Study on Giant Intestinal Cystic Disease of Common carp Caused by

Thelohanellus kitauei in Northern Vietnam

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Abstract

Haiduong province is far from Hanoi center for 40 km in east, a large area for freshwater production in Northern Vietnam, Common carp is major cultural species. However, disease is a serious problem for fish farmers. Major disease is giant intestinal cystic caused by Myxobolus sp with low growth rate, high FCR, and high mortality. This paper provides information based on the survey from 212 fish farms in four districts, following with diseased fish were sampled for diagnosis. The results showed that major fish culture system in Haiduong is polyculture (99.61%) with ratio of Common carp was from 15.63-32.10% of total fish stocking. There are 31.91% of diseased fish ponds. Non disinfected ponds could be infected with 4.28 times higher compared to that of disinfected ponds. Infected fish often black colour, swollen abdomen and intestine contains liquid and white cystics (92 cystics) with size of cystic is up to 5.3x3.7 cm. Myxobolus sp. was identified as Thelohanellus kitauei.

Keywords: Common carp, Haiduong, Thelohanellus kitauei.
1. Introduction

Common carp is a traditional freshwater fish species commonly found in the northern of Vietnam due to high quality of meat, nutritious taste, good tolerance in cold water. It can be cultured in a lot of water bodies such as in rice fields, ponds, rivers or reservoirs and in many farming systems such as extensive, semi-intensive or intensive fish farms (Kim & Nguyen, 2012; 2013; Kim, Phan & Nguyen, 2013).

In the past, Common carp was mainly stocked with low stocking density of less than 10% of total stocking fish (Kim & Tran, 2010). However, today, Common carp has become the main target species with high percentage in polyculture. Many diseases occurred in Common carp such as openning gill disease caused by Centrocestus formosanus (Kim et al., 2012), KHV disease caused by the Herpesvirus and the most recently giant intestinal cystic disease in Common carp resulted in reduction of growth rate, high food conversion ratio (FCR) and mortality in cultured fish.

Myxobolus usually parasites on fins, skin, gills and viscera of the carp (Kim, 2014). Cystic disease caused a lot of damage to Common carp when they parasited in the gills, making it difficult for breathing, causing intestinal obstruction that does not absorb food when ingested in the gut (Lingtong et al., 2017). Haiduong is a provinces with the largest freshwater fish production in the Northern Vietnam with total area of aquaculture is 10,900 ha, total product is 64,859 tons (Department of Haiduong Fisheries, 2015), in which Common carp production is one of the main targets of the local. In this article will present some results of the study on the characteristics of giant intestinal cystic disease in Common carps in Haiduong province.
2. Materials and Methods

2.1 Materials

The study was conducted by surveying information of 212 Common carp cultured Households in four districts: Binhgiang, Camgiang, Ninhgiang and Thanhmien of Haiduong province. The ponds are known to be diseased by giant cystic when the fish in the pond are sick or die with clinical signs of the disease. Thirty five specimens of Common carp were collected from diseased fish ponds in the area for the purpose of collecting sample, synthesizing signs, lesions, cyst number counts, and measurement of intestinal tumor size using Panmer ruler error of 0.1mm; 30 specimens of cystic in Common carp intestine were implanted, stained with Crystal violet 2% for 1 minute, morphological and microscopic examination of 10x100 magnification at the Aquatic Disease Laboratory, Faculty of Fisheries, Vietnam National University of Agricultural. The study was conducted between January and July 2017.

2.2 Methods

Primary data collection method: The study was conducted on the basis information (inherited from books, newspapers, magazines, local government reports and research reports) and directing survey of fish pond farmers.

Survey method: The directed interview method based on a set of questionnaire which was prepared before to interview 212 of fish pond cultured households at 4 districts in Haiduong province. Interviewing and questionnaires were constructed on the basis of cross-sectional questionnaires and odds ratio (Ors) in the epidemiological study of fish farmers (Phan et al., 2010). Content in questionnaire contains information such as number of ponds, pond size of each household, fish species in polyculture, percentage of Common carp stocking, feeding and look after fish pond. The questionnaire was
developed after a trial of 5 households and adjusted accordingly to formally interview 212 fish farmers.

Methods of sample collection, symptom descriptions, pathology and analysis of diseased specimens were carried out according to the parasite study method of Ha and Te (2007); Arthur and Te (2006).

Data analysis and processing: Interview data was coded and processed on Excel 2010 software. Statistical descriptive of software: mean ± standard deviation, maximum, smallest, percentage (%) and sample tests.

3. Results and Discussion

3.1 Status of fish pond culture and giant intestinal cystic disease in Haiduong

3.1.1 Status of fish pond culture in Haiduong

The results of survey on 212 fish farms with 257 ponds in 4 districts in Haiduong province are summarized in Table 1. The fish ponds in the surveyed location have an average area of 3224.60 m², the smallest pond area is 100 m², the largest 18000 m². In general, the area of ponds is suitable for semi-intensive and intensive culture in Haiduong (Kim & Tran, 2010). The resulted survey shows that the most fish pond culture in Haiduong is polyculture ponds. The percentage and species of polyculture are shown in Table 2.
Table 1. Status of fish pond culture in Haiduong province

| №  | Location   | № of HH | № of ponds | Average of pond area (m²) | Monoculture | Polyculture |
|----|------------|---------|------------|--------------------------|-------------|-------------|
|    | (District) |         |            |                          | No of ponds | No of ponds |
|    |            |         |            |                          | Ratio (%)   | Ratio (%)   |
| 1  | Binhgian   | 48      | 55         | 2914.60 ± 2360.19        | 0           | 55          |
|    |            |         |            |                          | 0%          | 100%        |
| 2  | Camgian    | 32      | 52         | 3156.90 ± 1831.55        | 0           | 52          |
|    |            |         |            |                          | 0%          | 100%        |
| 3  | Ninhgian   | 65      | 81         | 3806.30 ± 3309.26        | 0           | 81          |
|    |            |         |            |                          | 0%          | 100%        |
| 4  | Thanhmi    | 56      | 69         | 2839.83 ± 1382.89        | 1           | 68          |
|    |            |         |            |                          | 1.45%       | 98.55%      |
|    |            |         |            |                          |             |             |
|    | **Total**  | **212** | **257**    | **3224.60 ± 2129.71**    | **1**       | **256**     |
|    |            |         |            |                          | **0.39%**   | **99.61%**  |

Table 2. The percentage of polyculture species in fish ponds in Haiduong province

| № | Location | Major carp | Silver carp | Grass carp | Common carp | Tilapia carp | Other species |
|---|----------|------------|-------------|------------|-------------|--------------|---------------|
| 1 | Binhgian | 15.58      | 9           | 41.20      | 32.10       | 0            | 1.12          |
| 2 | Camgian  | 22.96      | 3.81        | 42.06      | 27.81       | 0            | 3.36          |
| 3 | Ninhgian | 3.75       | 2.33        | 7.47       | 15.63       | 70.82        | 0             |
| 4 | Thanhmien| 3.17       | 2.83        | 6.89       | 17.63       | 69.48        | 0             |
3.1.2 Status of giant intestinal cystic disease in Common carp

Based on a survey of 212 fish farms in 4 key aquaculture districts in Haiduong province with 257 fish ponds, the occurrence of cystic disease on Common carp is quite serious. The result of survey is summarized in Table 3.

The result of survey showed that nearly one third (31.91%) of fish ponds in Haiduong were infected with giant intestinal cystic disease. The diseases occurred in fish ponds throughout the location and ranged from 27.27 to 42.31% of fish ponds. The disease occurred most in fish ponds in Cam giang district where Common carp was stocked with high percentage of polyculture (27.81%). Among the diseased fish ponds, only appeared on Common carp without appearing in other species, although the fish ponds were polyculture. Farhaduzzaman et al., (2010) showed that in Baladesh the cystic disease occurred in major carp. During the investigation of the disease occurring on Common carp, an important consideration was whether or not disinfection in relation to the disease. Follow-up results are shown in Table 4.

Table 4 shows the odds ratio (OR) = (4 * 171) / (78 * 2) = 4.38. By OR (= 4.38)> 1, it can be seen that when fish ponds are not disinfected with water, the risk of disease is 4.38 times higher than the fish ponds are disinfected before culture. However, disinfection of water can not eliminate the risk that the fish ponds have cystic disease.
Table 3. The status of cystic disease in Common carp Haiduong province

| No | Location (District) | No of fish ponds | No of infected fish ponds | Prevalence of fish ponds (%) |
|----|---------------------|------------------|---------------------------|----------------------------|
| 1  | Binhgiang           | 55               | 15                        | 27.27                      |
| 2  | Camgiang            | 52               | 22                        | 42.31                      |
| 3  | Ninhgiang           | 81               | 26                        | 32.10                      |
| 4  | Thanhmien           | 69               | 19                        | 27.54                      |
|    | Total               | 257              | 82                        | 31.91                      |

Table 4. The relation between disinfected ponds to the cystic disease

| Factors         | The disease occurred in fish ponds | Total |
|-----------------|------------------------------------|-------|
|                 | Yes      | No     |       |
| Risk            | Non-disinfection | 4     | 2    | 6    |
|                 | Disinfection   | 78    | 171  | 251  |
| Total           | 82       | 173    | 257  |

3.2 Clinical signs and symptom of giant intestinal cystic disease in Common carp

3.2.1 Clinical signs

During the investigation of diseased Common carp in Haiduong province, 35 specimens were collected to monitor the symptoms and clinical signs. Monitoring results are summarized in Table 5; Fig. 1 and 2.
Clinical signs of black body was also mentioned by the author Vo The Dung and Vo Thi Dung (2016) on *Synanceia verrucosa* was infected *Myxobolus* sp. (like cystic) in the gallbladder.

Table 5. The characters of intestinal cystic disease in Common carp (n=35)

| No | Clinical signs                                      | No of fish | Percentage |
|----|-----------------------------------------------------|------------|------------|
|    | with clinical signs (fish)                          |            |            |
| 1  | Black body, slow simming on surface water           | 35         | 100        |
| 2  | Jump up to surface water                            | 31         | 88.57      |
| 3  | Swollen abdomen                                     | 35         | 100        |
| 4  | Loss scales                                         | 7          | 20         |
| 5  | Opening and Red anal with yellow liquid             | 5          | 14.29      |
| 6  | Died standing like swimming                         | 13         | 37.14      |
Figure 1. Swollen abdomen in diseased Common carp (Black body; Loss scales)

Figure 2. Opening and red anal with yellow liquid run off

3.2.2 Histopatology of giant intestinal cystic disease in Common carp

The diseased fish samples were collected and operated showed that all diseased fish samples contained liquid in internal organs. The intestine remained many white tumors (cystics) make the thinning intestinal wall. The results of the examination are shown in Table 6; Fig. 3 and 4.

The examination of 35 diseased fish samples contained cystics, that was counted and measured of cystics was summarized in Table 7. In this case study, no cystic was found in other organ except intestine. Zhang et al., (2010), reported that cystic of Myxobolus wulii was found in hepatopancreas of diseased Common carp in China and Japan.
Average of number and size of cystics in diseased fish with clinical signs and symptoms were 16.74 cysts / fish, 2.65 cm x 1.2 cm, respectively. The highest number was up to 92 cysts and the largest cyst was 3.7 cm × 5.3 cm. The size of the cystic in this case is equivalent to that of diseased Common carp caused by *Thelohanellus kitauei* (2.0 cm x 3.6 cm) by Lingtong *et al*., 2017. The cysts in diseased Common carp contained in gut are larger than the cysts in gill (Kim, 2014). The shape and size of spores which taken from cystic in gut of diseased Common carp was smeared, stained, measured and compared with standard samples from other authors in Chinese and Japanese. The results of the measurement and analysis are shown in Table 8, the image of the spore is shown in Fig. 5.

Based on the results on morphology and size of the spore which samples obtained in Haiduong province and compared with standard samples of Chinese authors (Yanhua *et al*., 2016; Lingtong *et al*., 2017) and Japan (Syuzo and Kenji, 1981) showed that diseased Common carp in Haiduong province had giant intestinal cystics caused by *Thelohanellus kitauei*.

![Figure 3. The thinning intestinal wall and contain a lot of cystics and liquid](image)
Figure 4. The number and size of cystic in diseased Common carp

Figure 5. The shape and structure of spore parasites in gut and gill of Common carp
Table 6. Histopathological characters

| №  | Histopathological characters         | Nº of infected fish | Percentage (%) |
|----|--------------------------------------|---------------------|----------------|
| 1  | Thinning intestinal wall             | 35                  | 100            |
| 2  | Many cystics in the gut              | 35                  | 100            |
| 3  | Gut contain liquid                   | 29                  | 82.86          |
| 4  | Broken gut                           | 3                   | 8.57           |
| 5  | Swollen other organs                 | 35                  | 100            |
| 6  | Cysts in other organs                | 0                   | 0              |

Table 7. Number and size of cystic in intestine of diseased Common carp

| №  | Number and size of cystic | Nº of cystic/fish (n₁ = 35) | Size of cystic (n₂ = 30) |
|----|---------------------------|-----------------------------|--------------------------|
|    |                           | (Length (cm))               | (Wide (cm))              |
| 1  | Max                       | 92                          | 5.3                      | 3.7          |
| 2  | min                       | 7                           | 0.4                      | 0.3          |
| 3  | Average                   | $16.74 \pm 13.99$           | $2.65 \pm 1.39$          | $2.04 \pm 0.87$ |
Table 8. Size of spores was taken from the cystic of diseased Common carp (n=30)

| N° | Characters          | Size of spore from the studying samples (μm) | Size of Thelohanellus kitauei * (μm) |
|----|---------------------|---------------------------------------------|--------------------------------------|
| 1  | Length of shell (D_{TB}) | 30.9 ± 2.78                               | 38.41 ± 2.45                          |
| 2  | Length of spore (D_{TB}) | 24.02 ± 1.84                               | 25.98 ± 0.95                          |
| 3  | Lenth of top spore (D_{TB}) | 16.52 ± 1.84                               | 14.73 ± 0.92                          |
| 4  | Wide of shell (R_{TB}) | 13.85 ± 2.22                               | 13.3 ± 0.87                           |
| 5  | Wide of spore (R_{TB}) | 9.11 ± 2.08                                | 8.72 ± 0.51                           |
| 6  | Wide of top spore (R_{TB}) | 5.85 ± 0.98                               | 6.82 ± 0.45                           |
| 7  | Lenth of filament (CDR) | 280.05 ± 51.46                             | Filament with 8-10 circles            |

Note: *: Identificated by Lingtong et al., (2017)

4. Conclusions and recommendations

Freshwater fish was cultured in Hai duong provine, Vietnam mainly is polyculture accounting for 99.61% of ponds, of which Common carp is stocked from 15.63 to 32.1% of the 257 surveyed ponds, 31.9% of the ponds were infected with intestinal cystics. The disease often occurs in non disinfected ponds, which are 4.38 times higher than those in the disinfected ponds. Fish with symptoms of floating, black body, jumping on the surface water, swollen abdomen, thin intestinal wall contains many white cysts of size 2.04 cm x 2.65 cm. The spore is identified as Thelohanellus kitauei.
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