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The Effect of Consumption of Raw Chicken Meat on Humoral Immunity against *Campylobacter jejuni* in veterinarians and workers in a chicken processing plant

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INTRODUCTION

Campylobacter jejuni and *C. coli* are the leading cause of enteric infections in Japan and many other developed countries, and the public health burden of campylobacteriosis is increasing [1]. Although the epidemiological data in Japan is based on passive surveillance, approximately 2,000 to 3,000 cases per each year have been reported as a foodborne infection since 1982. Many risk factors for *Campylobacter* transmission have been identified. Handling and consumption of poultry meat are often causing of infection [2, 3]. Since Japanese have a food habitant to eat fresh raw "free-range" chicken meat and liver, the risk for infection with campylobacters may be high [4]. However, little is known about the relationship between consumption of raw chicken meat and humoral immunity against *C. jejuni* in humans. When people had been exposed to campylobacters contaminated in water or foods, it has been reported that their antibodies were rising [5]. This study was conducted by analyzing the antibody level against *C. jejuni* with questionnaires from 74 veterinarians who worked as a meat inspector and 181 workers from a chicken processing plant.

MATERIALS AND METHODS

Bacterial strains and antigen preparation. *C. jejuni* strain 81-176 was used as an antigen prepared by an acid extraction method as described elsewhere. In brief, bacteria were grown in Brucella agar in a microaerobic atmosphere at 37 °C for 48 h. Cells were harvested in 10 mM phosphate buffered saline (PBS, pH 7.2) and centrifuged at 8,000 x g for 10 min for three times and added acid glycine buffer (pH 2.2). Suspensions were stirred with a magnetic stirrer for 30 min and then centrifuged at 13,000 rpm for 10 min. The supernatant was neutralized by a buffer exchanged method. The oncentration of the soluble antigen was determined by Bradford protein assay and the antigens were stored at -20°C until use.

Collection of sera. Sera from 255 persons were collected from two groups, i.e., veterinarians and workers in a chicken processing plant in Miyazaki Prefecture, Japan who agreed with this investigation.

Questionnaires. Collaborators answered questionnaires based on parameters of risk factors as follows: (a) raw chicken meat intake (yes or no); (b) frequency of intake; (c) amount of intake; (d) intake duration; (e) gender (male or female); (f) age (young: 20-39 years old, adult: 40-70 years old); (g) pet owner.

ELISA procedures. Optimal conditions for ELISA assay were predetermined and the protocol was established before testing. Antigen was diluted in PBS and the protein concentration of 17.5 µg/ml was added into each well of a 96-well ELISA microplate. The volume of 75 µl was added to each well and incubated for 60 min at 37°C and then placed at 4°C for overnight. After washing the plate, PBS containing 1% of bovine serum albumin (BSA) was added into each well and incubated at 37°C for 2 h. The wells were then washed 5 times using PBS containing 0.05% Tween 20 (PBS-T). Each serum was diluted in PBS-T containing 1% BSA. Serum dilution was 1:200 for both IgG and IgA detections. The volume of 75 µl of diluted serum was added to each well and incubated at 37°C for 1 h. After washing the wells, peroxidase-conjugated goat anti-human IgG γ chain (1:5000) or goat anti-human IgA α chain (1:5000) in PBS-T containing 1% BSA was added and incubated at 37°C for 1 h. Following washing, the substrate *o*-phenylenediamine in a citric acid-sodium phosphate buffer (pH 5.0) was added to each well and incubated at 37°C for 15 min in the dark. The reaction was stopped by adding 75 µl of 2.5 M sulfuric acid and the absorbance was measured at 492 nm with an ELISA plate reader.

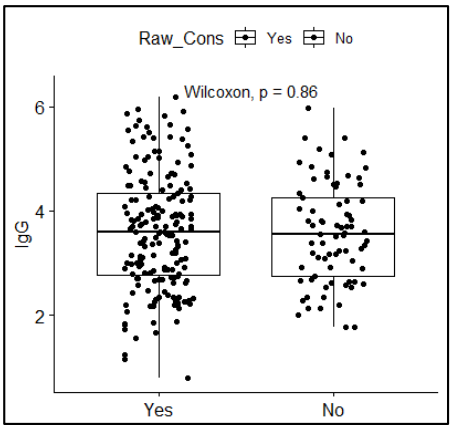
Statistical analysis: Each ELISA data was compared by a Wilcoxon test assessing differences between

respondents eating raw chicken meat (Yes) and those never eating (No) in each questionnaire. Statistical significance was defined as $P<0.05$.

RESULT AND DISCUSSION

A total of 255 of the 264 persons responded questionnaires. Compared the immunoglobulin class, the mean value of IgG was higher than IgA regardless of eating raw chicken meat (Fig. 1). However, there were no significant differences in both of IgG and IgA level between respondents eating raw chicken meat and those never eating (Fig.1).

(a)



(b)

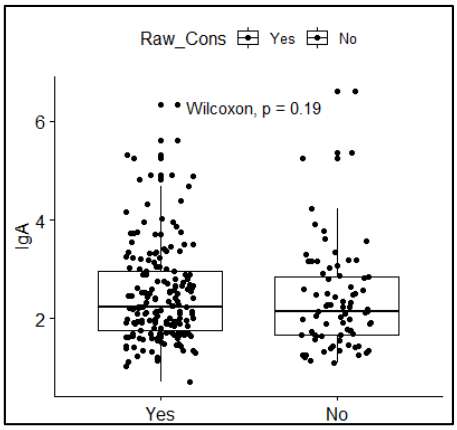
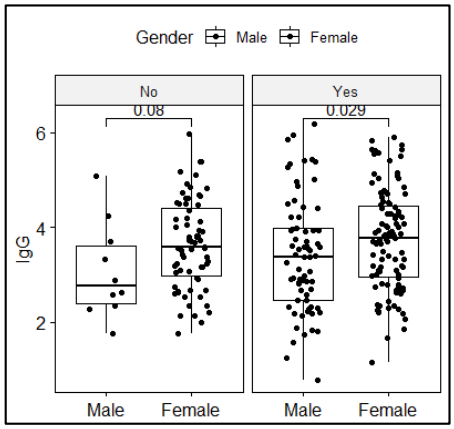


Fig. 1. Comparison of antibody level in IgG (a) and IgA (b) between respondents eating raw chicken meat (Yes) and those never eating (No)

When we focused on gender of persons eating raw chicken meat, IgG but not IgA level in female was significantly higher than that in male (Fig.2).

(a)



(b)

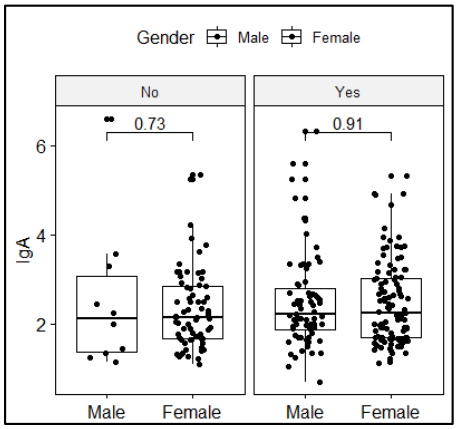
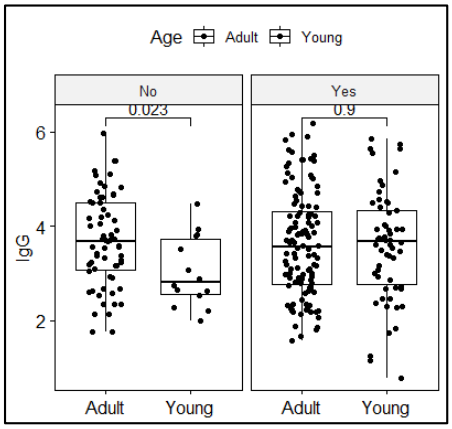


Fig. 2. Comparison of antibody level in IgG (a) and IgA (b) between male and female eating raw chicken meat (Yes) or not eating (No)

Next, when we focused on the age, the young respondents never eating raw chicken meat showed that IgG but not IgA level in young people was significantly lower than that in old people (Fig.3). However, there were no significant differences among other factors such as frequency and amount of intake, intake duration, and pet owner.

(a)



(b)

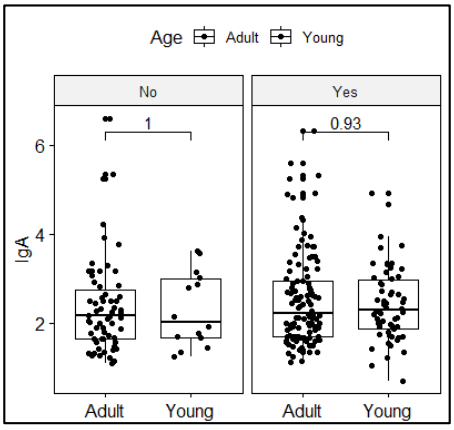


Fig. 3. Comparison of antibody level in IgG (a) and IgA (b) between young and old persons eating raw chicken meat (Yes) or not eating (No)

CONCLUSION

Although there were no significant differences in both of IgG and IgA level against *C. jejuni* between respondents eating raw chicken meat and those never eating, there was no confirmation that the people never eating raw chicken meat had not been exposed entirely by *C. jejuni* due to their job. However, IgG level against *C. jejuni* may be affected by some factors such as gender and age.

ACKNOWLEDGMENTS

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REFERENCES

[1] Ruiz-Palacios, G. M. 2007. The health burden of *Campylobacter* infection and the impact of antimicrobial resistance: playing chicken. *Cli. Infect.Dis.*, 44: 701-703

[2] Corry, J. E. and Atabay, H. I. 2001. Poultry as a source of *Campylobacter* and related organisms. *Symp. Ser. Soc. Appl. Microbiol.* 96S-114S

[3] Friedman, C. R., Neimann, J., Wegener, H. C. and Tauxe, R. V. 2000. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations, In: Nachamkin, I., Blaser, M.J. (Eds.), *Campylobacter* 2nd edition, ASM press, Washington D. C., pp. 121-138

[4] Latt, K. M., Urata, A., Shinki, T., Sasaki, S., Taniguchi, T., Misawa, N. 2018. Effect of morphological changes in feather follicles of chicken carcasses after defeathering and chilling on the degree of skin contamination by *Campylobacter* species. *J. Vet. Med. Sci.*, 80 (1): 49-54

[5] Monge, S., Teunis, P., Friesema, I., Franz, E., Ang, W., Pelt, W., Gras, L. M. 2018. Immune response-eliciting exposure to *Campylobacter* vastly exceeds the incidence of clinically overt campylobacteriosis but is associated with similar risk factors: A nationwide serosurvey in the Netherlands. *J. inf.* 1-7