



MINISTARSTVO POLJOPRIVREDE
Uprava za veterinarstvo i sigurnost hrane

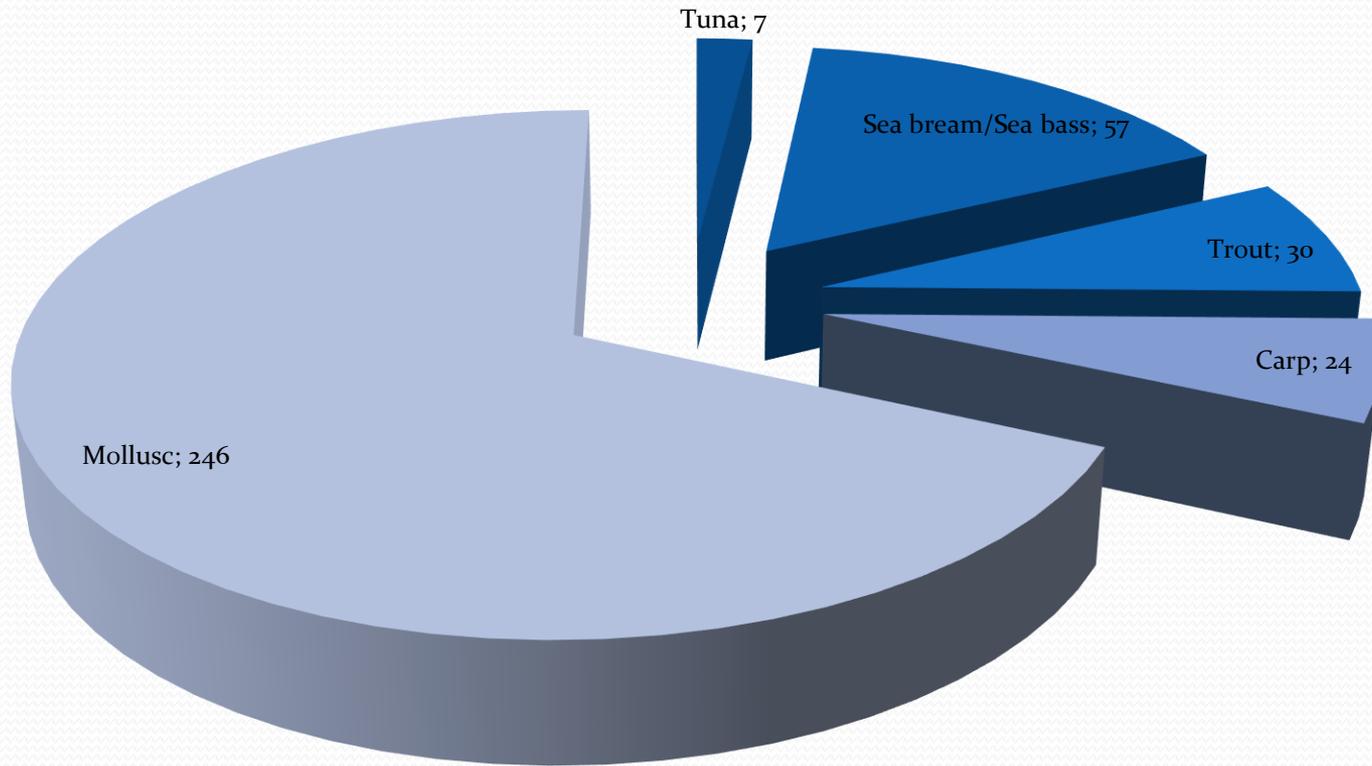
Hrvatski veterinarski institut

Aktualno stanje s bolestima riba u uzgoju u RH - prijetnje iz EU i trećih zemalja

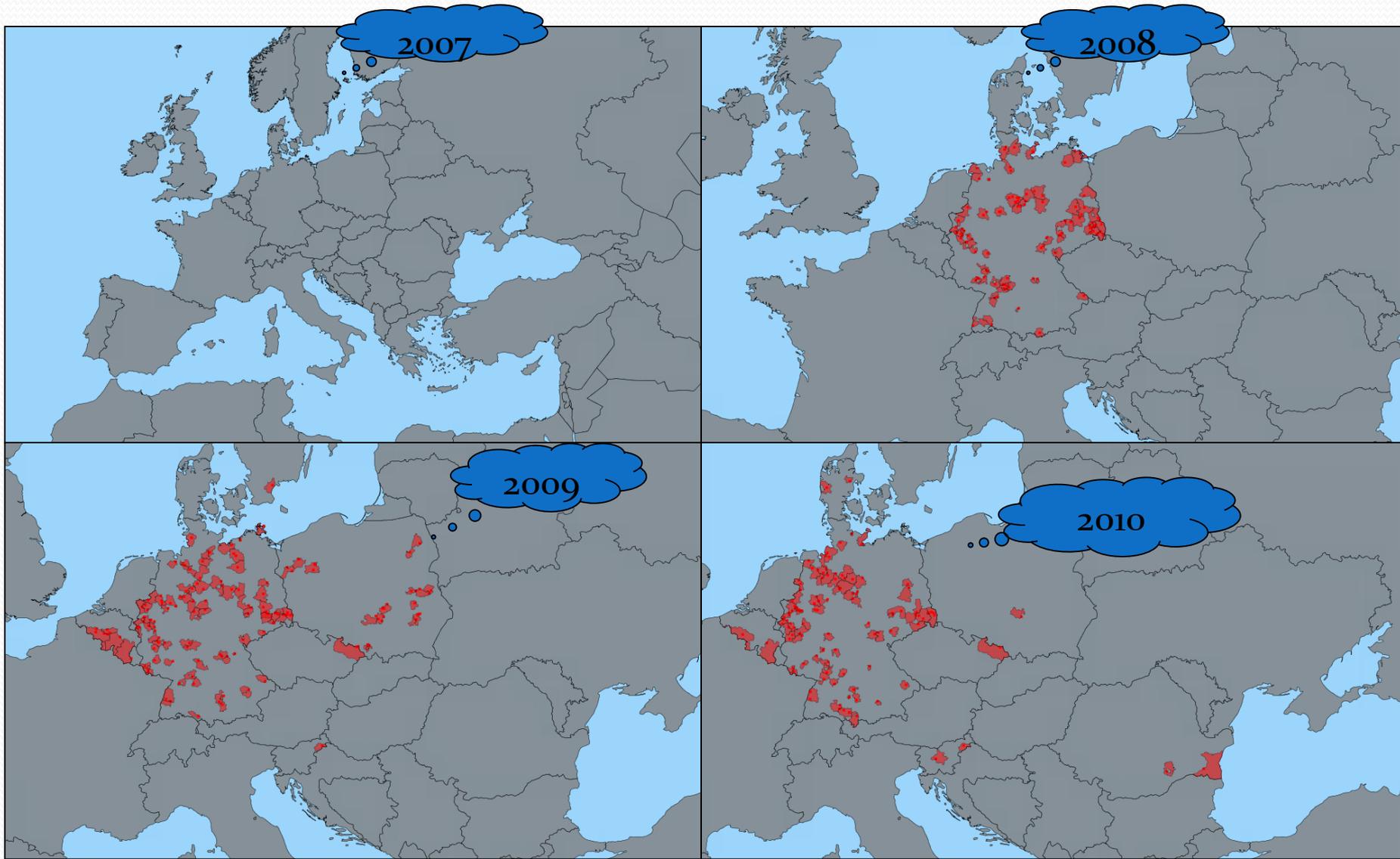
A map of Europe with the borders of countries outlined in black. Croatia is highlighted in a bright green color. The text is overlaid on the map.

**KHV, VHS, ZHN &
VER/VNN AND EMERGING DISEASES**

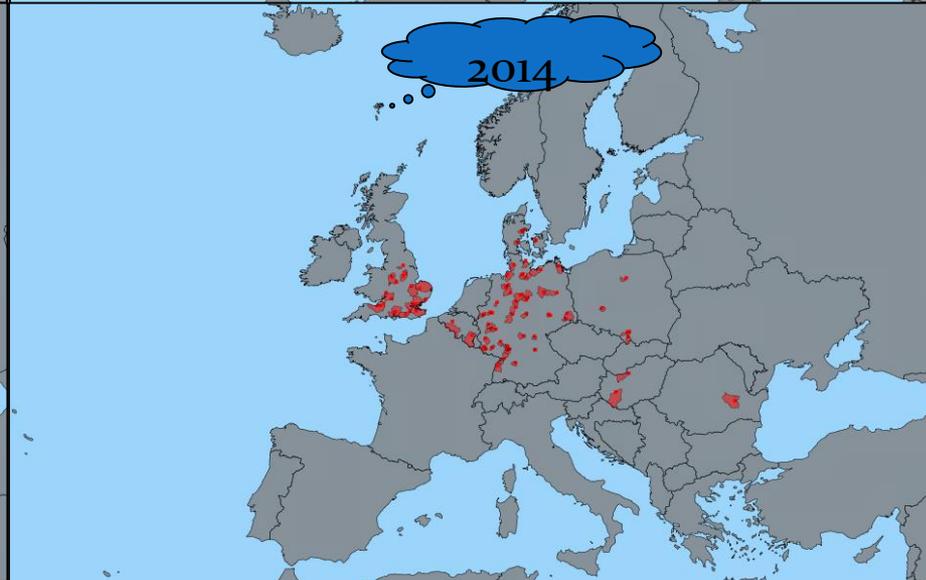
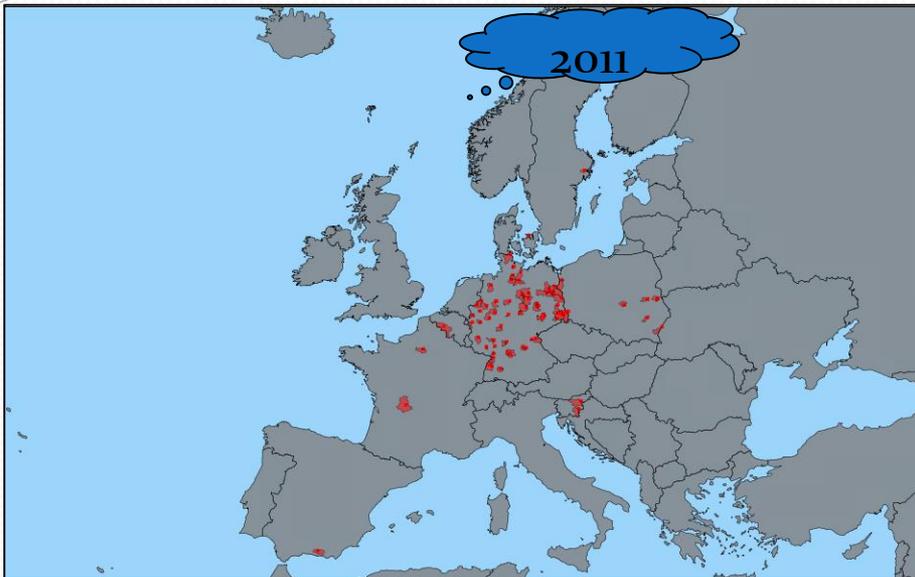
Farms registered in Croatia



KHV outbreaks in EU



KHV outbreaks in EU

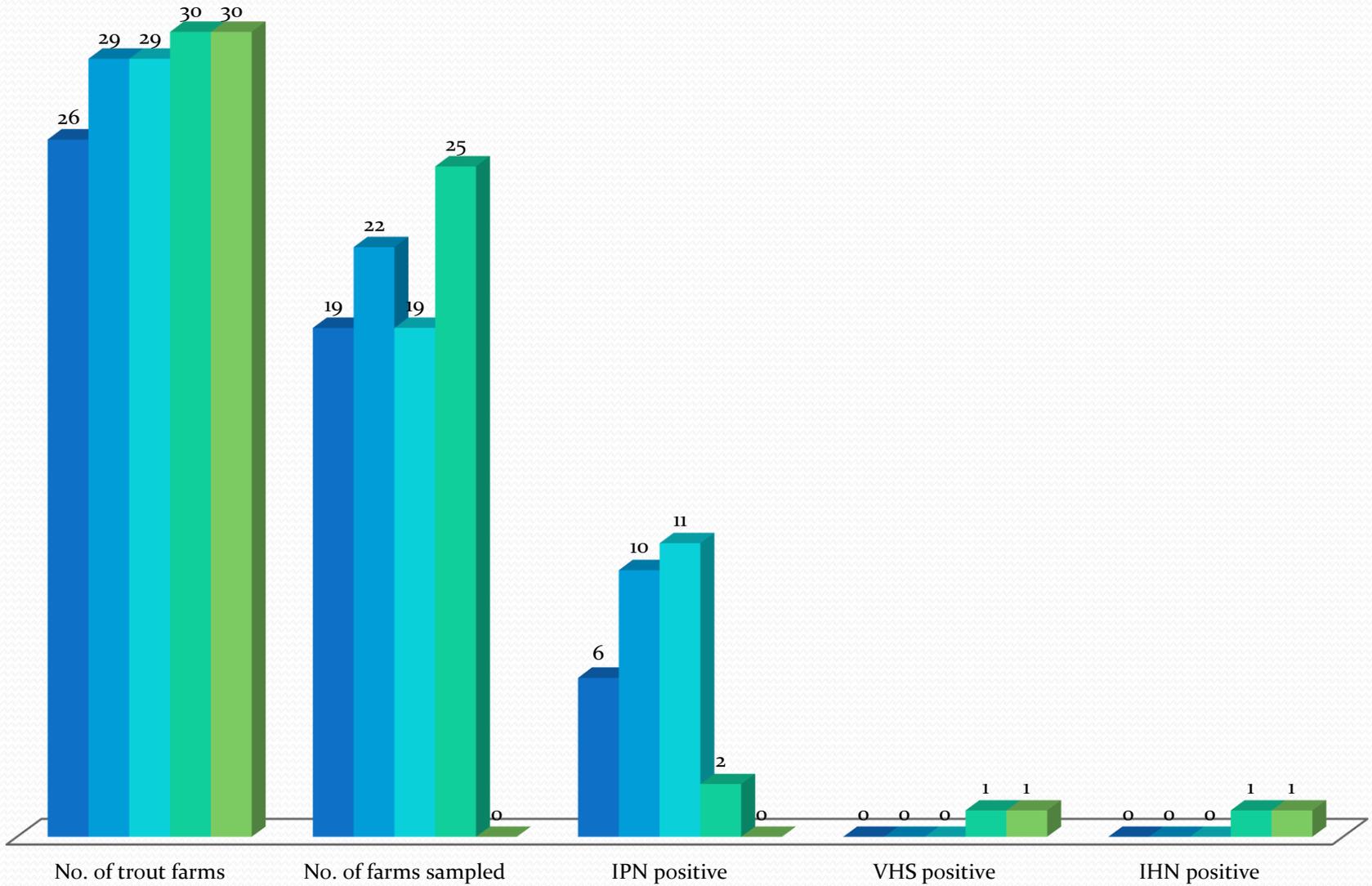


Surveillance programs

- Active
 - VHS/IHN surveillance program on all farm keeping susceptible species
 - KHV surveillance program on all farms keeping susceptible species

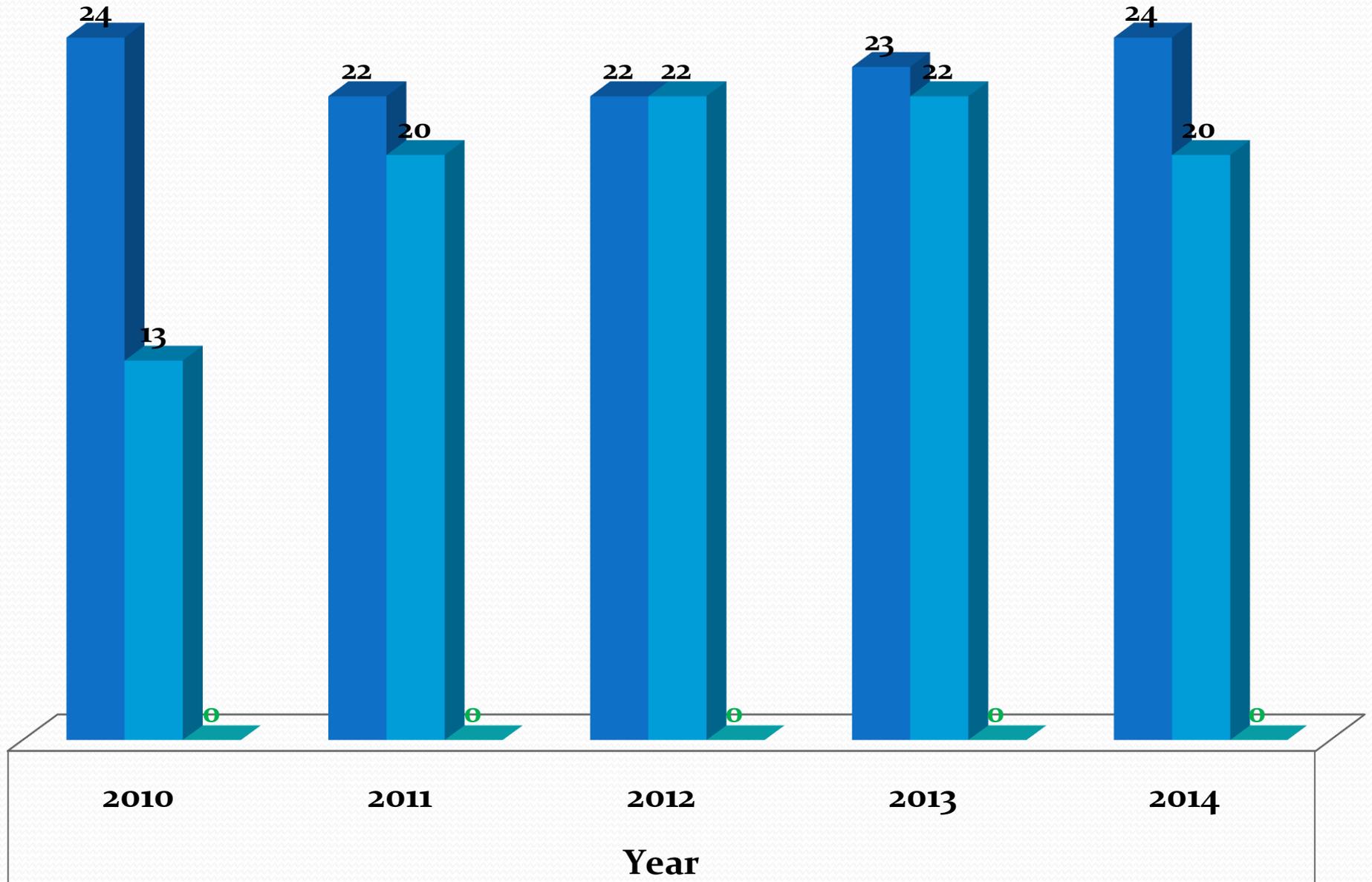
VHS/IHN Surveillance results

■ Year 2010 ■ Year 2011 ■ Year 2012 ■ Year 2013 ■ Year 2014



Results of KHV Surveillance programs

■ No. of carp farms ■ No. of farms sampled ■ KHV positive



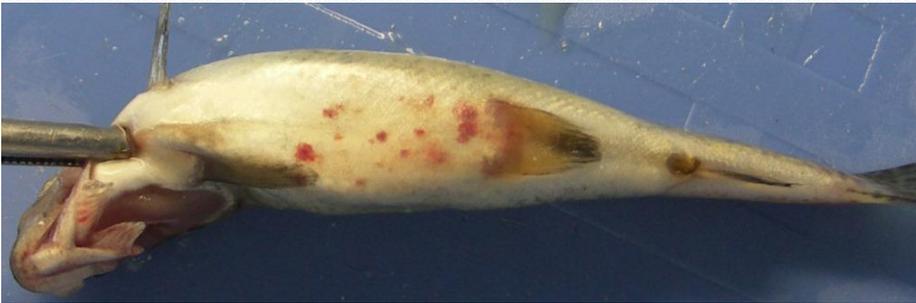
VHS/IHN outbreaks - JULY 2013



- 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



INTERNAL CHANGES



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

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

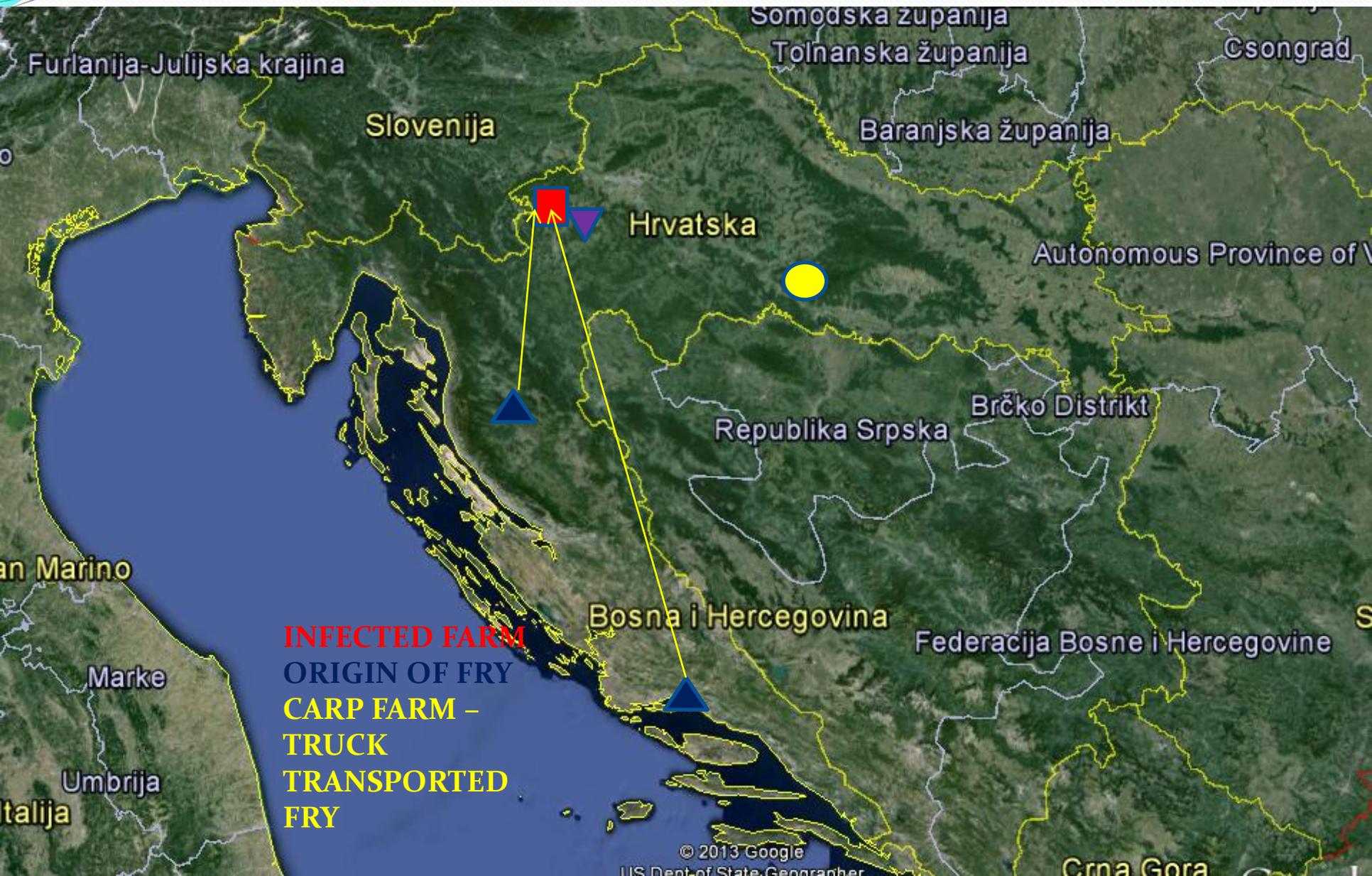
RESULTS OF LABORATORY EXAMINATION

- 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

- 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



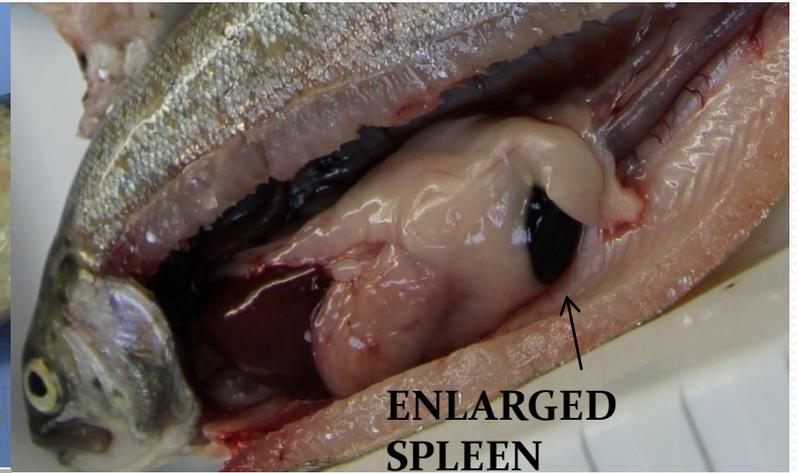
DECEMBER 2013

- 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

- 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
- Virological examination of samples from suspicious farms was negative



Findings

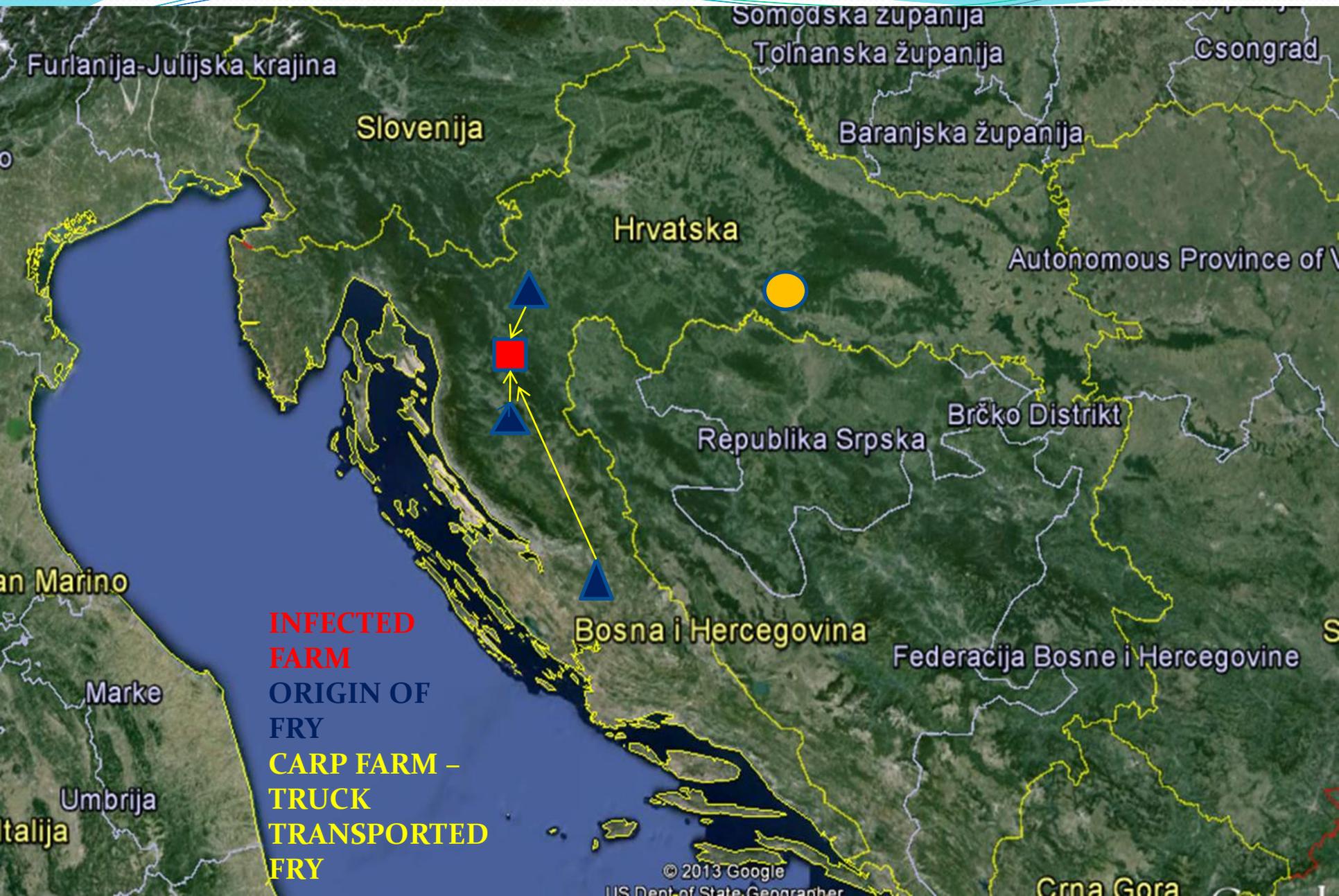


HAEMORRHAGES
IN MUSCULATURE



HAEMORRHAGES

MOVEMENT OF FISH AND VEHICLE

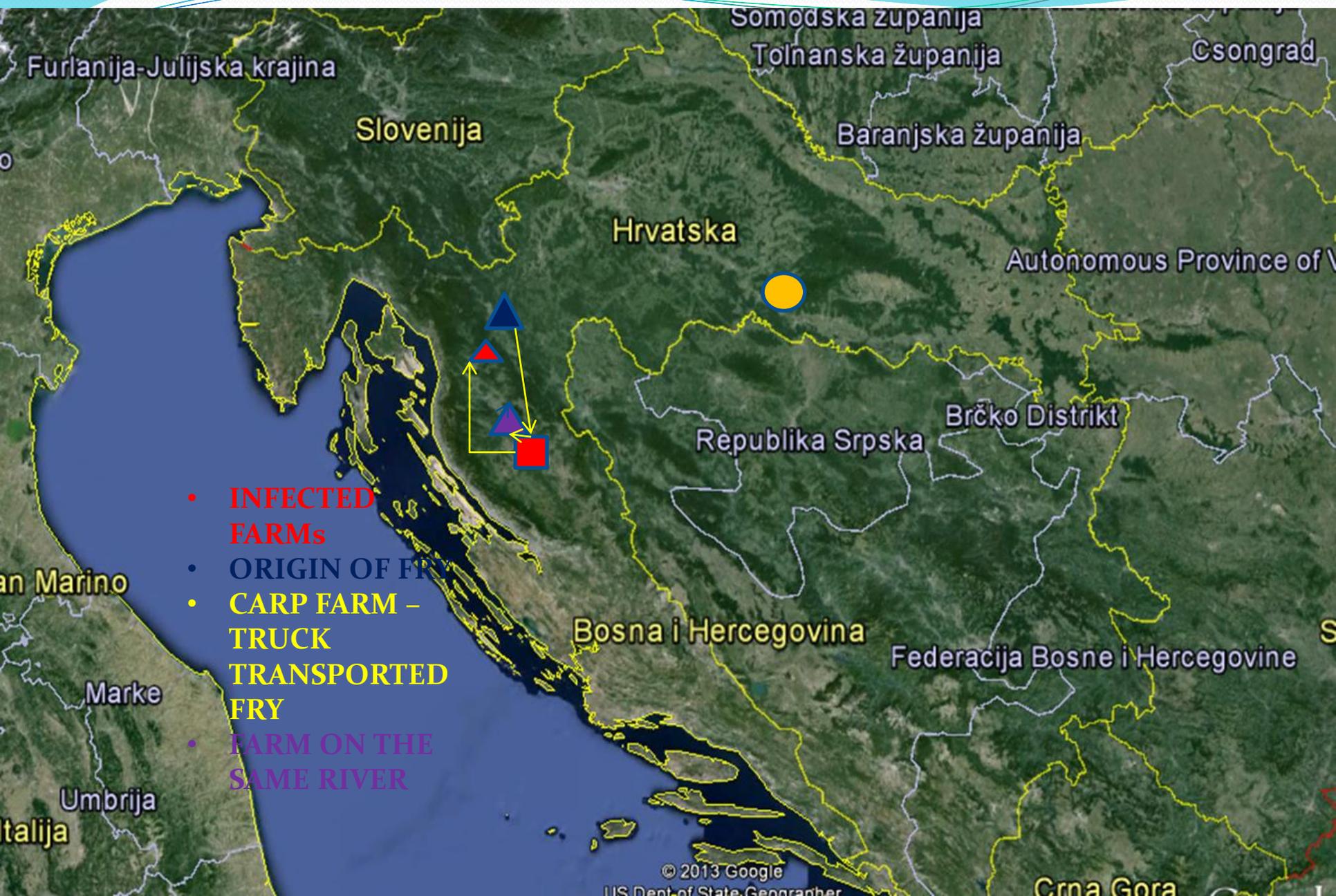




Samples were collected for routine surveillance programme

- 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



- **INFECTED FARMS**
- **ORIGIN OF FISH**
- **CARP FARM – TRUCK TRANSPORTED FRY**
- **FARM ON THE SAME RIVER**

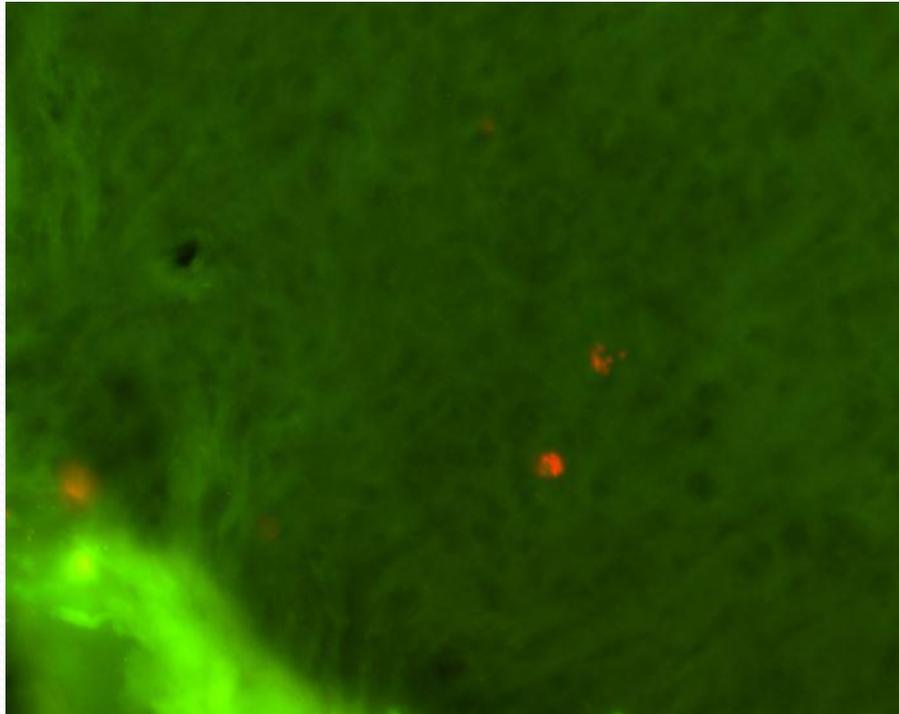
Current and future activities

- Declaration of KHV free country or zones/compartments
- Declaration of Bonamia free production areas
- Declaration of VHS free compartments
- Eradication of VHS in infected zones
 - Testing of fish in wild waters - Kostanjevac, Gacka, Dretulja

NON LISTED & EMERGING DISEASES

- December 2013 – notification of Mt and unusual behavior (uncoordinated, corkscrew swimming) of sea bass fry imported to farm in September
- Corneal opacity and brain hyperemia
- Bacteriological, virusological examination on SSN-1 cell line was negative, qRT-PCR was negative





PCR using primers for
Piscirickettsia
salmonis – POSITIVE
FITC brain histology -
POSITIVE

RICKETTSIA LIKE ORGANISM

- Summer 2014., mid June
- Import of sea bass fry from Italian hatchery and delivered to (?!) 5 marine farms along the coast
- Unusual behavior , uncoordinated, corkscrew swimming, reluctance to eat, eventually die
- Samples submitted to laboratory for diagnostic
- Absence of macroscopic symptoms except congestion of brain and meningeal hyperemia in June
- Brain and eyes tissue on SSN 1 cell line revealed presence of virus, „real time” qRT-PCR for beta noda virus POSITIVE (ct= 11,6)





HVALA NA POZORNOSTI