Journal of Aquaculture and Fish Health Vol. 12(3) - September 2023
P-ISSN: 2301-7309 / E-ISSN: 2528-0864
doi : 10.20473/jafh.v12i3.40256

Viral Nervous Necrosis and Vibriosis in Sunuk (*Plectropomus leopardus*) Grouper: A Case Study

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Received : 2022-10-31
Accepted : 2023-05-19

Keywords :
Groupers; Vibriosis; Viral Nervous Necrosis

Abstract

Viral Nervous Necrosis (VNN) and Vibriosis are contagious diseases that can affect groupers from the larval to adult stages and cause significant economic losses for farmers. *Sunuk* (*P. leopardus*) grouper is a grouper fish commonly found in NTB waters and has been widely cultivated. This study aims to provide information regarding suspected viral nervous necrosis and vibriosis that may occur in *sunuk* grouper. This case study was obtained from the fish health inspection process at BKIPM Mataram, involving one of the grouper cultivators in NTB. According to the owner's explanation, five *sunuk* grouper were caught by a fisherman and then cultivated in floating net cages. Two *sunuk* groupers showed injuries in body area and decreased appetite. For several days, two *sunuk* grouper showed an inactive swimming condition and swam sideways. The result of the physical examination was as follows: fish were swimming limply in an upside-down position and had wounds on various body parts. Virology and bacteriology test confirmed that the sick *sunuk* grouper was infected with Vibriosis and VNN. *Sunuk* grouper can be infected with two diseases in this case study, which can occur upon their sick condition from first-day arrival in floating net cages accompanied by stress condition, decreased appetite, that leads to a furher decline in the fish's health and the immune system not functioning optimally. This condition makes *sunuk* grouper susceptible to VNN and Vibriosis in this study case.

INTRODUCTION

Aquaculture has seen very rapid development in providing food of animal origin, along with the poultry and livestock sectors. This rapid development can help increase the availability of food of animal origin and improve the socio-economic sector in various countries (Dadar *et al.*, 2017). Grouper is one of the essential fish commodities with high economic value in the Asia-Pacific region, influenced by the distinctive taste of meat and export-oriented, with a high selling

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value in both domestic and international markets. This influenced grouper cultivation, increasing the production of Indonesian mariculture commodities (Das *et al.*, 2021). Statistical data from the Ministry of Marine Affairs and Fisheries shows that the total production of grouper in Indonesia in 2019 reached 23 million tons and in 2020, it reached 7 million tons (KKP, 2021).

One of the problems faced in grouper cultivation is the problem of diseases that can infect fish. Disease problems in grouper consist of infectious and non-infectious diseases that cause illness, death, and economic losses for farmers (Dadiono *et al*., 2020). One of the obstacles in the implementation of grouper cultivation is the death caused by infectious diseases. Infectious diseases that often cause losses to farmers are caused by viral, bacterial, parasitic, and fungal infections (Mahardika *et al.*, 2020).

A bacterial infection that is quite common in grouper is vibriosis. Vibriosis is a bacterial disease in fish, shrimp, and other aquatic animals due to infection with *Vibrio* sp. This disease can cause outbreaks with severe economic losses (Amalina *et al.*, 2019). Vibriosis in fish can be indicated by the presence of ulcer lesions in the skin area, erosion of the fin, an abnormal swimming pattern, inflammation, and loss of appetite, and causes death to more than 80% of fish cultivated in floating nets (Ilmiah *et al.*, 2012; Huzmi *et al.*, 2019).

Viral Nervous Necrosis (VNN) is one of the nervous system diseases in fish induced by *Betanodavirus* infection. Since 1980, cases of VNN disease have infected fish and caused the deaths of more than 39 fish species, accompanied by significant economic losses (Juniar *et al*., 2018). Cases of VNN infection in fish have been recorded in China, Japan, Korea, Malaysia, Australia, Indonesia, Vietnam, several countries in the Americas, and Europe (Ziarati *et al.*, 2020). Grouper is one fish species that the VNN virus can infect. Cases of VNN infection in grouper occur at the larval, juvenile, and adult stages, causing mortality in fish of up to 100% (Khumaidi *et al.*, 2019). Clinical symptoms of VNN in fish are influenced by differences in distribution time, age of fish, route of infection, and fish immune systems. Clinical symptoms shown by groupers infected with VNN include the movement of fish that looks passive, pale body color, swimming irregularly, and spinning upside down (Sembiring *et al.*, 2018).

Diagnosis of bacterial and viral disease cases in grouper can be done by observing clinical symptoms, performing a physical examination of the fish, and conducting laboratory investigations. Vibriosis in fish can be diagnosed using standard techniques such as selective cultures, gram stains, and biochemical testing for screening and early detection of bacterial presence. Molecular methods based on detecting a nucleic acid sequence that targets specific bacterial genes are another technique for detecting vibriosis in fish(Azwai *et al.*, 2016; Amalina *et al.*, 2019). According to OIE (2019), diagnostic methods used for the detection of VNN in fish can use Polymerase Chain Reaction (PCR), Immunofluorescence (IF), Immunohistochemistry (IHC), and ELISA. The most frequent method used for the diagnosis of VNN in grouper is the PCR method (Kurniawati *et al*., 2019).

Sunuk grouper (*P. leopardus*) is one of the grouper fish species found in the waters of West Nusa Tenggara and has been extensively cultivated due to relatively high prices and wide market demand. *Sunuk* grouper are known to be relatively more sensitive than other species of grouper fish, which cause frequent injuries and decreased appetite. This condition causes *sunuk* grouper to be more susceptible to infectious disease (Suwirya and Giri, 2010). Cases of diseases caused by microorganism infections still occur in grouper cultivation. One of which is VNN disease. According to (Rahmawanti *et al*., 2021), cases of VNN were found in samples of cantang grouper cultured in floating cages

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in the Penyambuan Village, North Lombok district. This article aims to provide information related to cases of vibriosis and VNN that occurred in *sunuk* grouper with disease diagnosis techniques based on observation of clinical symptoms, physical examination, and laboratory examinations at BKIPM Mataram.

METHODOLOGY

Ethical Approval

The Research Ethics Commission, University of Brawijaya, has carefully studied the research design and provided ethical approval (253-KEP-UB-2022).

Place and Time

This case study was carried out from July to August 2022. Fish samples were obtained from one of the farmers in East Lombok. Laboratory examinations were conducted at the Fish Quarantine Center for Quality Control and Fisheries Outcomes (BKIPM) Mataram.

Research Materials

The fish sample, in this case, study was five *sunuk* (*P. leopardus*) grouper (average weight range 0.7-0.8 kg and average length range 36.5–37.8 cm) obtained from one of the farmers in East Lombok (latitude 8°29'53.0" S and longitude 116°40' 28.8"E). The laboratory examination consisted of bacteriological and virological examinations Laboratory examination consists of bacteriological examination and virological examination. Bacterial inoculation from *sunuk* grouper was carried out on bacterial growth media consisting of Tryptic Soy Agar (TSA) 2% (Merck®, Germany), Thiosulfate Citrate Bile Salts Sucroses Agar (TCBS) 2% (Merck®, Germany), Triple Sugar Iron Agar (TSIA) (Merck®, Germany), media MR-VP (Glucose Phosphate Broth) (Merck®, Germany), Simmons Citrate Agar (Merck®, Germany), Urea Agar Base (Merck®, Germany), Bile Aesculin Agar (Merck®, Germany), Indole Nitrate Medium (Merck®, Germany), Oxidative/ Fermentative (Basal Medium), Nutrient Gelatin, Phenylalanine Agar, the sugar medium and bacterial tests accompanied with catalase test, oxidase test, and gram bacterial test using 3% KOH.

The virological examination used the conventional Polymerase Chain Reaction (PCR) The viral RNA extraction steps were carried out using RLT buffer (Qiagen®, Germany), β-mercaptoethanol (Sigma-Aldrich®, USA), 70% ethanol, RW 1 buffer (Qiagen®, Germany), RPE buffer (Qiagen®, Germany), and Buffer RNase Free Water (Qiagen®, Germany).

The amplification stage was carried out in two stages consisting of the first stage for 2 hours 30 minutes for the process of converting RNA into cDNA and the second stage for 1 hour 25 minutes for the process of multiplying viral cDNA in the nested reaction to being able to visualize it on gel electrophoresis. Electrophoresis steps using 1.5% agarose concentration with Syber green dye (Sigma-Aldrich®, USA).

Research Design

The method used in this study is a case study accompanied by a diagnosis of the disease based on anamnesis, and clinical symptoms, and supported by laboratory tests. The results of laboratory tests in determining the diagnosis of the disease are then compared with the literature.

Work Procedure

Signalment and Anamnesis

Signalment is a record of the identity of an animal or patient that serves as identification. Anamnesis is a conversation with the animal's owner concerning the animal's past. The history includes the animal's symptoms, the time and duration of the incident, and the animal's condition when it was discovered (Duguma, 2016).

Five *sunuk* grouper (*P. leopardus*) were taken from a grouper cultivator in East Lombok to check the health status of the fish. Three samples of *sunuk* grouper were taken from healthy fish plots and two

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samples from sick fish. Information received from the owner explained that one *sunuk* grouper showed a slight wound in the small abdominal area, and fishermen caught this fish. Fish that show these wounds are isolated in a special place for sick fish. During the rearing process, fish with minor wounds on the abdomen showed injuries in several areas of the body, such as the operculum, several areas of the body, and fins, and swam obliquely. When the samples were brought to BKIPM for examination, the fish were swimming upside down.

Physical Examination

Physical examination is a method to obtain information about the health status of animals (Duguma, 2016). The results of an examination of the length and body weight of *Sunuk* grouper showed that the average length ranged from 36.5–37.8 cm with a body weight ranging from 0.75–0.8 kg. Physical examination of five *sunuk* grouper groupers revealed that three fish had no injuries to their bodies and were healthy. One *sunuk* grouper showed injuries to the body area of the abdomen and fins and one *sunuk* grouper showed reverse swimming conditions with lesions in the mouth, body, and tail areas with small sizes up to Figure 1 depicts the results of a physical examination of one of the *sunuk* groupers.

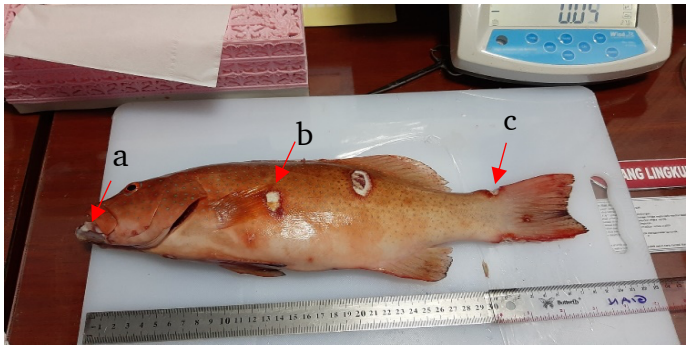

Figure 1. Physical examination in *sunuk* grouper. Description: (a) lesion in the mouth area, (b) lesions on the body, (c) lesion in the tail area.

Necropsy

Necropsy is carried out to observe the external and internal conditions of the fish body, which can indicate changes that the presence of disease conditions can cause. The stages of a necropsy on fish begin with euthanization and physical examination to observe changes in external body parts, blood collection and organ sample collection (Blazer *et al.*, 2018). Necropsy, in this case, the study was performed for bacteriological and virological tests were carried out to diagnose cases of the disease in *sunuk* grouper. A total of two samples of *sunuk* grouper were necropsied for bacteriological and virological examination. The eye and brain organs were taken for the virological examination, which was then stored in a sample container containing 70% ethanol.

Bacteriological Test

Bacteriological testing is an examination technique in determining the presence of bacterial microorganisms, both pathogenic and non-pathogenic, contained in a tested sample. The bacteriological examination can be carried out by planting samples on general, selective, differential, and biochemistry accompanied by bacterial gram testing, catalase testing, oxidase, and another bacteriological method testing (Leboffe, 2011).

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A bacteriological examination in this case study was carried out by identifying bacteria in the skin area that showed an ulcer and anterior kidney. Bacterial samples were taken by swab in the area around the skin tissue with ulcer lesions and the anterior kidney. Bacterial inoculation was carried out on TSA 2%, TCBS 2%, TSIA, MR-VP media, Simmons Citrate Agar, Urea Agar Base, Bile Aesculin Agar, Indole Nitrate Medium, Oxidative/ Fermentative, Nutrient Gelatin, Phenylalanine Agar, sugar medium accompanied by catalase, oxidase, and bacterial gram tests using 3% KOH.

Virological Test

Viral disease testing in aquatic animals can be carried out using four methods consisting of tissue culture, Immunochemistry (ICC), ELISA, and PCR methods (Kurniawati *et al*., 2019). According to OIE (2019), fish can be used as a sample that shows abnormal swimming behavior associated with loss of appetite and a progressive change in skin pigmentation. Disease diagnosis in the laboratory can be through PCR with the tested samples from the eyes or brains of fish.

A virological examination was carried out in this case study to diagnose a suspected viral nervous necrosis infection in one of the *sunuk* groupers, which showed upside-down swimming and the presence of injuries to the body area. The virological examination used the conventional PCR method. The virological examination used the conventional PCR method.

The examination procedure begins with taking samples of the brain and eyes of the two *sunuk* grouper through necropsy. The viral RNA extraction steps were carried out using RLT buffer, β-mercaptoethanol, 70% ethanol, RW1 buffer, RPE buffer, and Buffer RNase Free Water.

The amplification stage was carried out in two stages consisting of the first stage for 2 hours 30 minutes for the process of converting RNA into cDNA and the second stage for 1 hour 25 minutes for the process of multiplying viral cDNA in the nested reaction to being able to visualize it on gel electrophoresis. Electrophoresis steps using 1.5% agarose concentration with Syber green dye. The tested sample markers were inserted sequentially, then electrophoresed with an electric power of 100 volts for 45 minutes, and then the agar gel was observed under UV light.

Data Analysis

Data analysis in this case study is to diagnose disease in *sunuk* grouper based on the results of physical examination, clinical symptoms, and laboratory tests accompanied by a study of journal literature and e-books.

RESULTS AND DISCUSSION

Infectious disease problems in grouper cultivation can occur due to interactions between the host, pathogenic agents, and environmental influences. Changes in environmental conditions parameters such as temperature, salinity, dissolved oxygen, to the presence of pathogenic agents can make fish susceptible to infection by disease (Vijayan *et al*., 2015). Laboratory examination results showed that three *sunuk* grouper were healthy, one sunuk grouper was diagnosed with Vibriosis and one *sunuk* grouper in this case study was infected by VNN and *Vibrio*.

The results of bacterial testing from skin tissue and the anterior kidney of the *sunuk* grouper are presented in Table 1. Clinical signs from two *sunuk* grouper included ulcer lesions on the mouth, operculum, abdomen, and fishtail, reversed swimming, decreased appetite, and inactive mobility. According to Amalina *et al.* (2019), the clinical symptoms of bacterial disease in groupers are fish experiencing a decrease in appetite, swimming slowly, the presence of lesions in the body area, to swimming sideways due to loss of balance.

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Vibrio sp. assay was identified through growth on TCBS media. This test was carried out based on clinical symptoms shown by groupers that lead to Vibriosis. The growth results on the media showed that the bacteria had yellow bacterial colonies (Figure 2), which indicated the presence of *Vibrio* sp. According to Leboffe (2011), *Vibrio* sp. in this medium has yellow and blue colonies, distinguished by their ability to ferment the sucrose contained in the media. *Vibrio* species capable of fermenting sucrose will change the color of the medium to yellow.

Table 1. Bacteriological examination results.

Bacterial Test	Result
TSA (Tryptic soy agar)	Positive (White colony with small circular size, entire edge and flat elevation)
TCBS Agar	Positive (The bacterial colonies yellow)
TSIA (Triple sugar iron agar)	
Butt	Acid (Yellow)
Slant	Acid (Yellow)
H_2S	Negatif
Gas	Positive (Media elevated)
Indol	Positive (Red ring)
Oxidative/Fermentative Media	
Parafin	Fermentative (Bacteria capable of fermenting glucose)
	Fermentative (Bacteria capable of fermenting glucose)
Non-Parafin	
Gelatin	Negative (Media after being put in the refrigator freezes)
MR (Methyl red) Test	Positive (Red after methyl red reagent is given)
VP (Voges-Proskauer) Test	Negative (Orange ring wasn't formed after give alpha napthol and KOH 40%)
Urease	Positive (Bacteria can ferment urea and change the color of the media to pink)
Citrate	Negative (Bacteria are not able to ferment citrate)
Bile Aesculin	Positive (There is a black color around the bacterial colony)
Phenylalanine	Negative (There is no change in the color of the media to green)
Sugar media	
Arabinose	Negative (Medium is yellow)
D-cellobiose	Positive (Medium is red)
Glucose	Positive (Medium is red)
Lactose	Negative (Medium is yellow)
Manitol	Positive (Medium is red)
Salicin	Negative (Medium is yellow)
Sorbitol	Negative (Medium is yellow)
Sucrose	Positive (Medium is red)
Trehanolse	Positive (Medium is red)
Xylose	Positive (Medium is red)
Catalase test	Hydrolyze H_2O_2 and form gas bubbles
Oxidase test	Positive (the test strip of the oxidase paper changes color to violet after being given a bacterial colony)
Gram test	Gram-negative indicated by bacterial colonies that were slimy and formed fine threads after being given KOH

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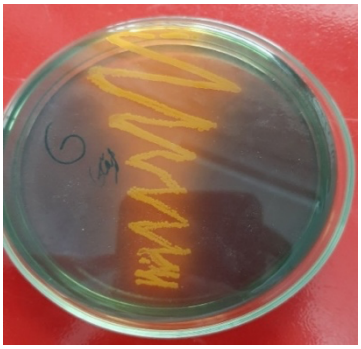

Figure 2. *Vibrio* sp. growth results on TCBS agar media.

The results of bacterial identification in *sunuk* grouper showed that the fish were infected with *V. carchariae*, which caused Vibriosis. According to Nicolas *et al.* (2002) *V. carchariae* is a Gram-negative bacteria with curved rod-shaped morphology, motile bacterium, and does not have spores and capsules. The growth results of *V. carchariae* on TCBS media were yellow, the urease medium was positive, the catalase test was positive, mannitol and sucrose sugar tests were positive, and arabinose and sorbitol sugar were negative. This bacterium has an almost identical affinity with *V. harveyi* and is still included in the subspecies of the bacterium.

V. carchariae was first known to cause vibriosis in aquaculture animals by sampling dead sandbar sharks and lemon sharks (Zhang *et al*., 2020). *V. carchariae* infection in grouper was first reported to occur in *Epinephelus coloides* with clinical symptoms of gastroenteritis accompanied by the presence of yellow fluid (Lee *et al*., 2002).

Clinical signs in *sunuk* grouper cases with vibriosis were indicated by ulcer lesions from the mouth to the tail, slower mobility, and decreased appetite. According to Hastari *et al*. (2014), clinical symptoms shown by grouper experiencing vibriosis indicate changes in behavior such as slow fish movement, morphological changes indicated by ulcers on the skin area that extend to muscle tissue, hemorrhage in the gill area, mouth area, fins, and scales, eyes have exophthalmia, and an enlarged abdomen filled with fluid. Other clinical symptoms of *V. carchariae* are indicated by causing gastroenteritis accompanied by yellow fluid in the intestine (Lee *et al*., 2002).

Sunuk grouper suffers from a vibriosis infection due to contact between sick fish from the sick shelter. This is shown by the *sunuk* grouper, which initially had a slight wound in the abdominal area, but when it was put in a particular place, the sick fish experienced injuries in several areas of the body. These findings are supported by fish taken from the sick shelter area that tested positive for vibriosis during laboratory tests, as well as infected fish with vibriosis and VNN. According to Mohamad *et al.* (2019), *Vibrio* sp. transmission is complicated and influenced by the connection between pathogenic agents, hosts, and environmental circumstances. Vibriosis is a waterborne infection that is transmitted horizontally between sick and healthy fish via contact with open lesions or secretory processes such as feces. Inadequate water quality, such as changing salinity and strong ambient temperature fluctuations, can encourage the growth of *Vibrio* sp. (Ina-Salwany *et al.*, 2019).

The presence of lesions in the body area of the *sunuk* grouper is one factor that makes it easier for *V. carchariae* to infect fish. The occurrence of damage to the epidermis area of the skin affects the occurrence of stress conditions, and the performance of the immune system through mucus and physical defenses does not work optimally. *Vibrio* sp. can enter the fish body through the skin, gills, and gastrointestinal tract (Mohamad *et al.*, 2019). Bacteria in the fish's body can cause infection and tissue damage through their virulence

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factors. *Vibrio* sp. virulence factors consist of extracellular products (ECPs), potent toxins, and membrane proteins that help in adhesion, colonization, and invasion of host tissues to outer membranes and cell walls that can induce inflammation (Ina-Salwany *et al.*, 2019).

Virus examination through conventional PCR method on the eye and brain organs of *sunuk* grouper is shown in Figure 3.

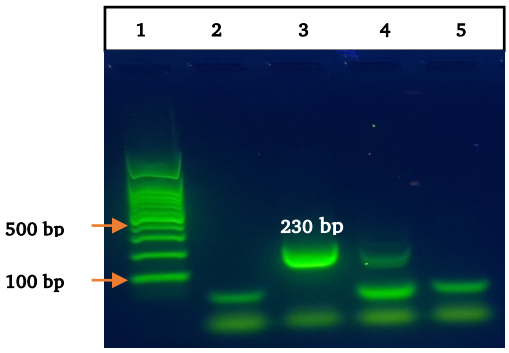

Figure 3. Conventional PCR-VNN examination showed positive results. Description: 1 (DNA Markerii 100 bp) i, 2i (Negative Control), 3 (Positive Control), 4 (Sample of *sunuk* grouper 1) show a positive result, and 5 (Sample of *sunuk* grouper 2) shows negative result.

The results of the virus examination showed that the *sunuk* grouper was infected by VNN. Positive results are indicated by the sample band that aligns with the positive control at 230 bp. Diagnosis of VNN that occurs in fish can be carried out through observation of clinical symptoms, physical examination, and laboratory examination. Laboratory examination can be done through ELISA and PCR (Binesh, 2014; Hazreen-Nita *et al.*, 2019). According to Kurniawati *et al*. (2019), the reading of positive results of fish samples infected with VNN through the conventional PCR method was indicated by the band of the tested samples aligned with the positive control at 230 bp.

Viral nervous necrosis is a disease of nervous system disorders caused by *Betanodavirus* infection in fish. This virus has infected more than 55 species of fish worldwide, with a mortality of up to 100%. The case of VNN that infects fish will cause significant economic losses for farmers (Juniar *et al.*, 2018; Ariff *et al.*, 2019). VNN is one of the viral diseases that often infects groupers. VNN infects groupers and occurs in the larval, juvenile, and adult stages(Sulistiyono *et al*., 2020).

The clinical symptoms of VNN shown by *sunuk* grouper in this case study consisted of decreased appetite and reverse swimming and *sunuk* grouper can be infected with VNN which can be caused by horizontal transmission due to contact with sick fish or environmental influences. According to Ariff *et al.* (2019), the clinical symptoms of grouper infected with VNN consisted of impaired swimming process, decreased appetite, spinal relocation, abdominal distension, lesions on the skin area, skin pigmentation, and damage to the fin area. Infection in the area of the central nervous system and retina causes fish to swim uncoordinated and causes lesions in the body area due to fish hitting walls or nets where groupers are kept. Horizontal transmission of *Betanodavirus* can be through fish infected with VNN with healthy fish, fish feed that has been infected, and environmental influences. The occurrence of horizontal transmission can be influenced by the lack of application of biosecurity in preventing VNN cases (Bandín and Souto, 2020).

VNN infection can occur in *sunuk* grouper it is suspected that *sunuk* grouper has experienced illness due to vibriosis,

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which can affect the occurrence of decreased physiological processes, appetite, and stress conditions for fish. Barbosa *et al.* (2020) explained that stress conditions could influence a decrease in the performance of the immune system in fish due to unacceptable environmental conditions, the presence of pathogenic agents, and reduced appetite. Decreased appetite can affect the decline in the performance of the immune system in fish. Nutrient components of protein to vitamins are needed by the body to help improve the performance of the immune response. Thus, the lack of nutrients obtained can cause the fish's immune response to not work optimally against the presence of pathogenic agents.

CONCLUSION

The analysis shows that the incidence of disease cases in *sunuk* grouper can occur due to a decrease in the fish body's immune response. The case of *Viral nervous necrosis* and Vibriosis diseases in one *sunuk* (*Plectropomus leopardus*) grouper was the first case analyzed at BPKIM Mataram. Further research can be conducted on infecting to observe the morphology of the bacteria and observations of the immune response possessed by grouper fish to add information about the effect of infection on two diseases and the need for good biosecurity for aquaculture sites to prevent pathogen spread.

CONFLICT OF INTEREST

The author declares there is no conflict of interest regarding the publication of this paper.

AUTHOR CONTRIBUTION

All authors have contributed to the final manuscript. The contribution of each author is as follows, Dr. Uun Yanuhar and Prof. Muhammad Musa have directed and guided the implementation of this case study; Gian Suryanatha Hartawan collected data, compiled manuscripts, compiled drawings, compiled main conceptual ideas, and critically revised articles; drh. Amira Baihani; guide for a laboratory test and assist in the diagnosis of the disease; Yusuf Arif Wahyudi; Assist in discussion related to disease cases; Choirul Huda; compiling manuscripts and compiling drawings; Nico Rahman Caesar; compiling manuscripts and compiling drawings.

ACKNOWLEDGMENT

Thanks to the Directorate of Research Technology and Community Service, Directorate General of Higher Education, Research and Technology, Ministry of Education, Culture, Research, and Technology for Funding Master Thesis Research (PTM) in 2023 in accordance with Research Contract Number: 1144.2/UN 10.C10/TU/2023.

The authors would like to thank the employees of the Mataram Quarantine Center for Quality Control and Fishery Products Safety (BKIPM), Surabaya I Quarantine Center for Quality Control and Fishery Products Safety (BKIPM), and the lecturers of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, for providing advice, information, facilities, and assistance for the writing of this article.

REFERENCES

Amalina, N.Z., Dzarifah, Z., Amal, M.N. A., Yusof, M.Z, Zamri-Saad, M., Alsaari, N., Tanaka, M., Mino, S., Sawabe, T. and Ina-Salwany, M.Y., 2019. Recent update on the prevalence of *Vibrio* species among cultured grouper in Peninsular Malaysia. *Aquaculture Research*, *50*(11), pp.3202-3210. https://doi.org/10.1111/are.14275

Ariff, N., Abdullah, A., Azmai, M.N.A., Musa, N. and Zainathan, S.C., 2019. Risk factors associated with viral nervous necrosis in hybrid groupers in Malaysia and the high similarity of its causative agent nervous necrosis virus to reassortant red-spotted grouper nervous necrosis vi-

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rus/striped jack nervous necrosis virus strains. *Veterinary World*, *12*(8), pp.1273-1284. https://doi.org/10.14202/vetworld.2019.1273-1284

Azwai, S.M., Alfallani, E.A., Abolghait, S.K., Garbaj, A.M., Naas, H.T., Moawad, A.A., Gammoudi, F.T., Rayes, H.M., Barbieri, I. and Eldaghayes, I.M., 2016. Isolation and molecular identification of *Vibrio* spp. by sequencing of 16s rDNA from seafood, meat and meat products In Libya. *Open Veterinary Journal*, *6*(1), pp.36-43. https://doi.org/10.4314/ovj.v6i1.6

Bandín, I. and Souto, S., 2020. Betanodavirus and VER disease: A 30-year research review. *Pathogens*, *9*(2), 106. https://doi.org/10.3390/pathogens9020106

Barbosa, L.M.G., Moraes, G., Anibal, F.F. and Marzocchi-Machado, C.M., 2020. Effect of environmental thermal fluctuations on innate immune responses in pacu *Piaractus mesopotamicus* juveniles. *Aquaculture Reports*, *17*, 100303. https://doi.org/10.1016/j.aqrep.2020.100303

Binesh, C.P., 2014. Elevation of temperature and crowding trigger acute viral nervous necrosis in zebra fish, *Brachydanio rerio* (Hamilton-Buchanan), subclinically infected with Betanodavirus. *Journal of Fish Diseases*, *37*(3), pp.279–282. https://doi.org/10.1111/jfd.12080

Blazer, V.S., Walsh, H.L., Braham, R.P. and Smith, C., 2018. Necropsy-based wild fish health assessment. *Journal of Visualized Experiments*, *2018*(139), pp.1-11. https://doi.org/10.3791/57946

Dadar, M., Dhama, K., Vakharia, V.N., Hoseinifar, S.H., Karthik, K., Tiwari, R., Khandia, R., Munjal, A., Salgado-Miranda, C. and Joshi, S.K., 2017. Advances in aquaculture vaccines against fish pathogens: Global status and current trends. *Reviews in Fisheries Science & Aquaculture*, *25*(3), pp.184-217. https://doi.org/10.1080/23308249.2016.1261277

Dadiono, M.S., Widodo, M.S. and Wijaya, R., 2020. Broodstock health management for cantang grouper (*Epinephelus* sp.) in BBRBLPP Gondol Bali. *Journal of Aquaculture Development and Environment*, *3*(2), pp.152-155. http://dx.doi.org/10.31002/jade.v3i2.3210

Das, S.K., Xiang, T.W., Noor, N.M., De, M., Mazumder, S.K. and Goutham-Bharathi, M.P., 2021. Temperature physiology in grouper (Epinephelinae: Serranidae) aquaculture: A brief review. *Aquaculture Reports*, *20*, 100682. https://doi.org/10.1016/j.aqrep.2021.100682

Duguma, A., 2016. Practical manual on veterinary clinical diagnostic approach. *Journal of Veterinary Science & Technology*, *7*(4), 1000337. https://doi.org/10.4172/2157-7579.1000337

Hastari, I.F., Sarjito and Prayitno, S.B., 2014. Characterization causative agent of vibriosis and histology tiger grouper fish (*Epinephelus fuscoguttatus*) from floating net cages Teluk Hurun, Lampung. *Journal of Aquaculture Management and Technology*, *3*(3), pp.86–94. https://ejournal3.undip.ac.id/index.php/jamt/article/view/5805

Hazreen-Nita, M., Azila, A., Mukai, Y., Firdaus-Nawi, M. and Nur-Nazifah, M., 2019. A review of Betanodavirus vaccination as preventive strategy to viral nervous necrosis (VNN) disease in grouper. *Aquaculture International*, *27*, pp.1565-1577. https://doi.org/10.1007/s10499-019-00410-5

Huzmi, H., Ina-Salwany, M.Y., Natrah, F.M.I, Syukri, F. and Karim, M., 2019. Strategies of controlling vibriosis in fish. *Asian Journal of Applied Sciences*, *7*(5), pp.513-521. http://dx.doi.org/10.24203/ajas.v7i5.5933

Ilmiah, Sukenda, Widanarni and Harris, E., 2012. Isolation and characterization of pathogenic *Vibrio* on tiger

Cite this document as Hartawan, G.S., Yanuhar, U., Musa, M., Baihani, A., Wahyudi, Y.A., Huda, C. and Caesar, N.R., 2023. Viral Nervous Necrosis and Vibriosis in Grouper Fish: A Case Study. *Journal of Aquaculture and Fish Health*, *12*(3), pp.334-345.

grouper *Epinephelus fuscoguttatus*. *Jurnal Akuakultur Indonesia*, *11*(1), pp.28-37. https://doi.org/10.19027/jai.11.28-37

Ina-Salwany, M.Y., Al-saari, N., Mohamad, A., Fathin-Amirah, M., Mohd-Aris, A., Amal, M.N.A., Kasai, H., Mino, S., Sawabe, T. and Zamri-Saad, M., 2019. Vibriosis in fish: A review on disease development and prevention. *Journal of Aquatic Animal Health*, *31*(1), pp.3-22. https://doi.org/10.1002/aah.10045

Juniar, E., Kurniasih and Sumiarto, B., 2018. Risk factors of a viral nervous necrosis disease in grouper (*Epinephelus* spp.) cultured in Bintan district, Indonesia. *Veterinary World*, *11*(5), pp.1558-1563. https://doi.org/10.14202/vetworld.2018.1558-1563

Kementerian Kelautan dan Perikanan [KKP], 2021. *Data statistik produksi perikanan ikan kerapu*. http://statistik.kkp.go.id

Khumaidi, A., Fadjar, M., Iranawati, F., Kilawati, Y. and Yanuhar, U., 2019. Mass mortality associated with viral nervous necrosis of hybrid grouper (*Epinephelus* sp.) cultured in city of grouper. *AIP Conference Proceedings*, *2120*, 070007. https://doi.org/doi.org/10.1063/1.5115724

Kurniawati, M.D., Sumaryam and Hayati, N., 2019. Application of conventional polymerase chain reaction (PCR) and real time-PCR for detection of VNN (viral nervous necrosis) virus in tiger grouper (*Epinephelus fuscoguttatus*). *Techno-Fish*, *3*(1), pp.19-30. https://doi.org/10.25139/tf.v3i1.1629

Leboffe, M.J. and Pierre, B.E., 2011. *A photographic atlas microbiology for the laboratory 4th edition*. Morton Publishing, USA, p.13-15, p.173.

Lee, K.K., Liu, P.C. and Chuang, W.H., 2002. Pathogenesis of gastroenteritis caused by *Vibrio carchariae* in cultured marine fish. *Marine Biotechnology*, *4*, pp.267-277. https://doi.org/10.1007/s10126-002-0018-9

Mahardika, K., Mastuti, I., Roza, D., Syahidah, D., Astuti, W.W., Ismi, S. and Zafran, 2020. Pemantauan insiden penyakit pada ikan kerapu dan kakap di pembenihan dan keramba jaring apung di Bali Utara. *Jurnal Riset Akuakultur*, *15*(2), pp.89-102. http://dx.doi.org/10.15578/jra.15.2.2020.89-102

Mohamad, N., Amal, M.N.A., Yasin, I.S.M., Saad, M.Z., Nasruddin, N.S., Al-saari, N., Mino, S. and Sawabe, T., 2019. Vibriosis in cultured marine fishes: a review. *Aquaculture*, *512*, 734289. https://doi.org/10.1016/j.aquaculture.2019.734289

Nicolas, J.L., Basuyaux, O., Mazurié, J. and Thébault, A., 2002. *Vibrio carchariae*, a pathogen of the abalone *Haliotis tuberculata*. *Diseases of Aquatic Organisms*, *50*, pp.35-43. https://doi.org/10.3354/dao050035

Office International des Epizooties [OIE], 2019. Chapter 2.3.12: Viral encephalopathy and retinopathy. *Manual of Diagnostic Tests for Aquatic Animals*, pp.1–20. https://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/chapitre_viral_encephalopathy_retinopathy.pdf

Sembiring, S.B.M., Wibawa, G.S., Mahardika, K., Widiastuti, Z. and Haryanti, 2018. Prevalensi infeksi viral nervous necrosis (VNN) dan iridovirus pada hatcheri dan budidaya ikan laut. *Media Akuakultur*, *13*(2), pp.83-90. https://doi.org/10.15578/ma.13.2.2018.83-90

Sulistiyono, A., Kurniasih and Untari, T., 2020. Detection of nervous necrosis virus of grouper fish in Lombok Island, Indonesia based on serological and molecular studies. *Egyptian Journal of Aquatic Biology and Fisheries*, *24*(1), pp.329-335. https://doi.org/10.21608/ejabf.2020.70593

Vijayan, K.K, Rajedran, K.V., Sanil, N.K. and Alavandi, S.V., 2015. Fish

Cite this document as Hartawan, G.S., Yanuhar, U., Musa, M., Baihani, A., Wahyudi, Y.A., Huda, C. and Caesar, N.R., 2023. Viral Nervous Necrosis and Vibriosis in Grouper Fish: A Case Study. *Journal of Aquaculture and Fish Health*, *12*(3), pp.334-345.

health management in cage aquaculture. *5th International symposium on Cage aquaculture in Asia CAA5*, *94*(12), pp.95-103. http://eprints.cmfri.org.in/id/eprint/10589

Zhang, X.H., He, X. and Austin, B., 2020. *Vibrio harveyi*: A serious pathogen of fish and invertebrates in mariculture. *Marine Life Science & Technology*, *2*, pp.231–245. https://doi.org/10.1007/s42995-020-00037-z

Ziarati, M., Zorriehzahra, M.J., Kafilzadeh, F., Kargar, M. and Ghasemei, F., 2020. An overview of Betanodavirus and perspective of Viral Nervous Necrosis (VNN) disease in Iranian southern waters. *Iranian Journal of Fisheries Sciences, 19*(5), pp.2214-2233. https://doi.org/10.22092/ijfs.2020.122274

Cite this document as Hartawan, G.S., Yanuhar, U., Musa, M., Baihani, A., Wahyudi, Y.A., Huda, C. and Caesar, N.R., 2023. Viral Nervous Necrosis and Vibriosis in Grouper Fish: A Case Study. *Journal of Aquaculture and Fish Health*, *12*(3), pp.334-345.