First Record of Anguillid Herpesvirus 1 Linked to a Mass Mortality Event in Shortfin Eel (*Anguilla bicolor*) in Indonesia

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Abstract

Anguillid herpesvirus 1 (AngHV-1), a member of the Alloherpesviridae family, is known to cause high mortality in both wild and farmed eels. Notably, no cases of AngHV-1 infection in Indonesia until June 2023, when a significant mortality rate exceeding 75% among cultured glass eels was documented in Bogor, Indonesia. This study investigated the outbreak by collecting 30 diseased fish from multiple cultured tanks to examine clinical symptoms, histopathological changes, and viral presence through PCR targeting the viral DNA polymerase gene. Hemorrhagic lesions in the abdomen and anal regions were the primary clinical symptoms. Histopathological examination revealed hyperplasia, fusion, and epithelial lifting of the gill secondary lamellae. PCR, using 394 bp primer specific for AngHV-1, confirmed 100% infection among the collected samples, indicating rapid viral transmission within the rearing environment. Phylogenetic analysis of partial DNA polymerase amino acid sequences showed that Indonesian AngHV-1 isolate is genetically diverse and shares similarities with strains from China, Taiwan, Canada, and several European countries, suggesting the emergence of a novel strain. This study highlights the urgent need for enhanced biosecurity measures to curb AngHV-1 spread in the Indonesian eel aquaculture sector.

Keywords: Anguillid herpesvirus 1, molecular detection, Indonesia, phylogenetic, histology

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INTRODUCTION

The shortfin eel, *Anguilla bicolor*, holds significant economic importance in Indonesia (Mahardika *et al*., 2023). This species comprises two distinct subspecies: *A. bicolor bicolor*, found in the western waters of Sumatra and southern Java, and *A. bicolor pacifica*, located around Sulawesi (Arai and Taha, 2021; Fahmi *et al*., 2015). Most of Indonesia's eel exports come from wild-caught glass eels, resulting in relatively low production. Another critical challenge for eel farming is disease prevalence, leading to high mortality rates and, subsequently, cultivation failure. Eels diseases are caused by viral, bacterial, parasitic, and fungal pathogens (Andriyanto *et al*., 2021). Viruses, in particular, represent an emerging threat to aquatic species compared to bacteria (Meurens *et al*., 2021). Viral diseases in eels include Anguillid herpesvirus-1 (AngHV-1), rhabdovirus eel virus European X (EVEX), and aquabirnavirus eel virus European (EVE) (Ruiz de Ybáñez *et al*., 2023).

AngHV-1 belongs to the genus Cyprinivirus within the Alloherpesviridae family, a group of Herpesvirus affecting fish and amphibians (Donohoe *et al*., 2021; Streiff *et al*., 2023). The virus causes hemorrhagic disease and has significantly increased mortality rates in eels (Guo *et al*., 2022; Kempter *et al*., 2014). In China, AngHV-1 has led to mortality rates reaching 30% among cultured *A. rostrata* stocks (Guo *et al*., 2022). Over the last two decades, primary infections of AngHV-1 have been reported in Korea, Poland, Ireland, and Vietnam (Kempter *et al*., 2014; Kim *et al*., 2012; McConville *et al*., 2018; Panicz *et al*., 2021). Until 2023, AngHV-1 had not been detected in Indonesia. This study

presents the first molecular detection of AngHV-1 and histopathological examination related to a recent mass mortality event of *A. bicolor* in Indonesia.

MATERIALS AND METHODS

Ethical Approval

The dissection and use of *A. bicolor* as experimental subjects in this study were conducted in accordance with the ethical guidelines established by the National Research and Innovation Agency Ethical Committee. Ethical approval was granted under protocol number 089/KE.02/SK/04/2024.

Study Period and Location

In June 2023, a significant mortality even among glass eels was observed at an eel-rearing facility in Bogor, Indonesia, with estimated losses exceeding 75% of the total population. The mass mortality occurred approximately three weeks after the eels were introduced into the culture tank, and affected individuals exhibited surface-swimming behavior prior to death.

Sample Collection

Thirty diseased eels (0.1–0.3 g) were collected from the Bogor eel-rearing facility in June 2023. Following anesthesia in 4°C water, glass eels were sacrificed, enclosed in plastic bags, and frozen at -20°C for viral genome extraction and species identification. Additionally, five eels were preserved in a 10% buffered neutral formalin (BNF) solution for histopathological examination. All procedures were conducted at the Laboratory for the Development of Industrial Technology for Agriculture and Biomedic (LAPTIAB)-BRIN in Tangerang Selatan.

Viral DNA Extraction, PCR Amplification, and Sequencing

Viral DNA was extracted directly from 20 mg of head tissue using the phenol-chloroform method described by Sambrook *et al*. (1989). Thirty DNA samples were amplified using primer pairs HVAPOLVPSD (5’-GTG TCG GGC CTT TGT GGT GA-3’) and HVAPOLOOSN (5’-CAT GCC GGG AGT CTT TTT GAT-3’) targeting the DNA polymerase for AngHV-1 detection, producing a 394 bp fragment (Rijsewijk *et al*., 2005). For AngHV-1 phylogenetic analysis, one sample was selected, and new primer pairs AngHV pol F (5’-TGA GGG AGA AAG TGT GCG TC-3’) and AngHV pol R (5’-CGA GCCA AGC TGA AGG AGA A-3’) were designed to amplify a 1500 bp fragment of the DNA polymerase gene. PCR was performed using MyTaq™ HS Mix (Bioline) with the following thermal cycling conditions: AngHV-1 Detection: Initial denaturation at 94°C for 10 min; 40 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 45 s, and extension at 72°C for 1 min; final extension at 72°C for 7 min. 1500 bp amplification: Initial denaturation at 94°C for 10 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s, extension at 72°C for 1 min; final extension step at 72°C for 7 min. PCR products were electrophoresed in a 1% (w/v) agarose/TBE gel containing 0.002% (v/v) SYBR™ Safe DNA Gel Stain at 100 V for 30 min, and visualized using a UV transilluminator. Sanger sequencing was conducted by 1st BASE using an Applied Biosystems genetic analyzer.

Host Species Identification

DNA barcoding was used to identify the eel species. Extracted DNA was amplified with primer pairs FF2d (5'-TTC TCC ACC AAC CAC AAR GAY ATY GG-3') and FR1d (5'-CAC CTC AGG GTG TCC GAA RAA YCA RAA-3') targeting the CO1 gene (Ivanova *et al*., 2007). PCR amplification was performed under the following conditions: pre-denaturation at 94°C 2 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 40 s, and extension at 72°C for 1 min; final extension at 72°C for 10 min. Sequencing was performed as previously described.

AngHV-1 Bioinformatic Analysis

The DNA sequences obtained were aligned between the forward and reverse strands and compared with sequences in the GeneBank using Basic Local Alignment Search Tools (BLAST)

(https://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine sequence homology with AngHV-1. Phylogenetic analysis was conducted using Molecular Evolutionary Genetic Analysis (MegaX) software, with a tree constructed based on translated amino acid sequences using the UPGMA method with 1000 bootstrap replicates (Hua *et al*., 2017; Kumar *et al*., 2018). Eleven sequences of AngHV-1, three Cyprinid herpesvirus, and one Atlantic cod herpesvirus sequence from the GeneBank were used for comparison.

Histopathological Observation

Histopathological preparations were carried out following a modified protocol from Nuryati *et al*. (2023), which included tissue dehydration, rehydration, clearing, and embedding. Sections (5 µm) were prepared using a Leica semiautomatic microtome and stained with hematoxylin and eosin. Stained tissues were observed using an Olympus CX-23 binocular microscope.

Data Analysis

The results of this study are presented descriptively.

RESULTS AND DISCUSSION

DNA barcoding using the CO1 gene was performed to confirm the eel species as *A. bicolor*, as verified by BLAST results. This finding aligns with Taufiq-Spj *et al*. (2022), who also identified *A. bicolor* through DNA barcoding of eels in West and Central Java. The nucleotide sequence of the CO1 was deposited on GeneBank with accession number PP892205.

Figure 1. Clinical symptoms of naturally diseased *Anguilla bicolor* glass eel. Haemorrhage is observed in the abdominal and anal areas (white arrows).

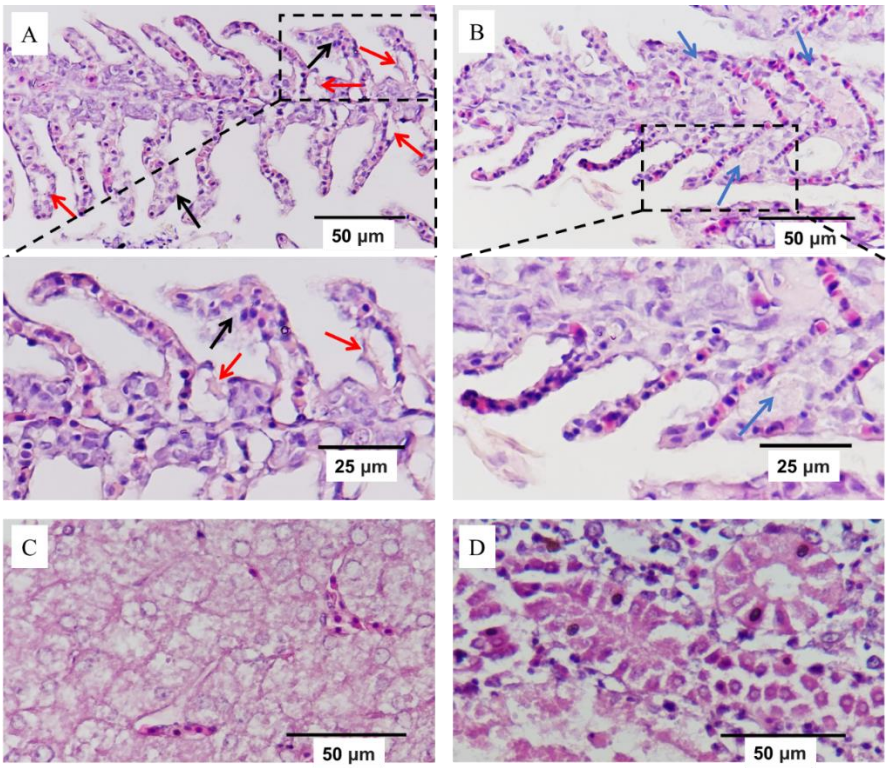

Figure 2. Histopathological changes in the gill of diseased glass eel. (A) The gills exhibit epithelial lifting (red arrow), and hyperplasia in the secondary lamellae (black arrow). (B) Fusion of the secondary lamellae (blue arrow). No histopathological changes were detected in (C) the liver and (D) kidney.

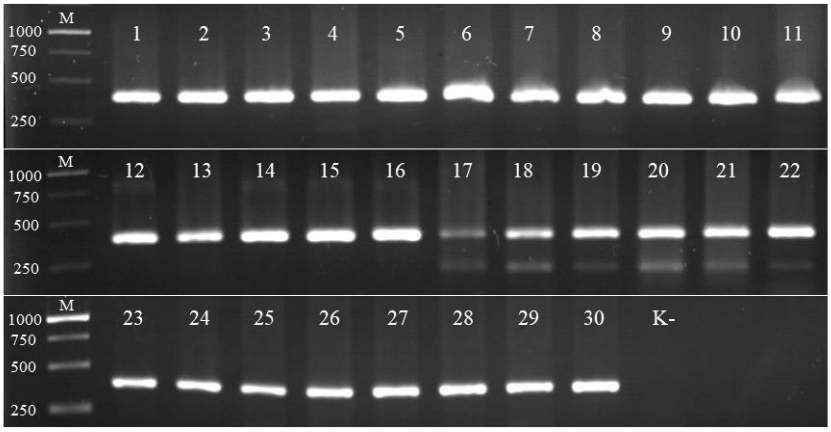

Figure 3. PCR amplification result of AngHV-1 DNA polymerase gene. Lanes 1–30 display a 394 bp amplicon, confirming the presence of AngHV-1 in the samples. A 1 kb marker (M) provides size reference, with lane K- serving as a negative control.

Table 1. Percentage similarity of AngHV-1 isolate IDPL2 with BLAST top five hits. Based on DNA polymerase sequences

Sequence	Accession Number	Query Cover	Percent Identity
AngHV-1 strain FC 2021	OM649903	100%	99.87%
AngHV-1 isolate 16023	OM936983	100%	99.87%
AngHV-1 strain UK N080	MW580855	100%	99.87%
AngHV-1 strain HVA 486123	MW580854	100%	99.87%
AngHV-1 strain DK-206116-1	MW580853	100%	99.87%

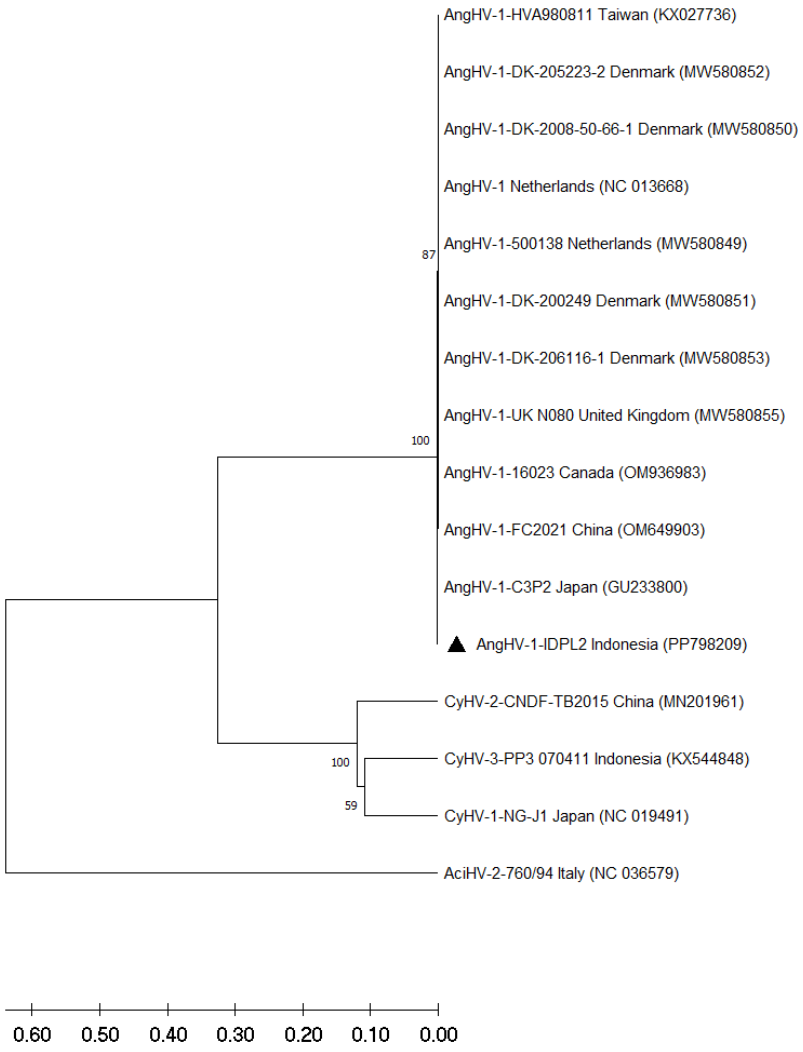

Figure 4. Phylogenetic tree of AngHV-1 isolate IDPL2 constructed using partial DNA polymerase amino acid sequences via the UPGMA method. AngHV: Anguillid herpesvirus; CyHV: Cyprinid herpesvirus; AciHV: Acipenserid herpesvirus. GeneBank accession numbers are listed in brackets. A black triangle denotes the AngHV-1 IPDL2, with the scale bar indicating substitutions per amino acid site.

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                                                    110       120       130       140
AngHV-1-IDPL2            Indonesia    (PP798209) ENLYTILLHKSVETGWTRFTKYKSSSLRHYLSMRNKYKGDM
AngHV-1-FC2021           China        (OM649903) ....................T.T................
AngHV-1-16023            Canada       (OM936983) ....................T.T................
AngHV-1-UK N080          UK           (MW580855) ....................T.T................
AngHV-1-DK-206116-1      Denmark      (MW580853) ....................T.T................
AngHV-1-DK-200249        Denmark      (MW580851) ....................T.T................
AngHV-1-500138           Netherlands  (MW580849) ....................T.T................
AngHV-1                  Netherlands  (NC013668) ....................T.T................
AngHV-1-C3P2             Japan        (GU233800) ....................T.T................
AngHV-1-DK-2008-50-66-1  Denmark      (MW580850) ....................T.T................
AngHV-1-HVA980811        Taiwan       (KX027736) ....................T.T................
AngHV-1-DK-205223-2      Denmark      (MW580852) ....................T.T................
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Figure 5. Alignment of the translated amino acid sequence showing two unique amino acid substitutions in the AngHV-1-IDPL2 isolate from Indonesia compared to other sequences in GeneBank. GenBank accession numbers are included in brackets for reference.

The clinical symptoms in diseased glass eel included marked haemorrhage in abdominal and anal regions (Figure 1). Similar symptoms were observed by Guo *et al*. (2022), who reported red abdomen and congestion in adult *A. rostrata* infected by AngHV-1 in China. Park *et al*. (2012) documented comparable haemorrhages in the operculum and abdominal area of *A. bicolor* infected with AngHV-1 in Korea. In Vietnam, juvenile giant mottled eel (*A. marmorata*) infected with AngHV-1 exhibited haemorrhages on the skin near the operculum and internal organs like the liver and gut (Panicz *et al*., 2021). AngHV-1 has been associated with eel mortality rates between 1% to 10%, which can rise to 50% under stress condition (Kempter *et al*., 2014). In certain cases, the mortality rate has been reported to reach 12% in Vietnam and up to 30% in China (Guo *et al*., 2022; Panicz *et al*., 2021). In this study, eel mortality exceeded 75%, likely due to the increased susceptibility of glass eels compared to older life stages, exacerbated by mechanical stress, handling, and environmental adaptation (Josset *et al*., 2015).

Histopathological observation revealed multiple alterations in the gills, including secondary lamellae hyperplasia, secondary lamellae fusion, and epithelial lifting (Figure 2). These findings align with other studies documenting secondary lamellae hyperplasia and fusion in AngHV-1-infected eel cultured in several countries, including Netherland and Korea (Hangalapura *et al*., 2007; Kim *et al*., 2012; Park *et al*., 2012). Epithelial lifting has also been reported in common carp gills following exposure to the carp edema virus (Adamek *et al*., 2021). Structural damage such as secondary lamella hyperplasia, may impair gas exchange, resulting in hypoxia (Adam *et al*., 2019; Rani *et al*., 2022; Novindasari *et al*., 2024). Under certain conditions, hypoxia can lead to significant mortality in cultured fish populations (Wang *et al*., 2023). In this study, hypoxia was evident, as affected fish were observed swimming near the surface prior to death. Unlike studies on eels cultured in China and Korea, no histopathological alterations were found in the liver or kidneys (Guo *et al*., 2022; Park *et al*., 2012; Safira *et al*., 2022), suggesting that the virus had a milder impact on these organs compared to the gills. Previous studies indicate that AngHV-1 infection initially affects the skin and stomach, followed by the gills, and then internal organs like the intestines and liver, with gill hyperplasia often preceding kidney necrosis (Hangalapura *et al*., 2007).

PCR analysis confirmed the amplification of a 394 bp segment of AngHV-1 DNA polymerase in all the samples (Figure 3), indicating rapid viral spread within the tank-reared fish population. Recent studies have demonstrated that viruses can disseminate swiftly in high-density environments, such as aquaculture ponds. Dharan *et al*. (2022) highlighted that viral exposure in such settings can lead to substantial fish mortality, underscoring the significance of horizontal transmission in viral proliferation. Additionally, a longer segment of DNA polymerase (~1500 bp) was successfully amplified for phylogenetic analysis, yielding 1486 nucleotide bases that encode 494 amino acids. This sequence has been deposited at GeneBank under the name AngHV-1 isolate IDPL2 with accession number PP798209 and protein ID XAV45685.

A nucleotide homology analysis using BLAST revealed a 99.87% sequence similarity with multiple AngHV-1 DNA polymerase sequences deposited in GeneBank (Table 1). In Alloherpesviridae, sequence identity ≥ 90% is indicative of conspecificity with the reference virus (Zhang *et al*., 2023). Phylogenetic analysis of partial DNA polymerase amino acid sequences, performed using the UPGMA method, showed that AngHV-1 IDPL2 diverges from strains originating from Europe, Canada, Taiwan, and China (Figure 4). Additionally, two distinct amino acid substitutions were identified in the partial DNA polymerase sequence, with threonine replaced by lysine (Figure 5).

The family Alloherpesviridae includes herpesviruses infecting fish (genera *Salmonivirus*, *Ictalurivirus*, and *Cyprinivirus*) and amphibians (genus *Batrachovirus*). Unlike other cypriniviruses, AngHV-1 exhibits a slower evolutionary rate and lower positive selection, likely due to the solitary and single-spawning

nature of eels, which restricts continuous viral transmission chains. Conserved genes, including the DNA polymerase, in Alloherpesviridae, adapt to host immune responses and habitats (Donohoe *et al*., 2021; Garver *et al*., 2018; Fikri *et al*., 2024). The identification of a novel amino acid mutation in the DNA polymerase of AngHV-1 enhances the understanding of the virus's molecular evolution. Nevertheless, current knowledge about the effects of such mutations on viral replication and pathogenicity within the Alloherpesviridae remains limited. In a related virus, herpes simplex virus (HSV), belonging to the same order (Herpesvirales), similar mutations in the DNA polymerase and thymidine kinase genes have been linked to resistance against antiviral drugs, offering a potential parallel for understanding AngHV-1 mutations (Andrei *et al*., 2007). Further research on DNA polymerase mutations in AngHV-1 is needed to understand their roles in pathogenicity and drug resistance fully.

CONCLUSION

This study successfully identified the AngHV-1 infection as the cause of mass mortality in glass eel reared in Bogor, marking the first documented case of this virus in Indonesia. The phylogenetic analysis and observed amino acid mutations within the DNA polymerase gene suggest that the detected AngHV-1 may represent a novel strain. To confirm the existence of a new strain and facilitate a comprehensive analysis of AngHV-1 in Indonesia, future studies will require whole-genome sequencing. The clinical symptoms and histopathological changes documented in eels infected with this virus provide valuable insight into disease manifestation and progression. These findings serve as an early warning for eel farms in Indonesia, emphasizing the critical importance of biosecurity measures to prevent the spread of this virus and mitigate the potential economic impact on the eel farming industry.

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AUTHORS' CONTRIBUTIONS

EIR: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Writing - Original Draft. HW: Conceptualization, Methodology. AA: Resources, Methodology. NM: Validation, Investigation. ARA: Resources, Data Curation. AFL: Visualization, Investigation. KMD: Resources, Data Curation. AF: Resources. IC: Supervision and Writing – Review & Editing. WS: Supervision and Writing – Review & Editing. DY: Supervision and Writing – Review & Editing. TB: Manuscript Review. RSA: Supervision and Writing – Review & Editing. SS: Validation, Writing – Review & Editing.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Adam, M. A., Maftuch, M., Kilawati, Y., & Risjani, Y. (2019). The effect of cadmium exposure on the cytoskeleton and morphology of the gill chloride cells in juvenile mosquito fish (*Gambusia affinis*). *Egyptian Journal of Aquatic Research*, 45(4), 337–343.

Adamek, M., Teitge, F., Baumann, I., Jung-Schroers, V., El Rahman, S. A., Paley, R., Piackova, V., Gela, D., Kocour, M., Rakers, S., Bergmann, S. M., Ganter, M., & Steinhagen, D. (2021). Koi sleepy disease as a pathophysiological and immunological consequence of a branchial infection of common carp with carp edema virus. *Virulence*, 12(1), 1855–1883.

Andrei, G., Fiten, P., Froeyen, M., De Clercq, E., Opdenakker, G., & Snoeck, R. (2007). DNA

Polymerase Mutations in Drug-Resistant Herpes Simplex Virus Mutants Determine In Vivo Neurovirulence and Drug-Enzyme Interactions. *Antiviral Therapy*, 12, 719–732.

Andriyanto, S., Novita, H., Sumiati, T., & Taukhid. (2021). Isolation and identification of bacteria and parasites in glass eel (*Anguilla* spp.). *E3S Web of Conferences*, 322, 02012.

Arai, T., & Taha, H. (2021). Contrasting patterns of genetic population structure in tropical freshwater eels of genus Anguilla in the Indo-Pacific. *Heliyon*, 7(5).

Dharan, V., Khoo, L., Phelps, N. B. D., Kumar, G., Steadman, J., Bosworth, B., & Aarattuthodi, S. (2022). An investigation into the pathogenesis of blue catfish alloherpesvirus in ictalurid catfish. *Journal of the World Aquaculture Society*, 53(2), 384–400.

Donohoe, O., Zhang, H., Delrez, N., Gao, Y., Suárez, N. M., Davison, A. J., & Vanderplasschen, A. (2021). Genomes of Anguillid Herpesvirus 1 Strains Reveal Evolutionary Disparities and Low Genetic Diversity in the Genus Cyprinivirus. *Microorganisms*, 9(998), 1–26.

Fahmi, M. R., Solihin, D. D., Shao, Z., Pouyaud, L., & Berrebi, P. (2015). Population Genetic Structure of the Tropical Eel *Anguilla bicolor* in Indonesian Waters Based on Microsatellite Markers. *Folia Zoologica*, 64(2), 87–96.

Fikri, F., Wardhana, D. K., Purnomo, A., Khairani, S., Chhetri, S., & Purnama, M. T. E. (2022). Aerolysin gene characterization and antimicrobial resistance profile of *Aeromonas hydrophila* isolated from milkfish (*Chanos chanos*) in Gresik, Indonesia. *Veterinary world*, 15(7), 1759.

Garver, K. A., Leskisenoja, K., Macrae, R., Hawley, L. M., Subramaniam, K., Waltzek, T. B., Richard, J., Josefsson, C., & Tellervo Valtonen, E. (2018). An alloherpesvirus infection of european perch perca fluviatilis in Finland. *Diseases of Aquatic Organisms*, 128(3), 175–185.

Guo, R., Zhang, Z., He, T., Li, M., Zhuo, Y., Yang, X., Fan, H., & Chen, X. (2022). Isolation and Identification of a New Isolate of Anguillid Herpesvirus 1 from Farmed American Eels (*Anguilla rostrata*) in China. *Viruses*, 14(12), 2722.

Hangalapura, B. N., Zwart, R., Engelsma, M. Y., & Haenen, O. L. M. (2007). Pathogenesis of Herpesvirus anguillae (HVA) in juvenile European eel *Anguilla anguilla* after infection by bath immersion. *Diseases of Aquatic Organisms*, 78(1), 13–22.

Hua, G. J., Hung, C. L., Lin, C. Y., Wu, F. C., Chan, Y. W., & Tang, C. Y. (2017). MGUPGMA: A Fast UPGMA Algorithm With Multiple Graphics Processing Units Using NCCL. In *Evolutionary Bioinformatics*, 13(1).

Ivanova, N. V., Zemlak, T. S., Hanner, R. H., & Hebert, P. D. N. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, 7(4), 544–548.

Josset, Q., Trancart, T., Mazel, V., Charrier, F., Frott, L., Acou, A., & Feunteun, E. (2015). Pre-release processes influencing short-term mortality of glass eels in the French eel (*Anguilla anguilla*, Linnaeus 1758) stocking programme. *ICES Journal of Marine Science*, 73(1), 150–157.

Kempter, J., Panicz, R., & Bergmann, S. M. (2014). First Detection of anguillid herpesvirus 1 (AngHV1) in European eel (*Anguilla anguilla*) and imported American eel (*Anguilla rostrata*) in Poland. *Bulletin European Association of Fish Pathology*, 34(3), 87–94.

Kim, H. J., Yu, J. H., Kim, D. W., Kwon, S. R., & Park, S. W. (2012). Molecular evidence of anguillid herpesvirus-1 (AngHV-1) in the farmed Japanese eel, *Anguilla japonica* Temminck & Schlegel, in Korea. *Journal of Fish Diseases*, 35(4), 315–319.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549.

Mahardika, V., Nurilmala, M., Pertiwi, R. M., Nurjanah, & Nugraha, R. (2023). Characterization and Effect of Processing on Parvalbumin Content in Indonesian Shortfin Eel (*Anguilla bicolor bicolor*). *Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology*, 18(3), 194–201.

McConville, J., Fringuelli, E., Evans, D., & Savage, P. (2018). First examination of the Lough Neagh European eel (*Anguilla anguilla*) population for eel virus European, eel virus European X and Anguillid Herpesvirus-1 infection by employing novel molecular techniques. *Journal of Fish Diseases*, 41(12), 1783–1791.

Meurens, F., Dunoyer, C., Fourichon, C., Gerdts, V., Haddad, N., Kortekaas, J., Lewandowska, M., Monchatre-Leroy, E., Summerfield, A., Wichgers Schreur, P. J., van der Poel, W. H. M., & Zhu, J. (2021). Animal board invited review: Risks of zoonotic disease emergence at the interface of wildlife and livestock systems. *Animals*, 15(6).

Novindasari, B. B. M., Nurrahmi, I. A., Andrian, K. N., & Haryanto, A. (2024). Molecular Fish Sexing on Taisho Sanshoku Koi (*Cyprinus carpio*) Based on ArS. 9–15 Gene Amplification using PCR Method. *Jurnal Medik Veteriner*, 7(2), 255–263.

Nuryati, S., Yanti, M., Rahman, & Wahyuwardani, S. (2023). Kidney and liver histopathology of sea bass *Lates calcarifer* infected with black body syndrome-associated bacteria. *Jurnal Akuakultur Indonesia*, 22(2), 170–178.

Panicz, R., Eljasik, P., Nguyen, T. T., Vo Thi, K. T., & Hoang, D. Van. (2021). First detection of Herpesvirus anguillae (AngHV-1) associated with mortalities in farmed giant mottled eel (*Anguilla marmorata*) in Vietnam. *Journal of Fish Diseases*, 44(6), 847–852.

Park, S.-W., Jung, E.-B., & Kim, D.-W. (2012). Outbreak of Anguillid herpesvirus-1 (AngHV-1) infection in cultured shortfin eel (*Anguilla bicolor*) in Korea. *Journal of Fish Pathology*, 25(3), 151–158.

Rani, C. A. M., Safira, A., Suryadiningrat, M., Fikri, F., Wardhana, D. K., & Purnama, M. T. E. (2022). Characterization of Tilapia collagen-loaded chitosan nanofibers synthesized by electrospinning method for wound dressing. In *IOP Conference Series: Earth and Environmental Science*, 1036(1), 012034.

Rijsewijk, F., Pritz-Verschuren, S., Kerkhoff, S., Botter, A., Willemsen, M., Nieuwstadt, T. Van, & Haenen, O. (2005). Development of a polymerase chain reaction for the detection of Anguillid herpesvirus DNA in eels based on the herpesvirus DNA polymerase gene. *Journal of Virological Methods*, 124(1–2), 87–94.

Ruiz de Ybáñez, M. R., del Río, L., Flores-Flores, C., Muñoz, P., Berriatua, E., Rubio, S., & Martínez-Carrasco, C. (2023). Monitoring for *Anguillicoloides crassus*, Anguillid herpesvirus 1, aquabirnavirus EVE and rhabdovirus EVEX in the European eel population of southern Spain. *Journal of Fish Diseases*, 46(4), 417–431.

Safira, A., Rani, C. A. M., Puspitasari, R. A., Ayuningtyas, A. K. P., Mahendra, Y. A., Purnomo, A., Chhetri, S., & Purnama, M. T. E. (2022). Amino Acid and Proximate Analysis of Type-1 Collagen from Sea Cucumber and Tilapia-Skin and its Potential Application as Artificial Tendon. *Pharmacognosy Journal*, 14(4).

Sambrook, J., Fritsch, E., & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual* (2nd ed.). Cold Spring Harbor Laboratory Press.

Streiff, C., He, B., Morvan, L., Zhang, H., Delrez, N., Fourrier, M., Manfroid, I., Suárez, N. M., Betoulle, S., Davison, A. J., Donohoe, O., & Vanderplasschen, A. (2023). Susceptibility and Permissivity of Zebrafish (*Danio rerio*) Larvae to Cypriniviruses. *Viruses*, 15(3), 768.

Taufiq-Spj, N., Santosa, G. W., Trianto, A., Sugianto, D. N., Wirasatriya, A., Budimawan, B., Yusuf, M., Indarjo, A., Helmi, M., Sembiring, A., Pertiwi, N. P. D., & Ighwerb, M. I. (2022). Comparison in the

phylogenetic pattern of Java eel (*Anguilla bicolor bicolor*) from Java to Western Indian Ocean: Monophyletic fact and migration loop possibility. *Egyptian Journal of Aquatic Biology & Fisheries*, 75–91.

Wang, Z., Pu, D., Zheng, J., Li, P., Lü, H., Wei, X., Li, M., Li, D., & Gao, L. (2023). Hypoxia-induced physiological responses in fish: From organism to tissue to molecular levels. In *Ecotoxicology and Environmental Safety*, 267(1).

Zhang, W., Wang, R., Gu, C., Yang, Q., He, M., Xiao, W., He, L., Zhao, M., Zou, X., & Yu, Z. (2023). Comparative genomic analysis of alloherpesviruses: Exploring an available genus/species demarcation proposal and method. *Virus Research*, 334.

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