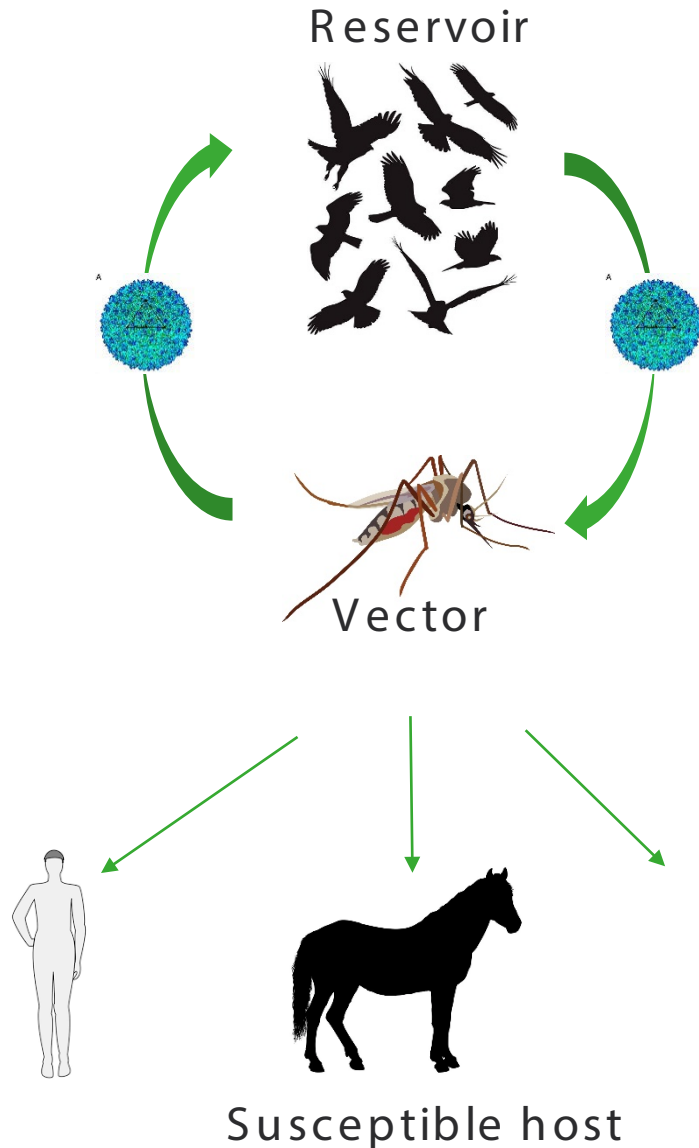




West Nile virus monitoring in Flanders (Belgium) during 2022-2023 reveals endemic Usutu virus circulation in the wild bird population

Sohier Charlotte

Transmission cycle WNV en USUV



Mosquito-borne viruses - Flaviviridae

Enzootic cycle

Both viruses circulate between birds and mosquitoes, mainly members of the genus *Culex*

⇒ For Belgium: *Culex pipiens* s.l., *Culex modestus*, *Culex torrentium*

Incidental (dead end) hosts

Humans, horses, and other mammals can get infected when bitten by infected mosquitoes

...

⇒ usually do not develop levels of viraemia high enough to start a productive infection in a competent vector

Epidemiological aspects

WNV

First isolated in Uganda in 1937

Circulating in Europe since 1950s,
never detected in Belgium

9 known genetic lineages,
Lin 1 & Lin 2 in Europe (humans)

Notifiable in Europe (category E, AHL)

Asymptomatic in many avian species,
often fatal for corvids and raptors illness

USUV

First isolated in South Africa in 1957 (near Usutu river)

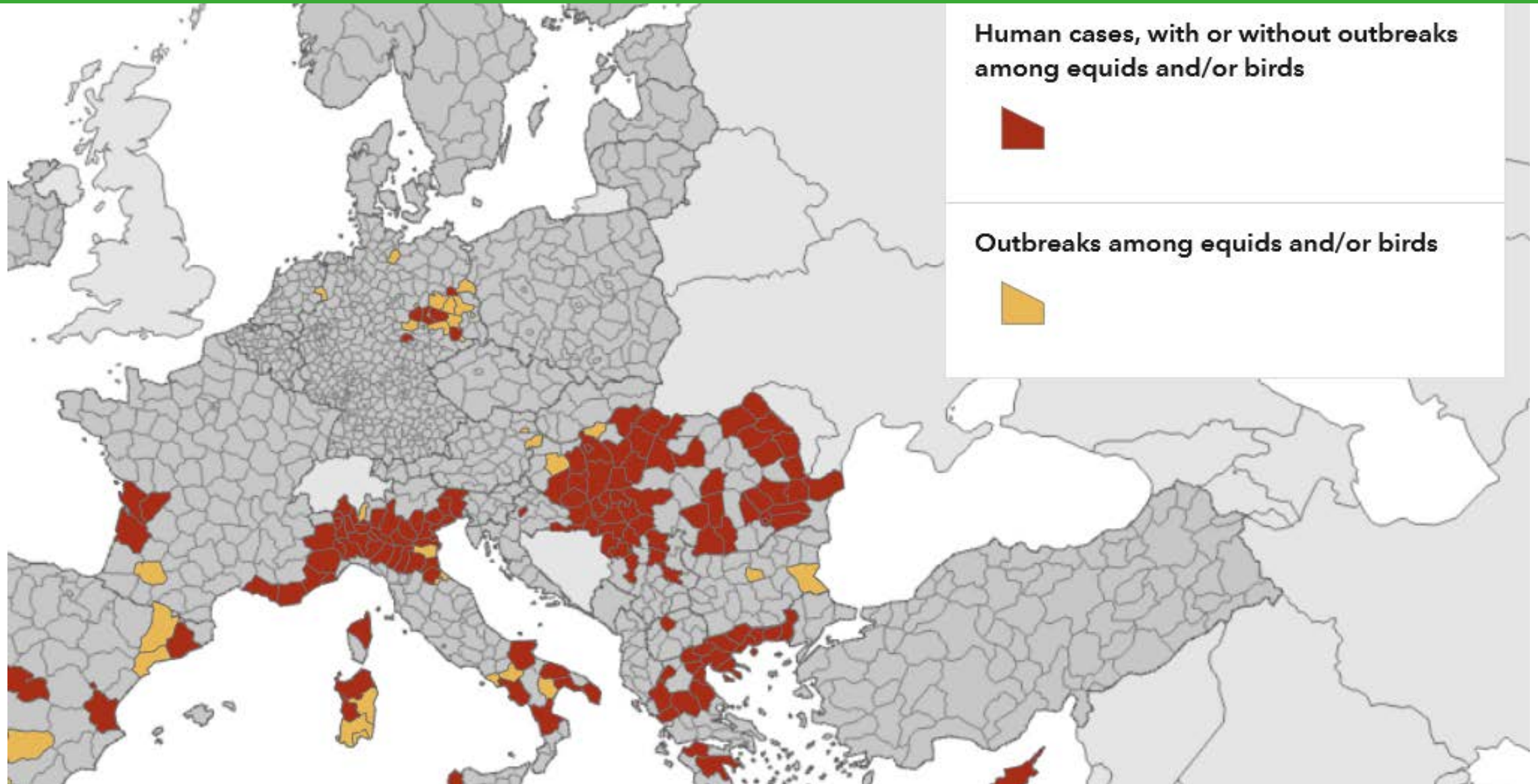
**USUV first detected in Europe in 1996 (Italy),
in Belgium in 2012**

8 lineages (Africa 1–3 and Europe 1–5),
in Belgium Lin Eu1, Eu3 en Af3

Not notifiable

Highly pathogenic and fatal for several bird species,
especially blackbirds and great grey owls

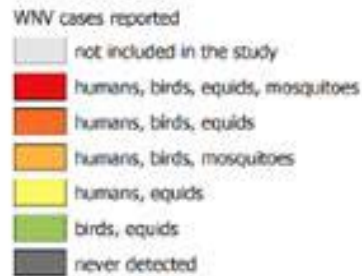
Geographic spread of WNV in 2023 in birds, equids, humans



Geographic spread of WNV from 2012 to 2021 in birds, equids, humans & mosquitoes

17 EU/EEA countries reported
WNV

Belgium currently free from
West Nile Virus?



Countries not visible
in the main map extent



Geographic spread of USUV from 2012 to 2021

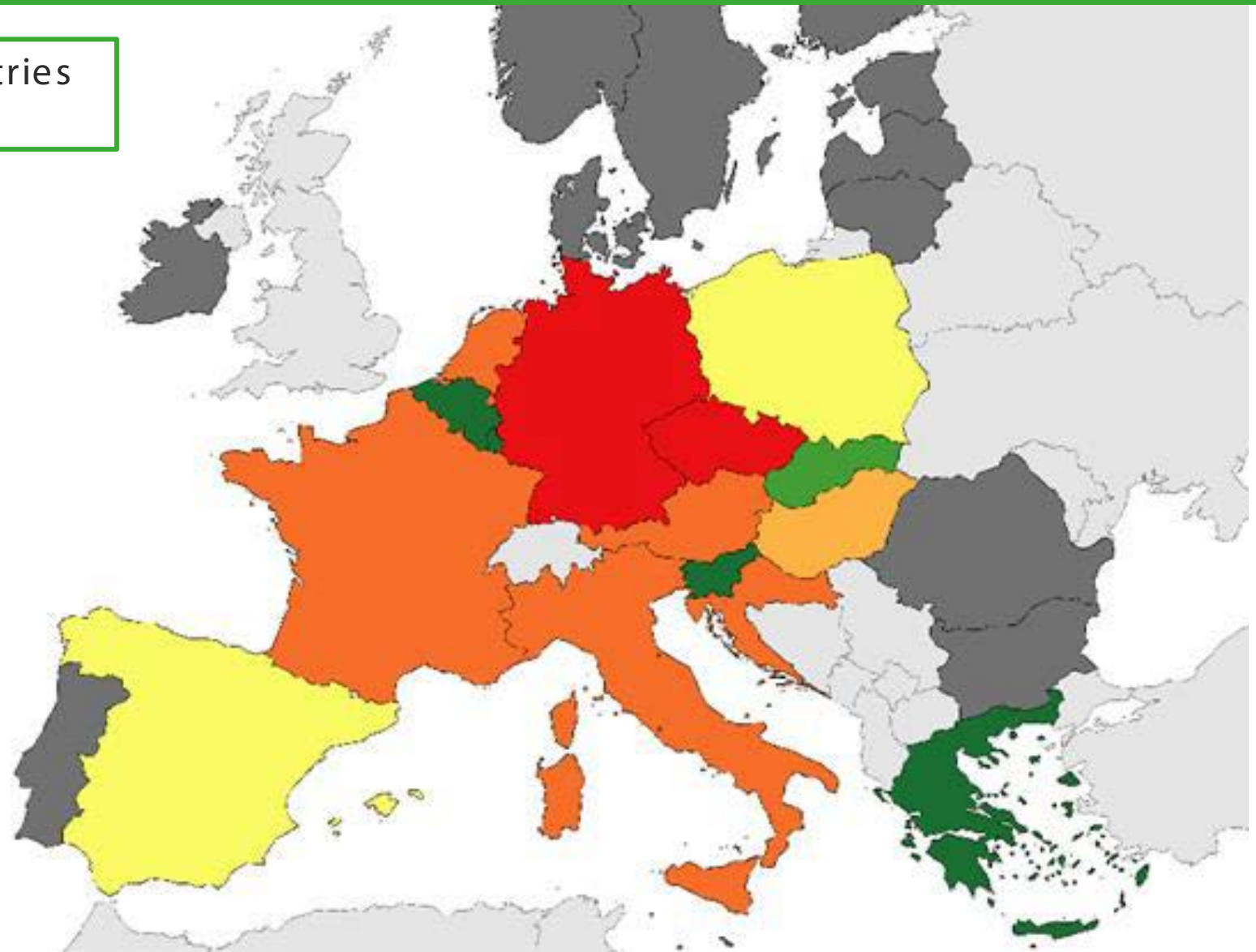
in birds, equids, humans & mosquitoes

15 EU/EEA countries reported USUV

- USUV cases reported
- not included in the study
 - humans, birds, equids, mosquitoes
 - humans, birds, mosquitoes
 - humans, birds
 - birds, equids
 - birds, mosquitoes
 - birds
 - never detected

Countries not visible in the main map extent

Luxembourg



Timeline USUV Belgium

- 2012**
First detection in birds
- 2016**
widespread bird mortality
- 2018**
further detection in birds & bats
- 2019-2020**
USUV antibodies in wild boar
- 2022-2023**
Endemic ?

Surveillance options in animals & mosquitoes



Birds

1. Passive surveillance through swabs or organs of death birds
2. Active surveillance through swabs/blood/serum from living birds

- rt-PCR
- Virus isolation
- VNT

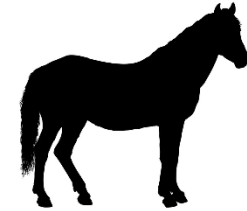


Mosquitoes

2 strategies:

1. all captured specimens of adult female mosquitoes
2. only *Culex pipiens* females

- rt-PCR
- Virus isolation



Horses

1. Passive surveillance after clinical symptoms or dead animals
2. Active surveillance

- Mostly ELISA
- VNT
- rt-PCR

- WNV surveillance is more established in the EU compared to USUV, though USUV infections are emerging
- An integrated surveillance system (humans, animals, and mosquitoes) for WNV in place in some European countries

Difficulties with laboratory diagnosis

Serology

Commercial serological test (ELISA)
Often cross reaction



VNT for different flaviviruses

- Labour intensive
- Time consuming
- Technical requirements

Virology

Genetic variation between strains
rt-PCR often cross reaction with
other flaviviruses



perform multiple PCR's to detect all
lineages and avoid false positive
results

We perform 4 different rt-PCR's to
differentiate in birds (USUV & WNV)
In horses also rt-PCR for TBEV

Depends on species &
epidemiological situation

Surveillance in birds in 2022 & 2023

Passive surveillance in birds

Limited monitoring program in Flanders for WNV (USUV included) in dead birds

Ongoing since 2016

Swabs or organs of dead birds => rt PCR

2022: 11 samples

2023: 28 samples



Active surveillance in birds

Limited monitoring for WNV (USUV included) in birds submitted to bird rescue centers (in Flanders)

New from 2022

Only swabs => rt PCR

2022: 36 samples from 1 bird rescue center, only Corvids & raptors

2023: 222 samples from 2 bird rescue centers,

Corvids, raptors & songbirds

Conclusions limited monitoring 2022-2023

- **no WNV detected yet in Belgium**

- a real absence or a consequence of underdiagnosis due to limited monitoring?

No new detections since 2020 in the Netherlands (only 1 season)
No detections in North of France

} Belgium currently
free from WNV?

- **USUV detected in both years**

- geographic spread across the surveyed area
- Among the USUV-positive birds, 75 % were Eurasian Blackbird
- Africa 3 lineage was isolated (was also detected in 2017)



Future perspectives

- Continue **passive** monitoring in dead birds
- **Active** monitoring in **wild birds (black birds & birds of prey)**
European project (OH4 surveillance) with 11 countries in 2024 – 2025 - 2026
 - Bird rescue centers in Flanders, Wallonia and Brussels
 - Collaboration with the regions and ULiège
 - 750 swabs/year

=> Early detection of an introduction - monitor the geographic distribution/spread to new areas
- For both monitoring: **differentiate** between West Nile virus and Usutu virus

Thanks for your attention !

Thanks to

- **Service Avivir (Sciensano)**
- **ANB, Muriel Vervaeke**
- **VOC Brasschaat-Kapellen**
- **VOC Opglabbeek**

