



SURVEILLANCE PROGRAMME

Based on the National legislation

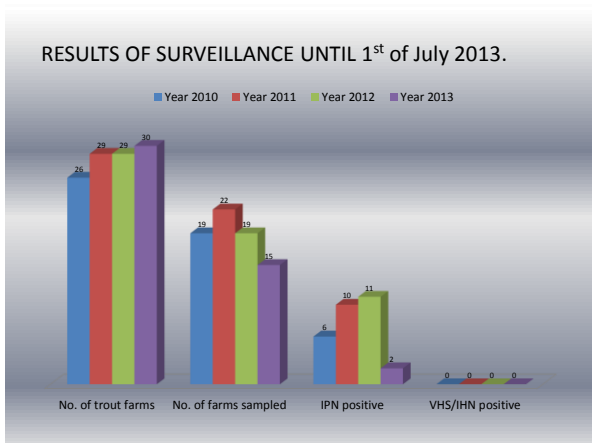
- Ordinance on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals (OG 42/08, 36/10) which is fully aligned with **Council Directive (EC) No 88/2006**
- "Order on measures to protect animals from infectious and parasitic diseases and the financing thereof", issued by ministry of Agriculture, Veterinary Directorate and published yearly in Official Gazette

Purpose of programme

- Achieving disease free status on all farms
- To assure conditions for free trade of live fish
- Protection of aquaculture capacities in Croatia

Salmonid farms

- Viral Hemorrhagic Septicemia, Infectious Hematopoietic Necrosis
- Financed from national budget
- In 2013 most farms had conditions for disease free status



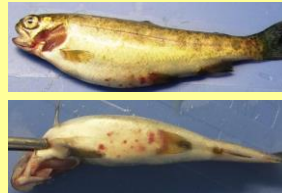
JULY 2013



- Small family run farm producing 23 t of rainbow trout
- Selling fish to the restaurant in region and to Zagreb
- Preparing and selling fish on farm

- Owner noticed high mortalities in two raceways with fry weighing about 60 grams
- He notified official vet who sampled affected fish and sent samples to NRL
- Samples were submitted to standard laboratory procedure; external examination, parasitological investigation, necropsy and internal examination, bacteriological and virological examination, fixation of tissues for histology

EXTERNAL SIGNS



- ✓ exophthalmia
- ✓ pale gills
- ✓ distended abdominal wall with hemorrhages on the skin
- ✓ hemorrhages in the eye

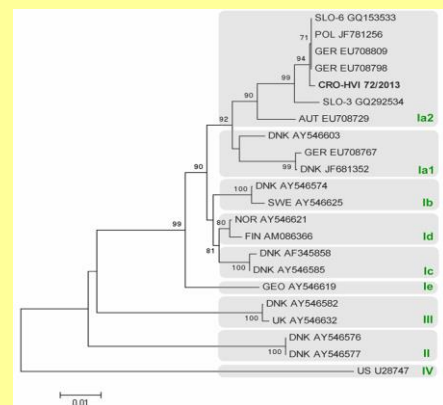
INTERNAL CHANGES



- ✓ hemorrhages on the liver
- ✓ pyloric caeca
- ✓ swimbladder intestine
- ✓ dorsal musculature
- ✓ enlarged spleen

RESULTS OF LABORATORY EXAMINATION

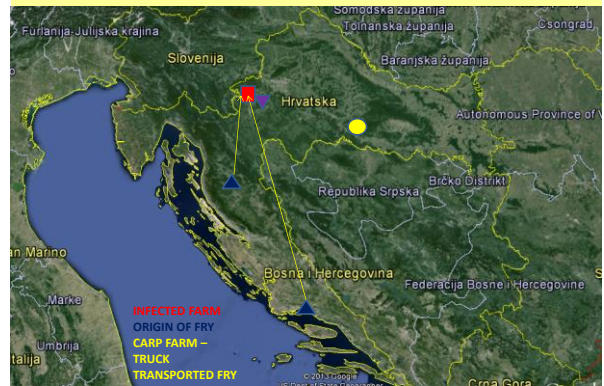
- Bacteriological examination was negative
- Material from organs was tested on EPC and BF 2 cell lines and CPU appeared 5th day after inoculation
- Supernatant was tested by VHS, IHN and IPN ELISA ;
- **VHSV positive, finding confirmed by RT-PCR**
- **Isolate characterized by partial sequencing of nucleocapsid (N) gene and glycoprotein (G) gene**
- Results were confirmed by EURL and IZSVE (full length G-gene)



EPIDEMIOLOGICAL INVESTIGATION

- Diagnosis notified to CVO
- Case reported to OIE, EC
- Immediate epidemiological investigation: fry bought from two Croatian farms (both with brodstock and hatchery)
- On the river, flowing into - another small farm
- Measures lifted on all farms: sampling, movement restriction and control, inspection of vehicle transported fish – determining routes of movement, disinfection records, sampling of susceptible species

MOVEMENT OF FISH AND VEHICLE



ALL SAMPLES FROM FARMS WITH MEASURES LIFTED
EXCEPT INFECTED ONE WERE NEGATIVE.

WHAT WAS THE ORIGIN OF INFECTION?



ILLEGAL TRADE???

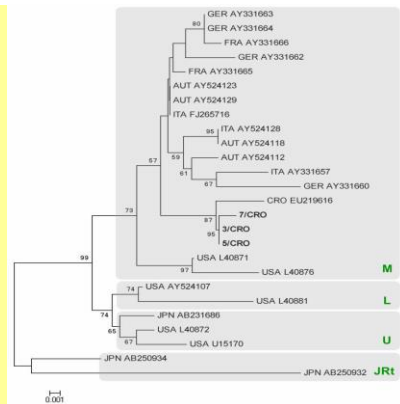
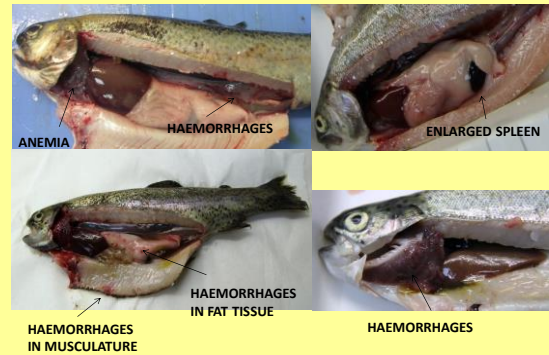
DECEMBER 2013

- Samples from newly established farm were submitted through surveillance programme
- 2 out of 15 pools were positive on cell cultures, subsequent VHS, IHN, IPN ELISA revealed presence of IHN virus
- Diagnosis was confirmed by RT-PCR
- isolate characterisation by sequencing extended mid G region (Kolodziejek et al. 2008)

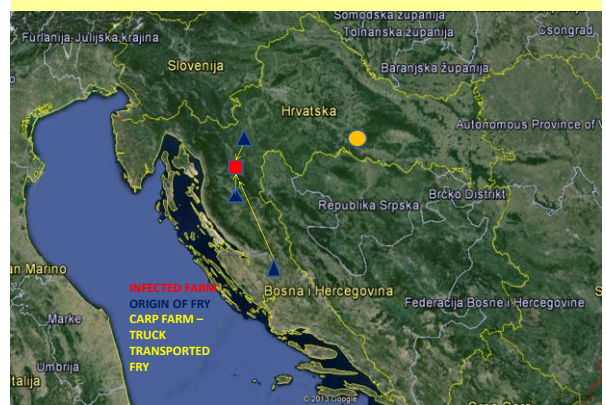
- Inspection on the farm
- Mortalities in rainbow trout weighing 160 -200 g
- Farm manager tried to cure fish by antimicrobial treatment
- Epidemiological investigation was performed and same measures lifted as in previous outbreak of VHS



Findings



MOVEMENT OF FISH AND VEHICLE



- Virological examination of samples from suspicious farms was negative
- Again: what is the source of infection???
- **Illegal movement or something else???**
- Sequencing revealed that IHNV isolate from 2013. (CRO-7), 2004. (CRO-5) and Slovenian isolate (CRO-3) showed similarity

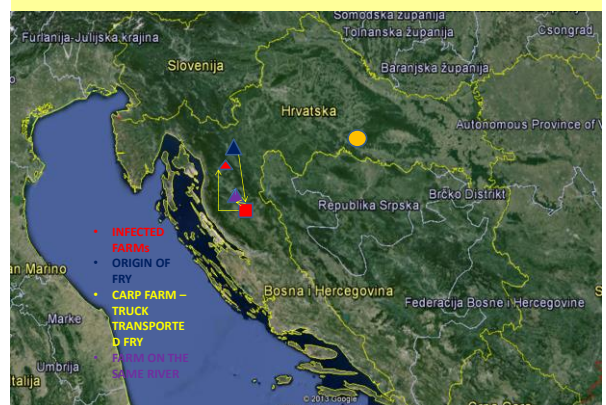
MAY 2014.



Reprocenter for repopulation of beautiful kartsic river with long tradition of angling local brook trout
Samples were collected for routine surveillance programme

- **3 out of 15 pools were positive on cell cultures**, subsequent VHS, IHN, IPN ELISA revealed presence of **double infection with IHN and VHS** viruses
- There were no clinical signs except gill anemia and exophthalmia
- **Diagnosis was confirmed by RT-PCR**
- Epidemiological investigation – all movements of fish into farm and from farm were studied (fig.)
- Measures were lifted

MOVEMENT OF FISH AND VEHICLE

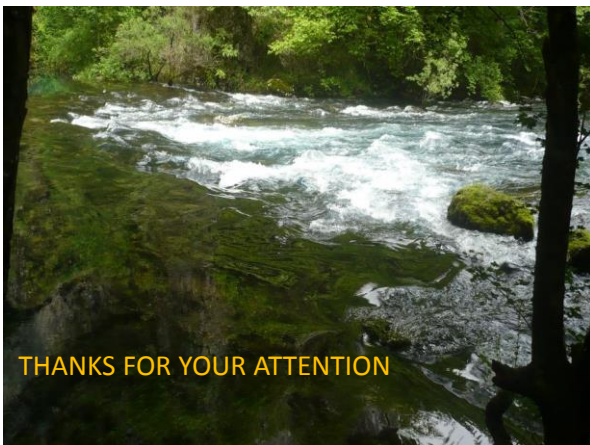


LINKAGE OF ALL OUTBREAKS

- Same truck owner in all cases ???
- Fish transported by same vehicle and the same driver!

ERADICATION PLANS

- Farm owners show interest for eradication – planned for summer 2014
- Financial aspect of outbreak – all applied measures and eradication should be covered by owner



THANKS FOR YOUR ATTENTION