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The Current Status of Viral Nervous Necrosis Disease in Türkiye

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ABSTRACT

The agent of Viral Nervous Necrosis (VNN) disease is betanodavirus and is a viral fish disease and VNN disease has been reported in many fish species in Türkiye and around the world. It is known to cause high mortality rates in aquatic animals living in both marine and fresh water. It has been reported that the RGNNV and RGNNV/SJNNV genotypes of the virus, which has four genotypes, were detected in Turkey by the end of 2023. RGNNV genotype was detected in sea bass (*Dicentrarchus labrax*) for the first time in our country in 2011 and after that has since been found in other fish species such as sea bream (*Sparus aurata*), red mullet (*Mullus barbatus*), and garfish (*Belone belone*) in the Mediterranean region. RGNNV genotypes have also been reported in sea bass (*D. labrax*) in the Black Sea, and in sea bass (*D. labrax*) and RGNNV/SJNNV genotypes have been reported sea bream (*S. aurata*) in the Aegean Sea. In this study, studies on VNN in Turkey were reviewed and it was aimed to discuss the current status of the disease as a whole.

Keywords: Betanodavirus, Türkiye, RGNNV, RGNNV/SJNNV

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Viral Nervöz Nekrozis Hastalığının Türkiye'deki Durumu

Öz: : Etkeni betanodavirus olan Viral nervöz nekrozis (VNN) hastalığı, Türkiye'de ve dünyada birçok balık türünde bildirimi yapılmış viral balık hastalığıdır. Hem deniz hemde tatlı sularda yaşayan sucul canlılarda yüksek mortaliteye neden olmaktadır. Dört genotipi bulunan virusun, Türkiye'de 2023 yılı sonuna kadar RGNNV ve RGNNV/SJNNV genotiplerinin tespit edildiği bildirilmiştir. Ülkemizde ilk defa 2011 yılında levrek (*Dicentrarchus labrax*) balığında RGNNV genotipi tespit edilmiştir. Sonrasında Akdeniz'de levrek (*D. labrax*), çipura *(Sparus aurata),* barbun *(Mullus barbatus)* ve zarganada (*Belone belone*) RGNNV virüs bulunmuştur. Karadeniz' de levrekte *(D. labrax)* RGNNV ve Ege denizinde levrekte (*D. labrax*) RGNNV ve çipurada *(S. aurata*) RGNNV/SJNNV genotipleri bildirilmiştir. Bu çalışmada, Türkiye'de VNN konusunda yapılmış çalışmalar taranmış ve Türkiye'de hastalığın güncel durumunun bir bütün olarak ele alınması amaçlanmıştır.

Anahtar kelimeler: Betanodavirus, Türkiye, RGNNV, RGNNV/SJNNV

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Introduction

Betanodaviruses, belonging to the Nodaviridae family, are known to cause viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) (Muroga 2001). VNN is a significant viral disease that affects marine fish farming worldwide. The disease causes neuropathological effects by attacking the central nervous system and retina through nerve degeneration and cellular vacuolation. While the virus can cause disease in adult fish, it is particularly devastating in fish larvae and juveniles (Totland et al. 1999; Johansen et al. 2004). Initially, VNN was thought to be specific to marine fish, but in recent years, cases of the illness have been reported in freshwater fish (Shetty et al. 2012; Costa and Thompson 2016). The VNN virus affects both farmed and wild fish living in marine and freshwater environments, and has been reported to infect more than 173 fish species (Bandin and Souto 2020).

The four genotypes of the viral nervous necrosis virus, Striped Jack Nervous Necrosis Virus (SJNNV), the Tiger Puffer Nervous Necrosis Virus (TPNNV), the Red-spotted Grouper Nervous Necrosis Virus (RGNNV), and the Barfin Flounder Nervous Necrosis Virus (BFNNV), were classified based on the T4 variable region of the RNA2 segment (Nishizawa et al. 1995; Panzarin et al. 2016). Turbot nervous necrosis virus (TNNV) has been proposed as a potential fifth genotype, but it remains unofficially classified (Johansen et al. 2004).

Betanodavirus's segmented genome allows for reassortment, a process where genetic segments from different genotypes combine to form new viral strains. Reassortant viruses combining SJNNV and RGNNV genotypes (RGNNV/SJNNV or SJNNV/RGNNV) have been frequently reported in sea bream and sea bass (Panzarin et al. 2014; Toffan et al. 2017; Volpe et al. 2020; Kaplan et al. 2022b). VNN virus was divided into three main serotypes by neutralization tests with polyclonal antibodies; SJNNV and RGNNV/SJNNV genotypes were classified in serotype A, TPNNV genotype in serotype B and BFNNV, RGNNV and SJNNV/RGNNV genotypes in serotype C (Nishizawa et al. 1997; Mori et al. 2003; Iwamoto et al. 2004; Panzarin et al. 2016).

Clinical signs of VNN in fish include abnormal swimming behavior, lethargy, skin discoloration (darkening), anorexia, and symptoms of nervous system malfunction caused by retinal and brain injuries. Both young and adult fish are susceptible to the disease, but it progresses more slowly in older fish, leading to individual mortality, while in larvae and juveniles, it progresses rapidly and leads to cumulative mortality. Another clinical sign is hyperinflammation in the air sac of the fish (Maltese and Bovo 2007; Nopadon et al. 2009; Vendramin et al. 2013). Typical symptoms of the disease include spiral swimming, abnormal response to stimulation, spinal deformation, exophthalmos, and opacification in the eyes (Gomez et al. 2009; Nopadon et al. 2009; Vendramin et al. 2013). Clinical signs of VNN include abnormal swimming behavior, lethargy, skin discoloration (darkening), anorexia, and symptoms of nervous system malfunction brought on by retinal and brain injuries. Young fish, as well as adult fish, are more susceptible to the disease. In older fish, the disease progresses more slowly and individual mortality occurs, while in larvae and juveniles, the onset of the disease is hyperacute and cumulative mortality occurs (Vendramin et al. 2014).

In this study, researchers in Türkiye collected data on the VNN virus, including the types of fish investigated, genotypes identified, and areas studied, and summarized it as a whole.

Geographical Distribution of the Virus

Water temperature significantly influences how viruses interact with their hosts. When the water temperature is suitable, it can demonstrate its capacity to cause illness. The infectious agent's capacity to colonize the host can be altered by temperature. VNN exhibits this temperature dependence, with different genotypes thriving at specific ranges. BFNNV genotype 15-20 ºC, TPNNV genotype 20 ºC, SJNNV 20-25 ºC, RGNNV 25-30 ºC water temperatures transpire. Therefore, different VNN genotypes are found in regions with varying water temperatures (Nylund et al. 2008; Maltese and Bovo 2007).

VNN virus has a wide geographical distribution due to its different genotypes. There have been reports of RGNNV in several fish species, and it is the most prevalent genotype in temperate and tropical fish species. RGNNV is extensively dispersed in farmed fish as well as wild fish throughout the Mediterranean basin and along the coastlines of Asia and Australia, according to recent research conducted in various geographic locations (Moody et al. 2009; Ciulli et al. 2007; Gomez et al. 2010; Gomez et al. 2004; Liu et al. 2015). Tuna sturgeon (*Acipenser gueldenstaedtii*), also freshwater species in Europe, Asia and Australia (Athanassopoulou et al. 2004), Goldfish (*Carassius auratus*) (Jithendran et al. 2011), Catfish (*Tandanus tandanus*) (Munday et al. 2002), Chinese catfish (*Parasilurus asotus*) (Chi et al. 2003), pikeperch (*Sander lucioperca*) (Bovo et al. 2011) reported as the only genotype associated with outbreaks. The BFNNV genotype is restricted to cold waters, mainly Japan, America and northern Europe. BFNNV was isolated from haddock (*Melanogrammus aeglefinus*), Atlantic and Pacific cod (*Gadus macrocephalus*), barfin halibut and Atlantic halibut in farms, and Atlantic cod and different wild fish species in Scandinavian coastal waters (Nylund et al. 2008; Korsnes et al. 2017). In contrast, the TPNNV genotype appears to be a minor variant. Because it was isolated from only one species in Japan (Nishizawa et al. 1997). The SJNNV genotype was thought to be restricted to Japan, as it was isolated only in kingfish (*Pseudocaranx dentex*) and red bream (*Pagrus major*) from farmed fish in Japan. However, it has also been reported in farmed sea bream (*Sparus aurata*), rock bass (*Argyrosomus regius*) and Solea senegalensis, a flatfish, in the Iberian Peninsula of Spain (Cutrín et al. 2007; Lopez-Jimena et al. 2010).

Research Conducted in Türkiye

The first study on VNN virus in Türkiye was conducted by Özkan Özyer et al. (2012). They sampled a total of 20 hatcheries and 33 fish farms were sampled from sea bass (*Dicentrarchus labrax*)

and sea bream (*S. aurata*) farms in the Aegean region, especially during periods when the water temperature reached 25 ºC and above. The collected samples were diagnosed by virus isolation method in SNN-1 cell line, Indirect Fluorescent Antibody Test (IFAT) method and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) method. The researchers used conventional RT-PCR, which was performed using the primer set to amplify 427 bp of RNA2 (capsid protein gene), as reported by Nishizawa et al (1994). However, no positivity was obtained by all three methods (Özkan Özyer et al. 2012).

In 2011, 30 sea bass taken from a sea bass farm in Mersin province of the Mediterranean region and were tested for virüs isolation on SNN-1 cell line and CPE was observed in the 3rd passage, followed by immunofluorescent antibody test (IFAT) and RT-PCR technique. The results were positive and the first detection of VNN virus in Türkiye was reported. The primer set amplifying 427 bp of RNA2 (capsid protein gene) reported by Nishizawa et al. (1994) was used in the study. As a result of the sequencing, 99% RGNNV genotype according to polymerase protein (RNA-1) and 99% RGNNV genotype according to capsid protein (RNA-2) were determined (Özkan Özyer et al. 2014).

After the detection of the presence of the virus in 2011, the virus was detected again in fingerling sea bass and sea bream hatcheries that did not show symptoms. In 2014, the virus was detected in asymptomatic sea bass and sea bream fry, and the disease was clinically detected in fingerling-sized sea bass in a hatchery and marine facility with 5% and 10% mortality (Kalaycı et al. 2016). Until 2016, studies conducted in the Aegean and Mediterranean regions revealed the presence of VNN virus.

Since VNN virus is not a notifiable disease, there was not much data on its spread and prevalence in Türkiye (Kaplan 2019). The researchers investigated sea bass hatcheries (16) and (20) sea bass breeding facilities in Türkiye. Positivity was obtained in fingerling size fish from one hatchery. It was reported that the virus was not detected in the other fishes in the hatchery, due to the hatchery closed recirculating system, which provided the biosecurity; the positivity obtained was reported to be samples from the pools using direct seawater. Based on the RNA1 and RNA2 segments genome analysis, the positive isolate was shown to have the RGNNV genotype (Kaplan 2019). In the sea bass breeding farm, it was isolated from sea bass weighing between 3.2 and 10.4 g and 6-10 cm in length and the prevalence was reported to be 5%. The facility where it was found to be positive and the hatchery were found to be connected (Kaplan and Karaoğlu 2021a).

After the Mediterranean and Aegean regions, screening studies were conducted in the Black Sea region. In 2016, samples were collected from 6 fish farms and VNN virus positivity was obtained from one fish farming. It was determined that the virus obtained was in the RGNNV genotype and was close to the freshwater fish *Micropterus salmoides* (largemouth bass) and *S. lucioperca* in Italy. The analysis in terms of RNA2 also revealed close similarities with *D. labrax* (sea bass) in Cyprus and groupers in China, respectively. No positivity was obtained in the scans conducted in 2017-2019 (Kaplan et al. 2021b).

There are reports that molluscs may be a potential reservoir for aquatic viruses (Gomez et al. 2010; Panzarin et al. 2012). In Türkiye, between 2016 and 2020, samples were collected from carpet shell clam (*Ruditapes decussatus*) and black mussel (*Mytilus galloprovincialis*) stations in the North Aegean and Marmara Seas. Thirty carpet Shell clam and black mussels were taken from each station, and samples prepared from their hepatopancreas were tested by real Time RT-PCR, but no positivity was obtained (Kaplan et al. 2022a).

Until 2019, VNN virus was detected in all regions of the Aegean, Mediterranean and Black Sea regions and it was reported to be in the RGNNV genotype according to the sequence data but there were no reports on other genotypes. When the homogenates prepared from asymptomatic sea bream (*S. aurata*) weighing 90-100 g sampled from a fish farm in the Aegean region and samples were taken from the hatchery associated with this farm were analyzed, VNN virus was detected and it was reported to be RGNNV/SJNNV reassortant according to sequence data. In the study, RGNNV/SJNNV genotype was identified for the first time in Türkiye. While VNN virus strains detected between 2016 and 2019 were very similar among themselves, it was reported that isolates of the RGNNV/SJNNV genotype were very different genotipically (Kaplan et al. 2022b).

In addition, between 2019 and 2021, a total of 400 wild fish from 27 different species were sampled in the Mediterranean region. A high prevalence of VNN virus was detected in the garfish (*Belone belone*) sampled from Iskenderun Gulf in October 2019 and in the red mullet (*Mullus barbatus*) collected in Antalya Gulf in September 2021. According to the RNA1 segment, both isolates were identified to be RGNNV genotype, but according to the RNA2 segment, the isolate obtained from garfish was determined to be SJNNV and the isolate obtained from red mullet was determined to be RGNNV genotype. Therefore, while the isolate from garfish was reassortant RGNNV/SJNNV, the isolate from red mullet was determined as RGNNV genotype (Kaplan et al. 2023).

In a study on aquarium fish, a total of 180 fish samples were taken from koi (*Cyprinus carpio*) and

goldfish (*C. auratus*) species from 3 different aquarium farms operating in Antalya province. With this study, the presence of VNN virus in aquarium fish was investigated for the first time in Türkiye and positivity was not obtained (Oğuz et al. 2023). Information about the studies conducted in Türkiye is presented in Table 1.

Table1. Information on studies conducted in Türkiye

Fish species	Latin name	Status	Genotype	Methods	Country or Region	Year	References
Sea bass Sea bream	*Dicentrarchus labrax* *Sparus aurata*	Hatchery and Farm	Not determined	SNN-1 Cell IFAT, RT-PCR	Aegean	2010, 2011	Özkan Özyer et al. 2012
Sea bass	*Dicentrarchus labrax**	Farm	RGNNV	SNN-1 Cell , IFAT, RT-PCR	Mersin	2011	Özkan Özyer et al. 2014
Sea bass Sea bream	*Dicentrarchus labrax** *Sparus aurata**	Hatchery and Farm	RGNNV	SNN-1 Cell, IFAT, RT-PCR	Aegean	2012, 2014	Kalaycı et al. 2016
Sea bass	*Dicentrarchus labrax**	Hatchery and Farm	RGNNV	SNN-1 Cell, Real Time RT-PCR	Mediterranean and Aegean	2016,2017	Kaplan and Karaoğlu 2019
Sea bass	*Dicentrarchus labrax**	Farm	RGNNV	Real Time RT-PCR	Black Sea	2016	Kaplan et al.2021b
Carpet shell Mussel	*Ruditapes decussatus* *Mytilus galloprovincialis*	Farm	Not determined	Real Time RT-PCR	Aegean and Marmara Seas	2016, 2017, 2018, 2019,2020	Kaplan et al. 2022a
Sea bream	*Sparus aurata**	Hatchery	RGNNV/ SJNNV	Real Time RT-PCR	Aegean	2019	Kaplan et al. 2022b
Atlantic chub mackerel Lizzard fish Sea bream Bogue Goldbanded goatfish Round herring	*Scomber colias* *Saurida lessepsianus* *Sparus aurata* *Boops boops* *Upeneus moluccensis* *Etrumeus golanii*	Wild	Not determined	Real Time RT-PCR	Mediterranean	2019	Kaplan et al. 2023
Garfish Grey mullet Red mullet Annular seabream Bogue Sea bream	*Belone belone** *Mugil cephalus* *Mullus barbatus* *Diplodus annularis* *Boops boops* *Sparus aurata*	Wild	RGNNV Not determined	Real Time RT-PCR	Mediterranean	2020	Kaplan et al. 2023
Sea bass Sardine Common sole Lizzard fish	*Dicentrarchus labrax* *Sardinella aurita* *Solea solea* *Saurida lessepsianus*	Wild	Not determined	Real Time RT-PCR	Mediterranean	2020	Kaplan et al. 2023
Forkbeard Annular Seabream Meagre	*Phycis phycis* *Diplodus annularis* *Argyrosomus regius*	Wild	Not determined	Real Time RT-PCR	Mediterranean	2021	Kaplan et al. 2023

table-1 continue							
Zebrafish	*Diplodus cervinus*						
Saddled Seabream	*Oblada melanura*						
Salema	*Sarpa salpa*						
Common pandora	*Pagellus erythrinus*						
Yellow mouth barracuda	*Sphyraena viridensis*						
Mediterranean Horse mackerel	*Trachurus mediterraneus*	Wild	Not determined	Real Time RT-PCR	Mediterranean	2021	Kaplan et al. 2023
Red Sea goatfish	*Parupeneus forsskali*						
Crocodile toothfishes	*Champsodon nudivitis*						
Threadfin breams	*Nemipterus randalli*						
Slender rainbow sardine	*Dussumieria elopsoides*						
Morocco dentex	*Dentex moraccanus*						
Red cornetfish	*Fistularia petimba*						
Sardine	*Sardinella aurita*						
Lizzard fish	*Saurida lessepsianus*						
Red mullet	*Mullus barbatus**		RGNNV				
Common carp	*Cyprinus carpio*	Aquarium	Not determined	Real Time RT-PCR	Antalya	2023	Oğuz et al. 2023
Goldfish	*Carassius auratus*						

*VNN detected species.

Discussion and Conclusion

VNN virus has been detected in more than 173 species and has a very wide distribution. Approximately 90% of the detected VNN virus was genotyped and 86% of them were found to have the RGNNV genotype, 5.5% had the SJNNV genotype, while only 1.8% of VNN positive species had the BFNNV genotype (Bandin and Souto 2020). In Türkiye, RGNNV genotype was detected for the first time in 2011 and all detections made in the following years were of the same genotype (Özkan Özyer et al. 2014; Kalaycı et al. 2016; Kaplan 2019, 2021a; Kaplan et al. 2022b, 2023). Only in 2019, RGNNV/SJNNV was reported to be reassortant in a sample taken from a sea bream farm in the Aegean Sea and in garfish sampled from the Mediterranean Sea (Kaplan et al. 2022b, 2023).

The high prevalence of the RGNNV genotype in Turkish waters may be attributed to the warmer average sea temperatures compared to other regions. These temperatures might not be suitable for the survival or establishment of other VNN genotypes.

It is also commonly recognized that three distinct factors including the environment, host, and pathogen can affect an epidemic of a disease (Snieszko 1973). Recognizing that several environmental elements, such as stress, stocking density, and temperature, are predisposing factors for VNN epidemics is also important (Ma et al. 2015).

It has been shown that there is both horizontal and vertical transmission in various fish species. In studies conducted on Asian perch or barramundi (*Lates calcarifer*), European perch (*D. labrax*), bream (*S. aurata*), brown-marbled grouper (*Epinephelus fuscoguttatus*) and Senegal flounder, there was horizontal transmission from fish to fish or by water to fish. (Péducasse et al. 1999; Aranguren et al. 2002; Manin and Ransangan 2011; Hick et al. 2011; Souto et al. 2015). Vertical transmission has been reported in fish such as European sea bass (*D. labrax*), Asian sea bass (*L. calcarifer*) (Breuil et al. 2002; Azad et al. 2006). This occurs through viral shedding in the gonads, leading to infected eggs and seminal fluids (Valero et al. 2015; Nishizawa et al. 1994).

In studies conducted in Türkiye, it has been reported that horizontal transmission from water is the dominant mode. No detection of the virus in fish of different sizes from hatcheries with biosecurity measures supports this. However, the virus was found in areas that come into contact with seawater (Kaplan 2019; Kaplan and Karaoğlu 2021a; Kaplan et al. 2022b, 2023). On the other hand, there is no data or study on vertical transmission of the VNN. It has been reported that there have been significant increases in the number of Indo-Pacific fish through the Suez Canal, Atlantic fish through the Strait of Gibraltar, and species that find a chance to live in the Mediterranean through ship ballast water through both canals (Çınar and Bilecenoğlu 2015). In 2015, the Suez Canal was greatly expanded with the construction of a new canal, which was predicted to facilitate the introduction of species into the

Mediterranean. The Suez Canal is thought to be the entry point for 443 species of fish, macrophytes, and invertebrates into the Mediterranean, of which 89 have been documented in five or more nations (Galil et al. 2015). It has been reported that one of the first colonization areas of exotic species was the coasts of Türkiye due to its geographical location, and in the list reported in 2020, a total of 539 wild species were found in the seas of Türkiye, 404 of which were resident, while 105 species were invasive (Çınar et al. 2021).

Analysis of data from the General Directorate of Meteorology revealed significant differences in average seawater temperatures between 2010 and 2019 compared to 1970-1979. The Black Sea and Mediterranean Sea experienced the highest increase, with an average rise of 1.2 ºC. The Aegean and Marmara Seas saw increases of 0.9 ºC and 1.5 ºC, respectively (Kalıpcı et al. 2021). Warmer sea temperatures can create suitable habitats for certain non-native species, potentially facilitating their spread into the Mediterranean, Aegean, Marmara, and Black Seas. This trend aligns with reports of rapid population increases for species like pufferfish and lionfish, which were previously confined to the Mediterranean but have recently become more frequent in the Aegean (Kalıpcı et al. 2021).

It is believed that the rising temperatures of seawater in Türkiye may be caused by climate change. This, combined with the increased migration of fish through the Suez Canal and Strait of Gibraltar, could be contributing to the emergence of VNN in Turkish waters. The hypothesis is supported by the fact that VNN isolates found in Türkiye share a phylogenetic similarity with those isolated from Taiwan and Singapore, where VNN has previously occurred.

Encompassed by three seas and boasting abundant water resources (DSİ 2010), Türkiye heavily relies on a thriving aquaculture industry and fisheries sector. Fish health plays a critical role in ensuring the sustainability and economic viability of these sectors (Dönmez and Yılmaz 2018). Fish diseases pose a significant threat, particularly in aquaculture settings. They can hinder the sustainability of both aquaculture enterprises and natural fish populations. Viral diseases are one major category of concern (Dönmez and Yılmaz 2018).

As a result, all researchers emphasize that fish farms should take biosecurity measures, have their broodstock screened for diseases, disinfection of fertilized eggs with ozone or electrolyzed sea water, disinfection of the tools and equipment used in the farms, and the importance of vaccination practices. Although VNN is not classified as a notifiable disease in Türkiye, it poses a significant economic threat. Due to the lack of tracking, it is difficult to determine the current prevalence. Conducting extensive screening studies and research, particularly in freshwater ecosystems, is essential in mitigating the impact of this disease.

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