



Worm-free Cooking, Fish Safety: Recommendation to Strategy for Opisthorchiasis Prevention Program

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2020/v41i2230413

Editor(s):

(1) Dr. Wei Wang, Jiangsu Institute of Parasitic Diseases, China.

Reviewers:

(1) Andreia Juliana Rodrigues Caldeira, State University of Goiás, Brasil.

(2) Carlos Ramón Bautista Garfias, Cenid-Sai-Inifap, Mexico.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/64430>

Original Research Article

Received 25 October 2020

Accepted 30 December 2020

Published 31 December 2020

ABSTRACT

Fish-borne parasitic zoonosis such as Opisthorchiasis caused by *Opisthorchis viverrini* remains a major public health problem in many parts of Southeast Asia and Me Kong Basin region including Thailand. The focal point of Opisthorchiasis is located in north-eastern part of Thailand, along with high prevalence coincidence of cholangiocarcinoma, a major primary carcinoma of the liver with a very poor prognosis. *Opisthorchis viverrini* infection caused by developed cholangiocarcinoma (CCA). *O. viverrini* infection is acquired by eating raw or partially cooked fish. In endemic areas, several food preparations contain uncooked or raw freshwater fish. The most common local freshwater fish used for this recipe are Koi Pla, Pla Som, Lab Pla and Pla Yang or Grill fish. Raw fish dishes are known to be associated with the risk of liver fluke infection due to the consumption of cyprinoid fish that contains metacercaria of *O. viverrini*. Grill fish and Lab-Pla are among the famous Thai traditional food in the northeastern and northern part of Thailand. The consumption of raw Grill fish can lead to *O. viverrini* infection because cyprinoid fish is a second intermediate host of *O. viverrini*. This study investigated the literature of previous working for prevention and control of Opisthorchiasis and confirm worm-free cooking safety of cooking with consideration of time for

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the preparation of freshwater fish-grill for the prevention and control of Opisthorchiasis and Cholangiocarcinoma infection. The unique experiment experimental designed. The freshwater fishes grill within the group of 0,1,2,3,4 and 5 minutes with 10 fresh water fishes from fisherman in Nakhon Phanom and Sakhon Nakorn provinces which reported high *O. viverrini* infection among risk people in 2009. The another experiment using frozen freshwater fish at -10 degree for 5 days. The results showed that metacercariae remained active in control and 1-2 minutes experimental groups. The groups 3-5 minutes of grill fish partially cooked showed inactive metacercariae. The excretory bladder spread with unclear form. The conclusion suggested that worm-free cooking under review, and unique experiment of cook safety is a crucial basic knowledge leading to bringing knowledge, practically for the prevention and control Liver fluke infection. Recommendation information also let people better understand the concerns during health literacy program to stop transmission of liver fluke and need to be discussed among head villagers, public health agencies and teachers in the public participation process and school health program.

Keywords: *Opisthorchis viverrini* infection; cholangiocarcinoma (CCA); metacercaria of *O. viverrini*; effect of the cooking period in minutes in fish.

1. INTRODUCTION

In the Era 2020, science and technology played a crucial role in the operation of Health Sciences and Medical Science that encompass issues from operation to strategy. Information sharing of data on disease outbreaks among local, governments and international health organizations still require and push forward to strategize. In the 21st century, under the term of New Normal requires systems and approaches to redesign care practices and consider integrating local, regional, national, and global health information networks. The costs for disease prevention, treatment and eradication have so far frustrated the limited budgets of global public health systems. The WHO 1995 has estimate about the number of fish-borne trematode infection over 18 million, including people from developed countries, is more than half a billions. This phenomenal alert for public health significant of those diseases associated with poverty, traditional and culture, agriculture and environmental factors and control strategy [1],[2],[3] Fish-borne parasitic zoonosis caused by *Opisthorchis viverrini* remains a major public health problem in many parts of Southeast Asia including Thailand, Lao PDR, Vietnam and Cambodia. [1],[3] [4]. The strategy of prevention and control of fish-borne parasitic zoonosis is highly needed especially in the term of an outbreak, transmission in several countries such as in South East Asia, China and Korea. Reported data suggests that there are about 1.5 million people in Korea, 6 million people in China and over 5 million in Thailand infected with liver flukes, either *Clonorchis sinensis* or *Opisthorchis viverrini* [3].

There are more than fifty species with fish-borne parasitic zoonosis belonging to the

Heterophyidae and Echinostomatidae that have been reported from Korea [3], Thailand [5], [6] Fish-borne parasitic infections continue to be a major public health problem, with more than 50 million people infected throughout the world. Freshwater fish especially a cyprinoid fish dish that is commonly served raw is associated with Opisthorchiasis which is the major cause of Cholangiocarcinoma. The Opisthorchiasis or liver flukes have been a major health concern in many parts of southeast Asia, especially in Mekong basin areas, including Thailand. The chronic infections cause intrahepatic bile duct diseases such as cholangitis, hepatomegaly and cholangiocarcinoma. Thailand is going through socio-economic changes accompanied by rapid development. Indubitable trends include impressive advances in science and technology as well as the rapidly rising health education levels among all segments of the society.

The strategy of prevention and control programs dealing with fish borne liver fluke Opisthorchiasis has remained a serious concern for the public health system in Thailand. There are many factors which are influencing the survival and transmission rates of this parasite. In the case of opisthorchiasis, the consumption of raw or undercooked fish is the primary source of infection [7]. The prevalence of intermediate hosts such as freshwater snails and fishes are factors that have been contributing to high rates of infection especially in Northeast and North Thailand [8],[9]. In the northeast, several types of uncooked fish meals and are traditional foods have been culturally eaten by local people [10],[11]. The main of traditional food from Northeast were *Koi pla* (raw fish flesh mixed with chopped garlic, lemon juice, fish sauce, chilly,

roasted ground rice and vegetables) is probably the most popular, followed by fish preserved for less than seven days, *pla ra* and *jaewbhong* in which viable metacercariae are contaminated [12]. The investigators emphasized in the literature on fish safety and food safety undercooking process using Thai traditional style, important knowledge to realize practical solutions for opisthorchiasis prevention and control. The preliminary knowledge before applying new technology will benefit in epidemiological data and public health for awareness of the problem especially for guideline formulation for health agencies. Health literacy program to stop transmission of liver fluke is a basic need that needs to be discussed among head villagers, public health agencies and teachers in the public participation process and school health program for a better implementation of parasite management in an area.

2. METHODOLOGY

2.1 Worm-free or Fluke-free Cooking Experiment

1. Hot recipe: by Grill freshwater fish at several sizes
2. Cold recipe: Sudden freeze at minus 18 degree Celsius and man-made by using ice ball (zero degree Celsius)
3. Iceball mix with natural salt in a container (minus 3-5 degree Celsius)

2.2 The Study of Metacercaria Collection from Cyprinoid Fish by Digestion Technique

Fish collection. Several fish's species of freshwater fish were captured directly from water by the fishermen living nearby the reservoirs in Nakhon Phanom and Sakhon Nakorn provinces. Capture methods were done by a net and traditional methods.

2.3 Food Cooking under E-sarn Style [Grill]

The unique experiment by using posttest only experimental designed. The cyprinoid freshwater fish for the experimental of cooking with heat, the grill experiment used 10 fish with size varying from 8 -15 cm. In each group, fish samples were placed on the iron grill with an average heat of 100 degrees Celsius or even above charcoal stove with 150-180 degree Celsius including high peak at 270-300 degree Celsius. To ensure that the fish samples are properly cooked, it was turned back and forth every 10 seconds until 1, 2, 3, 4 and 5 minutes and control group. The grilled fish in each experimental group and control were digested with pepsin solution for metacercariae detection.

Treatment A Grilled fish in 1 minute, normal
Treatment B Grilled fish in 2 minute
Treatment C Grilled fish in 3 minute
Treatment D Grilled fish in 4 minute
Treatment E Grilled fish in 5 minute



Fig. 1. Showed the incubation of pepsin digestion in water bath

2.4 Metacercariae Collection from Cyprinoid Fish using Digestion Technique

Metacercariae collection followed the pepsin digestion technique [NaCl 0.85 %, 1% HCl and 1% pepsin power]. Each group of Fish in the experiment were placed in a pepsin digestion solution, incubated under 37 degrees Celsius for 1.30 hours followed by filtering and sedimentation with sedimentation jar. [Fig. 1]

2.5 Metacercaria Preparation and Identification

Identification and movement observe was done using a stereomicroscope.

3. LIMITATION

Fresh water fishes collected from natural reservoirs, cannot determine species and numbers of metacercaria. This study only the effect of heat to metacercariae stage.

4. RESULTS

The results showed that the treatment in each category group of grill 1,2,3,4 and 5 minutes gave changes on fish skin, body discolored until burned, and dry in treatments A, B, C, D, F. After 3 minutes of the experiment, the grilled fish showed burnt black color until 5 minutes [C-F].

The grilled fish in each treatment group was blended and digested with 1% pepsin solution then incubated for 1.5 hours at 37 degrees Celsius to obtain metacercariae and then

checked for the movement under a stereomicroscope. The results showed that metacercariae remained active in control and 1-2 minutes 'experimental groups [F-G]. The groups 3-5 minutes of grill fish partially cooked showed inactive metacercariae. The excretory bladder spread with unclear form, microvacuoles appear in the metacercaria during 3-5 minutes experiment group [H-J].

The morphological characteristics results showed number of active and inactive metacercariae in each experimntal group. In the group of control, 1 and 2 min showed the majority number of active metacercaria. After 3 minute showed majority of inactive metacercariae and excretory bladder disformal characteristics. [graph 1].

4.1 The Result of Cool Packaging

1. Frozen freshwater fish at -10 degree Celsius or 14 Fahrenheit from 5 days and over showed that the metacercariae started to die at 5 days which corresponds to WHO recommendation.
2. Freezing freshwater fish at -18 degree Celsius or 15.8 Fahrenheit for 3 days and over showed that the metacercariae started to die on day 3. This can be useful for fish suppliers and recommend the immediate deep freezing of fish before processing it as fermented freshwater fish locally called Pla Som (prepared with salt and stored for 3 days and eaten raw or uncooked) for commercial purpose.



Control

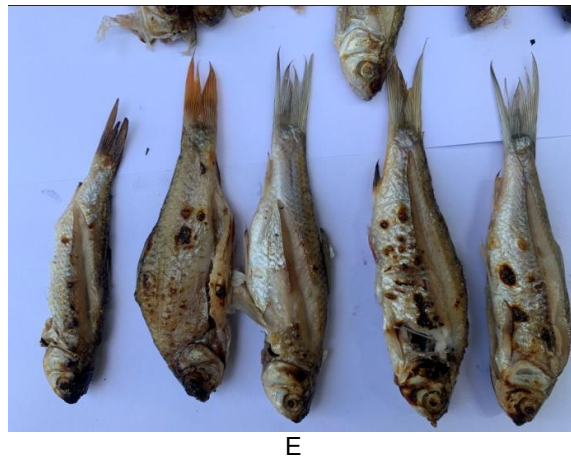
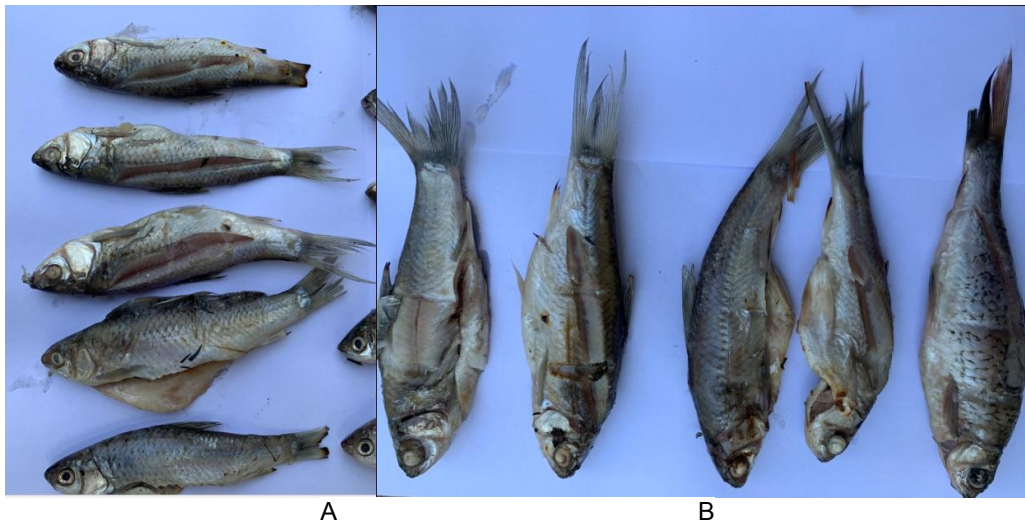
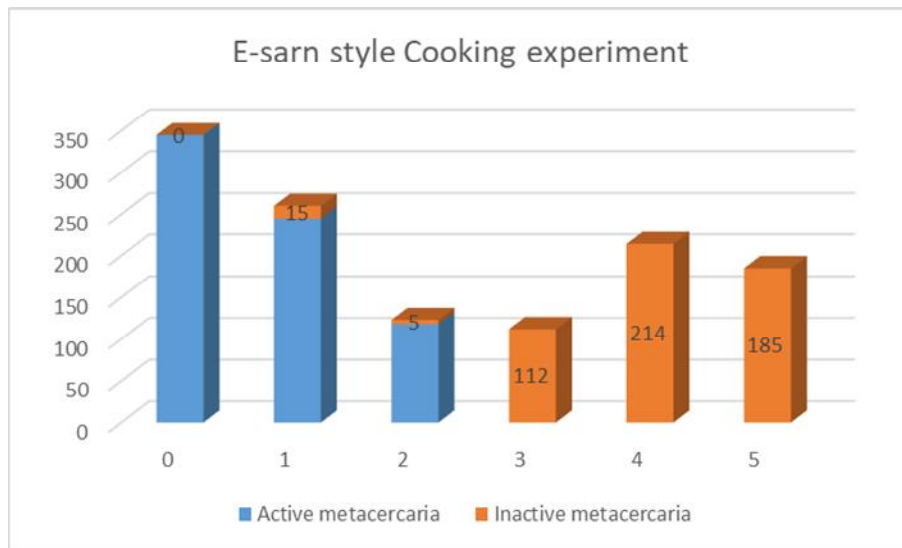


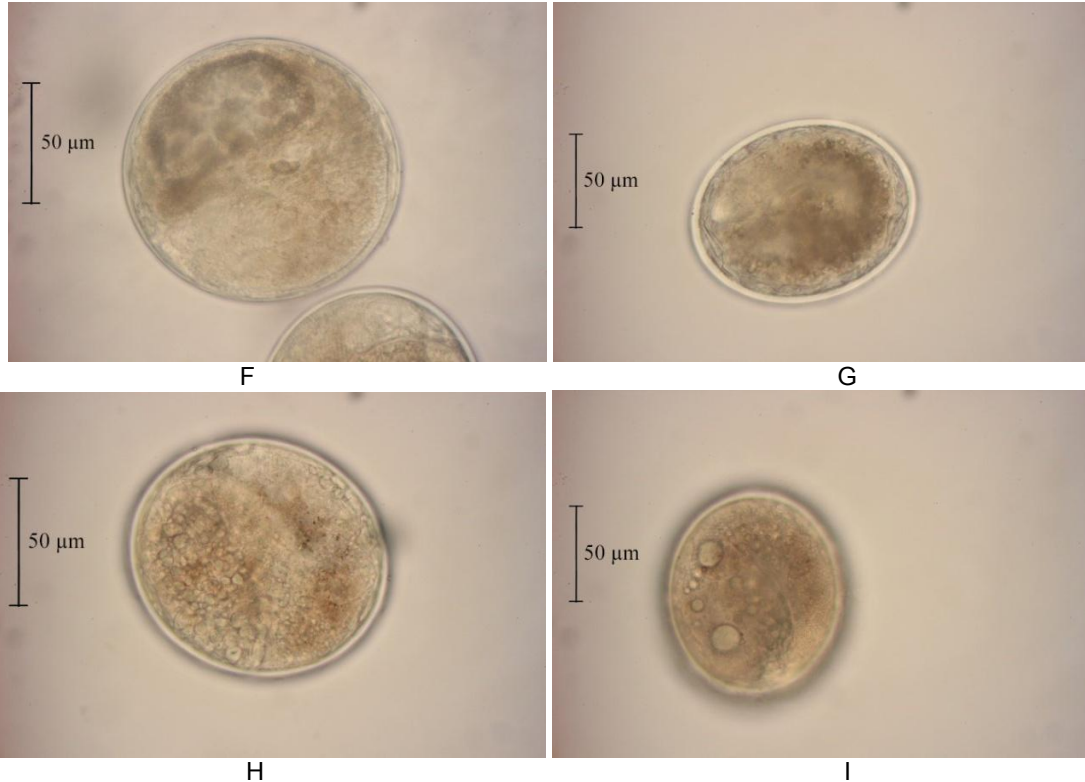
Fig. A. Grilled fish in 1 minute, normal, Fig. B. Grilled fish in 2 minute, Fig. C. Grilled fish in 3 minute, Fig. D. Grilled fish in 4 minute, Fig. E. Grilled fish in 5 minute

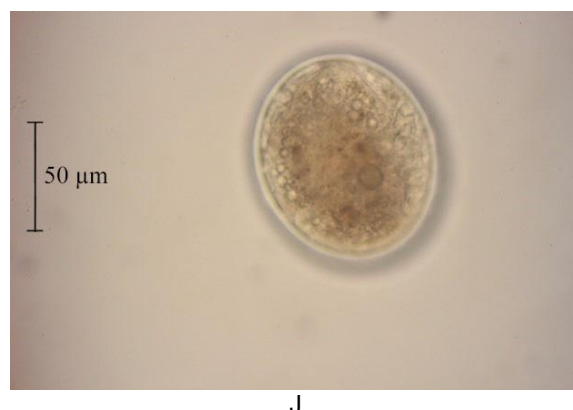


Graph 1. Showed number of metacercariae devided in to experiment times for cooking experiments

In local areas at the field make use of plain ice ball in boxes to preserve the fish and using ice ball mixed with salt, it preserves freshwater fish from approximately

minus 3 to 5 degree Celsius. This experiment showed that within 7 days the metacercariae starts to disperse the granule and inactive.





J

Fig. F. metacercariae at 1 minute, normal grilling, Fig. G. metacercariae at 2 minutes grilling, Fig. H. metacercariae at 3 minutes grilling, Fig. I. metacercariae at 4 minutes grilling, Fig. J. metacercariae at 5 minutes grilling

5. DISCUSSION AND CONCLUSION

The main outcome of fish grilling shows that cooking for more than 5 minutes by grill can bring safety into the fish by eliminating Opisthorchiasis. During the grilling process, heat destroys the metacercariae with inactive movement and disintegration of the excretory bladder. This crucial outcome can encourage the villagers to practice this method to help and promote eliminate opisthorchiasis as a result to reduce and Cholangiocarcinoma cases. Furthermore, this finding will be helpful in the health literacy program and strategy. 10 years' strategic plan for the elimination of the liver fluke and CCA running from 2016-2026. Traditionally, boiled and grilled fish are staple side dishes in Thailand and Mekong Basin. Under previous studies, many showed that preservation methods using spices affect fish safety because of metacercariae degeneration. The effect of spices from E-sarn food for metacercariae as the report by Chuboon *et al* 2005, showed that metacercariae remained active in all treated groups: fermented fish treated, fish sauce treated, chilli powder treated, ground roasted rice treated and complete mix treated [13]. The effect of the fermentation process and low temperature on viability and infectivity in hamster of *Opisthorchis viverrini* metacercariae showed that fermentation process in 1-4 days and fed hamsters had a worm recovery of 52%, 44%, 11.3% and 1% respectively, For the low-temperature experiment, worm recovery in 1-2 days showed 12.7% and 3.3 % respectively [14] Another study showed five different raw fish dishes that mimic traditional meals (2 fish dishes) prepared using the following methods: 1) left to dry at room

temperature; 2) frozen at -20° Celsius; 3) refrigerated at 4° Celsius; 4) marinated in saline (5% sodium chloride solution); and 5) marinated in 5% acetic acid solution to mimic traditional vinegar. Degeneration of the parasites was slowed by cooling; degeneration of all metacercariae took approximately 5 hours in the refrigerated or frozen fish, compared with 3 hours in all other dishes left at room temperature. [15]. According to the traditional Thai and local food style; Koi-Pla is easily made by unfermented raw fish. Koi pla is an unfermented raw fish dish in the northeastern region of Thailand. Unlike fermented fish dish such as Pla-Ra, viable metacercariae may be found resulting in high infection rate [16]. In the tradition of rural areas in the Mekong basin, Koi-Pla is a popular dish due to its good taste and short time preparation [16,17]. In a previous report, Koi-Pla was also crucial in the transmission of opisthorchiasis at the national level [18,19]. The endemic area of Opisthorchiasis like Nakhon Phanom in Thailand, the habit of consuming Koi- Pla is still the main risk factor of opisthorchiasis occurrence in the area. The villagers who consumed Koi-Pla were five-times more at risk to have opisthorchiasis infection [11]. The Culture and lifestyle of local people analysis of health behavior toward liver fluke infection concerning in the consumption of raw freshwater fish with white scales showed that Thai people consume papaya salad with raw fermented fish, raw fermented fish Jeaw Bong /raw fermented fish spicy dip, regularly consume pickled fish, and spicy raw fish salad. The cooks' practice and hygiene always showed they do not wash hands regularly with soap or water before preparing, cooking and eating fish. Moreover, cooking in sanitary facilities revealed that even

the atmosphere inside had never had a sanitary setting. The Recommendation for Worm-free Cooking, Eliminate Liver fluke and CCA, *Opisthorchis viverrini* is an important fish-borne zoonotic trematode which is widely distributed in South-East Asia, especially in China. Infections from human and animal reservoir hosts occur due to the consumption of raw or undercooked fish with *Opisthorchis viverrini* [11,19,20].

Assembly to Strategy under literature measure operating framework in each strategy. Strict policy, direction and supervision. Enhancing and push forward the measure in Opisthorchiasis prevention and control in strategy at each level. Improve systemically of Liver fluke infection and Cholangiocarcinoma policy with multilateral collaboration. Establish a monitoring and evaluation system. Motivate, boost the morale of local people to practice the practical method of eliminating fish parasites. Prevention measures of liver fluke infection and Cholangiocarcinoma elimination is classified as government policy. There is also a need to build concrete collaboration with partner's networks and university professors. It can be seen that the initiation establishes from the preparation of decade strategic plans. The establishment of the strategic plan committee can be monitored and evaluated in several sequences such as health promotion in hospital or primary health, community and district hospitals, districts and province public health offices. For public motivation, health promotion in hospital needs to collaborate with districts public health offices in many areas along with in organizing health promotion projects, awards can be presented for best practices, initiating and implementing activities together organized in integration together under village volunteer and community under the budget related to Departments and Ministry of Public Health.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors greatly appreciate the financial support and encouragement received from the following: The Bureau of General Communicable Diseases, Department of Disease Control,

Ministry of Public Health, Tropical and Parasitic Diseases Research Unit Faculty of Medicine, Mahasarakham University, and Dr. Vara Meesomboon from the Bureau of General Communicable Diseases, Thailand.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:

The peer review history for this paper can be accessed here:
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