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Effect of morphological changes in feather follicles of chicken carcasses after defeathering and chilling on the degree of skin contamination by *Campylobacter* species

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ABSTRACT. *Campylobacter jejuni* and *C. coli* are the leading causes of enteric infections in many developed countries. Healthy chickens are considered to act as reservoirs of campylobacters, as the organisms colonize the intestinal tract. Once infected birds enter a processing plant, contamination of chicken carcasses with campylobacters occurs over the entire skin during defeathering and evisceration due to leakage of crop and/or intestinal contents. Although the role of feather follicles in the contamination of chicken carcasses by campylobacters during processing is still debatable, it has been considered that the microorganisms would be entrapped and retained in the follicles due to the morphological changes resulting from defeathering and chilling. In the present study, we observed the morphology of feather follicles in chicken carcasses after defeathering and chilling. A total of 3,133 feather follicles were examined for morphological changes before and after chilling. Shortly after defeathering, most (91.5%) of the follicles were closed, whereas after chilling they were either closed (85.5%) or open (6%), although a small proportion of enlarged follicles became smaller or closed (2.6%). Moreover, 5.9% of the follicles that were slightly open became further enlarged after chilling. Furthermore, the proportion of enlarged feather follicles that became closed after chilling showed no discernible relationship with the degree of campylobacter contamination in different areas of the carcass skin, suggesting that campylobacters may not be confined to feather follicles as a result of the morphological changes attributable to defeathering and chilling.

KEY WORDS: *Campylobacter*, chicken carcass, contamination, feather follicle

Campylobacter jejuni and *C. coli* are the leading causes of enteric infections in many developed countries, and the public health burden due to campylobacteriosis is increasing [16]. Although many risk factors for *Campylobacter* transmission have been identified [10, 14], it is considered that the handling and consumption of poultry meat are the most important source of human campylobacteriosis [7, 9]. The epidemiological data for Japan are based on passive surveillance, but approximately 2,000 cases per year have been reported as foodborne infections since 1982 [8]. Since there is a preference in Japan for fresh raw "free-range" chicken meat and liver, this is likely to account for most cases of human campylobacteriosis.

Healthy chickens are considered to act as reservoirs of campylobacters, as the organisms colonize the intestinal tract. Once chickens enter a processing plant, contamination of the carcasses with *C. jejuni* and *C. coli* occurs over the entire skin during the defeathering and evisceration process due to expulsion and/or leakage of crop and intestinal contents [2, 12, 13, 15]. Berndtson *et al*. have demonstrated another possible mechanism of contamination, having isolated *C. jejuni* from subcutaneous scrapings of chicken carcasses, indicating that the organisms can reside in feather follicles [1]. They suggested that the scalding and defeathering procedures allow feather follicles to open, and that subsequent low-temperature chilling then closes the follicles again,

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thus trapping the microorganisms within them. However, the morphological changes in feather follicles in response to temperature during carcass processing have not been examined in detail. In the present study, we investigated the morphology of feather follicles in chicken carcasses after defeathering and chilling, and examined whether these morphological changes might play an important role in campylobacter contamination.

MATERIALS AND METHODS

Chicken carcasses

The present survey was conducted from May to July, 2011, at a processing plant in Miyazaki prefecture handling about 400–500 free-range chickens daily. A total of 15 4–5-month-old carcasses of both sexes were investigated after defeathering. After bleeding and scalding at 62°C for 80 sec, the carcasses were moved to a tank for defeathering using rubber fingers together with rotation in flowing tap water at 20°C for 70 sec. Then the carcasses were moved immediately in a chiller tank before evisceration.

Morphological observation of feather follicles

To observe the morphology of feather follicles after defeathering and after chilling of carcasses, different parts of the skin were photographed. To obtain photographs taken at the same angle after defeathering and after chilling, each part of the skin was marked with both sewing pins with a head (1 cm diameter) and branding with a heated iron. The following parts of the skin (with the number of feather follicles examined) were photographed: dorsal neck (n=960), abdominal region (n=851), thigh (n=684), back (n=391) and crotch (n=247). The chicken carcasses were then chilled in 15 *l* of ice water for 90 min. The subcutaneous temperature in the abdominal region was monitored using a digital thermometer (Sato Keiryoki MFG, Tokyo, Japan) during chilling, and each carcass was examined when the temperature had fallen below 8°C. After chilling, the same parts of the marked skin were photographed again from the same angle. A total of 3,133 feather follicles were examined for morphological changes after defeathering and after chilling. The areas of the feather follicles were measured using image analysis software (ImageJ, National Institutes of Health, Bethesda, MD, U.S.A., http://imagej.nih.gov/ij/) based on the diameter of the pin's head as a reference scale, and the area of each follicle after defeathering and after chilling was compared.

Enumeration of Campylobacter species naturally contaminating chicken skin after chilling

The number of contaminating campylobacter cells was determined by the most-probable-number (MPN) method. Ten-gram sample of skin from each of the dorsal neck, abdominal region, back, and thigh was removed after chilling from 3 chicken carcasses and a 10-fold serial dilution of each skin specimen (10^{-1} to 10^{-3}) was made in Preston enrichment medium (Oxoid, Basingstoke, U.K.) after homogenized using a stomacher for 90 sec. The samples were cultured at 37°C for 48 hr under microaerophilic conditions (80% N_2, 10% CO_2, 5% O_2 and 5% H_2), and then one loopful of each dilution was transferred to modified Cefoperazone Charcoal Deoxycholate agar (mCCDA; Oxoid CM0739) supplemented with CCDA selective supplement (Oxoid SR0155) for isolation. The number of campylobacter cells was calculated by applying the common 3-tube MPN procedure based on the number of bacterial colonies indicating PCR-positivity for campylobacter at each dilution. The specific PCR reactions for *Campylobacter* spp. were performed as described elsewhere [17].

Statistical analysis

The areas of feather follicles after feathering and after chilling were compared, and *Campylobacter* counts for different areas of chicken skin were compared by Fisher's exact two-tailed test using R version 3.1.3 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was defined as $P<0.05$.

RESULTS

Kinetics of subcutaneous temperature in carcasses after chilling

Three chicken carcasses were immersed in chilled water at 3.0°C, and the subcutaneous temperature was measured for 90 min. The initial mean temperature of the carcasses was 35.1°C and then gradually decreased with time (Fig. 1). The temperature of the carcasses fell below 8°C after 60 min of chilling, and this temperature was maintained until 90 min.

Morphological changes in feather follicles after defeathering and chilling

Based on the mean area of feather follicles after defeathering and chilling, 4 types of morphological changes were observed: i) closed→open, ii) slightly open→enlarged, iii) open→smaller or closed, and iv) closed→closed. The area of all closed follicles was considered to be zero. The "slightly open→enlarged " type was defined as a feather follicle with an area of more than zero after defeathering, and becomming larger after chilling. The "open→smaller or closed" type was defined as a feather follicle with an area of more than zero after defeathering, and becoming smaller or closed after chilling. Shortly after defeathering, most (91.5%) of the follicles were closed, whereas after chilling they were either closed (85.5%) or open (6%), although a small proportion of enlarged follicles became smaller or closed (2.6%). Moreover, 5.9% of the follicles that were slightly open became further enlarged after chilling (Table 1 and Fig. 2).

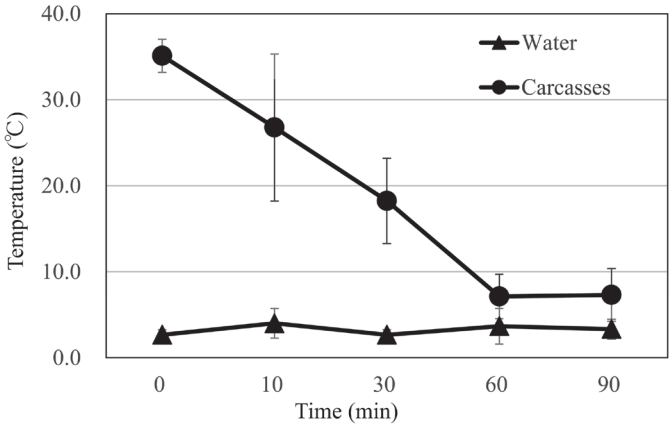

Fig. 1. Temperature kinetics (mean value ± SD) in the subcutaneous region of 3 carcasses after chilling.

Table 1. Morphological changes in feather follicles after defeathering and chilling

Skin region	No. of follicles examined	No. of morphological changes[a] (%)			
		Closed ↓ Open	Slightly open ↓ Enlarged	Open ↓ Smaller or Closed	Closed ↓ Closed
Dorsal neck	960	45 (4.7)	13 (1.4)	5 (0.5)	897 (93.4)
Abdominal	851	84 (9.9)	74 (8.7)	49 (5.7)	644 (75.7)
Thigh	684	28 (4.1)	12 (1.7)	8 (1.2)	636 (93.0)
Back	391	28 (7.2)	22 (5.6)	7 (1.8)	334 (85.4)
Crotch	247	2 (0.8)	63 (25.5)	14 (5.7)	168 (68.0)
Total	3,133	187 (6.0)	184 (5.9)	83 (2.6)	2,679 (85.5)

a) The area of all closed follicles is considered to be zero. The "slightly open →enlarged" type is defined as a feather follicle with an area of more than zero after defeathering, and becoming larger after chilling. The "open →smaller or closed" type is defined as a feather follicle with an area of more than zero after defeathering, and becoming smaller or closed after chilling.

Area of feather follicles after defeathering and chilling

Figure 3 shows the area of feather follicles after defeathering and chilling, omitting data for feather follicles that remained closed regardless of the processing steps. Compared to the mean area of feather follicles after each processing, the values after chilling were larger than those after defeathering for all of the skin regions examined.

Enumeration of Campylobacter spp. in different regions of carcass skin

The average counts of *Campylobacter* spp. from skin samples of the neck, abdominal region, back, and thigh were 0.79, 0.30, 0.78 and 0.30 log cfu/10 g, respectively (Fig. 4). The proportions of enlarged follicles that became closed in these skin areas after chilling were 0.52, 5.76, 1.79 and 1.17%, respectively. Although follicles in abdominal skin showed the highest proportion of closure (5.76%), the average count of *Campylobacter* spp. naturally contaminating the skin was the lowest in this region (0.30 log cfu/10 g).

DISCUSSION

As chicken carcasses are processed together with skin, the problem of microorganism control is well known [6]. Once organisms become attached to the skin surface, the effectiveness of disinfectant may be low, although data from various studies have been conflicting [11]. Moreover, the skin surface has many crevices or folds, and these areas may be difficult to decontaminate using disinfectants.

Berndtson *et al*. [1] demonstrated that *Campylobacter* spp. could be isolated from feather follicles by scraping samples of subcutaneous skin. They considered that feather follicles open after picking but then become closed when low-temperature chilling is performed, thus trapping campylobacter species within them. Since then, it has been considered that feather follicles play an important role in the bacterial contamination of chicken carcasses. However, Buhr *et al*. pointed out that as the samples examined were taken only after chilling, it was uncertain whether bacteria entered the follicles during defeathering or migrated into the skin while being chilled in water [3].

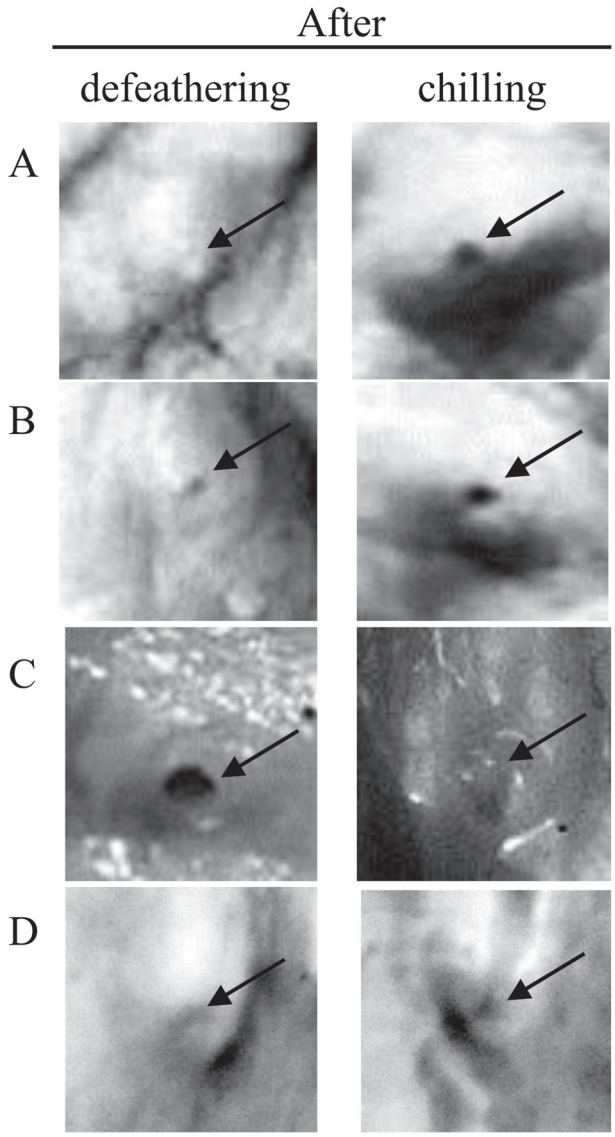

Fig. 2. Morphological changes in feather follicles examined after defeathering and chilling. Based on the mean area of feather follicles after defeathering and chilling, representative 4 types of morphological changes are photographed: (A) closed→open, (B) slightly open→enlarged, (C) open→smaller or closed, and (D) closed→closed. The area of all closed follicles is considered to be zero. The "slightly open→enlarged" type is defined as a feather follicle with an area of more than zero after defeathering, and becoming larger after chilling. The "open→smaller" type is defined as a feather follicle with an area of more than zero after defeathering, and becoming smaller or closed after chilling.

To clarify the role of feather follicles in bacterial contamination of chicken carcasses, Cason and colleagues performed a comparative study using genetically feathered and featherless broiler chickens [4]. They found no significant differences between the feathered and featherless broilers in terms of the numbers of aerobic bacteria, *Escherichia coli*, and *Campylobacter jejuni* in rinsed carcass samples immediately after defeathering, suggesting that feather follicles may make only a minor contribution to bacterial contamination.

In the present study, we observed morphological changes in feather follicles of chicken carcasses after scalding and chilling to determine if *Campylobacter* spp. were introduced into the enlarged follicles after picking and became entrapped in the closed follicles during chilling. As shown in Table 1, more than 90% of follicles were closed even immediately after defeathering. These closed follicles remained closed (85.5%) or opened again (6%) after chilling. In contrast, only a low proportion of enlarged follicles became smaller or closed (2.6%). These results suggested that most follicles became closed immediately after defeathering. Furthermore, the highest proportion of enlarged follicles that became smaller or closed after chilling was observed in the abdominal region, among the different areas of skin examined. However, the average *Campylobacter* spp. count was the lowest for the abdominal skin (Fig. 4). If the contamination mechanism proposed by Berndtson *et al*. occurred during processing, then the *Campylobacter* spp. count for the abdominal skin would have been higher than for other skin regions, suggesting that campylobacter species may not be confined to feather follicles as a result of the morphological changes occurring after defeathering

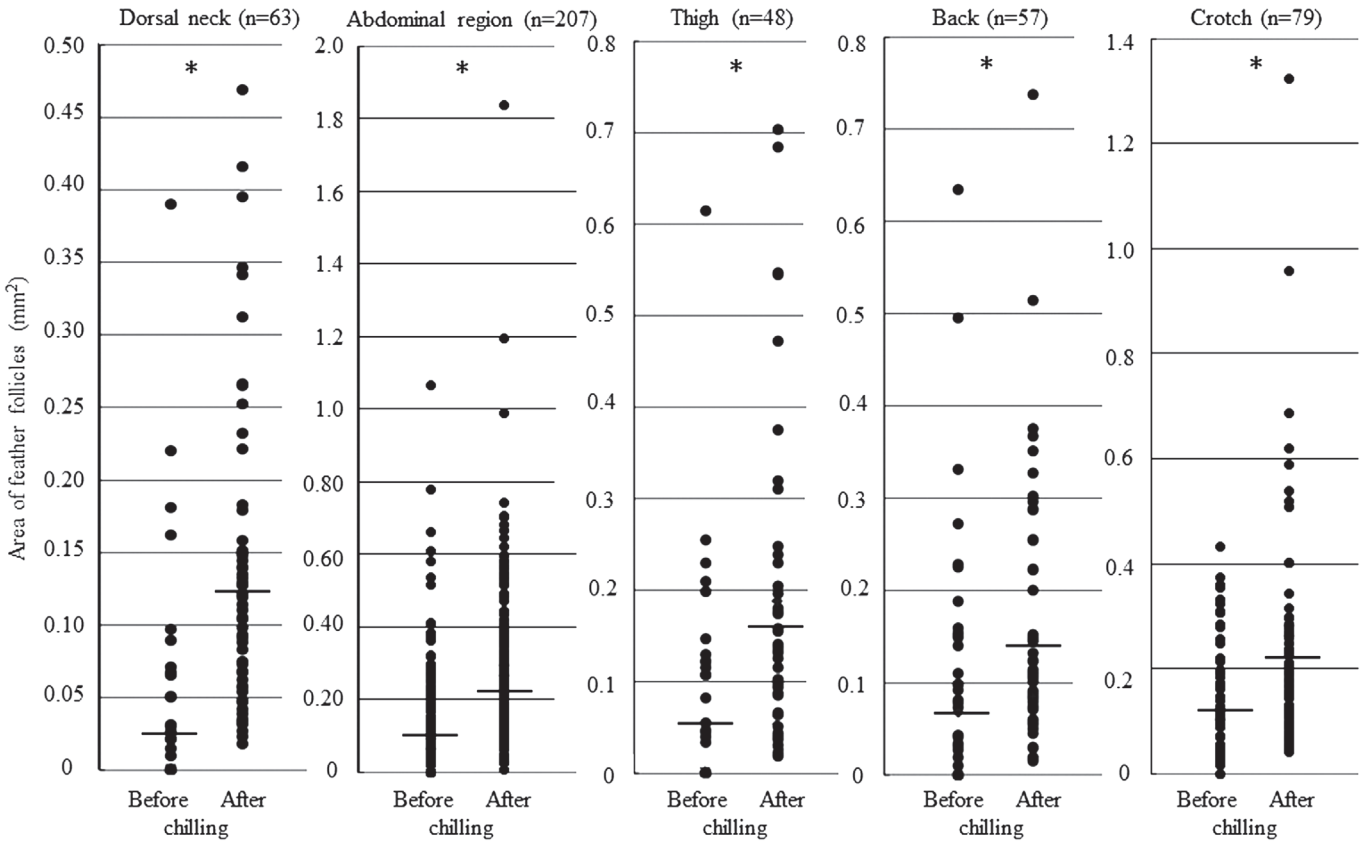

Fig. 3. Area of feather follicles after defeathering and after chilling. A total of 3,133 feather follicles of carcasses after defeathering and after chilling were photographed, and areas of the follicles were compared. The bar represents the average area of follicles examined. *P<0.01.

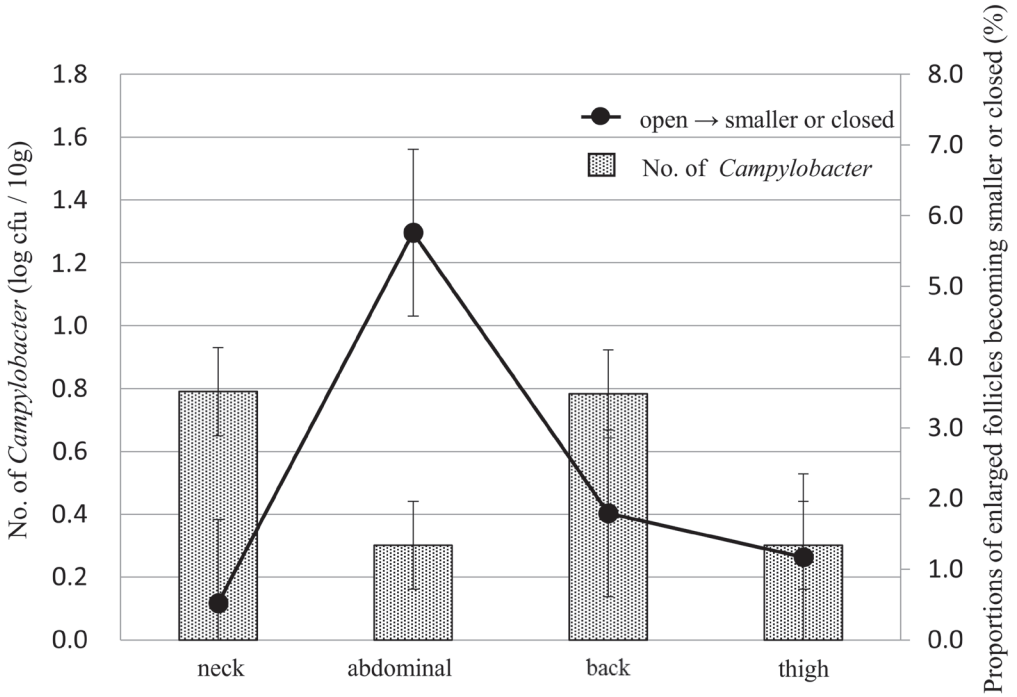

Fig. 4. Proportions of enlarged follicles that became closed and contamination by campylobacters in different skin areas of chicken carcasses after chilling. The number of contaminating campylobacter cells was determined by the MPN method. The number of *Campylobacter* represents the average from 3 chicken carcasses.

and chilling.

Chantaraponot *et al*. [5] examined the location of *C. jejuni* on chicken skin using green fluorescent protein (GFP)-labeled organisms. They reported that most viable cells were entrapped within feather follicles along with water, as well as in skin crevices. Since they inoculated a high number of GFP-labeled *C. jejuni* (10^8 to 10^9 cfu) on the outer surface of breast skin stored at −20°C before testing, their results may not have been representative of fresh skin naturally contaminated with *C. jejuni*.

Although the role of feather follicles in the contamination of chicken carcasses by campylobacters during processing is still debatable, further investigation is needed to determine whether the feather follicles of birds are able to open and close in response to physical factors including temperature, even in the postmortem period.

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