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Physical and molecular identification of fish and shrimp diseases in some farms of West Java, Banten and Jakarta, Indonesia

Identifikasi fisik dan molekuler penyakit ikan dan udang di beberapa tambak di Jawa Barat dan Jakarta, Indonesia

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ABSTRACT

Infectious disease is a major challenge in fish and shrimp farming systems caused by microorganisms including bacteria, viruses and parasites. Physical detection has limitations in detecting diseases in cultivated animals earlier, because of the varied clinical symptoms. The aims of this study was to identify the physical and molecular presence of infectious pathogens in fish and shrimp cultivated in several ponds in West Java, Banten and Jakarta. Monitoring was carried out in two stages, namely the period March–April 2021 and September 2021 by taking samples from several ponds in Bogor, Tangerang, Depok and Jakarta. The sample criteria used were fish and shrimp showing normal and clinical symptoms of being attacked by a disease which were collected by purposive sampling. The collected samples were examined in two stages, namely physical examination of clinical symptoms and molecular examination using PCR. The results showed that of the 24 species samples collected, 17 species showed normal clinical symptoms and seven species showed clinical symptoms. Of the 24 fish and shrimp samples, four samples were infected (2 samples from normal species and 2 samples with clinical symptoms). The infected normal samples were Osphronemus goramy and Litopenaeus vannamei. The infected samples with clinical symptoms were Carassius auratus by Aeromonas salmonicida and red zebra cichlid (Metriaclima estherae) by Red Sea Bream Iridoviral Disease (RSBIV). Physical testing supported by molecular detection of aquatic animals can be an effort to manage aquaculture systems in Indonesia.

Keywords: aquaculture, emerging disease, fisheries products, molecular assay

ABSTRAK

Penyakit infeksi adalah tantangan utama pada sistem budidaya ikan dan udang yang disebabkan oleh mikroorganisme termasuk bakteri, virus, dan parasit. Deteksi secara fisik memiliki keterbatasan dalam mengetahui lebih dini penyakit pada hewan budidaya, karena gejala klinis yang bervariasi. Tujuan dari penelitian ini adalah untuk melakukan identifikasi fisik dan molekuler keberadaan patogen infeksi pada ikan dan udang yang dibudidayakan di beberapa pertambakan di Jawa Barat, Banten, dan Jakarta. Pemantauan dilakukan dalam dua tahap yaitu periode Maret – April 2021 dan September 2021 dengan mengambil sampel dari beberapa pertambakan di Bogor, Tangerang, Depok, dan Jakarta. Kriteria sampel yang digunakan adalah ikan dan udang yang menunjukkan gejala klinis terserang oleh penyakit yang dikumpulkan secara purposive sampling. Sampel yang terkumpul diperiksa dalam dua tahap yaitu pemeriksaan fisik gejala klinis dan pemeriksaan molekuler menggunakan PCR. Hasil menunjukkan dari 24 sampel spesies yang terkumpul, sebanyak 17 spesies menunjukkan gejala klinis normal dan tujuh spesies menunjukkan gejala klinis. Dari 24 sampel ikan dan udang, terdapat empat sampel yang terinfeksi (2 sampel dari spesies normal dan 2 sampel dengan gejala klinis). Sampel normal yang terinfeksi adalah Osphronemus goramy dan Litopenaeus vannamei. Sampel yang terinfeksi dengan gejala klinis adalah Carassius auratus oleh Aeromonas salmonicida dan red zebra cichlid (Metriaclima estherae) oleh Red Sea Bream Iridoviral Disease (RSBIV). Pengujian fisik yang didukung dengan deteksi molekuler pada hewan budidaya dapat menjadi upaya manajemen sistem akuakultur di Indonesia.

Kata kunci: akuakultur, emerging disease, perikanan, pengujian molekuler

INTRODUCTION

Today, the aquaculture industry becomes one of the fastest growing industries not only in Indonesia but also in various countries in the world (Lee *et al*., 2022; Moreira *et al*., 2021). It may be caused by the reason of main source of protein and important nutrients needed by the wider community is from fish (Hayatgheib *et al*., 2020; Komolka *et al*., 2020; Vergis *et al*., 2021; Bedane *et al*., 2022; Purwanto *et al*., 2022; Yang *et al*., 2022). Likewise, ornamental fish is currently popular as pets and for business purposes (Becker *et al*., 2018). With the increasing public need for these fishery products, the industrialization of the fisheries sector continues to experience a significant increase, especially from intensive aquaculture activities (Soelistyoadi *et al*., 2019; Moreira *et al*., 2021; Kazangeldina *et al*., 2022).

However, an increase in the demand for and production of fishery products correlates with the risk of disease that may reduce the productivity of aquaculture products and thus reduce the quality of fishery products (Ador *et al*., 2022; Salem *et al*., 2020; Soelistyoadi *et al*., 2019). Diseases caused by pathogenic infections are one of the reasons for a decrease in the amount of production in aquaculture (Rahmawati *et al*., 2021; Salem *et al*., 2020). It is because fish and shrimp infected with pathogens will quickly spread their virulence factors to other individuals that causes mass mortality (Ador *et al*., 2022; Moreira *et al*., 2021; Rahmawati *et al*., 2021; Salem *et al*., 2020; Yang *et al*., 2022). In order to detect accurately early symptoms of infection accurately and prevent the spread of infection from spreading, it is necessary to monitor periodically by implementing fish and shrimp health checks.

The pathogens examined were viruses, bacteria and parasites. Viruses and bacteria are the most common infectious agents found in freshwater to seawater aquaculture activities (Ariff *et al*., 2019; Febrianti *et al*., 2021; Rahmawati *et al*., 2021; Salem *et al*., 2020; Soelistyoadi *et al*., 2019; Sukenda *et al*., 2020). As well as parasites that act as initial intermediaries for secondary infections (Riandi *et al*., 2021; Fira *et al*., 2021). Health checks are carried out by collecting samples in the form of fishery products and documenting test parameters such as clinical symptoms and pathogen detection results with a series of tests that have been implemented by official institutions (Ador *et al*., 2022; Lee *et al*., 2022). Examination of clinical symptoms was first carried out by looking at morphological differences and changes in behavior in aquaculture ponds (Moreira *et al*., 2021; Macaulay *et al*., 2022; Yang *et al*., 2022).

However, examination of clinical symptoms cannot be used as basic data. It is because the morphological changes that often occur are not clearly visible or some individuals show few or no clinical symptoms (Moreira *et al*., 2021; Chanu *et al*., 2022). On the other hand, several diseases caused by pathogens show similar clinical symptoms. For example, Epizootic Haematopoietic Necrosis Virus (EHNV), Infectious Haematopoietic Necrosis Virus (IHNV), Viral Nervous Necrosis (VNN), *Aeromonas salmonicida*, and *Vibrio* spp. have the same clinical symptoms that are difficult to distinguish, namely discoloration of the scales to darken, protruding eyes, enlarged abdomen, lethargy, and loss of appetite (Ahmadivand *et al*., 2017; Juniar *et al*., 2018; Moreira *et al*., 2021; Rahmawati *et al*., 2021; Rozi *et al*., 2018; Salem *et al*., 2020; Ziarati *et al*., 2022).

Therefore, testing the efforts with a molecular approach need to be used to detect the presence of infectious diseases in fishery products (Abdelsalam *et al*., 2022; Chanu *et al*., 2022; Kurniawati & Pursetyo, 2021; Yang *et al*., 2022; Yu *et al*., 2022). The Polymerase Chain Reaction (PCR) method is a molecular technique that has been widely used for the rapid diagnosis of pathogens found in fishery products but still with high sensitivity and specificity (Austin, 2019; Padr *et al*., 2022; Zorriehzahra *et al*., 2021). The PCR method works by duplicating certain nucleotide sequences so they can be detected, in this case the targeted nucleotide sequences can come from bacteria, viruses, or parasites (Kurniawati & Pursetyo, 2021).

Polymerase chain reaction (PCR) methods have been developed for species detection, fishery product authentication, measurement of immune gene expression, and the presence of pathogenic microorganisms in aquaculture system. However, reports regarding efforts to monitor the presence of pathogenic microorganisms in fishery products that are collected from ponds in several areas in West Java, Banten, and Jakarta, Indonesia. The purpose of this study was to determine the presence of infectious pathogenic contamination through periodic monitoring and to determine the relationship between clinical symptoms and pathogen infection in fishery products collected from West Java, Banten, and Jakarta.

MATERIALS AND METHODS

Study area and sampling methods

This research was conducted in two periods, in March-April 2021 and in September 2021. Monitoring and collection of fish and shrimp samples were carried out in 18 locations (first period) and 14 locations (second period). The monitoring locations consisted of ponds, farms and fish hatcheries spread across Tangerang Regency, Bogor Regency, Bogor City, Depok City, West Jakarta and South Jakarta. Samples of fish and shrimp in each pond in several Regencies/ Cities were determined using a purposive sampling method. The physical examination was carried out at the necropsy laboratory at the Fish Quarantine Center, Quality Control and Safety of Fishery Products Jakarta I (BBKIPM Jakarta I). The molecular examination for pathogenic bacteria was carried out at the Microbiology Laboratory of BBKIPM Jakarta I and molecular examination for viruses and parasites was carried out at the Virology Laboratory of BBKIPM Jakarta I.

Physical assay

Physical examination consisted of monitoring the fish and shrimp behavior at the monitoring location. Fish and shrimp showing diseased behavior were collected and put into sterile clear plastic filled with water and oxygen. The plastic bag was then stored in a cooler containing ice gel for further transportation to the laboratory. At the necropsy laboratory, fish and shrimp were immediately removed for euthanasia and their average length and weight were recorded. Then a second physical examination was carried out, namely morphological and anatomical examination of the internal organs of fish and shrimp (Wiradana *et al*., 2021a; Wiradana *et al*., 2022b). Each sample of fish and shrimp was labeled with a sampling location.

The external and internal organs of fishery products were examined carefully, the clinical symptoms that appear in the samples were recorded and documented. Each sample was taken for its organs to be extracted or cultured on a microorganism medium. As for bacterial examination, parts of the shrimp hepatopancreas, liver, intestine, kidney and fish spleen were collected in an Eppendorf tube containing semi-solid media for further culture. Meanwhile, for virus and parasite examination, parts of the hepatopancreas, prawn walking legs, swimming legs, tail, fins, gills, eyes and brain were collected in a 1.5 mL of microtube filled with 95% alcohol for further extraction. Molecular examination was carried out on all sample species with the target pathogen in each shown in Table 1.

Bacterial culture and propagation

Culture and propagation of *Aeromonas salmonicida* was carried out in the following way: swab samples of fish organs were cultured on blood agar (BA, Oxoid) and then incubated at 28°C. The growing bacterial colonies were then purified in tripton soya broth (TSB) media (Oxoid, England) and incubated at 28°C for 48 hours (Febrianti *et al*., 2021). *Edwardsiella ictaluri* culture and propagation was carried out in the following way: first, ose scrapes from fish kidney organs were cultured on brain heart infusion agar (BHIA) media (Lab M) and incubated at 28°C for 24 hours. Then, the growing bacterial colonies were purified three to four times using the multilevel loop scratch method on the same media (Purwaningsih *et al*., 2019).

Furthermore, culture and propagation of *V. parahaemolyticus* was carried out by scraping ose from the organ and culturing it on thiosulfate citrate bile salt sucrose (TCBS) medium (Difco, USA) and incubating at 37°C for 24 hours. The green bacterial colonies were then transferred to CHROMagar Vibrio (CHROMagar) media (Paris, France). Mauve colonies on CHROMagar Vibrio were then purified on tryptone soy agar (TSA) media (Difco, Becton- Dickinson Co.) and repeated three times.

DNA and RNA extraction

Bacterial DNA extraction was carried out from the culture stock obtained in the previous stage. Briefly, 500 µL of bacterial culture stock were centrifuged in 1.5 mL microtubes (Cleaver Scientific Ltd., UK) at 15,000 ×rpm for five minutes. The supernatant was then discarded and 200 µL of nuclease-free water (NFW) (Qiagen, Germany) was added and then vortexed. The resulting vortex solution was then heated in a thermoblock (Labnet, China) at 95°C for six minutes. Next, the solution was recentrifuged at the same speed for five minutes. The supernatant obtained was then stored at -20°C for use in the next stage (Hasanah *et al*., 2022).

Extraction of DNA, RNA virus and Hepatobacter penaei was carried out by adding 0.02 grams of target organs and placing them in a microtube filled with 400 µL of phosphate

Table 1. Types of fishery products and target pathogens detected in this study.

No.	Species	Common name	Pathogen		
			Virus	Bacterial	Parasite
1	*Litopenaeus vannamei*	Pacific white shrimp	WSSV, TSV, IHHNV, IMNV, YHV, CMNV	AHPND, NHP	EHP
2	*Metapenaeus monceros*	Speckled shrimp	WSSV, TSV, IHHNV, IMNV, YHV, CMNV	AHPND, NHP	-
3	*Penaeus monodon*	Giant tiger prawn	WSSV, TSV, IHHNV, IMNV, YHV, CMNV	AHPND, NHP	-
4	*Pangasius sp.*	Iridescent shark	-	*Edwarsiella ictaluri*	-
5	*Carrasius auratus*	Goldfish	KHV, SVC	*Aeromonas salmonicida, Edwarsiella ictaluri*	-
6	*Cyprinus carpio*	Common carp	KHV, SVC	*Aeromonas salmonicida*	-
7	*Poecilia reticulate* (juvenile)	Guppy (juvenile)	SVC, VNN	-	-
8	*Poecilia reticulate* (adult)	Guppy (juvenile)	SVC, VNN	-	-
9	*Poecilia reticulata* (fingerlings)	Guppy (fingerlings)	SVC, VNN	-	-
10	*Osphronemus gouramy*	Gourami	RSBIV	*Aeromonas salmonicida*	-
11	*Rasbora sp*.	Rasbora	RSBIV	-	-
12	*Paracheirodon innesi*	Neon Tetra	RSBIV	-	-
13	*Betta sp.*	Siamese fighting fish	RSBIV	-	-
14	*Pterophyllum scalare*	Manfish	RSBIV	-	-
15	*Apistogramma sp.*	Cichlid	RSBIV	-	-
16	*Paracheirodon axelrodi*	Cardinal Tetra	RSBIV	-	-
17	*Hemigrammus bleheri* (adult)	Red Nose Tetra (adult)	RSBIV	-	-
18	*Hemigrammus bleheri* (juvenile)	Red Nose Tetra (juvenile)	RSBIV	-	-
19	*Labidochromi caeruleus*	Lemon Cichlid	RSBIV	-	-
20	*Andinoacara pulcher*	Blue Electric	RSBIV	-	-
21	*Metriaclima estherae*	Red Zebra Cichlid	RSBIV	-	-
22	*Oreochromis niloticus*	Nile Tilapia	SVC, VNN, TiLV	*Aeromonas salmonicida, Edwarsiella ictaluri*	-
23	*Clarias sp.* (juvenile)	Catfish (juvenile)	-	*Aeromonas salmonicida, Edwarsiella ictaluri*	-
24	*Clarias sp*. (fingerlings)	Catfish (fingerlings)	-	*Aeromonas salmonicida, Edwarsiella ictaluri*	-

Note: WSSV (White Spot Syndrome Virus); TSV (Taura Syndrome Virus); IHHNV (Infection with infectious hypodermal and hematopoietic necrosis virus); IMNV (Infectious Myonecrosis Virus); YHV (Yellow Head Virus); CMNV (Covert Mortality Nodavirus); KHV (Koi Herpes Virus); SVC (Spring Viraemia of Carp; VNN (Viral Nervous Necrosis); RSBIV (Red Sea Bream Iridovirus); TiLV (Tilapia Lake-Virus); AHPND (Acute Hepatopancreatic Necrosis Disease); NHP (Necrotising Hepatopancratitis); EHP (*Enterocytozoon hepatopenaei*).

buffered saline (PBS) solution. The samples were then crushed and centrifuged at 5,000 ×rpm for five minutes. The supernatant was then collected and continued with the addition of Viral Nucleic Acid Kit II (Genaeid, Taiwan) according to the company work protocol. Total DNA was put in 50µL nuclease free water (NFW) (Qiagen, Germany) and stored at -20°C (Hasanah *et al*., 2022; Kumalasari *et al*., 2022).

DNA amplification

The PCR reaction was carried out on a MultiGene Optimax Thermal Cycler (Labnet International, USA) with a total reaction volume of 25µL consisting of 1× My Taq HS Red Mix (Meridian Bioscience, USA), 0.2µM forward and reverse primers, 1 unit of enzyme AMV reverse transcription (Promega, USA) (only for genomic RNA), 2 µL of nucleic acid from sample and Nuclease Free Water. DNA amplification was carried out under PCR cycle conditions referring to the work protocol at BBKIPM Jakarta 1. The types of primers and gene targets used are listed in Table 2.

PCR product electrophoresis

The PCR products were then electrophoresed on 1.5% agarose gel (Biorad, USA) with 1% gel-red staining (Milipore, USA) on Mupid-one (Advance, Japan) at 100 Volts for 30 minutes. Then, visualized with UV-transilluminator (Cleaver Scientific, UK) and documented with the UVITEC-Cam-Bridge application (UVITEC, UK).

RESULTS AND DISCUSSION

Result

Results of physical inspection of fishery products

Based on the results of physical examination taking into account the clinical symptoms appeared, of the 24 fishery product species collected, as many as 10 clinical symptoms were found in seven fish species and were not found in shrimp (Table 3). Of the seven species, there were three species with normal clinical symptoms in the first and second examination periods. In fact, two species of them, Tilapia (*Oreochromis niloticus*) and Manfish come from the same farm. However, they had different clinical symptoms compared between the first and second periods.

When viewed from all the clinical symptoms obtained, this finding confirmed that there were several species with the same clinical symptoms. As seen in Figure 1, the clinical symptoms found in different species were weak swimming activities, decreased appetite, and loose scales. Meanwhile, other clinical symptoms were only found in one species. To see whether these clinical symptoms were related to pathogen infection, further examination was carried out using a molecular method, namely PCR.

Molecular examination

Molecular examination of all samples whether showing clinical symptoms or not had been carried out. Out of a total of 7 species that had clinical symptoms, only two species were found to be positively infected by the pathogen, namely goldfish (*Carassius auratus*) that was positively infected by *Aeromonas salmonicida* with a prevalence of 66.67% and Red Zebra Cichlid (*Metriaclima estherae*) that was positively infected with RSBIV with a prevalence 20% (Table 4). The interesting thing about this finding is that there were two positive cases that came from a species that had no clinical symptoms, namely *A. salmonicida* infection in gourami (*Osphronemus goramy*) and YHV infection in white shrimp (*Litopenaeus vannamei*) with a prevalence of 20% each.

Discussion

This study revealed the presence of *A. salmonicida* infection in two types of fish showing different clinical symptoms. Gurami fish showed positive for *A. salmonicida* infection did not show any clinical symptoms. It is similar to a report submitted by Rozi (2018) that stated that several gourami that were positively infected with *A. salmonicida* showed a number of different symptoms, ranging from no symptoms to more than two clinical symptoms. *Aeromonas spp. bacteria* group known to have different pathogenicity depending on the stress level of the host (Hayatgheib *et al*., 2020). If the habitat environment supported host growth, *Aeromonas* spp. bacterial infection showed no clinical symptoms or was asymptomatic (Hayatgheib *et al*., 2020; Ziarati *et al*., 2022; Zorriehzahra *et al*., 2021). Conversely, if the habitat environment does not support host growth, it will cause the host to become stressed and experience clinical or symptomatic symptoms (Ziarati *et al*., 2022; Zorriehzahra *et al*., 2021).

Several environmental factors in aquaculture sites that may cause fish to become stressed are poor water quality, weather, climate change, and

Table 2**.** Primary oligonucleotides used in molecular assays with PCR for the detection of bacterial, viral and parasitic infections in fishery products.

Pathogens	Primers sequences (5'-3')	Base pairs	Gene target	Reference
Aeromonas salmonicida	27F (AGAGTTTGATCMTGGCTCAG) 1492R (TACGGYTACCTTGTTACGACTT)	1,400	16S rRNA	(Dos Santos *et al*., 2019)
Edwardsiella ictaluri	IVS (TTAAAGTCGAGTTGGCTTAGGG) IRS (TACGCTTTCCTCAGTGAGTGTC)	2,000	23S rRNA	(Williams & Lawrence, 2010)
Vibrio parahaemolyticus	Tdh-F (CCACTACCACTCTCATATGC) Tdh-R (GGTCTAAATGGCTGACATC)	199	Tdh	(Mulya *et al.*, 2022)
	Trh-F (GGCTCAAAATGGTTAAGCG) Trh-R (CATTTCCGCTCTCATATGC)	250	Trh	
Hepatobacter penaei	NHPF2 (CGTTGGAGGTTCGTCCTTCAGT) NHPR2 (GCCATGAGGACCTGACATCATC)	379	16S rRNA	(OIE, 2019a)
WSSV	146 F (ACTACTAACTTCAGCCTATCTAG) 146 R (TAATGCGGGTGTAATGTTCTTACGA)	1,447	PmNOB III	(Claydon *et al*., 2004)
	146NF (GTAACTGCCCCTTCCATCTCCA) 146NR (TACGGCAGCTGCTGCACCTTGT)	941		
TSV	9992F (AAGTAGACAGCCGCGCTT) 9195R (TCAATGAGAGCTTGGTCC)	321	VP2	(Aulia *et al*., 2019)
IHHNV	389F (CGGAACACAACCCGACTTTA) 389R (GGCCAAGACCAAAATACGAA)	389	ORF1	(Aulia *et al*., 2019)
IMNV	4587F (CGACGCTGCTAACCATACAA) 4914R (ACTCGGCTGTTCGATCAAGT)	139	ORF1	(Aulia *et al*., 2019)
	4725NF (GTAACTGCCCCTTTCACTTCCA) 4863NR (TACGGCAGCTGCTGCACCTTGT)			
YHV	10F (CCGCTAATTTCAAAAACTACG) 144R (AAGGTGTTATGTCGAGGAAGT)	135	ORF1	(OIE, 2021b)
CMNV	7F1 (AAATACGGCGATGACG) 7R1 (ACGAAGTGCCCACAGAC)	619	RNA polymerase (RdRp) gene	(Zhang *et al*., 2014a; Zhang *et al.,* 2018b)
	7F2 (CACAACCGAGTCAAACC) 7R2 (GCGTAAACAGCGAAGG)	165		
KHV	For (GGGTTACCTGTACGAG) Rev (CACCCAGTAGATTATGC)	409	outer primer thymidine kinase	(Novita & Koesharyani, 2009)
SVC	SVCV F1 (TCTTGGAGCCAAATAGCTCARRTC) SVCV R2 (AGATGGTATGGACCCCAATACATHACNCAY)	714	glycoprotein	(Kim, 2012)
VNN	VNN-F (CAACTGACAACGATCACACCTTC) VNN-R (CAATCGAACACTCCAGCGACA)	230	CP	(Kumalasari *et al*., 2022)
RSBIV	F (CCCGCACTGACCAACGTGTCC) R (CACAGGGTGACTGAACCTCAGGTCG)	191	MCP	(Rifai *et al*., 2019)
TiLV	112F (CTGAGCTAAAGAGGCAATATGGATT) 112R (CGTGCGTACTCGTTCAGTATAAGTTCT)	112	Segmen 3	(Aich *et al*., 2022)

high stocking densities in ponds (Dastin *et al*., 2021; Macaulay *et al*., 2022; Salem *et al*., 2020). Apart from causing fish to become stressed, a poor habitat environment can also cause pathogens, especially parasites and bacteria, to live and reproduce properly (Salem *et al*., 2020). Positive findings of *A. salmonicida* in gourami have been commonly reported, given that this fish has a slow growth rate, it makes the susceptible to the infection from pathogens such as bacteria (Febrianti *et al*., 2021; Dastin *et al*., 2021; Rozi *et al*., 2018). Previous research revealed that gourami cultivation with good maintenance management had a lower chance of being infected with *A. salmonicida* compared to gourami with slow growth due to poor management (Febrianti *et al*., 2021). Furthermore, gouramy with fast growth (addition of immunostimulants, probiotics, and phytobiotics) is known to have better resistance to *A. salmonicida* compared to gourami with slow growth (Febrianti *et al*., 2021; Dastin *et al*., 2021).

It is possible that if the development of gourami seeds with fast growth is carried out, it will produce gouramy that is completely resistant to *A. salmonicida*. In contrast to infections found in gourami, infections in ornamental fish such as goldfish showed many clinical symptoms and a high prevalence rate. The discovery of *A. salmonicida* infection in goldfish confirmed with clinical symptoms has also been reported

Table 3. Results of physical examination of fish and shrimp species in clinical symptoms in the first and second periods.

Species	Period	Source	Clinical signs
Osphronemus gouramy	1	Bogor Regency	Lumpy mucus
Pterophyllum scalare	1	Bogor City	Experiencing buds, red rashes on the fins and tail, lumpy mucus
Oreochromis niloticus	1	Bogor City	Wounded fins and body parts have moss
Carrasius auratus	1	West Jakarta	Opened *Operculum*
Paracheirodon innesi	1	West Jakarta	Weak swimming activities
Carrasius auratus	2	Bogor City	Many mortality, lack of appetite, little mucus production, white on head, pale gills, all scales off, gasping for breath, webbed but no ulcers, pale white body color before death
Pterophyllum scalare	2	Bogor City	Abnormal swimming, white fins, red mouth
Oreochromis niloticus	2	Bogor City	Weak or lethargic swimming, lack of appetite, dark color
Cyprinus carpio	2	West Jakarta	Exfoliated scales
Metriaclima estherae	2	West Jakarta	Weak swimming activities

Table 4. PCR Fish products that are positively infected by PCR examination pathogens.

No.	Species	Clinical signs	Pathogens name	Prevalence (%)
1.	*Carassius auratus*	exist*	*Aeromonas salmonicida*	66.67
2.	*Metriaclima estherae*	exist*	RSBIV	20
3.	*Osphronemus gouramy*	-	*Aeromonas salmonicida*	20
4.	*Litopenaeus vannamei*	-	YHV	20

Note: *Clinical symptoms found in the species are shown in Table 3.

in goldfish collected from aquariums in South Korea but with clinical symptoms that were different from those found, namely lethargy, weakness, and abnormal swimming. These different clinical symptoms showed the level of pathogenicity or severity of the infection. Clinical symptoms such as weak swimming activity, lethargy and decreased appetite were included as symptoms with low pathogenicity. Meanwhile, clinical symptoms such as floating on the surface, releasing air bubbles, and the mouth opening and closing quickly were clinical symptoms with high pathogenicity (Rozi *et al.*, 2018).

It is similar to the findings of this study, that the clinical symptoms of a rapidly opening-closing mouth in goldfish with the highest prevalence and mortality. In addition to infections caused by bacteria, this study also found two infections caused by viruses namely red sea bream iridovirus (RSBIV) on red zebra cichlid *(Metriaclima estherae)* and Yellow-Head Virus (YHV) on Pacific white shrimp *(Litopenaeus vannamei)*. Red Zebra Cichlid *(M. estherae)* is a type of ornamental fish commodity, so the discovery of positive cases of RSBIV in Red Zebra Cichlid fish adds to the list of ornamental fish positively infected with pathogens in this study. RSBIV is a virus in the Megalocytivirus genus that is known to be susceptible to infecting freshwater fish such as those from the Poeciliidae, Cichlidae and Osphronemidae groups (Johan & Zainathan, 2020).

Although it is known to be a threat to ornamental fish, not many studies have reported RSBIV infection in ornamental fish, especially Red Zebra Cichlids. However, it does not rule out that infection in these fish may have occurred but not many have reported it officially. The clinical symptoms resulting from Megalocytivirus infection are similar to the clinical symptoms produced by other pathogens. Some of them are that the fish body looks dark, has decreased appetite, is lethargic, swims weakly, and separates from the group (Johan & Zainathan, 2020; Rifai *et al*., 2019). RSBIV infection in this study showed the same clinical symptoms, namely weak swimming in Red Zebra Cichlids that were also found in Neon Tetras (*Paracheirodon innesi*) and Tilapia (*Oreochromis niloticus*) although the two fish did not show any positive indications of pathogen infection.

It confirms that it is difficult to determine disease-causing pathogens based solely on their clinical symptoms. The findings of YHV infection in this study occurred in Pacific white shrimp (*Litopenaeus vannamei*) that did not show clinical symptoms. YHV infection is commonly found in Asia Asia, one of which is Indonesia and the Pacific white shrimp is a type of shrimp that can be a host besides *Penaeus monodon* and *P. stylirostris* (Aulia *et al.,* 2019; OIE, 2021b). YHV infection in Pacific white shrimp may cause no clinical symptoms although it is known to cause a high mortality rate (Lee *et al*., 2022).

CONCLUSION

This study obtained four positive samples in farmed fish due to *A. salmonicida*, RSBIV, and YHV. There are four positive cases that cause clinical symptoms and those that don't. It proves that examination relying solely on physical examination cannot be used as the only approach. So it is necessary to do another approach, namely molecular examination. Regular training for fish and shrimp farmers on aquaculture management must also be carried out by important stakeholders to reduce productivity failure due to infectious diseases. Further research on the spread of antibiotic use and the incidence of resistance is needed to identify the scope of this threat in the aquaculture sector. Also, there is a need to further investigate antibiotic residues in feedstuffs and water in several aquaculture ponds in Indonesia.

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