



# Overview of the Koi sleepy disease in France

## The CEVIRAL project



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# The Koi sleepy disease

- 1<sup>st</sup> description in Japan  
(*Hosoya and Suzuki 1976*)
- Intense worldwide dissemination on koi and common carp
- Caused by Carp edema virus (CEV)

## Au Liban, des tonnes de poissons morts dans un lac pollué

Liban

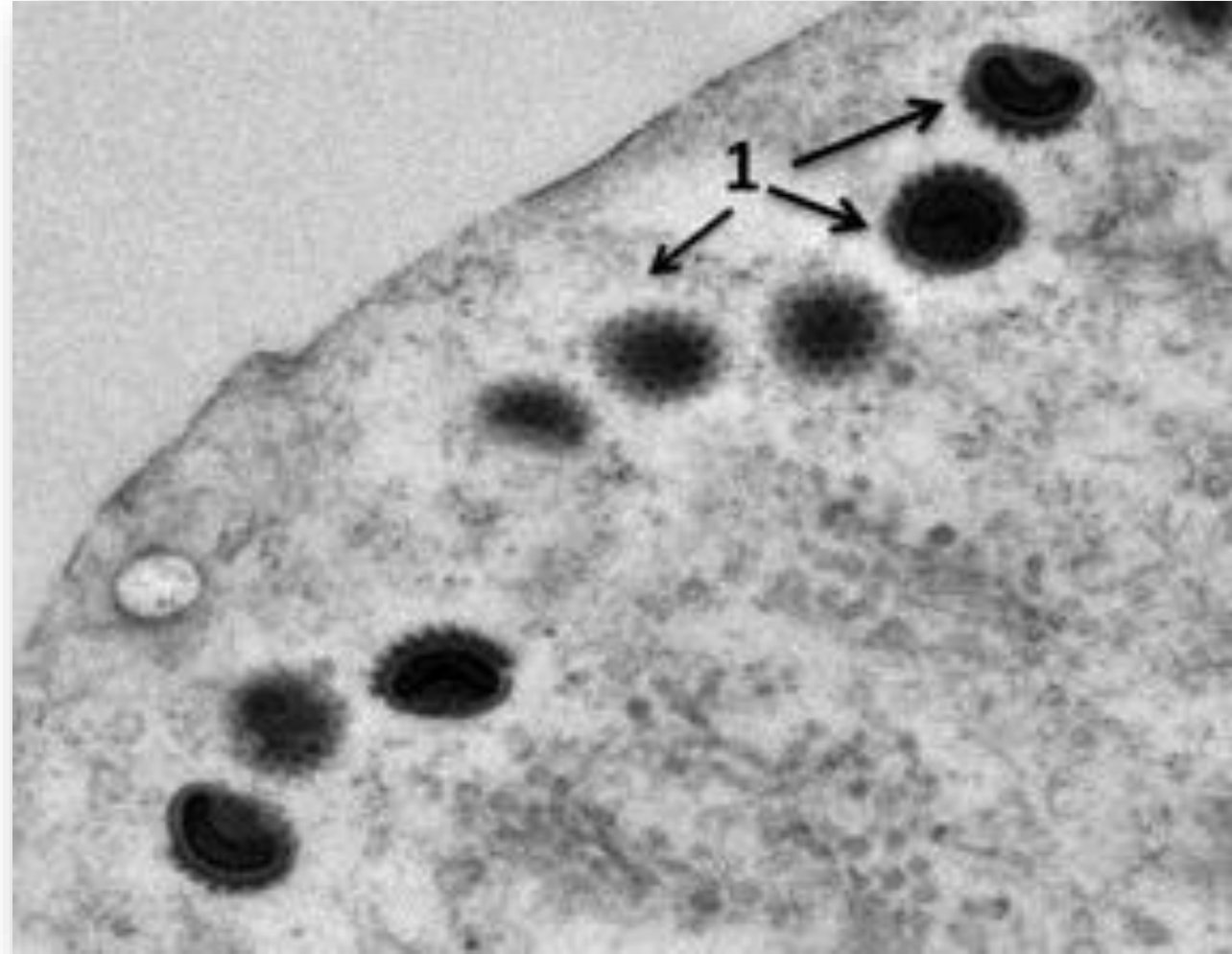
SUIVRE CE THÈME



Des carpes échouées sur une rive du lac Qaraoun, dans l'est du Liban, le 29 avril 2021 © AFP/JOSEPH EID

# Carp Edema Virus (CEV)

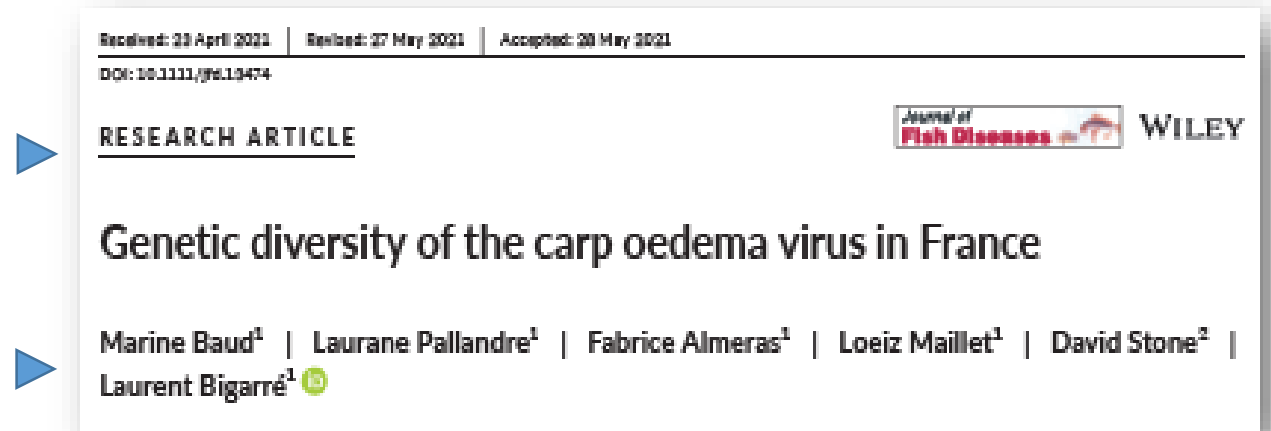
- *Poxviridae* Family
- Very large DNA genome, many genes unidentified (*Mekata et al., 2021*)
- 2 genogroups (gI, gII) and a lot of variants (partial P4a gene)
- Diagnostic by PCR (no cell culture)



*Way et al., 2017*

# The situation in France

- CEV in France since (at least) 2013  
(*Bigarré et al., 2013*)
- Yearly outbreaks in farms and ponds
  - Koi and common carps affected
  - CEV gI -> common carps
  - CEV gII -> Koi
- Primers for partial P4a gene of 890-893 bp
- One haplotype from common carp:  
possibly a recombinant

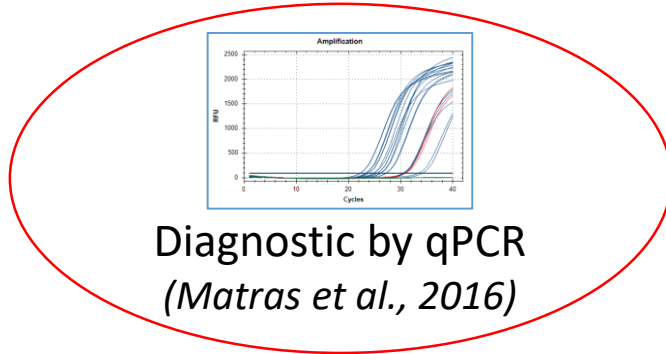


*Baud et al., 2021*

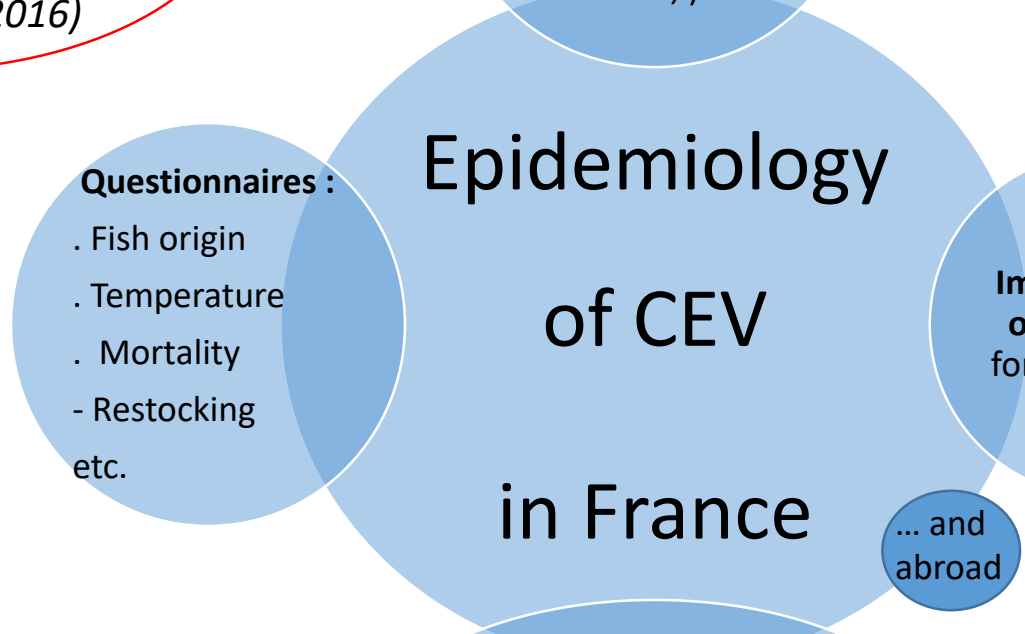
## TRACING OUTBREAKS: priorities and issues

- Epidemiological data ?
- Genetic links between outbreaks ?
- Mixed infections ?
- Genetic evolution of the virus ?

# The CEVIRAL project (European funds EMFAF)



**Many stakeholders...**  
(vets, producers, garden centers, dpt labs, research labs, )



**Questionnaires :**

- . Fish origin
- . Temperature
- . Mortality
- Restocking etc.

**Improvement of PCR tools for genotyping**

**Universal primers for :**

- a few complete genes
- gl and glI

**Genetic diversity**

- \* Phylogeny
- \* Molecular markers



# An example... tracing outbreaks in the 'Tarn' region



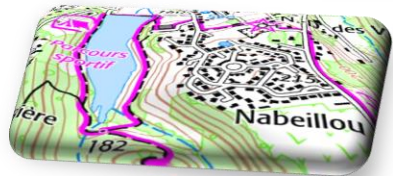
2022 : 4 lakes, without connections



3

## Lake 'Bézan'

- 6<sup>th</sup> -13<sup>th</sup> April 2022
- 150-200 of dead carps
  - Fish stocking by supplier X (dec 2021)
  - CEV +



1

## Pond 'Nabeillou'

- 30<sup>th</sup> march 2022
- 1 hundred dead carps
- Fish stocking by supplier X (dec 2021)
- No diagnostic !



2

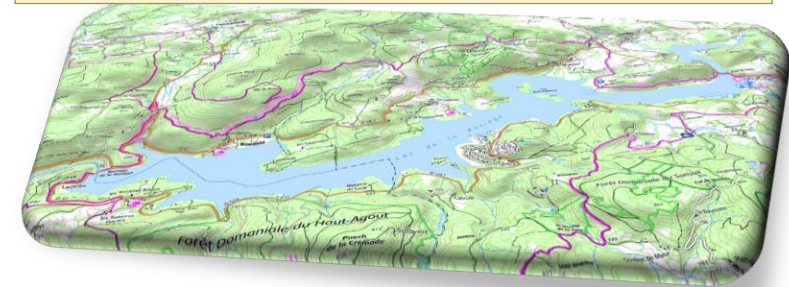
## Pond 'Aquaval'

- 4<sup>th</sup> April 2022
- About 300 of dead carps
- Fish stocking by supplier X (dec 2021)
- CEV +

4

## Lake 'la Ravière'

- 27<sup>th</sup> May 2022
- About 900 kg of dead carps
- No fish stocking
- CEV +



# CEV in the Tarn

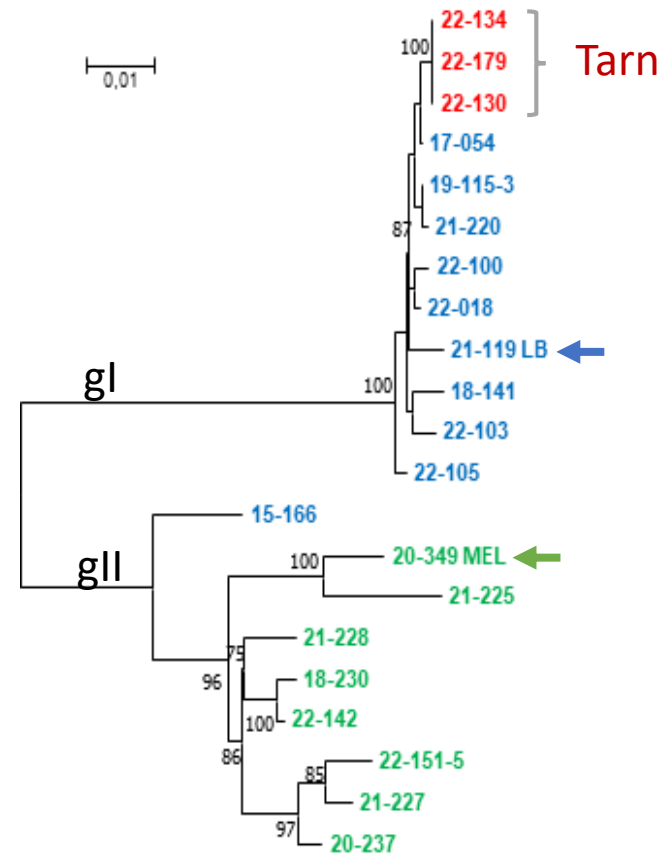
## Comparing the complete P4a gene

21 sequences compared:

- 19 from France (including 3 from Tarn)
- 1 from Lebanon
- 1 from Melanesia

### Results:

- ✓ All sequences in 2 genogroups : I and II
- ✓ Lebanon: gI ←
- ✓ Melanesia: gII ←
- ✓ Tarn : 100% identity between 3 sequences  
-> same origin !

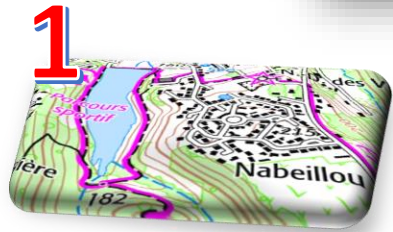


In blue and red: common carp  
In green: koi

# An example... tracing outbreaks in the 'Tarn' region



2022 : 4 lakes, without connections



Same provider

Same sequence

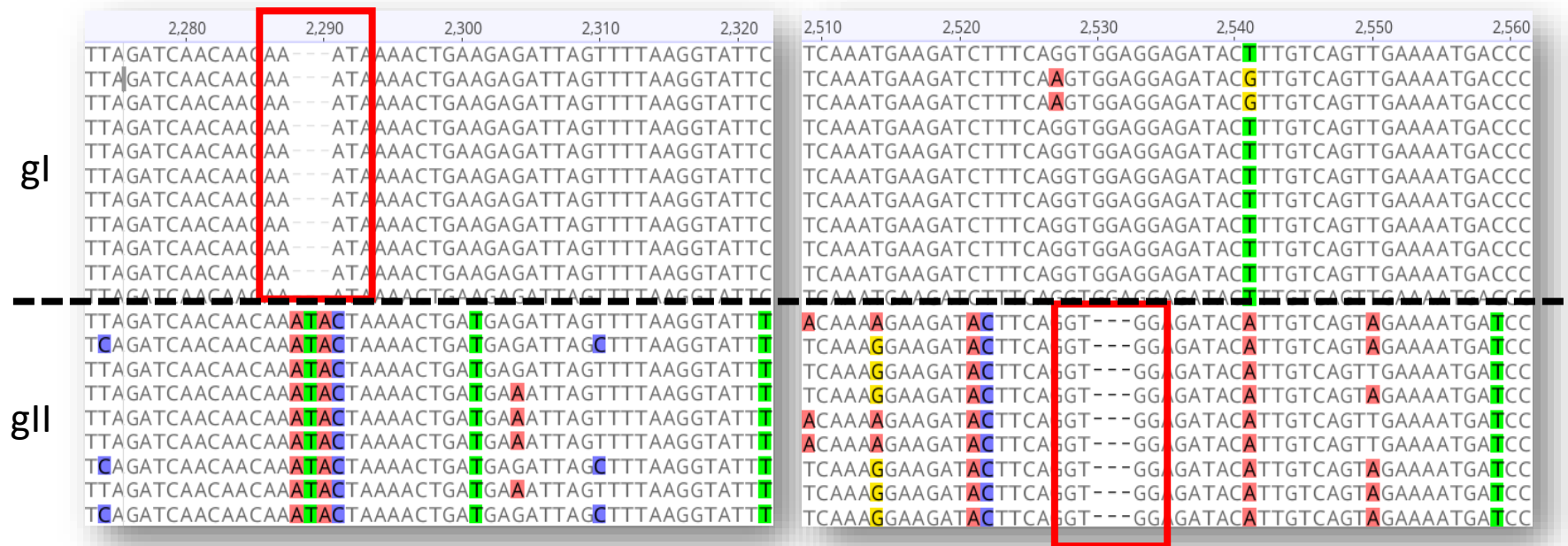
Probably same epizooty





# New molecular markers in complete P4a gene

- Previously known in the partial gene: one indel (3nt) in a microsatellite
- In the complete gene, 2 new indels, both genogroup-specific




# Conclusions

- New primers recognize complete P4a of CEV gI and gII
- First sequences from new geographical regions (Melanesia and Lebanon); consistent with spread of known genotypes
- A 'French' database enriched with sequences from 75 samples
  - 2021 to 2023, including new sites
    - Partiel P4a gene (890-893 bp)  $\approx$  75 samples
    - Complete P4a gene (3474-3477 bp)  $\approx$  30 samples

➡ Same genetic clustering gI and gII using **partial** or **complete** p4a phylogeny, but the most informative domain of this gene remains the one previously described (~890 bp)

➡ New markers in the complete P4a gene (indel of 3 nucleotides): function?

# Perspectives

- A new series of cases in spring 2023 under investigation in the North of France (4 sites of CC) 
- CEV transfer from Koi <-> CC ?  
2 sites under investigation
- More genetic markers to come...(full ORFs, substitutions and large indels)



# Thank you for your attention



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