JIPK. Volume 16 No 2 November 2024
Sinta 1 (Decree No: 158/E/KPT/2021)
e-ISSN:2528-0759; p-ISSN:2085-5842
Available online at https://e-journal.unair.ac.id/JIPK

JIPK
(JURNAL ILMIAH PERIKANAN DAN KELAUTAN)
Scientific Journal of Fisheries and Marine

Research Article

Susceptibility and Target Organ of Lymphocystis Disease Virus Infection in Giant Gourami (*Osphronemus goramy*), Hybrid Tilapia (*Oreochromis* sp.), Siamese Fighting Fish (*Betta splendens*), and Hybrid Catfish (*Clarias* sp.)

Nur Lailatul Fitrotun Nikmah, Alim Isnansetyo, Indah Istiqomah, and Murwantoko*

Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, 55281. Indonesia

OPEN ACCESS

ARTICLE INFO

Received: April 11, 2024
Accepted: June 24, 2024
Published: July 21, 2024
Available online: Oct 23, 2024

*) Corresponding author:
E-mail: murwantoko@ugm.ac.id

Keywords:
Lymphocystis Disease Virus
Major Capsid Protein
Predilection
Real-time PCR
Viral load

Abstract

Lymphocystis disease has a broad host range and has been reported to enter Indonesia. However, information regarding its susceptibility and predilection organs in fish is lacking. This study examined the susceptibility of four important fish species in Indonesia, namely, giant gourami (*Osphronemus goramy*), hybrid tilapia (*Oreochromis* sp.), Siamese fighting fish (*Betta splendens*), and hybrid catfish (*Clarias* sp.). The fish were infected with virus filtrate by intraperitoneal injection and immersion. The postinfection observation period was 60 days. Viral load was quantified by qPCR and expressed as major capsid protein (MCP) copy number/mg tissue. Mortality was observed in all fish species, with the highest recorded in hybrid catfish and the lowest in Siamese fighting fish. All the fish species showed changes in their clinical symptoms, such as anorexia and separation from schools. However, only giant gourami showed internal change seven days after injection (dpi), with white lesion detected in the liver. Viral load quantification showed that LCDV had different predilection organs in the four fish species. The highest viral load of giant gourami (1.7×10^4) was observed in the liver at 7 dpi, hybrid tilapia (7.5×10^3) was observed in the fins at 21 dpi, Siamese fighting fish (8.4 x 103) was observed in the fins at 14 dpi, and hybrid catfish (1.2×10^3) were observed in the fins and gills at 7 and 14 dpi. The findings indicated that giant gourami, hybrid tilapia, Siamese fighting fish, and hybrid catfish were susceptible to LCDV infection with different predilection organs.

Cite this as: Nikmah, N. L. F., Isnansetyo, A., Istiqomah, I., & Murwantoko. (2024). Susceptibility and Target Organ of Lymphocystis Disease Virus Infection in Giant Gourami (*Osphronemus goramy*), Hybrid Tilapia (*Oreochromis* sp), Siamese Fighting Fish (*Betta splendens*), and Hybrid Catfish (*Clarias* sp). *Jurnal Ilmiah Perikanan dan Kelautan*, 16(2):389-398. http://doi.org/10.20473/jipk.v16i2.56685

1. Introduction

Lymphocystis disease virus (LCDV) is the etiologic agent of lymphocystis disease, also known as cauliflower disease. This name comes from the typical clinical symptoms in the form of cauliflower-like nodules on the body surface and viscera of infected fish (Zhang *et al.*, 2023). Lymphocystis disease was first reported in flounder fish (*Paralichthys olivaceus*), with LCDV declared as the etiological agent and isolated in 1966 (Frasca *et al.*, 2018). Lymphocystis disease has the pathognomonic symptom of warts clustered on the body surface. These warts comprise hypertrophied dermal fibroblast cells containing replicative virions (Valverde *et al*., 2017).

Previous studies reported no mortality due to lymphocystis disease because the lymphocystis that appears can heal by itself (Zhang *et al*., 2023). Current studies, lymphocystis disease was associated with mass mortality in farmed fish (Rahmati-Holasoo *et al*., 2023) with incidence rate of up to 70% and was linked to economic losses in fish farming (Borrego *et al*., 2015). Lymphocystis disease has also begun to spread in Tunisia on the African continent since 2011, resulting in significant mortality of up to 45% of the total population of cultured gilthead sea bream (*Sparus aurata*) on local semi-intensive fish farms in Italy (Dezfuli *et al*., 2012) as well as 20–45% mortality of gilthead sea bream in Tunisia (Haddad-Boubaker *et al*., 2013). In addition, lymphocystis disease caused the mortality of 10% of the population of a yellow bar angelfish (*Pomacanthus maculosus*) farm in Iran (Rahmati-Holasoo *et al*., 2023) and 53% of the population of john snapper (*Lutjanus johnii*) on a farm in Malaysia with a total loss of USD 4,000 (Nurliyana *et al*., 2023).

Since the first report of LCDV from the Baltic Sea in Germany, this virus has spread throughout the continent (Borrego *et al*., 2015). To date, lymphocystis disease has been reported in 26 countries on four continents, including Europe (Benkaroun *et al*., 2022), North Africa (Cherif *et al*., 2020), East Africa (Abowei *et al*., 2011), Australia (Ashburner, 1975), China (Xu *et al.*, 2014), Japan (Kawato *et al.*, 2021), Spain (Valverde *et al*., 2017), Malaysia (Nurliyana *et al*., 2023), Indonesia (Murwantoko *et al*., 2022), Uruguay (Doszpoly *et al*., 2020), Turkey (Pekmez *et al*., 2022), Iran (Rahmati-Holasoo *et al*., 2022), Brazil (de Lucca Maganha *et al*., 2020), China (Zheng *et al.*, 2016), India (Shahi *et al*., 2020), Israel (Pekmez *et al*., 2022), Germany (Benkaroun *et al.*, 2022), Taiwan (Cheng *et al*., 2023), Korea (Cheng *et al*., 2023), Egypt (Aly *et al*., 2018), Singapore (Sheng *et al*., 2020), Virginia (Wolf, 1962), France (Cano *et al*., 2010), Tunisia (Cano *et al*., 2010), Portugal (Cano *et al*., 2010), and America (Benkaroun *et al*., 2022).

Lymphocystis disease has become endemic in the North Sea and the Mediterranean zone, affecting snakeskin gourami (*Trichogaster pectoralis*) in Jerusalem (Paperna *et al*., 1987); gilthead sea bream (*Sparus aurata*) in Spain (Basurco *et al*., 1990), Aqaba (Paperna *et al*., 1982), and Portugal (Menezes *et al*., 1987); common dab (*Limanda limanda*) in the North Sea (Dethlefsen *et al*., 2000); and Sennegalese sole (*Solea senegalensis* Kaup) and blackspot sea bream (*Pagellus bogaraveo*) in Spain (Alonso *et al*., 2005). This infection has also been reported in Asian waters, including in bastard halibut (*Paralichthys olivaceus*) in Hime, Japan (Matsuoka *et al*., 1995); Korean rockfish (*Sebastes schlegeli*) in Hokkaido, Japan (Tanaka *et al*., 1984); Korean rockfish (*Sebastes schlegelii*) in Korea (Chun, 1998); bastard halibut (*Paralichthys olivaceus*) in China (Xu *et al*., 2000); Indian glassy fish (*Parambassis ranga*), three spot gourami (*Trichopodus trichopterus*), and pearl gourami (*Trichopodus leerii*) in Yeosu, Korea (Hossain *et al*., 2008); paradisefish (*Macropodus opercularis*) in Guangdong, China (Xu *et al*., 2014); and giant grouper (*Epinephelus lanceolatus*), orange-spotted grouper (*Epinephelus coioides*), and brown-marbled grouper (*Epinephelus fuscoguttatus*) in Hainan and Fujian, China (Huang *et al*., 2015).

In Indonesia, lymphocystis disease infects clownfish (*Amphiprion percula*) (Lam *et al*., 2020; Murwantoko *et al*., 2022) and snakehead (*Channa striata*) (Sihananto *et al*., 2019; Nikmah *et al*., 2024). Indonesia has economically important fish and mainstay commodities, some of which are giant gourami (*Osphronemus goramy*), hybrid tilapia (*Oreochromis* sp.), Siamese fighting fish (*Betta splendens*), and hybrid catfish (*Clarias* sp.) (BPS-Statistics Indonesia, 2023). The production value of giant gourami was 875.594 tons in 2018, hybrid catfish was 993.768 tons in 2020, and hybrid tilapia was 1.172.633 tons in 2020 (BPS-Statistics Indonesia, 2023). Meanwhile, Siamese fighting fish are classified as ornamental fish, which shows potential utilization in Indonesia (Afnan *et al*., 2019). Therefore, this study aims to determine the possibility of LCDV infection, identify the predilection organs, and quantify the viral load in these important fish.

2. Materials and Methods

2.1 Materials

The equipment used in this study are centrifuge sorval legend 21R (ThermoScientific, USA), nylon syringe filters (Milipore, USA), NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), and CFX96 Touch Real-Time PCR Machine (BioRad,

USA). The materials used are Tryptic Soy Agar (Oxoid, USA), FavorPrepTM Tissue Genomic DNA Extraction Mini Kit (Favorgen, Taiwan), ethanol (Merck, germany), and 2x SensiFAST SYBR® No-ROX Mix (Meridian Bioscience, USA).

2.1.1 Ethical approval

The dissection and utilization of experimental animals in this study were performed under the supervision of ethical clearance no. 00025/04/LPPT/X/2022.

2.2 Wart Tissue Collection and Virus Filtrate Preparation

Wart tissue for virus infection was collected from sick with an average length of 15 cm and weight of 150 grams from an earth pond in Jejangkit Village, Barito Kuala Regency, South Kalimantan, Indonesia. In brief, three grams of wart tissues were homogenized in 27 mL of PBS and centrifuged at 3,000 xg for 10 minutes at 4°C. The supernatant was filtered using 0.22 µm nylon syringe filters (Millipore, USA) (Sigma-Aldrich, US). The virus filtrate was inoculated into Tryptic Soy Agar to ensure they were free of bacteria.

2.3 Experimental Infection

Giant gourami *Osphronemus gouramy* (length of nine cm), hybrid tilapia *Oreochromis* sp. (length of seven cm), Siamese fighting fish *Betta splendens* (length of three cm), and hybrid catfish *Clarias* sp. (length of seven cm) were collected from a commercial farm. Each fish species was kept in a separate tank at a density of one fish per two liters of water, with a change of 15% of the total volume every three days and continuous aeration in The Laboratory of Fish Health and Environment, Universitas Gadjah Mada. The fish were acclimatized for two weeks and given commercial feed ad libitum in the morning and evening.

The fish were anesthetized by immersion in water at 4°C (AVMA, 2020) before infection until their movements slowed down. Infection was induced on a minimum of 30 fish for each species. Giant gourami, hybrid tilapia, and hybrid catfish were intraperitoneally injected with 0.1 mL of virus filtrate containing 2 x 10^9, and Siamese fighting fish was immersed in one liter of virus filtrate containing 2x10^{10} for one hour. The control group for each species was treated with PBS as a substitute for viral filtrate. After infection, the fish were maintained on a recirculation system at a density of one fish per two liters of water, with a change of 10% once a week and continuous aeration. Commercial feed was given twice a day ad libitum. Behavioral observations were carried out every day, and sampling for virus quantification was performed at 2, 4, 7, 14, 21, 30, and 60 days after injection (dpi). The spleen, liver, kidney, fins, gills, and brain were collected and preserved in ethanol for further analysis.

2.4 DNA Isolation

The preserved tissues were washed with PBS and subjected to DNA isolation using FavorPrepTM Tissue Genomic DNA Extraction Mini Kit (Favorgen, Taiwan) following the manufacturer's protocol. The purity and quantity of extracted DNA were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) (García-Alegría *et al*., 2020). Good quality DNA with an absorbance value of 260/280 ranging from 1.8 to 2.0 was stored at −20°C until use.

2.5 Preparation of Recombinant Plasmid

The recombinant plasmid in this study used a partial major capsid protein (MCP) gene, amplified with specific primers, and cloned in a T-vector. The recombinant MCP plasmid was isolated using an alkali lysis minipreparation method (Sambrook and Russell, 2001). Its concentration and molecular mass were determined using https://scienceprimer.com/copy-number-calculator-for-realtime-pcr. The recombinant plasmid was subjected to a tenfold serial dilution from 10^1 to 10^8 copies/µL and used for virus quantification.

2.6 Quantification of LCDV MCP Copy Number

SYBR-based real-time PCR was used to quantify the viral load of the virus filtrate from the wart and from the spleen, liver, kidney, fin, gill, and brain using the CFX96 Touch Real-Time PCR Machine (BioRad, USA). The specific primers were MCP-F: CCTCGATTTCTGGTCCGCCT and MCP-R: ACCGTTGGAATCGACGGGTT with an amplicon target of 106 base pairs. The final volume of the qPCR reaction was 20 µL, consisting of 10 µL of 2x SensiFAST SYBR® No-ROX Mix (Meridian Bioscience), 0.8 µL of forward primer (400nM), 0.8 µL of reverse primer (400nM), 1 µL of DNA template, and 1.8 µL of ddH_2O. qPCR amplification was carried out as follows: an initial of 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 15 seconds. The melting curve was analyzed using a gradual temperature increase of 65°C to 95°C, rising by 0.5°C per cycle. Ct values converted to MCP copy numbers using a standard curve were employed for viral load quantification.

3. Results and Discussion

3.1 Results

The behavioral changes that occur after infection differed in each species. The first change observed was anorexia, which occurred in giant gourami starting at 6 dpi and in hybrid tilapia and hybrid catfish starting at 4 dpi. However, this change was not observed in Siamese fighting fish. The second change was swimming at the top edge of the tank away from the fish schools, which was observed in giant gourami at 20 dpi, in hybrid tilapia at 5 dpi, and in hybrid catfish at 3 dpi. This change was not observed in Siamese fighting fish. The third change was erratic swimming and staying at the bottom of the tank, which was observed in Siamese fighting fish at 15 dpi but was not exhibited by giant gourami, hybrid tilapia, and hybrid catfish. The control group did not show any changes in behavior throughout the observation period. Meanwhile, the total mortality of giant gourami was 14% of the total population, and that of hybrid tilapia was 36% of the total population (Figure 2). The control group had no mortality throughout the observation period.

The viral load of LCDV in the liver, spleen, kidney, gills, fins, and brain of each species was quantified at different times. All fish species showed an increase in viral load. An increasing pattern of viral load in giant gourami occurred from 7 dpi to 21 dpi. Initial observation results (7 dpi) showed that the highest viral load was in the liver (1.7×10^4). Over time, the highest viral load differed in each organ (Figure 3A). The highest viral load in the brain (1.2×10^4), liver (1.7×10^4), and kidney (2.7×10^5) occurred at 14, 7, and 30 dpi, respectively. The highest viral loads in the

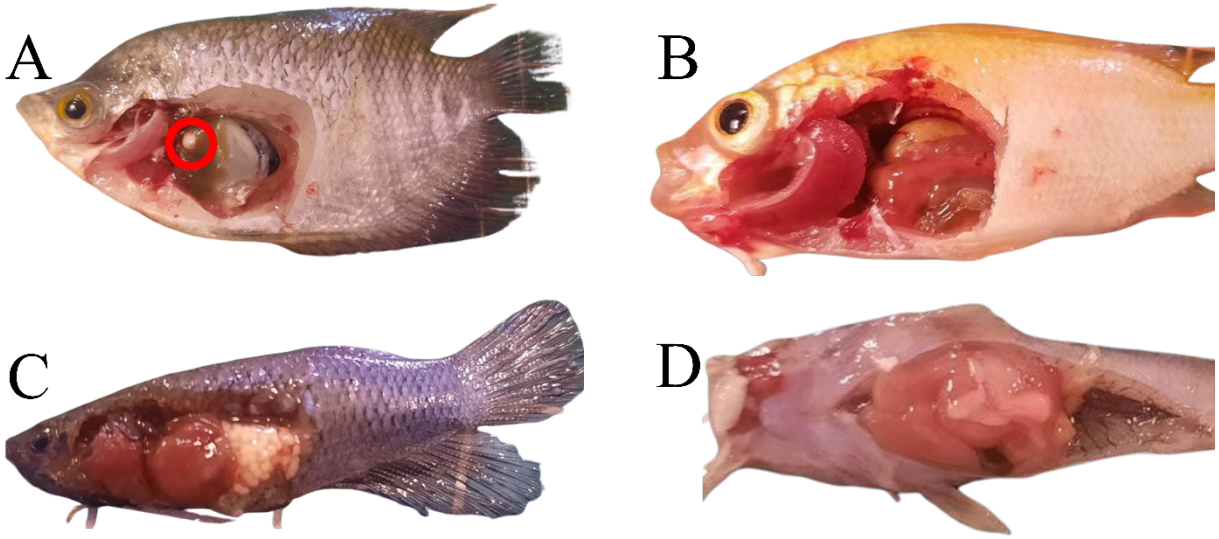

Figure 1. External and viscera appearance of fish at 7 days post injection of lymphocystis disease virus: A) giant gourami (*Osphronemus goramy*) with white nodule in the liver (red circle), B) hybrid tilapia (*Oreochromis* sp.), C) Siamese fighting fish (*Betta splendens*), and D) hybrid catfish (*Clarias* sp.).

No external morphological changes after infection were observed in giant gourami, hybrid tilapia, Siamese fighting fish, and hybrid catfish until the end of the observation. Meanwhile, internal morphological changes after infection were observed in giant gourami at 7 dpi, specifically a white nodule in the liver (Figure 1A), but not in hybrid tilapia (Figure 1B), Siamese fighting fish (Figure 1C), and hybrid catfish (Figure 1D) until the end of the observation. The control group did not experience external or internal changes throughout the observation period.

Mortality was observed in each species at different dpi. The first mortality of giant gourami, hybrid tilapia, Siamese fighting fish, and hybrid catfish was observed at 4, 1, 8, and 1 dpi, respectively (Figure 2). The highest total mortality was observed in hybrid catfish at 42% of the total population and the lowest in Siamese fighting fish at 5.7% of the total population.

spleen, fins, and gills were 1.0×10^6, 7.0×10^3, and 1.5×10^5, respectively (Figure 3A). An increasing pattern of viral load in hybrid tilapia was observed in the brain (1.3×10^3) and spleen (4.3×10^2) at 30 dpi, fins (7.5×10^3), gills (1.0×10^3), and liver (4.1×10^2) at 21 dpi, and kidney (6.1×10^2) at 7 dpi (Figure 3B). Meanwhile, an increasing pattern of viral load in Siamese fighting fish was detected in the brain (3.9×10^3) and fins (8.4×10^3) at 14 dpi and viscera at (3.9×10^3) at 7 dpi (Figure 3C). The highest increasing pattern of viral load in hybrid catfish was found in the brain (7.4×10^2), kidney (8.4×10^2), and spleen (1.6×10^2) at 1 dpi, fins (1.2×10^3) at 7 dpi, and gills (1.2×10^3) and liver (2.8×10^2) at 14 dpi (Figure 3D).

3.2 Discussion

Viral infection resulting in anorexia has been reported in salmon (*Salmo salar*) infected with IPNV,

characterized by increased expression of anorexia genes in the brain and decreased lipid content (Muñoz *et al*., 2021). This study showed that giant gourami, hybrid tilapia, and hybrid catfish experienced anorexia, which started at different periods after lymphocystis disease infection. Research on animal species infected with diseases and inherited infections has shown reduced their connectivity to their social groups as an instinct to prevent the disease from spreading widely (Lee *et al*., 2015; Lopes *et al*., 2016). The present work also observed a similar behavior among giant gourami, hybrid tilapia, and hybrid catfish, i.e., swimming

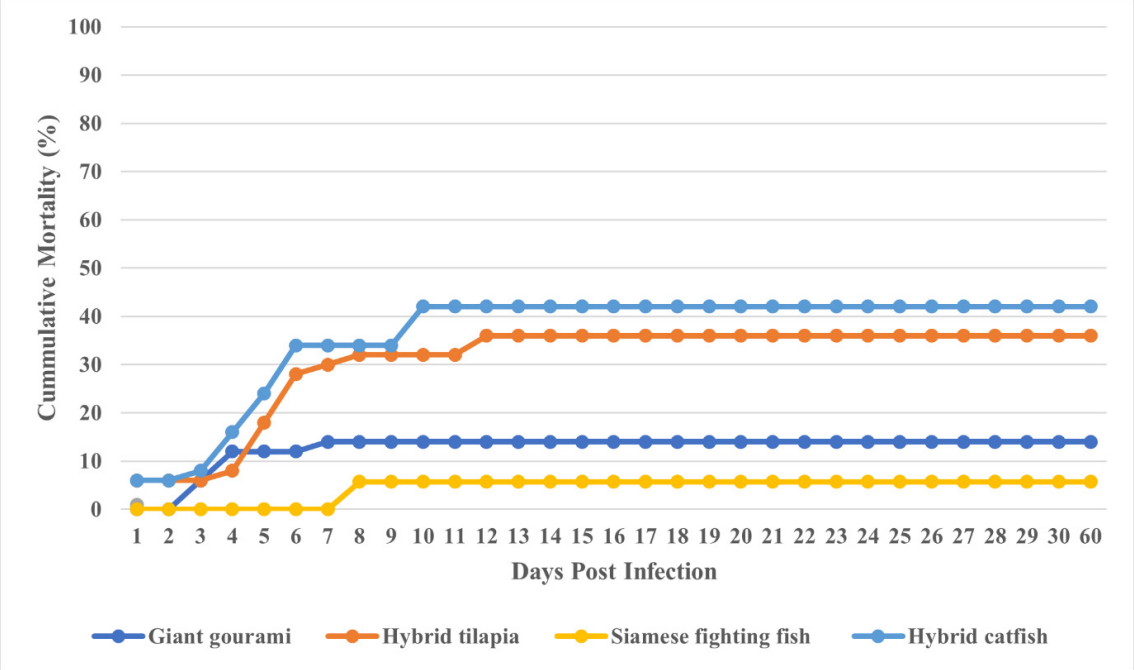

Figure 2. Cumulative mortality (%) of giant gourami (*Osphronemus goramy*), hybrid tilapia (*Oreochromis* sp.), Siamese fighting fish (*Betta splendens*), and hybrid catfish (*Clarias sp*.)

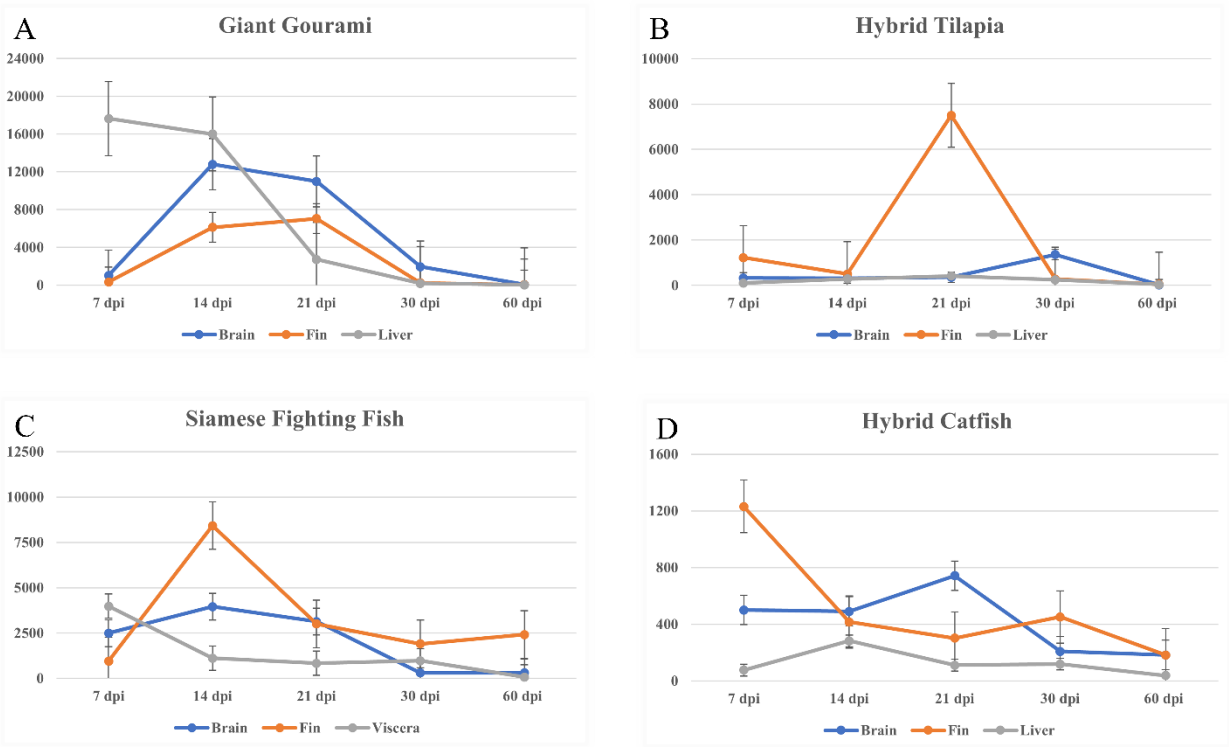

Figure 3. LCDV viral load on various tissues from the brain, liver, and fin of giant gourami (*Osphronemus goramy*) (A), hybrid tilapia (*Oreochromis* sp.) (B), Siamese fighting fish (*Betta splendens*) (C), and hybrid catfish (*Clarias* sp.). (D). Viral load in tissue is described as the mean ± SE of the number of MCP copies in 1 mg of tissue.

at the top of the tank away from the schools. Meanwhile, Siamese fighting fish swim erratically and stay at the bottom of the tank after being infected with lymphocystis disease.

Lymphocystis disease has typical clinical symptoms of multinodular tumor-like masses with diameter of up to 1 mm on the surface of the skin and, in some cases, viscera (Hick *et al*., 2016; Zhang *et al*., 2023). In this study, the same symptoms at seven days post-infection were observed in moribund giant gourami with white nodules in the liver (Figure 1A) but not in hybrid tilapia, Siamese fighting fish, hybrid catfish and the entire control group.

Lymphocystis disease resulted in 45% mortality of *Sparus aurata* in Italy and Tunisia (Dezfuli *et al*., 2012; Haddad-Boubaker *et al*., 2013), 10% mortality of *Pomacanthus maculosus* in Iran (Rahmati-Holasoo *et al*., 2023), and 53% mortality of *Lutjanus johnii* in Malaysia (Nurliyana *et al*., 2023). This study showed that mortality was observed in all four fish species, with the highest mortality of 42% in hybrid catfish, followed by 14% in giant gourami and 36% in hybrid tilapia, and the lowest mortality in Siamese fighting fish at 5.7% of the total population during the 60-day observation period. This result is in agreement with the behavioral and internal changes in giant gourami and the lack of internal changes in hybrid tilapia and hybrid catfish having a high total mortality rate. Siamese fighting fish do not experience anorexia in proportion to the lowest total mortality. Mortality without clinical symptoms has been observed in fish infected with susceptible viruses, but species-specific variations causing clinical symptoms are not clearly described (Jansen *et al*., 2019).

A study on gilthead seabream showed high viral loads in the fins, kidney, and brain (Valverde *et al*., 2017). The current research showed a high viral load in all organs of each fish species. The highest viral load was observed in the fins (7.5×10^3) at 21 dpi for hybrid tilapia (Figure 3B), in the fins (8.4×10^3) at 14 dpi for Siamese fighting fish (Figure 3C), and in the fins and gills at 1.2×10^3 for hybrid catfish (Figure 3D). These findings showed that fins with a high viral load are one of the target organs for LCDV infection in hybrid tilapia, hybrid catfish, and Siamese fighting fish. Nevertheless, the viral load fluctuations may indicate problems related to uneven sampling.

Giant gourami (*Osphronemus goramy*) has the most apparent infection pattern among the fish species. The increase in viral load in giant gourami occurred from 7 dpi to 21 dpi (Figure 3A). In contrast to the other species, its highest viral load was observed in the liver (1.7×10^4) at 7 dpi (Figure 3A). Paperna *et al*. (1987) first reported lymphocystis disease in ornamental gourami fish. Giant gourami is the most susceptible among the species in this study as indicated by the changes in its anorexic behavior, separation from the schools, presence of white nodules on its liver at 7 dpi (Figure 1A), and a high mortality rate reaching 14% (Figure 2). According to its development pattern, LCDV infection occurs from 7 dpi to 21 dpi in accordance with the increasing viral load in giant gourami (Figure 3A).

LCDV infection occurs in most commercially farmed fish, encompassing at least nine orders of teleost fish including the families Centratchidae, Cichlidae, Chetodontidae, Osphronemidae, Gobiidae, Pleuronectidae, Pomacentridae, Sciaenidae, and Serranidae (Huang *et al*., 2015; Cherif *et al*., 2020). The Channidae and Cyprinidae families were included in the LCDV host list in 2019 and 2020 (Sihananto *et al*., 2019; Shahi *et al*., 2020; Nikmah *et al*., 2024). This research showed that on the basis of the behavioral, external, and internal changes and the viral loads in several organs, the families Osphronemidae (giant gourami and Siamese fighting fish), Cichlidae (hybrid tilapia), and Claridae (hybrid catfish) must be included in the list of fish susceptible to LCDV infection.

The widespread of infectious diseases is due to intensive cultivation systems, introduction of fish to new areas, and international trade in live fish (Mugimba *et al*., 2021). International fish trade is associated with the spread of pathogens, especially viruses, in the same host species or new species that are highly susceptible in that geographic area (Go *et al*., 2006). The spread of pathogens in distant geographical regions has been reported for encephalopathy and retinopathy virus infections (Jithendran *et al*., 2011), ranavirus (George *et al*., 2015), cyprinid herpesvirus-2 (Sahoo *et al*., 2016), and ISKNV (Girisha *et al*., 2021). Similar to other viral infections, the global spread of LCDV is speculated to be due to international trade (Haddad-Boubaker *et al*., 2013). This research evaluated the susceptibility to lymphocystis disease of four fish species with economic value and extensive trade routes in Indonesia. Attention must be paid to fish transportation and quarantine to reduce the spread of infection.

4. Conclusion

The behavior, external and internal changes, mortality, and presence of virus confirmed that giant gourami, hybrid tilapia, Siamese fighting fish, and hybrid catfish are susceptible to LCDV infection. The high viral loads in one or several organs suggested that LCDV may have different organ predilections in each of the studied fish species. On the basis of the research findings, fish trade traffic must be tightened to reduce

the possibility of spreading the lymphocystis disease virus.

Acknowledgment

This work was supported by a grant from The Ministry of Education Culture and Research of Republic of Indonesia on "Program Magister menuju Doktor untuk Mahasiswa Unggul (PMDSU) No. B/277/D3.2/KD.02.00/2019."

Authors' Contributions

NLFN: developed the methods, conducted laboratory and field experiments, handled the experimental animal, analyzed the data, designed the figures, and improved the manuscript. AI and II: revised the article. Mw: provided the main conceptual ideas, developed methods, analyzed the data, earned funds for the laboratory and field activities, and finalized the manuscript. All the authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare no competing interest.

Funding Information

This work was supported by a grant from The Ministry of Education Culture and Research of Republic of Indonesia on "Program Magister menuju Doktor untuk Mahasiswa Unggul (PMDSU), Molecular Characterization of Emergence Fish Virus in Aquaculture, No. B/277/D3.2/KD.02.00/2019".

References

Abowei, J. F. N., Briyai, O. F., & Bassey, S. E. (2011). A review of some viral, neoplastic, environmental and nutritional diseases of hybrid fish. *British Journal of Pharmacology and Toxicology*, 2(5):227-235.

Afnan, M. A., Sutarjo, G. A., & Hariyadi, Y. (2019). Analysis of freshwater ornamental fish production (goldfish, koi, and betta fish) in Tulungagung District, East Java, Indonesia. *Indonesian Journal of Tropical Aquatic,* 2(20):80-87.

Alonso, M. C., Cano, I., Garcia-Rosado, E., Castro, D., Lamas, J., Barja, J. L., Borrego, J. J., & Bergmann, S. M. (2005). Isolation of lymphocystis disease virus from sole, Solea senegalensis kaup, and blackspot sea bream, *Pagellus bogaraveo* (Brunnich). *Journal of Fish Diseases,* 28(4):221-228.

Aly, S., Mansour, S., & Thabet, R. (2018). Pathological and molecular characterization of lymphocystis disease virus (LCDV) in sea bream fish in Egypt. *Suez Canal Veterinary Medical Journal,* 23(2):109-126.

American Veterinary Medical Association (AVMA). (2020). AVMA guidelines for the euthanasia of animals: 2020 Edition.

Ashburner L. D. (1975). Letter: Lymphocystis in paradise fish (Macropodus opercularis) in Australia. *Australian Veterinary Journal,* 51(9):448-449.

BPS-Statistics Indonesia. (2023). Aquaculture production by main commodity (tons), 2019-2020. [in English].

Basurco, A., Marcotegui, M. A., Rueda, A., Tidana, A., Castellanos, A., Tarazona, J. V., Muñoz, N. J., & Coll, J. M. (1990). First report of lymphocystis disease in *Sparus aurata* (Linnaeus) in Spain. *Bulletin of the European Association of Fish Pathologists*, 10(3):71-73.

Benkaroun, J., Bergmann, S. M., Römer-Oberdörfer, A., Demircan, M. D., Tamer, C., Kachh, G. R., & Weidmann, M. (2022). New insights into lymphocystis disease virus genome diversity. *Viruses*, 14(1):1-11.

Borrego, J. J., Valverde, E. J., Labella, A. M., & Castro, D. (2015). Lymphocystis disease virus: Its importance in aquaculture. *Reviews in Aquaculture*, 9(2):1-15.

Cano, I., Valverde, E. J., Lopez-Jimena, B., Alonso, M. C., Garcia-Rosado, E., Sarasquete, C., Borrego, J. J., & Castro, D. (2010). A new genotype of lymphocystivirus isolated from cultured gilthead seabream, *Sparus aurata* L., and Sene-galese sole, *Solea senegalensis* (Kaup). *Journal of Fish Diseases*, 33(8):695-700.

Cheng, M. C., See, M. S., Wang, P. C., Kuo, Y. T., Ho, Y. S., Chen, S. C., & Tsai, M. A. (2023). Lymphocystis disease virus infection in clownfish *Amphiprion ocellaris* and *Amphiprion clarkii* in Taiwan. *Animals*, 13(1):1-12.

Cherif, N., Amdouni, F., Maatoug, K., & Zaafran, S. (2020). Case report: First occurrence of Lymphocystis disease virus 3 (LCDV-Sa) in wild marine fish in Tunisia. *Annals of Marine Science*, 4(1):024-029.

Chun, S.K. (1998). Studies on lymphocystis disease in Sebastes schlegeli. *The Korean Society of Fish Pathology*, 1(2):73-76.

de Lucca Maganha, S. R., Cardoso, P. H. M., de Carvalho Balian, S., de Almeida-Queiroz, S. R., Fernandes, A. M., & de Sousa, R. L. M. (2020). Detection and molecular characterization of lymphocystivirus in Brazilian ornamental fish. *Brazilian Journal of Microbiology*, 51(2):531-535.

Dethlefsen, V., Lang, T., & Köves, P. (2000). Regional patterns in prevalence of principal external diseases of dab Limanda limanda in the North Sea and adjacent areas 1992-1997. *Diseases of Aquatic Organisms,* 42(2):119-132.

Dezfuli, B. S., Lui, A., Giari, L., Castaldelli, G., Mulero, V., & Noga, E. J. (2012). Infiltration and activation of acidophilic granulocytes in skin lesions of gilthead seabream, *Sparus aurata*, naturally infected with lymphocystis disease virus. Developmental and Comparative *Imunology*, 36(1):174-182.

Doszpoly, A., Kaján, G. L., Puentes, R., & Perretta, A. (2020). Complete genome sequence and analysis of a novel lymphocystivirus detected in whitemouth croaker (Micropogonias furnieri): Lymphocystis disease virus 4. *Archives of Virology*, 165(5):1215-1218.

Frasca, S., Wolf, J. C., Kinsel, M. J., Camus, A. C., Lombardini, E. D. (2018). Osteichthyes. In Terio, K.A., D. McAloose, J. St. Leger. (Eds.), Pathology of wildlife and zoo animals. (pp. 953-1001). Academic Press. ISBN 9780128053065.

García-Alegría, A. M., Anduro-Corona, I., Pérez-Martínez, C. J., Guadalupe Corella-Madueño, M. A., Rascón-Durán, M. L., & Astiazaran-Garcia, H. (2020). Quantification of DNA through the nanodrop spectrophotometer: Methodological validation using standard reference material and sprague dawley rat and human DNA. *International Journal of Analytical Chemistry*, 2020(1):1-9.

George, M. R., John, K. R., Mansoor, M. M., Saravanakumar, R., Sundar, P., & Pradeep, V. (2015). Isolation and characterization of a ranavirus from Koi, *Cyprinus carpio* L., experiencing mass mortalities in India. *Journal of Fish Diseases*, 38(4):389-403.

Girisha, S. K., Kushala, K. B., Nithin, M. S., Puneeth, T. G., Naveen Kumar, B. T., Vinay, T. N., Suresh, T., Ajay, S. K., Venugopal, M. N., & Ramesh, K. S. (2021). First report of the infectious spleen and kidney necrosis virus (ISKNV) infection in ornamental fishes in India. *Transboundary and Emerging Diseases,* 68(2):964-972.

Go, J., Lancaster, M., Deece, K., Dhungyel, O., & Whittington, R. (2006). The Molecular epidemiology of iridovirus in murray cod (Maccullochella peelii) and dwarf gourami (Colisa lalia) from distant biogeographical regions suggests a link between trade in ornamental fish and emerging iridoviral diseases. *Molecular and Cellular Probes*, 20(3-4):212-222.

Haddad-Boubaker, S., Bouzgarou, N., Fakhfakh, E., Khayech, M., Mohamed, B. S., Megdich, A., & Chéhida, N. B. (2013). Detection and genetic characterization of lymphocystis disease virus (LCDV) isolated during disease outbreaks in cultured gilt-head sea bream Sparus aurata in Tunisia. *Fish Pathology*, 48(3):101-104.

Hick, P. M., Becker, J. A., & Whittington, R. J. (2016). Iridovirus of Fish (Chapter 8). In F. S. B. Kibenge, & M. G. Godoy (Eds.), Aquaculture Virology. (pp.127-152). 1st Ed. Amsterdam: Elsevier Inc.

Hossain, M., Song, J. Y., Kitamura, S. I., Jung, S. J., & Oh, M. J. (2008). Phylogenetic analysis of lymphocystis disease virus from tropical ornamental fish species based on a major capsid protein gene. *Journal of Fish Diseases*, 31(6):473-479.

Huang, X., Huang, Y., Xu, L., Wei, S., Ouyang, Z., Feng, J., & Qin, Q. (2015). Identification and characterization of a novel lymphocystis disease virus isolate from cultured grouper in China. *Journal of Fish Diseases*, 38(4):379-387.

Jansen, M. D., Dong, H. T., & Mohan, C. V. (2019). Tilapia lake virus: A threat to the global tilapia industry?. *Reviews in Aquaculture*, 11(3):725-739.

Jithendran, K. P., Shekhar, M. S., Kannappan, S., & Azad, I. S. (2011). Nodavirus Infection in freshwater ornamental fishes in India: Diagnostic histopathology and nested RT-PCR. A*sian Fisheries Science*, 24(1):12-19.

Kawato, S., Nozaki, R., Hirono, I., & Kondo, H. (2021). Genome sequence of lymphocystis disease virus 2 LCDV-JP_Oita_2018, isolated from a diseased japanese flounder (*Paralichthys olivaceus*) in Japan. *Microbiology Resource Announcements*, 10(3):1-2.

Lam, C., Khairunissa, I., Damayanti, L., Kurobe, T., The, S. J., Pfahl, S. J., Rapi, S., Jenetski, N., & Baxa, D. V. (2020). Detection of a new strain of lymphocystis disease virus (LCDV) in cap-

tive-bred clownfish *Amphirion percula* in Soiuth Sulawesi, Indonesia. *Aquaculture International*. 28(1):2121-2137.

Lee, S. B., Choe, Y., Chon, T. S., & Kang, H. Y. (2015). Analysis of zebrafish (Danio rerio) behavior in response to bacterial infection using a self-organizing map. *BMC Veterinary Research*, 11(269):1-12.

Lopes, P. C., Block, P., & König, B. (2016). Infection-induced behavioural changes reduce connectivity and the potential for disease spread in wild mice contact networks. *Scientific Reports*, 6(1):1-10.

Matsuoka, S. (1995). Occurrence of viral, parasitic, and other non-bacterial diseases in cultured marine fin-fish in ehime prefecture from 1961 to 1993. *Aquaculture*, 43(4):535-541.

Menezes, J., Ramos, A., & Pereira, T. G. (1987). Lymphocystis disease: An outbreak in *Sparus aurata* from Ria Formosa, South Coast of Portugal . *Aquaculture*, 67(1):222-225.

Mugimba, K. K., Byarugaba, D. K., Mutoloki, S., Evensen, Ø., & Munang'andu, H. M. (2021). Challenges and solutions to viral diseases of finfish in marine aquaculture. *Pathogens (Basel, Switzerland)*, 10(1):1-21.

Muñoz, D., Fuentes, R., Carnicero, B., Aguilar, A., Sanhueza, N., San-Martin, S., Agurto, C., Donoso, A., Valdivia, L. E., Miguez, J. M., Tort, L., & Boltana, S. (2021). Viral infection drives the regulation of feeding behavior related genes in *Salmo salar*. *International Journal of Molecular Sciences*, 22(11391):1-17.

Murwantoko., Wijayanti, E., Agustatik, S., Valianti, H., Yulanda, L. K., & Nukmah, N. L. F. (2022). Detection and genotype determination of lymphocystis disease virus infecting orange clownfish, *Amphiprion percula* farmed in Batam, Indonesia. *Indonesian Aquaculture Journal*, 17(2):165-172.

Nikmah, N. L. F., Sihananto, B. S., Isnansetyo, A., Istiqomah, I., & Murwantoko. (2024). Identification and characterization of lymphocystis disease virus from snakehead (Channa striata) in South Kalimantan, Indonesia. *Aquaculture*, 558(1):1-7.

Nurliyana, M., Jumria, S., Lukman, B., Ina-Salwany, M. Y., Zamri-Saad, M., Annas, S., Keong, M. K., Norhariani, M. N., Rozi, R., & Amal, M. N. A. (2023). An outbreak of lymphocystis disease virus infection in cultured juvenile John's snapper (*Lutjanus johnii*) in Malaysia. *Aquaculture*, 571(739502).

Paperna, I., Ventura, T., & Matos, A. A. (1987). Lymphocystis infection in snakeskin gourami, *Trichogaster pectoralis* (Regan), (Anabantidae). *Journal of Fish Diseases*, 10(1):11-19.

Paperna, L., Sabnai, I., & Colorni, A. (1982). An outbreak of lymphocystis in Sparus aurata L. in the Gulf of Aqaba, Red Sea. *Journal of Fish Diseases*, 5(1):433-437.

Pekmez, K., Kaplan, M., Cagirgan, A. A., Arslan, F., Kafa, B., & Kalayci, G. (2022). Molecular characteristic of lymphocystis disease virus detected from sea bream in Turkey. *Bulletin of The European Association of Fish Pathologists*, 42(2):1-13.

Rahmati-Holasoo, H., Ghalyanchilangeroudi, A., Kafi, Z. Z., Marandi, A., Shokrpoor, S., Bahram, I., & Mousavi, H. E. (2023). Detection of lymphocystis disease virus (LCDV) from yellowbar angelfish (*Pomancanthus maculosus* Forsskål 1775) in Iran: Histophatological and phylogenetic analysis. *Aquaculture,* 562(738862).

Sahoo, P. K., Swaminathan, T. R., Abraham, T. J., Kumar, R., Pattanayak, S., Mohapatra, A., Rath, S. S., Patra, A., Adikesavalu, H., Sood, N., Pradhan, P. K., Das, B. K., Jayasankar, P., & Jena, J. K. (2016). Detection of goldfish haematopoietic necrosis herpes virus (Cyprinid herpesvirus-2) with multi-drug resistant Aeromonas hydrophila infection in goldfish: First evidence of any viral disease outbreak in ornamental freshwater aquaculture farms in India. *Acta Tropica*, 161(1):8-17.

Sambrook, J., & Russell, D. (2001). Molecular clonning. A laboratory manual. 3rd Ed. New York, USA: Cold Spring Harbor.

Shahi, N., Mallik, S. K., Kala, K., Joshi, N., Patiyal, R. S., Chandra, S., Singh, S., & Sarma, D. (2020). Seasonal emergence of benign epidermal tumor in farm-reared adult grass carp(*Ctenopharyngodon idella*) caused by lymphocystis disease virus at Uttarakhand, India. *Aquaculture*, 526 :(1):1-9.

Sheng, X., Zhong, Y., Zeng, J., Tang, X., Xing, J., Chi, H., & Zhan, W. (2020). Lymphocystis disease virus (Iridoviridae) enters flounder (*Paralichthys olivaceus*) gill cells via a caveo-

lae-mediated endocytosis mechanism facilitated by viral receptors. *International Journal of Molecular Sciences*, 21(13):4722.

Sihananto, B. S., Novita, H., Wianty, S., & Lusiastuti, A. (2019). A case study of lymphocystis virus disease in farmed giant snakehead (*Channa striata*) in Mandiangin, South Kalimantan. *Indonesian Aquaculture Journal*, 14(2):75-81.

Tanaka, M., Yoshimizu, M., Kusukari, M., & Kimura, T. (1984). Lymphocystis disease in kurosoi *Sebastes schlegeli* and Hirame *Paralichthys* olivaceus in Hokkaido, Japan. *Bulletin of The Japanese Society of Scientific Fisheries*, 50(1):37-42.

Valverde, E. J., Borrego, J. J., Sarasquete, M. C., Ortiz-Delgado, J. B., & Castro, D. (2017). Target organs for lymphocystis disease virus replication in gilthead seabream (*Sparus aurata*). *Veterinary Research*, 48(21):1-11.

Wolf, K. (1962). Experimental propagation of lymphocystis disease of fishes. *Virology*, 18(1):249-256.

Xu, L., Feng, J., & Huang, Y. (2014). Identification of lymphocystis disease virus from paradise fish *Macropodus percularis* (LCDV-PF). *Archives of Virology*, 159(9):2445-2449.

Zhang, H., Sheng, X., Tang, X., Xing, J., Chi, H., & Zhan, W. (2023). Transcriptome analysis reveals molecular mechanisms of lymphocystis formation caused by lymphocystis disease virus infection in flounder (*Paralichthys olivaceus*). *Frontiers in Immunology,* 14(1):1-18.

Zheng, F., Liu, H., Guo, X., & Wang, Bo. (2016). Isolation and identification of a new isolate of lymphocystis disease virus isolated from black rockfish (Sebastes schlegelii) in China, *Aquaculture*, 451(1):340-344.

.