

Abstract of Dissertation

Applicant

Doctoral Program in Animal and Food Hygiene
Graduate School of Animal Husbandry
Obihiro University of Agriculture and Veterinary Medicine
Student ID: 24329
Signature of Applicant: SHYAKA ANSELME

Title : Molecular epidemiology and comparative genomics of *Campylobacter jejuni* isolates from wild birds

(野鳥由来カンピロバクター属菌の病原性と分子疫学解析に関する研究)

Abstract:

Campylobacter spp. are the leading bacterial causes of gastroenteritis worldwide. Moreover, the disease caused by this bacterium, campylobacteriosis, can in some cases trigger deadly complications such as the Guillain-Barré syndrome and the reactive arthritis. Campylobacteriosis is mainly associated with consumption or contact with meat, poultry mainly, milk and water. However, sporadic cases are associated with other pathways including environmental sources and wild animals. For an effective control of human gastroenteritis caused by *Campylobacter*, it is crucial to evaluate the entire potential sources and their contributions to the *Campylobacter* spp. occurring in nature. Hence, *Campylobacter* prevalence in wild birds must be explored thoroughly, especially in countries with high human *C. jejuni* infection. In fact, wild birds come into contact with humans, livestock, pet animals and their environment including water. Since *Campylobacter* spp. have a broad range targets, it is believed that wild birds can act as a vehicle transmitting the bacterium from one host to another by the means of direct contact and environmental contamination by their droppings.

The aim of the current study was firstly to establish the prevalence of *Campylobacter* spp. in wild birds in Tokachi area, eastern Hokkaido, Japan and evaluate the virulence of obtained isolates. Secondly, to use the multilocus sequence typing and

determine the genotypes of *C. jejuni* from wild birds and establish the probable implication of wild birds in the transmission of campylobacteriosis in Japan using MLST method. Thirdly, to provide whole genomes of *C. jejuni* from wild birds and initiate a comparative genomics that evaluates virulence and unique features of these isolates.

Chapter one describes an investigation on the carriage of *Campylobacter* spp. in wild birds in Hokkaido, Japan and the virulence of the obtained isolates using *in vitro* tests. We have found that overall, 34 of 173 birds were positive, representing 19.7% of the total birds sampled. The species identification showed that most of the isolates were *C. jejuni* (32/34) but also, one isolate each, for *C. coli* and *C. fetus* was seen. This highlights a finding of public health importance as *C. jejuni* and *C. coli* are the main bacterial causes of gastroenteritis in humans. Moreover, *C. fetus* is of veterinary medicine importance, but may also, in particular conditions, infect humans. The virulence of isolates from wild birds using a human epithelial cell line, Caco-2, showed that isolates possess ability to invade and multiply inside cells *in vitro*, although the full expression of their virulence was hindered by unknown mechanisms. Supported by Real-time PCR results, we have found that 7 out of 32 tested isolates harbored all the virulence-related genes investigated. Lastly, the swarming motility on soft agar was reduced for all tested isolates, except for one isolate from crow. This shows that some of the *C. jejuni* introduced in the environment by wild birds may be fully virulent.

In chapter two, we used multilocus sequence typing, MLST, as an ideal epidemiological tool to infer similarity between isolates from our study and those from human, poultry and cattle in Japan. Our study found 19 sequence types, 13 of which are newly reported from the results of our study. In addition, some STs could be assigned to two known clonal complex; ST-179 and ST-952 complex, commonly reported by various studies in wild birds and environmental samples, implying host-specificity of isolates from wild birds. We have not found relationship between genotypes of isolates from wild birds and those from human, poultry and cattle in Japan, suggesting that wild birds are less likely involved in the transmission of campylobacteriosis in Japan. Yet, given that some STs and both clonal complex were found in other countries in host other than wild birds, we suggest that these isolates need a continuous monitoring as potential

source of *Campylobacter* infection.

The chapter three describes the sequencing of eight whole-genome sequences of *C. jejuni* from wild birds were generated using Next-generation sequencing technology. An endeavor was made to use generated genomes in the comparative genomics. We have provided first whole genomes of *C. jejuni* isolates from wild birds and deposited them at GenBank. Moreover, the obtained genomes were automatically annotated using RAST server and a comparative genomics done. The current study has shown that some virulence genes such as CDT operon, flagella-associated genes and iron-acquisition genes, have undergone important alterations. We have reported acquisition by some of the genomes from crow, of insertion elements similar to known *C. jejuni* prophages, *tetO* gene and a type-VI secretion system. Moreover, the isolates from pigeons harbor a filamentous hemagglutinin adhesin (FHA) that is reported to enhance cell binding. The observed plasticity of these genomes from wild birds may be related to evolution required for a safe colonization of wild birds.

In conclusion, our study showed a relatively high occurrence of *Campylobacter* spp. in resident wild bird in Tokachi area, Hokkaido, Japan. The species identified, showed that the campylobacters obtained were of public importance as *C. jejuni*, *C. coli* and *C. fetus* were found in sampled wild birds. Moreover, the results of the in vitro testes showed that some of the isolates of *C. jejuni* were fully virulent. No clear similarity was found between isolates of the current study and those previously reported in humans, poultry and cattle in Japan, using MLST method. Wild birds should however, be taken as a potential source of campylobacteriosis since some genotypes of our study was reported overseas in clinical outbreaks. However, additional research experiments should be carried out to isolate *Campylobacter* from human and livestock and to fully conclude on the potential hazard caused by the prevalence of this pathogen in wild birds. Lastly, we have sequenced and deposited whole genomes of *C. jejuni* and they will contribute to the understanding of the diversity of this pathogen.

This current study on the prevalence, molecular typing and genomic characterization of *C. jejuni* isolates from wild birds will serve as a benchmark for more researches to improve the understanding of the impacts and importance of the prevalence of *C. jejuni* in wild animals. Moreover, it will help provide valuable genomic

data on this pathogen, whose pathogenesis is still poorly understood.

- Notes
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