Clinical Case Study of Non-infectious Dropsy in Koi (Cyprinus carpio koi)

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Abstract

Dropsy is one of the clinical symptoms of the disease that is commonly found in fish. These symptoms are often associated with the incidence of infection with microorganisms. The study aims to report the dropsy case where no indications were found due to microorganism infection. An experimental koi fish in an aquarium had symptoms of pineapple-like scales with a previous clinical history of koi herpesvirus (KHV) infection.

Histopathological examination was carried out by collecting all organs and staining them with hematoxylin-eosin. A molecular microbiological examination was conducted to detect the presence of koi herpesvirus (KHV), iridovirus, *Streptococcus agalactiae*, *Francisella* spp., and *Aeromonas* sp. by collecting samples of gills, kidney, and aspirated fluid from the coelomic cavity. The histopathological examination showed abnormalities in muscle, gills, kidney, heart, spleen, intestine, liver, pancreas, brain, and eye. However, no bacterial colony or viral inclusion body was found in the examined organs. The findings align with the PCR examination, which showed negative results for koi herpesvirus (KHV), iridovirus, *Streptococcus agalactiae*, *Francisella* spp., and *Aeromonas* sp... This condition indicates a suspected cause of dropsy which is suffered due to kidney malfunction, which causes osmolarity disturbances and ascites in the coelomic cavity.

Keywords: ascites, clinical case, *Cyprinus carpio koi*, dropsy, koi, non-infectious

1. Introduction

Koi fish (*Cyprinus carpio koi*) belongs to the Cyprinidae family and is an ornamental fish with high economic value and stable market selling value fluctuations. Due to their phenotypic diversity, koi have been domesticated and cultivated for a long time. Hobbyists favor this freshwater fish in national and international markets, including Indonesia (Arifudin *et al*., 2020). However, the disease is one of the inhibiting factors in cultivating and maintaining koi in the pond, which can be economically detrimental. The disease may cause the growth of koi fish to be retarded, and it can even cause mass mortality (Chattopadhyay, 2016). Clinical symptoms need to be known so that the fish get immediate treatment and can reduce the disease risk.

One of the clinical symptoms that are often found in koi fish is dropsy. Dropsy is commonly used in coelomic distention, caused by ascites or effusion and fluid accumulation in the coelomic cavity. Dropsy is a sign of the ongoing process or initial stage of other diseases, often multisystemic ones, and affects the organs and tissues of the coelom. Dropsy is caused by various infectious and non-infectious agents and is often associated with

microorganism infections, such as viruses, fungi, and bacteria (Smith, 2019). The diagnosis of dropsy can be marked by swelling in the fish body, precisely in the fish tissues and organs, such as the kidney. The swelling occurs due to kidney malfunction. Physiologically, fish excrete some of the water naturally in the body. The kidney is an organ that plays a role in osmoregulation. In normal conditions, the water naturally in the kidney can be excreted, but in cases of renal failure, water cannot be excreted, so it accumulates in the organs, and eventually, swelling occurs (Vajargah, 2022). In dealing with dropsy, treatment choice or control depends on which agent causes dropsy (Smith, 2019). Therefore, further studies related to dropsy need to be carried out so that fish affected by dropsy cases can be treated appropriately according to the factors that cause it.

This study aims to evaluate dropsy cases in koi through histopathological and PCR examination. The study is conducted to complete the reporting gap and increase the knowledge related to dropsy in koi fish so that aquatic veterinarians can serve good therapeutic management according to the prognosis.

2. Materials and methods

Anamnesis

An experimental koi fish in an aquarium had symptoms of pineapple-like expanding scales with a previous clinical history of artificial infection with the koi herpesvirus (KHV). Four other fish were in the same aquarium with the same clinical history, but no clinical symptoms such as dropsy were found.

Anatomical pathology and histopathological examination

Anatomical pathology examination was carried out by examining the external conditions of each organ and visceral site to evaluate any abnormalities or gross signs that could be found. A histopathological examination was performed by making

histological preparations. Organ samples were fixed in formalin phosphate-buffered saline (FPBS) for trimming, inserted into tissue cassettes, dehydrated with an automatic tissue processing machine (Tissue-Tek VIP® 5 Jr, Sakura Finetek Japan Co., Ltd), and embedded into liquid paraffin using an embedding machine (Tissue-Tek TEC™, Sakura Finetek Japan Co., Ltd). Organs in solid paraffin were cut with a thickness of ±5 μm using a rotary microtome (Accu-Cut® SRM™, Sakura Finetek Japan Co., Ltd). Tissue samples were stained using hematoxylin-eosin (HE) (modification of Bancroft and Gamble, 2008).

Molecular examination with Polymerase Chain Reaction (PCR)

PCR examination was performed for koi herpesvirus (KHV), iridovirus, *Streptococcus agalactiae*, *Francisella* spp., and *Aeromonas* sp. as some microbial causes associated with dropsy in fish. According to the manufacturing procedure, the extraction method of nucleic acid from gills, kidneys, and coelomic fluid samples was the IQ2000 DTAB-CTAB extraction kit (GeneReach, Taichung City, Taiwan). The PCR master mix was used GoTaq® Master Mixes (Promega, Madison, USA), the use of which was adjusted to the manufacturer's instructions. PCR procedures for detecting iridovirus, *Streptococcus agalactiae*, *Francisella* spp., and *Aeromonas* sp. were performed according to Table 1. PCR detection for KHV using the Nested PCR IQ2000 KHV Detection and Prevention System Kit (GeneReach, Taichung City, Taiwan) according to the manufacturer's instructions targeting the amplicon at 320 bp and 550 bp.

The amplification results were then electrophoresed on 1.5% agarose gel (BIORON, Römerberg, Germany) in Tris-Borate-EDTA (TBE) 1X running buffer solution with a voltage of 110V for 35 minutes. Electrophoresis results were visualized on the UVITEC Cambridge Gel Documentation System (Uvitec Ltd,

Cambridge, UK), while a 1 kb marker (New England Biolabs Ltd., UK) was used as the standard base size.

Table 1 PCR procedure for detecting iridovirus, *Streptococcus agalactiae*, *Francisella* spp., and *Aeromonas* sp.

Pathogens	Primers 5′ → 3′	Total master mix volume (μL)	Amplification Cycle	Target amplicon and gene
Iridovirus	Forward primer iri f: ATGTCTGCGATCTCAGGTGCGAACG Reverse primer iri r: CCAATCATCTTGTTATAGCCAGACT GTTTGC (Modification of Dong *et al*., 2017)	24	Initial denaturation: 94°C, 2 min Denaturation: 94°C, 30 sec Annealing: 60°C, 30 sec Elongation: 72°C, 30 sec	449 bp; Major Capsid Protein (MCP)

			35 cycles	
Streptococcus agalactiae	Forward primer F1: GAGTTTGATCATGGCTCAG Reverse primer IMOD: ACCAACATGTGTTAATTACTC (Martinez *et al.*, 2001)	24	Initial denaturation: 94.4°C 2 min Denaturation: 92°C 1 min Annealing: 55°C 1 min Elongation: 72°C 1 min 30 sec 25 cycles	220 bp; 16S rRNA
Francisella sp.	Forward primer Fr153ForU: GCCCAYYWGWGGGGATACC Reverse primer Fr1281RevU1 : GGACTAMGASTRSCTTTMTGRGA	23.75	Denaturation: 95°C, 1 min Annealing: 60°C, 1 min Elongation: 72°C, 1 min	1170 bp; 16S rRNA

	Fr1281RevU2 : GGACTAMGASTRSCTTTMTGRGT (Jeffery *et al.*, 2010)		40 cycles	
Aeromonas sp.	Forward primer *Aer*-f: CCAAGGGGTCTGTGG-CGACA Reverse primer *Aer*-r: TTCACCGGTAACAGGATTG (Pollard *et al*., 1990)	24	Initial denaturation: 94°C, 2 min Denaturation: 94°C, 1 min Annealing: 55°C, 90 sec Elongation: 72°C, 2 min 30 cycles (Gustafson *et al*., 1992)	430 bp; Aerolysin

3. Results and discussion

Anatomical pathology and histopathological examination

The results of anatomical pathology examination showed symptoms of

pineapple-like scales in all body areas, thickening of the abdominal muscle, accumulation of fluid in the coelomic cavity, and morphological abnormalities in the size of the heart, pericarditis, and pale liver (Fig 1). No reddish lesion was found on the skin or fins. Based on the histopathological analysis, the organs that showed abnormalities were the muscle, gills, kidney, heart, spleen, intestine, liver, pancreas, brain, and eyes. The muscle had a severe degree of myositis and myocoagulation. The gills showed moderate eosinophilic granule cell (EGC) infiltration and mild monogenean infection.

Moreover, the heart had severe pericarditis, myocarditis, and mild hyaline accumulation. The spleen showed severe splenitis and moderate hyaline accumulation, and the kidney had severe nephritis and mild amyloid and hyaline accumulation. Moderate enteritis and a mild degree of protozoa were found in the intestine. The liver showed a severe degree of hydropic degeneration and a mild degree of necrosis. The pancreas had a mild degree of necrosis.

Furthermore, the brain had severe meningeal congestion, a moderate degree of malacia, and a mild degree of gliosis, and the eye showed moderate inflammation. The accumulation of melanomacrophage centers (MMCs) was found in the gills, spleen, kidney, intestine, liver, brain, and eyes. No bacterial colony or viral inclusion body was found in all organ samples (Figs 2–3).

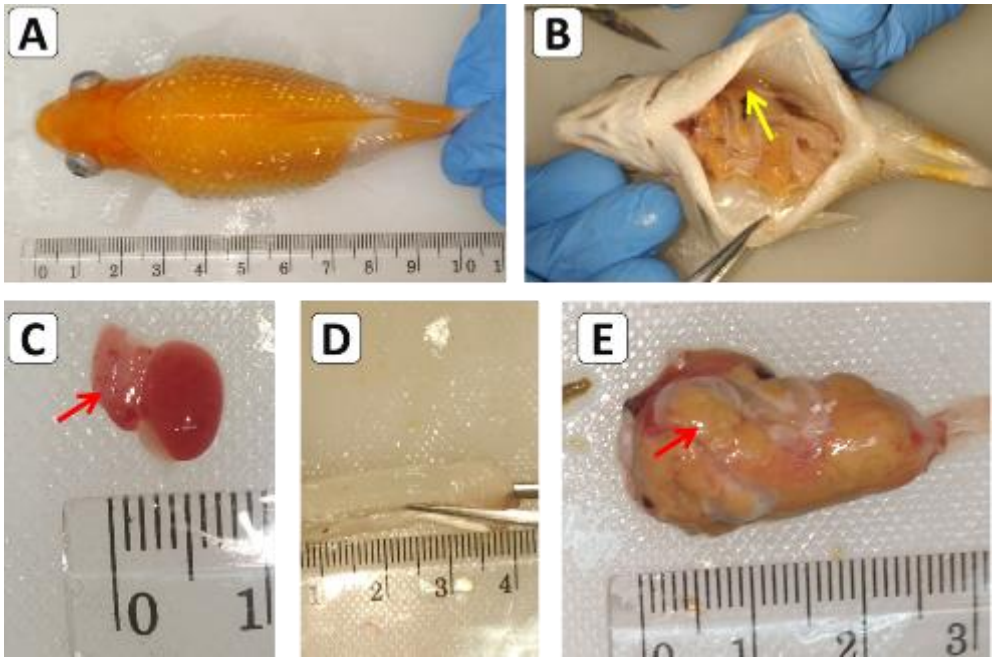

Figure 1: Anatomical pathology examination results.

A. Clinical symptoms of pineapple-like scales all over the body. B. There was an accumulation of fluid in the coelomic cavity (yellow arrow). C. Presence of morphological abnormalities in the size of the heart and pericarditis (red arrows). D. Thickening of the abdominal muscle. E. Pale liver (red arrow).

Dropsy is a clinical symptom usually associated with infection by microorganisms, such as bacteria or viruses. The infection can occur in the peritoneal area, including the kidney, causing fluid accumulation. Fluid in the body accumulates and causes dropsy and its scales to protrude and take on a pineapple-like shape (Sanil and Vijayan, 2008). This infection is not only single but can also be accompanied by double infections from different bacteria or viruses. Lee and Wendy (2017) reported an incident of *Aeromonas hydrophila* co-infection accompanied by the presence of *Edwardsiella tarda*, which can cause clinical symptoms, including dropsy in red tilapia. Ramírez-Paredes *et al*. (2019) also

found a double infection between Infectious Spleen and Kidney Necrosis Virus (ISKNV), a species of megalocytivirus family Iridoviridae, and *Streptococcus agalactiae* in tilapia can also cause dropsy which is accompanied by a darker change in body color and abnormalities in swimming movements. The incidence of dropsy due to viral hemorrhagic dropsy (VHD) in the three-spot gourami (*Trichogaster trichopterus*) caused by an iridovirus infection has also been reported by Paperna *et al*. (2001). Symptoms of dropsy may be accompanied by congestion and enlargement of the spleen, exophthalmia, hemorrhage, and the discovery of yellow fluid in the coelomic cavity. Dropsy symptoms in goldfish (*Cyprinus carpio*) are also reported to be caused by *Raoultella ornithinolytica* bacterial infection (Al-Shammari *et al.*, 2019). Öztürk and Altýnok (2014) reported that *Oncorhynchus mykiss* could suffer dropsy due to *Aeromonas caviae* infection. Oğuz (1999) reported that an infection could also cause suspected dropsy with the *Myxobolus* sp. parasite, which causes liver damage in the form of cysts.

The infection process by microorganisms has an essential role in the occurrence of dropsy, but non-infectious factors can also trigger this condition. Poor water quality and inadequate feed nutrition can also cause dropsy due to organ damage, such as renal failure or infection of the liver. Age can also affect the incidence of dropsy, where old fish are very susceptible to renal failure. Excessive eating patterns, excessive use of dry feed, and unhealthy live feed for fish can trigger problems in the digestive system and renal failure, resulting in dropsy (Vajargah, 2022).

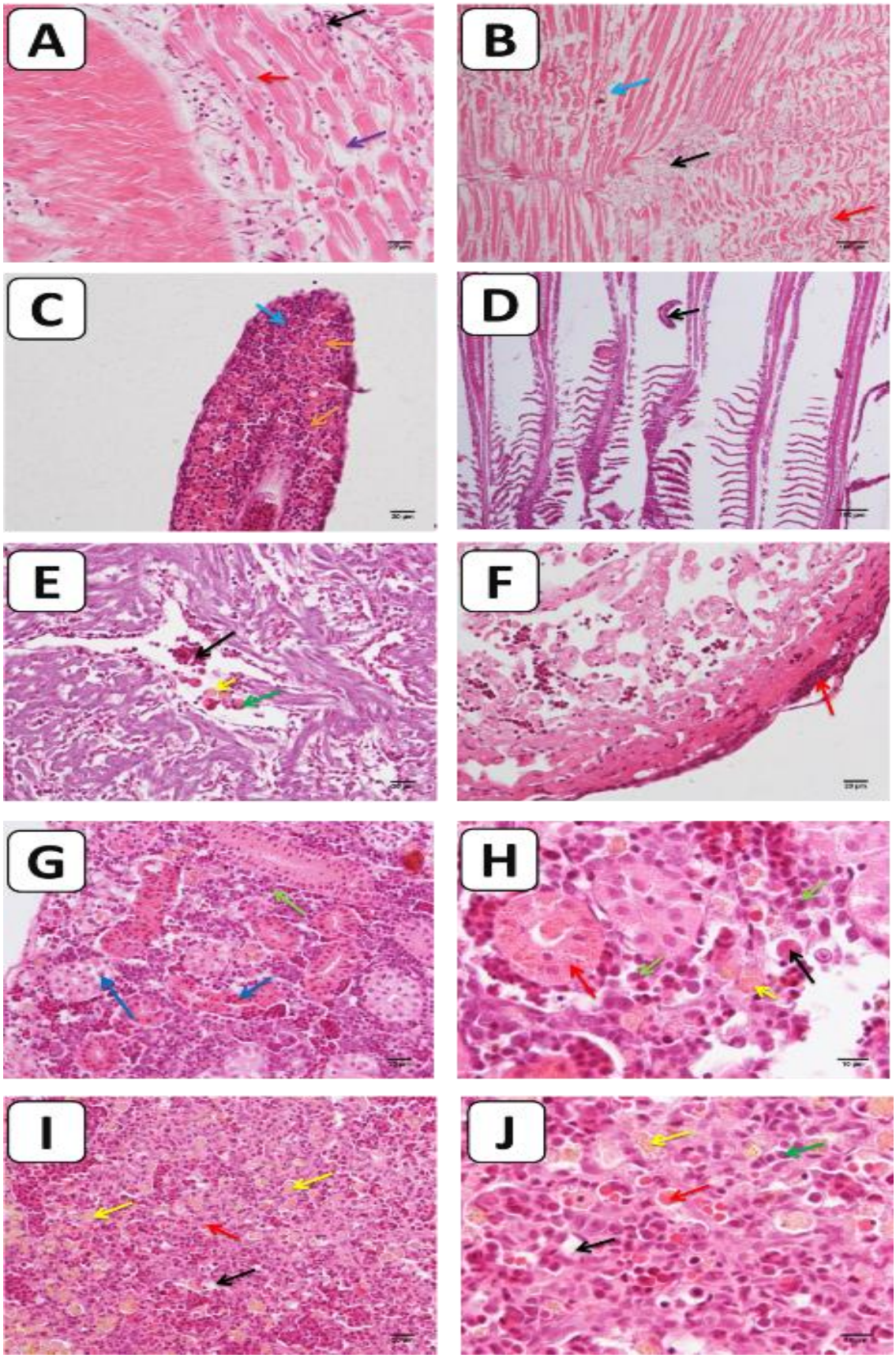

Figure 2: Results of histopathological examination of muscle, gills, heart, kidney, and spleen.

A. Muscle showed severe inflammatory cell proliferation (black arrows), severe myocoagulation (red arrows), and severe necrosis (purple arrows) (HE stain, Bar = 20 µm). B. Existence of severe interstitial cell stretching (blue arrow), severe myocoagulation (red arrow), and severe inflammatory cell proliferation (black arrow) in muscle (HE stain, Bar = 100 µm). C. Gills showed proliferation of mild inflammatory cells (blue arrows) and moderate degrees of eosinophilic granular cells (orange arrows) (HE stain, Bar = 20 µm). D. Mild degree of monogeneans (black arrows) in gills (HE stain, Bars = 100 µm) E. Cardiac bulbus arteriosus showed severe inflammatory cell proliferation (black arrow), a mild degree of hyaline (green arrow), and a mild degree of MMCs accumulation (yellow arrow) (HE stain, Bar = 20 µm). F. The heart also showed severe pericarditis (red arrows) (HE stain, Bar = 20 µm). G. Kidney showed a mild degree of necrosis (blue arrows) and severe inflammatory cell proliferation (green arrows) (HE stain, Bar = 20 µm). H. Kidney showed the presence of hyaline (red arrow), amyloid (black arrow), MMCs (yellow arrow), and inflammatory cells (green arrow) (HE stain, Bar = 10 µm) I. Spleen showed the accumulation of MMCs severe (yellow arrows), severe lymphoid follicle depletion (black arrows), and moderate degrees of hyaline (red arrows) (HE stain, Bar = 20 µm). J. Spleen showed inflammatory cell proliferation (green arrows), depletion of lymphoid follicles (black arrows), MMCs (yellow

arrows), and hyaline (red arrows)

(HE stain, Bar = 10 µm).

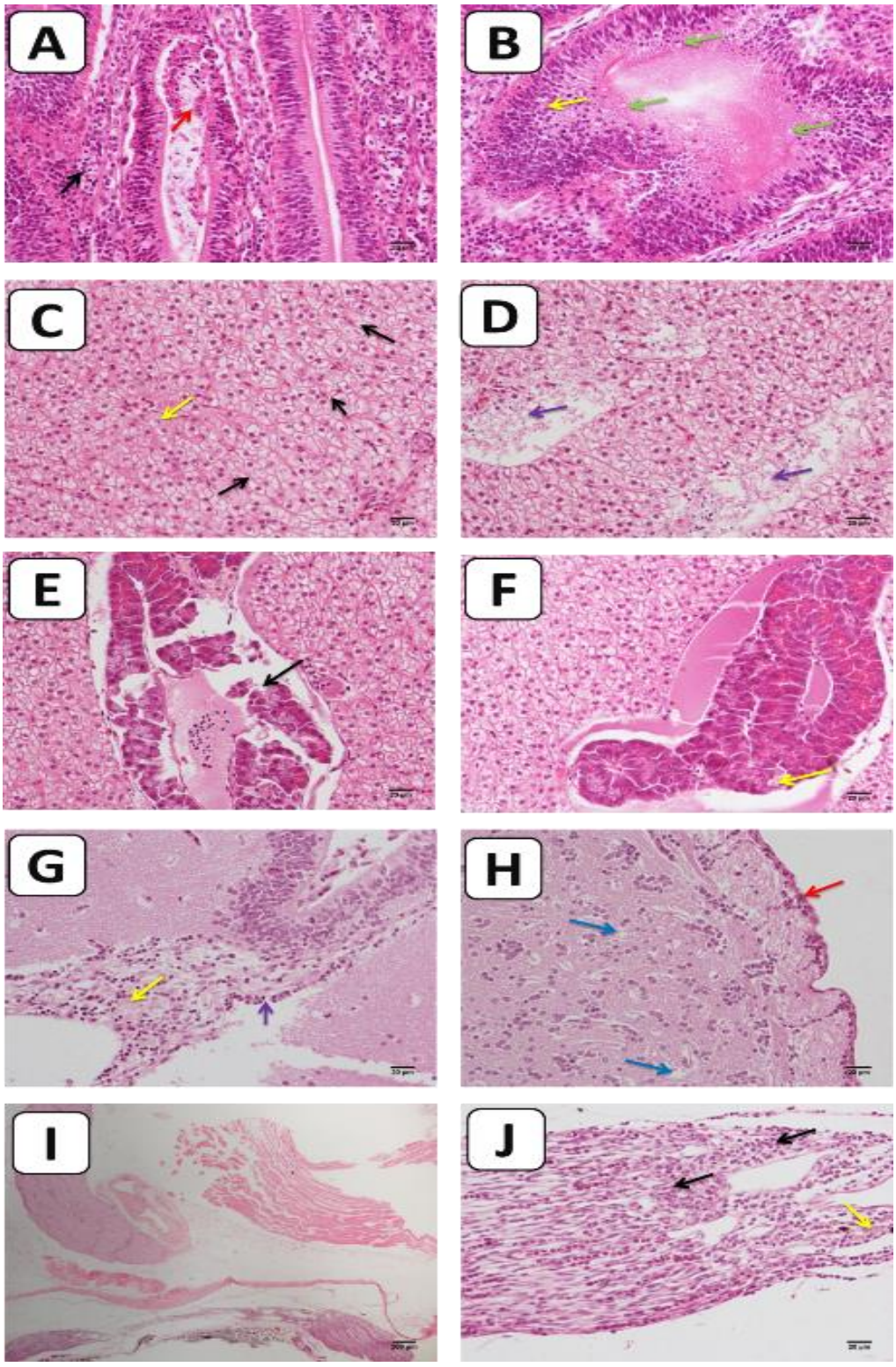

Figure 3: Histopathological examination of the intestine, liver, pancreas, brain, and eyes. A.

Intestinal histopathology showed mild necrosis (red arrows) and severe inflammatory cell proliferation (black arrows) (HE stain, Bar = 20 µm). B. Mild presence of protozoa (green arrows) and mild accumulation of MMCs (yellow arrows) in the gut (HE stain, Bar = 20 µm). C. Liver showed mild MMCs accumulation (yellow arrow) and severe hydropic degeneration (black arrow) (HE stain, Bar = 20 µm). D. The liver had a slight degree of necrosis (purple arrow) (HE stain, Bar = 20 µm). E. Pancreas shows mild necrosis (black arrow) (HE stain, Bar = 20 µm). F. Pancreas also showed a mild degree of MMCs accumulation (yellow arrow) (HE stain, Bar = 100 µm) G. Brain showed the presence of mild gliosis (purple arrows) and mild MMCs accumulation (yellow arrows) (HE stain, Bar = 20 µm) H. Presence of moderate degrees of malacia (blue arrows) and severe meningeal congestion (red arrows) in the brain (HE stain, Bar = 20 µm) I. Histopathological appearance of the eye with HE staining (Bar = 200 µm). J. Eye showed a mild degree of accumulation of MMCs (yellow arrow) and moderate proliferation of inflammatory cells (black arrow) (HE stain, Bar = 20 µm).

Based on the histopathological examination results, infectious disease agents were not found in all the visceral organs examined, such as bacterial colonies or viral inclusion bodies. However, amyloid and hyaline can be found in the kidney. Amyloid is a protein substance that can be a monoclonal antibody light chain, protein A, non-immunoglobulin protein, prealbumin, and β2-microglobulin. Amyloid appears homogeneous and

amorphous under the light microscope and is stained pink with hematoxylin-eosin staining. Hyaline is a substance with a homogeneous texture of white color, shiny, dense, and smooth with hematoxylin-eosin staining. Amyloid and hyaline are lesions that can indicate damage to the process of protein metabolism in individuals. Both of these findings in the kidney may indicate signs of nephritis or be a suppurative process response due to chronic antigen-infected stimulation (Kyle, 2001). However, the kidney damage which triggers inflammatory signs is not only caused by infectious agents. Ko *et al*. (2017) stated that high protein consumption could cause an increase in intraglomerular pressure and glomerular hyperfiltration. These conditions can trigger damage to the glomerular structure leading to chronic kidney disease (CKD) or exacerbating the CKD condition.

The anatomical pathology condition of the thickened muscle was also confirmed on histopathological examination, which showed a severe degree of interstitial muscle stretching. This is presumably due to fluid can accumulate in muscle tissue, causing this condition. The accumulation of melanomacrophage centers (MMCs) in the gills, spleen, kidney, intestine, liver, brain, and eyes indicated a defense response against foreign bodies. The MMCs are a pigmented aggregate produced from the phagocytosis of macrophages as part of the immune system in fish and can be found in the cranial kidney, spleen, and liver (Steinel and Bolnick, 2017).

Molecular examination with Polymerase Chain Reaction (PCR)

Based on the results of the molecular examination of koi herpesvirus (KHV), iridovirus, *Streptococcus agalactiae*, *Francisella* sp., and *Aeromonas* sp. showed that the five pathogens were not detected in the examined samples. The electrophoresis results are presented in Fig 4.

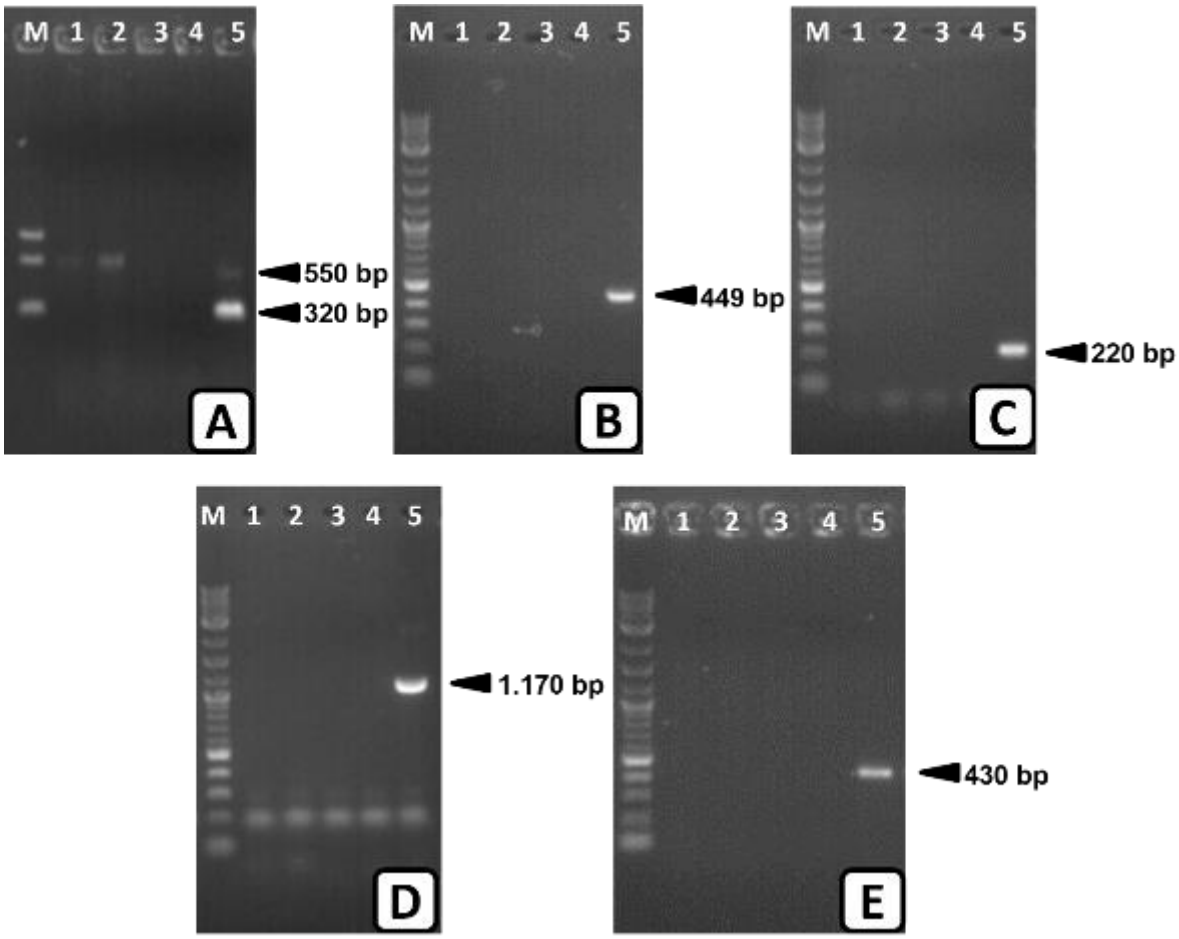

Figure 4: Molecular examination results on koi fish samples with dropsy. A. Koi herpesvirus (KHV). B. Iridovirus. C. *Streptococcus agalactiae*. D. *Francisella* spp... E. *Aeromonas* sp... M. Markers. 1. Gill. 2. Kidney. 3. Coelomic fluid. 4. Negative control. 5. Positive control.

Histopathologically, no infectious agent was found that could potentially cause dropsy. Negative PCR results also confirmed it for KHV, iridovirus, *Streptococcus agalactiae*, *Francisella* sp., and *Aeromonas* sp... Dropsy finding in koi fish in this study is suspected to be sterile or non-infectious due to kidney damage which triggers changes in osmolarity so that fluid can accumulate in the coelomic cavity and cannot be adequately excreted through the urine. This presumptive was also supported

because four other koi in the same aquarium did not suffer dropsy.

The accumulation of inflammatory cells in several internal organs can be caused by sterile necrosis and inflammation. This condition can occur, suggesting the blood urea nitrogen (BUN) level was increased and recirculated to the vascular flow. The renal failure to filter blood caused acute injury, and the tissues were damaged to be necrotic. This necrotic condition can spread cytokine signals that attract inflammatory cells to the center of inflammation so that inflammatory cells can accumulate in visceral organs and muscle tissue. Lau and Vaziri (2016) stated that urea can induce cell apoptosis in the vascular endothelial cells and endothelial dysfunction; it stimulates oxidative stress and dysfunction in adipocytes, leading to insulin resistance conditions. Furthermore, an increase in urea can indirectly cause a carbamylation reaction caused by isocyanic acid (a product of urea catabolism), which can change the structure and function of protein in the body.

Vajargah (2022) reported that antibiotics such as tetracycline, chloramphenicol, neomycin sulfate, penicillin, and nalidixic acid could effectively treat ascites symptoms. However, dropsy that is non-infectious due to irreversible damage to organs such as the kidney is difficult to treat with antibiotics, especially tetracyclines, which are dangerous for individuals with renal failure (Phillips *et al*., 1974). The use of diuretic drugs, such as furosemide, ethacrynic acid, and hydrochlorothiazide, may be considered for koi with dropsy since Nishimura (1977) reported that these three drugs could cause a mild diuretic effect on the kidney of freshwater catfish (*Ictalurus punctatus*). However, the study regarding the effect and appropriate dosage of diuresis drugs in koi still needs further research.

Based on the anamnesis, clinical symptoms, anatomical pathology,

histopathology, and PCR examination, it can be concluded that dropsy in the koi fish in this study was sterile or non-infectious and caused by liver and kidney damage, so the fish could not maintain the body osmolarity. This was based on histopathological and PCR examination, which showed no signs of an infectious agent that can cause dropsy in fish. The prognosis of dropsy found in this study is infausta due to the severity of the kidney damage in fish.

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