論 文 の 内 容 の 要 旨

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論文題目　Freshwater fish production and clonorchiasis in the Red River delta of Vietnam
（ベトナム紅河デルタの淡水魚生産と肝吸虫感染リスク）

Clonorchiasis is an infectious disease caused by the liver fluke *Clonorchis sinensis*, which often accompanies serious symptoms such as hepatic dysfunction and liver cancer. High prevalence of *C. sinensis* infection has been increasingly reported in many parts of Vietnam. *C. sinensis* uses freshwater snails of the genus *Bithynia* as its primary hosts and freshwater fish species as its secondary hosts, and human infection occurs when metacercariae in flesh or on skin of freshwater fish are ingested by human hosts. The disease transmission is influenced by various cultural and socioeconomic factors such as distribution of intermediate host snails, contamination of aquatic environment by human excreta, culturing methods of freshwater fish, a habit of raw fish consumption, and host-parasite relationship. It is thus important to develop an area-specific control approach by understanding the existing transmission cycle of *C. sinensis* in each local setting and identifying the point of intervention where the transmission cycle can be effectively cut off.

Since freshwater fish is the major transmission media of *C. sinensis* infection to human, expansion of freshwater aquaculture has been implicated as a potential cause of high clonorchiasis frequency in east and Southeast Asia. However, due to the lack of substantive information to confirm the association between the recent rapid development of freshwater aquaculture sector and the prevalence of *C. sinensis*, no control strategies have been established targeting at freshwater fish production. As a result, the national control program of foodborne trematode infections in Vietnam has only focused on mass drug treatment and health education.

The present study aimed at elucidating the association between the prevalence of *C. sinensis* infection and freshwater fish consumption/production in Vietnam, and thereby recommending a control strategy to produce hygienically safe freshwater fish while evading associated health risks rather than to change people' tradition and cultural behavior by external intervention. The study focused on the Red River delta of northern Vietnam where *C. sinensis* infection is reportedly the most prevalent in the country.

In **Chapter 2**, the association between the prevalence and the intensity of *C. sinensis* infection by age, gender and a habit of eating raw fish was assessed in the area of high-prevalence and low-prevalence of *C. sinensis* infection in northern Vietnam.

Stool examination and questionnaire survey were conducted in Kim Son district of Ninh Binh province where consumption of raw freshwater fish had been widely known (the first study) and Luong Son district of Hoa Binh province where such consumption had not been reported at the time of the survey (the second study), targeting at 1,155 and 155 villagers respectively.

In the first study, 648 out of 1,155 individuals (56%) reported the habit of eating raw fish and the prevalence of infection was 26% (301). In the second study, 35 out of 155 (23%) individuals reported the same habit and the infection prevalence was 1.3% (2). The extremely low number of positive cases made odds ratio analysis impossible in the second study. In the first study, however, the infection prevalence was 3-fold higher in males than in females, and the habit of eating raw fish increased the risk of *C. sinensis* infection by 53-fold. The strong association of raw fish consumption with the prevalence and the intensity of *C. sinensis* infection were thus confirmed in this chapter.

Freshwater fish is one of the most important protein sources for Vietnamese citizens, especially in non-coastal regions. Freshwater fish production in the Red River delta has

been steadily growing since 1990 in terms of both production and area. **Chapter 3** attempted to investigate the association between the production systems of freshwater fish and the prevalence of *C. sinensis* infection in the Red River delta, Vietnam. The types of freshwater aquaculture in the Red River delta were found to be 3-fold: small-scale aquaculture, large-scale aquaculture and rice-cum-fish culture. The most dominant in the delta was small-scale aquaculture, accounting for approximately half of the water surface used for freshwater aquaculture, followed by large-scale aquaculture. This study revealed a tendency that the larger the area of water surface used for aquaculture in provincial land was, the higher the reported provincial prevalence of *C. sinensis* infection was. The infection prevalence was particularly high where small-scale aquaculture was extensive. In large-scale aquaculture and rice-cum-fish culture systems, commercial pellet feed is commonly used. In the meantime, small-scale aquaculture was further sub-divided into a feed-based system and an fertilizer-based system, and in the latter case, animal manure and human excreta are reused to fertilize fish ponds in place of commercial feed. This led author to speculate that the direct use of animal manure and human excreta in fertilizer-based systems, which accounted for a substantial portion of small-scale aquaculture, caused harboring of various pathogens including *C. sinensis* in these fish ponds, infected the farmed fish in the ponds with *C. sinensis*, and resultantly led to the high prevalence of *C. sinensis* infection in the provinces where small-scale aquaculture was prevalent.

In **Chapter 4**, the prevalence of *C. sinensis* infections in relation to engagement in aquaculture and more specifically, the use of animal manure and/or human excreta in aquaculture were assessed in order to confirm the public health concern on this waste reuse in freshwater aquaculture. Stool examination and questionnaire survey were carried out in Kim Son district of Ninh Binh province where the first survey of Chapter 2 was performed and all the participants of the study who were found to be infected with *C. sinensis* received treatment with praziquantel. The re-infection rate of the study population was 9.2% (19 positive cases out of 206). While engagement in agriculture did not contribute to increased risk of *C. sinensis* infection, aquaculturists were found to show 2.9-fold higher infection risk than non-aquaculturists ($p<0.001$). Contrary to author's speculation that the use of animal manure and/or human excreta in aquaculture led to the high prevalence of *C. sinensis* in the provinces where small-scale aquaculture was prevalent, the use of neither of animal manure nor human excreta increased the

infection risk (OR 0.4, p=0.21 for animal manure; OR 0.2, p=0.06 for human excreta). Besides, composting of animal manure and/or human excreta did not reduce the infection risk (p=0.48 by Fisher's exact test for animal manure; p=0.08 by Fisher's exact test for human excreta). This indicated that farmed fish were equally parasitized by *C. sinensis* regardless of the use of animal manure or human excreta. It was suggested that there were other factors that contributed to parasitization of freshwater fish by *C. sinensis* in fish farming areas.

For freshwater fish to be parasitized by *C. sinensis*, excreta of *C. sinensis*-infected humans or animals need to enter the water body where the fish inhabits, and snail hosts also need to inhabit in the same water body. **Chapter 5** aimed at comparing the fecal contamination level of water environment of different aquaculture systems, namely excreta-based systems (using animal manure) and feed-based systems (using commercial feed), in the Red River delta of Vietnam. *Escherichia coli* (*E. coli*) was used as an indicator of fecal contamination. Concentrations of *E. coli* in fish pond water and feedwater (irrigation water) as well as those on the skin surface of the farmed fish (Nile Tilapia *Oreochromis niloticus*) were enumerated. *E. coli* counts on the skin surface of tilapia were strongly correlated with those in pond water (R^2=0.90, 9<0.05). The *E. coli* counts of both the fish pond water and on skin surface of the farmed fish were significantly higher in fertilizer-based systems than in feed-based systems. This is presumably due to the direct use of fresh animal manure in fertilizer-based systems. According to multiple regression analysis, the number of pigs kept per m^2 pond area was found to be an important determinant of the fecal contamination level not only of pond water but also of fish skin. The *E. coli* level of feedwater did not significantly differ between the two systems since both fertilizer-based and feed-based ponds co-existed in the study area and the same irrigation water was fed into the both systems. In the study area, not only the *E.coli* counts in the pond water of both systems but also those of the local irrigation canal exceeded the microbiological quality standards of wastewater-fed aquaculture recommended by WHO (1.0×10^3 *E.coli* per 100ml water sample) and that of water for fisheries designated by the Japanese government (1.0×10^3 *E.coli* per 100ml water sample). This implied that the fecal contamination of the canal water occurred due to the fact that both excreta-based and feed-based ponds co-existed in the area, from which pond water is equally discharged back to the canal two to three times a month.

Chapter 6 provided an overall discussion of the results presented in Chapters 2 to 5. On the basis of the findings, the potential transmission mechanism of *C. sinensis* in aquaculture-active areas was speculated as follows: firstly, the reuse of pig manure to fertilizer fish ponds in fertilizer-based systems contaminated both pond water and farmed fish in the system; secondly, discharge from the fertilizer-based systems into the local irrigation canal contaminated the irrigation water; thirdly, this irrigation water was fed into fish ponds of feed-based systems then contaminated both pond water and farmed fish in the system. Accordingly, it was conclude that inappropriate management of the fertilizer-based aquaculture systems was causing parasitization of farmed fish by *C. sinensis* in both systems.

Conventional control strategies of *C. sinensis* infection are comprised of the following two approaches: 1) drug treatment of patients with praziquantel for eliminating human host reservoir; and 2) health education for a promotion of cooked fish consumption for preventing infection. It is, however, widely acknowledged that mass treatment of patients combined with health education alone is not sufficient to eradicate the infection, as people's cultural behavior and food habits cannot be changed easily. Establishment of a clonorchiasis control strategy targeting at freshwater fish production is therefore expected.

The livestock-integrated aquaculture systems in Vietnam are known to be highly cost-effective, environmental-friendly as it effectively recycles agricultural wastes, and has contributed to poverty reduction of poor farmers in Vietnam. In view of the rapid growth of aquaculture sector in Vietnam, this chapter recommended strategic measures to produce hygienically-safe freshwater fish by reducing the health impacts associated with this system, such as pretreatment of feedwater before intake or that of pond water prior to discharge, and eradication of snail hosts in both aquaculture ponds and irrigation canals by use of molluscicide or introduction of their natural predators, and to ultimately promote it as a model for sustainable fish production.