UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF VIRAL NERVOUS NECROSIS (VNN) AND IRIDOVIRUS INFECTIONS IN MARINE CAGE-CULTURED FOOD FISH AT PULAU KETAM SELANGOR, MALAYSIA

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NURUS SA'ADATUL A'ABADIAH HUSSIN

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APPROVAL

It is hereby certified that we have read this project paper entitled "Prevalence Of Viral Nervous Necrosis (VNN) And Iridovirus Infections In Marine Cage-Cultured Food Fish At Pulau Ketam Selangor, Malaysia", by Nurus Sa'adatul A'abadiah bt Hussin and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 - Project.

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DEDICATIONS

This project paper is dedicated to

My parents

My family

My friends

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LIST OF ABBREVIATIONS

%	Percent
=	Equal
+	Positive
AUP	Animal Utilisation Protocol
cm	Centimetre
DNA	Deoxyribonucleic acid
DOF	Department of Fisheries Malaysia
DPX	Distyrene Plasticizer Xylene
GIV	Grouper Iridovirus
g	gram
H&E	Haematoxylin & Eosin
IACUC	Institutional Animal Care and Use Committee
K	Thousand
kg	kilogram
MT	Metric tonnes
nm	nanometre
PCR	Polymerase Chain Reaction
RM	Ringgit Malaysia
RNA	Ribonucleic acid
STL	Standard Total Length
VNN	Viral Nervous Necrosis

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999-Projek

PREVALENS JANGKITAN SARAF VIRAL NIKROSIS (SVN) DAN IRIDOVIRUS (GIV) DALAM KALANGAN IKAN LAUT TERNAKAN SANGKAR DI PULAU KETAM SELANGOR, MALAYSIA

Oleh

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Jangkitan Saraf Viral Nekrosis (SVN) dan Iridovirus (GIV) adalah jangkitan biasa yang boleh menyebabkan kematian secara berskala besar dan kerugian kepada ekonomi. Risiko penyakit tersebut perlu dikaji bagi mencegah kerugian yang akan berlaku. Kajian ini berobjektif untuk membuktikan prevalens jangkitan Saraf Viral Nikrosis (SVN) dan Iridovirus (GIV) dalam kalangan ikan laut ternakan sangkar di Pulau Ketam, Selangor, Malaysia. Sejumlah 15 ekor ikan bersaiz dewasa terdiri daripada ikan kerapu (*Epinephelus coides)* (n=3), ikan merah (*Latjanus campechanus)* (n=6) and ikan siakap Asia *(Lates calcarifer)* (n=6) telah diambil sampel secara rawak dari sangkar masing-masing. Jumlah berat badan dan panjang badan

setiap ekor ikan telah diukur dan kewujudan tanda-tanda klinikal dan abnomal telah diperiksa dan direkod. Bahagian otak dan hati ikan diambil dan diperbaiki dalam larutan 70% alkohol untuk pengesanan virus menggunakan PCR, sementara sampel otak, mata, buah pinggang dan hati diproses untuk tujuan histopatologi. Tanda-tanda klinikal seperti lesi di kulit iaitu kehilangan sisik ikan berlaku dalam kalangan ikan siakap, manakala untuk ikan kerapu pula, terdapat berlakunya lesi di bahagian kulit beserta dengan kewujudan tompok hitam di badan. Hasil daripada ujian makmal menunjukkan bahawa prevalens jangkitan SVN adalah 27% (4/15) sementara jangkitan GIV adalah 0% (0/15) daripada jumlah ikan. Prevalens pada jangkitan VSN pada ikan kerapu adalah yang tertinggi dengan 66% (2/3) Seperti diketahui, ikan kerapu boleh bertindak sebagai pembawa semula jadi virus. Sebaliknya, sebanyak 0% jangkitan Iridovirus pada ketiga-tiga spesies mungkin disebabkan oleh penggunaan kit PCR yang hanya mengesan Irido-Megalocytivirus, bukan Iridovirus (GIV). Secara histopatologinya, degenerasi vakuolatif lapisan retina dan lapisan granular otak diperhatikan pada ikan VNN + ve. Kajian ini mendapati bahawa jangkitan SVN dan GIV adalah berpotensi untuk wujud terhadap ikan laut ternakan sangkar di Pulau Ketam Selangor, Malaysia

Kata kunci : Saraf Viral Nekrosis (SVN), Grouper Iridovirus (GIV), Ikan merah, Ikan siakap asia, Ikan kerapu

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Project.

PREVALENCE OF VIRAL NERVOUS NECROSIS (VNN) AND IRIDOVIRUS INFECTIONS IN MARINE CAGE-CULTURED FOOD FISH AT PULAU KETAM SELANGOR, MALAYSIA

By

NURUS SA'ADATUL A'ABADIAH HUSSIN

2020

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Co-supervisor: Associate Professor Dr. Hassan Haji Mohd Daud
Dr Mohd Fuad Matori

Viral Nervous Necrosis (VNN) and Iridovirus (GIV) infections are common infections that can cause mass mortality and economic losses. The risk of the viral disease needs to be studied to prevent the losses. The objective of the current study was to determine the prevalence of Viral Nervous Necrosis (VNN) and Iridovirus (GIV) in a marine cage-cultured fish farm at Pulau Ketam, Selangor, Malaysia. A total of 15 grow-out fishes consisted of grouper (*Epinephelus coides)* (n=3), Red snapper (*Latjanus campechanus)* (n=6) and Asian seabass *(Lates calcarifer)* (n=6) were randomly sampled from the farm. The total body weight and body length of individual fish were measured and presence of clinical signs and abnormalities were noted. The brain and spleen were taken and fixed in 70% alcohol for virus detection using PCR, while brain, eyes, kidney and spleen samples were processed for histopathology. Clinical signs such as skin lesions i.e. loss of scale were observed in Asian seabass, while in grouper, skin lesion

together with darkening of body. Laboratory results showed that the prevalence of VNN infection was 27% (4/15) while GIV infection was 0% (0/15) in total fish. The prevalence of VNN infection in grouper was the highest with 66% (2/3). As known, grouper could act as a natural reservoir of the virus. On other hands, 0% prevalence of Iridovirus infection in all three species could be due to the use of PCR kit that only detects Irido-Megalocytivirus, not (GIV). Histopathologically, vacuolative degeneration of the retina layer and in brain granular layer were observed in VNN+ve fish. This study found that VNN and GIV infection were at latent infection level in marine cage-cultured food fish at Pulau Ketam, Selangor, Malaysia.

Keywords: Viral Nervous Necrosis (VNN), Grouper Iridovirus (GIV), Red snapper, Asian seabass, Grouper

1.0 INTRODUCTION

Human population nowadays prefer a healthy diet. Among various kinds of foods from animals and plants, fish are the one of preferable choices as a healthy source of protein (Von Goh, 2018). In a previous study, about 1.93 million metric tonnes (MT) of fish produce is needed in this year (2020) as estimated by National Agro-food Policy (FAO) (2011-2020). In Malaysia, strategies are being planned by the Department of Fisheries Malaysia (DOF) to meet the supply and demand in this industry. Malaysia was blessed with a vast source of water and land, making Malaysia a potential country in development of the aquaculture industry. Thus, it can fulfil the demand as estimated by the Food and Agriculture Organization in the future. Furthermore, the aquaculture industry plays a major role in exportation of fish and fish products to other countries such as Hong Kong, Singapore, Australia and China (Von Goh, 2018).

Marine fish such as Asian seabass, Black pomfret, Sardine anchovies, Indian mackerel are in top 15 popular fishes consumed by Malaysian (Von Goh, 2018). Asian Seabass was placed at 13^{th} with annual per capita weight for total population (kg) of 1.16kg. Thus Asian seabass is one of the common fish that is cultured in the country. Furthermore, Grouper and Red snapper were also included in common fish cultured in Malaysia.

However, issues such as increasing production cost, lack of skilled labour, threat of disease and food safety make the mariculture development in tough situations. Viral diseases such as Viral Nervous Necrosis (VNN) and Iridovirus infection are major problems in mariculture that result in significant economic losses in farms and hatcheries (Harikrishnan et al., 2011).

PCR is used in this study as it is a powerful and popular method to diagnose VNN and Iridovirus also it an effective method to detect the virus, as recommended by the OIE, World Organisation for Animal Health (2019). By practicing this method, detection of early signs of the viral disease in the fish will be faster and accurate during an outbreak or high mortality.

The objective of this study is to determine the prevalence of two important marine fish viruses which are VNN and Iridovirus in floating cage-cultured food fish at Pulau Ketam, Selangor in selected species of farmed marine fish through PCR examination and histology of selected organs. By using this basic information on prevalence of the viruses, farmer will know at much earlier stage (eg. Latent infection) and do mitigation measures before possible outbreak.

2.0 LITERATURE REVIEW

2.1 Aquaculture

According to the Department of Fisheries (2018), Aquaculture is defined as propagation of fish seed or the raising of fish through husbandry during the whole or part of its life cycle. In our country, this sector has become one of the critical activities as a vital source of growth and toward to be mainstream of the nation's economy. Marine capture fisheries, inland fisheries and aquaculture contributed about 1,780,168 miliion MT with a value of RM 10,598 million to the economy (Yusoff, 2015). Aquaculture can be divided into three sub-industry which is freshwater, brackishwater and seaweed aquaculture. In 2018, total aquaculture was 381,465.16 million MT with a value of RM 3,057 million to the country. Marine fish aquaculture contribute about 45% to the brackishwater aquaculture. The production was 49,767.94 million MT (RM 1,062,618.96 K) in 2018 (DOF, 2018). But this amount was reduced in both production and value compared to 2017. In some extent, production of aquaculture contributes for about 0.9% to the nation economy, while 12.5% to the nation's agriculture.

2.2 Marine Fish

Marine food fish such as Asian Seabass (*Lates calcarifer),* Grouper (*Epinephelus coioides*), Red Snapper (*Latjunas campechanus*) and Pomfret (*Pampus argentus)* were commonly cultured in Asian countries (Harikrisnan, 2011) because of high market value. In Malaysia, Asian seabass, grouper and red snapper are the most popular fish that are cultured (Yusoff, 2015). According to the Department of Fisheries (2018), the production of Asian

seabass was 9,682 MT with value of RM 134,995.68 thousand, followed by Red snapper (8,305MT with value of RM 202,815.62 thousand) and Grouper with production of 7,798 MT (RM 299,362.32 thousand). These three fish were produced more than 7,000 MT each compared to other marine fish that were culture in Malaysia such as Mangrove snapper (6,489MT). These fish become popular in the aquaculture industry because of their characteristics. As stated by Harikrishnan et al. (2011), these fish have fast growth criteria, efficient feed conversion and also high market value. Jerry (2015) also mentions that these fish are able to tolerate a wide range of salinity, because of it, these fish become popular in aquaculture.

2.3 Viral Disease

Fish stock depletion, disease, media influences towards aquaculture industry, non-compliance towards Halal aquaculture, poor interaction between stakeholders (Fathi et al., 2018) and climate change (Hamdan et al., 2015) are the issue and challenge faced by the aquaculture sector. Disease has become one of the threats in the aquaculture industry. Disease can be caused by bacteria, virus and parasite commonly faced by farmers. Viral diseases are considered more important compared to other agents. This is because of persistence of infection and also have lack of recovery from the infection (Ivan et al., 2019). In Malaysia, Viral Nervous Necrosis (VNN) and Iridovirus infections are the most common viral infection that occurred (Azilla & Mohd-Syafiq, 2017).

2.3.1 Viral Nervous Necrosis

Viral Nervous Necrosis (VNN) also known as Fish Encephalitis or Viral Encephalopathy and Retinopathy (Masheh et al., 2012) is caused by an agent called betanodavirus. Betanodavirus is a single stranded RNA (ssRNA), non-enveloped virus with icosahedral capsid with range from 25 to 34 nm in diameter. This virus infected more than 120 species of both marine and freshwater fish (Azilla and Mohd-Syafiq, 2017). The virus in particular affects larval and juvenile stages of fish. In the marine aquaculture industry, infection by this virus is one of the main reasons for great economic losses (Zorriehzahra et al., 2019). The clinical signs such as erratic swimming (Furusawa et al., 2006), abnormal body posture, enlarged abdomen, bilateral exophthalmos and haemorrhagic petechiae was observed in a study (Nazari et al., 2012). Retina of infected fish also will show vacuolar lesion on histopathology examination (Mauida et al., 2011)

2.3.2 Iridovirus Infection

Iridovirus infection is another viral disease that was commonly reported in Malaysia (Azilla & Mohd-Syafiq, 2017). This virus is a non-enveloped double-stranded DNA (dsDNA) that also produces large icosahedral virions ranging from 125 to 200 nm. This virus replicates in the cytoplasm of an infected cell. Iridoviridae family consists of three different genera with different clinical signs, which is *Lymphocytivirus, Megalocytivirus* and *Ranavirus* (Ivan et al., 2019). Grouper iridovirus infection (GIV) is a disease that is commonly reported in grouper farms and this disease is caused by marine Ranavirus

(Hazeri et al., 2016). In a study on Iridovirus infecting grouper species, clinical sign such as abnormal swimming, sloughing of epidermis, dermal ulceration, necrosis of gills and darkening of tail was reported in it (Hazeri et al., 2016). The pathogen of this infection causes high mortality rate in few days post infection in marine fish aquaculture (Sah Putra et al., 2016).

2.4 Economic Loss of Viral Infection

Production of aquaculture can be totally affected if an outbreak occurs. Those outbreaks can cause huge loss in the aquaculture industry. Both VNN and Iridovirus infection can cause mass mortality in the marine fish farm. A study about VNN infecting Guppy fish (*Peocilia reticulta)* was done to observ the pathogenicity of the virus. From the study, fish infected with VNN virus achieve 100% mortality 30 days post infection (Nazari et al., 2012). While in 2018, a VNN outbreak was reported. From that outbreak, the mortality was high within a few days post infection. A total loss for about RM 131,400 was recorded from the outbreak (Ivan et al., 2019). Another outbreak caused by Iridovirus infection was also reported in 2019. The outbreak caused mortality up to 100% to the farm. From the outbreak also, for about RM 50,000 was lost in a farm every for a cycle (Ivan et al., 2019). From this outbreak, a huge amount of profit was lost. If this continues, both protein supply and economic income for both farmer and country will be affected.

3.0 MATERIALS AND METHODS

3.1 Fish Sampling

This study was approved by the Institutional Animal Care and Use committee (IACUC, UPM) with the AUP number UPM/ IACUC/ AUP-U052/2020. Total of 15 marine fishes were retrieved in this study comprising of 6 Asian Seabass (*Lates calcarifer)*, 6 Snapper (*Latjunas camppechanus*) and 3 of Grouper (*Epinephelus coioides)* from a floating sea cage fish farm at Pulau Ketam, Selangor. Body weight, standard body length (STL) and body length of the fishes is measured and recorded using a ruler and weighing scale.

3.2 Organ Sampling and Fixation

A thorough physical examination was done on each fish before it was euthanized. Any abnormality and lesions were recorded. The fish were euthanized by pithing severing the cervical vertebrae column with a sterilized blade. Next, post mortem was performed to observe and record for any lesion seen on the organ. Then, the eyes, brain, spleen and kidney were extracted from the carcass through dissection. The organs were fixed in 2 different solutions which is, 70% alcohol for preservation of nucleic acid extraction and 10% buffered formalin for preservation of histological processing.

3.3 Molecular Detection by PCR

Organs that were fixed in 70% alcohol was used for molecular detection. Brain samples was used for VNN detection while spleen samples was used for Iridovirus detection. In order to detect the presence of viral, the total of nucleic acid of organs were extracted by using IQ Plus^{TM} Extraction Kit (GeneReach

Biotechnology Corp. Taiwan), according to the manufacturer's instruction. PCR reaction procedure was performed using IQ Plus™ VNN Kit and IQ Plus™ Irido-M Kit (GeneReach Biotechnology Corp. Taiwan), according manufacturer's instruction then analyse in POCKIT™ nucleic acid analyser machine within one hour. The result of diagnosis test are shown on the monitor of the machine.

3.4 Preparation of Histology Slides

All organs samples were place in 10% buffered formalin fixatives with the ratio of 1:10 for 24 hours. After that, the samples were processed in the tissue processor. Besides that, the organs sample that was fixed in 10% buffered formalin was left until the tissue processing stage. Next, the samples were embedded in paraffin wax. After cooling and trimming process, the samples were sectioned at 4µm, dried and stained by using Haematoxylin & Eosin (H&E). Mounting of glass slide using Distyrene Plasticizer Xylene (DPX) was done after staining process and let it dry overnight. Finally, the slides are ready to be viewed.

3.5 Histology and morphological observation

All the slides were viewed under the light microscope equipped with a digital camera (Image Analyzer (Olympus® BX51) and the pathological changes in the samples were take noted. Each slide will be viewed with 10 field views under microscope. For VNN lesion detection, brain and eyes samples were examined, while Iridovirus lesion detection, spleen and kidney were examined.

3.6 Data Analysis

The prevalence of viral infection was calculated. To determine the prevalence of viral infection in marine fishes, using formula :

$$\text{Prevalence (\%)} = \frac{\text{number of positive sample}}{\text{total number of fish sample, N}} \times 100\%$$

4.0 RESULTS

A bit about the farm background. This fish farm is located at Pulau Ketam, Selangor near Pulau Ketam Ferry Jetty, with a coordinate of 3º00'57 N 101º14'54E. This area of this farm is about 2500 m^{2}. This farm practices polyculture which include, Red Snapper, Mangrove jack, Grouper, Asian Seabass, Sweet lips. From the fish samples that were sampled from the farm, the weight, TBL in centimetre (cm), were taken from each individual fish during necropsy. The weight of the fish is varies. The weight is from 50g up to 300g each. The STL is from 11.0 cm up to 22.5 cm and the total body length is from 13.0 cm up to 26.0 cm.

4.1 Gross Lesion

Clinical sign such as skin lesion example, ulceration of skin, tail erosion, epidermis sloughing, and darkening of skin colouration (Figure 1) were observed in Grouper, while only ulceration of skin was observed in Asian seabass (Figure 2) were noted during external examination.

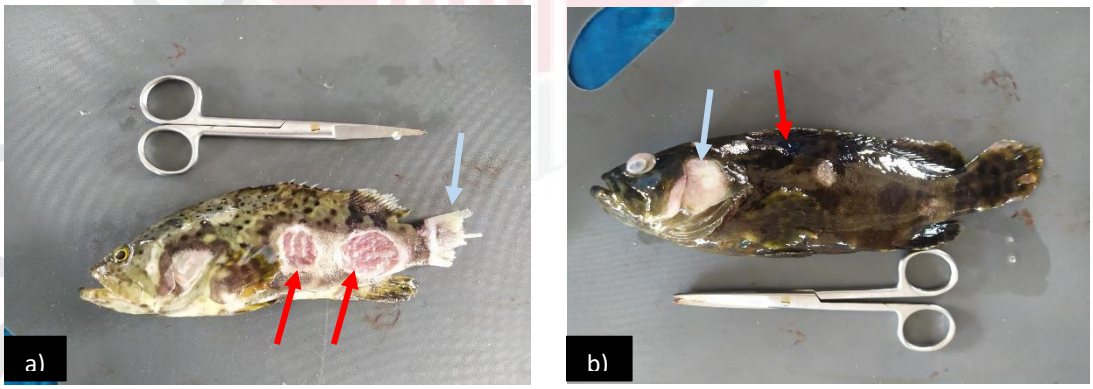

Figure 1: Gross lesion of Grouper. Gross lesion that was observed in grouper samples during necropsy: a) Ulceration of skin (red arrow) and tail erosion (blue arrow), b) Skin darkening (red arrow) and epidermis sloughing (blue arrow).

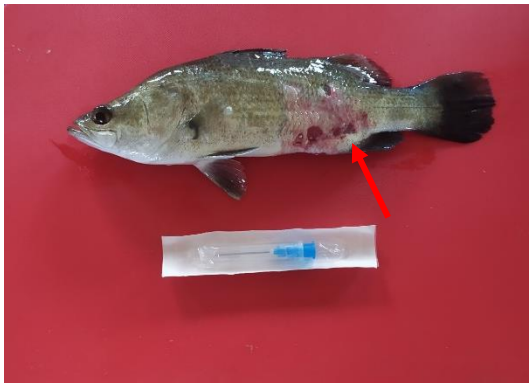

Figure 2: Gross lesion of Asian seabass. Ulceration of skin (red arrow) was observed during external examination of Asian seabass

4.2 Molecular Test (PCR)

Molecular test using PCR was done for both VNN and Iridovirus detection in brain and spleen, respectively as recommended by OIE. Results showed that only one fish has presence of VNN virus in the sample for both Asian seabass and Red snapper, while 2 out of 3 fish were detected with VNN virus in grouper (Table 1). No presence of Iridovirus was detected from all samples.

Table 1: Result of molecular test (PCR) of VNN detection in sample of brain (n=15).

	No. of fish			
FISH	VNN		Iridovirus	
	Positive	Negative	Positive	Negative
Asian Seabass	1	5	0	6
Red Snapper	1	5	0	6
Grouper	2	1	0	3
Total	**4**	**11**	**0**	**15**

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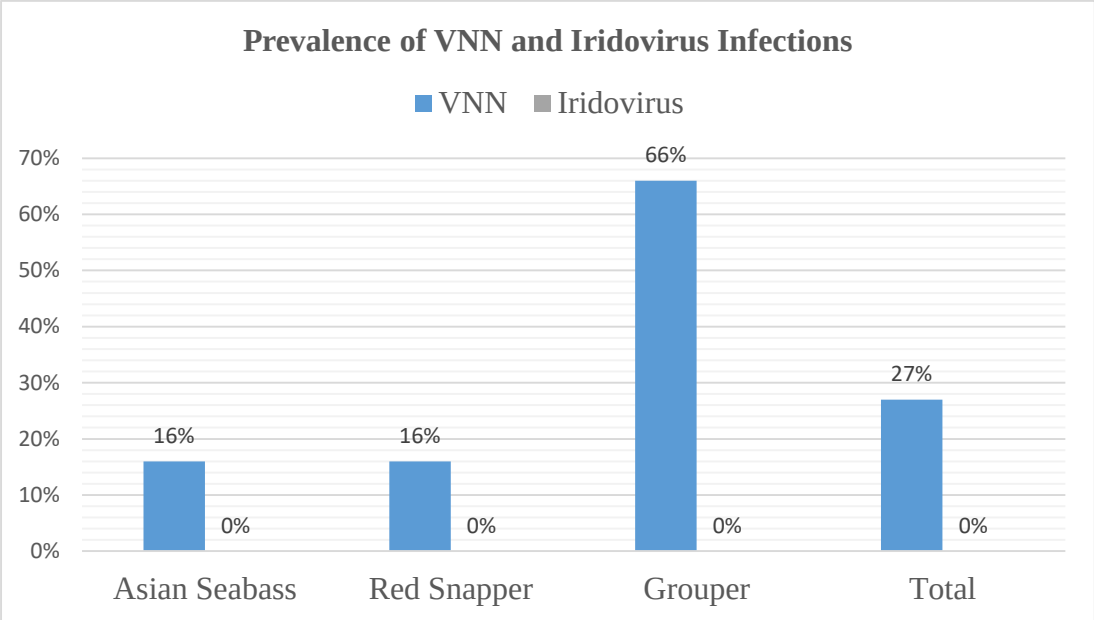
4.3 Prevalence

The prevalence of VNN and Iridovirus infections were shown in Figure 3 by using formula stated in methodology.

Figure 3: The prevalence of VNN and Iridovirus Infection in the farm (n=15).



4.4 Histology Examination

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In this study, fish that showed positive results by the molecular test were examined based on histopatholgoy. Histopathological changes associated with VNN infection were often detected in the eyes and brain. VNN+ve fish showed evidence of vacuolar lesion in both retina layer (Figure 4) and granular layer of brain, while hydropic degeneration was observed in the brain (Figure 5), thus, this might be evidence of necrosis of tissue and cause abnormalities to the fish. No obvious changes of spleen and kidney of infected fish.

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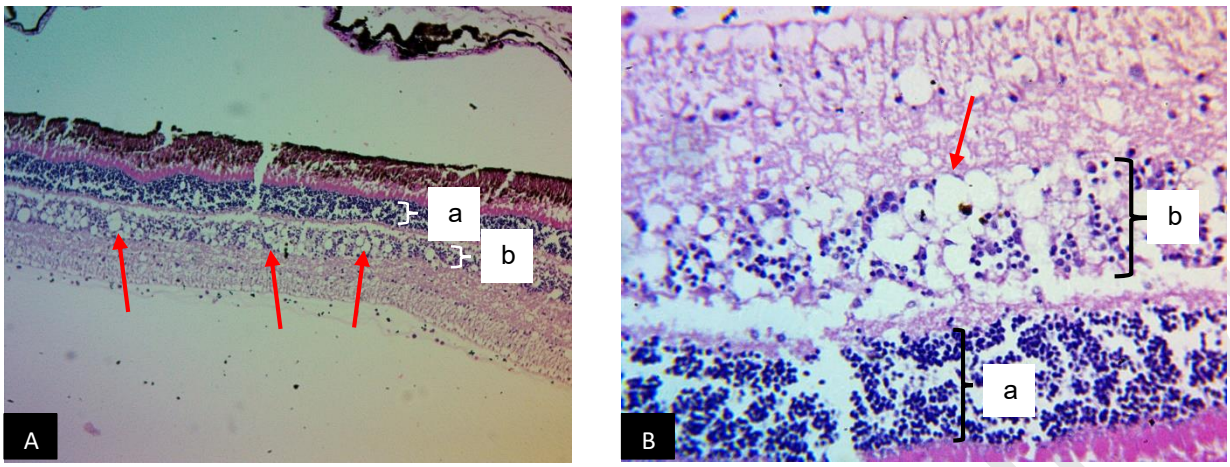

Figure 4: Histological examination of retina layer of grouper fish staining with H&E stain. **A)** Vacuolar lesion along the retina layer, under 100x magnification, **B)** The arrow shown is cellular vacuolation as a lesion in the retina layer under 400x magnification

*a: Outer nuclear layer
b: Inner nuclear layer

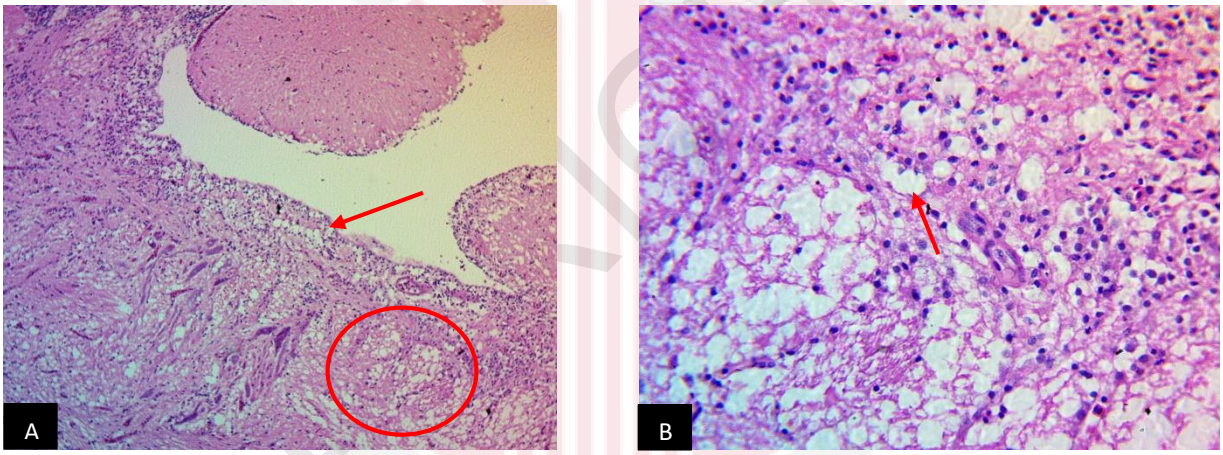

Figure 5: Histological examination of brain of Asian seabass fish staining with H&E stain. **A)** Tissue vacuolation (arrow) and hydropic degeneration (circle) were observed, under 100x magnification, **B)** Cellular vacuolation (arrow) was observed in the brain that lead to necrosis and malfunction of organ, under 400x magnification.

5.0 DISCUSSION

Viral Nervous Necrosis (VNN) was successfully detected from the 15 fish samples including 6 Asian seabass, 3 Grouper, and 6 Red snapper. Within this study with a prevalence of 4/11 (27%) positive for VNN, with the Grouper fish (2/3, 66%) has the highest prevalence compared to Asian Seabass (1/6, 16%) and Red Snapper (1/6, 16%). In this study also, Grouper has the highest prevalence compared to other fish, might be due to grouper can act as a potential natural reservoir for the virus (VNN) (Ma et al., 2012). This due to the juvenile stage of grouper culture seems to be natural to have VNN during that stage (Ma et al., 2012). Besides, according to Ransangan et al. (2013), 60.98% is an alarming level of betanodavirus infection, while for this study, indicate that prevalence of total samples was below an alarming level. Hereby we can indicates that the farm has low prevalence of VNN. While, the prevalence of Iridovirus infection from that farm was 0%, 0/11 and no iridovirus was detected from the fish sample taken from the farm. The non-existence of Iridovirus infection in this study could be due to the use of a PCR kit that only detects Irido-Megalocytivirus, not Grouper-Iridovirus (GIV). Most of the Iridovirus infection that occurs in cultured marine fish was infected by GIV. Iridovirus-Megalocityvirus usually infecting ornamental marine fish.

In both Grouper and Asian seabass, several gross lesions were observed on the several fishes which include skin lesions such as ulceration of skin, darkening of skin, epidermis sloughing and tail erosion. In a study of VNN infection in hybrid grouper that was done by Ariff et al. (2019), the infected grouper showed several lesions that include darkened skin pigmentation, backbone deviation, abdominal distention, skin lesion and fin erosion. Furthermore, Hazeri et al. (2016) also reveal the clinical sign of

Iridovirus infection in hybrid grouper that include ulceration of the operculum near to caudal fin with hemorrhages, necrosis of caudal fin, sloughing of epidermis, dermal ulceration and pop eyes. These signs were also found within this study. Hereby, we can conclude that the fish samples from the farm in Pulau Ketam, Selangor might have ongoing infection either caused by VNN and Iridovirus.

Histologically, vacuolar lesion was observed in the inner nuclear layer (INL) of retina layer of grouper which showed positive results by the molecular test as shown in Figure 5 while cellular vacuolation was observed in Figure 6. Apart from the retina layer, histopathology of the brain has also been observed. A similar situation occurred in betanodavirus experimental infection in freshwater ornamental guppies. Some of the histopathology of the infected fish were vacuolation in the ganglion cell layer of retina, while multifocal vacuolation and necrosis of forebrain (Mauida et al., 2011). Junlar et al. (2018) also stated that positive VNN fish by molecular fish showed the presence of vacuolation on both brain and eyes in study of VNN disease infecting grouper cultured in Bintan district, Indonesia. Thus this supports the hypothesis in current study.

Presence of betanodavirus in the farm might be indicative of an ongoing outbreak of VNN incorporating very low mortality rate and can reduce the production of fish (Sah Putra et al., 2020). We can deduce the presence of this infection can come from two transmission, which is horizontal and vertical transmission (Khairiah et al., 2019). Horizontal transmission usually occurs from survived fish, wild carrier or infected live feed. While vertical transmission occurs from parents. Khairiah et al. (2019) also mention that, high rate (60%-100%) of fry will be detected positive VNN if at least one parent is positive VNN. In this study, the infection that occurs might come from horizontal transmission due to this farm feeding them with trash fish since trash fish can become a source of infection of VNN (Pena et al., 2011). From the

finding, an outbreak might occur if predisposing factors occur. Fish mortality depends on the fish's health and status of water quality (Junlar et al., 2018), thus maintaining good practice can improve and maintain the health of fish and good water quality.

6.0 CONCLUSION AND RECOMMENDATIONS

As for the conclusions, we can justify that the prevalence of VNN in cage-cultured marine food fish at Pulau Ketam is at control level. The presence of VNN in cage-culture marine food fish at Pulau Ketam can be an indication of ongoing infection or maybe latent infection with low mortality. Aside from that, absence of Iridovirus detected in cage-cultured marine food fish at Pulau Ketam might be due to false-negative results. Lastly, microscopic changes such as vacuolar lesion of eyes and brain can be observed in infected fish. Thus, lead to malfunction of eyes and brain then lead to abnormal swimming behaviour and blind that lead to loss of appetite and lethargic, and result in weight loss and proceed with death. Outbreaks can happen if predisposing factors occur and can cause huge loss to both fish farmers and the nation in terms of production and profit toward the economy.

For future studies, we highly recommend to increase the sample size and sampling from several cage-cultured farms to have better results to represent a population. In addition to this, if possible, data sampling could be done during an outbreak or high mortality to support findings on morphological changes. Furthermore, it is recommended to take sample from trash fish and wild caught fish also to understand better the transmission that may occurred. As for the fish farmers, proper aquaculture cage cultured management must be practiced to avoid high mortality or outbreak, especially for infection and disease.

REFERENCES

Annual Fisheries Statistic. (2018). Annual Fisheries Statistic, Department of Fisheries Malaysia, Ministry of Agriculture and Agro-Based Industry, Malaysia.

Ariff N, Abdullah A, Azmai MNA, Musa N, Zainathan SC (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 virus/striped jack nervous necrosis virus strains, *Veterinary World*, 12(8): 1273-1284.

Azilla, A. & Mohd-Syafiq, M.R, (2017). Bacteria and viral diseases in marine cage-cultured fish in Malaysia. *Fishmail: Publication of Malaysian Fisheries Society*, 23(2017): 14-15

Fathi, S., Harun, A. N., Rambat, S., & Tukiran, N. A. (2018). Current Issues in Aquaculture:Lessons from Malaysia. *Advanced Science Letters*, *24*(1), 503-505.

Furusawa, R., Okinaka, Y., & Nakai, T. (2006). Betanodavirus infection in the freshwater model fish medaka (Oryzias latipes). *Journal of general virology*, *87*(8), 2333-2339.

Hamdan, R., Othman, A., & Kari, F. (2015). Climate change effects on aquaculture production performance in Malaysia: an environmental performance analysis. *International Journal of Business and Society*, *16*(3).

Harikrishan, R., Balasundaram, C., & Heo, M.S (2011). Fish health aspect in grouper aquaculture. *Aquaculture* 320 (2011): 1-21

Hazeri, M, Hassan, M.D, Abba, Y, Omar, A.R, Allaudin. Z.N, Solatani. M, Hamdan. R.H, Nora Faten, A.M, Sharifah Raina M. & Sadegh Vishkaei, M (2016). Histopathological evaluation and molecular detection of natural Iridovirus infection in cultured grouper fish in Malaysia. *Comparative Clinical Pathology* 25(2016): 965–971

IQ Plus™ VNN Kit and IQ Plus™ Extraction Kit User Manual (2015/09). GeneReach Biotechnology Corp. Taiwan

IQ Plus™ Iridovirus Kit and IQ Plus™ Extraction Kit User Manual (2015/09). GeneReach Biotechnology Corp. Taiwan

Ivan, K. M. C., Andrew, M. S., & Yin, S. L (2019). The Significance of Major Viral and Bacterial Diseases in Malaysian Aquaculture Industry. *Pertanika J. Trop. Agric. Sc*. 42 (3)(2019): 1023 – 1047

Juniar E, Kurniasih K, Sumiarto B (2018). Risk factors of a viral nervous necrosis disease in grouper (Epinephelus spp.) cultured in Bintan district, Indonesia, *Veterinary World*, 11(11)(2018): 1558-1563

Khairiah, A.A, Amal, M.N.A., Saad, M.Z., Murni, M, Abdullah, A, Mustafa, S., & NIk Yusof, N.H (2019). Prevalence, Risk Factors and Transmission of Nervous Necrosis Virus in A Hatchery Producing Hybrid Grouper (Epinephelus lanceolatus × Epinephelus fuscoguttatus) Fry. *Pertanika J. Trop. Agric. Sc*. 42 (1)(2019): 125 - 138

Ma, H., Xie, J., Weng, S., Zhou, T., & He, J. (2012). Co-infection of megalocytivirus and viral nervous necrosis virus in a very severe mass mortality of juvenile orange-spotted groupers (Epinephelus coioides). *Aquaculture*, *358*, 170-175.

Masheh, S., Santosh, K.S., Venugopal, M.N, & Karunasagar, I (2012). Betanodavirus of Marine and Freshwater Fish: Distribution, Genomic Organization, Diagnosis and Control Measures. *Indian J Virol* 23(2)(2012): 114–123.

Nazari, A., Hassan, M. D., Bovo, G., Zorriehzahra, M. J., Azmi, T. I., & Arshad, S. S. (2014). Pathogenicity of viral nervous necrosis virus for Guppy fish, Poecilia reticulata. *Iranian Journal of Fisheries Sciences*, *13*(1), 168-177.

de la Peña, L. D., Suarnaba, V. S., Capulos, G. C., & Santos, M. N. M. (2011). Prevalence of viral nervous necrosis (VNN) virus in wild-caught and trash fish in the Philippines. *Bull. Eur. Assoc. Fish Pathol*, *31*, 129-138.

Ransangan, J., Manin, B.O, Lal, T.M.M., Lu, K.C., Sade, A., & Azila, A. (2013). Betanodavirus Infection in Marine Fish Aquaculture in Malaysia. *Research Journal of Animal, Veterinary and Fishery Sciences* 1(7) (2013): 10-15

Sah Putra, B., Hick, P. M., Hall, E., Whittington, R. J., Khairul, R., & Becker, J. A. (2020). Prevalence of infectious spleen and kidney necrosis virus (ISKNV), nervous necrosis virus (NNV) and ectoparasites in juvenile Epinephelus spp. farmed in Aceh, Indonesia. Pathogens, 9(7), 578.

Von Goh, E. (2018). *The status of fish in Malaysian diets and potential barriers to increasing consumption of farmed species* (Doctoral dissertation, University of Nottingham).

World Organisation for Animal Health. (2019). Red Sea Bream Iridoviral Disease. Retrived March, 05, 2020 from https://www.oie.int/index.php?id=2439&L=0&htmfile=sommaire.htm

Yusoff, A. (2015). Status of resource management and aquaculture in Malaysia. International Workshop on Resource enchacement and Sustainable Aquaculture Practice in Southest Asia (2014): 53-65

Zorriehzahra, M., Hassantabar. F., Ziarati, M., Goharrizi, Y.L., Seidgar, M., Radkhah, K. & Asadi, S.M (2019). Impact of Viral Nervous Necrosis (VNN) Disease as a New Threat to Global Fisheries and Aquaculture Development- *A Review*. *Iranian Journal of Virology* 13(2)(2019): 42-57

Zorriehzahra, M.J, Adel, M, Dadar, M, Ullah, S. & Ghasemi M (2016). Viral nervous necrosis (VNN) an emerging disease caused byNodaviridae in aquatic hosts: Diagnosis, control and prevention: A review. *Iranian Journal of Fisheries Sciences* 18(1)(2019) 30-47