A Serological Survey on Infectious Pancreatic Necrosis Virus (IPNV), Viral Hemorrhagic Septicemia Virus (VHSV) and Infectious Hematopoietic Necrosis Virus (IHNV) from Rainbow Trout in Turkey

Cüneyt Tamer[1], Yüksel Durmaz[3], Hasan Sercan Palancı[1], Emre Özan[2], Hamza Kadı[3], Zafer Yazıcı[1], Harun Albayrak[1]

[1]Ondokuz Mayıs University, Veterinary Faculty, Department of Virology, Samsun, Turkey
[2]Ondokuz Mayıs University, Veterinary Faculty, Department of Veterinary Experimental Animals, Samsun, Turkey
[3]Samsun Veterinary Control Research Institute, Samsun, Turkey

Geliş Tarihi / Received: 23.08.2019, **Kabul Tarihi** / Accepted: 10.10.2019

Abstract: Infectious pancreatic necrosis (IPN), viral hemorrhagic septicemia (VHS) and infectious hematopoietic necrosis (IHN) are the most significant viral diseases of salmonid species. This study examined the seroprevalence of infectious pancreatic necrosis virus (IPNV), viral hemorrhagic septicemia virus (VHSV) and infectious hematopoietic necrosis virus (IHNV) in rainbow trout cultured in Turkey. A total of 597 serum samples of adult trout were obtained from 18 commercial trout farms in the Middle and Eastern Black Sea regions of Turkey and were then examined by the virus neutralization test. As a result of the test, VHSV and IHNV antibodies were not detected. However, IPNV antibodies were found in 11 of 18 trout farms (61.1%) and 45 of 597 serum samples (7.5%). Three viral agents characterized as causing persistent infections were serologically screened for the first time in Turkey. The high seropositivity rate against IPNV was namely caused by asymptomatic carrier broodstock fish.

Key words: IHN, IPN, Serology, Trout, VHS.

Türkiye'deki Gökkuşağı Alabalıklarında Enfeksiyöz Pankreas Nekrozu Virüsü (IPNV), Viral Hemorajik Septisemi Virüsü (VHSV) ve Enfeksiyöz Hematopoetik Nekroz Virüsü'nün (IHNV) Serolojik Olarak Araştırılması

Özet: Enfeksiyöz pankreas nekrozu (IPN), viral hemorajik septisemi (VHS) ve enfeksiyöz hematopoetik nekroz (IHN), salmonid türlerinin en önemli viral hastalıklarıdır. Bu çalışmada, Türkiye'de yetiştirilen gökkuşağı alabalıklarında enfeksiyöz pankreas nekrozu virüsü (IPNV), viral hemorajik septisemi virüsü (VHSV) ve enfeksiyöz hematopoetik nekroz virüsü (IHNV) seroprevalansı incelenmiştir. Türkiye'nin Orta ve Doğu Karadeniz bölgelerinde bulunan 18 ticari alabalık çiftliğinden toplam 597 adet alabalığı kan serumu örneği alınmış ve daha sonra virüs nötralizasyon testi ile incelenmiştir. Test sonucunda VHSV ve IHNV antikorları tespit edilmedi. Ancak, IPNV antikorları 18 alabalık çiftliğinin 11'inde (%61,1) ve 597 serum numunesinin 45'inde (%7,5) bulundu. Türkiye'de kalıcı enfeksiyonlara neden olan üç viral ajan serolojik olarak tarandı. IPNV'ye karşı yüksek seropozitiflik oranı, yani asempomatik taşıyıcı yavru balıklarından kaynaklanmıştır.

Anahtar kelimeler: Alabalık, IHN, IPN, Seroloji, VHS.

Introduction

Intensively culturing fish in a high population density increases the incidence of infections and thus facilitates the spreading, settlement and longer duration of diseases. Also, infectious diseases are held responsible for economic loses that affect the development of aquaculture. Infectious pancreatic necrosis (IPN), viral hemorrhagic septicemia (VHS) and infectious hematopoietic necrosis (IHN) are the most significant viral diseases of salmonid species (Crane and Hyatt, 2011). Infections are typically spread internationally by the transportation of hard roe and fry fish, and by the migration of anadrom fishes (Albayrak and Özan 2010).

In Turkey, the fishery sector has developed rapidly and has led to the emergence of many industrial areas. A total of 537,345 tons of aquatic products have been produced in our country as of 2014 and 43.7% of this production is from aquaculture. 48.3% (113.593 thousand tons) of aquaculture production is trout production (GTHB, 2016). The fishery industry has become a significant industry

Yazışma adresi / Correspondence: *Cüneyt Tamer (ORCID: 0000-0003-3240-8425), Ondokuz Mayıs Universtiy, Faculty of Veterinary Medicine, Virology Depart-ment, Atakum Samsun/Turkey E-mail: cuneyt_tamer@hotmail.com*

in Turkey and continues to grow. Aquaculture production comprised 43.7% of total fishery production in 2014. Also, trout aquaculture comprised 48.3% of aquaculture production (Gürcay et al., 2013). The amount of research on viral infections and their effect on economic loss in the trout farming in Turkey, is quite scarce. Although the presence of IHNV and VHSV was reported (Değirmenci et al. 2008; Gürcay et al. 2013; Işıdan and Bolat 2011; Işıdan and Kutlu 2014; Kalaycı et al. 2006), there is no research which provides evidence of antibodies against these viruses.

IPNV belongs to the *Aquabirnavirus* genus of the *Birnaviridae* family. IPNV has icosahedral symmetry and is an enveloped virus having two segments and double-stranded RNA. IPNV has two segments which are called segment A, which has 2,5 kb RNA, and segment B, which has 2,3 kb RNA (Albayrak and Özan 2010).

VHS has wide host range. This virus is isolated from different regions of the world and causes economic losses worldwide (Ammayappan et al., 2010, Rexhepi et al., 2009). *Piscine novirhabdovirus* is an enveloped virus which has non-segmented RNA. VHS is caused by *Piscine novirhabdovirus* of the *Novirhabdovirus* genus of the *Rhabdoviridae* family (Einer-Jensen et al. 2014; ICTV. 2016; Işıdan and Kutlu 2014; Kima et al. 2015).

IHN, which is characterized as part of the *Salmonid novirhabdovirus* species of the *Novirhabdovirus* genus of *Rhabdoviridae* family, causes high mortality and acute-systemic infection in fish. The *Salmonid novirhabdovirus* genome contains approximately 11 kb and single-stranded RNA. According to filogenetic analysis results, *Salmonid novirhabdovirus* is divided into five big genogroups: U, M, L, E and J (Ammayappan et al. 2010; ICTV. 2016; Jia et al. 2014, Nishizawa et al. 2006).

These serological assays are important for the detection of asymptomatic transporters because of the persistence of these three viruses (Albayrak and Özan 2010; Candan 2002).

Material and Methods

Serum samples

Fishes collected the serum samples was aproximately 200-250 grams rainbow trout from 18 different commercial trout farms in the Middle and Eastern Black Sea regions of Turkey. Serum samples were collected from trout farms in Samsun, Ordu, Tokat, Giresun, Trabzon and Rize provinces between 2011 and 2012. Each sample (597) was inactivated at 42°C for 30 minutes and was stored at -20°C.

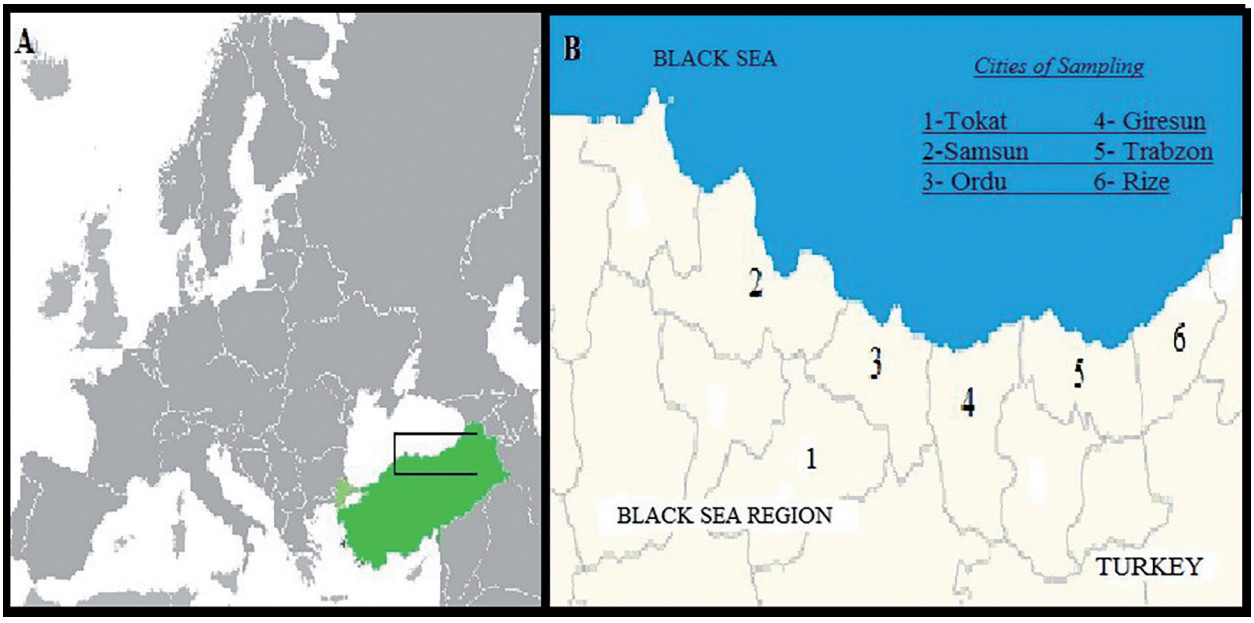

Figure 1. Areas in the Middle and Eastern Black Sea where rainbow trout were collected for viral examination

Cell Lines and Virus Isolates

IPN 1054 (sp serotype), VHS Bolu (Ie genotype) and IHN-ref virus (Albayrak and Özan 2010); Albayrak et al. 2018), which were recieved from the Samsun Veterinary Control Institute, were used as assays. The RTG-2 cell line was used to produce the viruses and as the neutralization assay. A Leibovitz's L-15 medium which contained 1% penisilin (10,000 U/ml) - streptomycin (10 mg/ml) - amphotericin B (0.025 mg/ml), 1,5 mM hepes and 10% fetal calf serum was used to produce the cell line and viruses.

Virus Titration Assay

Titration was conducted by the microtitration method in 96 well-plates. The serum was diluted from 10^{-1} to 10^{-12} and four wells were used for each dilution, having a total volume of 100 µl. 50 µl of cell suspension which contained 300,000 cells in one milliliter was added into each well and the plates were incubated at 15°C. The cytopathic effect was checked daily. After 7 days of incubation at 15°C, the $TCID_{50}$ value was calculated according to Reed and Muench (1938). This method was conducted three times for each virus (Darling et al., 1998).

Virus Neutralization Assay

Trout serums were diluted at 1/10 and samples were put in two wells for each serum at 50 µl. 50 µl virus solutions of 100 $TCID_{50}$ ml^{-1} were added into the wells. After that 50 µl the cell solution containing 300,000 cells in one mililiter was added into each well and plates were incubated at 15°C for 7 days. This method was done three times for each virus (Albayrak and Özan 2010).

Serum Neutralization Assay

Positive samples obtained according to the virus notralization assay results were diluted as log_2 in 96 well plates. Virus solutions of 100 $TCID_{50}$ ml^{-1} were added into the wells. Cell solutions containing 300,000 cells in one mililiter, were added into solutions and were incubated at the 15°C incubator. ND_{50} rate was calculated after seven days of incubation (Reed and Muench 1938).

Results

For IPNV, 45 samples (7.5%) of 597 samples were detected as having antibodies against IPNV (Table 1). All of the 597 samples were detected as having no antibodies against VHSV and IHNV. Antibodies against IPNV were detected in 11 (61.1%) of 18 enterprises in 6 cities. Evaluation results and ND_{50} values are shown in Table 2.

Table 1. Seropositivity disturbition of IPN virus according to provinces

Provincies	Total positives in enterprises	Total Samples	Positives Samples	Prevalence (%)
Samsun	2 / 2	97	16	16.4
Ordu	4 / 2	110	2	1.8
Tokat	3 / 2	112	2	1.7
Giresun	3 / 2	102	14	13.7
Trabzon	3 / 2	90	9	10
Rize	3 / 1	86	2	2.3
Total	18 / 11	597	45	7.5

Table 2. ND_{50} assessment results according to the provinces of serums

Provincies	Dilutions Rate of Serums between ½ to 1/256							
	1/2-1/4	1/4-1/8	1/8-1/16	1/16-1/32	1/32-1/64	1/64-1/128	1/128-1/256	1/256 and above
Samsun				1		1	1	13
Ordu								2
Tokat								2
Giresun			1			2	2	9
Trabzon					3			6
Rize								2
Total			1	1	3	3	3	34

Discussion

VHS is the most important viral disease among trouts in Europe. VHSV was also reported in various sea fish in North America and Europe (Hedrick et al. 2003). In Turkey, the first report and isolation of VHSV was made from 20-25 day-old turbot at the Black Sea Aquaculture Research Center in 2004, and the calculated mortality rate was 99%. VHSV was firstly isolated in trout in 2006 at Mudurnu, Bolu with a 90% mortality rate (Kalaycı et al. 2012). Although VHSV was reported as en-

demic in the Black Sea region (Nishizawa et al. 2006b), VHSV was not detected in other research conducted between 2007 and 2009 (Işıdan and Bolat 2011). However, antibodies against VHSV in aquaculture facilities in the same region were not detected in this study. Consequently, VHSV, which was reported as endemic in turbot fish in the Black Sea region, was not detected in rainbow trout in the same region.

Although presence of IHNV was reported in various regions of the world including North America, Europe, Australia and East Asia, IHNV has not been reported in Turkey (Albayrak and Özan 2010). In this study, antibodies against IHNV were not detected as previously reported (Albayrak and Özan 2010).

In Turkey, IPNV was first reported in 2002 in trout (Candan 2002). IPNV was transmitted via water to other trout enterprises which contained the same water source. It was transmitted through eggs and fry to other trout enterprises which used different water sources (Akhlaghi and Hosseini 2007; Albayrak and Özan 2010; Gurcay et al. 2013).

In another study, IPNV and IHNV were investigated by Polymerase Chain Reaction (PCR) in 32 rainbow trout enterprises in the Middle and Eastern Black Sea regions. IHNV was not detected but IPNV was detected at 10 rainbow trout farms and the prevalence of IPNV infection was determined as 44% (Albayrak and Özan 2010). IPNV was isolated from 26 (10.69%) of 243 isolation materials in various regions including the Central Anatolian, Eastern, Southeast and Mediterranean regions of Turkey. In this study, IPNV contamination was detected in each city that was sampled. Seroposivity against IPNV was detected in 11 enterprises (61.1%) of 18 enterprises. In addition, IPNV was detected in 15 samples (7.5%) of 597 serum samples. The findings obtained in this study are the same as other research in Turkey.

The prevalence of IPNV was 11% in another study and 70% of the sampled trout facilities was contaminated with IPNV in Kosovo (Rexhepi et al. 2009). In this study, the prevalence of IPNV was found to be 16.4% in Samsun, 13.7% in Giresun, 10% in Trabzon, 2.3% in Rize, 1.8% in Ordu and 1.7% in Tokat provinces. As a result of serum neutralization tests, antibody titers were calculated 1/8 - 1/16 in 1 enterprise, 1/16 - 1/32 in 1 enterprise, 1/32 - 1/64, 1/64 - 1/128, 1/128 - 1/256 in 3 enterprises, antibody titers of 1/256 and above were detected at 34 enterprises. Thus, IPNV is endemic in the Black Sea Region and should not be ignored.

Consequently, commercial trout farms in Turkey are highly infected with IPNV, although they are not infected with VHSV and IHNV. Therefore, economic losses are likely to occur, especially in trout breeding. It is important to identify infected broodstocks, which are the main source of control, in the spread of the disease and remove them from enterprises. Controlled production with healthy broodstocks will prevent the loss of juvenile fish, making it possible for farmers to eradicate IPNV contamination.

References

1. Akhlaghi M, Hosseini A. (2007). First report on the detection of infectious pancreatic necrosis virus (IPNV) by RT-PCR in rainbow trout fry cultured in Iran. *Bull Euro Ass Fish Pathol.* 27(5), 205.
2. Albayrak H, Özan E. (2010). Gökkuşağı alabalıklarında (*Oncorhynchus mykiss* Walbaum, 1792) infeksiyöz pankreatik nekrozis ve infeksiyöz hematopoietik nekrozis virus enfeksiyonlarının varlığının araştırılması. *Ankara Üniv Vet Fak Derg.* 57, 125-129.
3. Albayrak H, Isidan H, Kalayci G, Ozan E, Vakharia VN. (2018). Genetic analysis of the complete G gene of viral hemorrhagic septicemia virus (VHSV) genotype Ie isolates from Turkey. *Iran J Fish Sci.* 17(1), 67-73.
4. Ammayappan A, LaPatra ES, Vakharia NV. (2010). A vaccinia-virus-free reverse genetics system for infectious hematopoietic necrosis virus. *J Virol Methods.* 167, 132-139.
5. Ammayappan A, Kurath G, Thompson MT, Vakharia NV. (2011). A reverse genetics system for the Great Lakes strain of viral hemorrhagic septicemia virus: the NV gene is required for pathogenicity. *J Mar Biotechnol.* 13, 672–683.
6. Candan A. (2002). First report on the diagnosis of infectious pancreatic necrosis (IPN) based on reverse transcription polymerase chain reaction (RT-PCR) in Turkey. *Bull Euro Ass Fish Pathol.* 22(1), 45-48.
7. Crane M, Hyatt A. (2011). Viruses of Fish: An overview of significant pathogens. *Viruses.* 3, 2025-2046.
8. Darling JA, Boose AJ, Spaltro J. (1998). Virus assay methods: Accuracy and validation. *Biologicals,* 26, 105-110.
9. Değirmenci U, Nemli E, Çağırgan H. (2008). Türkiye'nin değişik bölgelerinden infeksiyöz pankreatik necrozis virusu izolasyonu. I. Ulusal Alabalık Sempozyumu. Turkey. Isparta. November.

10. Einer-Jensen K, Harmache A, Biacchesi S, Bremont M, Stegmann A, Lorenzen N. (2014). High virulence differences among phylogenetically distinct isolates of the fish rhabdovirus viral hemorrhagic septicaemia virus are not explained by variability of the surface glycoprotein G or the nonvirion protein Nv. *J Gen Virol.* 95, 307-316.

11. Gurcay M, Turan T, Parmaksız A. (2013). Türkiye'de kültürü yapılan gökkuşağı alabalıklarında (*Oncorhynchus mykiss Walbaum*, 1792) infeksiyöz pankreatik nekrozis virus varlığının tespiti üzerine bir araştırma, *Kafkas Universitesi Veteriner Fakültesi Dergisi,* 19(1), 141-146.

12. Gıda Tarım ve Hayvancılık Bakanlığı (GTHB). 2016. Su ürünleri istatistikleri 2016 yılı raporu, 1-12.

13. Hedrick RP, Batts WN, Yun S, Traxler GS, Kaufman J. Winton JR. (2003). Host and geographic range extensions of the North American strain of viral hemorrhagic septicemia virus. *Dis Aquat Organ.* 55. 211–220.

14. ICTV. (2016). ICTV Master Species List 2016. Retrieved from https://talk.ictvonline.org/files/master-species-lists/m/msl/6776.

15. Işıdan H, Bolat Y. (2011). A survey of viral hemorrhagic septicemia (VHS) in Turkey. *Turk J Fish Aquat Sc.* 11, 507-513.

16. Işıdan H, Kutlu I. (2014). Viral hemorajik septisemi virüs genotip 1e suşlarının çipura (*Sparus Aurata*) ve levrek (*Dicentrarchus Labrax*) balıkları üzerinde patojenitelerinin belirlenmesi. *Yunus Araştırma Bülteni,* 2, 49-53.

17. Jia P, Zheng CX, Shi JX, Shi-Fu KD, Jin-Jin WB, Jun-Qiang HE, Ning-Yi J. (2014). Determination of the complete genome sequence of infectious hematopoietic necrosis virus (IHNV) Ch20101008 and viral molecular evolution in China. *Infection, Genetics and Evolution,* 27, 418–431.

18. Kalaycı G, Incoglu S, Ozkan B. (2006). First isolation of viral haemorrhagic septicaemia (VHS) virüs from turbot (*Scophthalmus maximus*) cultured in the Trabzon coastal area of the Black Sea in Turkey. *Bull Euro Ass Fish Pathol.* 26, 157-161.

19. Kalaycı G, Inçoğlu Ş, Özyer ÖB, Kucukali Y. (2012). Türkiye'de infeksiyöz pankreatik nekrozis ve viral hemorajik septisemi hastalıklarının durumu. *J of Bornova Vet Cont Res Inst.* 34(48), 31-38.

20. Kima HS, Yusuff S, Vakharia NV, Evensen O. (2015). Interchange of L polymerase protein between two strains of viral hemorrhagic septicemia virus (VHSV) genotype IV alters temperature sensitivities in vitro. *Virus Research*. 195, 203–206.

21. Nishizawa T, Kinoshita S, Kim WS, Higashi S. Yoshimizu M. (2006). Nucleotide diversity of Japanese isolates of infectious hematopoietic necrosis virus (IHNV) based on the glycoprotein gene. *Dis Aquat Organ.* 71, 267–272.

22. Nishizawa T, Savaş H, Işıdan H, Üstündağ C, Iwamoto H. Yoshimizu M. (2006). Genotyping and pathogenicity of viral hemorrhagic septicemia virus from free-living turbot (*Psetta maxima*) in a Turkish Coastal Area of the Black Sea. *Appl Environ Microbiol.* 72, 2373-2378.

23. Reed LS, Muench H. (1938). A simple method of estimating fifty percent endpoints. *J Tro Med.* 27, 493-497

24. Rexhepi A, Scheinert P, Bërxholli K, Hamidi A, Sherifi K. (2009). Occurrence of infectious pancreatic necrosis virus (IPNV) in farmed rainbow trout (*Onchorhynchus mykiss*) in Kosovo. *Veterinaria.* 58(1-2), 47-53.