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RESEARCH ARTICLE

Comparative Study of Different Methods for Viral Nervous Necrosis (VNN) Diagnosis in Fish

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ABSTRACT

The consumption of fish as useful animal protein is on the rise around the world, and the transport of fish is being considered as a means of trade between countries around the world. Every year, the occurrence of the Viral Nervous Necrosis (VNN) disease in fish, as their main host, causes great damage to the fisheries industry. Undoubtedly, one of the ways to succeed in the aquaculture industry of the future will be the rapid diagnosis and prevention of aquatic diseases, which are currently the biggest cause of losses in the aquaculture industry. Failure to definitively diagnose the pathogen in aquatic animals has led to an increase in the use of antibiotics and, while it may not lead to definitive treatment, has also led to the development of drug resistance. Therefore, prolonging the time of identification and diagnosis of the pathogen causes more prevalence of the disease, more losses and damages in fish farms and the resulting costs. OIE documents and various pieces of evidence introduce valid tools and methods in the diagnosis of betanodavirus that can be used according to the type of sample and available facilities. In this regard, the selection of a valid and rapid diagnosis method will make a significant contribution to improving methods to control, prevent and combat the spread of this worldwide disease. Therefore, the purpose of this study is to compare different methods of VNN diagnosis and evaluate their advantages and disadvantages.

INTRODUCTION

One of the most important infectious diseases that affect fish around the world is Viral Nervous Necrosis, which has been identified as a neuropathogenic disease called VNN or VER. The VNN virus is a member of the nodaviridae family and a genus of betanodaviruses. The envelopes and polyhedral virus consist of two positively polarized single-stranded RNA molecules [1,2]. VNN disease mainly affects the larval and juvenile stages of fish [3] and ranges from acute infection with 100% clinical symptoms and mortality to chronic and persistent infection worldwide. The main symptoms of the disease are a variety of neurological disorders such as abnormal swimming behavior, rapid and sudden swimming, and other outward symptoms such as swollen bladder dilation, dull skin and lethargy are also observed [2,4]. Various studies have shown that infected fish with VNN can transmit the virus horizontally and vertically to future generations, and fish, as a harbor of the virus, transmit the infection to other fish [2,5]. One of the most important economic fish in the Persian Gulf region is the genus *Epinephelus* or grouper, whose high sensitivity to VNN has also been confirmed [6]. There are various methods for diagnosing betanodavirus from fish with clinical signs and subclinical fish. In description of

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subclinical situation, it can be a disease or injury, without signs and symptoms that are detectable by physical examination or laboratory test; not clinically manifest. In diagnosis, where some criteria are met but not enough to achieve clinical status. While choosing a suitable test method for diagnosing the disease and valid results, it is done based on some considerations, which can be: the type of sample, facilities and equipment available, international acceptance of the method and its obsolescence, method efficiency (sensitivity and specificity of diagnosis and analysis), cost and time of experiments, reproducibility of the method, etc. [2]. In this regard, to identify VNN as a serious threat to the world's aquatic life [7] various methods have been evaluated and introduced, in this article, the advantages and disadvantages of each have been evaluated separately. Concerning to Sensitivity and Specificity definition it can be described that mathematically describe the accuracy of a test which reports the presence or absence of a condition, in comparison to a 'Gold Standard'. In a diagnostic test, sensitivity is a measure of how well a test can identify true positives and specificity is a measure of how well a test can identify true negatives [8].

MATERIALS AND METHODS

OIE documents and various articles introduce valid tools and methods in the diagnosis of betanodavirus that can be used depending on the type of sample and the facilities available. In a study conducted from 2016 to 2020 to detect betanodavirus in wild and farmed fish in the Persian Gulf region, various methods were tested and evaluated, including rapid detection kit, PCR molecular methods, Nested-PCR, qPCR and the Cell Culture method [2].

RESULTS AND DISCUSSION

In this study, which performed various methods to distinguish betanodavirus from fish with clinical signs and subclinical fish, the advantages and disadvantages of each method have been evaluated and discussed.

Rapid diagnosis kit

This method is one of the fastest and most accessible methods for detecting the virus in fish. Advantages of this method: easy access, high speed in obtaining results, no need for advanced equipment and low cost, but its disadvantages include its lack of sensitivity and specificity to all viral genotypes [9].

PCR

In this method, a pair of oligonucleotide primers are used to amplify a small fragment of the infectious agent genome, and the sensitivity of the method is relatively high with detection of at least 100 to 1000 copies. The specificity of the method is also high, but both sensitivity and specificity

are improved by the Nested-PCR method. One of the most important limitations of this method is the high risk of contamination during work [10]. However, sometimes PCR is not specific enough and involves non-specific DNA fragments and therefore additional bands may occur [11]. On the other hand, some PCR results require accurate and skilful interpretation [12]. Other disadvantages are the time-consuming method [13].

Nested PCR

In this method, two replication cycles are performed with 4 primers called internal and external primers, which provide much higher sensitivity and specificity than PCR. The sensitivity of the method is usually >10 copies of the infectious agent genome, and due to the use of 4 oligonucleotides in specific binding to the target sequence, the specificity of the method is also high [14,15]. The disadvantage of this method is the possibility of contamination when transferring the first stage production to the second microtube to perform the second stage and it is time-consuming [16].

Real-time

This is a type of amplification method that during the amplification cycle, the reaction products are identified directly using a set of specific colors. This method has advantages compared to PCR or Nested-PCR methods, such as: using a pair of primers to improve the sensitivity of the method, increase speed, reduce costs and risk of contamination, do not use hazardous substances such as ethidium bromide, the possibility of a small method to detection, use of colors indicating virus replication, no need for agarose gel. Despite its advantages, there are disadvantages such as the high cost of materials and equipment and rarely primer dimer formation [17].

Cell culture

This method is considered the Gold Standard for Virus Detection by the OIE. The two cell lines used in the above method are E11 and SSN-1. This method is very sensitive in detecting the virus from asymptomatic and subclinical fish, but it is time-consuming and costly [2]. All findings in present study can be concluded in table 1.

CONCLUSION

Betanodavirus has been identified as one of the most serious threats to many marine fish species and causes great economic losses in the aquatic industry [18] and on the other hand asymptomatic infected fish as the main source of infection transmission [7]. Therefore, screening marine fish susceptible to this virus is very important. In general, among the methods used to detect the virus, it should be noted that the rapid detection kit and PCR method is the fastest and most accessible virus detection method in clinically infected fish and PCR Nested and Real-time is a useful method for virus

Table 1: Comparison of the three evaluated methods of the present study (high: +++; medium: ++; low: +).

Diagnosis Method	Detection speed	Sensitivity and specificity	Cost	Gold Standard	Choice priority
Rapid Diagnosis Kit	+++	++	+	++	++
PCR & Nested-PCR	++	++	++	++	+++
Real-time	+++	+++	+++	++	+++
Cell Culture	+	+++	+++	+++	+

detection in sub clinically infected fish (Fish are carriers). Typically, molecular methods for diagnosing viral diseases have advantages such as short time, speed, sensitivity and high specificity. In this regard, despite the appropriate method of cell culture, due to its time-consuming, less used and recently molecular methods due to speed, sensitivity and specificity are very much considered. However, virus isolation by cell culture following molecular detection is still considered the Gold Standard method [2]. It should be noted that fish play a major role in the spread of the disease to new areas, so the monitoring and care program is important [8]. On the other hand, due to the economic importance of Persian Gulf fish species and regional fisheries development programs in the field of fish farming in cages, screening of asymptomatic fish by rapid diagnostic methods as well as other accurate diagnostic methods in cases of possible infection due to control and prevention that could be supported aquatic resources in the southern waters of the country can be considered by researchers and experts in various fields of fisheries.

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