別紙様式第４

学　位　論　文　要　旨

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[論文題名]

Work-related increases in titer of *Campylobacter jejuni* antibody among workers at a chicken processing plant in Miyazaki prefecture, Japan, independent of individual ingestion of edible raw chicken meat（宮崎県内の食鳥処理場従事者におけるカンピロバクター・ジェジュニに対する抗体価は鶏肉の生食とは関係なく、処理場の作業内容に関連して上昇する）

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[要　旨]

Introduction: *Campylobacter jejuni* is one of the most important causes of bacterial foodborne illness in humans worldwide. Consumption of raw and/or undercooked chicken meat contaminated with this organism has been considered to be a main route of transmission to humans. *C. jejuni* colonizes the intestinal tract of livestock animals, especially chickens, with a high prevalence in the range of 10^3-10^9 cfu/g. Such high levels allow bacteria to spread easily in the environment of a chicken processing plant, resulting in contamination of chicken carcasses, equipment, and work surfaces. Therefore, workers in poultry abattoirs may be frequently exposed to *Campylobacter jejuni*. However, since such individuals rarely develop campylobacteriosis, some specific immune responses due to frequent exposure to low levels of *Campylobacter* may effectively protect them against disease manifestations. In this context, however, another parameter may be potentially relevant, i.e. the relationship between the immune response and the location in a processing plant where work is performed. Since a previous microbiological survey of *Campylobacter* contamination in chicken carcasses has demonstrated a significant difference in contamination levels among various steps of processing, we hypothesized that the levels of immune response among workers may differ according to location, due to the differing degrees of exposure to this pathogen. In the present study, we aimed to evaluate the levels of immunoglobulin G (IgG) and IgA against *C. jejuni* in serum samples from workers at a chicken processing plant who were occupationally exposed to *Campylobacter* spp. contamination on chicken carcasses throughout the year. A questionnaire, including items on age, consumption of raw chicken meat, work locations within the plant, length of employment, etc., was completed by each worker to evaluate the factors that influenced the detectable antibody responses. Furthermore, specific antigens of *C. jejuni* recognized in the workers' sera were also identified.

Material and Methods: A total of 65 broiler carcasses were obtained from a commercial chicken processing plant in Miyazaki prefecture, Japan, to examine the level of *Campylobacter* spp. contamination. The number of campylobacters was obtained by applying the common 3-tubes most probable number (MPN) procedure at each dilution. A total of 123 workers in the same chicken processing plant described above were invited to participate in this study. The questionnaire included the following items: gender, age in years (30, 30-50 or >50), length of employment (<1 year or ≥1 year), work locations in the plant (slaughtering, evisceration, cutting, deboning, trimming, packaging or shipping), habitual ingestion of raw and/or undercooked chicken meat or its product(s), amount in one meal (>100 g, 50-100 g or <50 g) and frequency (weekly to monthly or yearly), and food poisoning symptoms after eating raw and/or undercooked chicken meat. Of the 123 workers who consented to participate, 104 were female and 19 were male. To avoid any statistical bias, only serum samples from female workers were used for analysis. Acid extracts of four *C. jejuni* strains representing the genotypes most dominant in Miyazaki were used as antigens. Enzyme-linked immunosorbent assay (ELISA) was conducted to assess the IgG and IgA responses against *C. jejuni* and Western blotting to detect the specific protein(s) recognized by antibodies in the sera of workers. The protein identification using Nano-ESI-IT-TOFMS.

Results: *Campylobacter* was recovered from the back skin (65/65; 100%) and breast skin (64/65; 98.5%), with an average yield of 1.33 log MPN/10 g and 1.05 log MPN/10 g, respectively. The highest numbers of *Campylobacter* contaminating the carcasses were observed in August, being 2.62 log MPN/10g and 2.55 log MPN/10g for back and breast skin, respectively. The mean optical density (OD) values were 0.623 and 0.372 for IgG and IgA, respectively. The highest OD value for IgG was observed in workers located at the evisceration processing step, followed in order by packaging, deboning, trimming, and cutting. For IgA levels, the highest OD was observed in workers located at the evisceration step, followed in order by cutting, trimming, packaging, and deboning. Furthermore, the IgG and IgA levels in workers at the evisceration step were significantly higher than those at other locations in the plant. The groups with the highest and lowest ODs for IgG and IgA ELISA bound to AEs showed a range of 17-95 kDa and 40-95 kDa, respectively. Several common bands were recognized in sera for both the highest- and lowest-OD groups for IgG and IgA, while others appeared to be unique to a specific individual. A strong positive band with an apparent molecular mass of 43 and 60 kDa was detected for both IgG and IgA in the highest-OD serum samples. Similarly, two bands of 43 and 60 kDa were also recognized for both IgG and

IgA in the lowest-OD serum samples. The strongest spots shown by Western blotting by IgG and IgA antibodies showing the highest ODs in the ELISA were identified as flagellins: flagellin A (FlaA, 59.6 kDa) and flagellin B (FlaB, 59.7 kDa).

Discussion: In the present study, we demonstrated that consumption of raw or undercooked chicken meat did not affect the humoral levels of both IgG and IgA. Furthermore, none of the participants stated that they had suffered symptoms suggestive of enteritis after ingestion of such meat. One possible explanation is that all the participants may have acquired protective immunity conferring resistance to campylobacteriosis because of exposure to *C. jejuni* in the chicken processing plant. However, since eating raw and/or undercooked chicken meat is considered the most significant risk factor for *C. jejuni* infection, it is strongly recommended that chicken meat should only be eaten after thorough cooking. For the relationship between work location in the plant and individual levels of antibodies to *C. jejuni*, the results showed that individuals who worked at the evisceration step had levels of both IgG and IgA that were significantly higher than those of workers at the other processing steps. The evisceration process is known to have a high potential for campylobacter contamination due to rupture and/or leakage of intestinal contents containing the organism, Here, we demonstrated that after the evisceration process, 100% of carcasses were contaminated with *Campylobacter*, indicating that workers could be easily exposed with the bacterium during work. The results for Western blotting showed that two major antigens of 43 and 60 kDa were recognized by antibodies in sera of workers using the five samples showing the highest and lowest OD values. One protein (60kDa) among the positive spots was identified as flagellin using two-dimensional gel electrophoresis (2-DE) followed by Nano-LC-ESI-IT-TOFMS. Flagella of *C. jejuni* are considered to be one of the most important virulence factors that help the organism to invade into the intestinal cells of the host and have potential to elicit an immune response. However, spots within the range 40-43 kDa were not clearly detected by Western blotting after 2-DE, possibly due to the low concentration of each protein. Therefore, we were unable to identify these proteins. Our present findings suggest that titers of IgG and IgA against *C. jejuni* in processing plant workers are increased by exposure to *Campylobacter*, regardless of individual consumption of raw chicken meat, even though the antibody level varies according to work location in the plant. We have also demonstrated that a long-term exposure of workers to *C. jejuni* may elicit protective immunity against campylobacteriosis.