IHN outbreak in rainbow trout farmed in Saltwater in Croatia

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FARMING OF RAINBOW TROUT IN SALTWATER

- several attempts to establish farming of rainbow trout in the Adriatic Sea, mostly unsuccessful
- during the summer temperatures are up to 25°C causing low oxygen saturation
- Velebitski Kanal was evaluated as one of several suitable sites for this project
- area with an abundant inflow of submerged rivers supplying marine environment with cold freshwater making this site suitable for rainbow trout farming

Lesson learnt from past:

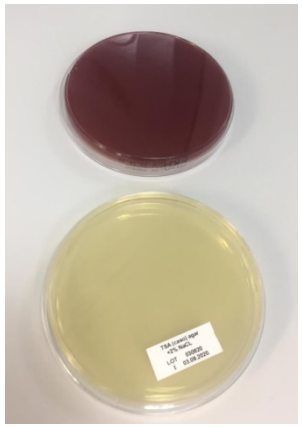
- rainbow trout should be placed into cages during the favourable weather during winter time (no wind, low sea temperature
- very good growth rate
- Desirable flavour of the market size fish
- Depletion of oxygen during the summer months – trigger for different bacterial diseases

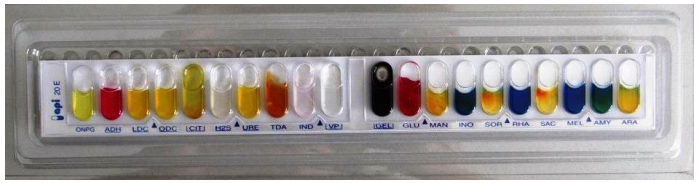
POLAND

DENMARK

DIAGNOSTICS

Bacteriology

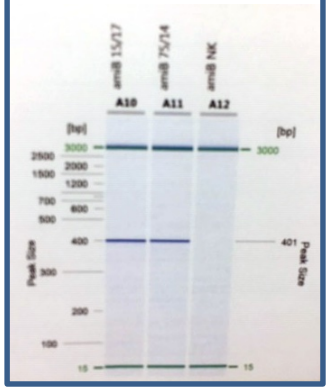




Vibrio spp.

Bacterial DNA was isolated from pure culture using NucleSpin Microbial DNA kit (Macherey Nagel, Germany) according to the manufacturer instructions

amiB gene



16SrRNA + sequencing

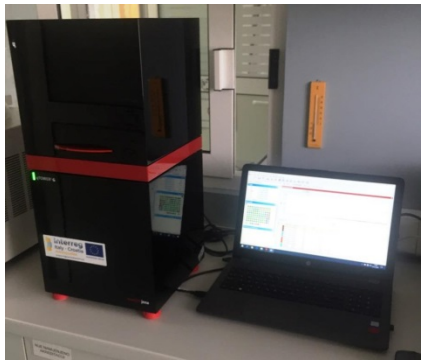
Vibrio anguillarum

Virology

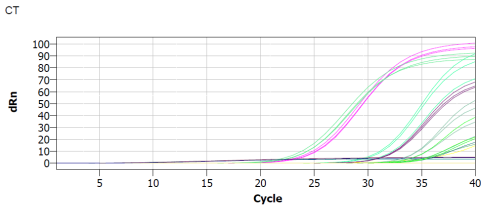
Viral RNA was extracted on KingFisher Duo Prime Purification System (Thermo Scientific) using MagMAX CORE Nucleic Acid Purification Kit following the manufacturers simple workflow instructions for processing animal swab samples

RT-q-PCR for detection of VHSV according to Commission decision 2015/1554

VHSV negative



Modified Purcell et al. (2013) single step RT-q-PCR for detection of IHNV



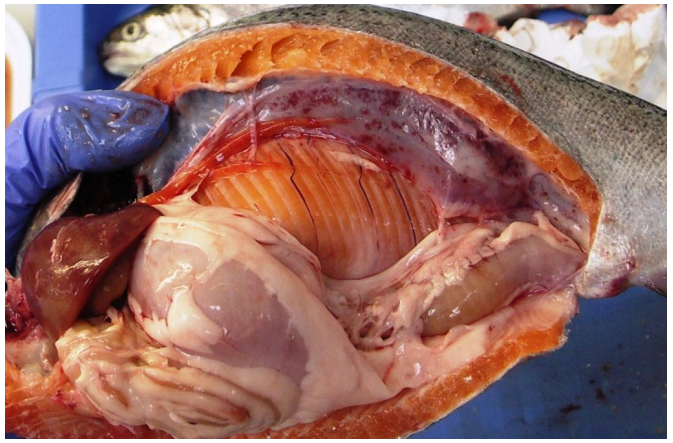
C11	PK IHN 18/3 p.t	Positive control	FAM		24.53	24.35
A10	57/20-5 bris	Unknown	FAM		31.62	30.9
A11	57/20-5 bris	Unknown	FAM		30.68	30.9
A12	57/20-5 bris	Unknown	FAM		30.42	30.9
E7	57/20-1 bris	Unknown	FAM		31.9	31.91
E8	57/20-1 bris	Unknown	FAM		32.01	31.91
E9	57/20-1 bris	Unknown	FAM		31.83	31.91
F7	57/20-2 bris	Unknown	FAM		23.57	23.43
F8	57/20-2 bris	Unknown	FAM		23.64	23.43
F9	57/20-2 bris	Unknown	FAM		23.09	23.43
G7	57/20-3 bris	Unknown	FAM		34.03	34.34
G8	57/20-3 bris	Unknown	FAM		34.9	34.34
G9	57/20-3 bris	Unknown	FAM		34.09	34.34

Notification of IHNV presence in saltwater enviroment to CA

- Official visit to the farm and sampling of fish in different cages; C1(10), C2(7), C3(8),C4(5)
- Specimens submitted to the laboratory weighed 50-300 grams

Hemorrhages on the skin of the opercula, vent, fin bases

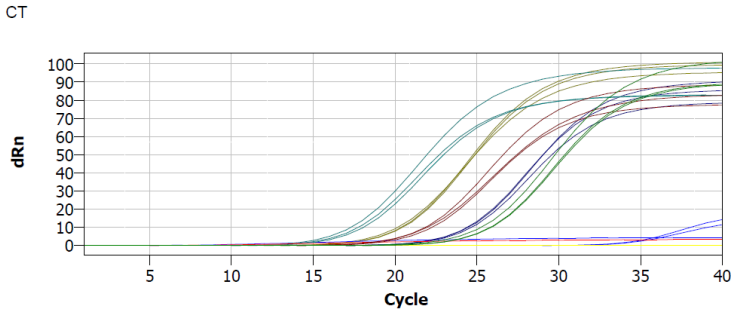
In the mouth



Dark gills and liver, hemorrhages in pyloric caeca, liver, enlarged spleen, swim bladder

DIAGNOSTIC PROCEDURES:

- Fish from each cage were considered as a pool
- Material from organs was innoculated on EPC and BF 2 cell lines and CPE appeared 6^{th} day after inoculation for sample C1, & C4 on EPC and sample C3 on both EPC and BF2 while sample C2 was negative even after subcultivation in new cell culture
- Positive supernatants were tested for the presence of VHSV, IHNV and IPNV using commercial ELISA kits (TestLine, Czech Republik; BioX, Belgium)
- Samples C1 & C4 tested positive for IHNV while C3 tested positive for IPNV
- ELISA IHNV positive samples were tested using RT-qPCR (single step modified protocol by Purcell et al. 2013)
- ELISA IPNV positive sample was tested using end-point PCR targeting VP2/NS junction region (Heppell et al. 1992)

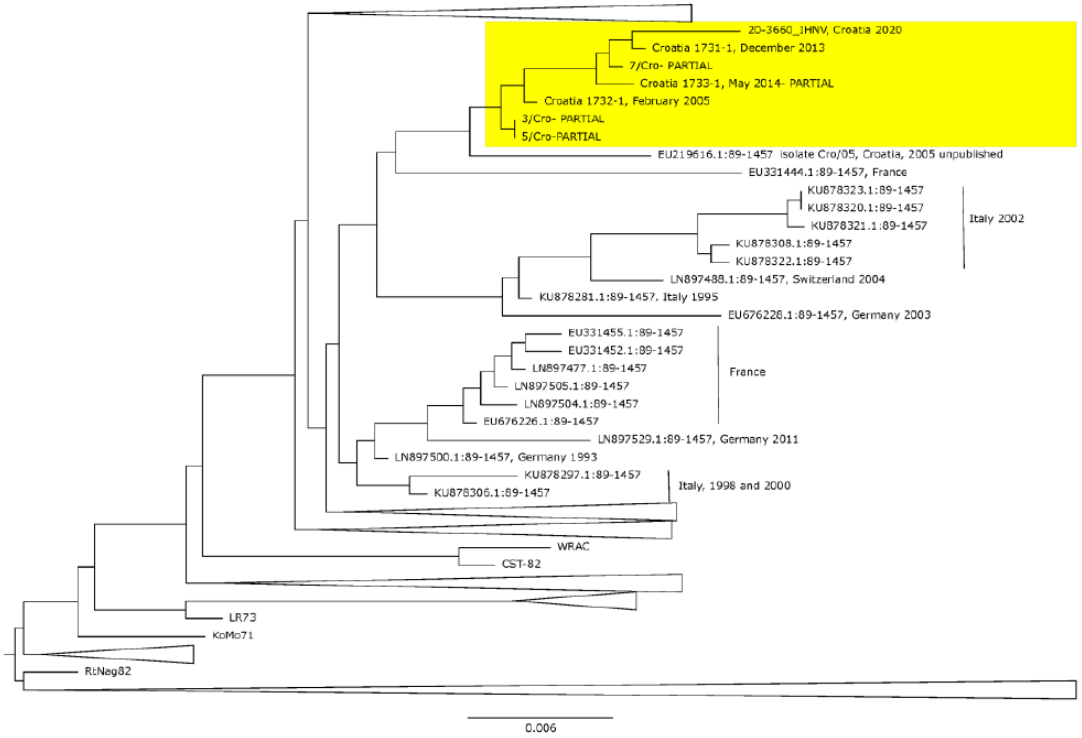


Well		Sample name	Sample type	Dye	Gene	Ct	Mean Ct
A1		61/20-1 E	Unknown	FAM		19.03	19.22
A2		61/20-1 E	Unknown	FAM		19.27	19.22
A3		61/20-1 E	Unknown	FAM		19.34	19.22
B1		61/20-3 E	Unknown	FAM		No Ct	
B2		61/20-3 E	Unknown	FAM		36.38	36.65
B3		61/20-3 E	Unknown	FAM		36.92	36.65
C1		61/20-4 E	Unknown	FAM		23.29	23.39
C2		61/20-4 E	Unknown	FAM		23.53	23.39
C3		61/20-4 E	Unknown	FAM		23.34	23.39
D1		61/20-1 B	Unknown	FAM		16.17	16.6
D2		61/20-1 B	Unknown	FAM		16.63	16.6
D3		61/20-1 B	Unknown	FAM		16.99	16.6
E1		61/20-3 B	Unknown	FAM		No Ct	
E2		61/20-3 B	Unknown	FAM		No Ct	
E3		61/20-3 B	Unknown	FAM		No Ct	
F1		61/20-4 B	Unknown	FAM		20.7	20.83
F2		61/20-4 B	Unknown	FAM		21.11	20.83
F3		61/20-4 B	Unknown	FAM		20.7	20.83
G1		NTC	NTC	FAM		No Ct	
G2		NTC	NTC	FAM		No Ct	
G3		NTC	NTC	FAM		No Ct	
H1		PK	Positive control	FAM		24.11	24.52
H2		PK	Positive control	FAM		24.71	24.52
H3		PK	Positive control	FAM		24.73	24.52

- amplification and sequencing of „mid-G“ region of the G gene of the IHN virus (Kolodziejek et al. 2008) confirmed the identification and showed similarity to CRO/05 in Genbank

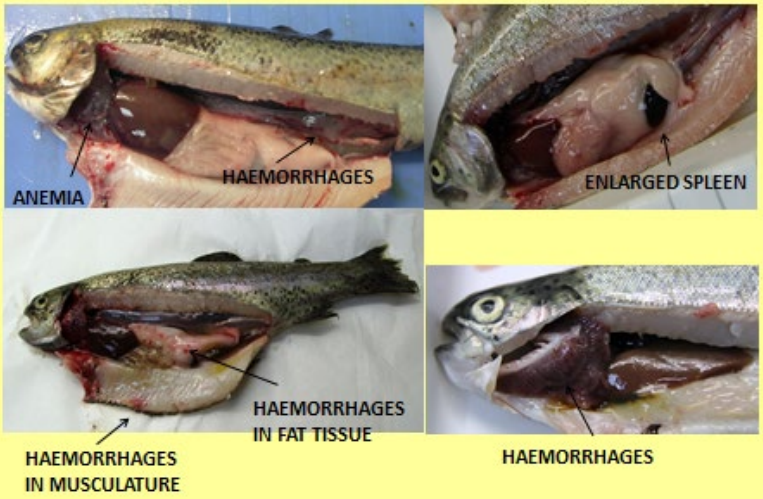
Results of sequencing and phylogeny

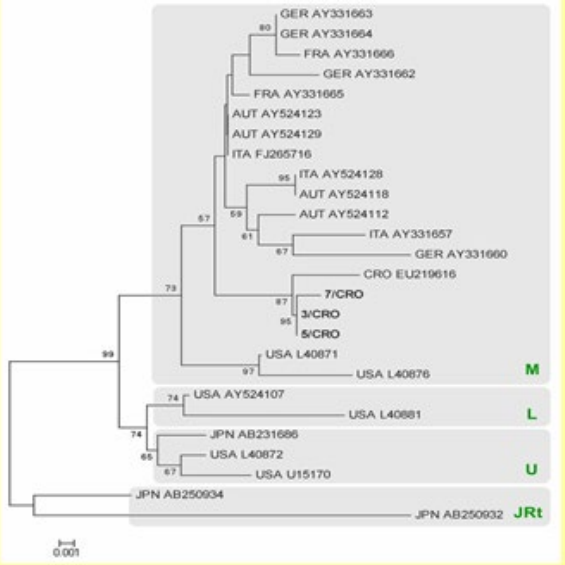
- Different samples (organ suspension, supernatant from EPC cell lines) were sent to EURL for confirmation – all sequences were the same – IHNV genotype E



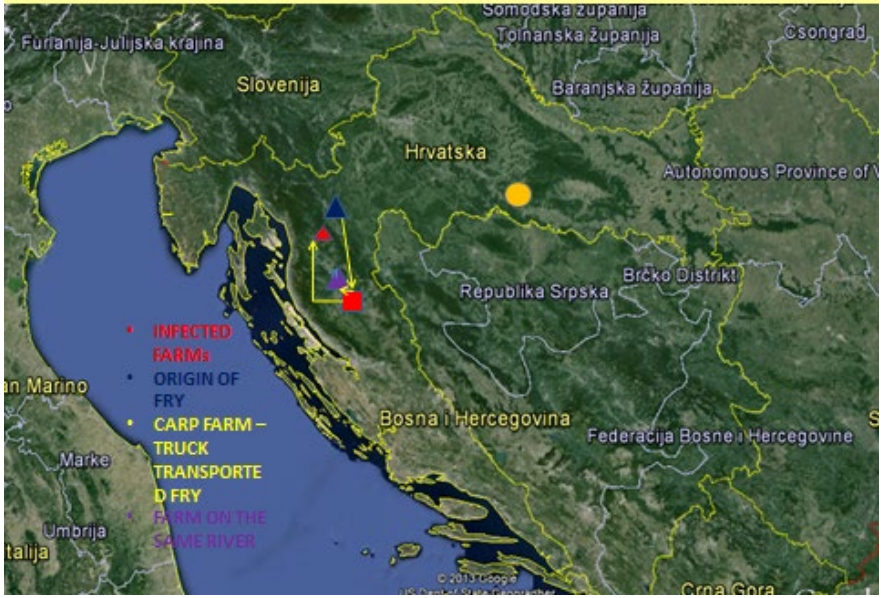
- Comparison of sequences available in Genbank database and sequences form unpublished Croatian outbreaks in 2005, 2013 &2014 confirmed this isolate is the closest to CRO/05 but also closely correlates with other national isolates

Findings



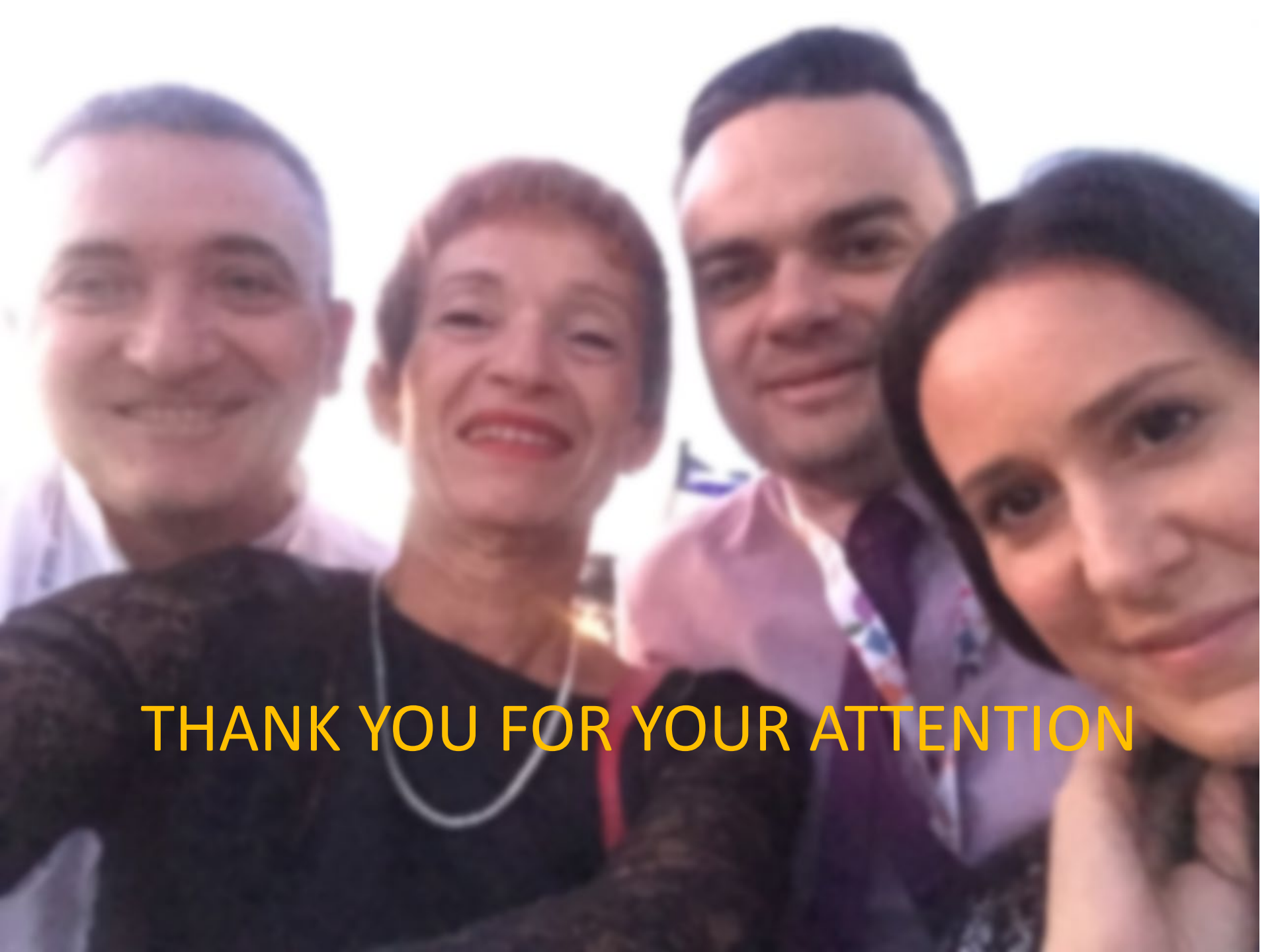


MOVEMENT OF FISH AND VEHICLE



Conclusion:

Based on the data obtained by partial sequencing of glycoprotein gene are suggesting the possibility of circulation of the virus within the country rather than being imported from other countries


THANK YOU FOR YOUR ATTENTION