Numerical findings on crucial viral pathogens in Rainbow trout (*Onchorhynchus mykiss*) farms of northern Iran

M Khosravi[1*], P Shohreh[2], R Khoshbakht[1]

[1]Department of Pathobiology, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran

[2]Department of Clinical Science, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran

Received: September 2019 **Accepted:** November 2019

Abstract

Viral hemorrhagic septicemia virus (VHSV), Infectious hematopoietic necrosis virus (IHNV), and infectious pancreatic necrosis virus (IPNV) are documented as the most considerable viral pathogens in Rainbow trout (*Onchorhynchus mykiss*). This study aimed to evaluate the frequency of these pathogens in 65 farms with suspected clinical signs in northern Iran from March 2016 to February 2018. Logistic regression analysis used to assess the effect of several determinant factors on the occurrence of these pathogens. In total, 19 (29.23%) farms were positive by a reverse transcription-polymerase chain reaction (RT-PCR). The frequency of VHSV, IHNV, and IPNV was reported 18.5%, 6.2%, and 4.61%, respectively. The most affected farms (78.95%) used river water. Furthermore, our results revealed that using river water raised the chances of viral disease by 5 times (OR = 5.02; P = 0.01). Thus, using river water was a risk factor for the occurrence of viral pathogens. From four weight groups (A to D); fishes in groups A (fish < 1 gr) and B (1 to 20 gr) were more affected but not statistically significant (P>0.05). This study has provided insight into the frequency of these targeted viruses. Collectively, establishing routine rapid diagnostic programs and setting up basic educational practices can be valuable to design the prevention and control strategies.

Keywords: Rainbow trout, Viral hemorrhagic septicemia virus, Infectious Hematopoietic necrosis virus, Infectious pancreatic necrosis virus, Frequency

Introduction

From the middle of the 1900s, viral pathogens have been considered as one of the most severe threats for the aquaculture industry and causes significant economic losses in trout producing farms (Crane & Hyatt 2011; OIE 2018; Rasmussen 1965). Two Fish Rhabdoviridae

*Correspondence M Khosravi, Department of Pathobiology, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran (e-mail: mojtaba.kh.dvm@gmail.com).

[DOR: 20.1001.1.2345315.2019.5.2.6.9]

[DOI: 10.29252/ijaah.5.2.22]

viruses, Viral hemorrhagic septicemia virus (VHSV) and Infectious hematopoietic necrosis virus (IHNV), and one Birnaviridae virus, Infectious pancreatic necrosis virus (IPNV), are considered as the most serious viral agents of wild and farmed Rainbow trout (*Oncorhynchus mykiss*). (Bootland & Leong 2009; OIE 2019; Dobos 1995).

They are responsible for outbreaks with mass mortality in Rainbow trout (*Oncorhynchus mykiss*) farms in the world and Iran (Bootland & Leong 2009; OIE 2019; Enzmann, Castric, Bovo, Thiery, Fichtner, Schütze & Wahli 2010; Meyers & Winton 1995; Guerrero, Herrera, Salinas, Torres, Montero & Barrón 2008; Soltani, Rouholahi, Zargar, Abdi, Mohamadian & Ghajari 2014; Soltani, Rouholahi, Ebrahimzadeh Mousavi, Abdi, Zargar & Mohamadian 2014; Haghighi Khiabanian asl, Bandehpour, Sharifnia & Kazemi 2008; Fallahi, Soltani, Karegar, Zorriehzahra, Shchelkunov, Hemmatzadeh & Nouri 2003). VHS and IHN diseases can occur at any stage of fish life, but it seems that juvenile fishes are most susceptible and the viruses may cause losses in several percent in large fish to 100% in fry (OIE 2019; LaPatra 1998; Smail & Snow 2011). Rainbow trout fries are more susceptible to IPNV and it may induce mortalities as high as 90% in them (Crane & Hyatt 2011). Horizontal transmission largely has been described for VHSV and IHNV, while in case of IPNV vertical route has been proved as a major route (Crane & Hyatt 2011; Bootland, Dobos & Stevenson 1991; Mutoloki, Jøssund, Ritchie, Munang'andu & Evensen 2016). VHS disease was first described in German Rainbow trout farms as early as 1938 (Schäperclaus 1938). Latter, VHSV has isolated over 80 marine and freshwater fishes and showed the ability and high intensity of virus to invade new hosts (OIE 2018; Enzmann et al. 2010; Gagné, Mackinnon, Boston, Souter, Cook-Versloot, Griffiths & Olivier 2007). Another Rhabdovirus (IHNV) was first recognized from western North America and the latter disease expand to other geographical regions (OIE 2019; Dixon, Paley, Alegria-Moran & Oidtmann 2016). In North America in the 1950's Wood and colleagues reported the first isolation of IPN disease in freshwater trout (Wood, Snieszko & Yasutake 1995). Then, IPNV has been isolated in Europe since the early 1970's (Ball, Munro, Ellia, Elson, Hodgkiss & McFarlane 1971). It seems that these viral pathogens are endemic in many countries in North America, Asia, and Europe such as France, Italy, Switzerland, UK, Scotland, Germany, Japan, South Korea, Mexico, and Canada (OIE 2019; Enzmann et al. 2010; Meyers & Winton 1995; Roberts & Pearson 2005; Stone, Ferguson, Tyson, Savage, Wood, Dodge, Woolford, Dixon, Feist & Way 2008; Guerrero et al. 2008; Bain, Gregory & Raynard 2008; Panzarin , Holmes , Abbadi , Zamperin, Quartesan, Milani, Schivo, Bille, Pozza, Monne & Toffan 2018; Abbadi, Fusaro , Ceolin , Casarotto , Quartesan , Dalla Pozza , Cattoli, Toffan, Holmes & Panzarin 2016; Cieslak, Mikkelsen, Skall, Baud, Diserens, Engelsma, Haenen, Mousakhani, Panzarin, Wahli, Olesen & Schütze 2016). Until the middle of the 2000s, there were not any data about the detection of these viral agents in

[DOR: 20.1001.1.2345315.2019.5.2.6.9]
[DOI: 10.29252/ijaah.5.2.22]

salmonid farms of Iran. Later, the detection of all these mentioned viruses was confirmed in various parts of Iran (Akhlaghi & Hosseini 2007; Fallahi et al. 2003; Haghighi Khiabanian asl et al. 2008).

Applying extensive surveillance and major efforts to prevent the occurrence of a new outbreak has caused a massive reduction of new incidents associated with these pathogens in Europe. For example, in 2014, only one farm in Italy (of 901) and 28 farms in Slovenia (of 321) were infected with IHNV (Dixon et al. 2016).

There are many Rainbow trout farms in Iran. In the year 2017, from 300 thousand tons of total aquaculture roughly 160 thousand tons was regarding *Oncorhynchus mykiss* (IFO 2018; Zorriehzahra, Kakoolaki, Mehrabi, Sepahdari, Ghasemi, Yarmohammadi & Ghiasi 2018). According to the Iranian Fisheries Organization (IFO), the northern part of the country hosts many commercial Rainbow trout farms. Proper geographical conditions including freshwater sources and climate conditions have prepared good opportunities to establish and extend trout husbandry (Soltani et al. 2014; Fattahi, Akhlaghi, Mohammadi, Soltanian, Shahbazian 2019; IFO 2018).

Regarding the highest rate of infectivity, mortality, simple transmission routes as well as, economic impacts of these causative agents, the prevalence, incidence, and distribution of these selected agents should have been considered annually. Little information has been presented about these viral pathogens distribution in different regions of northern Iran. This study was done to determine the greater details about the frequency of mentioned viral agents in trout farms, suffering from clinical manifestations of these mentioned diseases, in this part of the country. In addition, we assessed several determinant factors on the occurrence of these pathogens.

Materials and Methods

Sampling

This cross-sectional study was conducted to determine the frequency of IHNV, VHSV, and IPNV in some Rainbow trout farms located in the north of Iran for two years from March 2016 to February 2018. Samples were selected from moribund fish of 65 farms in various areas. The sampling area was divided into six stations. From each farm, fishes with clinical signs associated with these target pathogens including exophthalmia, petechial hemorrhage around the eyes, intestine, and muscles, and swollen abdomen were chosen. The month of sampling and water source and temperature of all studied farms were considered. According to the weight, all samples were categorized into 4 groups, including group A (fish < 1 gr), group B (fish with 1 to 20 gr), group C (20 < fish < 40 gr), and group D (40 < fish < 300 gr). All samples were kept at -70^{o}C as soon as possible after capture.

RNA extraction from organ samples

The target organs of each fish (spleen, kidney, gills, brain, and muscle) were aseptically pooled and submerged in 10% phosphate-buffered saline (PBS) with pH 7.4. (OIE 2019; Brudeseth, Castric & Evensen 2002). Viral RNA was extracted with Viral Gene-spin™ Viral DNA/RNA Extraction Kit (iNTRON,

[DOR: 20.1001.1.2345315.2019.5.2.6.9]
[DOI: 10.29252/ijaah.5.2.22]

South Korea) according to the manufacturer's instructions. Approximately, 25 to 35 mg of pooled samples were homogenized in the presence of lysis buffer and then incubated at 55^{o}C for 10 min. Total RNA was eluted in 50 ul of elution buffer and stored at -80 °C until used.

Reverse transcription and PCR amplification (RT-PCR)

Synthesis of first strand cDNA was performed by using the Maxime™ RT PreMix kit (iNTRON, South Korea) according to the manufacturer's instructions: briefly, 5 µl total RNA was added to a lyophilized tube containing random hexamer primers and Reverse Transcriptase M-MuLV enzyme. The thermal program was applied based on company instruction. In the next step for tracing and detecting of IHNV, VHSV, and IPNV, a set of primers were served (Table 1). PCR amplification was conducted in final volume of 25 ul consisting 3 ul of cDNA, 20 µM of each primer, 0.2 mM each deoxynucleoside triphosphate (Sinaclone, Iran), 1.5 mM magnesium chloride (Sinaclone, Iran), 2.5 µl of reaction mix (10X), 2.5 Uof Taq polymerase (Sinaclone, Iran) and distilled water. All PCR reactions were carried out by a thermal cycler (Biorad, United States) machine. PCR profile procedure was performed using the following conditions: an initial denaturation step at 94 °C for 4 min, followed by 35 cycles consisting of 40 s at 94 °C for DNA denaturation, 40 s for primers annealing temperature (Table 1), and 50 s at 72 ^{O}C for DNA extension. PCR amplicons were electrophoresed on 1.5 % agarose gel (Sinaclone, Iran) and stained with ethidium bromide. Then, gels were visualized under ultraviolet transillumination (Emmenegger, Meyers, Burton & Kurath 2000; Santi, Vakharia & Evensen 2004).

Table 1. Primer sequences used for detecting target viral pathogens in this study

Name of primer	Sequence (5` to 3`)	Target gene	Annealing temperature	Product size (bp)	Reference
VNF	ATGGAAGGAGGAATTCGTGAAGCG	N gene	60°C	512	[OIE 2018]
VNR	GCGGTGAAGTGCTGCAGTTCCC				
IHNF	AGAGATCCCTACACCAGAGAC	G4 gene	56°C	693	[Emmenegger et al., 2000]
IHNR	GGTGGTGTTGTTTCCGTGCAA				
IPNF	GAGTCACAGTCCTGAATC	polyprotein gene	56°C	1093	[Santi et al. 2004]
IPNR	AGCCTGTTCTTGAGGGCTC				

Statistical analysis

All results were expressed as absolute and relative frequency. Logistic regression was implemented for univariable and multivariable analyses of qualitative variables by using the SPSS version 25 software (SPSS Inc., Chicago, IL, USA). For all analyses, P<0.05 was considered as statistically significant (Dohoo, Martin & Stryhn 2003).

Results

During this study, 65 farms with clinical manifestations (septicemia symptoms) were screened for three viral pathogens with RT- PCR method (Figure 1). VHSV and IHNV were found in 18.5% (95% CI = 9 to 27.9), 6.20% (95% CI = 0.3 to 12), respectively, where 4.61% (95% CI = 0 to 9.7) were positive for IPNV.

[DOR: 20.1001.1.2345315.2019.5.2.6.9]
[DOI: 10.29252/ijaah.5.2.22]

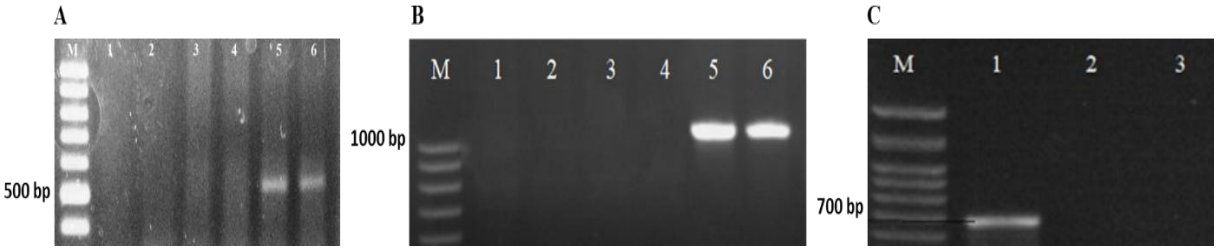

Figure 1. Agarose gel electrophoresis of PCR products of selected viral pathogens in this study. (A): M; 100bp molecular size marker; Lane 1: negative control; Lane 2 to 4: negative samples; and lane 5 and 6: VHSV positive samples. (B): M; 100bp molecular size marker; Lane 1: negative control; Lane 2 to 4: Negative samples; and lane 5 and 6: IPNV positive samples. (C): Lane 1: M; 100bp molecular size marker; IHNV positive sample; lane 2: Negative control; and Lane3: negative sample.

Table 2 presents the descriptive results of these targeted viral agents according to several independent variables including, years of study, water sources, stations of sampling, and weight groups. The frequency of VHSV and IPNV was higher in March 2016 to March 2017, while the most IHNV occurrence was documented in April 2017 to February 2018. The study showed that farms were located in station 1 mostly affected by VHSV and IHNV. For IPNV, the highest frequency was observed in station 6. Farms using river water were mostly affected with all three viral pathogens rather than those have been supplied by well as the water source. To determine that fishes in which weight groups are more affected by these pathogens, all of the collected fishes were fallen into 4 weight groups (A to D) as mentioned above, including group A (n: 19), group B (n: 25), group C (n: 14), and group D (n: 7). The majority of positive samples for VHSV and IHNV were related to group B, whereas for IPNV was settled in group A.

Table 2. The result of the absolute and relative frequency of three viral pathogens related to several independent variables

Variable / Viral disease	Category	VHS		IHN		IPN	
		Positive (%)	**Negative (%)**	**Positive (%)**	**Negative (%)**	**Positive (%)**	**Negative (%)**
year	2016-2017	8 (66.7)	24 (45.3)	1 (25)	31 (50.8)	3 (100)	29 (46.8)
	2017-2018	4 (33.3)	29 (54.7)	3 (75)	30 (49.2)	0 (0.0)	33 (53.2)
	Total	12 (100)	53 (100)	4 (100)	61 (100)	3 (100)	62 (100)
Station	1	6 (50)	14 (26.4)	3 (75)	17 (27.9)	1 (33.33)	19 (30.6)
	2	1 (8.3)	4 (7.5)	0 (0.0)	5 (8.2)	0 (0.0)	5 (8.1)
	3	0 (0.0)	8 (15.1)	0 (0.0)	8 (13.1)	0 (0.0)	8 (12.9)
	4	2 (16.7)	8 (15.1)	0 (0.0)	10 (16.4)	0 (0.0)	10 (16.1)
	5	1 (8.3)	7 (13.2)	1 (25)	7 (11.5)	0 (0.0)	8 (12.9)
	6	2 (16.7)	12 (22.6)	0 (0.0)	14 (23)	2 (66.7)	12 (19.4)
	Total	12 (100)	53 (100)	4 (100)	61 (100)	3 (100)	62 (100)
Water source	well	3 (25)	26 (49.1)	1 (25)	28 (45.9)	0 (0.0)	29 (46.8)
	River	9 (75)	27 (50.9)	3 (75)	33 (54.1)	3 (100)	33 (53.2)
	Total	12 (100)	53 (100)	4 (100)	61 (100)	3 (100)	62 (100)
Weight group(g)	A	3 (25)	16 (30.2)	1 (25)	18 (29.5)	2 (66.7)	17 (27.4)
	B	6 (50)	19 (35.8)	3 (75)	22 (36.1)	1 (33.3)	24 (38.7)
	C	1 (8.3)	13 (24.5)	0 (0.0)	14 (23)	0 (0.0)	14 (22.6)
	D	2 (16.7)	5 (9.4)	0 (0.0)	7 (11.5)	0 (0.0)	7 (11.3)
	Total	12 (100)	53 (100)	4 (100)	61 (100)	3 (100)	62 (100)

Regarding the incidence of the mentioned viral pathogens, prior studies have been suggested that the lower temperature are favorable for both VHSV and IPNV, while in the

[DOR: 20.1001.1.2345315.2019.5.2.6.9]
[DOI: 10.29252/ijaah.5.2.22]

case of IHNV increasing the temperature (above 10 °C) ideal for onset of infection.

According to table 3, our results revealed that VHSV, IHNV, and IPNV were frequently detected in February, April, and November, respectively. In the present study, VHSV, IHNV, and IPNV were found in the temperature range of 5 -16 °C, 6-16 °C, and 13-18 °C, respectively.

Table 3. Summary of several information about positive samples for all studied viruses

Sample ID	Sampling date	water temperature (°C)	Water resource
VHSV isolates			
RT.A2	Mar 2016	7	River
RT.A7	Apr 2016	10	River
RT.A14	Jan 2016	8	River
RT.A18	Feb 2016	5	River
RT.A20	Mar 2017	8	River
RT.A33	Feb 2018	7	River
RT.B5	Feb 2016	16	Well
RT.N5	Apr 2017	11	River
RT.N10	Feb 2018	7	River
RT.C8	Dec 2017	11	River
RT.T5	Feb 2016	15	Well
RT.T6	Feb 2016	14	Well
IHNV isolates			
RT.A3	Mar 2016	7	River
RT.A22	Apr 2017	12	River
RT.A37	Feb 2018	6	River
RT.C6	Apr 2017	16	Well
IPNV isolates			
RT.A14	Nov 2016	18	River
RT.T4	Nov 2016	13	River
RT.T14	Nov 2016	13	River

A univariable logistic regression test was served to determine the number of variables that were significantly correlated with the occurrences of viral pathogens in the present study. The frequency distribution of positive and negative samples, odds ratio (OR), and the significance level of the independent variables in the univariable logistic regression model are listed in Table 4. Based on the results of the analysis, variables with P<0.2 including, Weight group, sampling area (Station), and type of water source were entered into multivariable analysis.

Table 4. Univariate analysis of several effective factors that influencing on the occurrence of these three viral pathogens

variables	Category	Positive (%)	Negative (%)	[b]OR (95% CI)	[c]P-value
Station	[a]1	10 (52.6)	10 (21.7)	-	-
	2	1 (5.3)	4 (8.7)	0.25 (0.02-2.64)	0.25
	3	0 (0.0)	8 (17.4)	[d] NC	NC
	4	2 (10.5)	8 (17.4)	0.25 (0.04-1.48)	0.12
	5	2 (10.5)	6 (13)	0.33 (0.05-2.06)	0.23
	6	4 (21.1)	10 (21.7)	0.40 (0.09-1.71)	0.21
Water source	aWell	4 (21.1)	25 (54.3)	-	-
	River	15 (78.9)	21 (45.7)	4.46 (1.28-15.52)	0.02
Weight group (g)	[a]A	6 (31.6)	13 (28.3)	-	-
	B	10 (52.6)	15 (32.6)	1.44 (0.41-5.06)	0.56
	C	1 (5.3)	13 (28.3)	0.16 (0.01-1.58)	0.12
	D	2 (10.5)	5 (10.9)	0.86 (0.12-5.81)	0.88

[a]reference group; [b]Odds ratio (confidence interval for OR); [c]P<0.05 was considered as statistically significant; [d] NC, not calculable.

[DOR: 20.1001.1.2345315.2019.5.2.6.9]
[DOI: 10.29252/ijaah.5.2.22]

Table 5 shows the results of multivariable logistic regression analysis obtained from univariable analysis with independent variables. Our results revealed that using river water increases the chances of viral diseases (OR = 5.02; 95% CI = 1.38 to 18.17; P = 0.01).

Table5: multivariable Logistic regression analysis of variables associated with occurrence of these three viral pathogens

variables	Category	Positive (%)	Negative (%)	[b]OR (95% CI)	[c]P-value
Water source	[a]Well	4 (21.1)	25 (54.3)	-	0.01
	River	15 (78.9)	21 (45.7)	5.02 (1.38-18.17)	

[a]reference group; [b]Odds ratio (confidence interval for OR); [c]P<0.05 was considered as statistically significant.

Discussion

Viral agents are one of the most hazardous pathogens that have caused drastic economic losses to the aquaculture industry. Increasing global trends in aquaculture productions resulting in new opportunities for the transmission and distribution of aquatic viral pathogens (OIE 2019; Cieslak et al. 2016). Studies over the past two decades have been documented the presence, occurrence, and distribution of these viruses in the different parts of Iran especially in the northern provinces of the country, as well as, the provinces located in the Zagros Mountain (Fallahi et al. 2003; Soltani et al. 2014; Haghighi Khiabanian asl et al. 2008; Soltani et al. 2014; Ahmadivand, Soltani, Mardani, Shokrpoor, Rahmati-Holasoo, Mokhtari & Hasanzadeh 2016; Fadaeifard, Raissy, Moumeni & Faghani 2012; Zargar, Soltani, Hematzadeh, Kazemi & Ebrahimzadeh Mousavi 2008).

The presence of the VHS virus was first confirmed in 2005 during a large outbreak in Guilan province (Rudsar city) (Haghighi Khiabanian asl et al. 2008). Subsequent investigations led to identify the other potential centers of the disease in Iran. Furthermore, a similar profile of identification and report of two other target viruses was observed in the middle of the 2000s in Iran (Akhlaghi & Hosseini 2007; Fallahi et al. 2003). VHSH, IHNV, and IPNV have led lethal diseases in salmonid and non-salmonid fishes (Bootland & Leong 2009; Dixon et al. 2016; McAllister, Newman, Sauber & Owens 1984). The prevalence of them has been described by many researchers in several parts of Iran. In the case of VHSV, Ahmadivand et al. During 2014-2015 showed that the VHS virus was responsible for mass mortalities (30 to 70%) of Rainbow trout farms in the north and center of Iran (Ahmadivand et al. 2016). In addition, the presence of IHNV and IPNV were confirmed in different regions of the country (Soltani et al. 2014; Zargar et al. 2008; Fallahi et al. 2006).

The present study was conducted to design more accurate pictures of the crucial viral pathogens such as VHSV, IHNV and IPNV frequencies in Rainbow trout farms in different regions of northern Iran. We also evaluated the influence of determinant factors on viral pathogens occurrences.

Our data confirmed the presence of all targeted pathogens in trout farms in this part of

[DOR: 20.1001.1.2345315.2019.5.2.6.9]
[DOI: 10.29252/ijaah.5.2.22]

the country. In total 29.23% of studied farms were positive for all targeted viral pathogens. The frequency of VHSH, IHNV, and IPNV was determined as 18.5%, 6.2%, and 4.61%, respectively, among 65 farms with clinical manifestations related to their diseases from March 2016 to February 2018. More specifically, in the period of the study, two different waves of epidemics were observed. As mentioned in the result, During the first years of monitoring (March 2016 to March 2017), VHSV and IPNV more frequently were detected, while the most IHNV infected farms were found in April 2017 to February 2018. This fact could be related to adopting preventive strategies such as increased water quality, proper nutrition programs and improved fish resistance against putative environmental stress.

Based on our data, VHSV had the highest occurrence (12/65) among other monitored viruses circulating in this area. Furthermore, VHSV has been detected from the most surveyed stations indicating the potential threatening role of it for Rainbow trout farms. However, these results are in agreement with those of prior studies that had been done in Iran (Soltani et al. 2014; Zargar et al. 2008; Fallahi et al. 2006; Ahmadivand et al. 2016).

River and well waters are commonly used in this part of the country. Using well water, springs, and other groundwater sources potentially are considered to be a preventive strategy (Scarfe 2006). Thus, it seems the consideration of the risk attributed to pathogen spread is important. This study showed a relatively high percent of affected farms are using the river water (78.9%). In addition, our data revealed that the river water increased the chances of viral disease by 5 times (OR= 5.02; P = 0.01). It has been proposed that establishing the farms with a short distance from each other, using the same water supplier, the lack of sufficient strict sanitary, applying improper preventive, controlling, and monitoring programs might have resulted in an increase of associated cases of these viral pathogens in our studied trout farms (Ghorani, Adel, Dadar, Langeroudi, Kamyabi, Vakharia & Einer-Jensen 2016). Therefore, the usage of well water might be considered as a protective factor against these viral agents occurrences. Despite, the potential preventive role of well water for viral transmission, in this study, 21.1% of farms (four farms out of twenty-one farms) have been supplying with this source of water were positive. It could be due to direct and indirect transmissions through introducing of infected fish, contaminated objects (such as equipment and vehicles), vectors, employees, and visitors (OIE 2019).

Mainly, first-feeding fry and fingerling life-stages are highly vulnerable to these viral pathogens (OIE 2019). To have a better knowledge of the affected fish population in this part of the country, all samples were categorized into four weight groups. As we expecting, the data revealed that groups A and B were more targeted by these viral agents. Briefly, IPNV was typically isolated from group A, while the majority of positive samples for VHSV and IHNV were related to group B, indicating that younger fishes more susceptible rather than older ones. But, our results did not

[DOR: 20.1001.1.2345315.2019.5.2.6.9]
[DOI: 10.29252/ijaah.5.2.22]

show any significant association between the occurrence of viral pathogens and the age of fish at the time of infection.

Water temperature also has important role in Rainbow trout casualties in the course of these target pathogens disease. Furthermore, low water temperature will support the viability, infectivity, and transmission of them. (Meyers & Winton 1995; Amend 1970; Tu, Spendlove & Goede 1975). In case of VHS virus has been shown that the disease outbreak mainly occur at temperature range 4- 14°C (Haghighi Khiabanian asl et al. 2008). In 2019, Fattahi et al. observed a higher mortality of VHS disease in periods of colder temperatures in Iran specially, during the fall and early spring months (Fattahi et al. 2019).as well as, Zorriehzahra et al (2018) founded VHS virus in water temperature of 13-15°C. The temperature spectrum from 8–14°C has been suggested for IHN occurrence (OIE 2019; Zorriehzahra et al. 2018). Prior research proved that temperature above 10 °C was ideal for onset of IHN infection (Zorriehzahra et al. 2018). Barja et al. (1983) revealed that IPN virus maintains its infectivity at 15 °C and 20°C up to 20 and 15 days, respectively (Barja, Toranzo, Lemos & Hetrick 1983). In comparison with the VHS and IHN viruses, the IPN virus occurrence can happen over a wide temperature range but lower temperature can lead to longer monition rates (Zorriehzahra et al. 2018). Our results revealed that a further temperature ranges for these target pathogens (table 3). They mainly detected in February, April, and November, respectively. It seems that the increase of positive cases in the mentioned months could be due to proper water temperature for virus viability that have has addressed in our data.

Recently, there are increasing trends in Iranian farmers to introduce eyed-eggs and fries from abroad countries. This issue provides an efficient route of viral transmission and isolation of genotypes of VHSV and IHNV circulating in European countries. (Ahmadivand et al. 2016; Ghorani et al. 2016; Mulei, Nyaga, Mbuthia, Waruiru, Xu, Evensen, Mutoloki 2019). Concerning the above-mentioned facts, it has been suggested that intensifying biosecurity practices for introducing eyed-eggs and fries from trout producing farms of abroad countries and using proper water filtering system suppliers. Besides, these pathogens such as IHNV and IPNV could develop persistent infections in recovered fishes and they are considered as potential reservoirs of infection in breeding farms (Crane & Hyatt 2011; Roberts & Pearson 2005).

In summary, due to the high importance of these diseases and economic losses resulting from them, it is essential to establish laboratories that take samples routinely and monitor the genotypes of viruses that are circulating at this part of the country. Moreover, due to the lack of any specific treatment for these diseases; setting up the basic educational programs and encouraging farmers to perform preventive strategies and report of similar manifestations attributed to these pathogens, are very helpful. The data presented in this study have further extended our general knowledge on the important role of water source in the occurrence of pathogens.

[DOR: 20.1001.1.2345315.2019.5.2.6.9]
[DOI: 10.29252/ijaah.5.2.22]

Furthermore, our research was limited only the farms with clinical symptoms associated with studied pathogens and further supplementary studies are needed. Collectively, our results recommended that putting firm laws to limit the freely access to farms, the application of strict surveillance, and quarantine programs ,as well as, fish movement restriction when the disease occurrence is confirmed, should be intensified to impede of the disease emerge into disease-free zones.

Conflicts of interest

None of the authors has any conflicts of interest to declare.

Acknowledgement

This research work has been supported by a research grant from the Amol University of Special Modern Technologies, Amol, Iran.

References

Abbadi M., Fusaro A., Ceolin C., Casarotto C., Quartesan R., Dalla Pozza M., Cattoli G., Toffan A., Holmes E.C. & Panzarin V. (2016) Molecular Evolution and Phylogeography of Co-circulating IHNV and VHSV in Italy. *Frontiers in Microbiology* 7, 1306.

Ahmadivand S., Soltani M., Mardani K., Shokrpoor S., Rahmati-Holasoo H., Mokhtari A. & Hasanzadeh R. (2016) Isolation and identification of viral hemorrhagic septicemia virus (VHSV) from farmed Rainbow trout (*Oncorhynchus mykiss*) in Iran. *Acta Tropica* 156, 30–36.

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. *Bulletin of the European Association of Fish Pathologists* 27(5), 205-210.

Amend D.F. (1970) Control of infectious Hematopoietic Necrosis virus disease by elevating the water temperature. *Journal of the Fisheries Research Board of Canada* 27(2), 265-270.

Bain N., Gregory A. & Raynard R.S. (2008) Genetic analysis of infectious pancreatic necrosis virus from Scotland. *Journal of Fish Diseases* 31(1), 37-47.

Ball H.J., Munro A.L.S., Ellia A., Elson K.G., Hodgkiss W. & McFarlane I.S. (1971) Infectious pancreatic necrosis in Rainbow trout in Scotland. *Nature* 234 (5329), 417-418.

Barja J.L., Toranzo A.E., Lemos M.L. & Hetrick, F.M. (1983) Influence of water temperature and salinity on the survival of IPN and IHN viruses. *Bulletin of the European Association of Fish Pathologists* 3, 47-50.

Bootland L.M., Dobos P. & Stevenson R.M.W. (1991) The IPNV carrier state and demonstration of vertical transmission in experimentally infected brook trout. *Diseases of Aquatic Organisms* 10, 13-21.

Bootland L.M & Leong J.C. (2009) Infectious haematopoietic necrosis virus. In: Fish diseases and disorders (ed. by P.T.K. Woo. & D.W.

[DOR: 20.1001.1.2345315.2019.5.2.6.9]
[DOI: 10.29252/ijaah.5.2.22]

Bruno), pp. 66−109. Wallingford, UK: CABI Publishing.

Brudeseth B.E., Castric J., Evensen Ø. (2002) Studies on Pathogenesis Following Single and Double Infection with Viral Hemorrhagic Septicemia Virus and Infectious Hematopoietic Necrosis Virus in Rainbow Trout (*Oncorhynchus mykiss*). *Veterinary Pathology*, 39 (2), 180-189.

Cieslak M., Mikkelsen S.S., Skall H.F., Baud M., Diserens N., Engelsma M.Y., Haenen O.L., Mousakhani S., Panzarin V., Wahli T., Olesen N.J. & Schütze H. (2016) Phylogeny of the Viral Hemorrhagic Septicemia Virus in European Aquaculture. *PLOS ONE* 11(10), e0164475.

Crane M. & Hyatt A. (2011) Viruses of Fish: An Overview of Significant Pathogens. *Viruses* 3(11), 2025–2046.

Dixon P., Paley R., Alegria-Moran R. & Oidtmann B. (2016) Epidemiological characteristics of infectious hematopoietic necrosis virus (IHNV): a review. *Veterinary Resea*rch 47 (1), 63.

Dobos P. (1995) The molecular biology of infectious pancreatic necrosis virus (IPNV). *Annual Review of Fish Disea*ses 5, 25–54.

Dohoo I., Martin W., Stryhn H. (2003) Logistic regression. In: Veterinary epidemiologic research (2nd edition), pp. 335-369. AVC Inc, Prince of Edward Island Canada.

Emmenegger E.J., Meyers T. R., Burton T.O. & Kurath G. (2000) Genetic diversity and epidemiology of infectious hematopoietic necrosis virus in Alaska. *Diseases of Aquatic Organisms* 40(3), 163-176.

Enzmann P.J., Castric J., Bovo G., Thiery R., Fichtner D., Schütze H. & Wahli T. (2010) Evolution of infectious hematopoietic necrosis virus (IHNV), a fish rhabdovirus, in Europe over 20 years: implications for control. *Diseases of Aquatic Organisms* 89 (1), 9-15.

Fadaeifard F, Raissy M, Moumeni M. & Faghani M. (2012) Evaluation of infectious hematopoietic necrosis, infectious pancreatic necrosis and viral hemorrhagic septicemia viruses in Iranian and imported Rainbow trout eggs: Across sectional study. *Journal of Veterinary of Research* 67 (4), 393-399. [In Persian]

Fallahi R., Soltani M., Karegar R., ZorriehZahra M.E.J., Shchelkunov I., Hemmatzadeh F. & Nouri A. (2003) Isolation and identification of the IHNV-like agent from farmed Rainbow trout (*Oncorhynchus mykiss*) from Iran. *Archives of Razi Insti*tute 56(1), 37-47.

Fattahi F., Akhlaghi M., Mohammadi A., Soltanian S., Shahbazian N. (2019) Phylogenetic analysis of viral hemorrhagic septicemia virus from recent outbreaks in some of the Iranian farmed Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). *Iranian Journal of Fisheries Sciences*. DOI: 10.22092/IJFS.2019.119829.

[DOR: 20.1001.1.2345315.2019.5.2.6.9]

[DOI: 10.29252/ijaah.5.2.22]

Gagné N., Mackinnon A.M., Boston L., Souter B., Cook-Versloot M., Griffiths S. & Olivier G. (2007) Isolation of viral haemorrhagic septicaemia virus from mummichog, stickleback, striped bass and brown trout in eastern Canada. *Journal of Fish Diseas*es 30 (4), 213-223.

Ghorani M., Adel M., Dadar M., Langeroudi A.G., Kamyabi R., Vakharia V.N. & Einer-Jensen K. (2016) Phylogenetic analysis of the glycoprotein gene of viral hemorrhagic septicemia virus from Iranian trout farms points towards a common European origin. *Veterinary Microbiology* 186, 97-101.

Guerrero L., Herrera E., Salinas J.,Torres J., Montero A.B. & Barrón B. (2008) Detection and Genotyping of an Infectious Pancreatic Necrosis Virus from Asymptomatic Rainbow Trout (*Oncorhynchus mykiss*) Facilities in Mexico. *Intervirology* 51, 285–292.

Haghighi Khiabanian asl A., Bandehpour M., Sharifnia Z. & Kazemi B. (2008) Diagnosis of viral haemorrhagic septicaemia (VHS) in Iranian Rainbow trout aquaculture by pathology and molecular techniques. *Bulletin of the European Association of Fish Pathologists* 28 (5), 170-175.

Iran Fisheries Organization (IFO). (2018) Annual report book. In: Administration (ed). Iran Fisheries Organization Serial Book, Tehran. [In Persian]

LaPatra S.E. (1998) Factors affecting pathogenicity of infectious hematopoietic necrosis virus (IHNV) for salmonid fish. *Journal of Aquatic Animal Health* 10 (2), 121–131.

McAllister P.E., Newman M.W., Sauber J.H. & Owens W.J. (1984) Isolation of infectious pancreatic necrosis virus (serotype Ab) from diverse species of estuarine fish. *Helgoländer Meeresunters* 37, 317-328.

Meyers T.R & Winton J.R. (1995) Viral hemorrhagic septicaemia virus in North America. *Annual Review of Fish Diseases* 1995; 5: 3–24.

Mulei I.R., Nyaga P.N., Mbuthia P.G., Waruiru R.M., Xu C., Evensen Ø., Mutoloki S. (2019) First detection and isolation of infectious haematopoietic necrosis virus from farmed Rainbow trout in Nyeri County, Kenya. *Journal of Fish Diseases* 42 (5), 751-758.

Mutoloki S., Jøssund T.B., Ritchie G, Munang'andu H.M. & Evensen Ø. (2016) Infectious Pancreatic Necrosis Virus Causing Clinical and Subclinical Infections in Atlantic Salmon Have Different Genetic Fingerprints. *Frontiers in Microbiology* 7, 1-10.

OIE. (2018) Viral haemorrhagic septicaemia. Available at: www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/chapi tre_vhs.pdf.

OIE. (2019) Manual of Diagnostic Tests for Aquatic Animals. Available at: www.oie.int/international-standard-setting/aquatic-manual/access online/.Accessed 2019.

[DOR: 20.1001.1.2345315.2019.5.2.6.9]
[DOI: 10.29252/ijaah.5.2.22]

Panzarin V., Holmes E.C., Abbadi M., Zamperin G., Quartesan R.,Milani A., Schivo A., Bille L., Pozza M.D., Monne I. & Toffan A. (2018) Low evolutionary rate of infectious pancreatic necrosis virus (IPNV) in Italy is associated with reduced virulence in trout. *Virus Evolution* 4(2), vey019.

Rasmussen C.J. (1965) A biological study of the Egtved disease (InuL). *Annals of the New York Academy of Sciences* 126(1), 427–460.

Roberts R.J. & Pearson M.D. (2005) Infectious pancreatic necrosis in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 28 (7), 383-390.

Santi N., Vakharia V. N. & Evensen Ø. (2004). Identification of putative motifs involved in the virulence of infectious pancreatic necrosis virus. *Virology*, 322(1), 31-40.

Scarfe A.D. (2006) Aquaculture Biosecurity Prevention, Control, and Eradication of Aquatic Animal Disease, Blackwell Publishing Professional, Iowa, USA.

Schäperclaus W. (1938) Die Schädigungen der deutschen Fischerei durch Fischparasiten und Fischkrankheiten. *Allg Fischztg* 41, 256–259.

Smail D.A. & Snow M. (2011) Viral haemorrhagic septicaemia. In: Fish Diseases and Disorders (ed by P.T.K. Woo, & D.W Bruno), pp. 110–142. Wallingford, UK: CABI Publishing.

Soltani M., Rouholahi S.h., Ebrahimzadeh Mousavi H.A., Abdi K., Zargar A. & Mohamadian S. (2014) Genetic diversity of infectious pancreatic necrosis virus (IPNV) in farmed Rainbow trout (*Oncorhynchus mykiss*) in Iran. *Bulletin of the European Association of Fish Pathologist*s 34 (5), 156-164.

Soltani M., Rouholahi S.h., Zargar A., Abdi K., Mohamadian S. & Ghajari A. (2014) Study of the distribution of infectious pancreatic necrosis (IPN) in Rainbow trout farms of Iran using RT-PCR. *Iranian Journal of Veterinary Medicine* 10 (2), 29-39. [In Persian]

Stone D.M., Ferguson H.W., Tyson P.A., Savage J., Wood G., Dodge M.J., Woolford G., Dixon P.F., Feist S.W. & Way K. (2008) The first report of viral haemorrhagic septicaemia in farmed Rainbow trout, *Oncorhynchus mykiss* (Walbaum), in the United Kingdom. *Journal of Fish Diseases* 31 (10), 775-784.

Tu K.C., Spendlove R.S. & Goede R.W. (1975) Effect of temperature on survival and growth of infectious pancreatic necrosis virus. *Infection and Immunity* 11(6), 1409–1412.

Wood E.M., Snieszko S.F. & Yasutake W.T. (1995) Infectious pancreatic necrosis of brook trout. *American Medical Association archives of pathology* 60 (1), 26-28.

Zargar A., Soltani M., Hematzadeh F., Kazemi B. & Ebrahimzadeh Mousavi H.A. (2008) Study on distribution of infectious hematopoietic necrosis (IHN) in five major province producing Rainbow trout (*Oncorhynchus mykiss*) fry in Iran by Indirect Fluorescence Antibody (IFAT) and Nested-RT

[DOR: 20.1001.1.2345315.2019.5.2.6.9]

[DOI: 10.29252/ijaah.5.2.22]

PCR techniques. *Journal of Veterinary of Research* 63 (3), 99-105. [In Persian]

Zorriehzahra M.J., Kakoolaki S., Mehrabi M.R., Sepahdari A., Ghasemi M., Yarmohammadi M. & Ghiasi M. (2018) Identification of some health indicators related to OIE notifiable viral diseases in Rainbow trout (*Oncorhynchus mykiss*) based on the strategic plan for producing Specific Pathogen Free (SPF) broodstock in Iran. *Iranian Journal of Aquatic Animal Health* 5(1), 71-82.

[DOR: 20.1001.1.2345315.2019.5.2.6.9]

[DOI: 10.29252/ijaah.5.2.22]

یافته‌های عددی در مورد بیماری‌های مهم ویروسی در مزارع قزل آلای رنگین کمان (*Onchorhynchus mykiss*) در شمال ایران

مجتبی خسروی[1*]، پولین شهره[2] و راحم خوشبخت[1]

[1] گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه تخصصی فناوری های نوین آمل، آمل، ایران

[2] گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه تخصصی فناوری های نوین آمل، آمل، ایران

[DOR: 20.1001.1.2345315.2019.5.2.6.9]

[DOI: 10.29252/ijaah.5.2.22]

چکیده

ویروس سپتی سمی خونریزی دهنده (VHSV)، ویروس نکروز عفونی بافت‌های خونساز (IHNV) و ویروس نکروز عفونی لوزالمعده (IPNV) در زمره قابل‌توجه‌ترین عوامل بیماریزای ویروسی در مزارع پرورشی ماهی‌های قزل آلای رنگین کمان مطرح گردیده اند. هدف از مطالعه حاضر، تعیین فراوانی این پاتوژن‌ها در ۶۵ مزرعه دارای علائم بالینی مشکوک در شمال ایران در بازه زمانی مارس ۲۰۱۶ تا فوریه ۲۰۱۸ بود. آزمون رگرسیون لجستیک برای ارزیابی اثر چندین فاکتور تعیین کننده در فراوانی این پاتوژن‌ها، استفاده شد. بطور کلی، ۱۹ مزرعه (٪۲۹/۲۳) پس از انجام آزمون RT-PCR مثبت بودند. فراوانی VHSV، IHNV و IPNV به ترتیب ۱۸/۵٪ ، ۶/۲٪ و ۴/۶۱٪ گزارش شد. اغلب مزارع آلوده (۷۸/۹۵ درصد) از آب رودخانه استفاده می‌کردند. علاوه بر این، نتایج نشان داد که استفاده از آب رودخانه شانس ابتلا به ویروس را ۵ برابر افزایش می‌دهد (OR= 5.02; P= 0.01). بنابراین استفاده از آب رودخانه یک عامل خطر در بروز پاتوژن‌های ویروسی می‌باشد. از چهار گروه وزنی (A تا D)؛ ماهی‌های گروه A (ماهی <۱ گرم) و B (۱ تا ۲۰ گرم) بیشتر تحت تأثیر قرار گرفتند اما از نظر آماری معنی‌دار نبود (P>۰/۰۵). در مجموع، ایجاد برنامه‌های مدون تشخیص سریع و تنظیم شیوه‌های آموزشی پایه می‌تواند برای طراحی استراتژی‌های پیشگیری و کنترل بسیار ارزشمند باشد.

کلمات کلیدی: قزل آلای رنگین کمان، ویروس سپتی سمی خونریزی دهنده، ویروس نکروز عفونی بافت‌های خونساز، ویروس نکروز عفونی لوزالمعده، فراوانی

***نویسنده مسئول:** mojtaba.kh.dvm@gmail.com