Description of Gill Histopathology And Water Quality Of Koi Fish (Cyprinus Koi fishio) Infected By Koi Herpes Virus (KHV)

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Abstract. One of disease that attacks in common koi fish until now is caused by infection of Koi Herpes Virus. Koi Herpes Virus (KHV) is one type of DNA virus that attacks the family of Cyprinidae. Based on the preliminary test using PCR techniques gills, of koi fish infected by KHV was positive. This study aimed to determine water quality analysis and histopathological gills of koi fish (Cyprinus Koi fishio Linn) that infected KHV. Water quality measurements and gills tissue of fish observation was conducted in three repetition. Histopathological observations of gills tissue used for PCR analysis. The results of PCR analysis showed light line DNA band with size of 292 bp. The results of temperature, brightness, pH, DO, CO₂ and COD are still in accordance with water quality standards, while values of ammonia showed 0.373-0.377 mg/l and BOD 3.986-4.729 mg/l that exceeding prescribed quality standards. Histopathological observations of gills tissue showed damage to the primary lamella form like edema, hyperplasia, atrophy, congestion, and necrosis than the type damage of secondary lamellae like edema, hyperplasia, fusion lamella, telangiectasis, congestion, and necrosis. Suspected KHV infection caused damage for gills of koi fish in histopathological.

1. Introduction
Water quality has an important role in the process of raising fish. water quality is a major environmental component that directly affects the life of organisms in water [1]. Thus, water quality must be managed properly, if the water quality is not managed properly, it will cause a decrease in water quality so that it can cause disease which can later become an obstacle in fish farming or maintenance. Koi fish (Cyprinus Koi fishio Linn) is a freshwater fish that has important economic value, so this fish is widely cultivated and can be maintained in certain ponds [2]. However, the problem is larvae stage of koi fish more susceptible to disease and most of them could be death. It can be caused by parasites, bacteria, fungi, and viruses [3]. Also, most frequent cause by virus such as KHV.

According to [4], One of the common diseases in koi fish now is a disease caused by Koi Herpesvirus infection. Based on the results of surveys in the field also found the symptoms of disease in koi fish, namely fish more often swim on the surface, slimy body, pale gills, and hemorrhagic body. The visible symptoms of the disease in koi fish indicate that the disease is caused by the herpes virus. In addition, the results of the diagnosis using the Polymerase Chain Reaction (PCR) technique in the preliminary test on koi fish showed that
the positive koi fish were attacked by Koi Herpes Virus (KHV) where positive organs were attacked by Koi Herpes Virus (KHV) from the diagnosis using the Polymerase Chain Reaction (PCR) technique is part of the gill organs in koi fish. For survival, koi fish must be very dependent on the quality of the water used as a place to live or their habitat. So, it is necessary to analyze the water quality in the maintenance ponds of koi fish infected with Koi Herpes Virus with the aim to determine the condition of water quality in koi fish maintenance ponds. Based on the results of the analysis using Polymerase Chain Reaction (PCR) with the gill organs in koi fish that have been positive for Herpes Virus Koi, to determine damage to the gills of koi fish (Cyprinus Koi fishio Linn), research is also needed on the picture of Histopathology of Koi fish Gills (Cyprinus Koi fishio Linn) infected with Koi Herpes Virus. The aimed of this study is to determine condition of the water quality and histopathological capture of Koi fish gills infected by Koi Herpes Virus (KHV) from cultivation ponds.

2. Materials and Methods

2.1. Research Materials
The material used in this study is the measurement of water quality with Physics parameters (Temperature and Brightness) and Chemical parameters (Temperature, DO, CO₂, Ammonia, BOD and COD) and histopathological features of koi fish gills.

2.2. Research Methods
The method used in this research is descriptive which is conducted by taking a sample of three repetitions in the koi fish rearing pond (Cyprinus Koi fishio Linn). Samples taken were koi fish (Cyprinus Koi fishio Linn) which showed symptoms of Koi Herpes Virus in koi fish rearing ponds and gill organs were analyzed using Polymerase Chain Reaction (PCR) then histopathological analysis was performed to determine damage to koi fish gills. The measured water quality parameters are Physics parameters (temperature and brightness) and Chemical parameters (pH, DO, CO₂, ammonia, BOD and COD).

3. Results and Discussion

3.1. Observation Location Description
The location of this study was in the Balai Benih Ikan (BBI) of Babadan Village, Wlingi District, Blitar Regency, East Java. Balai Benih Ikan (BBI), geographically located at 111 25' - 112 20' BT and 7 57'- 8 51 LS. Types of ponds used as koi fish rearing ponds are intensive ponds. Blitar Regency has an area of 1,588.79 Km with land use, namely paddies, fields, plantations, ponds, tegal, forests, fish ponds and so on [5].

3.2. PCR (Polymerase Chain Reaction) Analysis
Gills in koi fish that were positive for KHV infection based on test results obtained from the BPAP-Bangil Disease and Environmental Laboratory showed DNA band sizes of 292 bp using the primary sequence F292: 5'-GAC-ACC-ACA-TCT-GCA-AGG-AG-3' and R292: 5'-GAC-ACA-TGT-TAC-AAT-GGT-CGC-3'. Koi fish in the first, second and third observations can be said that the formation of a band in 292 showed positive results of being infected with KHV at a mild attack rate. According to [6], Sample criteria for koi fish infected with Koi Herpes Virus (KHV), namely mild infection of KHV, indicate clinical features. Head and eyes are normal, gills are not white, the body's skin is not hemorrhagic, and the lesion and PCR test results show a band of 290 bp.

3.3. Water Quality Analysis
The results of the analysis of water quality in the maintenance of koi fish (Cyprinus Koi fishio Linn) ponds can be seen in the following table 1.

Table 1. The result of water measurements

|                      | Week-1 | Week-2 | Week-3 | Water Quality Standards          |
|----------------------|--------|--------|--------|----------------------------------|
| Temperature (°C)     | 26     | 25     | 27     | 25-30 (SNI, 1999)                |
| Brightness (cm)      | 33     | 32.6   | 32     | >30 cm (SNI, 1999)               |
| pH                   | 8      | 8      | 8      | 6.5-8.5 (SNI, 1999)              |
| DO (mg/l)            | 7.43   | 7.77   | 7.09   | >5 mg/l (SNI, 1999)              |
| CO₂ (mg/l)           | 3.58   | 4.63   | 4.59   | 5-10 mg/l (PP No. 83 Tahun 2001) |
| Amonia (mg/l)        | 0.373  | 0.374  | 0.377  | <0.02 (SNI, 1999)                |
| BOD (mg/l)           | 3.986  | 4.662  | 4.662  | 3 mg/l (PP No. 83 Tahun 2001)    |
| COD (mg/l)           | 7.48   | 7.45   | 7.61   | 25 mg/l (PP No. 83 Tahun 2001)   |

In koi fish rearing ponds (Cyprinus Koi fishio Linn) a temperature of 25-27°C is obtained. This indicates that the range of temperature values under koi fish maintenance is still within the normal range. The temperature value of 25-30°C for koi fish maintenance ponds is a range of values suitable for koi fish maintenance [7]. For the value of the brightness in the pond maintenance of koi fish obtained values of 32-33 cm. The range of brightness values in the koi fish maintenance is still within the normal range. Brightness values>30 cm for koi fish maintenance ponds are a range of values suitable for koi fish maintenance [7]. Then for dissolved oxygen results obtained values of 7.095 mg/l - 7.43 mg/l. The range of dissolved oxygen values in the koi fish maintenance is still within the normal range. A dissolved oxygen value of 5 mg/liter for koi fish rearing ponds is a range of values suitable for raising koi fish [7]. In addition, pH value of 8 was obtained, the range of pH values in ponds under koi fish maintenance was within the normal range. The pH value of 6.5-8.5 for koi fish maintenance ponds is a range of values suitable for koi fish maintenance [7]. The value of CO₂ in the maintenance of koi fish obtained a value of 3.58-4.63 mg/l. This indicates the presence of CO₂ in the waters exceeds normal limits in accordance to Government Regulation Number 82 2001 concerning water quality management, the value of CO₂ in aquaculture ponds was 5-10 mg/l. Whereas the value of Ammonia obtained a value of 0.373-0.377, while according to [7], Ammonia value in good aquaculture ponds is <0.02 mg/l. Then for the COD value obtained a value of 7.45-7.61. According to [8] a good COD value in aquaculture ponds of 25 mg/l. While the BOD value obtained is 3,986-7,729. According to [8] good BOD value in aquaculture ponds of 3 mg/l. This indicates that BOD in koi fish maintenance ponds is more common in fish rearing ponds in the waters.

3.4. Histopathological Analysis

Here is a histopathological picture of the gills of koi fish (Cyprinus Koi fishio Linn) infected with KHV in the first, second and third observations that can be seen in Figure 1.
Figure 1A. gills looks normal. The parts of the gill structure are still complete and have not been damaged at all. According to [9], normal gills are one sheet of gills consisting of several primary lamellae and one primary Lamela consisting of several sheets of the secondary lamella. Figure 1B showed damage to the primary lamella in the form of edema, hyperplasia, congestion, and necrosis. Whereas in secondary lamellae appear edema damage, hyperplasia, lamella fusion, congestion, and necrosis. Then Figure 1C showed damage to the primary lamella in the form of atrophy. Whereas secondary lamellae appear edema damage, hyperplasia, lamella fusion, telangiectasis, and necrosis. Figure 1D histopathologically the damage to the primary lamella in the form of edema and damage to the secondary lamella in the form of edema, hyperplasia, lamella fusion, congestion, and necrosis. According to [10], edema is swelling in the tissues and accumulation of fluid in the body. According to [11], states hyperplasia occurs accompanied by an increase in the number of mucous cells at the base of the lamella and can eventually lead to lamella fusion. According to [10], Fusion is the joining of certain tissues or cells. Congestion is an abundance of blood in blood vessels so that blood capillaries swell [12]. According to [10], states that atrophy is shrinkage in cells and tissues so that it appears smaller than originally. According to [12], Necrosis is cell death, thought to occur as a result of sudden circulatory and ischemic disorders.

4. Conclusion
Based on the results of Water Quality in koi fish maintenance ponds (Cyprinus Koi fishio Linn) obtained the value of Physics parameters (temperature of 25-27°C and brightness of 32-33 cm). As for the results of chemical parameters (dissolved oxygen is around 7.095 mg / lt - 7.43 mg / lt, pH is 8, CO₂ is 3.58-4.63 mg/l, Ammonia is 0.373-0.377 mg/l, COD is 7.45-7.61 and BOD of 3.986-4.729. So it can be concluded that the value of ammonia and BOD exceeds the presence of water. In addition, histopathologically the gills infected with Koi Herpes Virus showed that at first observation there was damage to the primary lamella in the form of edema, hyperplasia, congestion, and necrosis. Whereas secondary lamella experiences such as edema, hyperplasia, lamella fusion, congestion, and necrosis. Then in the second observation histopathologically there was a primary lamella damage namely atrophy and in the secondary lamella edema, hyperplasia, lamella fusion, telangiectasis, and necrosis. Whereas the third observation was histopathologically the occurrence of edema, hyperplasia in primary lamellae and showed histopathologically the occurrence of edema, hyperplasia, lamella fusion, congestion and necrosis in secondary lamellae.

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