（別紙）研究成果の概要（英文）

Title of research project	Research for quantitative risk analysis of *Campylobacter* food poisoning in Japan
Research project number	1806
Research period	FY 2018 – 2019
Name of principal research investigator (PI)	Hiroshi Asakura, D.V.M., Ph.D.

Abstract/Summary

FSCJ conducted self-tasking risk assessment on Campylobacter jejuni and Campylobacter coli in chicken on 2009. Given the risk assessment report proposed the development of quantitative risk assessment for reducing the bacterial contamination in chicken throughout food chain, this study aimed at accumulating scientific evidences for quantitative risk assement of Campylobacter contamination in chicken meat.

Throughout the study, we adopted universal method ISO 10272-2:2017 for quantitative detection of *C. jejuni* and *C. coli* from a variety of samples. For the microbial detection from human clinical specimens, we simultaneously used real-time PCR approach as considered variation of bacterial survival conditions dependent on the samples. Toward poultry cecal samples, we used 16S rRNA pyrosequencing methods to elucidate dynamics of bacterial community structures during the experimental terms. Fluorescence-based immunochlomatography was constructed to examine its sensitivity and specificity for the detection of *C. jejuni* and *C. coli*.

To monitor spatiotemporal dynamics of *C. jejuni* in poultry caeca, *C. jejuni* 81-176 was experimentally administrated into SPF chicken at 2weeks age, and fed for up to 10 months. The bacterial numbers continuously increased for up to 4-8 weeks p.i., whereas thereafter decreased. At 6, 8, 10 months p.i., only one out of five birds were positive for *C. jejuni*. In parallel to the altered bacterial colonization fitness, bacterial community structures were also altered between 0-8 weeks p.i. and 4-10 months p.i., suggesting the association between these bacterial dynamics.

We also conducted the quantitative detection of the thermophilic *Campylobacter* at poultry slaughterhouses. At one processing plant that produced chicken-sashimi located on south Kyushu area, laying hen at old age were used as materials. Important issue was that the surface of the poultry carcasses were heated prior to cutting, which process clearly decreased the numbers of *Campylobacter* spp. At other slaughterhouses, we could obtain the quantitative data showing that chilling process drastically decreased the bacterial contamination on the poultry carcasses.

We also examined the estimation of the burden of foodborne campylobacterisis in Japan as well as its source attribution. Fluorescence immunochlomatography-based detection

system was also developed and its sensitivity and specificity were examined, revealing approximately 10^3 cells of *C. jejuni* 81-176 could be detected by this approach although the system exhibited variation of detection sensitivity dependent on bacterial strains.

A total of 289 clinical fecal samples originated from patients who suffered from campylobacteriosis were subjected quantitative detection assays. The real-time PCR assays showed greater sensitivity than the culture-based methods, and the data of which also exhibited association with the medication histry of the sources. We calculated correction formula to predict bacterial numbers based on the qPCR approach.

Together, we expect that our data would contribute to the development of risk assessment of *Campylobacter* contamination in chicken meats, provided by the FSCJ.

１． List of papers published on the basis of this research

1) Asakura H, Sakata J, Nakamura H, Yamamoto S, Murakami S. Phylogenetic diversity and antimicrobial resistance of *Campylobacter coli* from humans and animals in Japan. Microbes Environ. 2019;34(2):146-154.
2) Kakiuchi R, Ai R, Horiuchi Y, Asakura H, Chuma T. The Effect of heating surface on the reduction of microorganism: Contamination through chicken-sashimi processing in a small-size poultry processing plant. Jpn. J. Food Microbiol. 2019;36(2):105-109.
3) Kakiuchi R, Ai R, Horiuchi Y, Asakura H, Chuma T. Campylobacter Contamination of Torisashi (Chicken-Sashimi) and Chicken Meat for Cooking Retailed in Kagoshima. Jpn. J. Food Microbiol. 2019;36(4):165-168.
4) Kumagai Y, Pires SM, Kubota K, Asakura H. Attributing human foodborne diseases to food sources and water in Japan using analysis of outbreak surveillance data. J. Food Prot. In press. DOI: 10.4315/JFP-20-151.

２． List of presentations based on this research

1) Kakiuchi R, Shimoji Y, Duc VM, Chuma T. Prevalence of Campylobacter spp. in chicken-sashimi and chicken meats for heat-processing. The 39th Annual meeting of Japan Society for Food Microbiology. Osaka, Sep 2018.
2) Shimoji Y, Ai R, Kakiuchi R, Duc VM, Obi T, Chuma T. Quantitative detection of *Campylobacter* spp. from the materials of chicken-sashimi products. The 161th Annual meeting of Japan Society for Veterinary Medical Sciences. Ibaraki, Sep 2018.
3) Asakura H, Morita Y, Chuma T, Nakamura H. Control of *Campylobacter* spp. in chicken meats and application of next generation sequencer for tracking the contamination. The 161st Annual meeting of Japan Society for Veterinary Medical Sciences.

Ibaraki, Sep 2018.
4) Kawase J, Asakura H. Detection of foodborne pathogens by real-time PCR: its application for estimating the bacterial numbers. The 71st Annual meeting of Japan Society for Bacteriology, Chugoku-Shikoku area. Ehime, Oct 2018.
5) Kubota K, Amanuma H, Tamura M, Tamai K, Shimojima M, Shibuya S, Sakurai Y, Komatsu M, Kasuga F. Estimating the burden of foodborne illness for *Campylobacter*, *Salmonella* and *Vibrio parahemolyticus* in Japan, 2006-2015. International Association for Food Protection (IAFP) Annual meeting (Salt Lake City, US). July 2018.
6) Sasaki T, Igimi S, Asakura H. Cross contamination of *Camyplobacter* spp. at poultry slaughterhouse. The 11th Annual meeting of Japan Society for *Campylobacter* Research. Tokushima, Dec 2018.
7) Asakura H, Yamamoto S, Nakayama T, Sasaki T, Okamura M, Chuma T. Fitness of chicken intestinal colonization and the associated genomic alteration of *Campylobacter jejuni* from wild birds. The 11th Annual meeting of Japan Society for Campylobacter Research. Tokushima, Dec 2018.
8) Asakura H, Sakata J, Akase S, Yamamoto S, Kawase J. Distribution and phylogenic diversity of *Campylobacter jejuni* HS:19 in Japan. The 33th Annual meeting of Japan Society for Microbial Ecology. Yamanashi, Sep 2019.
9) Asakura H. Testing methods for the detection of *Campylobacter jejuni* and *coli* from poultry carcass and meat. The 12th Annual meeting of Japan Society for *Campylobacter* Research. Kagoshima, Sep 2019.
10) Sasaki T, Asakura H. Dynamics of Campylobacter contamination on poultry carcass at slaughter. The 12th Annual meeting of Japan Society for *Campylobacter* Research. Kagoshima, Sep 2019.
11) Nakayama T, Yamamoto S, Asakura H, Murakami S, Torii Y. Spatiotemporal dynamics of *Campylobacter* in poultry by experimental infection. The 40th Annual meeting of Japan Society for Food Microbiology. Tokyo, Nov 2019.
12) Hatanaka T, Hinenoya A, Yamasaki S. Quantitative detection of *Campylobacter* spp. on the surface of poultry carcasses during slaughter. The 40th Annual meeting of Japan Society for Food Microbiology. Tokyo, Nov 2019.

３．The number and summary of patents and patent applications

None

４．Others　(awards, press releases, software and database construction)

None