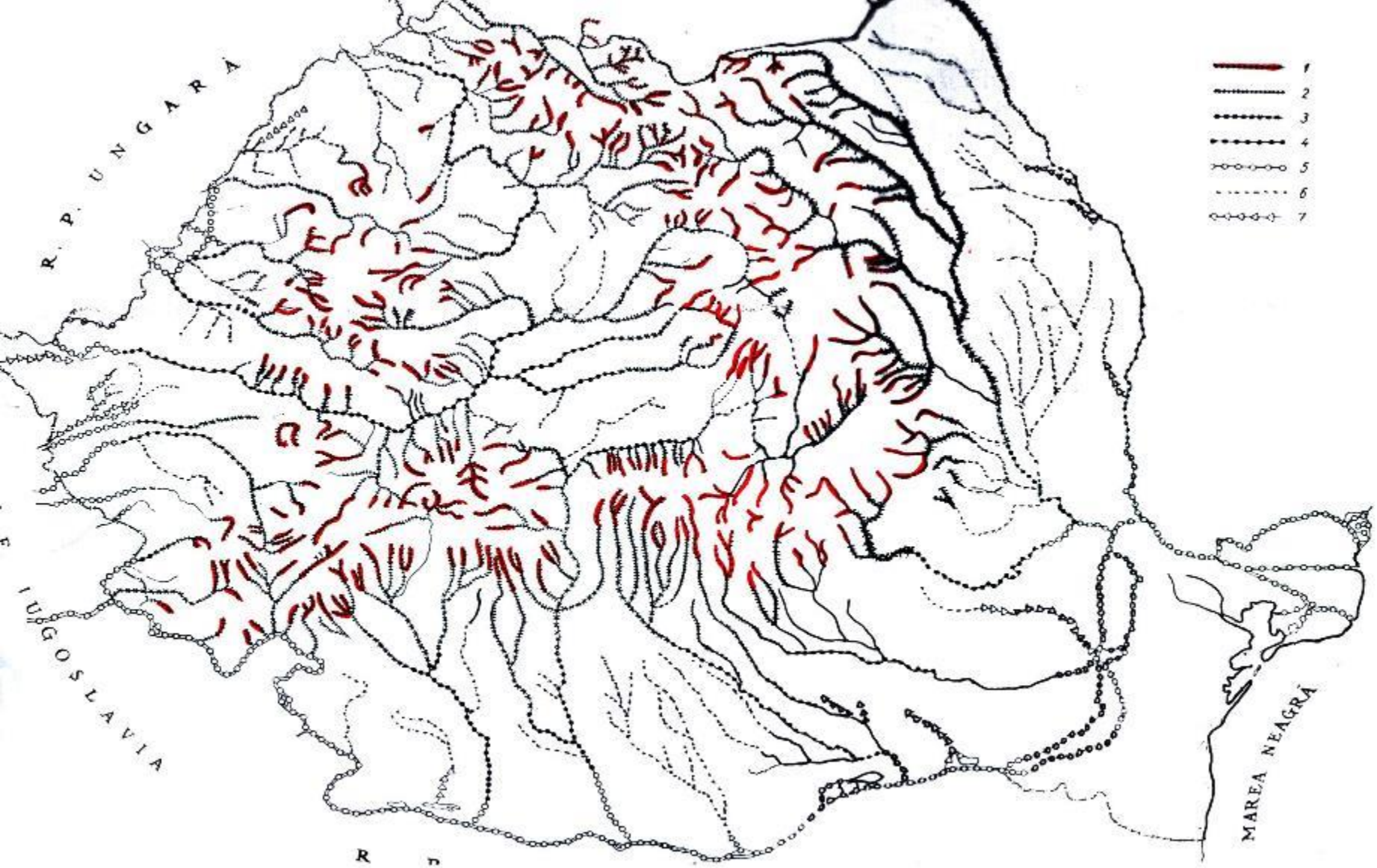


**Viral Hemorrhagic
Septicemia outbreaks in
Rainbow Trout
(*Oncorhynchus mykiss*) in
Romania**

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Romanian rearing trout data

- **Romanian trout farms are situated, in generally, in zones appropriate for salmonids rearing, at altitudes of 340-1450 meters**
- **The water source is ensured by river water, natural spring water or artificial lakes.**
- **Generally, in Romania, the rearing of salmonids is a semi-closed system and each unit has all categories of age, which provide the fry for the new repopulation.**



Natural area of trout – red lines

Salmonids species

- Beside rainbow trout (*Oncorhynchus mykiss*), other intensive reared salmonids species, are:
 - brown trout (*Salmo trutta fario*),
 - brook trout (*Salvelinus fontinalis*),
 - grayling (*Thymalus thymalus*) and
 - Danube salmon (*Hucho hucho*).

Historic

- **2007 – first case of VHS**
- **2016 - second case of VHS**

Data regarding the outbreak 1 (1)

- **In November 2021 a mortality started in one rainbow trout farm situated in the Buzau County**
 - **Farm with a semi-closed system of rearing,**
 - **Basca River as water source**
 - **Temperature of water 10 °C**
 - **10 out of 25 tanks were populated with fish**
 - **about 93000 fish and 50000 fry at the moment of outbreak**
 - **Four category of fish reared, according the weight:**
 - **trout about 200 g/fish,**
 - **trout about 50-60 g/fish,**
 - **Fingerlings (10-15 g/fish) and**
 - **fry.**

Sampling in outbreak 1

- **Sampling was performed on the 15th of November 2021 by the owner and specimens with apathy and/or abnormal swimming, skin darkening, exophthalmia and gill palness were taken for laboratory investigations in NRL.**
- **On the 19th of November a VHS suspicion notification were sent to the veterinary services of Buzau County by the Romanian NRL - IDAH .**
- **As a result, a second sampling was performed on the 22nd of November by official veterinary services and sample taken was sent to the NRL for VHS confirmation**

Laboratory examination – outbreak 1 (1)

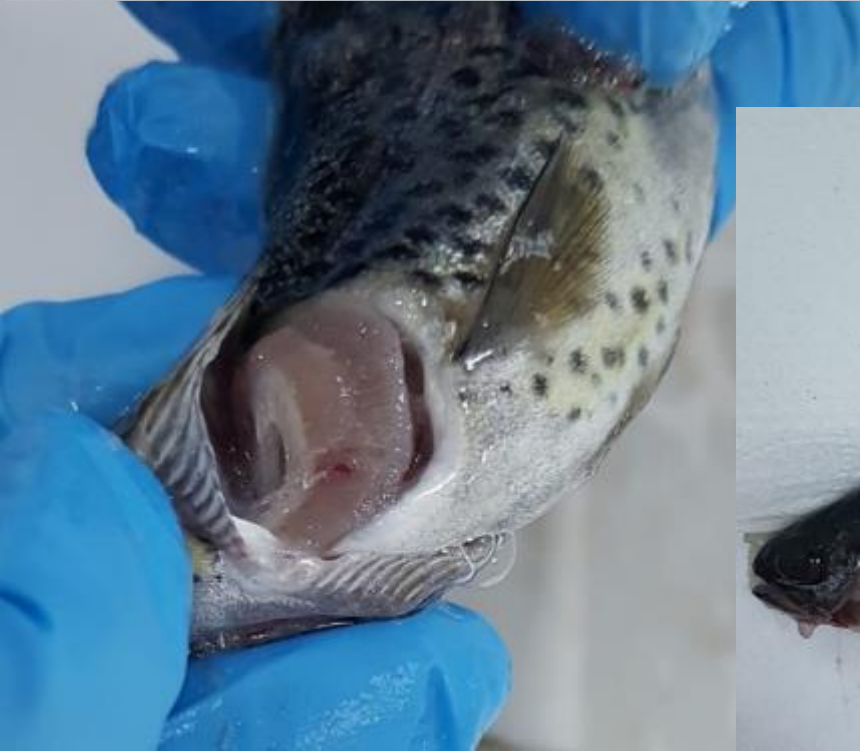
- **First sampling performed by owner:**
 - Anatomopathological examination,
 - bacteriological examination by inoculation on TSA agar and incubation at 25 °C for 72 h
 - virological examination.
- **Second sampling performed by veterinary service of Buzau County**
 - virological examination

Laboratory examination - outbreak 1 (2)

- For the virological examination, tissue pools (kidney, spleen, encephalon), were prepared, triturated and centrifuged;
- Aliquots of tissue homogenate, diluted 1:10, were inoculated on EPC and BF2 cell lines, according with the laboratory working instructions and the EURL for fish and crustacean diseases's Diagnostic manual: "Diagnostic methods for the surveillance and confirmation of infection with VHSV and IHNV, v2021.2";
- ELISA and IFAT tests (BIOX) were performed from the supernatant of cell culture where CPE was registered;
- Real time RT-PCR and PCR sequencing from cell culture supernatant were performed

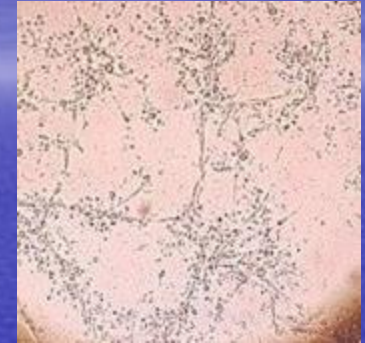
Results in outbreak 1

- **In both sampling, anatomopathological examination showed bleeding around the eyes and on the pectoral fin bases, paleness or white-grey gill with rare hemorrhages, liquid in the abdominal cavity, discolored liver, hemorrhagic spots on surface of the fatty tissue and on swim bladder, low firmness kidney, hemorrhagic spots in the muscles.**
- **Bacteriological examination showed no specific bacterial strain.**



Results of virological examination - first sampling, outbreak 1

Tests performed	Cytopathic effect	VHS	IHN
Virus isolation			
EPC cell line	-		
BF2 cell line	+++		
Identification from cell culture supernatants			
IFAT		+++	-



EPC on BF2 cell line,
x100, after 4 days of
inoculation

Results of virological examination - second sampling, outbreak 1

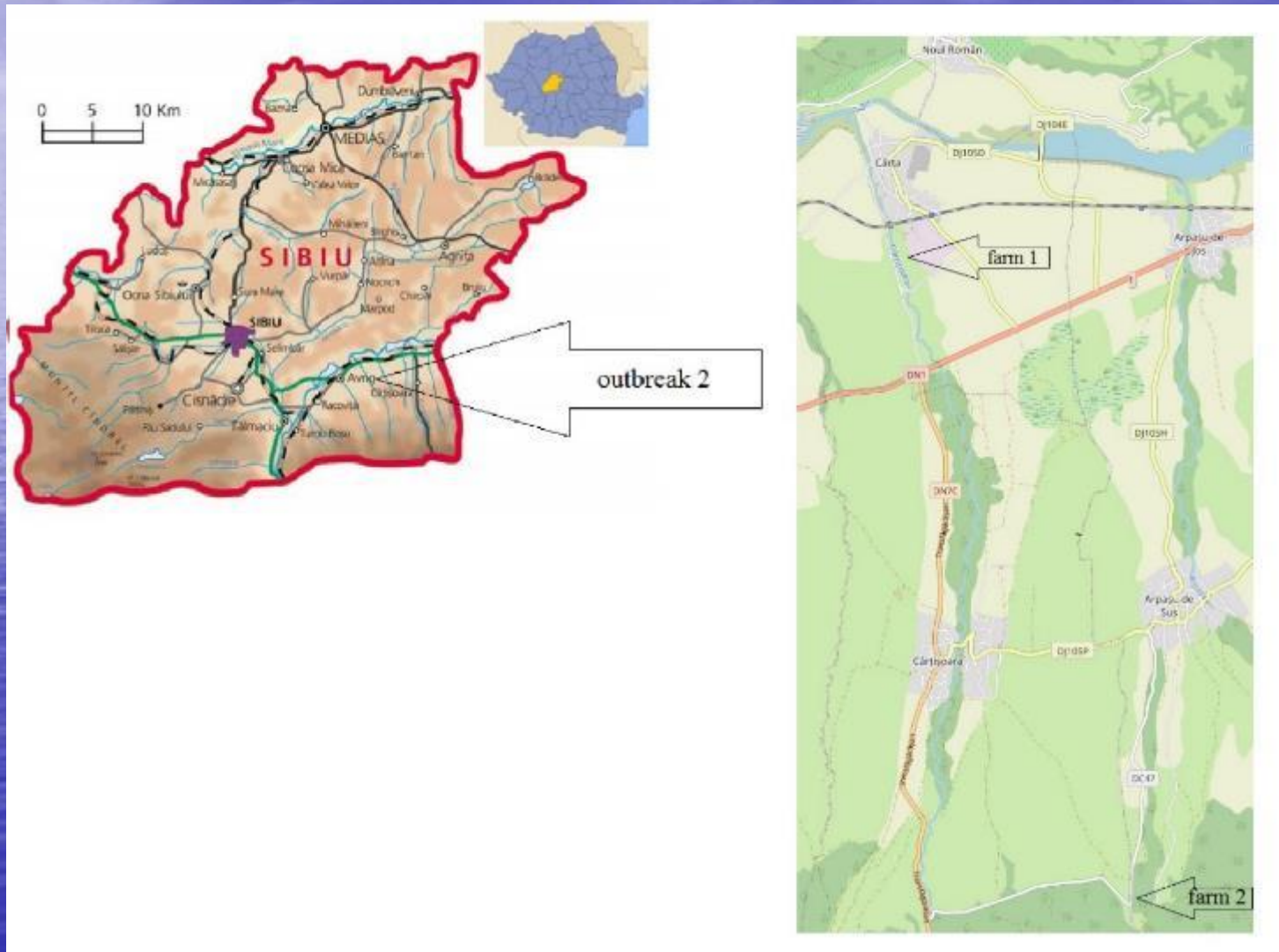
Tests performed	Cytopathic effect	VHS	IHN
Virus isolation			
EPC cell line	-		
BF2 cell line	+++		
Identification from cell culture supernatants			
ELISA		+++	-
IFAT		+++	-
real time RT-PCR		+++	
PCR and sequencing		+	

Data regarding the outbreak 2 (1)

At the end of January 2022, a mortality started in 2 close farms from Sibiu County, same owner

- Farm 1, with a semi-closed system of trout rearing;
- Water source is represented by Carta Spring River
- Temperature of water 8-10° C
- 6 tanks out of 8 were affected in the outbreak
- Four category of fish reared: broodstock trout, trout, fingerling and fry
- about 600000 fish, 700000 fry and 50000 eyed eggs at the moment of outbreak
- Farm 2, with a semi-closed system of trout rearing and sturgeons, has tanks for put and take fishing. Sturgeons were kept in a separate tank with distinct water flow;
- Water source is represented by Arpasel River;
- Temperature of water 6-8 °C ;
- 11 tanks out of 12 were affected in the outbreak,
- Two category of trout reared: trout and fingerling;
- About 74000 trout and 40 sturgeons

Sibiu County map



Sampling in outbreak 2

- **Sampling was performed on 2nd of March 2022 by the licensed veterinarian and specimens with apathy and/or abnormal swimming, skin darkening, exophthalmia and gill palness were taken from both farms, for laboratory investigations in NRL .**

Laboratory examination - outbreak 2 (1)

- **Sampling performed in farm 1**
 - **Anatomopathological examination,**
 - **bacteriological examination by inoculation on TSA and Ordal agar and incubation at 25 °C for 72 h**
 - **virological examination.**
- **Sampling performed in farm 2**
 - **Anatomopathological examination,**
 - **bacteriological examination by inoculation on TSA and Ordal agar and incubation at 25 °C for 72 h**
 - **virusological examination**
 - **Histology**

Results outbreak 2

- In both farm of outbreak 2, anatomopathological examination showed excess mucus on the skin and gill, reddening of mouth corner, palate and on the internal face of operculum, enlarged spleen, discolored liver, hemorrhagic spots on the liver and swim bladder, hemorrhagic spots in muscles.
- Bacteriological exams showed no *Yersinia ruckeri* or *Aeromonas salmonicida*, but a *Flavobacterium* sp. strain was isolated from gill.
- At histological examination were present hemorrhagic infiltration in the gills, gut, muscles, liver and pyloric ceca, accompanied by necrotic desquamation in glandular epithelium of pyloric ceca and gut epithelium, necrosis of gill epithelium, interstitial lymphohistiocytic nephritis.

Farm 1 -outbreak 2



Farm 2 - outbreak 2



Results of virological examination in outbreak 2 – both farms

Tests performed	Citopatic effect	VHS	IHN
Virus isolation			
EPC cell line	-		
BF2 cell line	+++		
Identification from cell culture supernatants			
ELISA		+++	-
IFAT		+++	-
real time RT-PCR		+++	
PCR and sequencing		+	

Epidemiological data(1)

- **Outbreak 1**
 - **The fish population was investigated by laboratory tests 2 months before the outbreak, with negative result for fish viruses.**
 - **Abnormal mortality was registered at the beginning of November 2021 in one tank of farm from Buzau County.**
 - **Rapidly the disease has spread in all populated tanks, including the incubation station.**
 - **The mortality increases over the first thirteen days when registered the pick corresponding to a 0.74% mortality percent.**
 - **No farms of fish upstream and downstream of outbreak, but wild species of trout, chub and grayling are present in the river.**
 - **Movements registered in the farm with 2 weeks before outbreak by introducing about 1279 fish from a Sibiu County farm, but without any mortality or clinical signs in fish population. Subsequent virological examination of the fish on the farm of origin was negative.**

Epidemiological data(2)

- **Outbreak 2**

- **At the end of January 2022, a mortality started in two close trout farms situated in the Sibiu County;**
- **Between farms 1 & 2 (same owner) from Sibiu County there were weekly movements of fish but no movements with other farms;**
- **The disease spread almost in the same time in both farms;**
- **The daily mortality in both farms varied from 0.1% to 0.7%.**
- **Farm 1 - 3 farms upstream and is close to Olt River. Water source passes through a locality before entering in the trout farm;**
- **Farm 2 - one farm down stream and is open to visitors by put and take activity;**
- **Wild species of trout are present in the river.**

Epidemiological data(3)

- **Possible routes of infection**
 - waterfowl are mentioned as a possible factor for both outbreaks in spread of VHS
 - The proximity of the Olt river in the case of outbreak 2
 - Carta Spring River (water source for farm 1 in outbreak 2) flows through a locality before to enter in farm

Prevention and control of disease

- **In farms, in the moment of disease suspicion, the activity of production and commercialization was stopped.**
- **Measures were taken to prevent and control the VHS disease according to the legislation in force;**
- **Samples taken from restricted zone were negative for VHS virus.**
- **For the eradication of disease the stamping-out was performed.**

Conclusions

- **The virological examination showed positive results for VHSV and negative results for IHNV in both sampling from Buzau County farm and in both farms from Sibiu County;**
- **Results of virological examination correlates with tissue changes and registered losses;**

Conclusions

- **VHS virus was confirmed by PCR sequencing in both outbreaks and phylogenetic analysis performed with “Fish pathogens Database” from EURL for fish and crustacean diseases website, identified genogroup I.a of VHSV.**
- **For tracing of origin of the VHS virus strains, the isolates from both outbreaks will be submitted to the EURL fish and crustacean diseases.**



**Acknowledge the
inputs of
Dan Catuneanu, Stelian
Mihalcea Ciprian
Botezatu, Adonis
Dragnea,
Rad Nutu,
Ittu Gabriel Dragos,
Flavius Octavian Flucus,
Serban Constantin
Tichindelean from
Sanitary Veterinary
Directions of Buzau and
Sibiu County**

**Acknowledge the
inputs
of Laura Elena Fartat,
Andrei Popovici and
Sorin Mihai from
Romanian NRL.**



Thank you for your attention !