



Overview of the fish disease situation in France in 2016-2017 (to date)

L. Louboutin, F. Almeras, M. Baud, L. Bigarré, J. Cabon, L. Pallandre, T. Morin

*French Agency for Food, Environmental and Occupational Health & Safety
Ploufragan-Plouzané Laboratory, Viral Fish Diseases Unit
Université Bretagne Loire, Plouzané, France*

In close relation with:

-Direction Générale de l'Alimentation,

Service des actions sanitaires en production primaire,

Sous direction de la santé et de la protection animales, Bureau de la santé animale

-Direction Départementale de la protection des populations du Département 57



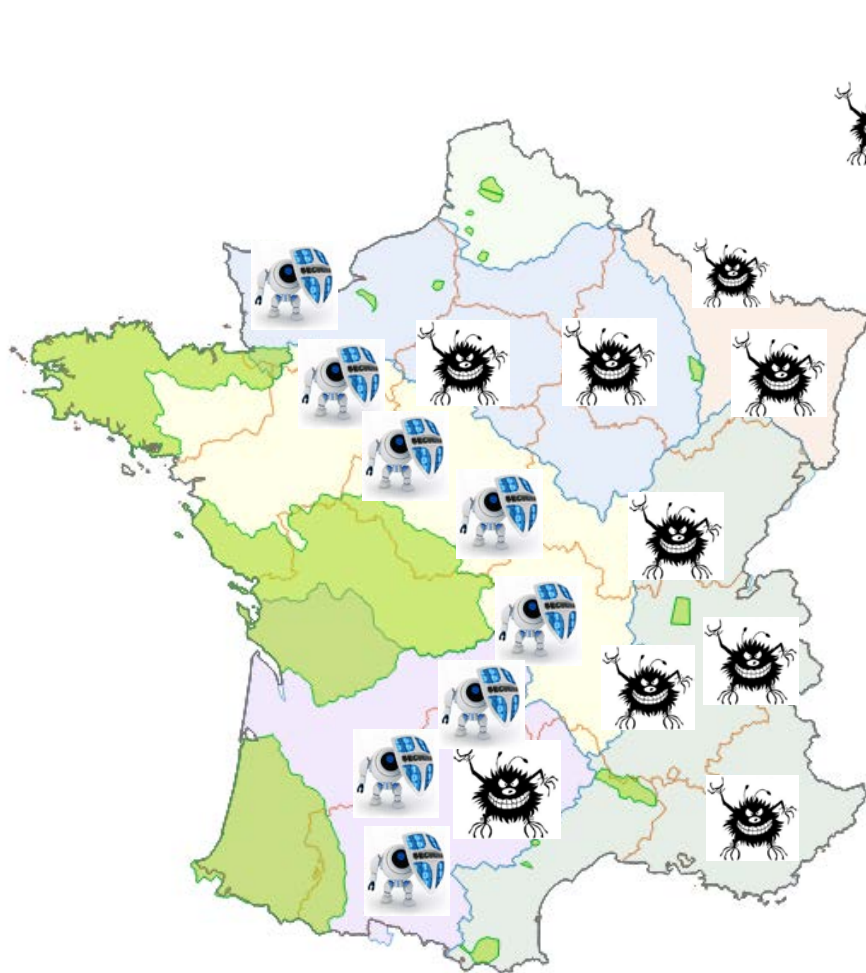
MINISTÈRE
DE L'AGRICULTURE
DE L'AGROALIMENTAIRE
ET DE LA FORÊT



Direction
départementale
de la protection
des populations

CONTEXT

France is not declared free from non exotic listed diseases VHS and IHN but...



... several **zones** have **VHS** and **IHN**
free status
(according to directive 2006/88)

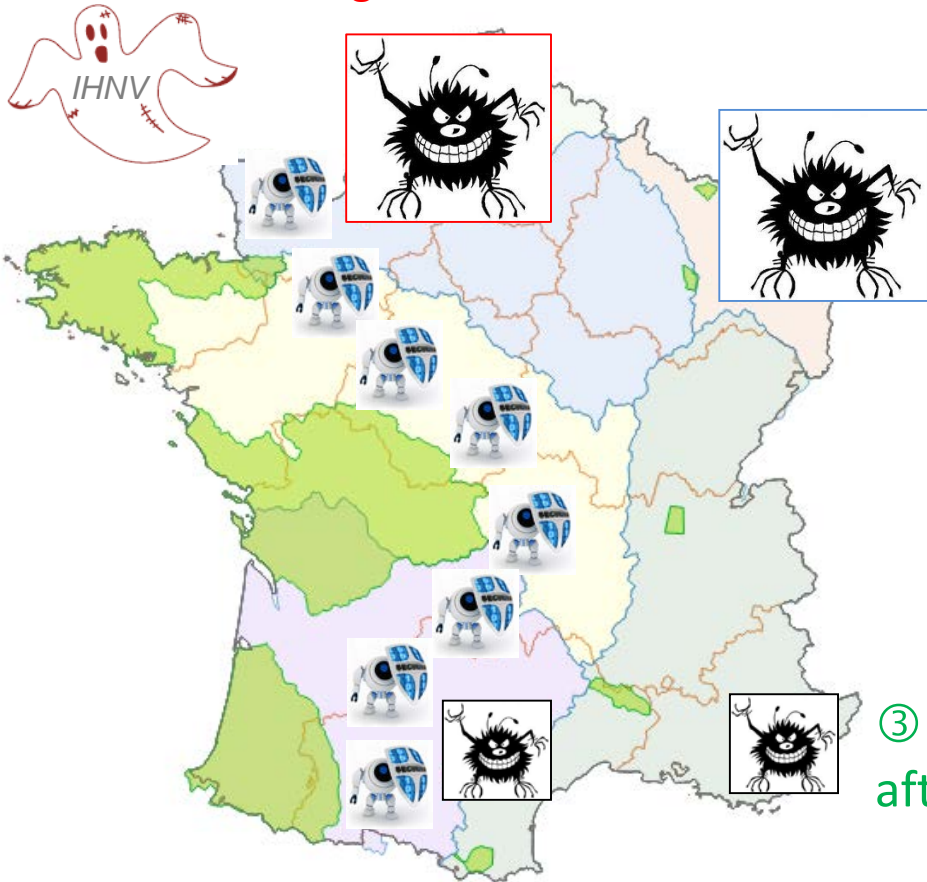


 Disease-free areas

Updated situation April 2017

↪ Listed disease outbreaks were detected in various areas

① The « ghost » IHN in Normandie...



② The recurrent VHS in Moselle...



③ KHV outbreaks after Koi importation ...



↪ Emergent viruses circulate on the French territory (CEV, Reovirus, ...)

① The « ghost » IHN in Normandie...

- An infected area containing some disease-free farms
- 6 geographical independent water catchment
- Several detection of IHNv since 2014 in this area



① The « ghost » IHN in Normandie...

- An infected area containing some disease-free farms
- 6 geographical independent water catchment
- Several detection of IHNv since 2014 in this area




Last case : **March 2017 / Normandy**

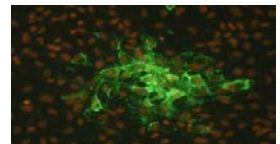
Fish farm subjected to surveillance program (category II)
Increased mortality on rainbow trout with haemorrhage
Bacteriology : presence of Yersinia and Flavobacterium



Sampling on rainbow trout and analysis by an accredited laboratory



IFAT : IHNv+ 
Confirmed by the NRL



Sequencing : partial G gene (570 bp, Emmenegger)



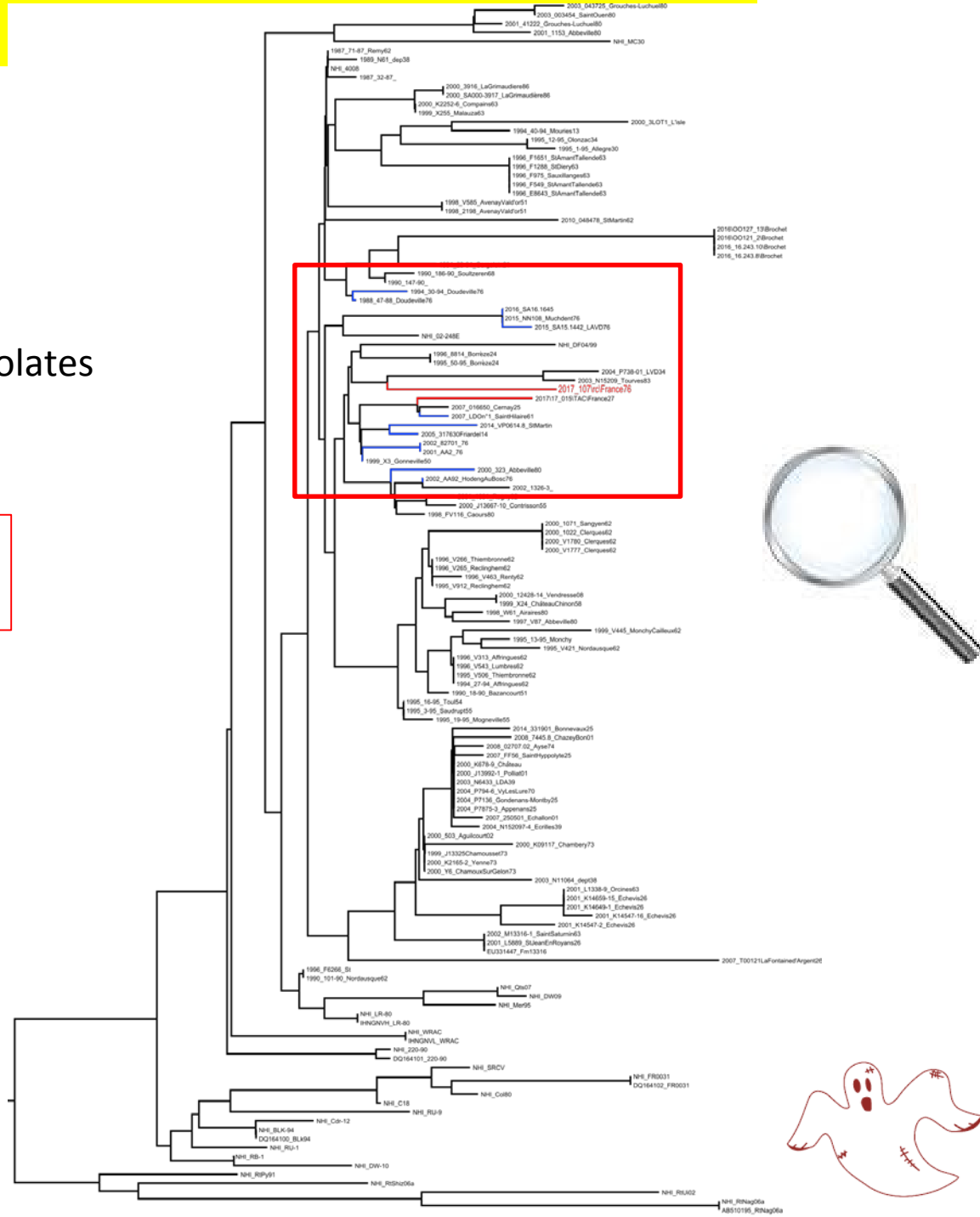
Sequencing Platform
(Dr Y. Blanchard et al.)



LABORATORY ANALYSIS

→ Comparison with other French isolates (from rainbow trout and brown trout)

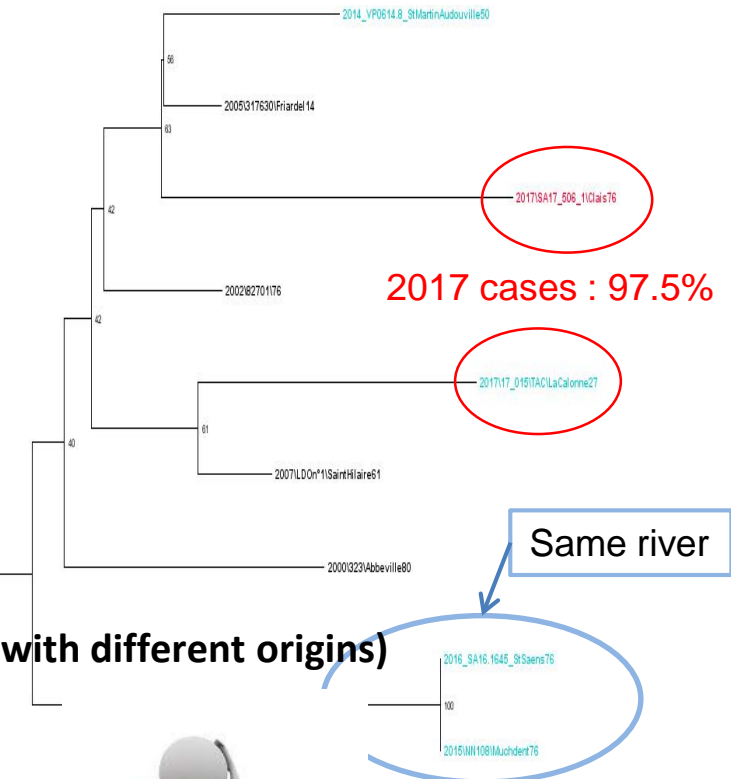
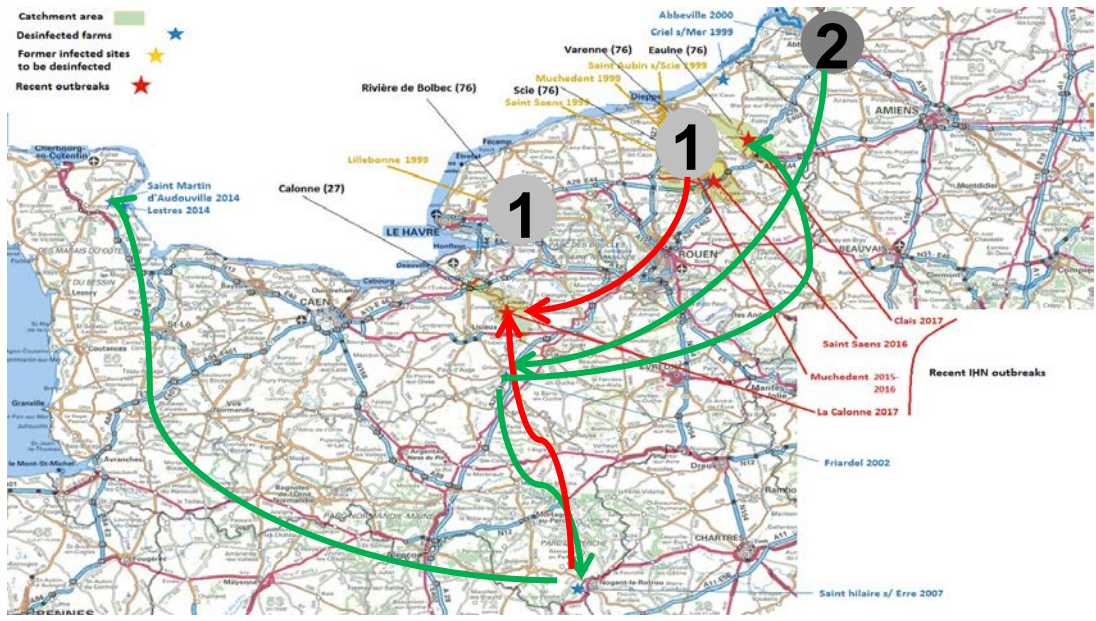
→ 136 sequences
→ Normand strains clustered in genotype E with other French isolates



SeaView analysis
BioNJ tree (500 bootstraps)



→ Focus on the “Normandy sequences”



- Hypothesis : Several strains circulating since 1999 (perhaps 2 different types, with different origins)
- No or inefficient disinfection = persistence of virus??
- Various unknown reservoirs??
- Virus spread: by wild animals or humans on longer distances ??



SeaView analysis
BioNJ tree (500 bootstraps)
red: sequence of Clais at Vatierville (76)
blue : recent isolates

% identity with other « Normand » strains :
Max = 100% (same river)
Min = 97.3%

② The recurrent VHS in Moselle...



- An infected area containing some disease-free farms
- Rich in ponds
- Several isolations of VHSv in **2 fish farms** since 2011

Last outbreak : **March 2016**

In the same 2 fish farms regularly infected (category V)

Every year, at the beginning of spring : increased mortality on rainbow trout

Sampling on rainbow trout and analysis by an accredited laboratory or by the NRL



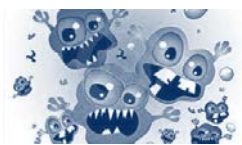
Cell culture / IFAT : **VHSv+**
RT-qPCR : **VHSv+**



Notification to the competent authority

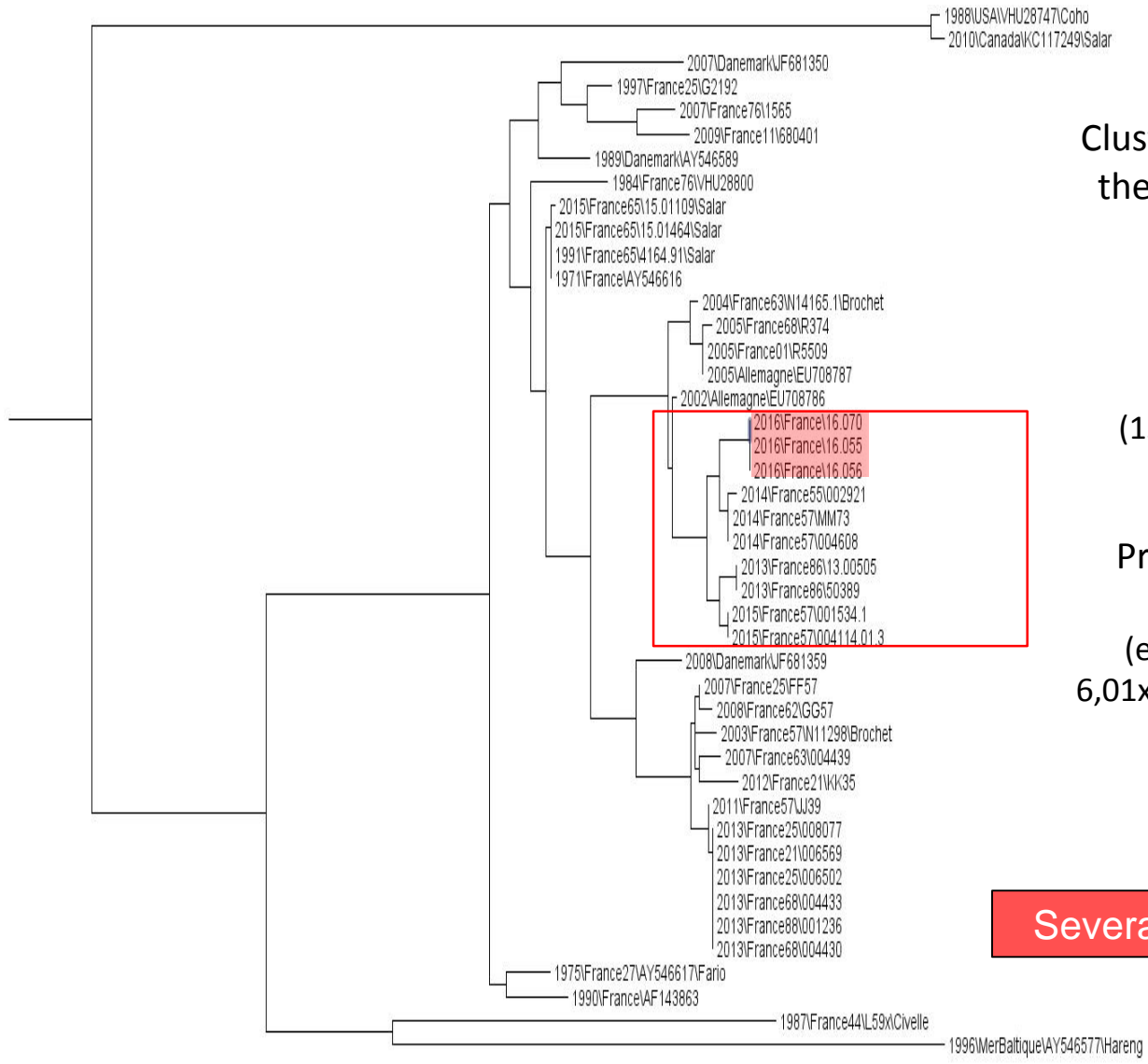


Strict surveillance measures



② The recurrent VHS in Moselle...

Characterization of the isolate by sequencing : full G gene (Einer-Jensen)



Cluster with previous isolates from the same fish farms (2013, 2014, 2015)

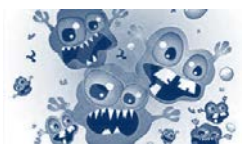
BUT

Many mutations observed (15 differences with 2015 isolates, 7 with 2014 isolates)

Probably not consistent with a natural evolution (estimated between $1,74 \times 10^{-3}$ et $6,01 \times 10^{-4}$ subs/site/year - 0,91 à 2,65 nt mutated / year in the G gene)



Several strains circulating in the area



Origin of the recurrence????

→ **Importation** of infected fish

- **Germany,**
- **The Netherlands,**
- **and Poland**

Support from European colleagues

	≠ with German isolates	≠ with Italian isolates
16/055 isolate (2016)	7	11
MM73 isolate (2014)	2	4

Not so far from German isolates but not totally identical

→ **Survival of the virus in the environment**
(in the river, in wild fish)

Electric fishing in the river / 60 brown trout



No VHSv detected

→ **Survival of viruses through reservoirs** (ponds containing numerous susceptible species)



Various and independent water systems
Managed by several fishermen



- Sampling from **Oct to Dec 2016**
- **9 ponds**
- **30 pikes** sampled in each pond
- **Analysis by the French NRL :**
 - cell culture (on **individual** fish)
 - RT-qPCR (on **pools**)

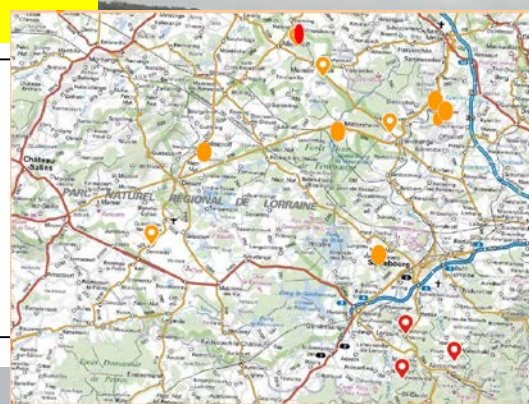


② The recurrent VHS in Moselle...



Water temperature :
10 to 14°C

Mean weight depending sample site :
from 200g to 2kg



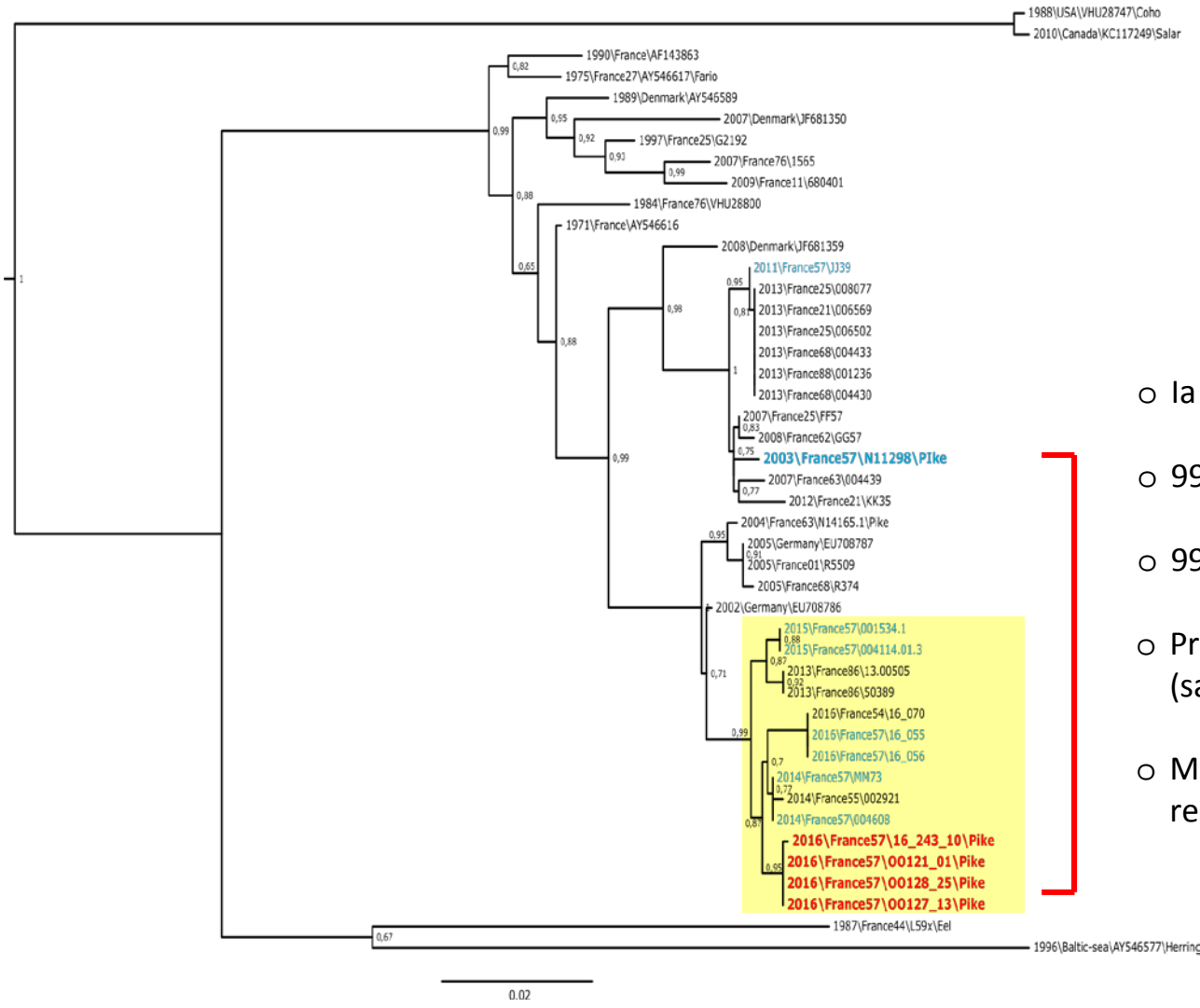
- Analysis results :
- 1 site /9 positive in **VHSv** AND **IHNv**
- 18/30 pikes VHSv+
- 7/30 pikes IHNV+ } 5 co-infections



↪ Selection of VHSv and IHNV isolates for characterization by G gene sequencing

② The recurrent VHS in Moselle...

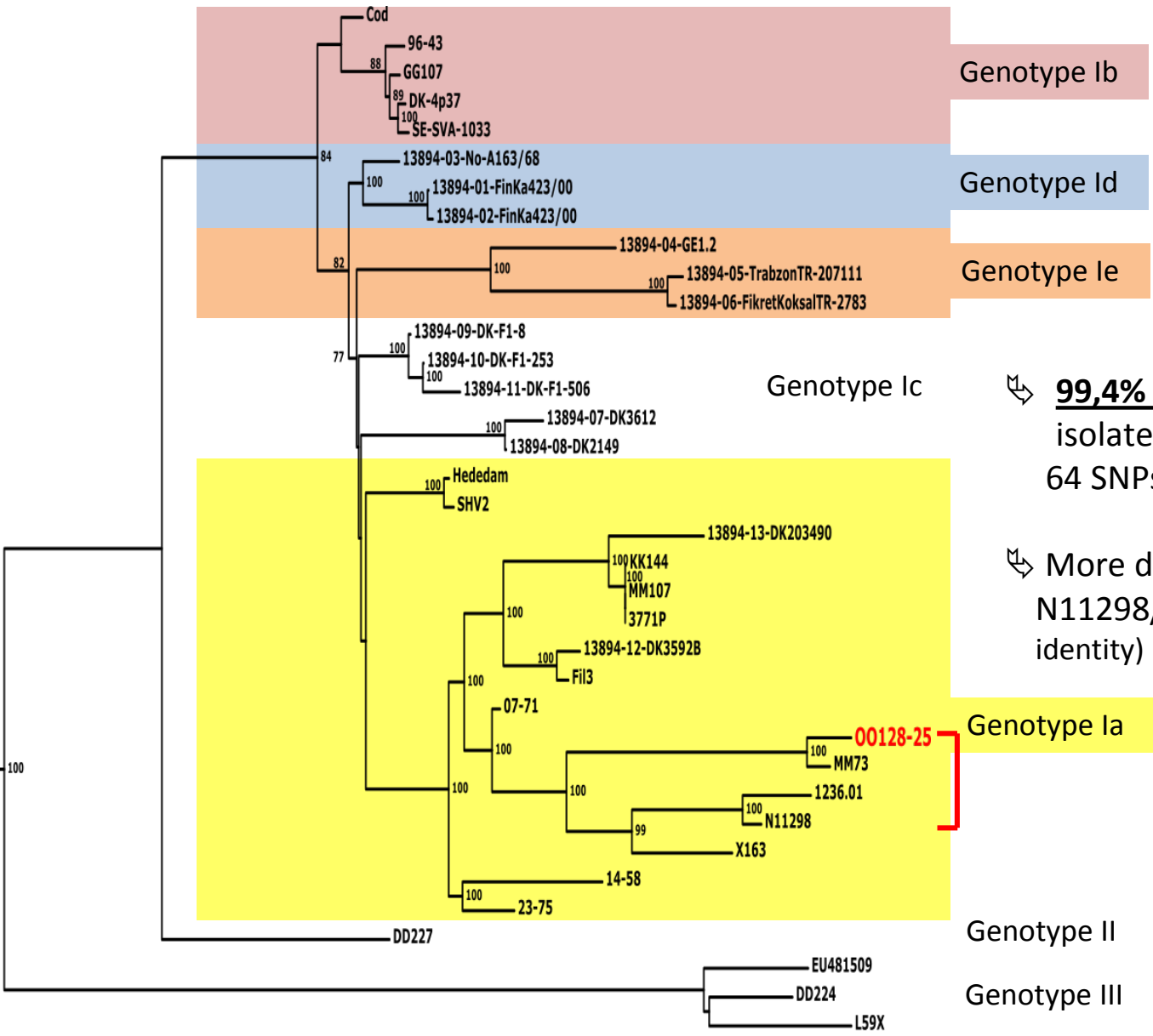
➤ VHSv / G gene :



- la genotype
- 99.5% identity shared with 2014 isolates
- 99% with 2016 isolates
- Previous isolation on pike in 2003 (same area) and 2004
- More distant : 96.5 and 98.4% identity respectively

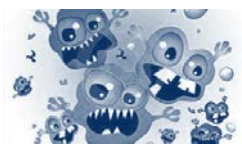
② The recurrent VHS in Moselle...

➤ VHSV / PhyML analysis after NGS :



↪ **99,4% identity with MM73**, isolated in the same farm in 2014 64 SNPs (10845 nt long)

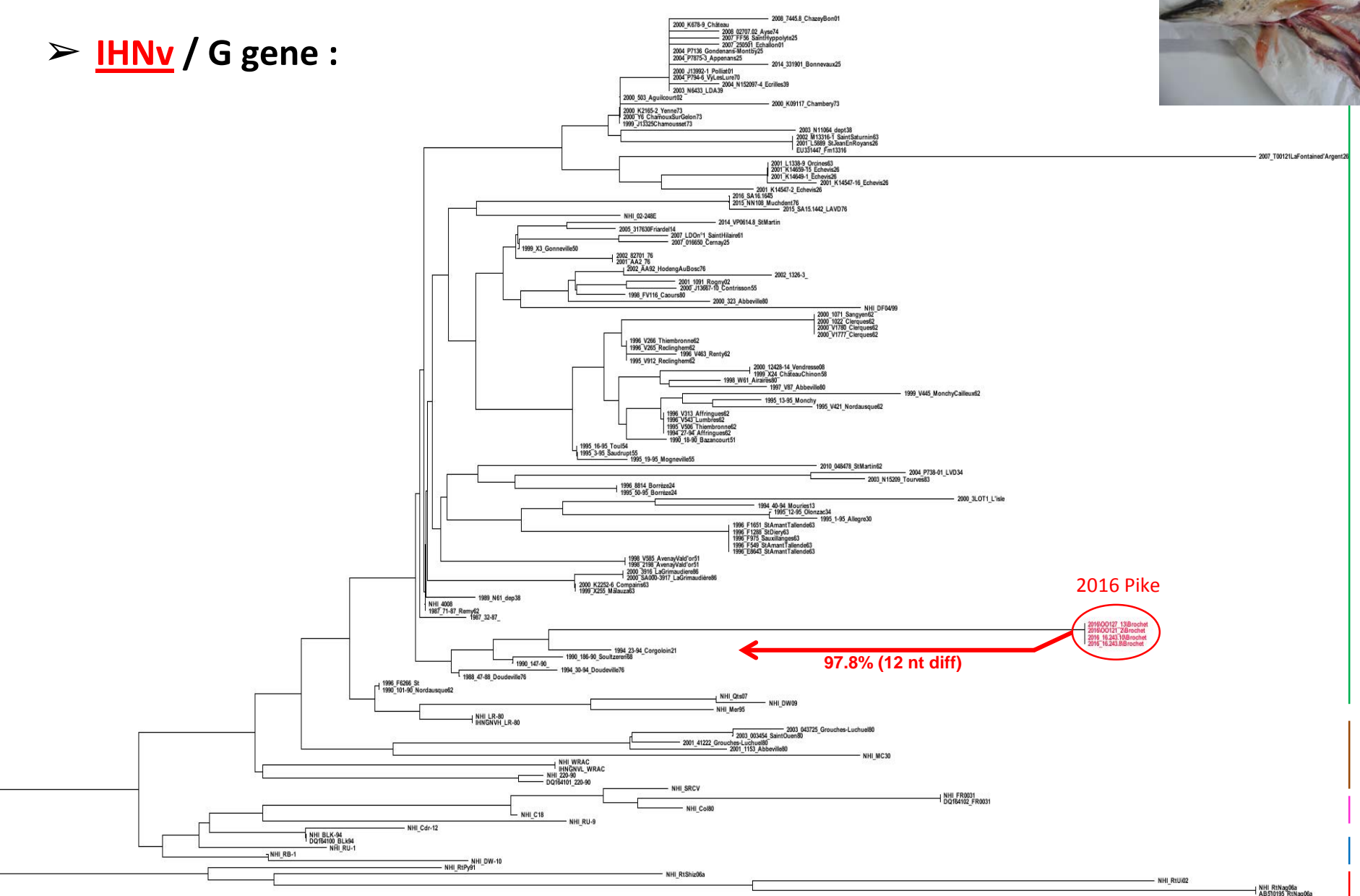
↪ More divergence with pike N11298/2003 isolate (96.6% nt identity)



② The recurrent VHS in Moselle...



➤ **IHNv / G gene :**



SeaView soft,
Distance method analysis BioNJ
Partial G gene (570 nt)

0.005

NH1_R19h06i
AB519192_R19h06i

E
M
L
U
J

② The recurrent VHS in Moselle...

➤ ***In vivo* experiments to test virulence on rainbow trout:**

VHSv and IHNV **+++ virulent**: respectively **95** and **85%** mortality after infection by bath (mean weight \approx 50g)



➤ **What happened next??**

The positive pond was declared infected, pike were sacrificed

Authorities are waiting for proof of disinfection (quite difficult to apply in a pond)

Following for more than 6 months

**No real answer for the origin of this VHSv...
Except that ponds may represent a potential reservoir...**

... but we should manage to get rid of these viruses soon...





Objectives:

- Acquisition of **status 1 (VHS and IHN free)** for the entire metropolitan territory
- Disappearance of outbreaks expensive for professionals and for the French state but also psychologically difficult to overcome
- Reduction in the risk of contamination allowing to establish a lower frequency for the tests dedicated to the qualification maintaining (EU Decision 2015/1554)
- Adaptation of targeted surveillance
- Improvement of the general health of farmed fish
- Emergence of a **national branch of ponds free for VHS and IHN** (carnivores and white fish)
- **Better value** of the sector at national and export levels
- **Simplification** of national movements
- Security of **trade** and **imports**

Project leader:



MINISTÈRE
DE L'AGRICULTURE
DE L'AGROALIMENTAIRE
ET DE LA FORÊT

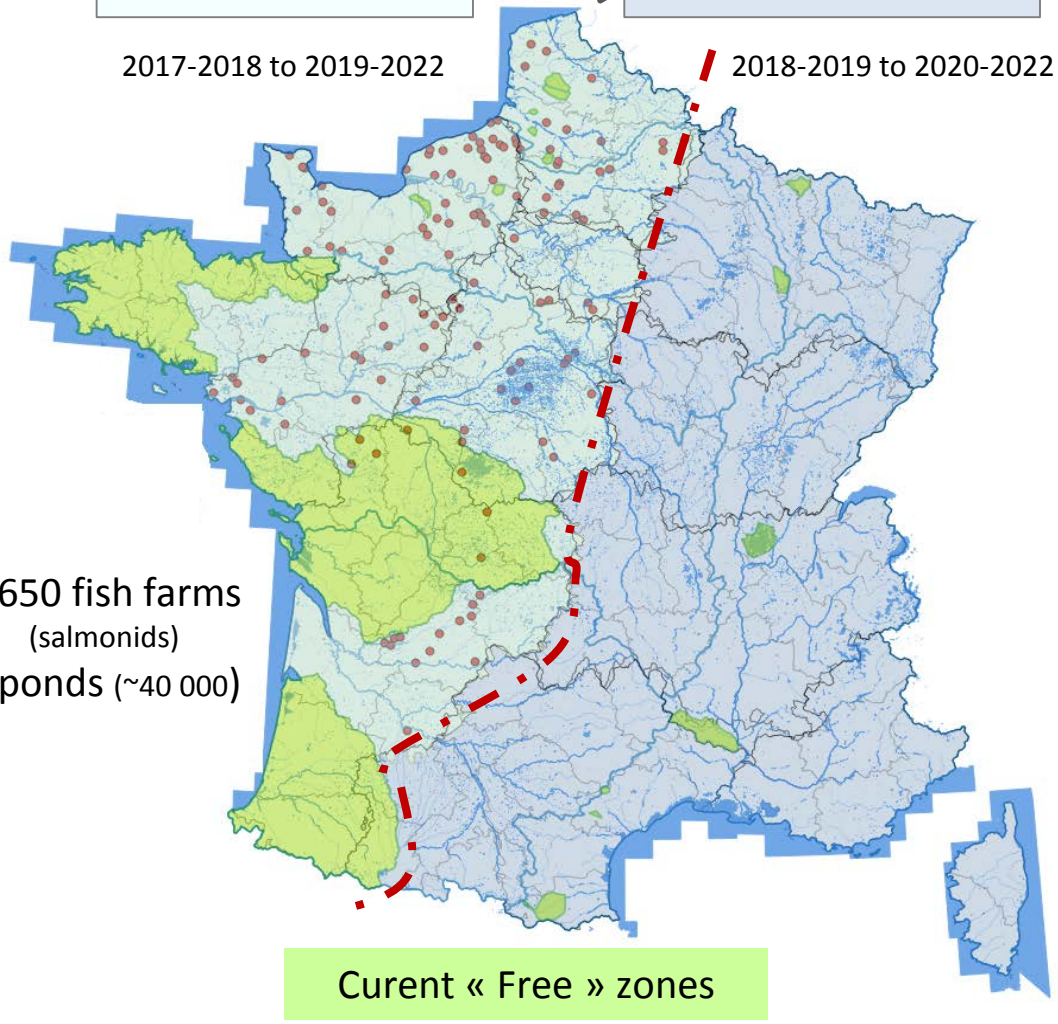
*Direction Générale
de l'Alimentation*

**Co-funded by European
Maritime and Fisheries Fund**



French Plan for Eradication and Surveillance (PNES) of VHS and IHN

Three-phases which overlap in time (2017-2022):



Qualification process:
clinical visits of aquaculture farms and sampling for analysis
Duration: 2-4 years/phase



Surveillance:
inspections
(function of the level of risk)

Who is involved ?

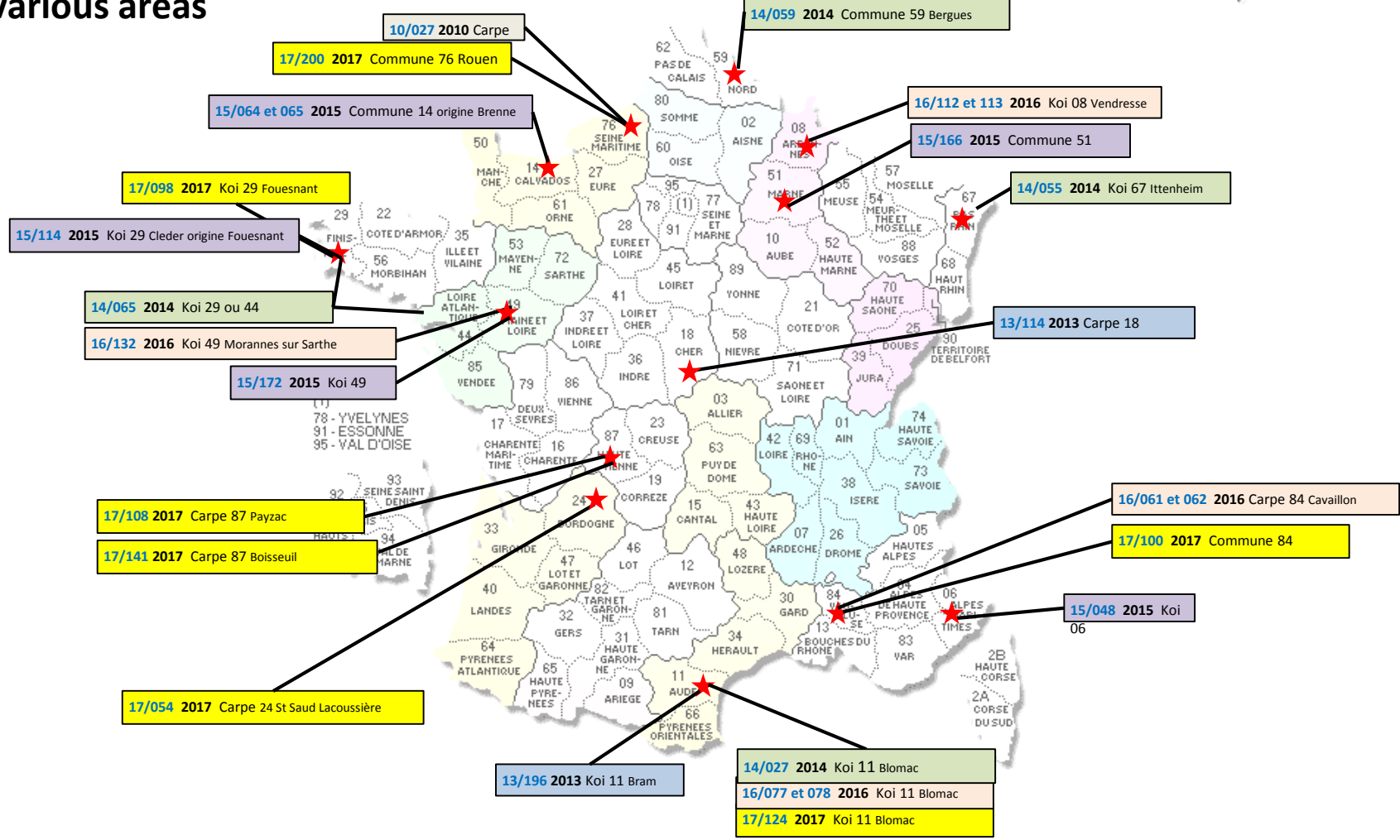
- Authorities (national and departmental levels)
- Professional representations, fish farmers, traders, associations
- Veterinarians
- NRL and its lab network
- ...



④ Emerging diseases : a focus on Carp Edema Virus ...

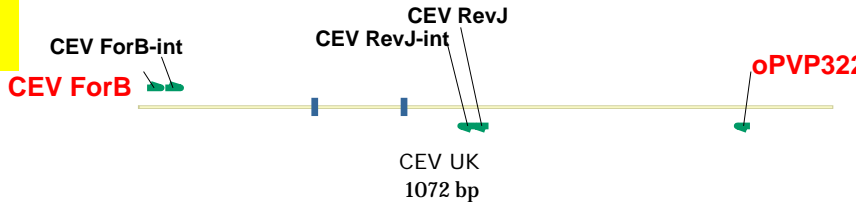


- > First case detected in **2010**
- > **Sporadic outbreaks**, on Koi but also on common carp
- > In **various areas**

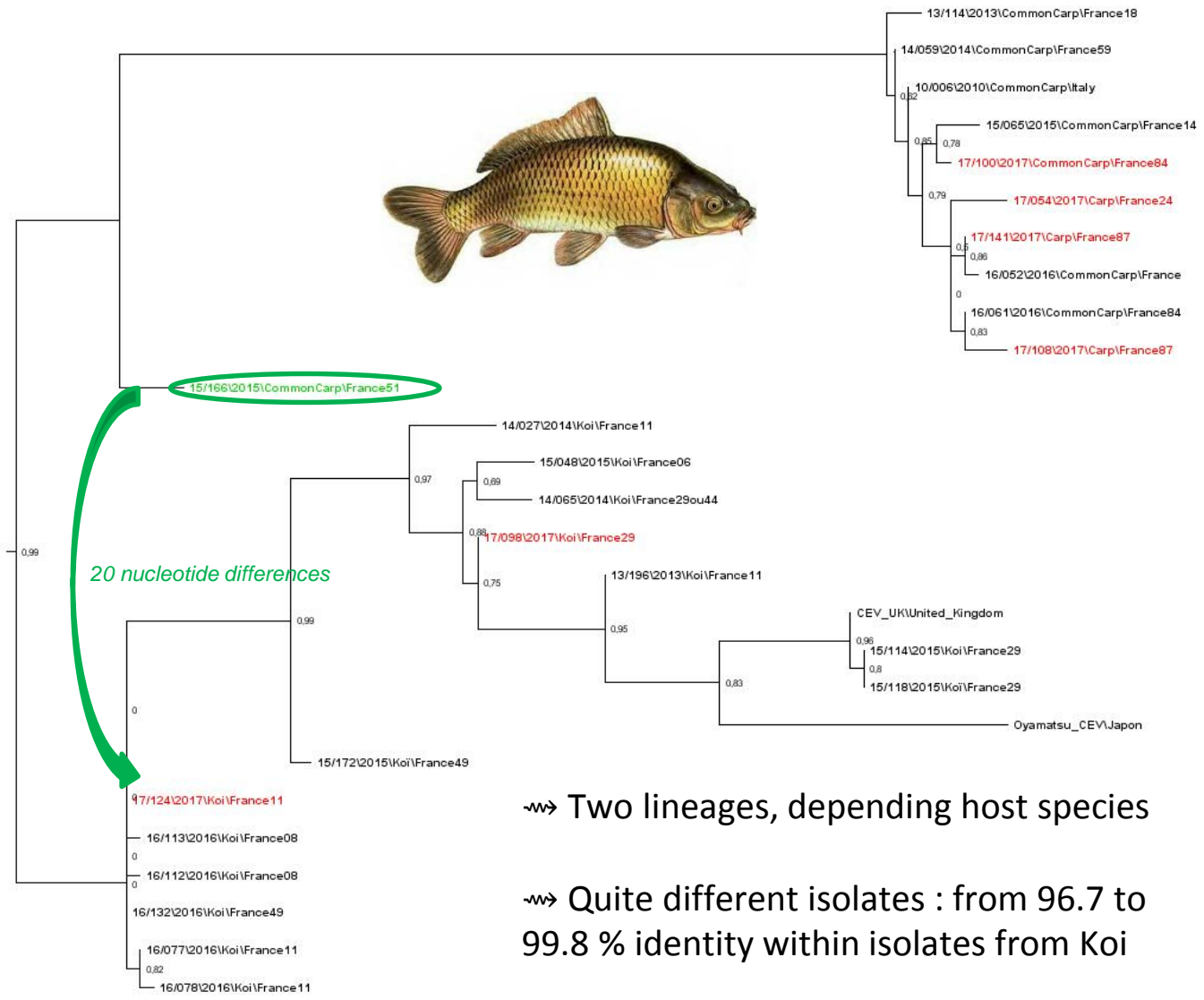


- > Since the beginning of 2017 : increasing number of outbreaks (7 in 7 different areas)
- > Information given to the authorities through our alert and vigilance system

④ Emerging diseases : a focus on Carp Edema Virus



Sequencing a 893 bp long fragment



Common carp

Koi



→ Two lineages, depending host species

→ Quite different isolates : from 96.7 to 99.8 % identity within isolates from Koi

CONCLUSION

- France has not a disease free status
- Viruses responsible for listed diseases circulate on the territory, with various outbreaks
- The National Plan for Eradication and Surveillance represents a huge work load...
But **if successful = no VHSV, no IHNv**
- Viruses responsible for emerging diseases will have still to be studied
- Importance of traditional **cell culture analysis** in parallel of molecular biology

Thank you very much for your attention!!





11th Annual Meeting EPIZONE
'Crossing barriers'

19-21 September 2017
Paris, France

Hosted by ANSES



<https://www.epizone-eu.net/en/Home/Annual-meeting.htm>

Deadlines:

**Abstract submission :
28/05/2017**

**Early bird registration :
07/07/2017**