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Immunohistochemistry of Gill and Brain Infected by *Viral Nervous Necrosis* (VNN) in Humpback Grouper (*Cromileptes altivelis*) Correlated with B-Actin Expression

Ach. Khumaidi*, Uun Yanuhar and Abdul Rahem Faqih
Fisheries and Marine Science Faculty, University of Brawijaya, Indonesia
*Corresponding author's e-mail: ach.khumaidi@gmail.com

ABSTRACT: β-actin is a protein which has important roles in the immune system such as clustering receptors, antigen internalization and managing vesicles turnover for antigen processing. There is increasing expression of β-actin at *Viral Nervous Necrosis* (VNN) infection in the gill and brain of humpback grouper (*C. Altivelis*). The observation on the percentage of β-actin's expression in the control group's fish gill was 31.9% and VNN treatment group's fish gill was 58.4%. The same happened in the control group's fish brain, which showed 31.4% and VNN treatment group's brain showed 72.6%. These increasing percentages showed that during VNN infection, fish body increased the expression of β-actin to improve immune system regulation when facing VNN infection. The result showed that when the VNN infection, the fish will improve B-actin expression to improve the immune response to eliminate VNN.

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INTRODUCTION

Humpback grouper is one of Indonesian main commodities in marine culture. This fish, which is called polka-dot fish, has quite a high selling price. In Indonesia, there have been a lot of fish farmers cultivating this humpback grouper, either by bamboo cages or floating nets [1]. In the cultivation process, there are a lot of problems faced by the fish farmers. One obvious problem is diseases' attack caused by intracellular parasite or known as viruses.

Viral Nervous Necrosis (VNN) is one of the biggest problems which causes most failures in marine culture. According to Yuwanita and Yanuhar [2], VNN attack to humpback grouper can cause network destruction such as hyperplasia, necrosis, vocalization and cause high mortality rates reaching 100%. This virus infects humpback grouper during larval and juvenile phase, and its target is the nerves in the eye and brain with special symptoms such as whirling, sleeping dead and abnormal behavior [3 and 4]. Regulation of mechanism in the immune system becomes very important for a successful fish immune system dealing with VNN infection. One important protein in immune system regulation is β-actin [5]. This protein is a component of cytoskeleton, which has an important role in cellular regulation, such as managing cells morphology, clustering receptor, antigen internalization and managing vesicles turnover for antigen processing. The decreasing regulation of actin cytoskeleton also gives impact like disrupting immune synapses forming, T cell and B cell activation [6]. Furthermore, β-actin is also involved in an important mechanism in nucleus such as transcription, mRNA export, and chromatin remodelling [7 and 8]. In the nucleus, β-actin is bound with RNA polymerase II [9] and RNA polymerase III [10].

Based on that function of β-actin, we acquired β-actin profile data in the infected organs of humpback grouper, the gill and the brain. The data acquired could be the indicator of the fish immune system's condition, because β-actin was the key regulator of cellular process [11], including immune system.

MATERIAL AND METHODS

VNN infection to humpback grouper (*C. altivelis*)

Before the treatment, fish had been acclimatized for 2 days. Infection was done by feeding the fish with the feed which had been mixed with the infected fish's tissue. Feeding was done *ad libitum* (little by little) at 9 a.m. and 3 p.m. local time. The fish received the treatment and reared for 2 weeks (14 days). On the fourteenth day, dissection was performed to extract the target organs, the gill and the brain.

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The gill and brain isolation of humpback grouper (C. altivelis)

In the organs isolation, fish which had been tested with in-vivo test was made unconscious using clove oil and sea water. After it was unconscious, dissection was performed and the gill and brain were extracted. The organs were then soaked in liquid nitrogen and kept in the liquid nitrogen tank until they were used for the next process.

Immunohistochemistry

Immunohistochemistry staining referred to Khan et al. [12]. Tissue piece was dehydrated with alcohol and cleaned with xylene. Peroxide endogenous enzyme was frozen with 3% hydrogen peroxide and methanol in the room temperature for 30 minutes with no light. Microwave antigen extraction was performed using 0.01 mol/L of sodium citrate buffer (pH 6,0). Then, it was incubated with β-actin monoclonal antibody (AC-15) for 16 hours in the 4 ^{0}C temperature with dilution 1:1000. After that, secondary antibody – IgG biotin conjugated was added for 30 minutes in the room temperature.

RESULTS

β-actin Existence analysis was focused on infected target organs, the gill and the brain. Immunohistochemistry images analysis using ImmunoRatio software resulted different DAB percentage between control group fish and VNN treatment group, either in the gill or in the brain. In normal gill DAB percentage was 31.9%, it showed that target gene (β-actin) existing in the normal fish was 31.9%. On the other hand, fish gill which had been infected by VNN as a treatment showed the percentage of target gene was higher about 58.4%. From these results, it was known that during VNN infection, there was a profile increase of existing β-actin.

Same thing happened in the brain as this organ also a target organ of VNN infection. Existing β-actin in the control group fish based on DAB Immunoratio was about 31.4%, while in the group treated with VNN was higher at 72.6%. From the data, there was a significant difference of β-actin profile in the control group fish and the fish with the treatment.

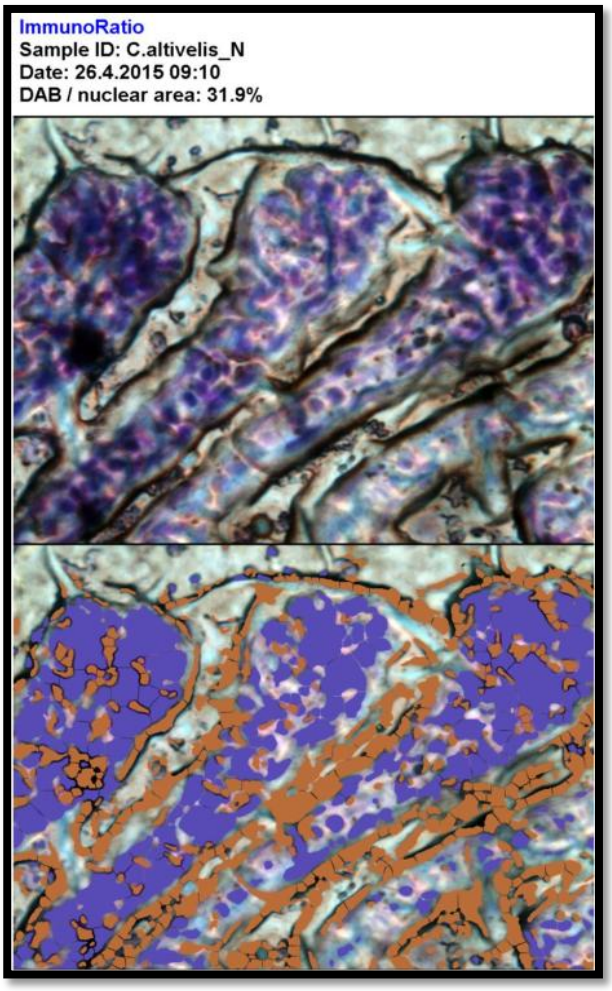

Figure 1. β-actin profile control fish gills

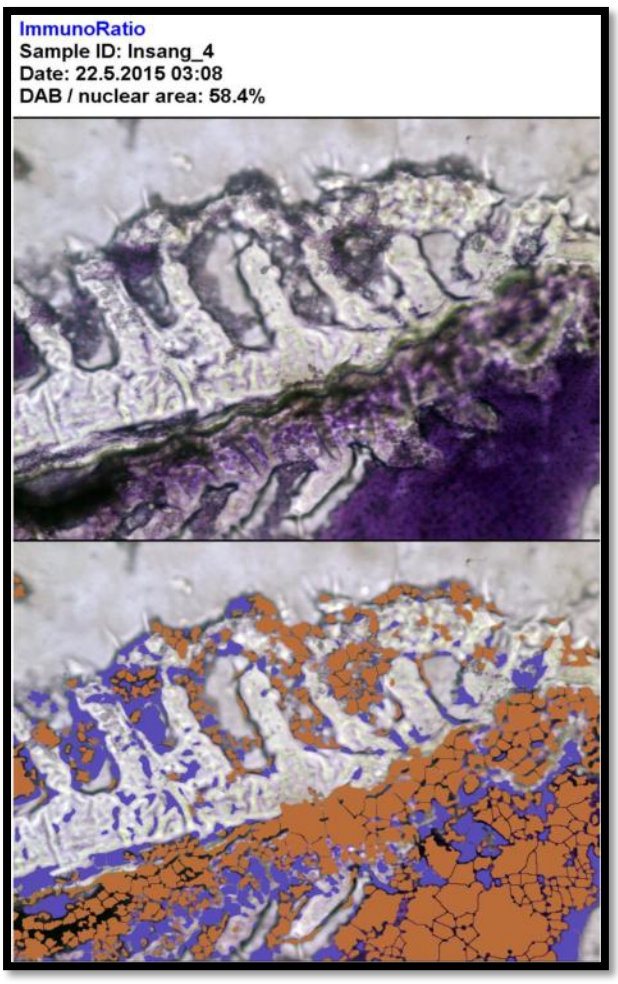

Figure 2. β-actin profile gills with treatment VNN

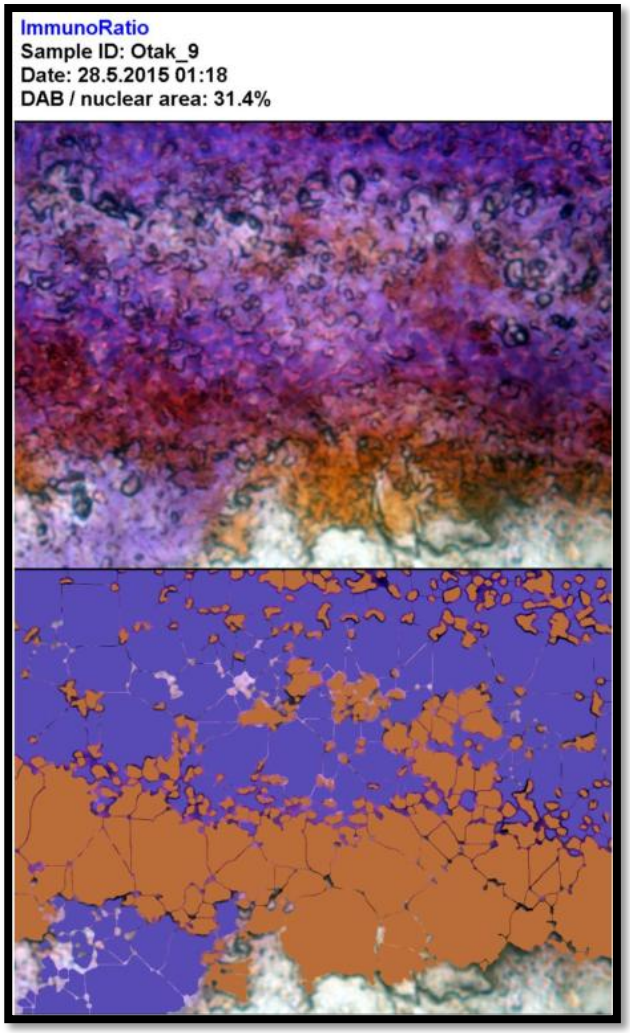

Figure 3. β-actin profile control fish Brains

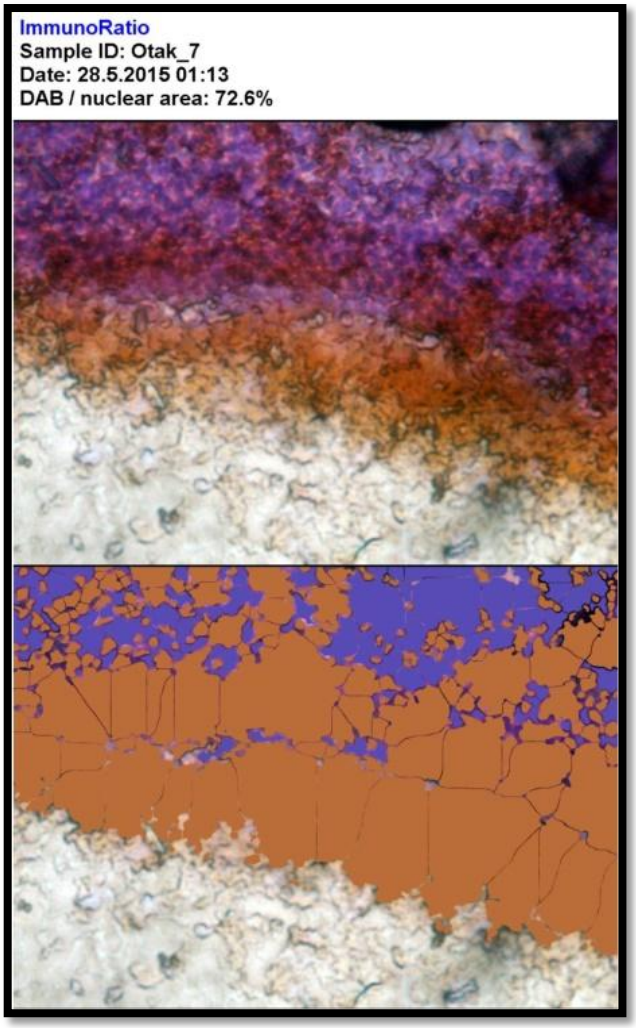

Figure 4. β-actin profile Brains with treatment VNN

DISCUSSION

β-actin is an important component in the cellular mechanism inside an organism. This gene is considered as the cell's internal controller gene [13]. This protein has important roles in the immune system toward pathogen infection such as clustering receptor, antigen internalization and managing vesicles turnover for antigen processing.

Based on observation results in Figures 1, 2, 3 and 4, there was different β-actin expression percentage between control group fish and the fish with treatment, where the fish treated with VNN infection showed a higher percentage than the control group fish. This β-actin expression increase was a response of the fish immune system when it faced VNN infection.

In figure 2 and 4, even there were increases of β-actin caused by the VNN treatment, there was still a difference. In the brain, there was a higher increase about 72.6% (Figure 4), while in the gill was only 58.4% (Figure 2). From the data, we can assume two things. First, infection happened in the fish brain was more severe than the infection in the gill, as β-actin expression in the brain was higher. The assumption was based on VNN infection pattern, as stated by Nguyen et al. [14] the brain was the main target of VNN infection, followed by eyes, gill, liver, kidneys, intestines and other organs. Second, the brain was actually able to express β-actin higher than the gill. It was stated by Zhang et al. [15] which said the β-actin expression level in the brain was higher than the gill of the orange-spotted grouper.

This increasing β-actin expression mechanism was triggered by *Toll-like receptor* (TLR) recognition of the virus [16]. This virus recognition produced transduction signal to create de novo and change the dynamics and expression of β-actin [17 and 18]. The increasing β-actin expression became the indicator of the immune response towards the virus.

The β - actin increase would improve immune system regulation of the fish. It happened because β-actin was the key regulator of cellular process [11]. According to Chow et al. [19], β-actin increase would change B cell

morphology. When β-actin expression increase happened, β-actin polymerization would happen and change B cell morphology. The change would give a positive impact to the immune system as the easier for B cell to capture antigen.

Furthermore, β-actin expression increase also gave important contributions in the antigen presentation process by *Major Histocompatibility Complex* (MHC) [20]. In the presentation process, β-actin bound MHC molecules and took them to cell membrane to present antigen to T cell. On the other hand, lessened β-actin would cut MHC signal and *T cell receptor* (TCR) so it would disrupt T cell proliferation and then would cut immune response [21]. From this result, it was shown that β-actin increase was a form of immune response of the fish towards VNN recognition.

CONCLUSION

Based on β-actin profile analysis results in the gill and brain of control fish and the fish with VNN treatment, it can be concluded that during the VNN infection, the fish would increase β-actin expression to improve system regulation and immune response in order to eliminate VNN.

Recommendation

For further research is necessary to study the correlation between the B-actin genes with an antiviral (such as interferon, P56, etc.).

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