

DETECTION OF MEGALOCITIVIRUS IN GOBY FISH (*Stiphodon* sp.) AT WAAI RIVER USING POLYMERASE CHAIN REACTION METHOD

Deteksi Megalocytivirus pada Ikan Goby (*Stiphodon* sp.) di Perairan Sungai Waai
Dengan Metode PCR Konvensional

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ABSTRACT

Megalocytivirus is an important viral pathogen affecting freshwater and marine fish, causing mass mortality and significant economic losses in aquaculture. Goby fish (*Stiphodon* sp.) have high ecological and economic value, particularly as ornamental fish and indicators of water quality, thus requiring early detection of viral diseases. This study aimed to detect Megalocytivirus infection in goby fish using conventional Polymerase Chain Reaction (PCR) targeting the major capsid protein (MCP) gene. Fish tissue samples were collected from February to April 2025 at the Maluku Fish, Animal, and Plant Quarantine Center. DNA extraction was performed using an RNA/DNA silica extraction kit, followed by PCR amplification and agarose gel electrophoresis. The results showed that all tested goby samples were negative for Megalocytivirus infection. These findings indicate that goby fish from Maluku waters were free from Megalocytivirus. Conventional PCR proved to be an effective and sensitive method for early detection of viral diseases in fish.

Keywords: Fish disease; Goby fish; Megalocytivirus; PCR

ABSTRAK

Megalocytivirus merupakan patogen virus penting pada ikan air tawar dan laut yang dapat menyebabkan kematian massal serta kerugian ekonomi dalam kegiatan akuakultur. Ikan goby (*Stiphodon* sp.) memiliki nilai ekologis dan ekonomis tinggi, khususnya sebagai ikan hias dan indikator kualitas perairan, sehingga diperlukan upaya deteksi dini terhadap penyakit virus. Penelitian ini bertujuan untuk mendeteksi keberadaan Megalocytivirus pada ikan goby menggunakan metode *Polymerase Chain Reaction* (PCR) konvensional dengan target gen *major capsid protein* (MCP). Sampel berupa jaringan ikan goby dikumpulkan pada periode Februari–April 2025 di Balai Karantina Hewan, Ikan, dan Tumbuhan (BK HIT) Maluku. Ekstraksi DNA dilakukan menggunakan *RNA/DNA silica extraction kit*, diikuti dengan amplifikasi PCR dan visualisasi hasil menggunakan elektroforesis gel agarosa. Hasil penelitian

menunjukkan bahwa seluruh sampel ikan goby yang diuji tidak terdeteksi Megalocytivirus. Hal ini mengindikasikan bahwa ikan goby dari wilayah perairan Desa Waai berada dalam kondisi sehat dan bebas dari infeksi Megalocytivirus. Metode PCR konvensional terbukti efektif dan sensitif sebagai alat deteksi dini penyakit virus pada ikan.

Kata Kunci: Ikan Goby, Megalocytivirus, PCR, Penyakit Ikan

INTRODUCTION

Gobies of the genus *Stiphodon* are a group of amphidromous fish with a wide distribution throughout the Indo-Pacific region, where they are closely associated with riverine ecosystems. These fish are ecologically significant as components of freshwater fish communities and can serve as biological indicators for the condition of aquatic environments (Keith *et al.*, 2014; Salindeho, 2021). Beyond their ecological value, some goby species also hold economic importance, serving as ornamental fish or as a local food source (Salindeho, 2021; Astuti, 2022; Khartiono *et al.*, 2025).

The health of fish in aquatic environments can be influenced by various factors, including environmental quality, interactions between organisms, and the presence of infectious agents (Sari *et al.*, 2025). Viral diseases are a major concern because infections may initially present without clear clinical signs, yet under certain conditions, they can progress into systemic diseases, leading to mass mortality (Sarjito *et al.*, 2013).

Megalocytivirus a group of viruses belonging to the family Iridoviridae is known to cause systemic infections, resulting in significant losses among both freshwater and marine fish. *Megalocytivirus* infections can affect internal organs, leading to health complications and mortality in fish, whether in aquaculture systems or wild populations (Yusliansyah *et al.*, 2019; Mosooli *et al.*, 2022; Yahya *et al.*, 2023). Fish infected with Megalocytivirus typically exhibit clinical signs such as a loss of appetite, weak swimming, and lethargy (Rifai *et al.*, 2019). Conventional Polymerase Chain Reaction (PCR) is a widely used molecular method for detecting fish pathogens, as it enables the specific amplification of target DNA fragments. In the case of Megalocytivirus, the major capsid protein (MCP) gene is frequently employed as a diagnostic target; it encodes the virus's primary structural protein and possesses characteristics that facilitate molecular identification (Go *et al.*, 2006; Yusliansyah *et al.*, 2019). PCR assays targeting the MCP gene can confirm the presence of viral genetic material in fish tissue samples, making them highly relevant for disease surveillance efforts.

Information regarding the presence of Megalocytivirus in goby fish within the waters of the Maluku region remains limited. Yet, fish of the genus *Stiphodon* are closely linked to river ecosystems and hold both ecological and economic value (Pasingi & Abdullah, 2018; Sahami *et al.*, 2019; Rauf *et al.*, 2024). Consequently, the early detection of significant pathogens in these fish is essential for establishing baseline data for aquatic health monitoring and biosecurity. This study aims to detect the presence of Megalocytivirus in goby fish (*Stiphodon* sp.) collected from river waters in Waai Village, Central Maluku Regency, using conventional PCR targeting the MCP gene.

RESEARCH METHODS

Place and Time

The research was conducted from February to April 2025 at the Maluku Animal, Fish, and Plant Quarantine Agency (BK HIT).

Research Sample

A total of 136 goby fish (*Stiphodon* sp.) were sampled from river waters in Waai Village, Salahutu District, Central Maluku Regency (Figure 1). The target tissues used for analysis included the liver, spleen, and kidneys, or the entire body in the case of smaller individuals.

DNA Extraction

DNA extraction was performed using an RNA/DNA silica extraction kit according to standard procedures. The extracted DNA was stored at -20°C until it was used for PCR analysis.

PCR Amplification

Amplification was performed using conventional PCR with primers specific to the Megalocytivirus MCP gene. The PCR reaction was carried out in a total volume of $25\ \mu\text{L}$, with thermal cycling conditions following the procedures used in the study. The amplification products were visualized using agarose gel electrophoresis.

Data Analysis

The data were analyzed using a qualitative descriptive approach based on the presence or absence of DNA bands at the target size. Samples were categorized as positive if a band was observed at the expected target size, and as negative if no target band was detected.

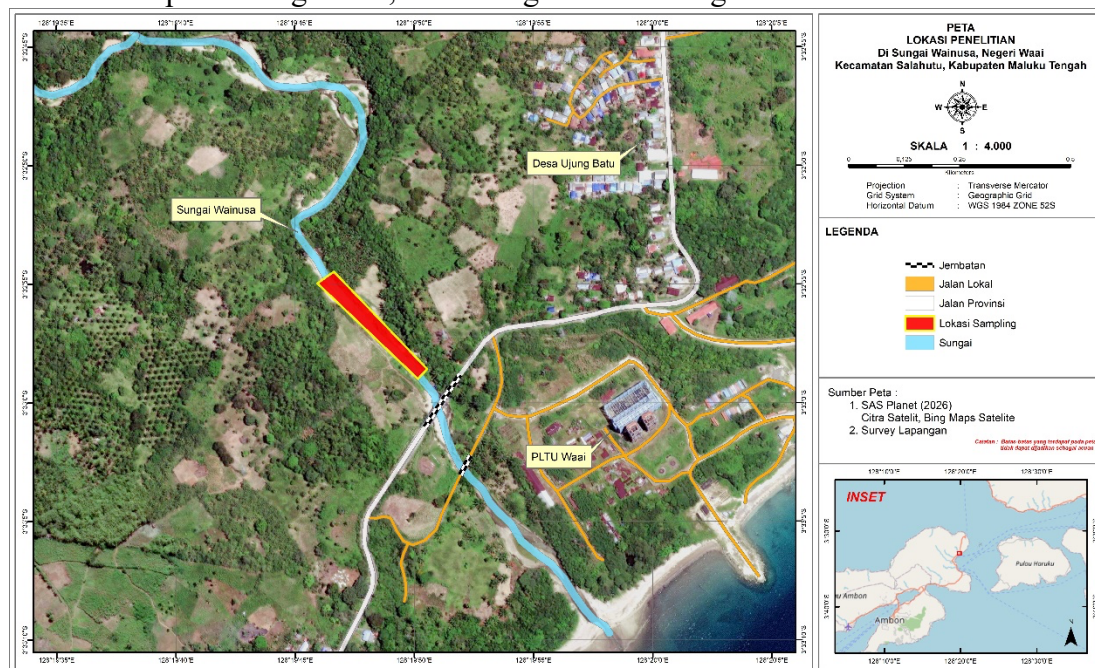


Figure 1. Locations of Research Sampling Points

RESULT

Megalocytivirus Detection Results

The PCR analysis results show that none of the analyzed goby fish samples exhibited a DNA band at the target gene size for the Megalocytivirus MCP gene (Table 1). Based on the interpretation criteria applied, all samples were categorized as negative for the presence of Megalocytivirus. Consequently, for the period and location of sample collection, this study found no molecular evidence of Megalocytivirus in the goby fish examined.

Table 1. Results of Megalocytivirus Testing in Goby Fish (*Stiphodon* sp.)

No	Date of entry	Type	Sample Size	Target	Result
1	02/02/25	Goby	8	Megalocytivirus	Negatif (-)
2	09/02/25	Goby	8	Megalocytivirus	Negatif (-)
3	14/02/25	Goby	8	Megalocytivirus	Negatif (-)
4	20/02/25	Goby	8	Megalocytivirus	Negatif (-)

No	Date of entry	Type	Sample Size	Target	Result
5	23/02/25	Goby	8	Megalocytivirus	Negatif (-)
6	26/02/25	Goby	8	Megalocytivirus	Negatif (-)
7	01/03/25	Goby	8	Megalocytivirus	Negatif (-)
8	08/03/25	Goby	8	Megalocytivirus	Negatif (-)
9	15/03/25	Goby	8	Megalocytivirus	Negatif (-)
10	22/03/25	Goby	8	Megalocytivirus	Negatif (-)
11	28/03/25	Goby	8	Megalocytivirus	Negatif (-)
12	06/04/25	Goby	8	Megalocytivirus	Negatif (-)
13	13/04/25	Goby	8	Megalocytivirus	Negatif (-)
14	21/04/25	Goby	8	Megalocytivirus	Negatif (-)
15	23/04/25	Goby	8	Megalocytivirus	Negatif (-)
16	28/04/25	Goby	8	Megalocytivirus	Negatif (-)
17	30/04/25	Goby	8	Megalocytivirus	Negatif (-)

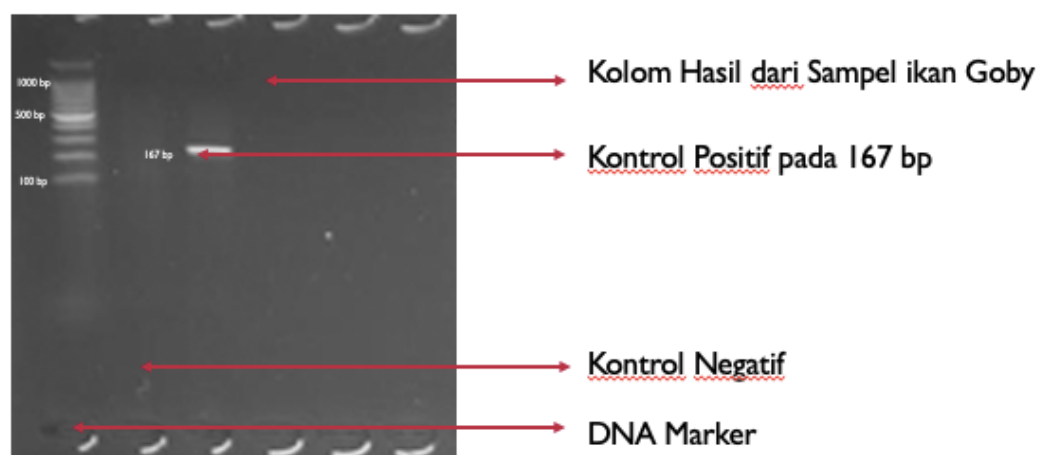


Figure 2. Electrophoresis results for Goby fish

DISCUSSION

Testing using primers specific to the major capsid protein (MCP) gene revealed no DNA bands at the target size for Megalocytivirus in any of the tested goby samples; consequently, all samples were classified as negative (Figure 2). These negative results should be interpreted strictly within the context of the surveillance conducted on the specific samples tested, rather than as evidence that the entire goby population across the Maluku region is free from Megalocytivirus. The absence of detectable target DNA may indicate either that the virus is not present in the samples examined or that the concentration of viral genetic material falls below the detection limit of the method under the given conditions (Fitri *et al.*, 2021). Therefore, the interpretation of these findings should be limited to the specific population, location, sampling period, and diagnostic procedures employed.

Gobies of the genus *Stiphodon* are amphidromous species that inhabit fast-flowing rivers with relatively high water quality; their presence is frequently used as a biological indicator of water quality (Keith *et al.*, 2014; Salindeho, 2021). From an epidemiological perspective, the risk of viral disease in fish can increase when they are subjected to environmental pressure and physiological stress. Factors such as high-density rearing conditions, declining water quality,

restricted circulation, and fluctuations in environmental parameters can adversely affect the physiological state and immune response of fish (Jeong *et al.*, 2008; Chinchar *et al.*, 2017; Guo *et al.*, 2025).

The river in Waai Village—the site where the samples were collected—is a flowing water body that has experienced relatively little anthropogenic pressure or intensive aquaculture activity (Wardono *et al.*, 2022). This well-preserved environmental condition likely contributed to the absence of Megalocytivirus in the goby population examined in this study. The failure to detect Megalocytivirus provides useful preliminary information, serving as baseline data on the health status of the goby population at the study site. A negative status for a single pathogen cannot be used as the sole parameter to conclude that an ecosystem is in good condition. A more robust assessment of water quality should integrate biological indicators, physicochemical water parameters, community structure, and indicators of pollution. The Major Capsid Protein (MCP) serves as a molecular target for detecting Megalocytivirus and its species, offering high sensitivity and specificity (Go *et al.*, 2006; Rifai *et al.*, 2019; Yusliansyah *et al.*, 2019). The MCP is a crucial marker for molecular identification, located between the outer envelope and the inner membrane (Dong *et al.*, 2011). As shown in Figure 1, the Goby fish collected from the waters of Waai Village tested negative for Megalocytivirus, as no bands aligned with the positive control (167 bp) were observed in the samples. A sample would be considered positive if a band appeared in alignment with the positive control (167 bp). The absence of the target band across all samples indicates a negative result based on the procedure applied.

The presence of Megalocytivirus is not solely determined by environmental conditions. The movement and trade of fish—particularly ornamental fish—can facilitate the spread of pathogens between regions. Go *et al.* (2016) demonstrated an epidemiological link between iridoviruses and the ornamental fish trade, while Yahya *et al.* (2023) identified viral infections as a significant threat to aquaculture operations in Indonesia. Consequently, even when research sites test negative for the virus, periodic surveillance remains necessary—especially in the event of increased fish trading activity, the introduction of fish from other regions, changes in environmental quality, or an increase in fish stocking density.

CONCLUSION

No molecular evidence of Megalocytivirus was found in the goby fish (*Stiphodon* sp.) examined from the river waters of Waai Village, Central Maluku Regency, during the study period from February to April 2025, based on conventional PCR amplification targeting the MCP gene. These findings indicate that all tested samples were negative for the presence of Megalocytivirus.

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