

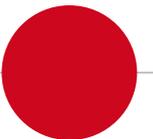


# Genomic surveillance of AIV: do we need more of it?

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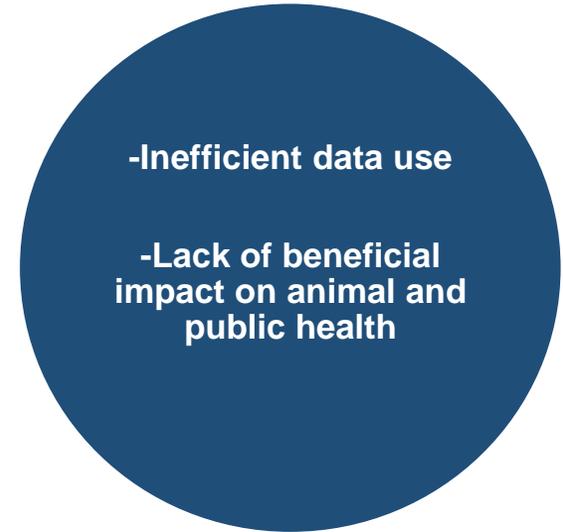
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## 2020-2021 HP H5Nx epidemic in EU MSs (5/10/2020–15/9/2021)

- 3627 Highly Pathogenic Avian Influenza (HPAI) virus outbreaks in EU MSs<sup>^</sup>
- 261 genome sequences available in GISAID (time needed for submission  $\mu$ @79 days)
- 114 genome sequences confidentially shared with the EURL

# Barriers to AIV sequence data generation and sharing



## Technical barriers

Limited capacity to perform sequencing

## Motivational barriers

No incentives

Scientific opportunities may be lost (data are not shared until articles are published)

## Economic barriers

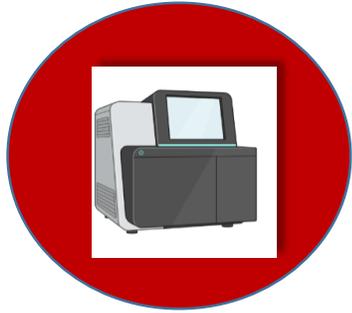
Lack of resources

## Political barriers

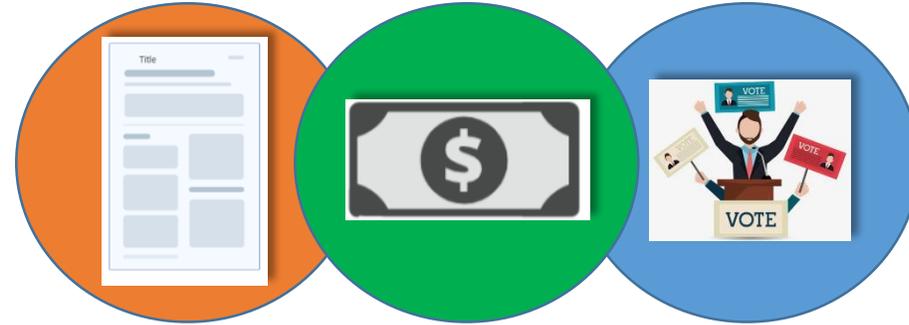
Lack of trust: potential misinterpretation, misuse or intentional abuse of data

Lack of guidelines: sharing not well regulated or standardized, resulting in protective policies

# Strategies to resolve specific barriers

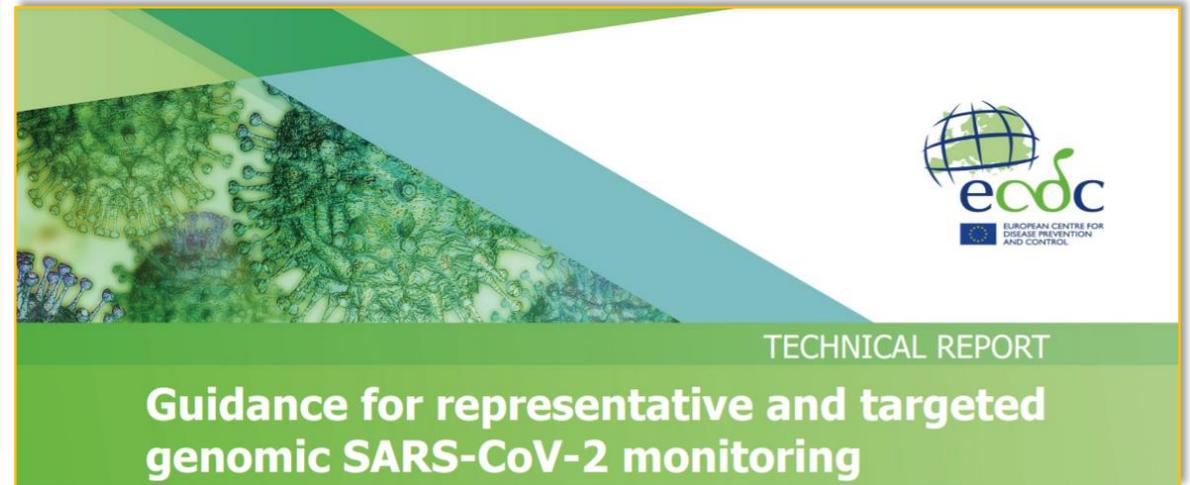
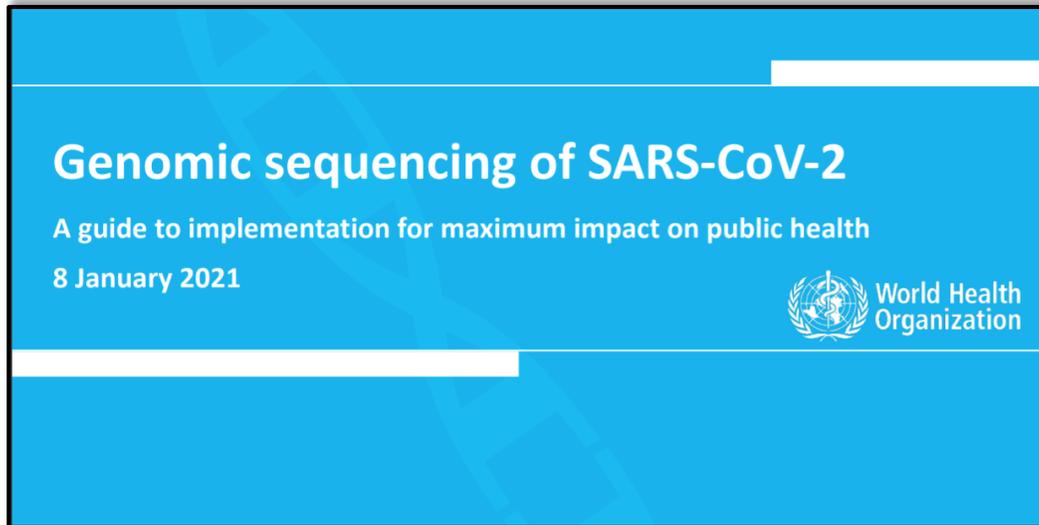


EURL available to  
provide support



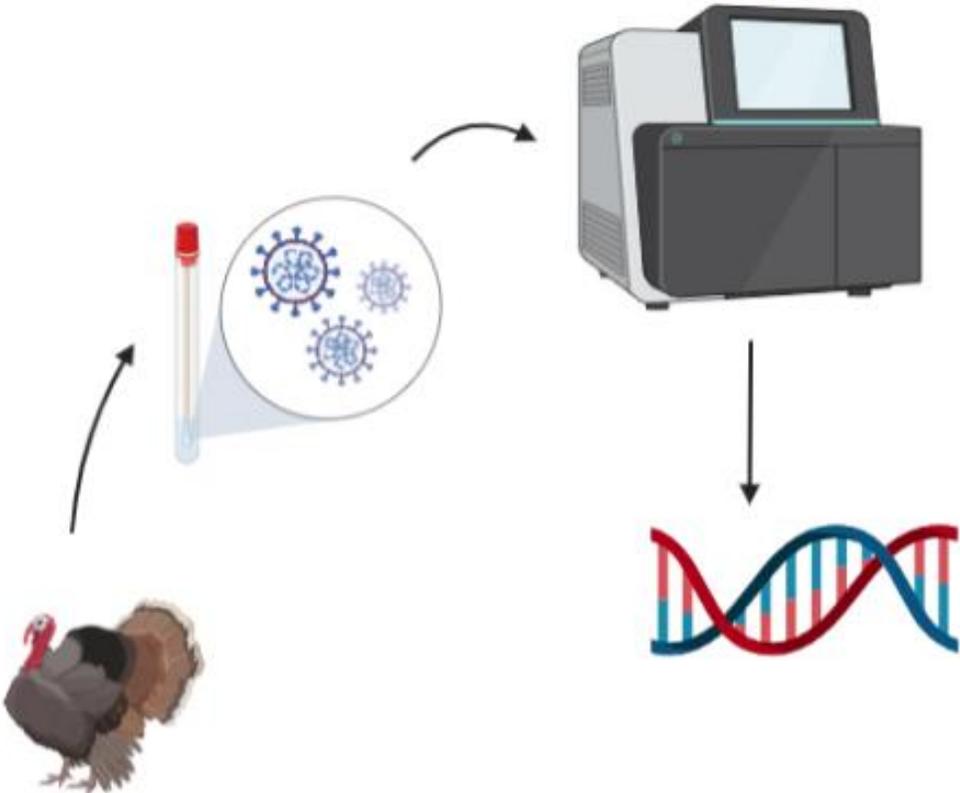
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# ● Strategies to resolve motivational, economic and political barriers



Is the provision of guidelines for genomic sequencing of AIV a possible solution?

# Genome sampling strategies



This block contains several diagrams illustrating different aspects of genome sampling strategies:

- Phylogenetic tree:** A tree showing relationships between different samples, with a legend for 'Bayes factor support' (100, 20-100, 10-20) and a color-coded legend for various regions (Africa, Central Asia, Europe, etc.).
- World map:** A map of the world with colored regions and arrows indicating movement or sampling locations.
- Chicken farm:** Three blue house icons with chickens, connected by dashed arrows, representing a farm or sampling site.
- PCR diagram:** A diagram showing the amplification of a DNA segment with primers (T, L) and a fluorescently labeled primer (F).
- Graph:** A graph showing the amplification of DNA over cycles, with a red curve for 'Positive' and a blue line for 'Negative', separated by a 'Threshold'.
- Animals:** Illustrations of a seal, a fox, and a cat.
- Human:** An illustration of a human torso showing the respiratory system (lungs and trachea).

**Depend on:**

- Answers being sought
- Resources available

Sampling strategy	Sequence Metadata	Objective
<b>Representative sampling of AIV positive cases obtained from national surveillance systems</b>	Species, Date and Location of sampling, Clinical information	Characterization of genetic diversity of AIV in poultry species
	Species, Date and Location of sampling, Clinical information	Characterization of genetic diversity of AIV in wild birds
<b>Targeted sampling from AIV outbreaks occurring in special settings or populations.</b>	Species, Date and Location of sampling, Clinical information	<ul style="list-style-type: none"> <li>-Unusual events (changes in pathogenicity, clinical signs, host range)</li> <li>-Evaluation of transmission drivers</li> <li>-Reconstruction of transmission dynamics within and between farms</li> <li>-Vaccine breakthrough infections</li> </ul>

**Sampling approach?  
 Number of samples to be sequenced?**

Sampling strategy	Sequence Metadata	Objective	Sampling Approach
<p><b>Representative sampling of AIV positive cases obtained from national surveillance systems</b></p>	<p>Species, Date and Location of sampling, Clinical information</p>	<p>Characterization of genetic diversity of AIV in poultry species</p>	<p>For outbreaks caused by HPAI viruses and LPAI viruses with zoonotic potential or increased virulence, it is recommended to sequence at least <b>one positive sample from each AIV outbreak</b>. In case of multiple outbreaks in a short timespan, at least <b>one positive sample</b> should be sequenced from <b>each cluster of infection</b> recognized through the epidemiological investigation.</p>
	<p>Species, Date and Location of sampling, Clinical information</p>	<p>Characterization of genetic diversity of AIV in wild birds</p>	<p>Sequencing is performed on <b>HPAI samples representative in terms of time, species, geography, subtype as well as in terms of disease severity</b>. In order to follow the evolution of the virus over time, sequencing of positive samples should be carried out periodically (at least every two weeks). The same sampling approach should be applied to emerging LPAI viruses with zoonotic potential.</p>
<p><b>Targeted sampling from AIV outbreaks occurring in special settings or populations.</b></p>	<p>Species, Date and Location of sampling, Clinical information</p>	<ul style="list-style-type: none"> <li>-Unusual events (changes in pathogenicity, clinical signs, host range)</li> <li>-Evaluation of transmission drivers</li> <li>-Reconstruction of transmission dynamics within and between farms</li> <li>-Vaccine breakthrough infections</li> </ul>	<p><b>Infection in mammals:</b> Dense sampling and sequencing of all or most of the infected individuals at the same location and time, whenever these events occur.</p> <p><b>Infection in domestic or wild birds:</b> dense sampling and sequencing of multiple samples from the same outbreak (e.g. in case of farms, one sample/epidemiological unit)</p>



# **Guidelines for genomic sequencing of AIV: proposed role of the EURL in the genomic surveillance of AIV**

## NRLs submitting samples for sequencing to the EURL

Provide the EURL with:

- material for nucleic acid extraction or material already extracted (RNA) or cDNA
- the Ct value of samples should be <28
- the cold chain should always be maintained during transport and RNA samples should be stored at  $\leq 70^{\circ} \text{C}$
- metadata.

## Sequence data generated at the NRLs

Provide the EURL with:

- Accession numbers of the sequences published by the NRLs
- unpublished consensus sequences generated by the NRLs
- metadata

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Genetic characterization of HPAI H5Nx viruses identified in Europe starting from October 16, 2020 (update March 03, 2021)

This report describes the genetic characteristics of the complete genomes of ninety-six European HPAI H5N8, H5N5, H5N1 and H5N3 viruses identified in seventeen European countries (Austria, Belgium, Croatia, Czech Republic, Denmark, France, Germany, Ireland, Italy, the Netherlands, Norway, Poland, Romania, Slovakia, Slovenia, Sweden, the United Kingdom) since October 2016 (Table 1). Analyses are based on sequences produced by the EURL and on sequences either provided by NRLs or deposited in GISAID by Member States (available on March 03, 2021), as well as sixty-one H5 viruses from Russia (59 H5N8, 2 H5N5) and seven H5N8 viruses from Kazakhstan available in GISAID, for a total of 164 genetically characterized viruses.

Please be aware that this report includes sequences not available in public databases and needs to remain confidential.

Table 1. Complete genomes of European HPAI H5N8, H5N5, H5N1 and H5N3 viruses analysed in this report.

Country	HPAI H5N8	HPAI H5N5	HPAI H5N1	HPAI H5N3	Total
Austria*	2	1			3
Belgium	3	1			4
Croatia	1				1
Czech Republic	14				14
Denmark	1	1		1	3
France*	4				4
Germany*	2	1		1	4
Ireland	1				1
Italy	11	1	2		14
Netherlands	8		6		14
Norway	1				1
Poland*	2				2
Romania*	3	2			5
Slovakia*	1	3			4
Slovenia*	1	1			2
Sweden*	3	4			7
United Kingdom	11	2			13
Total	59	17	8	2	96

\*The Polish NRL published the complete genome of 2 H5N8 viruses (reported in the table) in GISAID and provided the EURL with 21 HA gene and 12 NA (NS subtype) gene sequences (of which only 5 HA and 2 NA gene segments were submitted to GISAID).

\*Sequences of viruses from Austria, France, Germany, Romania, Slovakia, Slovenia and Sweden were provided by NRLs or produced by the EURL and are not yet publicly available.

Phylogenetic analysis

The phylogenetic analysis of the HA gene revealed that all the European HPAI H5 viruses form a single genetic group within clade 2.3.4.4B, and cluster with HPAI H5 viruses identified in Kazakhstan and in the Russian Federation starting from July 2020.

Reassortant genotypes

The characterization of the complete genome revealed that the HPAI H5 viruses, which have been circulating in Europe, Russia and Kazakhstan since July 2020, show distinct gene constellations, likely due to the occurrence of multiple reassortment events with LPAI viruses circulating in wild birds in Eurasia and



Ian Brown 04:00

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nomenclature  
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The results of the analyses are confidentially shared with NRLs and the EC through Mattermost platform. The release frequency of the EU genomic reports will be defined according to the epidemiological situation.

## ● Questions to ignite the discussion

- Are you aware of any other barrier preventing sequence data generation and sharing?
- Considering the sharing barriers you can see in your own country/laboratory, do you think guidelines could help you to manage genetic surveillance?
- Is sharing of the EU genomic reports prepared by the EURL in the Mattermost platform an acceptable option?



# Acknowledgments

A warm thank you goes to all the colleagues from the NRLs for their interest and motivation to share samples, metadata and sequence data with the EURL