New emerging viral disease on Giant Gourami (Osphronemus goramy Lac.) in Java, Indonesia

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Abstract. This study investigates the role of pathogens in the disease outbreaks and mass mortality affecting giant gourami (Osphronemus goramy) populations from 2018 to 2020, leading to significant economic losses. Presumptive diagnoses, based on clinical symptoms and references, implicated two viruses: Tilapia Lake Virus (TiLV) and Infectious Spleen and Kidney Necrosis Virus (ISKNV). Samples from four Indonesian provinces (West Java, Central Java, East Java and Yogyakarta) were tested molecularly for these viruses. The results revealed widespread viral pathogen involvement, with ISKNV prevalence ranging from 20% to 100% and TiLV prevalence consistently at 100%. These findings strongly suggest that the outbreaks were primarily caused by TiLV and ISKNV infections, potentially with other co-infecting pathogens. Bacterial pathogens, notably Aeromonas spp., Mycobacterium spp., and Pseudomonas spp., may have exacerbated the disease. This research highlights the emergence of viral diseases as a significant threat to giant gourami populations in Java, Indonesia.

1 Introduction

The giant gourami, Osphronemus goramy, is a freshwater fish native to Southeast Asia [1]. Global production of giant gourami was recorded at 145,000 tons in 2017, of which Indonesia contributed 98% of the total production, equivalent to 142,100 tons [2]. It was also noted that only five other countries reported production of this species (Thailand, Myanmar, Malaysia, Philippines, and Singapore). However, Vietnam and Laos are known to produce this fish but do not report it. Using conventional systems, the fish have been farmed in Indonesia for centuries, mainly in Central Java and West Java [3]. Giant gourami farming has grown rapidly in Java and Sumatra and has even spread to other areas such as Kalimantan, Sulawesi, Bali, and West Nusa Tenggara [4].

Giant gourami's community preference and economic value continuously increase and generally remain above the average of other freshwater-farmed fish species. These facts motivate farmers to promote more efficient production by changing the farming system from traditional to semi-intensive with higher stocking densities, using commercial feeds

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and shorter growing periods. As a result, as giant gourami farming becomes more intensive, disease issues have arisen as one of the challenges producers face. A parallel health management system has yet to accompany changes in the increasingly intensive farming system. In addition, giant gourami farming is generally implemented in an aquatic environment that is challenging to manage, where the process of infectious disease transmission is more difficult to control.

In 2018-2020, giant gourami farming in Indonesia experienced a disease outbreak that resulted in mass mortality, particularly in the production centers covering Java, with economic losses estimated at 40 billion rupiah and [5, 6, 7]. The impact of the disease outbreaks was reflected in the national production of this species. Production of giant gourami increased from 2011 to 2017; however, there was a significant decline of more than 30% from 2018 to 2020 [8]. Epidemiological studies related to disease outbreaks have not been conducted comprehensively, so the primary causes still need to be discovered and fully described, including the determinant factors of the disease [7].

This study aimed to investigate pathogen involvement in disease outbreaks and mass mortality of giant gouramis (O. goramy) in Java, Indonesia, during 2018-2020. Based on presumptive diagnosis (clinical symptoms, mortality patterns, and available references), this study highlighted the presence of two viral pathogens, Tilapia Lake Virus (TiLV) and Infectious Spleen and Kidney Necrosis Virus (ISKNV).

2 Materials and methods

2.1 Sample and data collection

The diseased giant gouramis were collected from West Java, Central Java, Yogyakarta, and East Java. The number and size of samples collected from each sampling point varied depending on the availability of the population at risk of disease and survivors in the recovery process. The number of sampling sites and the total number of samples collected in each province are shown in Table 1.

In addition to laboratory analysis, secondary information was collected from relevant resource persons (farmers and fishery health officers) through interviews guided by a simple questionnaire. The information collected in the questionnaire included the size of the affected fish, clinical signs, and mortality. Estimated losses due to the outbreak were obtained from the report prepared by the Fisheries Department in each area where samples were collected.

Table 1. The number and body weight of giant gourami (Osphronemus goramy) samples collected from each affected location.

<table>
<thead>
<tr>
<th>Province</th>
<th>Spot sampling</th>
<th>Number of samples</th>
<th>Size (gram)</th>
<th>Culture unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Java</td>
<td>4</td>
<td>40</td>
<td>50-100</td>
<td>Grow-out</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>400-600</td>
<td>Grow-out/broodstock</td>
</tr>
<tr>
<td>Central Java</td>
<td>2</td>
<td>9</td>
<td>2,500-4,000</td>
<td>Broodstock</td>
</tr>
<tr>
<td>Yogyakarta</td>
<td>2</td>
<td>5</td>
<td>300-550</td>
<td>Grow-out</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2,500-4,000</td>
<td>Broodstock</td>
</tr>
<tr>
<td>East Java</td>
<td>3</td>
<td>9</td>
<td>300-500</td>
<td>Grow-out</td>
</tr>
</tbody>
</table>
2.2 Identification of bacteria

The manufacturer's instructions prepared sterile media for isolating pathogenic bacteria. Bacterial isolation was performed primarily on samples with obvious clinical signs, such as ulcers and kidney abnormalities, liver, spleen, and muscle abnormalities. Bacteria from each organ were then isolated aseptically on tryptic soy agar (TSA), Brain Heart Infusion Agar (BHIA), and Lowenstein-Jensen medium. Incubations were 24-72 h at 28 °C, while *Mycobacterium* spp. were incubated for at least seven days.

Bacterial growth and morphology were observed. The bacterial analysis included Gram stain, motility, oxidase, Rimmler-Shott, catalase, O/F test, Triple Sugar Iron Agar (TSIA) test, and API 20E test (Biomereux). Bacterial identification was performed according to [9, 10, 11, 12].

2.3 Detection of virus

Virus analysis was conducted on two viral agents strongly implicated as potential primary etiological agents in emerging disease outbreaks. Specifically, these viruses were identified as TiLV and ISKNV. The fish specimens for this analysis were selected based on their manifestation of distinctive clinical symptoms indicative of the suspected viral infections.

2.3.1 Tilapia Lake Virus (TiLV)

The organs and tissues chosen to detect TiLV included the eye, brain, liver, spleen, and kidney [13]. Subsequently, these organ samples were meticulously preserved by immersion in RNA later solution or a solution comprised of absolute ethanol and glycerol in an 80:20 (v/v) ratio. The preserved samples were then securely stored at -70 °C until they were prepared for further analytical procedures.

The methodology employed for TiLV detection followed the procedure established by a prior study [14]. This involved using a semi-nested Reverse Transcription Polymerase Chain Reaction (RT-PCR) technique. In Step 1 of the nested RT-PCR, the specific primers were ext-1 (TATGCAGTACTTTCCCTGCC) and ME1 (GTTGGGCACAAGGCATCCTA). The target PCR amplicon size for this step was 415 base pairs. In Step 2, a semi-nested RT-PCR approach was utilized, and it featured the following primers: ME2 (TATCACGTGCGTACTTTCCCTGCC) and ME1 (GTTGGGCACAAGGCATCCTA). This step aimed to generate a PCR product with a size of 250 base pairs.

2.3.2 Infectious Spleen and Kidney Necrosis Virus (ISKNV)

The investigation involved the aseptic procurement of organs and tissues, specifically the liver, brain, spleen, and kidney, for the precise detection of ISKNV, as meticulously detailed in scholarly references [15, 16]. These biological specimens were individually harvested or arranged into various combinations and submerged in an appropriate preservative solution.

The analytical assessment of ISKNV was carried out in strict adherence to the rigorous methodological framework established by the authors in reference [17]. This method incorporated the judicious design of primers, derived from the nucleotide sequence of a 959-base pair Pst I restriction fragment, which was uniquely identified by its DDBJ accession number AB006954, originating from the genomic DNA of the Red Seabream Iridovirus (RSIV). These primers denoted as 1F (Forward Primer) with the sequence 5’CTCAAAACACTCTggCTCATC’3 and 1R (Reverse Primer) with the sequence...
5’GCACCAACACATCTCTATC’3, were thoughtfully tailored to specifically target a molecular weight of 570 base pairs within the RSIV genome DNA.

2.4 Analysis

The study's data and information underwent a comprehensive analysis encompassing qualitative and quantitative methodologies. Subsequently, the results were thoughtfully organized and presented in a meticulously structured tabular format, adhering to established academic standards.

3 Results and discussion

The giant gourami disease outbreak, from early 2018 to the mid-2020s, resulted in thousands of productive and candidate broodstock mortality. The extraction of data/information collected from the local fisheries office and the farmers is presented in Table 2. It was indicated that the size of giant gourami affected mainly by the disease and causing large economic losses at that time, was from the grow-out to broodstock sizes, with mortality rates of 20% - 60% with an estimated total loss of ≈ 48 billion rupiahs (US$ 3,139,307).

Table 2. Summary of data/information collected from affected areas in Java (West Java, Central Java, Yogyakarta, and East Java).

<table>
<thead>
<tr>
<th>Location</th>
<th>District</th>
<th>Stage</th>
<th>Size (gram)</th>
<th>Mortality rate (%)</th>
<th>Estimated loss (million rupiahs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Java</td>
<td>Bogor, Tasikmalaya, Banjar, Ciamis</td>
<td>Grow-out Brood stocks</td>
<td>100-600, 1,500-4,000</td>
<td>40-50, 20-25</td>
<td>1,500, 9,000</td>
</tr>
<tr>
<td>Central Java</td>
<td>Banyumas, Banjarnegara</td>
<td>Grow-out Brood stocks</td>
<td>300-700, 2,000-5,000</td>
<td>25-30, 50-60</td>
<td>5,351, 24,873</td>
</tr>
<tr>
<td>Yogyakarta</td>
<td>Bantul, Sleman, Kulon Progo, Gunung Kidul</td>
<td>Grow-out Brood stocks</td>
<td>100-600, 2,000-5,000</td>
<td>20-25, 30-35</td>
<td>1,000, 4,500</td>
</tr>
<tr>
<td>East Java</td>
<td>Tulung Agung, Kediri</td>
<td>Grow-out</td>
<td>100-450</td>
<td>20-25</td>
<td>2,000</td>
</tr>
<tr>
<td>Total estimated financial loss</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48,224</td>
</tr>
</tbody>
</table>

Behavioral observations made by farmers and direct observations in the field of diseased and moribund giant gourami generally show the following behavior a few days earlier: slow swimming movements, less responsiveness, and drastically decreased appetite. Concurrently, the clinical manifestations encompassed a spectrum of discernible symptoms, including but not limited to altered pigmentation characterized by a deepening of the body coloration, ocular anomalies such as endophthalmitis and cataracts, dermal irregularities such as scales appearing loose or skin exhibiting signs of peeling, as well as erosions and ulcerations. Most of these clinical symptoms are consistent with those described in tilapia exposed to TiLV as described by [13, 18, 19, 20, 21, 22].

Other clinical symptoms were also observed during the outbreak, including loss of equilibrium, excess mucus, bleeding petechiae on the skin, swimming upside down, abdominal distension, respiratory distress, darkening of body color, loss of scales,
enlargement and discoloration of internal organs, especially the spleen, and kidneys. These clinical signs were also reported in giant gourami infected by ISKNV, as described by [15, 16, 23].

During the examination of ailing specimens, we observed that when lifted, there was a noticeable excess of mucus and blood oozing from between the scales. To help clarify these observations, we've included visual representations of moribund giant gourami and those in the early stages of expiration in Figure 1. These images serve as visual aids to support our findings.

![Diseased giant gourami (Osphronemus gouramy) collected from the affected area in Yogyakarta (2019): pale/dark body color, scale protrusion, skin redness, and fin rot.](image)

**Fig. 1.** Diseased giant gourami (Osphronemus gouramy) collected from the affected area in Yogyakarta (2019): pale/dark body color, scale protrusion, skin redness, and fin rot.

Analysis of samples obtained from outbreak-afflicted regions spanning 2018 to 2020 unveiled a notable prevalence of bacterial infections. Mycobacteriosis and motile aeromonad septicemia (MAS) emerged as the principal afflictions affecting giant gourami. Additionally, diverse strains of bacteria were identified within the collected samples. In tandem with bacterial infections, the analysis also disclosed the presence of two viruses, TiLV and ISKNV, within the same set of test samples. The findings about identifying and detecting these pathogens have been systematically cataloged and delineated in Table 3.

**Table 3.** Bacteria identified and virus detected on giant gourami (Osphronemus gouramy) samples from affected areas in Java (West Java, Central Java, Yogyakarta, and East Java).

<table>
<thead>
<tr>
<th>No.</th>
<th>Location</th>
<th>(\Sigma) samples</th>
<th>Bacteria (Genus/species)</th>
<th>Prevalence (%)</th>
<th>Virus (Species)</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>West Java</td>
<td>40</td>
<td><em>Aeromonas</em> spp.</td>
<td>8.0</td>
<td>TiLV</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Mycobacterium</em> spp.</td>
<td>12.0</td>
<td>ISKNV</td>
<td>38.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Pseudomonas</em> spp.</td>
<td>8.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Central Java</td>
<td>9</td>
<td><em>Aeromonas</em> spp.</td>
<td>nd</td>
<td>TiLV</td>
<td>55.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Mycobacterium</em> spp.</td>
<td></td>
<td>ISKNV</td>
<td>20.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Pseudomonas</em> spp.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The tabulated data reveals the substantial impact of viral infections on the afflicted giant gourami population in the surveyed regions. Specifically, TiLV infection exhibited a remarkable prevalence of 100% in both West Java and East Java. In the neighboring regions of Central Java and Yogyakarta, the prevalence was 55.56%. In parallel, ISKNV infection was evident, with a prevalence of 38.46% in West Java and 20% in Central Java and Yogyakarta, while the prevalence reached 100% in East Java. As recognized worldwide, TiLV disease was initially known to affect only tilapia [13, 18, 19, 20, 22]. The spreading of the disease was very fast, and in 2020, [22] it was suspected that at least 16 countries across four continents had been exposed to TiLV. The virus has been detected in Indonesia [22, 24, 25]. Thus, it is likely that the virus was indeed present and widespread in tilapia farm areas in Indonesia [24, 25, 26]. However, the disease has generally not led to serious fatalities and losses compared to similar diseases in several other affected countries [21, 27, 28]. This is also based on the no reports of significant disease and mortality among tilapia farms, and this is also reflected in the national production of tilapia, which has been increasing steadily from 2010 to the present day [4].

The evidence of TiLV being able to infect giant gourami with significantly higher mortality rates than in tilapia is a serious concern to be aware of [29], until finally, it is relevant to the emergence of a disease outbreak in giant gourami culture, which closely coincided with the first detection of TiLV on tilapia in Indonesia [24, 25]. The outbreak of TiLV infection in giant gouramis is a new occurrence, and all samples collected from the affected areas revealed a high prevalence. The impact of disease outbreaks was reflected in the national production achievements for this species. The national production of giant gourami increased during 2011-2017; however, there was a significant decline of more than 30% from 2018-2020 [8]. To date, diseased fish showing the same clinical symptoms are still encountered with varying degrees of loss [30].

In all samples collected from the outbreak areas, ISKNV infection was also involved with a relatively lower prevalence than TiLV. ISKNV infection in giant gourami in Indonesia has been reported since a decade ago [15], as well as in other species of labyrinth fish [15, 31, 32]. However, the impact of the losses associated with this virus is less massive than after co-infection with TiLV as the outbreak occurred in 2018-2020. Outside Indonesia, the ISKNV infection has also been reported in giant gourami in India [16].

The heightened prevalence of both these viruses within the sampled population was further substantiated through molecular analysis. Notably, all samples from the affected regions returned positive results, as the data in Figures 2 and 3 corroborated. These findings underscore the substantial impact of these viral pathogens on the health of the giant gourami population under study.
further substantiated through molecular analysis. Notably, all samples from the affected areas revealed a high prevalence. The impact of disease outbreaks was reflected in the national production achievements for this species. The national production of giant gouramis increased during 2011-2017; however, there was a significant decline of more than 30% from 2018 onwards. As recognized worldwide, TiLV disease was initially known to affect only tilapia. However, in Indonesia, TiLV infection in giant gouramis is a new occurrence that coincided with the heightened prevalence of both these viruses within the sampled population. Thus, it is likely that the virus was indeed involved in the outbreaks, as the data in Figures 2 and 3 corroborated. These findings led to the involvement of other species of viruses in the disease and mortality among tilapia farms, and this is also reflected in the national production of tilapia, which has been increasing steadily from 2010 to the present day. In all samples collected from the outbreak areas, TiLV or ISKNV and co-infection with both species of viruses were involved. This suspicion is not only based on clinical symptoms and mortality patterns but also confirmed by the results of molecular diagnosis and available references [16, 28, 30]. The involvement of other...
pathogens belonging to the bacterial group also exacerbates the disease, particularly *Mycobacterium* spp. and *Aeromonas* spp.

Consequently, a strong and well-founded suspicion exists that Tilapia Lake Virus (TiLV) and Infectious Spleen and Kidney Necrosis Virus (ISKNV) are the primary etiological agents responsible for these infections. This suspicion is grounded in a comprehensive assessment encompassing anamnesis, clinical observation, and a thorough diagnostic analysis.

Furthermore, it is imperative to underscore that TiLV and ISKNV consistently manifest as the dominant pathogens, bearing substantial prevalence within the affected giant gourami population. This prevalence pertains to singular viral infections and co-infections with other microbial agents, such as *Mycobacterium* spp., *Aeromonas* spp., and *Pseudomonas* spp.

Determining the primary pathogenic agents of a newly emerging disease requires the completion of pathogenicity (Koch's postulates), virological (cytopathic effect/CPE), and histopathological assays. In this study, these diagnostics were not performed. Therefore, the gold standard diagnosis is still needed for confirmation. Nevertheless, base on this study, we posit with substantial confidence that both viral species, TiLV and ISKNV, act as primary pathogenic agents, harboring considerable potential as emerging diseases in giant gourami within Java and, conceivably, in gourami culture regions beyond the confines of Java Island.

Authors’ contributions

All authors have made substantial contributions to the refinement of the final manuscript. Taukhid Taukhid and Hessy Novita spearheaded the revision of core conceptual ideas, meticulously gathered and curated the data, drafted the manuscript, and designed the accompanying figures. In turn, Domenico Caruso, Tuti Sumiati, and Edy Barkat Kholidin provided invaluable critical revisions to the article.

Moreover, it is worth noting that all authors actively engaged in thorough discussions regarding the findings, significantly enriching the manuscript's overall quality. This collaborative effort culminated in developing the definitive version of the manuscript.

Conflict of interest

The authors declare the absence of competing interests that could influence the impartiality or integrity of the research and its associated findings.

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