*Genetics of Aquatic Organisms 9(3), GA935*
https://doi.org/10.4194/GA935

SHORT PAPER

# Detection of Anguilid Herpesvirus 1 in *Anguilla bicolor* Glass Eel Captured from the Estuary of Southern Java, Indonesia

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**How to Cite**

Romadhona, E. I., Simanungkalit, D. (2025). Detection of Anguilid Herpesvirus 1 in *Anguilla bicolor* Glass Eel Captured from the Estuary of Southern Java, Indonesia. *Genetics of Aquatic Organisms, 9(3), GA935*. https://doi.org/10.4194/GA935

**Article History**
Received 26 July 2025
Accepted 01 September 2025
First Online 24 September 2025

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**Keywords**
AngHV-1
Detection
*Anguilla bicolor*
Glass eel
Indonesia

## Abstract

Indonesia is a major global eel supplier, and its aquaculture still depends on wild-caught glass eels, which may carry pathogens such as Anguillid herpesvirus 1 (AngHV-1). This study aimed to detect AngHV-1 in healthy glass eels from Pangandaran, West Java. Using primers targeting a 394 bp of the DNA polymerase gene, 50% of 50 eel samples tested positive, while no virus was detected in the water sample. Despite the limited sample size and use of conventional PCR, these findings provide baseline data on AngHV-1 occurrence and underscore the need for continued surveillance to enhance disease prevention in eel aquaculture.

## Introduction

Indonesia plays a significant role in the global eel trade, particularly as a major supplier of live eels. In 2023, the country ranked as the fourth-largest exporter of live eels, with a total volume of 775.46 tons (WITS, 2023). To meet the demand, eel farming has been intensively developed across several regions, including Java, particularly for the cultivation of shortfin eel (*Anguilla bicolor*) (Hadie & Kusnendar, 2020). To increase production, eels are typically reared at high stocking densities. However, such high-density farming conditions, especially in aquaculture tanks, can elevate the risk of disease outbreaks caused by various pathogens, including fungi, bacteria, and viruses (Irshath et al., 2023). Among these, viruses are considered the most serious threat due to their rapid replication and potential to cause mass mortality in cultured fish populations (Valero & Cuesta, 2023).

*Cyvirus anguillidallo1*, also commonly known as Anguillid herpesvirus 1 (AngHV-1), is recognized as a significant pathogen affecting both wild and farmed eel populations. It belongs to the Alloherpesviridae (ICTV, 2024), a family under the order Herpesvirales that infects aquatic organisms. This virus is classified in the same genus as *Cyvirus cyprinidallo3* (koi herpesvirus), which is an important pathogen in the family Cyprinidae (Donohoe et al., 2021; Guo et al., 2022). AngHV-1 is known to cause severe hemorrhagic disease, often resulting in high mortality among the eels, with characteristic hemorrhagic lesions commonly observed on the skin and gills (Kempter et al., 2014; Kim et al., 2012). This virus has been reported in various regions worldwide, including Asia, Europe, and the Americas. In

Indonesia, the first documented infection of AngHV-1 occurred in 2023, with an estimated mortality rate exceeding 75% among reared glass eels (Romadhona et al., 2025).

Currently, eel farming in Indonesia still relies heavily on the capture of wild glass eels, with Pangandaran being one of the important capture sites (Fahmi et al., 2012; Samuel et al., 2025). Despite this, there have been no reports of AngHV-1 detection in glass eels captured directly from the wild, without prior exposure to aquaculture environments. This study aims to detect the presence of AngHV-1 among the wild-caught glass eels from Pangandaran, West Java. The findings are anticipated to confirm the presence or absence of latent AngHV-1 infection in wild-caught glass eels.

## Materials and Methods

### Eel Sampling

The sampling is conducted in June 2025 in Pangandaran regency, West Java, Indonesia (Figure 1.). A total of fifty healthy glass eels with a mean body weight of 0.11±0.01 g and mean body length of 5.46±0.3 cm (Figure 2.) were collected from the estuary. In addition, a 1 L water sample from a depth of 0.5 m was collected to assess the presence of viral particles in the water. The eels were transported alive to the laboratory in South Tangerang, Banten Province. Upon arrival, the eels were anesthetized using cold water at 4°C. Approximately 20 mg of tissue sample from the head region was excised using a sterile disposable cutter and transferred into a 1,5 mL tube. All samples were subsequently stored at −20°C for DNA extraction.

### Viral DNA Extraction, Amplification, and Sequencing

DNA extraction was conducted using the phenol-chloroform method according to Sambrook et al. (1989). Viral DNA was then amplified using primer pairs forward 5’ GTG TCGGGCCTTTGTGGTGA 3’ and reverse 5’ CATGCCGGGAGTCTTTTTGAT 3’, targeting 394 bp of the AngHV-1 DNA polymerase gene, as designed by Rijsewijk et al. (2005). The PCR system consisted of 0.5 µL each of the forward and reverse primers (10 nmol), 5 µL MyTaq™ HS Mix (Bioline), 3 µL dd$H_2O$, and 1 µL of the DNA sample. Amplification procedure included one initial cycle at 94°C for 10 minutes, followed by 40 cycles of 94°C for 30 seconds, 60°C for 45 seconds, and 72°C for 1 minute. The process concluded with a final step at 72°C for 7 minutes. PCR products were subjected to electrophoresis in a 1% agarose gel prepared with 1× TBE buffer and subsequently visualized using a UV


**Figure 1.** Geographic Location of Eel Sampling Site in Pangandaran Regency, West Java, Indonesia.

transilluminator gel doc. For DNA sequencing, one sample was selected as a representative, and then Sanger sequencing was conducted using the Applied Biosystems genetic analyzer.

**Bioinformatic Analysis**

The sequences obtained from Sanger sequencing were initially aligned by matching the forward and reverse reads in MegaX to generate a consensus sequence. This sequence was then analyzed using the BLAST (Basic Local Alignment Search Tool) to confirm the identity of the virus. A phylogenetic tree was subsequently constructed using an additional 17 AngHV-1 and one lymphocystis disease virus (LCDV) reference sequences retrieved from GeneBank. The analysis was performed using the Neighbor-Joining method with p-distance model and 1000 bootstrap replications in MegaX software (Kumar et al., 2018).

## Results

**AngHV-1 DNA Amplification**

The partial DNA polymerase gene of the AngHV-1 was successfully amplified through PCR, yielding approximately 394 bp amplicon (Figure 3.). Out of the total 50 eel tissues analyzed, 25 samples tested positive for AngHV-1, indicating a prevalence rate of 50% within the population. In contrast, no viral DNA was detected in the accompanying water sample, suggesting the absence or undetectable levels of viral particles in the water.

**Bioinformatic Analysis**

BLAST nucleotide alignment revealed that the sequenced DNA shared 100% identity with the top five AngHV-1 sequences in the database (accession numbers: MW580854, MW580851, MW580853, AF333066, HQ992949), thereby confirming the viral identity and indicating the absence of false positives. The sequence obtained in this study was deposited in GenBank under the name AngHV-1 isolate IDPG (accession number: PV933180). Phylogenetic analysis using the Neighbor-Joining method placed the sample within the AngHV-1 cluster, grouping it with strains from nearby countries, including Vietnam, Taiwan, Japan, and South Korea (Figure 4.). In contrast, the sequence was distinct from AngHV-1 strains originating from Japan, China, the United Kingdom, Canada, Denmark, and the Netherlands. Additionally, it differed from the previously reported AngHV-1 sequence from Bogor, Indonesia, suggesting the presence of intra-country genetic variation.

## Discussion

This study provides the first molecular evidence of *Anguillid herpesvirus 1* (AngHV-1) infection in healthy, wild-caught glass eels from an estuarine environment in Indonesia, highlighting the silent circulation of the virus within natural eel populations. AngHV-1 prevalence in this study was 50%, based on a sample of fifty individuals, which exceeds the recommended minimum for prevalence studies (15–45 individuals) and offers a sufficient basis for estimation with limited sampling (Sarabeev et al., 2025). In wild populations, previous studies have reported a wide range of prevalence, from 0% to 100%, indicating high variability across different environments and conditions (Kempter et al., 2014; Nzau Matondo et al., 2022; Tuan et al., 2016). The detection of the virus in clinically healthy individuals suggests a latent infection. This finding is consistent with previous research showing that members of the *Alloherpesviridae* family, including AngHV-1, are capable of persisting in host tissues without inducing observable symptoms (Quijano Cardé & Soto, 2024; van Nieuwstadt


**Figure 2.** Representative of the glass eels utilized in this study.

et al., 2001). Latency in 50% of the population may have serious implications, as the virus can potentially reactivate under stressful conditions, such as high stocking densities in aquaculture systems (Reed et al., 2017). If reactivation occurs, these latently infected individuals could serve as a source of infection, leading to rapid horizontal transmission and potentially resulting in 100% prevalence and a high mortality rate, as reported in a previous case in Bogor, Indonesia (Romadhona et al., 2025).

In this study, conventional PCR (cPCR) failed to detect viral DNA in water samples, suggesting either an absence or undetectable levels of viral particles. Given that eels migrate in dense groups, horizontal transmission via direct body contact may be the primary route of infection, even though immersion in contaminated water can also transmit the virus (Kroes et al., 2020). Moreover, the lack of evidence for vertical transmission of AngHV-1 indicates that glass eels likely acquire the infection during migration, potentially due to anthropogenic influences (Delrez et al., 2021). This finding highlights the limitations of cPCR. Nonetheless, cPCR is a reliable, simple, and cost-effective method for detecting target viral DNA, making it suitable for initial screening, particularly in resource-limited settings or when quantification is not necessary (Valones et al., 2009). For more advanced future research, quantitative PCR (qPCR) or droplet digital PCR (ddPCR) should be used to accurately detect low concentrations of viral genetic material in aquatic environments.

In addition to molecular detection, phylogenetic analysis using DNA polymerase provides valuable insights into the genetic variation and evolutionary relationships of AngHV-1. As a conserved core gene across AngHV-1 strains, DNA polymerase enables reliable cross-strain detection and provides strong resolution for broad-level phylogenies (Donohoe et al., 2021; Stephensen et al., 1999). In this study, phylogenetic analysis revealed that the Indonesian isolate AngHV-1 IDPG is closely related to strains from neighboring countries such as Vietnam and Taiwan, but genetically distinct from isolates reported in Japan, China, various European countries, and Canada. This pattern suggests that geographic proximity and regional environmental conditions may contribute to the genetic similarity of viral strains, while geographic barriers may

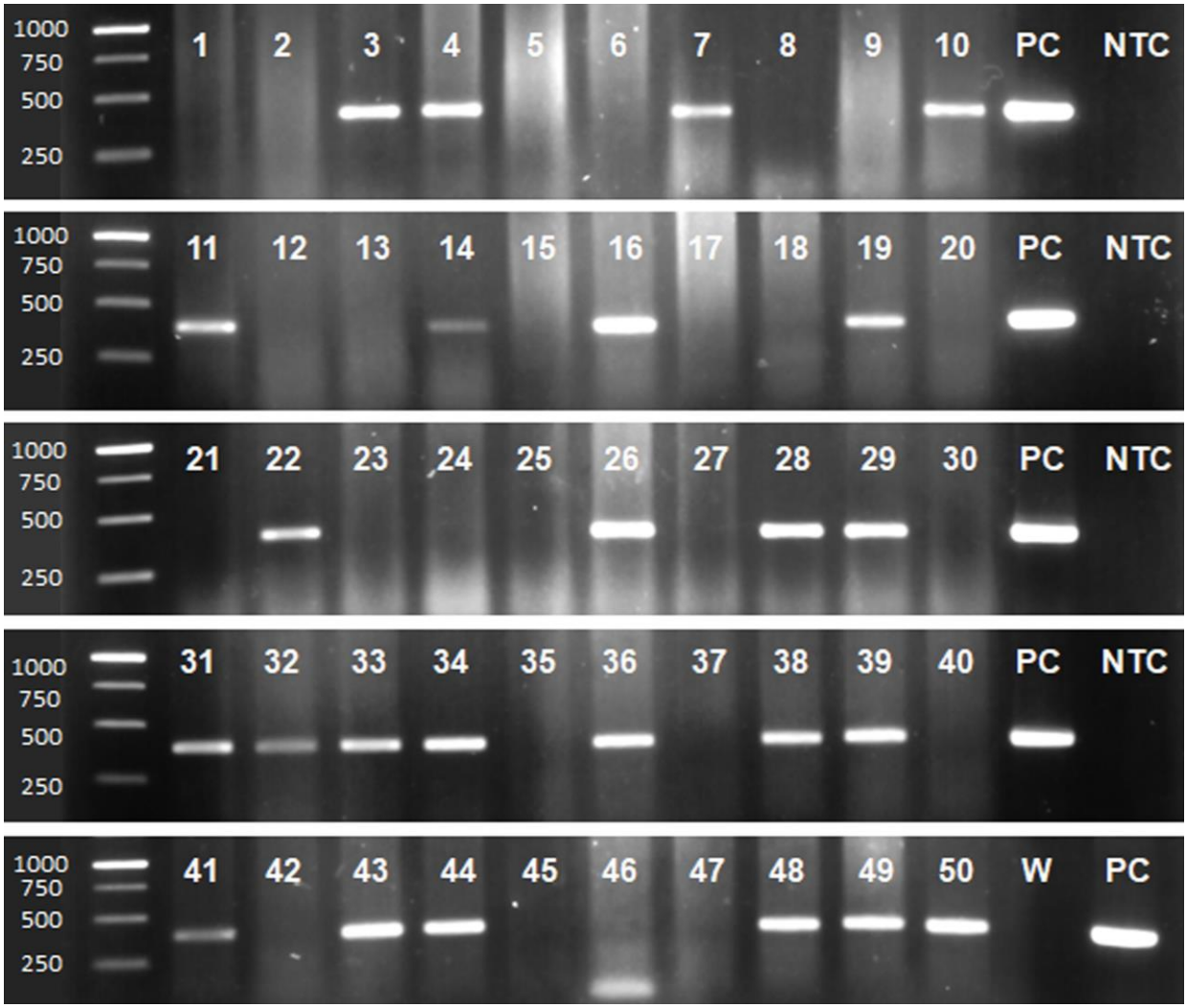


**Figure 3.** Amplification of the 394 bp AngHV-1 DNA polymerase gene. Lines 1-50 indicate the samples from the glass eels. PC indicates positive control. NTC indicates no template control. W indicates a water sample.

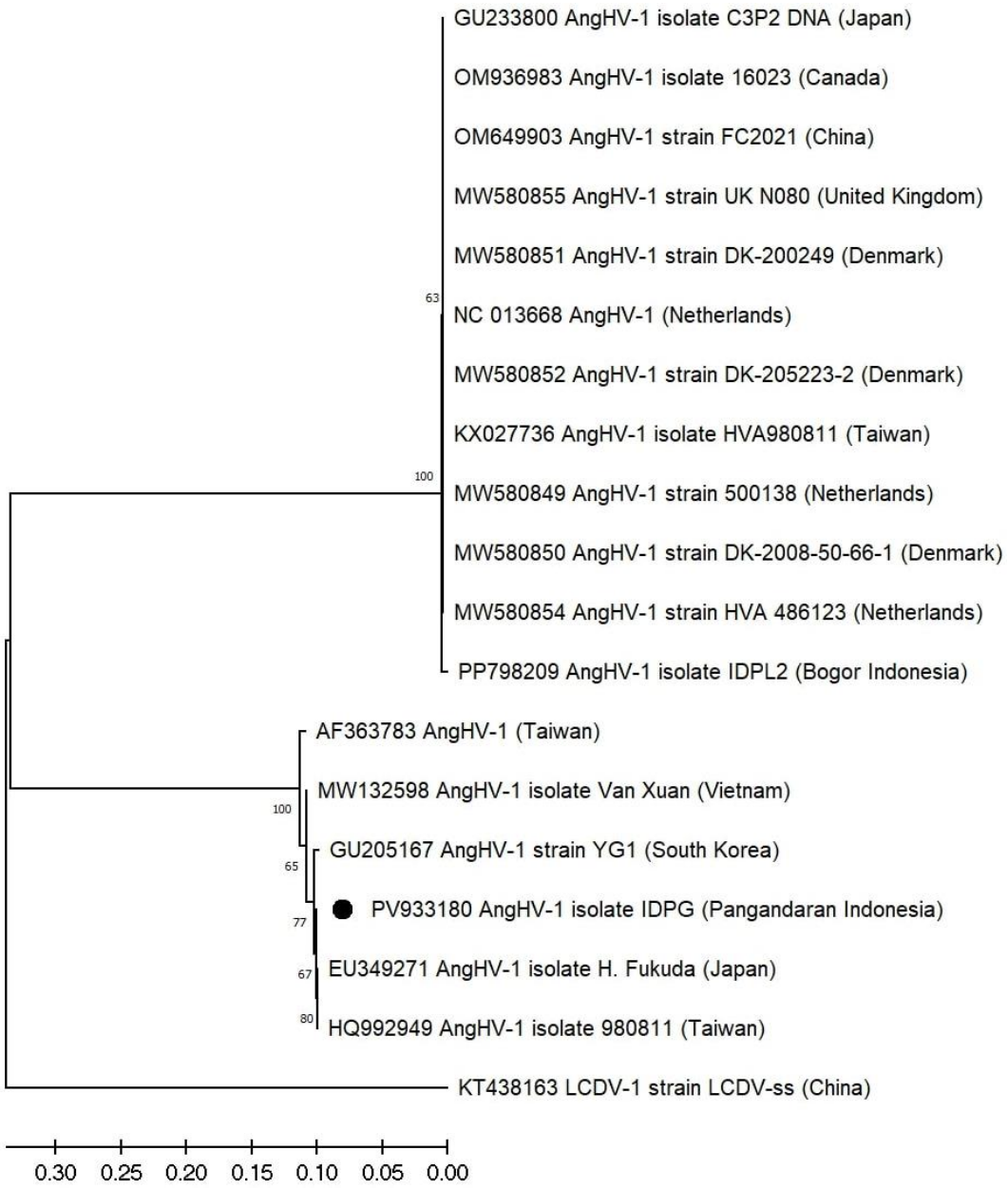


**Figure 4.** Phylogenetic tree of AngHV-1 isolate IDPG (●) constructed based on the partial DNA polymerase gene using the Neighbor-Joining method with p-distance evolutionary model and 1000 bootstrap replicates.

limit gene flow and promote divergence (Yang et al., 2025). Furthermore, intra-country variation was observed, as AngHV-1 IDPG from Pangandaran formed a separate clade from AngHV-1 IDPL2 isolated in Bogor, indicating the presence of multiple circulating lineages within Indonesia. Such genetic diversity may influence viral pathogenicity and could have implications for disease dynamics, potentially affecting aquaculture productivity and the sustainability of natural eel populations (Sanjuán & Domingo-Calap, 2021).

These findings highlight the need for continued surveillance to monitor the genetic diversity and spread of AngHV-1 in both wild and cultured eel populations. Although this study offers valuable insights, it was limited by a small sample size, partial gene analysis, and the use of conventional PCR instead of more sensitive methods like qPCR or ddPCR. Nonetheless, the results provide important early evidence of viral circulation in glass eels and suggest that wild-caught juveniles may serve as infection sources in aquaculture. Future research should include whole-genome sequencing to confirm strain differences, examine other life stages such as elvers and silver eels, and expand sampling to additional locations to better understand the virus's distribution, evolution, and epidemiological impact.

## Conclusions

This study successfully detected AngHV-1 in 50% of glass eels collected from the estuarine area of Pangandaran, Indonesia. Phylogenetic analysis revealed that the AngHV-1 isolate IDPG formed a distinct clade

from previously reported Indonesian isolates, indicating genetic variation within the country. These findings provide essential baseline data on the presence and diversity of AngHV-1 in wild eel populations and highlight the need for expanded surveillance and more comprehensive research to better understand the virus's transmission dynamics and to support effective disease prevention and control strategies in eel aquaculture.

## Ethical Statement

Eel dissection in this study was performed in compliance with the ethical standards set by the Ethical Committee of the National Research and Innovation Agency, with approval number 089/KE.02/SK/04/2024.

## Funding Information

The authors received no financial support for the research, authorship, and/or publication of this article.

## Author Contribution

First Author: Conceptualization, Data Curation, Formal Analysis, Writing – original draft; Second Author: Investigation, Writing – review and editing.

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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