SS. CYRIL AND METHODIUS UNIVERSITY IN SKOPJE
FACULTY OF VETERINARY MEDICINE - SKOPJE

RISING PREVALENCE OF INFECTIOUS HEMATOPOIETIC NECROSIS (IHN) IN NORTH MACEDONIAN TROUT FARMS: A SURVEILLANCE STUDY OF MAJOR VIRAL PATHOGENS IN AQUACULTURE (2015-2024)

ALEKSANDAR TRAJCHOVSKI[1], ZAGORKA POPOVA HRISTOVSKA[1], ANGELA GRUJOVSKA[2], ALEKSANDAR CVETKOVIKJ[1]

[1]*FACULTY OF VETERINARY MEDICINE-SKOPJE, SS. CYRIL AND METHODIUS UNIVERSITY IN SKOPJE, LAZAR POP-TRAJKOV 5-7, 1000 SKOPJE, NORTH MACEDONIA*

[2]*FOOD AND VETERINARY AGENCY, TRETA MAKEDONSKA BRIGADA 20, 1000 SKOPJE, NORTH MACEDONIA*

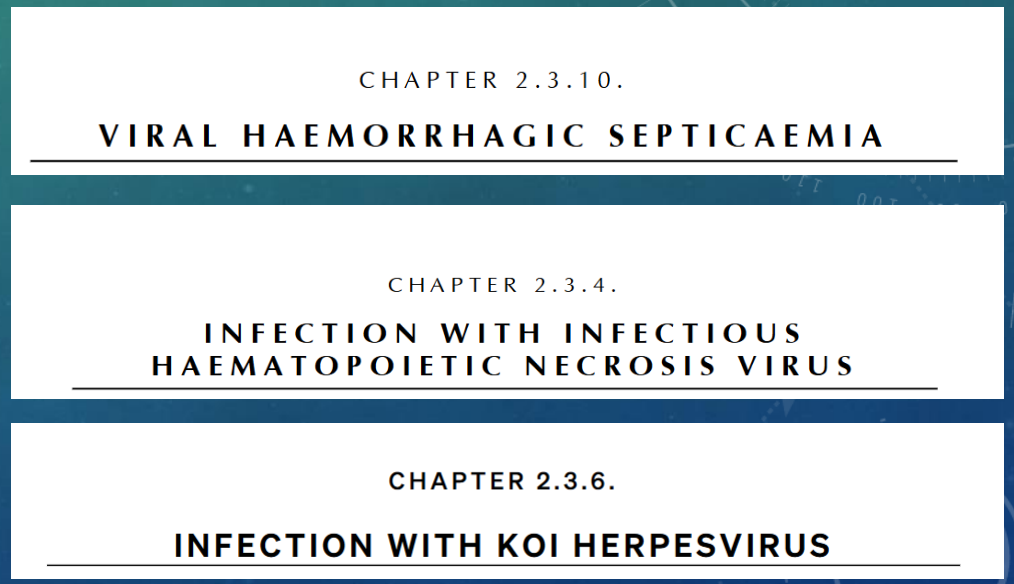
15th International Aquaculture Conference
2-4.4.2025
Vukovar, Croatia

1. INTRODUCTION

- The World Organization for Animal Health (WOAH)
- Reported diseases to the WOAH have a substantial impact on the international trade of aquatic animals and their goods

- Notifiable fish diseases in North Macedonia's aquaculture
  - Viral haemorrhagic septicaemia (VHS)
  - Infectious hematopoietic necrosis (IHN)
  - Koi herpesvirus disease (KHVD)

- Regular monitoring for these diseases was initiated in 2015
- We aimed to investigate the occurrence of VHS, IHN, and KHVD in aquaculture facilities across North Macedonia



1. INTRODUCTION

- IHN
  - North America
  - France, Germany, and Italy
  - Slovenia, Croatia, Kosovo and the Republic of North Macedonia
- VHS
  - Throughout Western Europe's history, freshwater salmonids have been closely associated with VHS
  - Germany, Italy , France and Denmark Bulgaria, Croatia and Slovenia
- KHVD
  - Israel, Germany , USA
  - Belgium, Denmark, England, Wales, France, Italy, Luxemburg, Netherlands, Switzerland, Sweden, Poland , Czech Republic
  - Romana, Slovenia , Croatia

Fig. 1 The distribution of VHS, IHN and KHVD

2. MATERIALS AND METHODS

- Fish were handled and sampled according to standardized methods
- Samples were collected from fish farms located in different regions of North Macedonia as part of the national surveillance program conducted between 2015 and 2024
- During this period, 57 trout fish farms and 43 carp fish farms were registered and were operating
- The number of farms fluctuated from year to year and not all had fish year-round

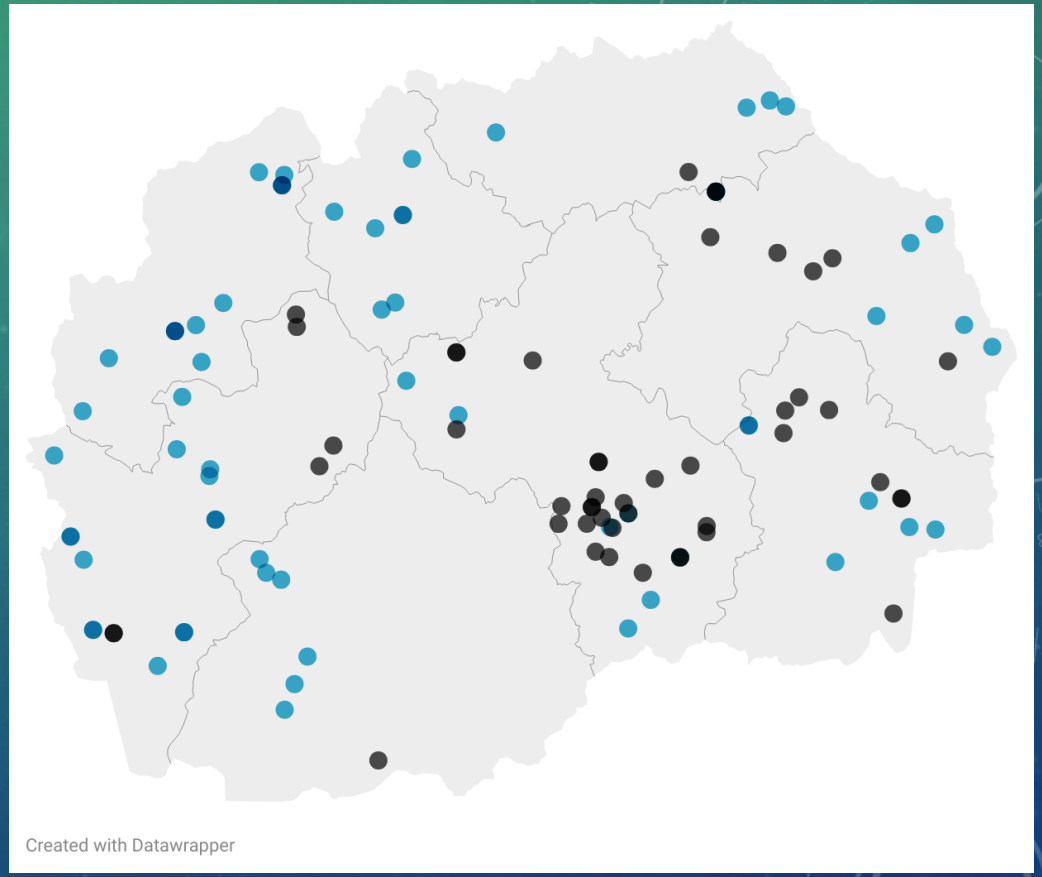


Fig. 2 Location of fish farms in North Macedonia, where blue dots represent cold-water fish farms and black dots represent warm-water fish farms

2. MATERIALS AND METHODS

Rainbow trout (*Oncorhynchus mykiss*)

- Water temperatures 8 to 15 °C
- VHS and IHN
- Three pooled samples from - 30 fish (heart, spleen, kidneys).
- 2015 and 2016 was conducted twice a year, once in spring and autumn.
- From 2017 to 2024, monitoring for VHS and IHN was conducted in autumn
- From 2015 to 2021, samples were collected from all fish farms across the country for IHN and VHS
- However, starting in 2022, samples were only taken from farms previously confirmed as negative for IHN and VHS. The remaining farms were considered positive for IHN and were excluded from sampling for both diseases
- The number of samples was reduced due to the adaptation of the program by the Food and Veterinary Agency
- 1,551 samples (for VHS and IHN) from 57 farms

Fig. 3 Collecting samples on the field

Fig. 4 Collecting samples in the field

2. MATERIALS AND METHODS

Common carp *(Cyprinus carpio)*

- 20 - 28 °C
- KHVD
- 15 samples (30 fish – gills and kidneys)
- 2017-2021, sampling in late summer and early autumn
- The number of sampled fish farms varied greatly from year to year
- 2022 - 2024, sampling was conducted only in early autumn
- 2,985 samples from 36 farms

Fig. 5 Carp farms in artificial lake

2. MATERIALS AND METHODS

- Testing Period (2015–2019)
  - Samples tested at the Institute of Veterinary Medicine of Serbia (NRL for fish disease)
    - Diagnostics performed using Bluegill fry-2 (BF-2) and Epithelioma papulosum cyprini (EPC) cell lines.
    - RT-qPCR also used for VHS and IHN detection
- Testing Period (After 2020)
  - Faculty of Veterinary Medicine - Skopje
    - Only RT-qPCR method used for virus detection

Tab.1. RT-qPCR for detection of VHS

Primer	Sequence of primer 5'-3 '	Publication
q VHS_F	AAACTCGCAGGATGTGTGCGTCC 3’	Jonstrup, S. P., Kahns, S., Skall, H. F., Boutrup, T. S., & Olesen, N. J. (2013). Development and validation of a novel TaqMan-based real-time RT-PCR assay suitable for demonstrating freedom from viral haemorrhagic septicaemia virus. Journal of Fish Diseases, 36(1), 9-23. https://doi.org/10.1111/j.1365-2761.2012.01416.x
q VHS_R	TCTGCGATCTCAGTCAGGATGAA 3’	
q VHS_Pro	FAM-TAGAGGGCCTTGGTGATCTTCT-BHQ1-3’	

Tab.2. RT-qPCR for detection of IHN (till May 2021)

Primer	Sequence of primer 5'-3 '	Publication
qIHNV_F	AGAGCCAAGGCACTGTGCG	Purcell, M. K., Thompson, R. L., Garver, K. A., Hawley, L. M., Batts, W. N., Sprague, L., Sampson, C., & Winton, J. R. (2013). Universal reverse-transcriptase real-time PCR for infectious hematopoietic necrosis virus (IHNV). Diseases of Aquatic Organisms, 106(2), 103-115. https://doi.org/10.3354/dao02644
qIHNV_R	TTCTTTGCGGCTTGGTTGA	
qIHNV_Pro	6FAM-AGCGGGACAGGRATGACAATGGTG- BHQ1	

Tab.3. RT-qPCR for detection of IHN (after May 2021)

Primer	Sequence of primer 5'-3 '	Publication
qIHNV_F	AGAGCCAAGGCACTGTGCG	Cuenca, A., Vendramin, N., & Olesen, N. J. (2020). Analytical validation of one-step realtime RT-PCR for detection of infectious hematopoietic necrosis virus (IHNV). Bulletin of The European Association of Fish Pathologists, 40(6), 261-272
qIHNV_R	TTCTTTGCGGCTTGGTTGA	
qIHNV_Pro	6FAM-TGAGACTGAGCGGGACA-NFQ/MGB	

2. MATERIALS AND METHODS

- Both institutions used only molecular diagnostic techniques for detecting KHVD

Tab.4. RT-qPCR for detection of KHV

Primer	Sequence of primer 5'-3 '	Publication
qKHV_89f	GACGCCGGAGACCTTGTG	Gilad, O., Yun, S., Zagmutt-Vergara, F. J., Leutenegger, C. M., Bercovier, H., & Hedrick, R. P. (2004). Concentrations of a Koi herpesvirus (KHV) in tissues of experimentally infected Cyprinus carpio koi as assessed by real-time TaqMan PCR. Diseases of Aquatic Organisms, 60(3), 179-187. https://doi.org/10.3354/dao060179
qKHV_163r	CGGGTTCTTATTTTTGTCCTTGTT	
qKHV_109pro	FAM-CTTCCTCTGCTCGGCGAGCACG-BHQ1	

3. RESULTS

- This study showed that VHS and KHVD were not present in the aquaculture facilities in North Macedonia up to 2024.
- IHN steadily increased in incidence

Tab.5 Results from analyzed fish samples for viral diseases (2015–2024)

Year	Total number of fish farms			Sampled fish farms n (%)			Positive fish farms n (%)			Positive non-sampled fish farms n (%)			Positive sampled fish farms n (%)		
	IHN	VHS	KHVD	IHN	VHS	KHVD	IHN	VHS	KHVD	IHN	VHS	KHVD	IHN	VHS	KHVD
2015	49	49	-	49(100%)	49(100%)	-	-	-	-	-	-	-	-	-	-
2016	50	50	-	50(100%)	50(100%)	-	-	-	-	-	-	-	-	-	-
2017	51	51	15	51(100%)	51(100%)	15(100%)	-	-	-	-	-	-	-	-	-
2018	50	50	14	50(100%)	50(100%)	14(100%)	2(4%)	-	-	-	-	-	2(4%)	-	-
2019	50	50	15	50(100%)	50(100%)	15(100%)	-	-	-	-	-	-	-	-	-
2020	47	47	18	47(100%)	47(100%)	18(100%)	17(36.17%)	-	-	-	-	-	17(36.17%)	-	-
2021	49	49	20	49(100%)	49(100%)	18(100%)	20(40.82%)	-	-	17(34.69%)	-	-	10(20.41%)	-	-
2022	50	50	11	30(60%)	30(60%)	11(100%)	27(54%)	-	-	20(40%)	-	-	7(23.33%)	-	-
2023	50	50	15	27(54%)	27(54%)	15(100%)	33(66%)	-	-	27(54%)	-	-	6(22.22%)	-	-
2024	50	50	13	15(30%)	15(30%)	13(100%)	36(72%)	-	-	33(66%)	-	-	3(20%)	-	-

3. RESULTS

- Since the first detection of IHN in 2018 there are fluctuating numbers of outbreaks, with peaks in 2020 and subsequent declines in later years, indicating ongoing challenges in managing the disease.
- Impact on Local Aquaculture
- The Ministry aims to double aquaculture production by 2030

Tab. 6 Number of new fish farms in North Macedonia where Infectious Hematopoietic Necrosis (IHN) was detected by year

Year	
2020	17
2021	3
2022	7
2023	6
2024	3

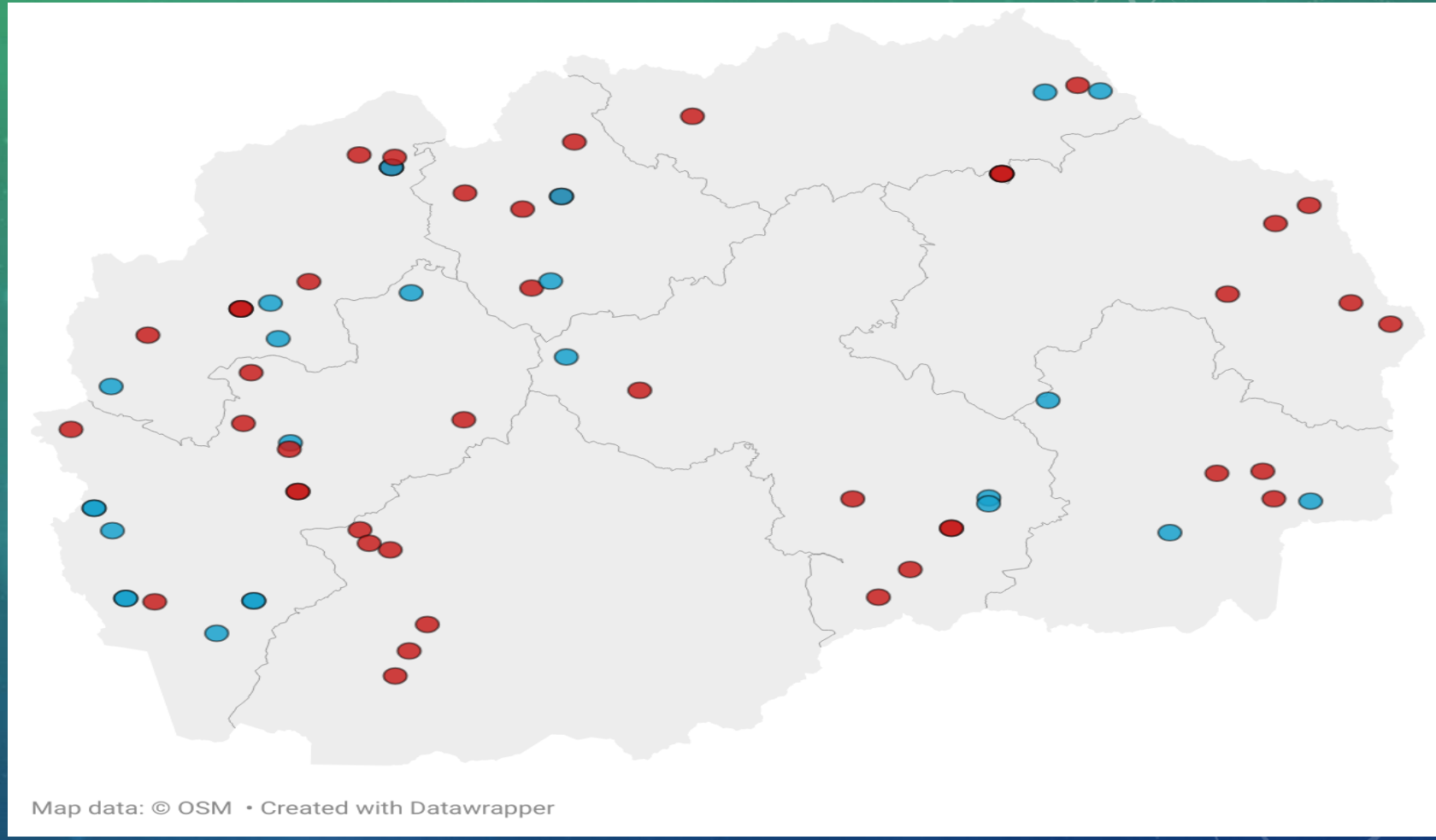


Fig. 6 Map of North Macedonia showing the distribution of fish farms in relation to the detection of Infectious Hematopoietic Necrosis (IHN). Red dots mark fish farms where IHN has been detected, while blue dots indicate farms where the virus has not been found.

Total: 36 IHN-infected fish farms out of 50

3. RESULTS

- The GenBank BLAST sequence was most similar to the IHN isolate MAKIHNV1, isolated from North Macedonia in 2018 (MN641902)
- Similarity of 99.74% (20-8197-6, 20-8197-5, 20-8197-1 and 20-8197-9) and 99.66%
- All isolates were placed in clade E-1 of European genogroup E
- Molecular tracing has identified the IHNV strain present in North Macedonia as part of the European E genogroup, specifically the E-1 clade. This classification aligns it with similar isolates from countries such as Germany, Italy, and Iran

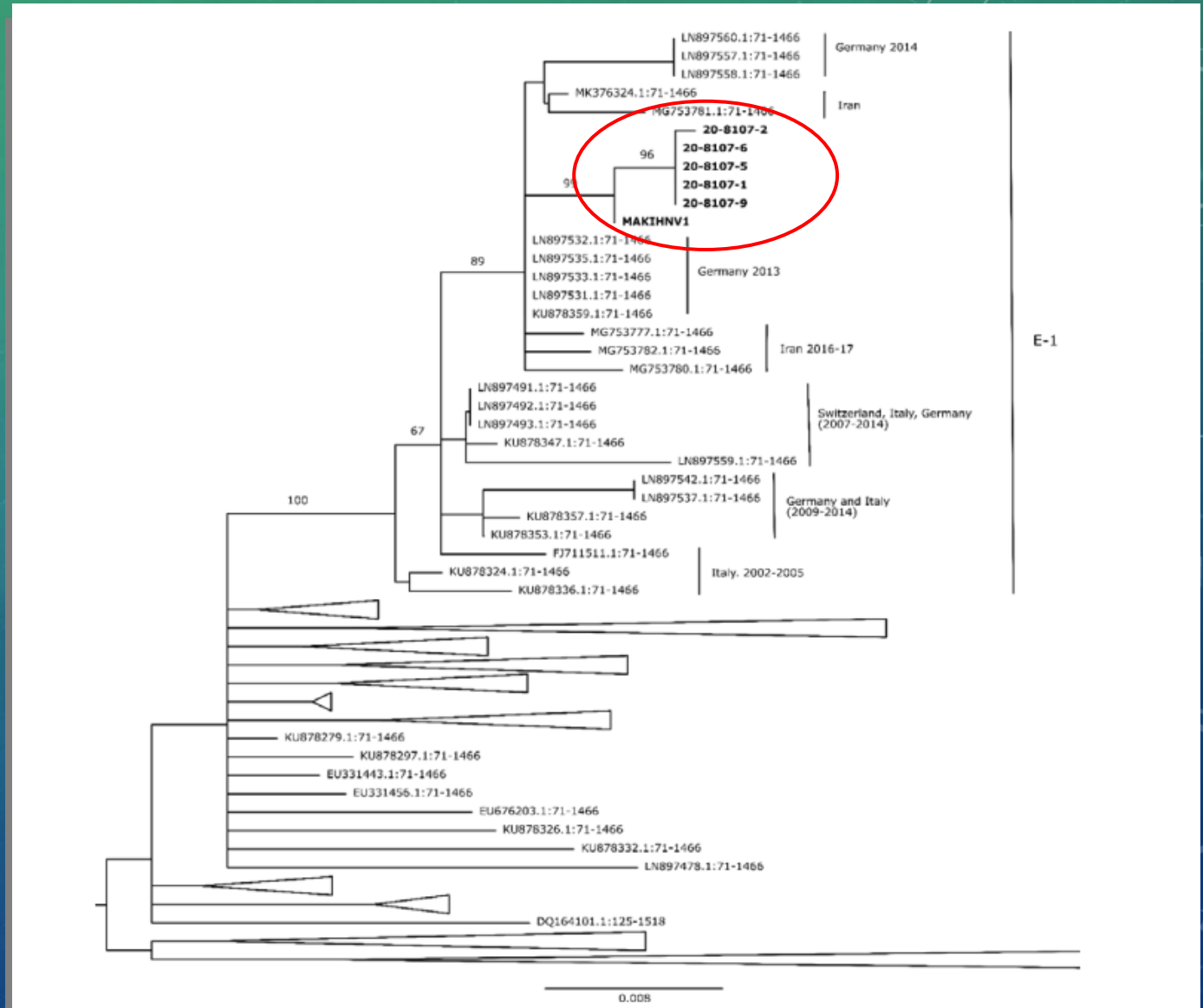


Fig. 7 Phylogenetic analysis of IHN isolates based on the G gene

4. CONCLUSION

- The distribution of IHN in the Republic of North Macedonia
- How did the virus enter?
- Poor suppression methodology
- Biosecurity measures

Fig. 8 Gross pathology of IHN

5. FUTURE CHALLENGES

- Increasing the sample size in future studies
- Continued research into the epidemiology of IHNV
- Genetic studies of IHN strains
- Impact of IHN on autochthonous species
- Monitoring open waters
- Collaboration between stakeholders - public awareness and education

Fig. 9 Collecting samples from fish in open waters


THANK YOU