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The incidence of *Campylobacter* contamination levels through Chicken-Sashimi Processing steps in A Small-scale Poultry processing plant applying the External stripping method

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ABSTRACT. This study aimed to analyze the incidence of *Campylobacter* in a small-scale chicken meat processing plant producing "chicken-sashimi", and determine the effectiveness of surface burning as a treatment during processing. The most probable number (MPN) method was used to analyze the load of *Campylobacter* in 48 samples from four different processing steps (de-feathering, chilling, surface burning, and final-products; 12 samples each). We found the highest load of isolated bacteria in chicken skin after de-feathering. *Campylobacter* was not detected after the surface burning step despite a large load of bacteria present in the cecum content. *Campylobacter* was absent in the final products. Adequate surface burning can avoid *Campylobacter* contamination of chicken sashimi in the processing plant by applying the external stripping method.

KEY WORDS: *Campylobacter,* chicken sashimi, poultry processing, surface burning

Campylobacter is one of four key global causes of diarrheal diseases. It is considered to be the most common bacterial cause of human gastroenteritis in the world [31]. Most *Campylobacter* infections are foodborne, although contaminated water and environmental exposure have also been implicated [5]. To date, the genus *Campylobacter* contains 35 species [16]. The two species most commonly associated with human disease are *C. jejuni* and *C. coli*. Of these, *C. jejuni* accounts for more than 80% of *Campylobacter*-related human illness, with *C. coli* accounting for up to 18.6% [12]. Campylobacteriosis is the most common infection, which can precede the onset of post-infectious Guillain-Barré syndrome, a severe demyelinating neuropathy, occurring in approximately three out of every 10,000 campylobacteriosis cases [24]. *C. jejuni* is the leading cause of bacterial diarrheal disease worldwide and is the most frequent antecedent to Miller Fisher syndrome [25].

In Japan, epidemiological investigations of *Campylobacter* food poisoning have shown that chicken meat and its products are the most important sources of infection, as is the case in other industrialized nations [30]. Some studies have found that chicken meat has a high probability of exhibiting *Campylobacter* contamination [20, 22, 23, 29]. Raw or undercooked poultry meat and meat products are known as the main causes of campylobacteriosis, and one-fourth of the total food poisoning is caused by *Campylobacter jejuni*/*coli* [9].

In Kagoshima Prefecture, there is a deep-rooted culture of eating chicken meat (mainly thigh and breast meat) after boiling or burning the surface. This meat is commonly sold under names such as "chicken sashimi" and "chicken tataki", and is widely popular. The chicken sashimi processed in this prefecture is mainly prepared from disused breeding chicken (spent broiler breeders that were approximately 450-days old), sourced from all over Japan. Currently, chicken sashimi is offered in restaurants in various regions of Japan. Various incidences of food poisoning caused by *Campylobacter* from chicken sashimi have been reported in the country; it is therefore imperative that each processing plant and provider (such as a restaurant) handle this product carefully and hygienically. In the Kagoshima and Miyazaki Prefectures, "sanitary standards for raw poultry meat" and "hygiene measures

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for raw poultry meat" have been independently formulated and are well known to the relevant companies. External stripping is a method of removing meat such as thighs, and breasts from the outside without making contact with the internal organs of the chicken. Since, the method does not damage the internal organs, they can be processed without contamination.

The Food Safety Commission created a guide in 2006 titled "Risk Profile for Food Health Impact Assessment-*Campylobacter jejuni/coli* in chicken, etc". Its recommendations may contribute to reducing the risk of contamination of chicken with bacteria at the production stage. According to the description in this guide, in addition to improving breeding hygiene management and establishing measures to suppress the colonization of *Campylobacter* in the chicken intestine, logistic slaughter has been recommended so that *Campylobacter* free chickens processed first [6]. However, a method that can reliably control *Campylobacter* contamination in commercially available chicken has not yet been established. Recently, the risk management measures for eating raw meat have been strengthened in Japan. Although it is well known that chicken meat is heated before its commercial distribution, in principle, no standards have been set.

Therefore, the main objectives of this study were to analyze the incidence of *Campylobacter* from small-scale chicken meat processing plants producing chicken sashimi, and the effect of surface burning on the reduction of *Campylobacter* in different processing steps.

A total of 120 cloacal swabs were collected from live chickens using sterile cotton swabs. The breed of chicken was Akadori-Satsuma, approximately 60 to 80 days-old and bred in a cage system. The samples were kept in 5 ml Preston broth, chilled with ice blocks during transport. Samples were delivered to the Laboratory of Veterinary Public Health, Kagoshima University, and cultured on the day of arrival. Specimens were incubated at microaerophilic conditions (mAc; 5% oxygen, 10% carbon dioxide, and 85% nitrogen) at 42°C for 48 hr. A loopful of the culture was streaked onto a selective Butzler agar (Oxoid) plate, which was incubated at 42°C for 48 hr in mAc. *Campylobacter* presence was confirmed using phase-contrast microscopy, and a direct colony PCR. Only chickens carrying *Campylobacter* in their cloaca were selected for subsequent experiments.

We selected a small-scale poultry processing plant that processes poultry in Kagoshima prefecture. The small-scale plant in the study processes the chicken in an external stripping style. The chilling system process comprised two steps. The chickens were first fresh chilled in 50 ppm sodium chloride salt for more than 2 min and then chilled in a chiller tank for more than 10 min. After being drained, the surfaces of the chicken was burned using a gas burner for 45 sec to 1 min until the surface turned brown.

The experiment design showed in Fig. 1.

The experiment were performed four times (November 2018, January 2019, June 2019, and August 2019). Twelve cecal contents were collected from 12 cloacal swab-positive chickens during one sampling occasion, and then skin from 3 chickens each after de-feathering, cooling, and surface burning, and final products derived from the remaining 3 chickens. Forty-eight samples including 36 skin samples and 12 meat samples were collected.

The colony count was obtained from the cecum content using the serial dilution method [1]. A total of 0.5 g chicken cecum content and 5 ml of buffered peptone water-sodium thiosulfate (BPW-ST) was mixed well for 10 sec to prepare a sample suspension (10-fold dilution). Next, a 10-fold serial dilution was prepared. The suspension at the same concentration (ten-, hundred-, thousand-, and ten-thousand-folds dilutions) was inoculated to mCCDA (Nippon Becton Dickinson Co-Ltd., Fukushima, Japan) clear agar by 50 µl per dish (two dishes per one concentration), and incubated in mAc at 42°C for 48 hr. *Campylobacter* presence was confirmed using phase-contrast microscopy and a direct colony PCR.

The load of *Campylobacter* from chicken skin or meat samples was estimated using the most probable number (MPN) by three-tube method [4, 10]. A total of 25 g of chicken skin or meat sample and 225 ml of Preston liquid medium (Oxoid, Hampshire, UK) were mix in stomach bag for 1 min to prepare a sample suspension. Next, three 10 ml suspensions were prepared; three 1 ml and 0.1 ml suspension solutions were inoculated into 9 ml and 9.9 ml of Preston liquid medium, respectively, and cultured as 10-fold,

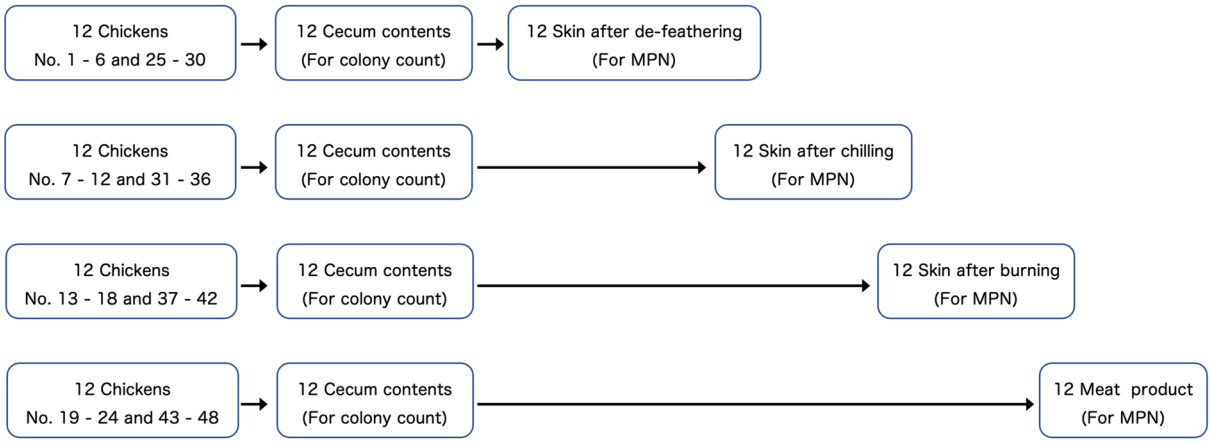

Fig. 1. Experiment design and the sampling collection sites. Chicken No. 1−24 were collected in winter (November, 2018 and January 2019), chicken No. 25−48 were collected in summer (June and August, 2019).

100-fold, and 1,000-fold diluted solutions of 10 ml. Three dilutions of suspensions were incubated in mAc at 42°C for 48 hr. Next, one loopful from each enrichment solution was spread onto Butzler agar, and incubated in mAc, at 42°C for 48 hr. The MPN analysis was conducted based on the growth of *Campylobacter* on Butzler agar, and calculated using the MPN standard table. *Campylobacter* was confirmed using phase-contrast microscopy and a direct colony PCR.

The strains of the isolates were identified by the multiplex PCR method [8, 15, 28] using *C. jejuni*-specific primers (VS15/VS16) and *C. coli*-specific primers (CC18F/CC519R). The total volume of the reaction was 20 µl, which consisted of four types of primer storage solutions (2 pmol/µl each; 2 µl each), 10 µl of Emerald Amp PCR Master Mix (TAKARA BIO, Kusatsu, Japan), and 2 µl of sterile distilled water. The reaction started after the addition of a loopful of *Campylobacter* [26]. DNA derived from *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559 were used as positive controls. The PCR reaction was performed in 35 cycles of 94°C for 30 sec, 56°C for 30 sec, and 72°C for 30 sec. The final PCR product was electrophoresed on a 1.5% agarose gel (AMRESCO, Solon, OH, USA) at 100 V for 40 min, and the presence/absence of amplified fragments and the molecular weight were confirmed after staining with ethidium bromide.

Student's *t*-test was performed to detect significant differences in the bacterial load across cecal contents, and between chicken skin sampled in winter and summer.

Our findings on the incidence of *Campylobacter* in chicken cloaca swabs are shown in Table 1. Overall, 69.2% of all tested chickens tested positive for *Campylobacter* (83 out of the 120 tested chickens). Out of these, *C. jejuni* was detected in 82; only one chicken had *C. coli*. The rate of positive samples detected in June and August (summer in Japan) was slightly higher than in November and January (winter).

Table 2 demonstrates that 22 out of 24 cecum content samples were positive for *Campylobacter* in the winter. There were differences in the load of *Campylobacter* detected among the different chicken processing steps. Five out of six chicken skin samples tested positive after de-feathering. The load of *Campylobacter* was between 4–23 MPN/10 g. Moreover, after chilling, we observed only one positive sample (23 MPN/10 g) out of six chicken skin samples. The other twelve samples tested (six chicken skin samples after surface burning; six chicken meat samples in the end-product step) were less than detection limit (<3 MPN/10 g) for *Campylobacter*.

Table 3 shows the load of *Campylobacter* from cecum content samples, chicken skin samples, and chicken meat samples from each processing step in the summer season (June and August). All six chicken skin samples carried *Campylobacter* after de-feathering, even though two of the cecum content had no *Campylobacter*. The load of *Campylobacter* in chicken skin (after de-feathering) in summer ranged between 93 and upwards of 1,100 MPN/10 g. All chicken skin sample tested positive for *Campylobacter* after chilling (4–23 MPN/10 g). After the surface burning step, we found one positive sample out of six chicken skin samples. In the final product step, all six chicken meat samples contained *Campylobacter* less than the detection limit (<3 MPN/10 g).

As observed in Tables 2 and 3, there was a significant difference in the bacteria load in cecal contents between winter and summer. In winter the mean ± standard error (SE) of samples 1 to 24 was $2.3 \times 10^4 \pm 4.1 \times 10^4$; in summer the mean ± SE of samples 25 to 48 was $4.8 \times 10^6 \pm 1.4 \times 10^7$. The load of *Campylobacter* in cecal contents was significantly higher in summer than in winter ($P<0.001$).

At the same processing step, the load of *Campylobacter* was significantly different between winter and summer except after the burning step, and at the end product step. After de-feathering the mean ± SE of samples 1 to 6 was 8.3 ± 3.1, whereas that of samples 25 to 30 was 342.2 ± 154.5. The load of *Campylobacter* from skin after de-feathering was significantly higher in summer than in winter ($P<0.001$). After the chilling step the mean ± SE of samples 7 to 12 was 6.3 ± 3.3, whereas that of samples 31 to 36 was 10.2 ± 3.2. The load of *Campylobacter* from the skin after chilling in summer was significantly higher than in winter ($P<0.05$). After the burning surface step, the mean ± SE of samples 13 to 18 was 3 ± 0, whereas that of samples 37 to 42 was 3.2 ± 0.2. The load of *Campylobacter* on skin after burning the surface was not significantly different between summer and winter.

Our results, as seen in Table 1, agree with other studies that have affirmed that *C. jejuni* is much more prevalent than *C. coli* in chicken [11, 18]. We also found similar results regarding the effect of seasonal factors on the rate of *Campylobacter* infection in chickens as a study by Humphrey, where it was found that there is generally a higher rate of colonization in summer than at any other time of year [14].

The results of the *Campylobacter* load tests between winter and summer in Tables 2 and 3 demonstrated that the load of *Campylobacter* in different processing stages was significantly higher in summer than in winter. This may be one of the reasons

Table 1. The incidence of *Campylobacter* in chicken cloaca swabs in Kagoshima, Japan

Sampling time (month)	No. of chickens tested	No. of chickens positive (%)	No. of *Campylobacter* species	
			C. jejuni	*C. coli*
November, 2018	30	18 (60.0)	17	1
January, 2019	30	20 (66.7)	20	0
June, 2019	30	21 (70.0)	21	0
August, 2019	30	24 (80.0)	24	0
Total	120	83 (69.2)	82	1

Table 2. Number of *Campylobacter* among each processing step in winter season

Sample	Cecum content (cfu/g)	Chicken skin after de-feathering (MPN/10 g)	Chicken skin after chiller (MPN/10 g)	Chicken skin after burning (MPN/10 g)	Chicken meat in products (MPN/10 g)
1	1.1×10^4	<3	-	-	-
2	6.6×10^4	7	-	-	-
3	7×10^2	4	-	-	-
4	4.2×10^3	9	-	-	-
5	1.1×10^3	4	-	-	-
6	5.4×10^3	23	-	-	-
7	2.3×10^4	-	23	-	-
8	3×10^2	-	<3	-	-
9	ND *	-	<3	-	-
10	1.1×10^5	-	<3	-	-
11	1.7×10^4	-	<3	-	-
12	7.5×10^3	-	<3	-	-
13	1×10^2	-	-	<3	-
14	6.9×10^3	-	-	<3	-
15	6.8×10^3	-	-	<3	-
16	2.3×10^3	-	-	<3	-
17	8.8×10^4	-	-	<3	-
18	3×10^4	-	-	<3	-
19	ND *	-	-	-	<3
20	1.6×10^5	-	-	-	<3
21	5×10^2	-	-	-	<3
22	1.6×10^3	-	-	-	<3
23	1.7×10^4	-	-	-	<3
24	1.5×10^2	-	-	-	<3

Chicken 1–3, 7–9, 13–15, and 19–21 were collected in November, 2018, and chicken 4–6, 10–12, 16–18, and 22–24 were collected in January, 2019. * Not detected (The number of *Campylobacter* less than 1×10^2 cfu/g). MPN: most probable number.

supporting the finding by of Torrung Vetchapitak and Naoki Misawa that *Campylobacter* food poisoning in Japan show seasonal fluctuation with a major peak in the rainy season (May to September) [30].

The process followed by the small-scale chicken sashimi processing plant under study included the following main steps: scalding, de-feathering, evisceration, crop removal, inside/outside wash, chilling, surface burning, and end-product. Steps such as scalding, de-feathering, evisceration, crop removal can all contribute to cross-contamination of *Campylobacter* by various means. Some studies have also reported that evisceration is one of the most critical steps causing cross-contamination [2]. Many in-plant studies have confirmed that the load of *Campylobacter* species significantly increases after this process [19]. In addition, a recent article about the recommended practices to eliminate *Campylobacter* from live birds and chicken meat in Japan [9] was comparable to the processing techniques in our study.

In 2002, Park *et al*. [21] reported that despite being thermophilic in their growth requirements, *C. jejuni* and *C. coli* are sensitive to high temperatures and, consequently, will not survive in food that has been pasteurized or adequately cooked. Minimizing *Campylobacter* contamination is very important because a large proportion of chicken meat already carries bacteria prior to the burning step. Thus, careful surface burning is essential when consuming chicken sashimi.

Some previous studies have reported the effect of various strategies for reducing the occurrence of *Campylobacter* in chicken meat, including using high temperatures, administration of vaccines [7, 13]. Although chilling treatment causes a decrease in bacterial load to some extent, it is believed that this method alone cannot fully control the load of bacteria [3, 17, 27]. As shown by our results in Table 3, *Campylobacter* remains present after the chiller step, although its load is reduced to between 4–23 MPN/10 g of chicken skin samples. *Campylobacter* contamination of chickens on farms can lead to the transmission of these bacteria along the chicken production chain. In addition, the incidence of *Campylobacter* in chicken meat varies between different processing steps. The reduction of *Campylobacter* contamination in chicken products is the most effective strategy to reduce the risk of human *Campylobacter* infection from chicken.

While various methods have been proposed, one which will reliably prevent *Campylobacter* contamination has not yet been established. Tables 2 and 3 show that only one skin sample from one chicken after the surface burning step was detected with *Campylobacter* at a load of 4 MPN/10 g, although the skin samples were taken from 12 chickens that carried *Campylobacter* in their cecum content. Our results suggest that surface burning in the process of preparing torisashi may be effective to reduce *Campylobacter* contamination under the condition of external stripping method.

Table 3. Number of *Campylobacter* among each processing step in summer season

Sample	Cecum content (cfu/g)	Chicken skin after de-feathering (MPN/10 g)	Chicken skin after chiller (MPN/10 g)	Chicken skin after burning (MPN/10 g)	Chicken meat in products (MPN/10 g)
25	4×10^5	290	-	-	-
26	ND *	120	-	-	-
27	ND *	210	-	-	-
28	1.3×10^4	93	-	-	-
29	9×10^5	>1,100	-	-	-
30	9×10^4	240	-	-	-
31	7.2×10^4	-	4	-	-
32	4.3×10^5	-	15	-	-
33	1.0×10^2	-	4	-	-
34	6.9×10^5	-	23	-	-
35	8×10^3	-	4	-	-
36	ND *	-	11	-	-
37	ND *	-	-	<3	-
38	5.5×10^4	-	-	<3	-
39	7.6×10^3	-	-	<3	-
40	5.2×10^7	-	-	<3	-
41	5×10^7	-	-	<3	-
42	2.9×10^4	-	-	4	-
43	2.6×10^4	-	-	-	<3
44	ND *	-	-	-	<3
45	4.3×10^5	-	-	-	<3
46	10^6–10^7	-	-	-	<3
47	10^5–10^6	-	-	-	<3
48	6×10^3	-	-	-	<3

Chicken 25–27, 31–33, 37–39, and 43–45 were collected in June, 2019, and chicken 28–30, 34–36, 40–42, and 46–48 were collected in August, 2019. *Not detected (the number of *Campylobacter* less than 1×10^2 cfu/g). MPN: most probable number.

CONFLICT OF INTEREST STATEMENT. The authors declare that this research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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