



MINISTARSTVO POLJOPRIVREDE
Uprava za veterinarstvo i sigurnost hrane

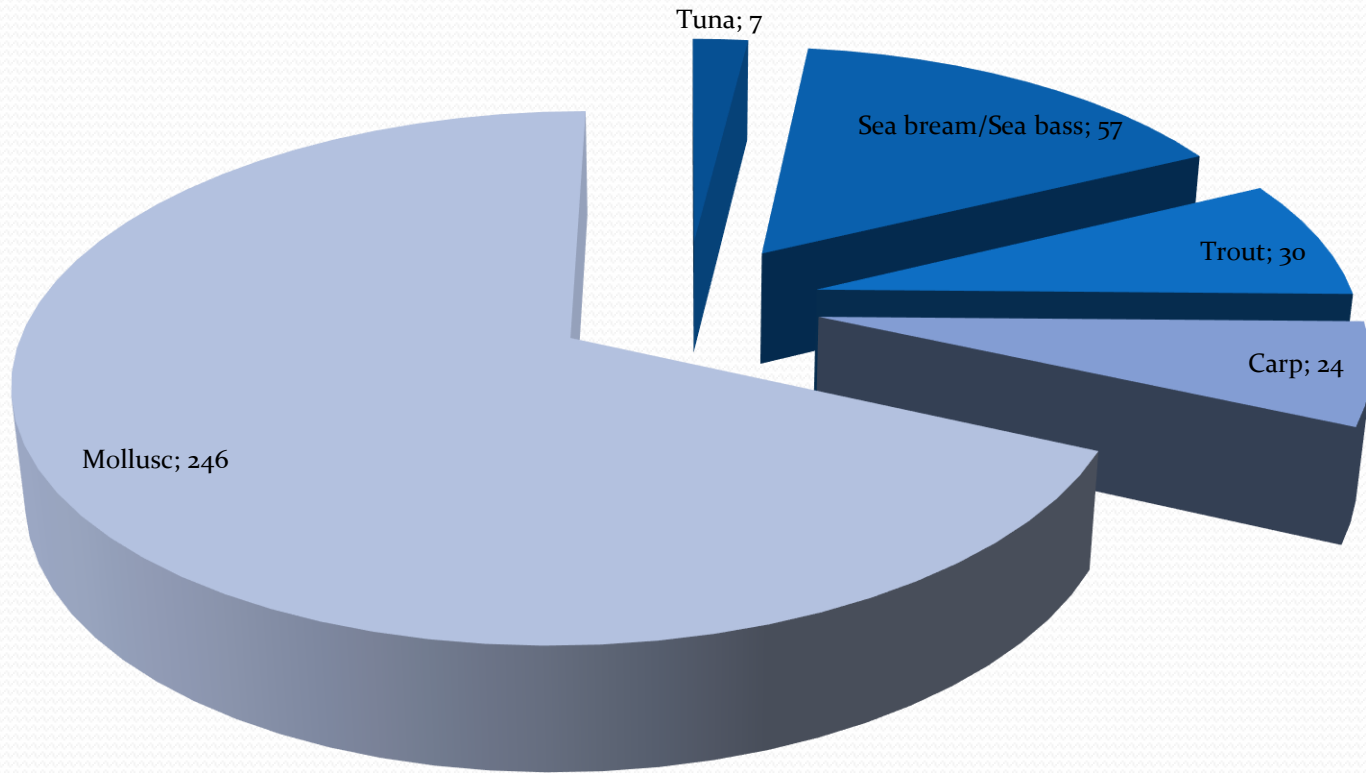
Hrvatski veterinarski institut

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

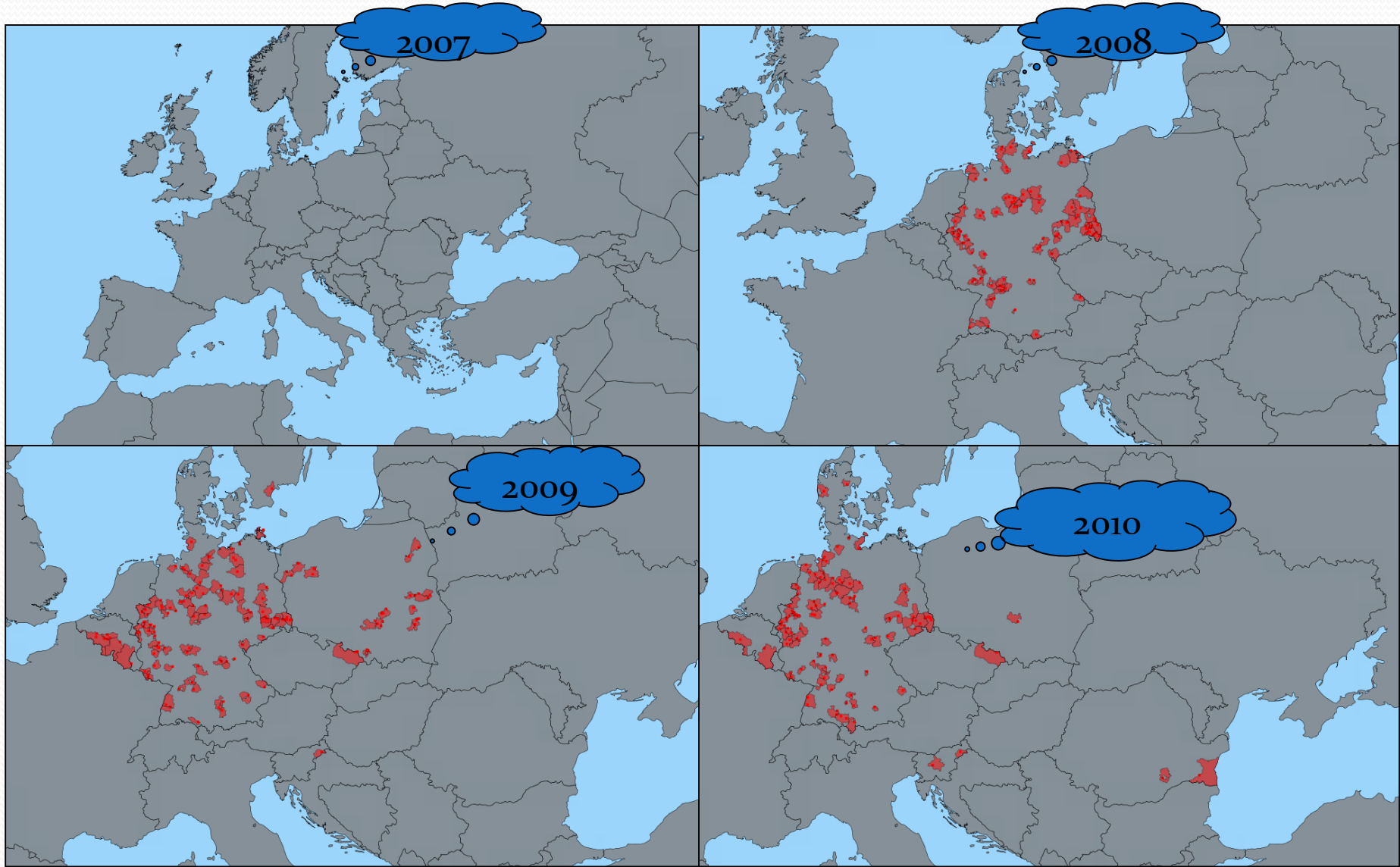
**KHV, VHS, ZHN &
VER/VNN AND EMERGING DISEASES**

A map of Europe with country borders shown in black. The landmasses are a light gray color, and the surrounding oceans are a light blue color. Croatia is highlighted in a bright green color, located in the Balkans region of Europe, between the Adriatic Sea to the west and the Black Sea to the east.

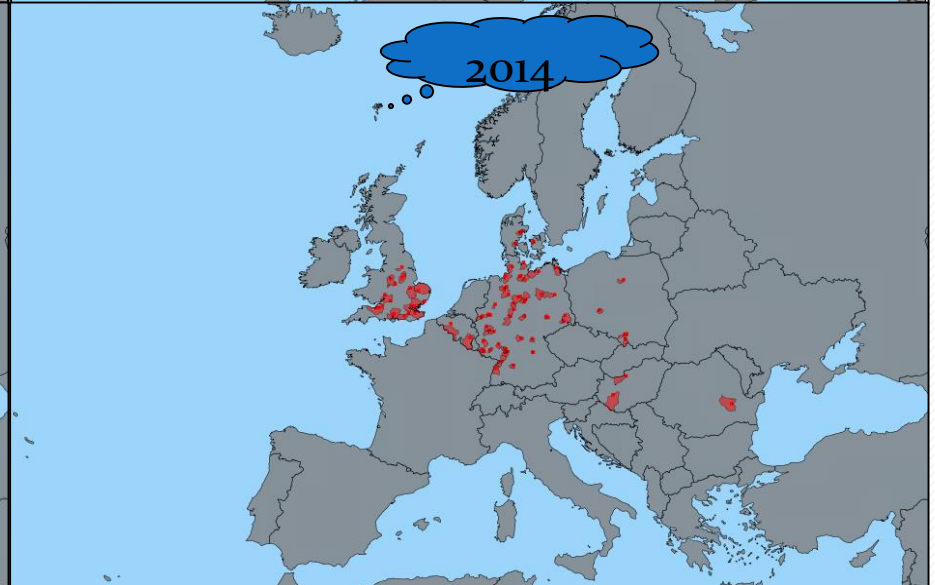
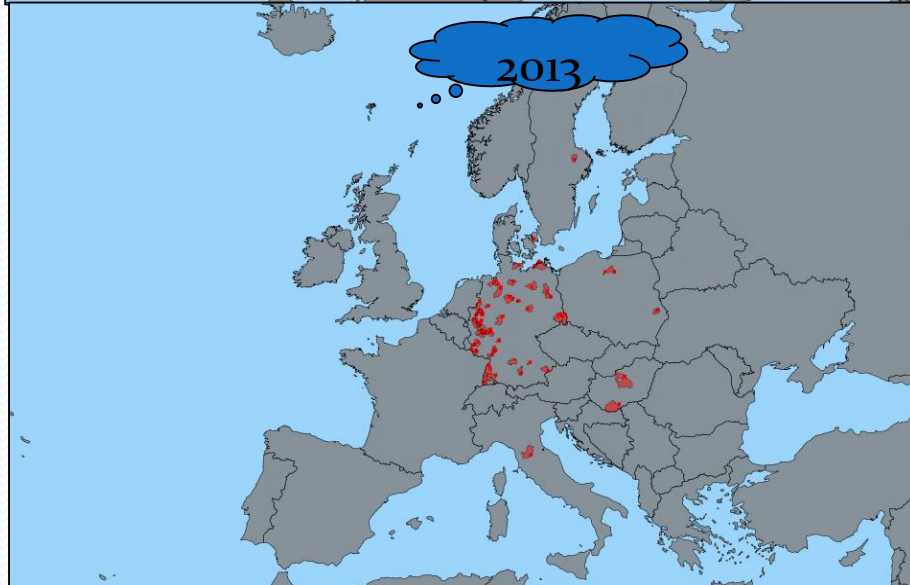
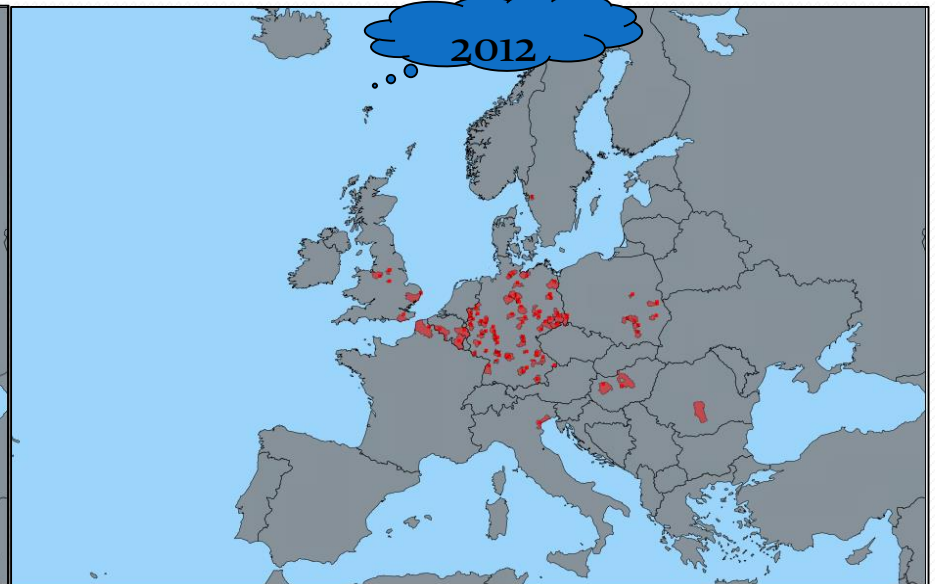
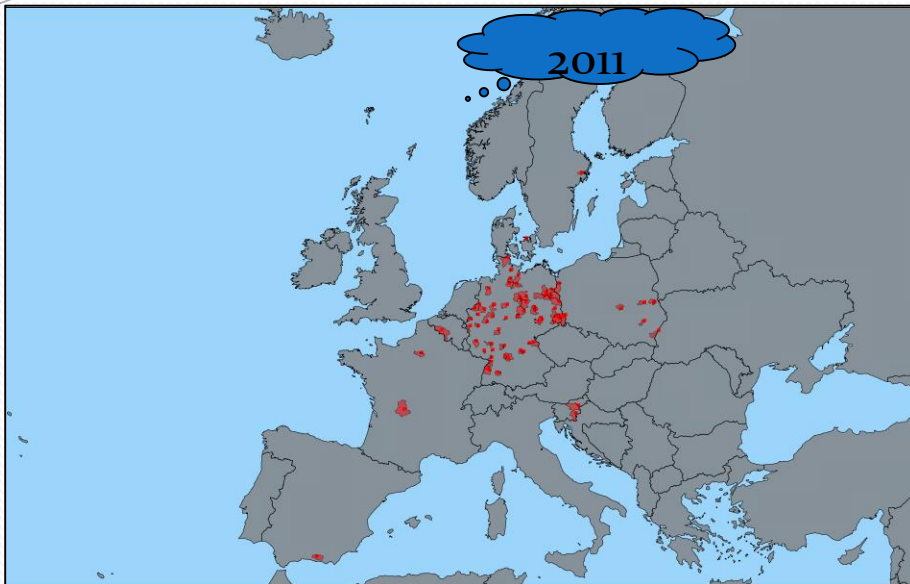
Farms registered in Croatia



KHV outbreaks in EU



KHV outbreaks in EU

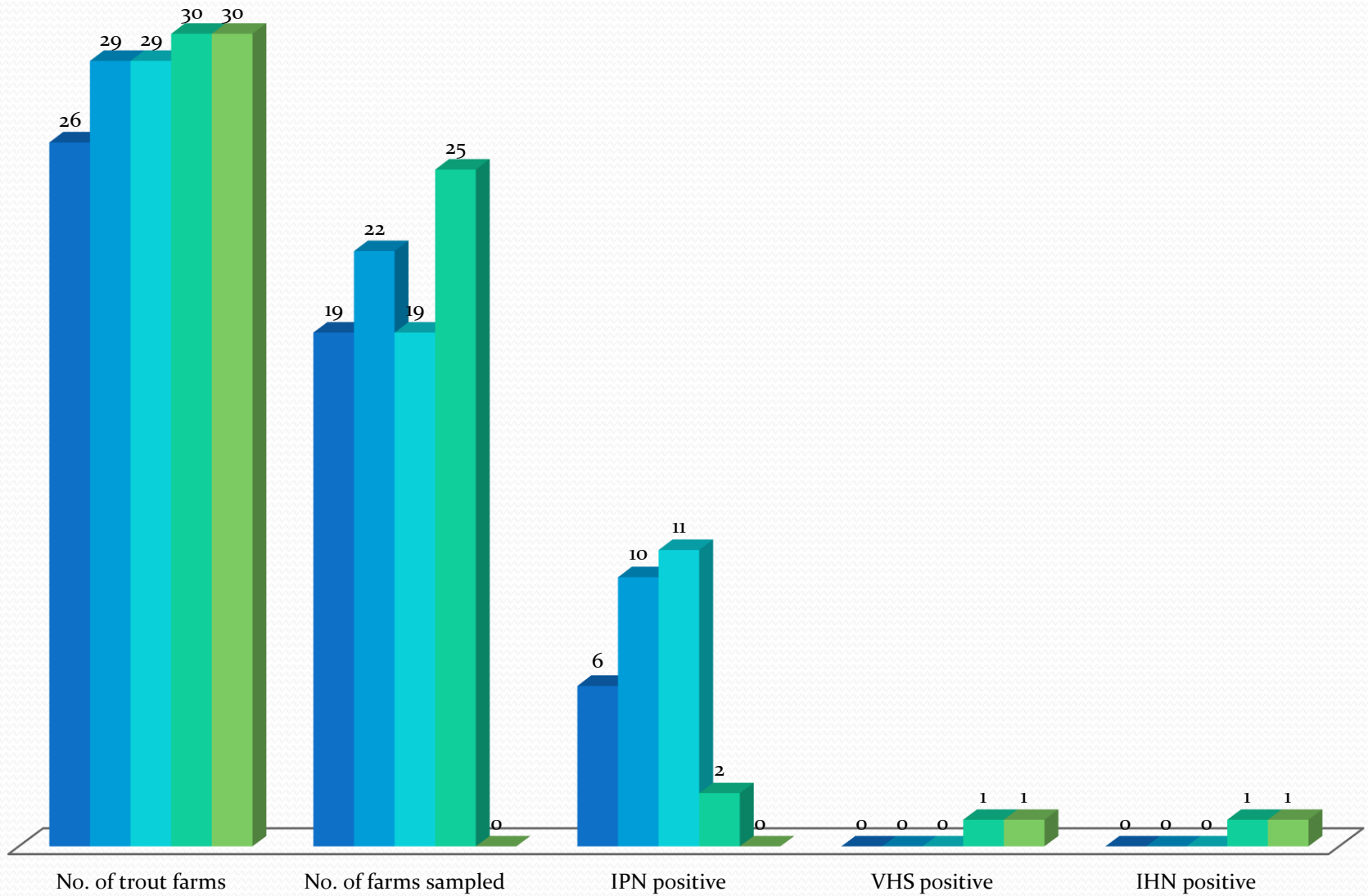


Surveillance programs

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

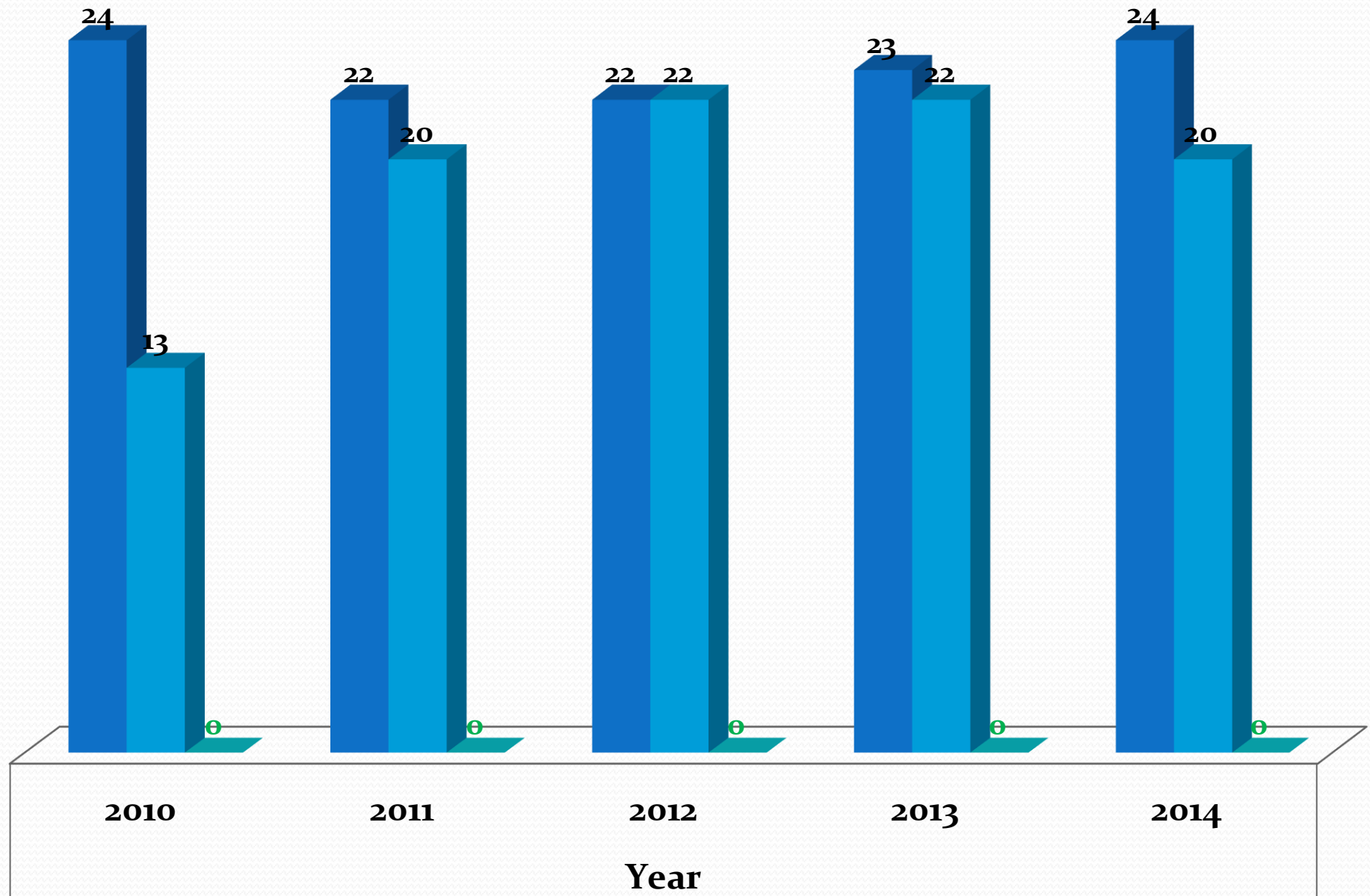
VHS/IHN Surveillance results

■ 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



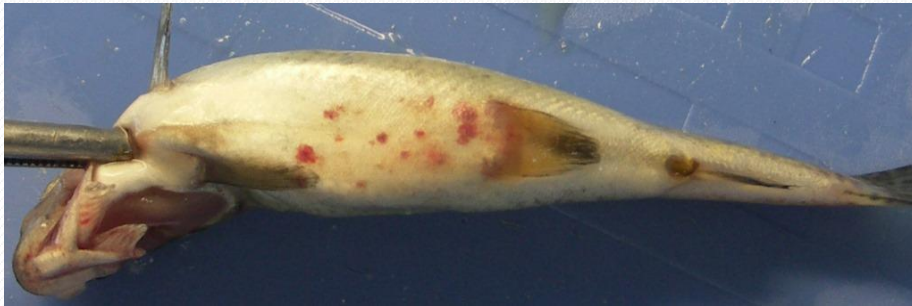
VHS/IHN outbreaks - JULY 2013



- 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



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

INTERNAL CHANGES



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

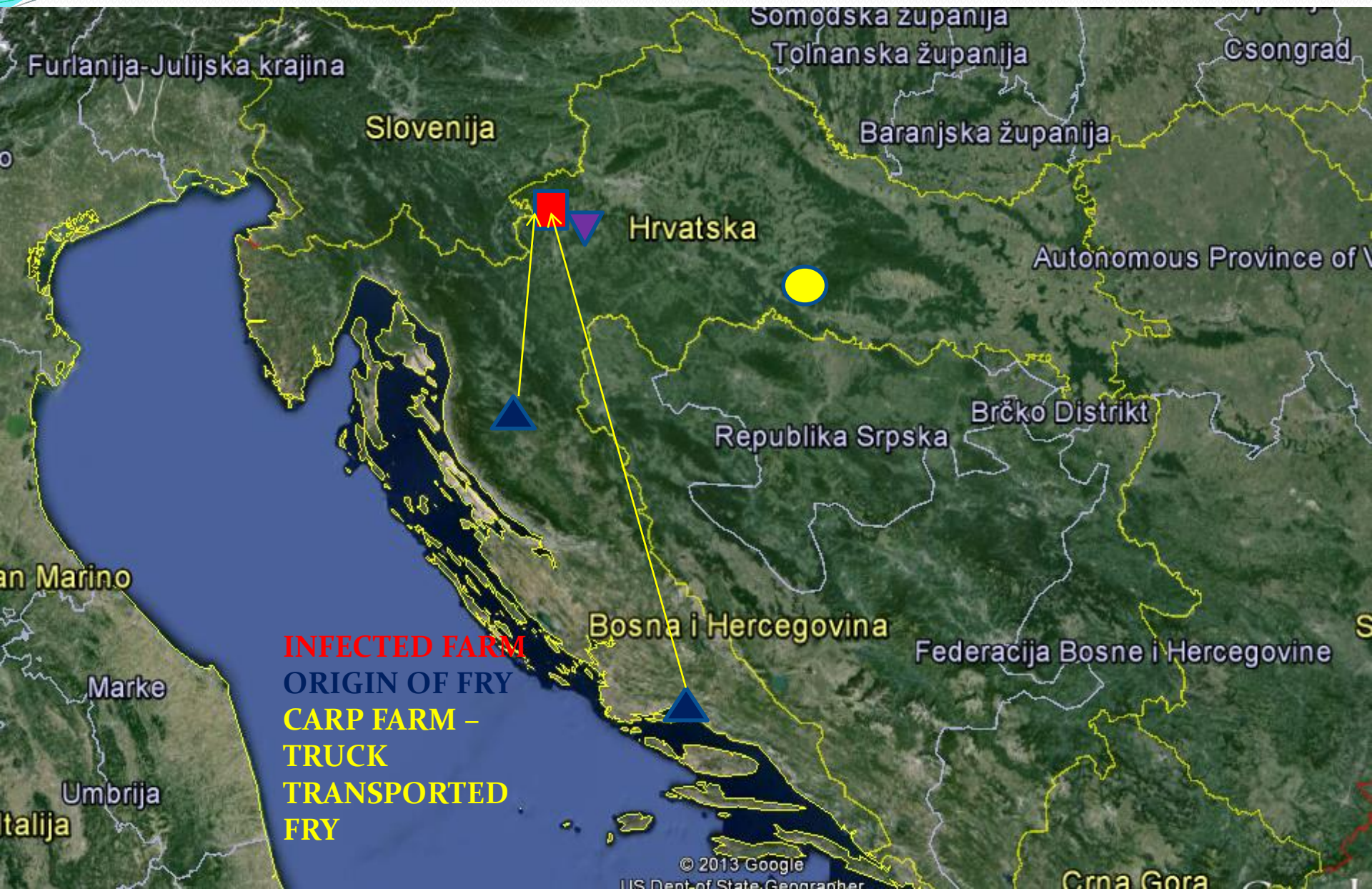
RESULTS OF LABORATORY EXAMINATION

- 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 lenght 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



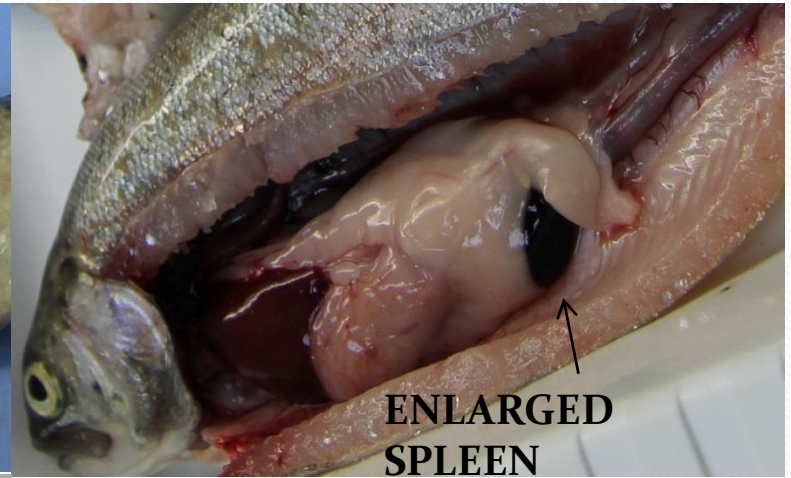
DECEMBER 2013

- 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

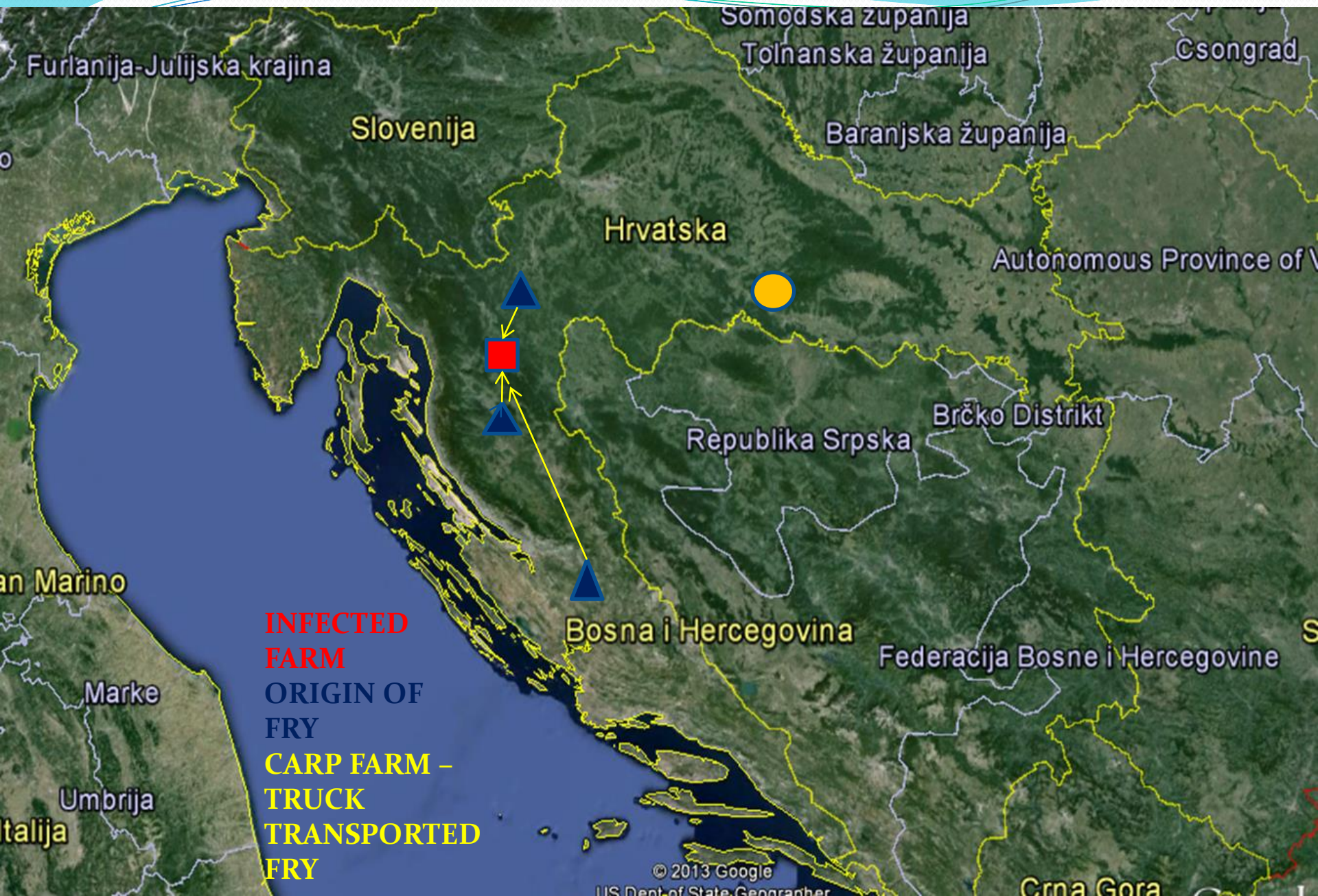
- Inspection on the farm
- Mortalities in rainbow trout weighing 160 -200 g
- Farm manager tried to cure fish by antimicrobial treatment
- Epidemiological investigation was performed and same measures lifted as in previous outbreak of VHS
- Virological examination of samples from suspicious farms was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



Findings



MOVEMENT OF FISH AND VEHICLE

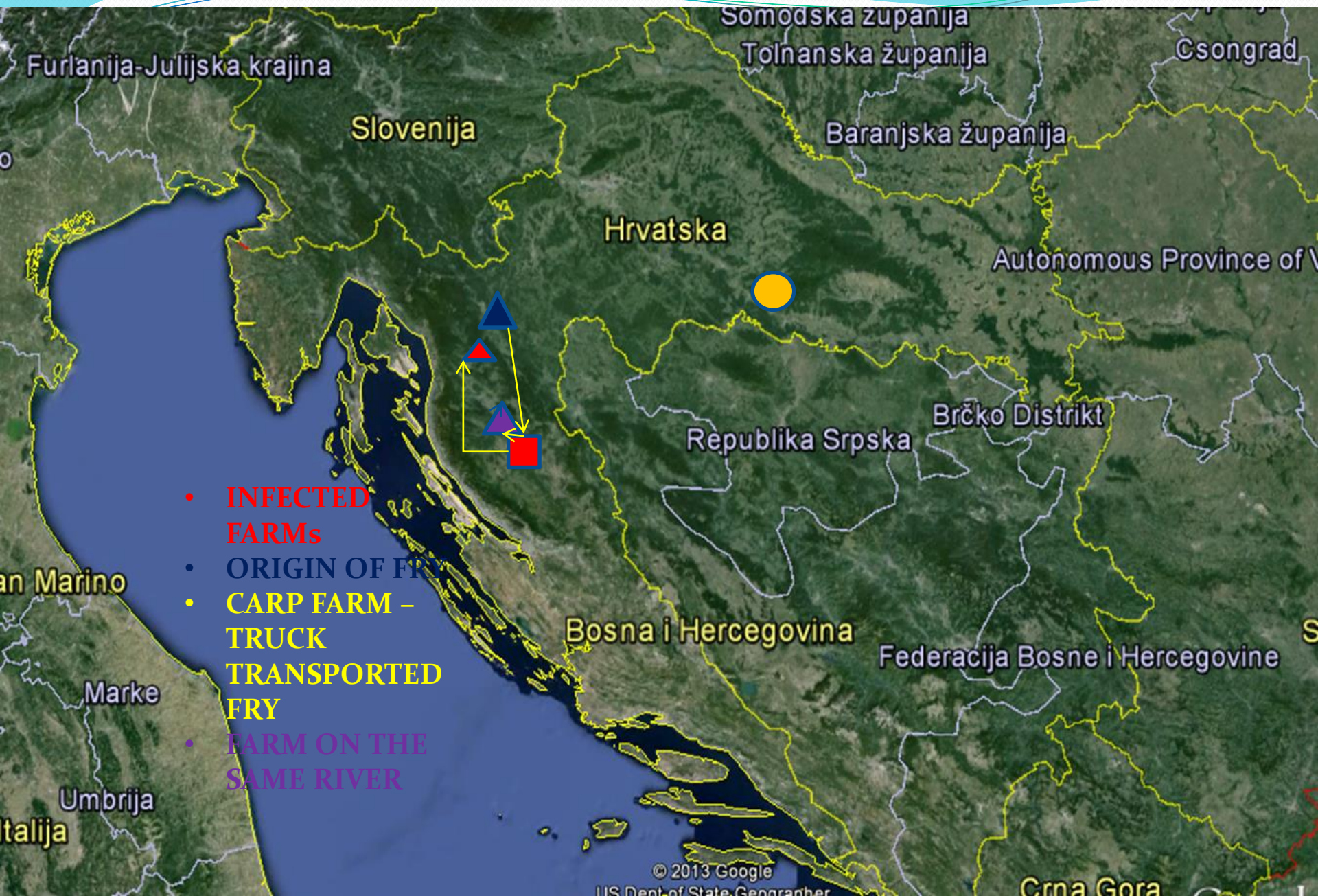




Samples were collected for routine surveillance programme

- 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





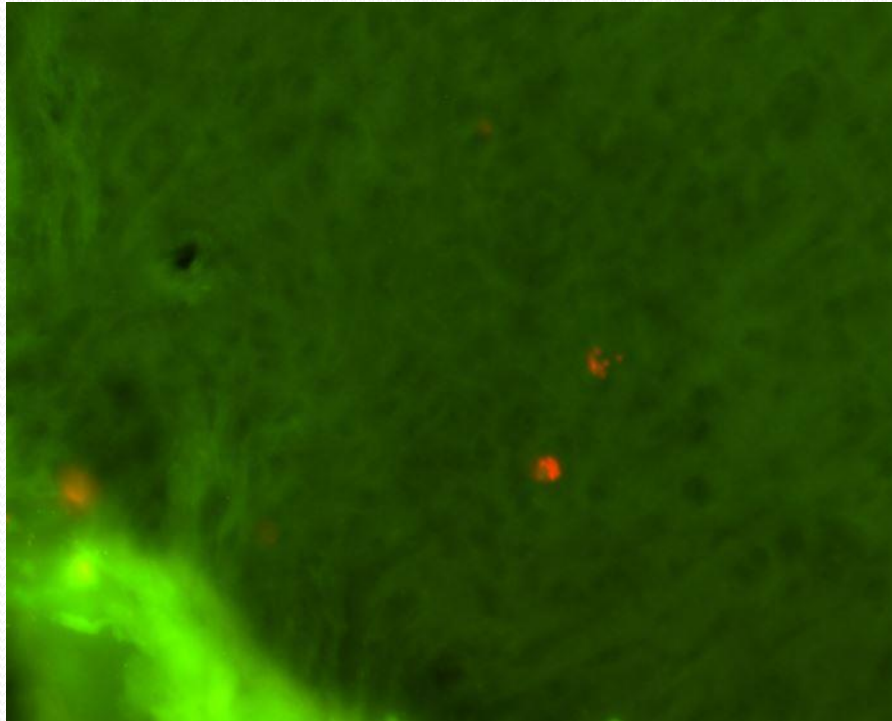
Current and future activities

- 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