevalence of *Campylobacter* was still high. In conclusion, the KAP of poultry handlers had insignificant effects on the prevalence of *Campylobacter* along the poultry production chain in Peninsular Malaysia.

Introduction

Foodborne disease outbreaks occur due to microbiological hazards from various species of foodborne pathogens, such as *Campylobacter* (ranging from 1.0 to 9.0%) (Humphrey *et al*., 2007; Scallan *et al*., 2011; EFSA and ECDC, 2016). Poultry is commonly considered a major reservoir for *Campylobacter* contamination, and eating raw or undercooked poultry is the main risk factor for campylobacteriosis (Scott *et al*., 2015). Human foodborne campylobacteriosis is the most commonly reported zoonosis in the European Union with over 200,000 confirmed cases in 2016 (Chlebicz and Śliżewska, 2018). A study in Japan revealed that more than 450 incidences of *Campylobacter* infections in patients were reported throughout 1999 to 2005; these infections were connected to the consumption of poultry products or poultry-related foods (Igimi *et al*., 2008). Campylobacteriosis typically presents an acute diarrhoeal illness that lasts up to seven days. Significant complications include sepsis, reactive arthritis, Guillain-Barré syndrome, bacteraemia with extraintestinal sites of infection, and septic abortion (Balen Topić *et al*., 2007).

Campylobacter is present in various foods, such as milk (4%) (Mansoureh *et al*., 2022), fresh vegetables and fruits (0.53%) (Mohammadpour *et al*., 2018), and chicken meat (30%) (Rossler *et al*., 2019) which is considered its dominant vehicle for contamination. This is because the gastrointestinal tracts of chicken contain *Campylobacter*, whereby

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infected viscera and meat from poultry frequently result in food poisoning (Skirrow, 1991). Cross-contamination from other carcasses and self-infection from their own faeces or feathers along the poultry production chain significantly impacts the levels of *Campylobacter* contamination in chickens (Hayama *et al*., 2011). According to the Food and Drug Administration (FDA, 2010), the presence of *Campylobacter* in retail chicken breast meat was more than 90.0% from 2002 until 2010. A study done in Germany reported the prevalence of *C. jejuni* in broiler carcasses to be 45.9%, thus indicating the possibility of contamination at poultry slaughtering plants (Atanassova and Ring, 1999). Next, a study in Belgium found the contamination rate of *Campylobacter* in broiler carcasses to be 38.8%, and attributed it to contamination during rearing on the farm, transport to the slaughterhouse, and carcass processing (Herman *et al*., 2003). These variations in prevalence are most likely related to the sampling method (Jørgensen *et al*., 2002) and seasonal influence (Baali *et al*., 2020).

Apart from Western countries, *Campylobacter* is also prevalent in Asia. From previous investigations in Thailand, the prevalence of *Campylobacter* in chickens before slaughter ranged between 48.0 to 64.0% (Meeyam *et al*., 2004; Padungtod and Kaneene, 2005; Boonmar *et al*., 2007), whereas the prevalence of *Campylobacter* in poultry meat at retailers was 52.0% (Vindigni *et al*., 2007). Furthermore, Ma *et al*. (2017) reported the contamination rate of *C. jejuni* from broiler carcasses to be 13.7% at the retail level in Tianjin, China. Several reports from Malaysia have recorded a very high prevalence of *Campylobacter* spp., especially *C. jejuni*, at poultry farms, as well as in poultry and poultry products from retail outlets (Son *et al*., 1996; Saleha, 2002). Rejab *et al*. (2012) determined that the overall *Campylobacter* contamination rate was 61.0% in chicken carcasses and at processing lines of modern processing plants. This was possibly caused by consistently leaving too little time between cleaning and disinfection prior to the next slaughter. This study conducted in six states of Malaysia showed extensive *Campylobacter* contamination in chicken carcasses and slaughterhouses. Mohamed-Yousif *et al*. (2019) identified the occurrence of *Campylobacter* in poultry (60.0%) and poultry environments (22.1%) in Selangor, Malaysia.

The prevalence rates of *Campylobacter* in chicken populations have been reported to reach up to 100.0% in some farms in Malaysia, which is in line with several studies from 1989 to 2002 that reported *Campylobacter* prevalence in various poultry species in Malaysia to range from 12.1 to 87.9% (Saleha, 2003). A study by Chai *et al*. (2009) found *Campylobacter* to occur in 57.1% of the poultry manure samples from vegetable farms in Selangor, Malaysia, thereby posing a potential risk for raw vegetable consumption in Malaysia, and also providing baseline data on *Campylobacter* contamination at the farm level. Choo *et al*. (2011) isolated *Campylobacter* (5.0%) from houseflies (*Musca domestica*) in a poultry farm in Selangor, Malaysia, which indicated that *Campylobacter* was shed in faecal materials; as such, flies could have picked up *Campylobacter* from the chicken litter. Furthermore, *Campylobacter* was found in samples of chicken (50.9%) and chicken meat (26.6%) in different districts of Selangor, Malaysia, which indicated that broiler chickens were colonised not only by the common *Campylobacter* species, but also by other *Campylobacter* species (Sinulingga *et al*., 2020). The prevalence of *C. jejuni* in broiler chicken farms in Kelantan, Malaysia, was 65.0%. The associated risk factors were the open house system and untreated water sources; accordingly, the researchers suggested that farmers should avoid those potential risk factors linked to the colonisation of *Campylobacter* (Wahab *et al*., 2021).

Campylobacter can contaminate birds through the transovarian channel from the breeder to the offspring (vertical transmission) (Tang *et al*., 2020), and through infection from the environment to a flock when unhygienic farming activities are practised (horizontal transmission) (Arsi *et al*., 2017). Overcrowding and a lack of biosecurity measures in poultry houses further increase the incidence of poultry contamination (Frederick and Huda, 2011). At poultry processing plants, the upper gastrointestinal tract, which usually contains *Campylobacter*, can cause cross-contamination in chicken carcasses (Byrd and Rand McKee, 2005). Contamination can also occur *via* improper handling of poultry carcasses by poultry workers who do not follow proper sanitation procedures, such as the use of sanitised utensils and gloves (Mazengia *et al*., 2015). In addition, meat handlers at retail markets who practice improper handling of poultry are considered to be a risk factor for cross-contamination of poultry meat. For instance, contamination could happen if the same chopping board were used for raw and ready-to-eat food (*e.g.*, vegetables) (Ravishankar *et al*., 2010).

Bhandari *et al*. (2013) proved that chicken handlers play a major role in maintaining sanitary conditions and preventing cross-contamination to ensure the safety and quality of chicken products. Additionally, food safety-related knowledge and attitudes are necessary for safer chicken production. For that reason, food safety-related knowledge, attitudes, and practices (KAP) are one of the key factors to achieving good control strategy for *Campylobacter* in the food chain. Poultry handlers in particular, can minimise *Campylobacter* prevalence at farms, processing plants, and retail outlets. However, a study about the prevalence of *Campylobacter* at each critical point along the poultry production chain has yet to be conducted. To the best of our knowledge, no study has ever investigated this association among workers along the poultry production chain in Peninsular Malaysia. To fill the information gap, the KAP of workers along the poultry production chain and their correlation with *Campylobacter* prevalence was investigated in the present work. The present work constituted a bigger study on the effects of KAP of poultry handlers on the prevalence of *Campylobacter*. The present work aimed to determine the effects of KAP of poultry handlers on the prevalence of *Campylobacter* along the poultry production chain in Peninsular Malaysia.

Materials and methods

Sample collection

Data collection was performed based on a cross-sectional study method involving five companies (A, B, C, D, and E) in Peninsular Malaysia (Figure 1).

Figure 1. Map of the study location (adapted from https://www.malaysiavisa.my/places-in-malaysia).

All companies had three components: farm, processing plant, and retailer; the supplier of the supply chain. The selected companies were chosen as suggested by the Department of Veterinary Services Malaysia based on their large-scale production (≥ 50,000 broilers produced per cycle). A total of 1,230 microbiological samples were collected from the farms (n = 460), processing plants (n = 170), and retailers (n = 600). The sample size was calculated based on the total sampling points collected at farms, processing plants, and retailers from all five companies. Samples from the farm were collected from the swabs of left- and right-boots, swabs of feeders, swabs of drinkers, and fresh faeces. Next, samples from the processing plant included swabs of transport crates, stunning water, swabs of slaughtering shackles, scalding water, swabs of de-feathering shackles, swabs of evisceration shackles, washing water, chilling water, swabs of left- and right-sides of the whole chicken surface, and swabs of deboning utensils. At the retailers, swab samples were collected from the left and right sides of the wings, thighs, and breasts of whole chickens. Sterile cotton swabs in 10 mL Letheen broth transport medium (Puritan, United States) were used in this study. All microbiological samples were transferred into sterile containers, stored at -20°C, promptly transported to the laboratory, and immediately analysed upon arrival. During sampling, survey questionnaires regarding the workers' KAP were distributed among the workers.

Sample processing

Campylobacter was isolated with slight modifications as described in the international standard method for *Campylobacter* isolation (ISO, 2006). Each microbiological sample was processed individually through the pre-enrichment procedure using the Bolton enrichment broth base medium (Oxoid, UK), and incubated at 42°C for 30 min. The pre-enriched samples were homogenised for 2 min. Then, they were serially diluted from 10-fold to 100-fold, and incubated in microaerophilic conditions at 42°C for 24 h. Next, a loopful of the broth culture was streaked onto the blood-free *Campylobacter* selective agar (Oxoid, UK), and incubated in microaerophilic conditions at 42°C for 24 to 48 h. Single colonies of presumptive *Campylobacter* isolates were further investigated.

Microbiological analyses

Isolated single colonies were analysed using the Gram-staining method, and catalase and oxidase tests. The findings were captured at 400× using a biological microscope (Interscience, France). Bacterial genomic DNA was extracted using the boiled-lysis method, and further confirmation of *Campylobacter* was made through the polymerase chain reaction (PCR). PCR amplification was performed in a thermocycler (Bio-Rad, USA) using a forward primer (5'-GGAGG CAGCA GTAGG GAATA TGACG GGCGG TGAGT ACAAG-3') targeting a genus-specific 16S rDNA encoding DNA region for *Campylobacter* spp. (1,041 bp) (Huang *et al*., 2021). The PCR amplification was optimised in a total reaction volume of 25 μL, consisting of master mix (12.5 μL, 1 μM), primer (1 μL, 10 μM), DNA template (1 μL), and sterile distilled water (9.5 μL). The components were mixed thoroughly, and the PCR amplification of the target sequence was done in a thermocycler programmed for 30 cycles of amplification. Each cycle consisted of three-step reactions: initial denaturation (94°C, 2 min) followed by 30 cycles of denaturation (94°C, 30 s), annealing (55°C, 30 s), extension (68°C, 45 s), and final extension (72°C, 5 min). The amplified PCR products and their sizes were determined *via* electrophoresis on 1.0% agarose gel (80 V, 35 min). A gel documentation system was used to capture the gel images (Bio-Rad, USA).

Questionnaire

The KAP study was conducted among the workers at the broiler farms, processing plants, and retailers. The questionnaire was adapted and modified from Gomes-Neves *et al*. (2007), Acikel *et al*. (2008), and Tokuç *et al*. (2009). The modifications aimed to make the questionnaire more suitable for the respondents who worked in the poultry industry. Pretesting was done among 30 respondents from a company based on their small-scale production (20,000 broilers produced per cycle) in Selangor. A total of 300 respondents answered the KAP questionnaire. The self-administered questionnaire was designed to evaluate the KAP of the poultry handlers regarding the hygiene and sanitation of the working area, handling of live birds and raw poultry meat, awareness of possible contaminants, and other factors. The questions were divided into four categories: Section A focused on the poultry handlers' sociodemographic characteristics (six questions); Section B evaluated the poultry handlers' knowledge in the handling of poultry and sanitation (ten questions); Section C assessed the poultry handlers' attitudes in the handling of poultry and sanitation (ten questions); and Section D evaluated the poultry handlers' practices in the handling of poultry and sanitation (ten questions). The questionnaire was written in the two most commonly used languages in Malaysia, namely Malay and English. Each respondent was fully informed about the study, and each interview lasted about 20 to 30 min.

Section A: Poultry handlers' sociodemographic characteristics

This section consisted of six items, which were poultry handlers' gender, nationality, age, educational level, working duration, and food safety training course attended. The respondents were grouped into age groups of 20 to 29, 30 to 39, 40 to 49, and above 50 years old. For educational level, the respondents were classified into four groups: primary school, secondary school, diploma, and degree. The respondents were also categorised into three groups based on working durations of less than a year, within one to five years, and more than five years. The question on attendance to a food safety training course had two options of either "yes" or "no".

Section B: Poultry handlers' knowledge in handling of poultry and sanitation

This section analysed the respondents' knowledge related to handwashing, proper attire, cleaning and sanitising, cross-contamination, and storage. This section contained ten items, and the respondents were required to choose either "true" or "false" as the answer. The chosen answers were converted to a score of 100.0%.

Section C: Poultry handlers' attitudes in handling of poultry and sanitation

Ten items were used to measure the poultry handlers' attitudes towards hand washing, proper clothing, sneezing and coughing techniques, equipment hygiene, smoking, wearing jewellery, and the location of the vehicles. The respondents were required to state whether they "agree" or "disagree" with the given statements. The chosen answers were converted to a score of 100.0%.

Section D: Poultry handlers' practices in handling of poultry and sanitation

In this section, the workers selected "yes" or "no" for the statements related to their practices, such as hand washing, wearing clean clothes and other protective equipment, cleaning and disinfecting, smoking, and the location of the vehicles. The answers were converted to a score of 100.0%.

Score interpretation

Table 1 shows the score marks and the level of KAP according to Ansari-Lari *et al*. (2010). A score below 50.0% is considered low level of KAP, whereas a score within the range of 50.0 to 74.9% is considered acceptable level of KAP. A score higher than 75.0% is considered high level of KAP.

Table 1. Subject classification based on scoring marks.

Range of scoring mark	Level of KAP
< 50%	Low
50 - 74. 9%	Acceptable
≥ 75%	Excellent

Validity and reliability of the instruments

The content validation of the questionnaires was performed by cross-referencing previous studies conducted by Gomes-Neves *et al*. (2007), Acikel *et al*. (2008), and Tokuç *et al*. (2009). Reliability for each set of questions in the questionnaires was tested with Cronbach's alpha, which fell within the range of acceptable limit (> 0.8) (Nunnally, 1994).

Statistical analysis

The data for broiler farms, processing plants, and retailers were analysed separately using the analysis of variance (ANOVA). The mean values reported were the values of percentage prevalence. Descriptive statistics were used to determine the frequencies of the poultry handlers' sociodemographic characteristics. The association between KAP levels of the poultry handlers and the prevalence of *Campylobacter* at the farms, processing plants, and retail markets were tested using the Chi-square test and Fisher's exact test.

Results and discussion

Prevalence of Campylobacter along poultry production chain

The presence of *Campylobacter* along the poultry production chain is summarised and presented in Table 2. A total of 313 from 460 samples (68.0%) were found positive for *Campylobacter* at all broiler farms. The percentage of prevalence of *Campylobacter* in broiler farms ranged from 22.8 to 93.5%, with the highest coming from broiler farm E. The most positive samples (more than 75.0%) in the farms came from fresh faeces. Kagambèga *et al*. (2018) noted a similarly high prevalence of *Campylobacter* in poultry faeces, whereby modern poultry farmhouses were commonly associated with the flock's litter, and acted as a pool and source for

Table 2. Analysis of variance (Pr > F)[1] of *Campylobacter* prevalence[2].

Company	Mean percent prevalence		
	Farm (*n* = 460)	**Processing plant (*n* = 170)**	**Retailer (*n* = 600)**
A	82.6[c] (76/92)	79.4[c] (27/34)	10.0[d] (12/120)
B	54.4[d] (50/92)	91.2[b] (31/34)	32.5[a] (39/120)
C	87.0[b] (80/92)	73.5[d] (25/34)	17.5[b] (21/120)
D	22.8[e] (21/92)	50.0[e] (17/34)	15.0[c] (18/120)
E	93.5[a] (86/92)	100.0[a] (34/34)	1.7[e] (2/120)

[1]Probability (Pr) > F-ratio. [2]Prevalence is mean value of positive PCR detection of *Campylobacter*. Means followed by different lowercase superscripts in the same column are significantly different ($p < 0.05$) based on ANOVA.

colonisation by *Campylobacter*. The occurrence of *Campylobacter* in poultry ranged from 6.3 to 38.1%, and in poultry environments ranged from 25.0 to 81.3%. However, our results of *Campylobacter* prevalence in Selangor contradicted the results obtained by Mohamed-Yousif *et al*. (2019), whereby we found *Campylobacter* to occur in both poultry (14.3%) and poultry environments (81.3%).

Campylobacter control programs should be based on an integrated approach that addresses effective litter management systems at poultry farms. The persistence of *Campylobacter* in animal and environmental reservoirs in poultry farms requires further investigations to change farming practices toward preventing such contaminations. *Campylobacter* transmission to poultry can occur *via* the environment and through horizontal transmission (flock-to-flock). Once *Campylobacter* successfully colonises a broiler flock, it can spread so quickly that eradicating it will be almost impossible. Georgiev *et al*. (2017) established fundamental methods to counteract bacterial colonisation of flocks at the farm level. The primary step in the effort to minimise contamination in the farm involves proper disinfection protocols as this could help to reduce broiler infections by this bacterium by up to 40% (Gibbens *et al*., 2001). Silva *et al*. (2011) claimed that the implementation of hygienic barriers between the internal and the external environments also reduced the risk of flock contamination at farms. Examples of these are the imposition of hygiene rules like handwashing and sanitising hands, and changing boots and coveralls regularly.

Newell and Fearnley (2003) stated that another important biosecurity measure was sanitising equipment, such as the buckets used to remove dead birds, and any other equipment brought into the slaughterhouse. Limited entrance access with an entrance order system depending on the age of the birds was among other measures (Sahin *et al*., 2015). For example, farmworkers should first enter the hatchery where the youngest birds are kept, before entering the grower house where the older birds live. This is mainly because the stronger immune system of the older birds makes them more resilient than the younger chicks to any possible pathogens. Huneau-Salaün *et al*. (2007) reported that colonisation typically occurs in broiler flocks aged two to three weeks. Colonisation before two weeks of age is very rare (Kuana *et al*., 2007; Hansson *et al*., 2010). Nevertheless, we did not compare the prevalence of *Campylobacter* between hatchery and grower houses since our samples came from only grower houses.

In the present work, the total distribution of *Campylobacter* isolate from the poultry processing plants was 78.8% (134/170). The incidence ranged from 50.0 to 100.0%, with the highest percentage of prevalence originating from the poultry processing plant. This finding was similar to the results reported by Rejab *et al*. (2011) in Malaysia who found that 61.1% of the chicken carcass samples from the poultry processing plant were contaminated with *Campylobacter*, and this contamination happened at several stages along the processing line. The poultry processing plant is commonly divided into a dirty zone, where slaughtering, bleeding, scalding, de-feathering, and evisceration processes take place, and a clean zone, where procedures are carried out at low temperatures and under strict hygiene controls. Cross-contamination could happen in the processing environment especially on the machines, knives, and chopping boards during the de-feathering and evisceration processes as a result of unhygienic poultry handling practices. McCarthy *et al*. (2018) and Harris *et al*. (2018) discovered that the massive cross-contamination occurring during scalding is likely due to improper monitoring of the pH (should be slightly acidic ~pH 6.5) and temperature (should be in the range of 51 - 53°C) of the scalding water. This corresponded with a study in China by Xiao *et al*. (2019) who reported that the chilling water used in the plant was contaminated with *Campylobacter*. Chilling is one of the critical processes, where several parameters like air temperature, movement, filtering, and relative humidity should be regularly monitored to limit the growth of foodborne pathogens (Stella *et al*., 2021). Our data showed that 80.0% of the carcass's washing water samples were contaminated by *Campylobacter*. Therefore, a stringent water management system should be applied throughout all processing steps to reduce *Campylobacter* load in a poultry processing environment. Micciche *et al*. (2018) reported that direct transmission of bacteria could occur if a contaminated water source was used.

The slightly higher prevalence of *Campylobacter* in the present work compared to previous research studies was possibly due to inadequate cleaning and disinfection at all sampling points in the processing plants. Several studies had also found that the slaughtering of infected broiler flocks could contaminate both the carcasses and the entire slaughtering line (Lillard, 1990; Corry *et al*., 2002; Olsen *et al*., 2003). Our study revealed that the slaughter utensils in all the processing plants were contaminated even before the slaughtering activities started. The cleaning and disinfection processes carried out before each slaughter activity were insufficient to eliminate the remaining *Campylobacter* contamination from the slaughter environment. Rasschaert *et al*. (2007) suggested that the slaughtering of healthy flocks should be done before the infected flock, and careful attention to critical points of cross-contamination in the line would help to reduce *Campylobacter*-positive flocks. Biswas *et al*. (2019) concluded that the primary measure to control contamination during poultry slaughtering and processing depended heavily on careful management practices to avoid colonisation, transmission, and cross-contamination.

Peyrat *et al*. (2008) stated that *C. jejuni* was able to survive overnight on equipment surfaces despite cleaning and disinfection procedures. Hence, the *Campylobacter* colonies probably contaminated carcasses during the slaughtering process. Four points in the poultry processing plant have been considered critical control points (CCP) where the birds are easily contaminated *via* cross-contamination: de-feathering, evisceration, scalding, and chilling (Buncic and Sofos, 2012). Our findings were consistent with that of Giombelli and Gloria (2014) who discovered *Campylobacter* to be a major contaminant at all the CCP. Therefore, serious measures to reduce *Campylobacter* dissemination along the poultry processing line should be taken. The implementation of Hazard Analysis and Critical Control Points (HACCP) in the poultry industry is extremely important because it involves constantly monitoring all steps of the processes to ensure product safety (Oloo *et al*., 2017).

The levels of *Campylobacter* contamination at retailers ranged from 1.7 to 32.5%, and retail outlet B was found to harbour the highest prevalence of *Campylobacter*. The total prevalence of *Campylobacter* in the retail samples was found to be slightly lower (15.3%) than those from the broiler farms (68.0%) and the poultry processing plants (78.8%). Tuncer and Sireli (2008) conducted a study regarding microbial growth on broiler carcasses stored at different temperatures. The processing plants usually maintain a temperature of about 10°C during carcass handling, while meat products in the retailers' fridges are stored at approximately 4°C. This study reported that the total viable counts developed more quickly at 10°C as compared to 4°C. After initial contamination, some bacteria can persist for up to ten days at refrigerated temperature during meat product storage. However, refrigeration by chilled air decreases the total viable count (approximately 1 log), and inhibits the multiplication of *Campylobacter* (Rouger *et al*., 2017). Therefore, survival temperatures and their microaerophilic phenotypes are factors that need to be considered when reducing bacterial prevalence at the retail level. A similar finding was noted in the study done by Sinulingga *et al*. (2020) who reported the retail market to have a *Campylobacter* contamination level of 14.3%. Due to unhygienic practices during the handling of raw chicken, most poultry become contaminated by *Campylobacter* along the processing line, starting from the primary production through to the final product (Kunadu *et al*., 2020). It is crucial to combat these pathogenic contaminations, especially those common among poultry such as *Campylobacter*, at the retail level before they reach the consumer, and cause serious public health issues. Wieczorek *et al*. (2015) suggested that the most effective and easy way to reduce the incidence of and inactivate *Campylobacter* is by freezing poultry at -15°C and lower. *Campylobacter* is unable to multiply in food at conditions below 30°C with high oxygen levels and dry conditions. However, the most effective way to avoid flock colonisation before slaughter is to implement stringent biosecurity controls (Abdul-Rahiman *et al*., 2021). Both physical and chemical interventions must be considered for specific post-slaughter interventions. Physical interventions include steaming or hot water sprays, electrolysed water, ozone water, irradiation, ultrasound, forced air chilling, crust freezing, and cold plasma treatment. Chemical interventions are chlorine-based washes, organic acid spray washes, essential oils, and phosphate-based treatments (Huss *et al*., 2018; Lu *et al*., 2019). Using a combination of physical and chemical measures can reduce *Campylobacter* contamination at the retail level even further.

Sociodemographic characteristics of respondents

The sociodemographic characteristics of all 300 respondents from companies A, B, C, D, and E were considered in this KAP study, and the results are shown in Table 3. Of all respondents, 246 (82.0%) were men and 54 (18.0%) were women. The majority of respondents, 210 (70.0%), were aged between 30 to 39 years old. Next, 172 (57.3%) respondents had achieved an educational level of secondary school. Of all respondents, 243 (81.0%) had been working in the poultry industry for less than a year. Regarding the training course, 254 (84.7%) respondents had attended regular food safety training courses provided by the companies to educate their workers on basic knowledge and awareness of food safety.

Table 3. Distribution of sociodemographic characteristics of respondents.

Variable	Total (*n* = 300)	Percentage (%)
Gender		
Male	246	82.0
Female	54	18.0
Nationality		
Malaysian	168	56.0
Others	132	44.0
Age group		
20-29	58	19.3
30-39	210	70.0
40-49	24	8.0
50	8	2.7
Educational level		
Primary school	127	42.3
Secondary school	172	57.3
Diploma	1	0.3
Degree	0	0
Working duration		
< 1 year	243	81.0
1 - 5 years	55	18.3
> 5 years	2	0.7
Training course		
Yes	254	84.7
No	46	15.3

KAP of poultry handlers along poultry production chain

As shown in Table 4, the mean KAP scores of farmworkers were 99.40 ± 1.26, 99.50 ± 0.97, and 99.60 ± 0.52, respectively. Overall, the poultry handlers at the farm demonstrated an excellent level of knowledge. Similarly, Abdullahi *et al*. (2016) reported that the farmworkers had good KAP regarding biosecurity at poultry farms. All the respondents in the poultry farms had excellent knowledge about the importance of proper clothing while working, such as the use of a cap, mask, and protective gloves. Thongpalad *et al*. (2019) stated that it is important to equip poultry handlers with knowledge about proper clothing; with the right perception, workers tend to put it into practice which consequently helps in reducing the risk of cross-contamination. Our findings showed that the average score of the workers' attitude in farms towards the safe handling of poultry and sanitation was excellent. However, 97.0% of workers stated that wearing jewellery (including rings and plain bands) and watches while working is acceptable. Therefore, workers need further education on the appropriateness of wearing these items while handling birds. Kusumaningrum *et al*. (2003) found that bacterial pathogens can survive on stainless steel surfaces for up to four days. Therefore, wearing jewellery can enhance the spread of microorganisms in birds. Hence, farmworkers must have an understanding of the risk of possible contamination that can occur if they wear jewellery when working.

Table 4. Association between prevalence of *Campylobacter* and KAP of poultry handlers in the poultry industry (n = 300).

Poultry production chain	Variable	Mean[1] ± Standard deviation	*P* value Fisher's exact test
Farm	Knowledge Acceptable, excellent	99.40 ± 1.26	0.87
	Attitude Acceptable, excellent	99.50 ± 0.97	0.36
	Practice Acceptable, excellent	99.60 ± 0.52	0.48
Processing plant	Knowledge Acceptable, excellent	99.50 ± 0.47	0.11
	Attitude Acceptable, excellent	99.31 ± 0.78	0.19
	Practice Acceptable, excellent	99.14 ± 0.94	0.28
Retailer	Knowledge Acceptable, excellent	94.40 ± 8.47	0.17
	Attitude Acceptable, excellent	82.00 ± 27.33	0.31
	Practice Acceptable, excellent	88.40 ± 9.88	0.40

[1]Mean score value after conversion to 100 points.

Next, the average practice score among farmers was excellent. Nevertheless, from our observation, the farmworkers did not perform the practices correctly in every aspect, especially those related to biosecurity at the farm. For example, the practice of dipping boots in disinfectant, not smoking in the farm area, and parking vehicles away from the farmhouse.

The mean KAP values of poultry handlers at the processing plants were 99.50 ± 0.47, 99.31 ± 0.78, and 99.14 ± 0.94, respectively. The average score in the knowledge section obtained by poultry handlers in all processing plants was shown to be at an excellent level. Most workers had the appropriate knowledge and correct information regarding safe poultry handling and sanitation. However, employers in the industry need to further emphasise the importance of handwashing to the workers. Based on our findings, there were respondents (1.0%) who were not aware of the presence of bacteria in a healthy person. According to Biswas *et al*. (2019), bacteria are easily transmitted through the dirt beneath the fingernails; thus, scrubbing hands thoroughly is important after being contaminated. Previously, Adesokan and Raji (2014) reported that the workers had good KAP towards safe poultry handling. The average attitude score of the workers in all processing plants towards the safe handling of poultry and sanitation was excellent. Nevertheless, based on our observation, they need to concentrate on matters of self-discipline. For instance, coveralls should not be used in any other places except the working area. Contaminated coveralls could potentially cross-contaminate other clean surfaces and increase the prevalence of *Campylobacter* in the production line (Djeffal *et al*., 2018). The average practice score among meat handlers in the poultry processing plants was excellent. Despite that, the employees claimed that the workers sometimes neglect handwashing, which indicated a poor level of hygiene. Employers should regularly inspect the activities of the workers and the cleanliness of the workers' hands because hands often harbour numerous foodborne disease microorganisms (Baş *et al*., 2006).

The mean KAP values of poultry handlers at retailer markets were 94.40 ± 8.47, 82.00 ± 27.33, and 88.40 ± 9.88, respectively. Overall, the meat handlers at all supermarkets had attained an excellent level of knowledge. Similarly, Tegegne and Phyo (2017) reported that the workers at retail markets had good knowledge about hygiene and the spread of pathogens to the public. All the respondents were aware of and had the correct information about the importance of the appropriate storage temperature to reduce the rate of meat spoilage and cross-contamination issues that would cause product spoilage. Based on our findings, the average attitude score of the workers in supermarkets towards the safe handling of poultry and sanitation was excellent. The workers had a positive attitude, and were committed at the workplace. They believed that smoking, rubbing hands on the face while working, and improper sanitation should be avoided as these actions would reduce the safety and quality of poultry meat. However, 22.0% of respondents disagreed with the prohibition of wearing jewellery at work as they believed wearing jewellery would not contaminate the carcass. A study conducted by Ingle *et al*. (2012) revealed that rings and watches were shown to increase the frequency of hand-related bacterial contamination. Additionally, the sharp edges of some jewellery can cause bruises on the skin. Hence, jewellery is not recommended in the workplace (Wambui *et al*., 2017). The average practice score among meat handlers in supermarkets was excellent. Yet, some workers (9.0%) still came to work even if they were sick. The standard protocol only allows healthy workers to handle meat in a food establishment (Marriott *et al*., 2006).

Association between KAP of poultry handlers and Campylobacter prevalence along poultry production chain

The present work found that the study population had high KAP levels of appropriate safe poultry handling, and this was insignificantly associated with the prevalence of *Campylobacter* (Table 4). KAP variables were used to study the association between KAP and the prevalence of *Campylobacter* along the chicken production chain. However, there was no significant relationship between the prevalence of *Campylobacter* and the excellent, acceptable KAP levels of chicken handlers in farms, processing plants, and retailers.

Based on the present work, the chicken handlers' KAP levels were insignificantly related to the prevalence of *Campylobacter*. From the KAP data obtained, most of the chicken handlers had excellent KAP for the safe handling of chicken and sanitation procedures. However, the prevalence of *Campylobacter* was high. The reason for this finding might be because the respondents had a tendency towards wrong reporting in self-reporting questionnaires. Therefore, the reported behaviour did not correspond with the actual behaviour. Respondents are often prone to giving socially desirable answers, possibly resulting in an overreporting of "good behaviour" (Mazengia *et al*., 2015). In addition, knowledge does not always translate to attitudes and practices. One limitation of the study was its relatively short study period which did not reflect the potential difference between seasons or years.

Conclusion

The present work was conducted to determine the effects of knowledge, attitude, and practice (KAP) of poultry handlers on the prevalence of *Campylobacter* along the poultry production chain in Peninsular Malaysia, from farm to retailer. *Campylobacter* was isolated from the farm (68.0%, *n* = 313/460), processing plant (78.8%, *n* = 134/170), and retailer (15.3%, *n* = 92/600) samples. The retailer samples had the lowest prevalence of *Campylobacter* in comparison with samples from farms and processing plants. *Campylobacter* isolates from retailers were associated with faecal contamination in the farm and processing plant, emphasising the need for improved measures for reducing carcass contamination along the poultry production chain. In contrast, the prevalence of *Campylobacter* was significantly high even though the overall KAP levels of the poultry workers were excellent. These insignificant results might be due to the tendency of respondents to report wrongly in self-reporting questionnaires causing the reported behaviour not correspond with the actual behaviour. Respondents are prone to giving socially desirable answers, possibly resulting in an overreporting of "good behaviour". *Campylobacter*-associated illness leads to health and economic burdens which involve productivity and cost losses. In the future, a comparison between seasons or years of *Campylobacter* samples and an observation study of KAP will provide more useful information. Moreover, continuous efforts to improve the safe handling of poultry are important at all critical control points of contamination by thorough and regular monitoring that is based on scientific information.

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