Guidance for genomic monitoring of Avian Influenza Virus (AIV)

Background

Genomic surveillance of Avian Influenza Virus (AIV) is essential to characterize its genetic diversity and to detect the emergence of viruses that can result in increased transmissibility, disease severity, or have impact on animal and public health as well as on control measures. In addition, it enables the rapid investigation of the epidemiological, evolutionary and transmission dynamics of influenza viruses at local, regional and international scales, thus allowing an appropriate risk assessment and management. Viral genome sequencing with a well-defined sampling and sequencing strategy should then be routinely integrated in surveillance systems to ensure representativeness and reliability of findings and provide timely and accurate genetic information on the viruses circulating in the affected areas.

Genome sampling strategies and study design

Genome sampling strategies depend on the answers being sought and, due to the high cost of sequencing, they are strictly bound to the available resources. The main application of AIV genome sequencing for European Member States (MS) is to:

- Explore the genetic diversity of the viruses circulating in the European territories;
- Promptly detect the occurrence of novel virus introductions;
- Understand the virus origin, spread and evolution;
- Identify the links among outbreaks and differentiate primary and secondary outbreaks in poultry;
- Identify molecular determinants for pathogenicity, transmissibility, host specificity and diagnostic failure.

Therefore, the EURL recommends two complementary sampling approaches:

1. Representative sampling of AIV positive cases obtained from national surveillance systems;
2. Targeted sampling from AIV outbreaks occurring in special settings or populations.

Considering that the costs and work involved in gene sequencing are substantial, the proportion of samples to be sequenced and metadata reported in Table 1 are the minimum required to describe the genetic diversity of circulating viruses.

Table 1. Sampling approaches for the genetic characterization of AIVs

Sampling strategy	Sequence Metadata	Objective	Sampling Approach
Representative sampling of AIV positive cases obtained from national surveillance systems	Species, Date and Location of sampling, Clinical information	Characterization of genetic diversity of AIV in poultry species	For outbreaks caused by HPAI viruses (and LPAI viruses with zoonotic potential or increased virulence), at least one positive sample should be sequenced from each AIV outbreak in poultry. In case of multiple outbreaks (>2) in a short period of time (<1 week), at least one positive sample should be sequenced from each cluster of infection recognized through the epidemiological investigation.

		Characterization of genetic diversity of HPAI in wild birds	To generate data that reflect the situation in the wild bird population in each country, it is important to ensure that sequencing is performed on samples representative in terms of time, species and geography as well as in terms of disease severity. Therefore, at least one positive sample should be sequenced from each HPAI subtype, species and location. In case of persistent circulation of HPAI, sequencing should be carried out periodically (at least every two weeks) to follow the evolution of the virus and the disease over time. The number of sequenced samples may vary according to the number of outbreaks: - <5 cases in 2 weeks from different locations: all samples suitable for sequencing should be sequenced - >5 cases in 2 weeks from different locations: representative samples (≥10%) should be selected for sequencing
Targeted sampling from AIV outbreaks occurring in special settings or populations		Unusual events (changes in pathogenicity, clinical signs, host range)	Infection in mammals: dense sampling and sequencing of all or most of the infected animals at the same location and time, whenever these events occur. Infection in domestic or wild birds: dense sampling and sequencing of a representative number of samples from the same outbreak. In particular, at least one sample for each epidemiological unit in case of poultry farms and at least one sample from each species involved in case of wild birds.
		Vaccine breakthrough infections (identification of escape mutants during emergency vaccination)	Dense sampling and sequencing of a representative number of samples from the same outbreak. Specifically, at least one sample for each epidemiological unit.

Sequence data generation and sharing

The European Union Reference Laboratory (EURL) is more than available to support European National Reference Laboratories (NRLs) in case of need (Figure 1). The quality of the sample and the viral load are crucial to obtain optimal sequencing results, and the laboratories turning to the EURL for genetic characterization should submit samples selected based on the above sampling strategy, taking into account the following instructions:

- provide enough material for nucleic acid extraction (at least 200ul); extracted RNA or cDNA (at least 30 µl) could also be provided;

- the Ct value of samples should be <28;

- provide selected sequencing materials in a timely manner;

- the cold chain should always be maintained during transport and RNA samples should be stored at ≤ 70° C;

- the submission form (Annex) should be completed with required metadata.

European NRLs having access to a sequencing facility are encouraged to promptly share the AIV sequences with the EURL (ideally no later than two weeks after the outbreak). In order to use the genomic surveillance data for animal health decisions, virus sequence data should be accompanied by their respective metadata (Annex).

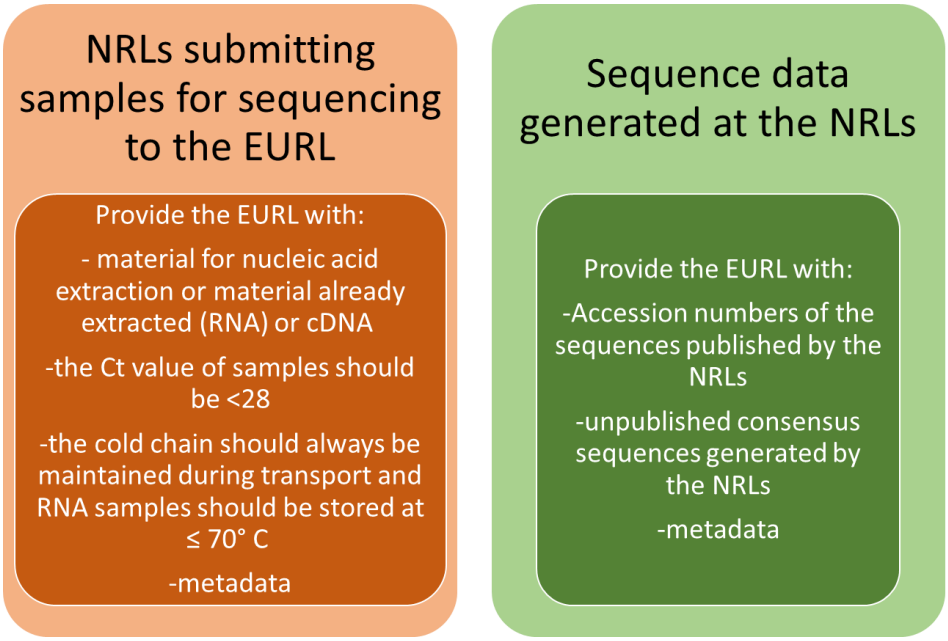

Figure 1. Sequence data generation and sharing

Data management, analysis and reporting

The EURL promotes the submission of sequence data to GISAID (https://www.gisaid.org) or Genbank. Accession numbers of the published sequences or sequence data generated by the NRLs from representative sequencing of AIVs should be shared with the EURL in a timely manner. The EURL and the European AI-ND NRL network have the responsibility to analyse all the data available to reconstruct the origin, transmission and spatial dynamics of AIV at European level, and to monitor the emergence of mutations of particular concern for animal and human health. The results of the analyses implemented by the EURL will be summarized in periodic reports which are going to be confidentially shared with the NRLs and the European

Commission (EC) via the IZSVe-EURL platform on *Mattermost Inc*. The reporting frequency will be adapted according to the epidemiological situation.