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The Conjecture of Causa Mortis In Jenkins' Whipray *Pateobatis jenkinsii* (Annandale, 1909): A Case Report

Dugaan Penyebab Kematian pada Ikan Pari Cambuk Jenkins *Pateobatis jenkinsii* (Annandale, 1909): Laporan Kasus

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Abstrak

Seekor ikan pari cambuk Jenkins jantan tangkapan liar ditemukan mati dalam tangki karantina dengan gejala klinis sebelum kematian adalah nafsu makan berkurang selama seminggu. Riwayat pengobatan yang diberikan adalah pemberian antibiotika enrofloxacin secara peroral. Periode terapi berlangsung selama sepuluh hari. Pengobatan terakhir adalah pemberian Hepavit® (ekstrak hati) dan injeksi intramuskular (IM) antibiotika enrofloxacin. Satu hari sebelum kematian, sampel darah dikoleksi dan kemudian diperiksa untuk mengetahui gambaran hematokrit dan beberapa parameter kimia darah. Hasil pemeriksaan darah ditemukan penurunan kadar *blood urea nitrogen* (BUN), alkaline phosphatase (ALP), dan alanine aminotransferase (ALT), peningkatan kadar glukosa, penurunan total protein dan kadar albumin, serta peningkatan kadar globulin. Pemeriksaan patologi anatomi ditemukan lesi pada ekor, di sekitar mata, dan *clasper.* Lesi hemoragik ditemukan di lapisan mukosa esofagus, lambung, dan kolon spiral. Gumpalan darah ditemukan di bawah lapisan tunika organ testis. Kerusakan organ hati secara makroskopis ditunjukkan dengan perubahan warna yang tidak homogen, pembengkakan organ, kongesti hati, dan konsistensi yang rapuh. Berdasarkan diagnosis morfologik, kausa kematian ikan diduga karena mengalami kondisi infeksi septikemia selama beberapa minggu sebelumnya.

Kata kunci: Elasmobranchii; ikan pari cambuk Jenkins; *Pateobatis jenkinsii***;** septisemia

Abstract

A wild-captive male Jenkins' whipray was found dead in a quarantine tank with a clinical sign before death was appetite decreased for a week. The treatment history was oral administration of enrofloxacin antibiotic tablets. The therapy period lasts for ten days. The last treatment was the administration of Hepavit® (liver extract) and intramuscular injection of enrofloxacin antibiotic. One day before the death, blood was collected and then examined for the hematocrit and some parameters of chemical blood. The results of blood examination were found a decrease in blood urea nitrogen (BUN), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) levels, increased glucose level, decreased total protein and albumin levels, and increased globulin level. Anatomical pathology examination was found lesions on the tail, around the eyes, and claspers. Hemorrhagic lesions were found in the mucous layer of the esophagus, stomach, and spiral colon. The blood clot was found under the tunica layer of testicular organs. Macroscopically, the liver is damaged by showing a non-homogeneous coloration,

organ thickening, congestion, and fragile consistency. Based on the morphological diagnosis, the fish is suspected died due to the condition of septicemia infection during the previous few weeks.

Key words : Elasmobranchs; Jenkins' whipray; *Pateobatis jenkinsii*; septicemia

Introduction

There are many types of marine creatures that live in the oceans on Earth. These creatures can be invertebrates to the mammals. Stingray is one type of fish that lives in the vast ocean. Until now there are approximately 220 species of stingrays which are classified into 10 families and 29 genera. One of the stingray species is *Pateobatis jenkinsii* or Jenkins' whipray. It was obtained from the catch of the Bengal fisheries steamer, "Golden Crown" (Annandale 1909). As for its classification, this stingray belongs to the kingdom of Animalia, phylum Chordata, class Chondrichthyes, order Mylibatiformes, family Dasyatidae, genus *Pateobatis*, and species is *Pateobatis jenkinsii*. There are several synonyms of the species, such as *Dasyatis jenkinsii, Dasyatis* (*Amphotistius*) *jenkinsii, Dasyatis* (*Himantura*) *jenkinsii, Dasyatis jenkinsii, Himantura draco, Himantura jenkinsii,* and *Trygon jenkinsii* (Pollerspöck & Straube 2009).

According to the data on the International Union for Conservation of Nature and Natural Resources (IUCN) Red List, the geographical distribution of Jenkins' whipray in nature is in the territorial sea of several countries in the world, including: Australia (North coast, Queensland, and West Australia), Bangladesh, Brunei Darussalam, India, Indonesia, Madagascar, Malaysia, Mozambique, Myanmar, Pakistan, Papua New Guinea, Philippines,South Africa, Sri Lanka, Thailand, and Yaman (Socotra area). This type of stingray lives in the sea at a depth of 50–90 m and was reported seen on coral reefs in Thailand. The maximum size of the disc from the Jenkins' whipray can reach a length of 150 cm with a total body length of 300 cm. The width of the discs in males ranges from 75–85 cm on average and at birth is 23 cm. The female reproduction is viviparous with histotropy and the estimated age of a generation of stingrays can reach 20 years in nature (IUCN 2016). According to the latest assessment data on May 12, 2015, Jenkins' whipray populations in the wild globally have been decreased as well as in the vulnerable category by IUCN Red List (Matsumoto et al. 2016). Uncontrolled fishing and excessive exploitation are thought to be the cause of the decline in Jenkins' whipray populations. Lost or degradation of its habitat as a decrease in the mangrove area is also another major threat to marine and coastal species. Jenkins' whipray is an important side catch for fishermen in Indonesia for its meat. The skin and cartilage have high economic value in the Southeast Asia region (IUCN 2016).

In addition to the threat of population decline caused by human activities, the presence of various diseases that can be suffered by the fish from the subclass of Elasmobranchii, such as sharks and rays, can also be one of the potential causes of the death. The elasmobranchs subclass can suffers various diseases, such as infectious or inflammatory diseases, nutritional disorders (emaciation), trauma, cardiovascular disorders (shock), and toxicity diseases. The infectious disease is the most common found. The infectious diseases that can attack stingrays, including: bacterial sepsis, fungal infections, nematode infestations (*Huffmanella* sp.), coccidiosis due to *Eimeria* spp., and cestoda infestations such as *Anthocephalum jensenae*, *Dollfusiella ocallaghani*, *Parachristianella* sp., *Proemotobothrium* sp., and *Trygonicola macroporus* (Garner 2013, Pollerspöck & Straube 2009). The existence of a disease that causes death can usually be easily found and traced its cause in stingrays that live in ex-situ conservation, such as in public aquariums. However, not many cases of the disease have been studied and there is still a lack of reference to assist in making the diagnosis of a disease in *Pateobatis jenkinsii* a challenge for aquatic veterinarians who handle it. The scarcity of reports of cases in aquatic animals found in the field, especially in *Pateobatis jenkinsii*, is very important to be reported as part of efforts to enrich the treasury of aquatic veterinary medicine and can be a reference for differential diagnoses in the future.

Materials and Methods

The material used in this clinical case was a Jenkins whipray (*Pateobatis jenkinsii*) that was found dead during treatment in quarantine tanks, syringe 3 mL, ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes, measuring tape ribbon, and a necropsy toolset (dressing forceps, toothed tissue forceps, operating scissors, scalpel and blade, and knife). The diagnostic methods were the analysis of the results of hematological and blood chemistry examination that were conducted with anatomical pathology findings. The blood examination was done by collecting the blood with syringe 3 mL from the caudal vein. Then, the blood sample was placed on EDTA anticoagulant tubes and was referred to the Clinical Diagnostic Laboratory, Division of Internal Medicine, Department of Clinical, Reproduction, and Pathology, Faculty of Veterinary Medicine, IPB University, Indonesia. The anatomical pathology methods were done by external examination for the first, include skin, scales, eyes, mouth cavity, and gills. Then, the abdominal cavity examination was done by making an incision in the ventral disc started from the cloaca and rotate around the abdominal cavity with the limit below the gills then return to the cloaca. After the abdominal cavity was opened, the visceral organ in the abdominal cavity was then inspected before was removed from the visceral site. Inspection, palpation, and incision were then carried out starting from the organs of the digestive tract in the form of the esophagus, stomach, spiral colon, liver, and cloaca. Then proceed with the examination of the lymphoreticular organ in the form of the spleen and genitalia organ in the form of testes. The organs and parts of the body that showed abnormalities or lesions were then documented and recorded.

Results and Discussions

Signalements

The species of animal is Jenkins' whipray (*Pateobatis jenkinsii*) (Figure 1). The gender is male. The age is unknown. The color is blackish brown. The body size are 125 cm (head to claspers), 135 cm (disc width), and 212 cm (total length – head to tail).

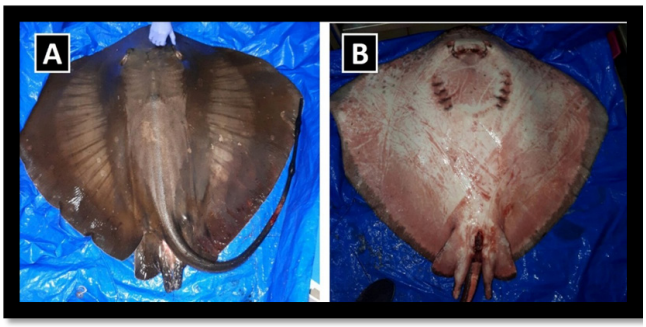

Figure 1 The individual appearance of Jenkins' whipray (*Pateobatis jenkinsii*) that was found dead in the Jakarta Aquarium's quarantine tank. (A). The dorsal portion of the disc. (B). The ventral portion of the disc.

Anamnese

A wild-captive male Jenkins' whipray (*Pateobatis jenkinsii*) was maintained on the Jakarta Aquarium's main tank was complained in the form of decreased appetite by aquarists for about a week. The history of treatment therapy on August 17–26th, 2017 was given enrofloxacin antibiotic tablets orally (peroral, PO) through feed once a day. On August 28th, 2017, the blood sample was taken using an EDTA anticoagulant tube and therapy was Hepavit® (liver extract) and an intramuscular (IM) injection of enrofloxacin antibiotics once a day. However, on August 29th, 2017 the fish was found dead in a quarantine tank.

Examination results

One day before death the whole blood sample had examined to see the hematological profile (hematocrit) and some blood chemical parameters. The blood examination results present in Table 1. After the stingray was found dead, then was done a necropsy. Based on the results of the external anatomical pathology examination was found several lesions and abnormalities in the visceral organs as follows. The external examination found any white patches on the dorsal disc and lesions on the tail, claspers, and under the eyes. The results of skin scrapings were taken from white patches on dorsal disc found ectoparasites in the form of copepods (Figure 2).

The coelomic cavity was found to have edema or *dropsy* (Figure 3). In the esophagus, the lesion was found in the form of mucosal discoloration that is not homogeneous (hemorrhagic) and

Table 1. Results of hematology and blood chemistry examination

No.	Parameters	Result	Reference	Unit	Information
1.	Hematocrit	24	15-25[a]	%	Normal
2.	Total protein	4,0	4,2-6,0[a]	g/dL	Low
3.	Sodium (Na^+)	>180	295,9-326,2[a]	mmol/L	Normal
4.	Potassium (K^+)	7,8	3,2-6,4[a]	mmol/L	High
5.	Calsium (Ca^{2+})	16,8	12,06-19,3[a]	mg/dL	Normal
6.	Phosphorus (P)	3,2	3,0-6,4[a]	mg/dL	Normal
7.	Blood urea nitrogen (BUN)	48	1.184,9-1.293,1[a]	mg/dL	Low
8.	Glucose	60	16,9-42,4[a]	mg/dL	High
9.	Creatinine	1,3	0,1-0,1[b]	mg/dL	High
10.	*Alkaline phosphatase* (ALP)	18	22-46[b]	U/L	Low
11.	Alanine aminotransferase (ALT)	12	13-25[c]	U/L	Low
12.	Total bilirubin	0,3	0,1-0,3[b]	mg/dL	Normal
13.	Albumin	0,0	0,5-0,8[b]	g/dL	Low
14.	Globulin	60	1,4-3,6[b]	g/dL	High
15.	Amylase	0	?	U/L	?

[a] Cain et al. 2004; [b]Ferreira et al. 2010; [c]Brito et al. 2015

there is yellowish mucus. In gastric was found hemorrhagic purpura in the mucosal layer. In the mucosa of the spiral colon was found hemorrhagic lesion. The examination of the cloaca was found in reddish mucosal color (hemorrhagic). In the spleen, there was a mass with soft consistency such as contained fluid in the edge of the spleen. In the testicular organs, lesion was found in blood clot below the tunica layer (Figure 4). The liver

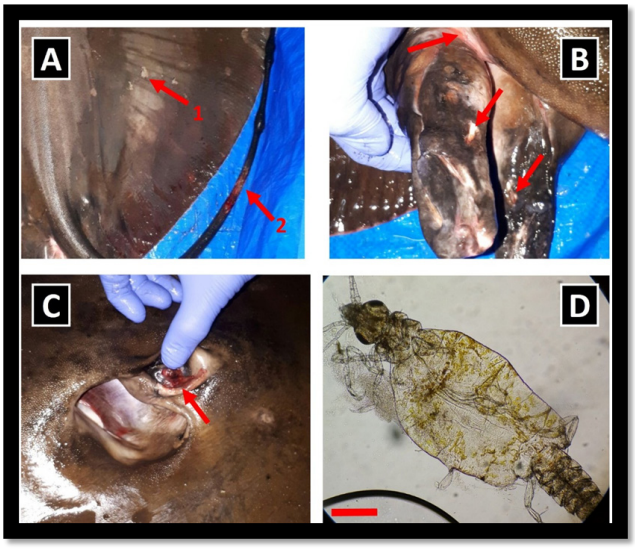

Figure 2 Results of the external anatomical pathology examination of the Jenkins' whipray (*Pateobatis jenkinsii*). (A). There are white patches on the dorsal disc (red arrow with number 1) and lesion on the tail (red arrow with number 2). (B). There were lesions in the claspers (red arrow). (C). There is a lesion under the eyes (red arrow). (D). Copepods ectoparasites were found in skin scrapings taken from the white patches in the dorsal disc. Objective lens 10X zoom in. Scale bar: 100 µm.

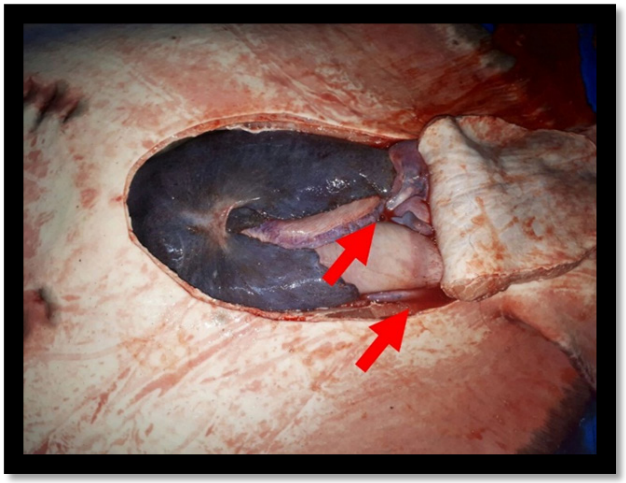

Figure 3 Incisions in the coelomic cavity. Examination results found a buildup of reddish-colored fluid (edema or *dropsy*) that are showed by a red arrow.

showed colors that were not homogeneous. The liver also showed other significant pathological changes, such as: also found fragile consistency, a thickening of the edge of the liver, and when an incision was made there was a condition of liver congestion was marked by the blood coming out when incised (Figure 5). The histopathological and microbiological examination was not done because there were obstacles in the laboratory referred to.

Discussions

Non-pathognomonic clinical symptom as well as a decreased appetite for about a week

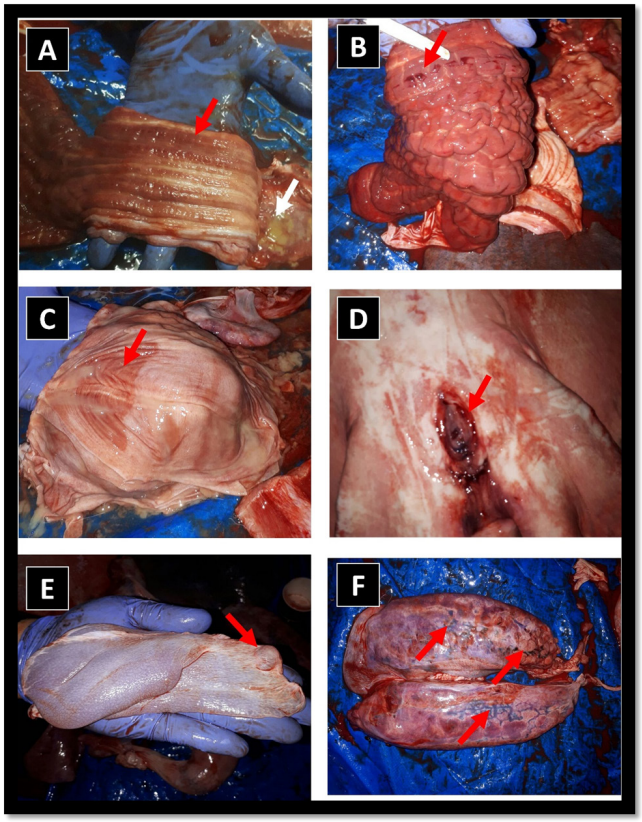

Figure 4 Anatomical pathology examination results of visceral organs which showed abnormalities. (A). Hemorrhagic lesions of the esophageal mucosal layer and found yellowish mucus (white arrow). (B). The hemorrhagic lesion with a purpura form in the gastric mucosal layer. (C). The hemorrhagic lesion in the spiral colon mucosal layer. (D). The hemorrhagic lesion in the cloaca. (E). There was a mass with soft consistency on the edge of the spleen. (F). Blood clot lesion under the tunica layel of the testis. The lesions are showed by a red arrow.

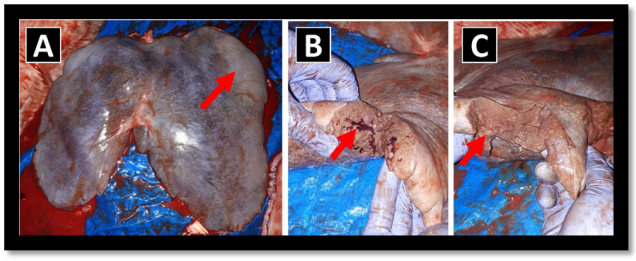

Figure 5 Results of examination of anatomical pathology of the liver. (A). The color of the liver is not homogeneous. (B). There was a thickening of the edge of the liver and found blood congestion when the incision. (C). The consistency of the liver becomes fragile on the other side of the liver. The lesions are showed by a red arrow.

was reported by the aquarists made several presumptions to the causes of the disease was suffered by a Jenkins' whipray. There was corpus alienum, infectious of microorganisms, and the disease causes toxicity into several presumptions were made. However, the results of a necropsy on the digestive tract examination did not reveal any corpus alienum. The results of the monitoring of water quality in the tank where the Jenkins' whipray was placed did not also indicate abnormalities. Strong suspicion of the cause of the disease was the presence of microorganism infections. The finding of lesions on the tail, around the eyes, and the claspers on the external examination was thought to be caused by a microorganisms infection, such as a bacterial infection. Copepods that were found in skin scrapings can worsen the condition of fish when the body was suffering an infection. Poecilostomatoida and Siphonostomatoida are two orders whose members which can infect many saltwater fish (Noga 2010).

A blood examination is done by comparing the results and normal ranges that refers to several species of stingrays because there is no specific study of the normal profile of hematology and blood chemistry in Jenkins' whipray (*Pateobatis jenkinsii*). The results showed that there was a decrease BUN level. The low BUN level in elasmobranchs shows an abnormal condition in the kidney organs. Normally, the BUN level always shows a very high level because it is to maintain the balance of osmolarity in the saltwater environment. High creatinine level are normal and are found in many marine fish species (Stoskopf et al. 2010). Blood chemistry also shows a decrease in ALT and ALP levels and an increase in blood glucose. Some cases of septicemia infections, such as bacterial infection like *Aeromonas spp.*, can decrease ALT level and increase glucose in the serum and some cases can cause anemia conditions which are indicated by a decrease in hematocrit, hemoglobin, and total erythrocytes count (Brenden & Huizinga 1986). In this case the hematocrit level is normal.

In blood examination results also found a decrease in total protein and albumin. Albumin is a major protein that is very important in maintaining osmotic pressure which regulates the movement of water and solutes in capillaries. Decreased or absent albumin level in serum can causes condition where water easily seeps out capillary vessels into interstitial tissue and is accommodated in body cavities, then causing edema conditions (Busher

1990). This condition was found at the first time of the incision when anatomical pathology examination was done. Albumin also plays a role in the transport of bilirubin, hormones, metals, vitamins, and drugs (Busher 1990). The absence of albumin is one of the other presumption that therapy using enrofloxacin antibiotic that had been given for ten days did not work well because it was not perfectly distributed in the body, other than the possibility of antibiotic resistance in bacteria that cause infection. Bacteria *Aeromonas spp.* which can cause septicemia can be found to be resistant to several antibiotics (Shotts et al. 1976, Dixon et al. 1990). Albumin is synthesized in the liver, if the liver suffers the damage or damage at the final level, it can decreases the concentration of albumin in the blood. The presence of liver damage was found, such as the thickening of the edges of the liver, changes in the color of the liver that were not homogeneous, the consistency of a fragile liver on some sides, and the presence of congestion in the liver was thought to be the cause of decreased albumin level. The condition of liver damage was thought to be caused by an infection with septicemia that had attacked the liver tissue. The increase in globulin in the results of blood chemistry examination showed that it is suspected that this also happened because of the condition of the infection. Globulin, like immunoglobulin, has an important role in the specific defense mechanism against microorganisms infection (Busher 1990). The amylase can not be discussed due to there is not any normal level references in Jenkins' stingray or other species for this parameter.

Septicemia is a bacterial infection condition that has spread systemically throughout the body. One of the bacteria that can cause septicemia in fish is *Aeromonas spp.* Infection of the genus *Aeromonas* usually causes disease in the form of motile aeromonad infection (MAI). Most MAI generally infects freshwater fish, but can sometimes be found in brackish or saltwater environments (Noga 2010, Hazen et al. 1978, Larsen & Jensen 1977). *Aeromonas hydrophila, A. jandaei, A. salmonicida,* and *A. sobria* are several species of *Aeromononas* that are pathogenic and can attack the fish (Toranzo et al. 1989, Esteve et al. 1993, Irianto 2005). *Aeromonas Salmonicida* is a cause of furunculosis in salmonids. However, some strains of the bacteria can infect freshwater fish. Septicemia which is caused by *A. salmonicida* will shows the clinical symptoms in the form of hemorrhage in the muscles of the body and other parts, ulcerative dermatitis, swelling of the spleen and kidneys, and ascites (Irianto 2005). Opportunistic bacteria, such as *Aeromonas spp*., in the environment can be found in stingray mucus so that it allows an easy invasion enter to the body. Opportunistic bacteria can become pathogenic if the individual's immune system declines due to several causes, such as environmental stress conditions that is caused by high fish populations (Domingos et al. 2011, Irianto 2005).

The clinical symptoms of MAI that can be found are very diverse ranging from superficial to deep skin infections. Gram-negative bacterial septicemia can be accompanied by skin lesions. The presence of hemorrhage and necrosis of the skin and fins, ulcers that turned progressively towards hemorrhagic septicemia, there is exophthalmos, stomach bulging liquid serosanguineous, hemorrhagic (petechiae), and the presence of hemorrhage and swelling in the digestive tract can be found in the results anatomical pathology examination of fish that have been suffered by MAI. Anorexia and darker body discoloration are common signs of systemic diseases (Noga et al. 2010). If it is continued on histopathological examination, MAI will show a skin lesion condition in the form of dermatitis or myositis which is acute to chronic. Septicemia can also causes depletion or necrosis of the kidneys to the spleen hematopoietic tissue, necrosis of the intestinal mucosal layer, focal necrosis of the heart, liver, pancreas, and gonads (Bach et al. 1978, Huizinga et al. 1979).

Septicemia is a condition in which there is a systemic inflammatory response due to an infection that involves a complex interaction between the host and the infectious agent. These interactions can increase the risk of death of individuals due to metabolic and cellular changes in the host's body. Molecularly, complement activation due to an abundance of anaphylatoxin C5a in the serum during the innate immune response stage and TLRs (toll-like receptors) family is a role in the incidence of septicemia

(Pop-Began 2014). Septicemia starts with the entry of bacteria which initially only causes a self-limited inflammatory response in the local area. If in normal conditions, the bacteria that enter can be eliminated by the innate immune system. However, if the inflammatory response cannot be overcome due to environmental stress conditions that cause a decreased immune response, then the bacterial infection will change beyond the body's compensation control limit in an effort to eliminate bacteria so that bacteria can leave the local area and begin to enter in the bloodstream. Bacteria can spread throughout the body's tissues through blood vessels. This condition can develop Multiple Organ Dysfunction Syndrome (MODS) due to compensatory anti-inflammatory response that is not running normally. MODS can increases the mortality of individuals who suffer septicemia (Bone 1992).

Conclusion

Considering the results of blood and anatomical pathology examinations, we conclude that the diagnosis leads to the infection with septicemia caused by bacteria. In spite of the management of fish welfare that had done as maximum as possible, the existence of individual stress factor during the process of adaptation to the new aquarium environment is can be thought to play a role in decreasing the immune system which results in changes opportunistic bacteria in environmental to be pathogenic which causes septicemia. The condition of septicemia is thought to have developed into MODS as a cause of death. These things were represented by the changes in the liver organ in the anatomical pathology examination and there was kidney organ abnormality as indicated by decreasing the BUN level in the blood chemistry examination.

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