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Betanodavirus Infections in Tilapia seed *(Oreochromis* sp.), in Indonesia

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ABSTRACT: Betanodavirus is the causative agent of the disease VNN (Viral Nerveous Necrosis) and has been reported in many cultured marine fish species worldwide and lately also attacked for freshwater species. In the present study, we described the betanodavirus infection in tilapia seed *(Oreochromis* sp.). The sample used (5-7 cm in total length) were taken randomly in Central Java, Indonesia. Brain and eyes of fish specimen were divided in two parts, for histopathological examination and for viral isolation using nested RT-PCR. The results showed that tilapia seed infected by VNN, and also showed a clear pathognomic VNN such as vacuolization and inclusion body in the brain and eyes, similar to the pattern of infection in naturally infected marine fish. By phylogenetic analysis, the isolates from tilapia seed belonged to RGNNV genotype (unpublished data). Although still very few reports and there is no outbreak of VNN in tilapia in Indonesia, but this study shows the possibility of that species could be the carrier or reservoir of this virus.
Key words: VNN, tilapia seed *(Oreochromis* sp.), histopathology, Indonesia

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INTRODUCTION

VNN is a disease that is common in marine fish commodities, especially snapper and grouper through the word such as Europe, North America, Asia, and Australia [1][1]. VNN usually causes high mortality especially at the larval and juvenile stage [2]. The fact that the VNN can infect several species of freshwater fish such as tilapia [3], goldfish and rainbow shark [4], guppy [5], and *Micropterus salmoides* [6], forcing us to be more aware of this disease. Tilapia into the Office International des Epizooties (OIE) list as one of the species that can be infected by VNN just like snapper, grouper, milkfish, and guppy [7].

In Indonesia, tilapia is one commodity that is widely cultivated and it has been introduced to all regions. Allegations that VNN that infect freshwater species derived from VNN that infect marine species [8], so that freshwater fish can act as a carrier of the virus and could be a carrier. Thus, it is necessary to laboratory examination every tilapia fry will be distributed, both among regions and between countries, so that the possibility of this disease spread can be avoided.

The purpose of this study was to determine the genomic VNN on tilapia seed with histopathology and PCR analysis in order to illustrate the virulence VNN and can be used as a reference for further research, particularly regarding VNN infection in freshwater fish species.

MATERIAL AND METHODS

Tilapia fry sample size of 5-7 cm in total length, were taken at random at the end of December 2014 in Central Java, Indonesia. The fish samples were then analyzed using histopathological and nested RT-PCR in the organs of the brain and eyes. PCR examination performed using Specific primers R3 (5'-CGA GTC ACG AAC GGT GAA GA-3 '), F2 (5'-CGT CAT GTC AGT GTG TCG CT-3'), NR3 (5'-GGA GCT GGG GAC TTT GCT CA -3 ') and NF2 (5' -GTT CCC TGT ACA ACG ATT CC-3 ')[7], and use a marker from Kappa Universal Ladder.

RESULTS

All samples were taken and tested, do not show many clinical symptoms such as that found in infected fish VNN in general like erratic swimming, only found a few fish that have black spots (Figure 1). The picture is in accordance with changes in the clinical symptoms caused by infection VNN according Thiery et al. [9], one of which can cause the body color is darker or blackish. Based on the results of tilapia seed samples by using nested RT-PCR method, resulting in a positive sample VNN of the brain and organs indicated by the parallel band in the positive control is 294 bp (Figure 2) for tilapia seed samples from Central Java, Indonesia. The results of the

histopathological examination of the brain organs and eyes, in addition showed mild vacuolation (Figure 3), there is also inclusion body (Figure 4). The brain and the eye is a target organ VNN, where pathognomic characteristic is the formation of vacuoles and Inclusion body or IB. Both disorders pathology shows that VNN not just replicate, but also cause pathological damage to the organ.

Figure 1. Tilapia seed (Oreochromis sp.) were infected VNN and showing black spots (arrows)

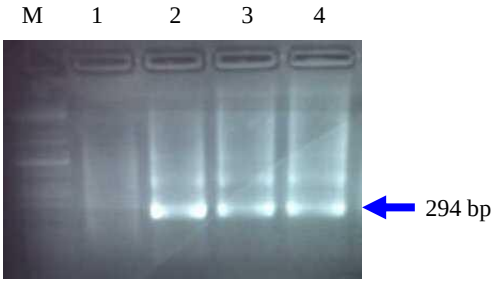

Figure 2. Results of nested RT-PCR in infected tilapia seed at 294 bp. (M = marker; 1 = negative control; 2 = positive control; 3 = brain; 4= eyes)

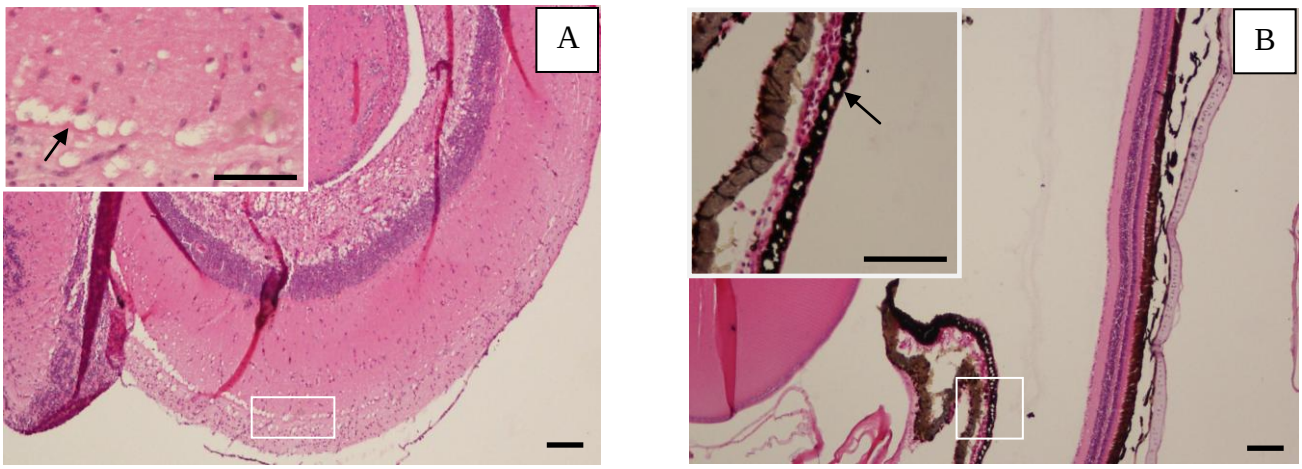

Figure 3. Results of histopathological using light microscopy at the organs of the brain (A) and eyes (B) shows vacuolation (arrow) in tilapia seed. H & E staining. Bar = 2µm, 100 µm (inset).

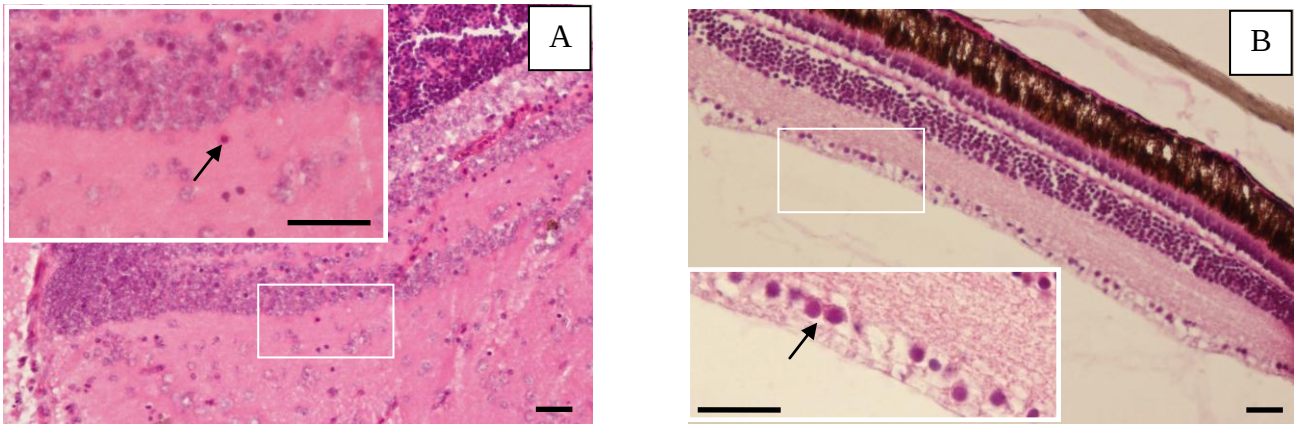

Figure 4. Results of histopathological using light microscopy at the organs of the brain (A) and eyes (B) shows the *inclusion body* inclusion body (arrow) in tilapia seed. H & E staining. Bar = 2µm, 100 µm (inset).

DISCUSSION

Although it did not reveal any abnormal swimming patterns, but still a little encountered clinical symptoms such as body color black, and it turns out tilapia seed potential VNN disease carrier. It is similar to previous research which states that VNN infected tilapia can be naturally and experimentally [4]. Tilapia seed samples were observed, not all cause clinical symptoms. Not the appearance of clinical symptoms are evident on tilapia seed that would later lead to pathogenicity, probably caused by these interactions lead to the emergence of the signal only in the cells of the body that is essential for the formation of the immune system, but it did not happen virus absorption. Schaulies [10] states that the interaction between the virus and the receptors despite not cause infection, but emit a signal transduction that can induce the secretion of cytokines such as interferon, which will have a major impact on the progression of the disease itself (pathogenicity). Further explained that, the signal transduction that is created as a consequence of virus binds to receptors of the host is an important mechanism that affects the cytopathogenicity virus and the immune response.

In organs that are VNN target organ, ie the brain and the eyes of the results of nested RT-PCR positive at 294bp. Likewise with histopathologic results showed pathognomik VNN with the formation of vacuoles in the organs of the brain and eyes, the same as the VNN infection that occurs naturally in grouper [11]. The brain and the eye is a target organ VNN, where pathognomic characteristic is the formation of vacuoles and Inclusion body (Figure 3 and 4). Both disorders pathology shows that VNN not just replicate, but also cause damage to the organ pathological brain and eyes [12]. These results are the same as previous studies carried out in European seabass fish [13; 14], Asian seabass (Lates calcarifer Bloch) [14], mangrove red snapper , Asian seabass [16] and tilapia [3]. The formation of vacuoles, IB, and necrosis of the tissue damage caused by VNN [9].

Almost the same results are also shown in research on mapping pathognomik on grouper larvae *(Cromileptes altivelis)* [17]. Research on betanodavirus infection using medaka fish (*Oryzias latipes*) as a model of freshwater fish showed that VNN has the ability to infect across species and adapt to a new host species conducted [18]. In addition, specific host on the most viral infections are usually controlled by the viral protein surface interactions with the appropriate receptors on the surface of target cells [19].

Tilapia seed has HSC70 receptor [20], just like receptors on grouper [21] specifically alleged that VNN can perform attachment and involved in an interaction that could lead to VNN into the body tilapia seed. Interactions between viruses and cellular receptors is a dynamic process chain that allows the entry of the virus into the cell [10]. During the seeds of tilapia have receptors that can bind to the attachment site(s) virus particles, then certainly virus will get into the host cell (tilapia seed), because it does not directly host cell have facilitated the virus to enter the cell [22]. Tilapia seed in this study also can experimentally infected VNN of nodavirus isolated from diseased grouper and nucleotide homology of new isolated nodavirus were 99% similar to genotype RGNNV (data not shown).

CONCLUSION

Seed tilapia can be infected betanodavirus seen from the results of histopathology (presence of vacuoles and inclusion body) and RT PCR (positive in 294 bp) from organ brain and eyes. Although still very few reports and there is no outbreak of VNN in tilapia in Indonesia, but this study shows the possibility of that species could be the carrier or reservoir of this virus.

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