APPLICATION OF HAZARD ANALYSIS CRITICAL CONTROL POINT (HACCP) AS A POSSIBLE CONTROL MEASURE FOR *OPISTHORCHIS VIVERRINI* INFECTION IN CULTURED CARP *(PUNTIUS GONIONOTUS)*

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Abstract. Opisthorchiasis due to *Opisthorchis viverini* and transmitted through infected freshwater cyprinoid fish (carps) affects more than 8 million people in Thailand, People's Democratic Republic of Lao, and Vietnam. The Hazard Analysis Critical Control Point (HACCP) -concept has been recommended by FAO and WHO to be included in programs to control foodborne trematode infections (FBT). HACCP is a multifactorial approach to control food hazards through surveillance of diseases, foods, and operations and education. This study describes the first attempt to apply HACCP to the prevention and control of *Opisthorchis viverrini* in pond culture carp *(Puntius gonionotus)*.

The experiment was designed and carried out by a multidisciplinary "HACCP team" including experts in the field of pulic health, parasitology, epidemiology, aquaculture, fisheries extension and fish inspection. The investigation was performed in two fish ponds in the District of Sun Pa Tong, Chiang Mai, Thailand. In the experimental pond, fish was cultured according to HACCP principles and compared with the control pond, which followed conventional aquaculture practices.

Water supply to the pond, fish fry, fish feed and pond conditions during the growing period were identified as critical control points (CCPs). Hazards were identified and analyzed, as well as control measures, critical limits, monitoring procedures, corrective actions, and record keeping developed for each one of the above CCPs. Complete pond preparation, particularly aiming to eliminate contamination of pond water with *O. viverrini* eggs, fish infected with parasite meacercariae and the first intermediate host (*Bithynia* spp), was conducted. After the pond was filled with water, *O. viverrini* metacercaria-free fry were released into the pond.

The preliminary results obtained indicate that HACCP-based principles applied to carp pond culture could be used as a strategy to prevent and control *O. viverrini* . Further studies should be undertaken aiming to confirm these preliminary results.

INTRODUCTION

Thailand is the most highly endemic country for opisthorchiasis due to *Opisthorchis viverrini* (WHO, 1995). According to the epidemiological survey carried out in 1991 by the Department of Communicable Diseases, Ministry of Public Health, Thailand, the overall prevalence of opisthorchiasis in the country was 15.2%.It was found that the prevalence was 24.0% in the northeast, 22.8% in the north, 7.3% in the center and only 0.3% in the south. According to the rates among different subpopulation groups and geographical areas, it is currently estimated that the approximate infected population throughout the country would be up to 8.6 million (Jongsuksuntigul and Imsomboon, 1993). In Chiang Mai area, in the northern part of Thailand, the disease has shown a high prevalence rate of 26.78% (Khamboonruang, 1993). Other studies carried in four districts (Sankampang, Sanpatong, Hang Dong and Mae Wong) in the Chiang Mai Area have reported a prevalence as high as 54% (Kaewvichit *et al*, 1993). The northerners are believed to acquire the infection from eating raw-fish dishes call "lahb-pla" and "pla-som" prepared from wild caught fish (WHO, 1995).

Since 1988, a national opisthorchiasis control program has been organized. The approaches used in the large pilot project carried out in seven provinces in the northeast Thailand included health education, introduction of community participation by employing health care approaches aimed at community self-reliance, annual selective treatment, and improvement of sanitation and nutritional status. The program has been implemented in all provinces in the northeast and is currently being extended to the northern provinces of Thailand. The program was centrally planned, with the participation of the provincial chief medical officers, local health authorities and individual members of the community (WHO, 1995).

Food control measures are particularly important in the struggle to control opisthorchiasis since the disease is definitely related to fish and fishery products being eaten raw or undercooked. In fact, despite the positive overall results of the National Opisthorchiasis Control Program which claimed that frequent consumption of uncooked fish dishes in northeast Thailand has decreased from 14% in 1990 to 7% in 1994, occasional consumption of raw fish dishes in the same region remains as high as 42% (Jongsuksuntigul and Imsomboon, 1995).

The prevention of fish becoming infected with *O. viverrini* depends on environmental control of surface waters where fish is caught or harvested (cultured fish) and the control of the first intermediate hosts (snails). There are many potential difficulties in the implementation of these measures. For example, monitoring and control of large bodies of surface waters (rivers, lakes, reservoirs) may be impracticable in Thailand. However, the improvement of aquaculture practices, widely spread in Thailand to exclude *O. viverrini*, may be possible with the cooperation and participation of the people involved. Prevention and control of opisthorchiasis could be reinforced by food safety procedures based on the principles of the Hazard Analysis Critical Control Point (HACCP)-system, which offers a systematic and sequential approach to the control of food hazards, including food (fish)-borne trematodes. This involves a complete new strategy of food-borne trematode control based on the comprehensive coverage of the origin of the fish up to its consumption. The proposed strategy is based on the assumption that the fish farmer must design and apply a HACCP plan for the production and marketing of fish safe from *O. viverrini* infection. The farmer would be assisted by local public health officers, fisheries officers and fish inspection officers. The whole process should involve the local community and reach village and retail markets, processing plants, restaurants, housewives and have the support of a well-coordinated health education and public information program (Lima dos Santos, 1995).

The National Opisthorchiasis Control Program in Thailand has sufficient data to convincingly demonstrate the public health importance of this food (fish)-borne disease, and to justify the priority for the intervention of other government institutions. However, control of opisthorchiasis and other fish-borne parasitic zoonoses has not been given priority by the areas of aquaculture and fish inspection and quality control of the Department of Fisheries of the Ministry of Agriculture. This Department plays a key role in the development of aquaculture and in the control of safety and quality of fishery products exported by the country. The safety of fishery products sold within the national market is the responsibility of the Ministry of Public Health. However, in regions of high prevalence of opisthorchiasis such as the north and northeast provinces of the country, the involvement of aquaculturists and fish inspectors, side by side with medical/public health officers is crucial. Furthermore, the increased production of cultured fish and shellfish has opened new domestic and international markets. International travels and increasing availability and interest in ethnic foods also contribute to spread the risk of infection with food-borne trematode infections (Dixon and Flohr, 1995). The potential economic losses that would result from banning the export of fish products from endemic areas need to be taken into account in economic planning and in deciding what priority should be given to the control of fish-borne trematode infections, in particular opisthorchiasis, by the Department of Fisheries.

The objective of the study was to assess the possibilities of successfully apply HACCP principles to prevent and control the infection of cultured fish with *O. viverrini* metacercariae. The work was planned and carried out by a multidisciplinary group of experts from different government institutions in Thailand in close collaboration with the Food and Agriculture Organization of the United Nations (FAO).

MATERIALS AND METHODS

HACCP

The HACCP system is based on the recognition that hazards to health exist at various points in the food chain (in the case of fish from catching or harvesting to the consumer), but that measures can be taken to control these hazards. Once the system is established, the main control efforts are directed towards the critical control points. The HACCP system comprises the following sequential steps:

1. Identify hazards and assess the severity and risk.
2. Determine critical control points (CCPs).
3. Establish critical control limits and tolerances at each CCP.
4. Monitor CCPs.
5. Take corrective action when monitoring indicates that criteria are not met.
6. Recording and record keeping.
7. Verify that the system is functioning as planned.

In the case of fish, the application of the HACCP concept to the control of opisthorchiasis would start with the assessment of the risk that certain species of fish (particularly those members of the Cyprinidae family, *ie* carps) caught or harvested in endemic areas are infected. A risk will exist if fish from endemic areas are consumed raw or inadequately processed. The HACCP concept is unique for every product and for every production unit: in each case a detailed study of the food producing/processing/marketing/ preparation flow is necessary to identify hazards and the critical control points.

Designing and implementation of HACCP plan

The formulation of the specific HACCP plan for the present study was based on the CODEX Guidelines for the Application of the Hazard Analysis Critical Control Point System (Codex Alimentarius Commission, 1993). The work was carried out by a multidisciplinary "HACCP team" including experts in public health, parasitology, epidemiology, aquaculture, fish biology, fisheries extension, and fish inspection and HACCP-based quality assurance programs.

The initial work included the selection of two appropriate fish ponds, one experimental pond and one control pond, to conduct the experiment. Members of the HACCP team have visited a number of fish ponds located within Chiang Mai districts recognized as endemic for *O. viverrini* and selected two fish ponds of the San Pa Tong Experimental Rice Farm of the Ministry of Agriculture and Cooperatives located in Sun Pa Tong district, Chaing Mai. The final selection was made taking into account of the following factors:

- confirmation of local endemic human opisthorchiasis;
- traditional fish farming practices in place;
- better possibility of efficient application of HACCP strategy; and
- proximity with RIHES laboratory facilities.

To confirm the prevalence of opisthorchiasis in the area where the selected ponds were located, feces were collected from all villagers and examined for helminthic eggs using the formalin-ether concentration technic (Richie, 1948).

The HACCP team decided to use a small carp called "Pla Ta Pien Kao" (*Puntius gonionotus*) in the experiment due to its reported incrimination as second intermediate hosts of *O. viverrini* (SEAMEO-TROPMED, 1988; WHO, 1995), as well as its common use as food fish in Chiang Mai area from local fish farms.

The experimental pond dimensions were 250 x 10m while the control pond was smaller, measuring only 40 x 4m. The ponds had a muddy bottom and had not been used for fish culturing for a considerable period of time prior to the experiment.

Four CCPs were identified in the experimental pond by the HACCP team, namely: water supply, fish fry, fish feed, pond conditions during growing.

For each one of the above CCPs the following hazards were identified and appropriate control measures established:

a) Water quality: Three significant hazards were identified by the HACCP team at this CCP, namely: (1) presence of *O. viverrini* eggs as a result of human and/or animal fecal contamination; (2) presence of fish infected with *O. viverrini* metacercariae; and, presence of infected or non-infected first hosts snails (*Bithynia* spp) with *O. viverrini* sporocysts. Control measures were taken against each of the above hazards. The first step consisted in draining the existing water from the experimental pond, which could not be completely done due to the prevailing raining season. Due to a moderately dense population of snails (*Bithynia* spp), a combination of molluscicides, including niclosamide (Bayluscide), copper sulfate and calcium oxide was sprayed at the pond bottom (base) and banks. Since the experiment was initiated during the raining season, the pond spontaneously refilled with permeated water from the surrounding environment. Three days after the treatment, approximately half the volume of the water was evacuated by a water pump regularly used by the farmers. The water level again was allowed to refill with permeated water.

Dried animal manure was added to the ponds twice, aiming at fertilizing the pond. Random samples of the dried manure were collected and analyzed for the presence of eggs of *O. viverrini* according to the formalin-ether concentration technic (Beaver, 1984).

b) Fish fry: The hazard of releasing fish fry infected with *O. viverrini* metacercariae in the pond was controlled by assuring that the fish fry supply was free from infection. Fry were randomly collected from the breeding place and inspected for metacercariae by the compression technic recommended by WHO (1995) for freshwater fish hosts of fishborne trematodes.

c) Fish feed: In the case of the fish feed, the presence of *O. viverrini* eggs as a result of human or animal fecal contamination was identified as a hazard. The feed consisted of "Charern Phokaphan" (CP) pellets which were packed in plastic bags and kept within the farmer's house. Fish was fed daily at 04:30 pm. Random feed samples were taken and analyzed for the presence of *O. viverrini* eggs by simple sedimentation technic.

d) Pond condition during culturing period: The HACCP team identified the contamination of pond water with *O. viverrini* eggs from human and/or animal sources, as well as the invasion of the experimental pond by infected snails as significant hazards at this CCP. Feces were collected from the farmer and its family members (3 persons in total) and examined for helminthic eggs using the formalin-ether concentration technic. The farmer's family had no pets (dogs, cats), therefore, it was not necessary to apply a similar control procedure. However, since loose dogs were present in the area, fencing was constructed around the experimental pond to prevent intrusion of animal reservoir hosts and, if flooding ever occurred, it would help to prevent contamination of pond water with possibly infected wild fish. Aquatic vegetation overgrowth on the experimental pond banks were constantly removed since leaves can serve as the best breeding place for intermediate snail hosts.

The farmer's house had a proper latrine and a second proper latrine was available close by the farmer's house for the use of personnel harvesting rice fields nearby.

Monitoring procedures

In the specific conditions of this study, since the water supply and the fish fry were added to the ponds only once, *ie at* the beginning of the experiment, monitoring procedures of established control criteria were limited to the third and fourth CCP.

Some parameters of water quality were monthly recorded in the experimental pond, aiming at controlling pond environment. The measurements included temperature of air and water, oxygen, pH, turbidity and conductivity. These measurements were done using a Water Quality Checker TOA-WQC-20A.

The criteria (critical limit) established for the control of presence of *O. viverrini* eggs in the water (absence of viable eggs) were monitored monthly by the modified WHO recommended method for the determination of trematode eggs in waste water used in agriculture and aquaculture (WHO, 1989). The possibility of fecal contamination from human and/or animal sources was also checked monthly through bacteriological analyzes (determination of coliform and fecal coliform bacteria) and biochemical oxygen demand (BOD) measurements according to Greenberg *et al* (1992).

The monitoring of the presence of infected and/or non-infected *Bithynia* spp snails in the ponds during the fish culturing period was performed by monthly collection of snails in the ponds, morphological selection of *Bithynia* spp specimens and examining the snails for the presence of *O. viverrini* sporocysts, according to Malek (1962).

The monitoring of the presence of metacercariae of *O. viverrini* in the fish during the culturing period was carried out by monthly sampling and inspection according to WHO recommended procedures for the determination of metacercariae in freshwater fish (WHO, 1995). Fish weight and length in both ponds were also recorded monthly.

RESULTS

Table 1 shows the results of the stool examination survey of the villagers living near the ponds carried out before the experiment. It shows that 13.9% of the villagers were infected with *O. viverrini*, confirming the endemic character of the parasite in the selected area.

Table 2 indicates the monthly records of water quality parameters relevant to proper fish culture.

Table 3 gives the results of the monthly water analysis carried out to monitor the presence of *O. viverrini* eggs. The results were all negative.

Table 4 also relates to the monitoring of water quality for the possible fecal contamination from human and/or animal origin: it gives the monthly results of microbiological analysis for the determination of coliforms and fecal coliforms in the water, as well as BOD monthly records. The results are all lower than the normal values for surface water according to National Requirements (Ministry of Health, 1989) and the WHO (1989) recommended coliform concentrations of less than 1000 per 100 ml for wastewater used for agriculture and aquaculture.

Table 5 gives the monthly results of the monitoring inspection conducted to determine the presence of infected and/or non-infected *Bithynia* spp snails in both pond boundaries. Results indicate that the snails were able to survive the molluscicide initial treatment and/or to invade the ponds. However, no inspected snail showed the presence of *O. viverrini* sporocysts.

Table 6 shows the negative results with the monthly sampling and inspection of fish to monitor the presence of *O. viverrini* metacercariae.

Table 7 indicates, instead, the increasing infection of fish with *Haplorchis* spp. metacercariae observed during the monthly fish sampling and analysis.

Table 8 indicates the monthly records taken of fish length and weight at both ponds.

Table 1

Stool examination of the villagers living near the ponds.

Parasite	Number positive (%)			
	Male	Female	Children	Total
Opisthorchis spp	18 (20.5)	3 (6.5)	-	21 (13.9)
S. stercoralis	20 (25.0)	3 (6.5)	-	23 ((15.2)
Teania spp	1 (1.1)	-,		- 1 (0.7)
Hookworm	1 (1.1)	-	-	1 (0.7)
G. lamblia	1 (1.1)	-	-	1 (0.7)
Negative	50 (56.8)	41 (89.1)	17 (100)	108 (71.5)

Table 2

Measurements of water quality in the experimental pond.

Collection time (Month after fry releasing)	Temperature		O_2	pH	Turbidity	Conductivity
	Air	Water				
0	27.1	27.7	5	7.4	170	0.3
1	26.5	27.8	4.8	7.2	177	0.3
2	25.8	28.8	3	6.4	248	0.2
3	22.7	23.7	4.1	7	230	0.2

Table 3

Examination of pond water for the presence of *O.viverrini* eggs.

Collection time (Month after fry releasing)	Control pond	Experimental pond
6 days	Negative	Negative
1	Negative	Negative
2	Negative	Negative
3	Negative	Negative

Table 4

Determination of pond water quality.

Collection time (month after fry releasing)	Coliform MPN/100 ml		Fecal coliform MPN/100 ml		BOD mg/l	
	Control pond	Experimental pond	Control pond	Experimental pond	Control pond	Experimental pond
Before fry releasing	> 240	> 240	240	96	5.3	6.3
1	> 240	> 240	240	124	7	> 7
2	94	172	15	20	3.3	4.7

Normal value for surface water
Coliform bacilli (MPN/100 ml) 5,000
Fecal coliform (MPN/100 ml) 1,000
BOD (mg/l) 1.5
From Laws and Standards on Pollution Control in Thailand, 2nd ed, 1989.

Table 5

Examination of *Bithynia* intermediate host snails for the presence of *O.viverrini* sporocysts.

Collection time (Month after fry releasing)	Control pond	Experimental pond	Pond boundary	
			Control	Experimental
-7 days	Negative (N = 57)	Negative * (N = 59)	Negative (N = 8)	Negative (N = 51)
1	Negative (N = 82)	Negative (N = 42)	Negative (N = 120)	Negative (N = 200)
2	Negative (N = 48)	Negative (N = 25)	Negative (N = 76)	Negative (N = 62)
3	Negative (N = 7)	Negative (N = 6)	Negative (N = 182)	Negative (N = 26)

Table 6

Examination of cultured fish for *O.viverrini* metacercariae.

Collection time (Month after fry releasing)	Control pond	Experimental pond
1	Negative (N = 48)	Negative (N = 50)
2	Negative (N = 50)	Negative (N = 50)
3	Negative (N = 30)	Negative (N = 30)

Table 7

Haplorchis spp metacercariae found in cultured fish.

Collection time (Month after fry releasing)	Control pond	Experimental pond
1	25% (N = 12/48)	12% (N = 6/50)
2	28% (N = 14/50)	10% (N = 5/50)
3	60% (N = 18/30)	17% (N = 6/30)

Table 8

Comparison of the size of fish in both ponds.

Collection time (Month after fry releasing)	Control pond		Experimental pond	
	Length	Weight	Length	Weight
0	3.74	1.57	3.74	1.57
1	8.3	7.74	12.66	36.65
2	12.25	28.78	15.86	66.18
3	13.34	31.01	18.3	87.4

DISCUSSION

The experiment is the first attempt to apply the HACCP-concept for the prevention and control of fish-borne trematodes. It is also the first collaborative investigation in the field involving the participation of a multi-disciplinary group of experts and institutions outside the public health sector. The exercise is a follow-up of specific recommendations made by the WHO Study Group on the Control of Foodborne Trematode Infections, held in Manila, Philippines, October 1993.

Results presented in the paper are not final, since the experiment is still in evolution. However, they confirm the positive contribution that the application of the HACCP concept can make for the control of opisthorchiasis and other fishborne trematode infections. If this approach is properly applied, no other system or method can provide such a degree of food safety. Its great advantage is that it is a systematic, multidisciplinary strategy that is both adaptable and cost-effective. It provides a comprehensive coverage of the fish-chain, from the origin of the fish (farm, river, lake, fish-pond) to consumption, and requires producers (fishermen, fish-farmers, fish processing plant owners) and traders to accept greater responsibility for food fish through their integration into a national plan of action (WHO,1995).

The prevailing satisfactory sanitary conditions in the farm where both ponds were located have substantially contributed to the positive introduction of HACCP principles, confirming that through appropriate aquaculture practices, contamination of the cultured fish and the risk of these infections can efficiently be reduced or eliminated. However, due to the originality of the study and the inexperience of those involved with the practical application of HACCP to aquaculture, the investigations have required considerable improvization and adaptation to the specific conditions faced in the selected ponds.

At each CCP identified by the HACCP team it was possible to successfully apply control measures to the hazards identified by the team with two exceptions: snail control and reservoir animal control. In fact, despite the control measures applied in the experimental pond, the snails were back one month after the chemical and physical control measures. However, no infected snail was found (when trematode eggs are not present in the ponds, the presence of the first intermediate host does not constitute a hazard "per se"). In the case of reservoir, a regular visual inspection monitoring visit showed the existence of holes in the experimental pond probably made by large size wild rats. These rats may not only contaminate the pond with parasite eggs but also facilitate the penetration of infected snails in the pond. The corrective measure taken included the physical obstruction of the rat holes, the use of chemical rat poison, rat-traps and hunting.

The investigation is scheduled to proceed until the moment when the pond-fish is considered ready for harvesting and marketing. Therefore, a number of monitoring procedures will be performed to assure that all criteria (critical limits) are under proper control. A parallel research will be carried out to confirm the susceptibility of the raised crap *(Puntius gonionotus)* as an intermediate host for *O. viverrini*.

The infection of fish with *Haplorchis* spp metacercariae was an unexpected result but it demonstrates the value of the application of HACCP for the control of food safety hazards in aquaculture:

1. The HACCP plan was specifically designed and implemented aiming to produce fish free from *O. viverrini* metacercariae. No attempt was originally made to control the fish infection with other fishborne trematode.

2. The finding of fish infected with *Haplorchis* spp metacercariae through the application of HACCP principles has led to the identification of a new fish-borne safety hazard in Chiang Mai area which must be further investigated. Efforts should be made to determine the first and second intermediate hosts in order to clarify the evolutive cycle of the parasite and to prevent human infection.

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