IAV-S circulation in Italy: update

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WOAH Reference Laboratory for swine influenza

Reference Centre World Organisation for Animal Health Founded as OIE

OFFLU SWINE INFLUENZA EXPERTS TECHNICAL MEETING
28 – 29 April 2026
WOAH Headquarters, Paris

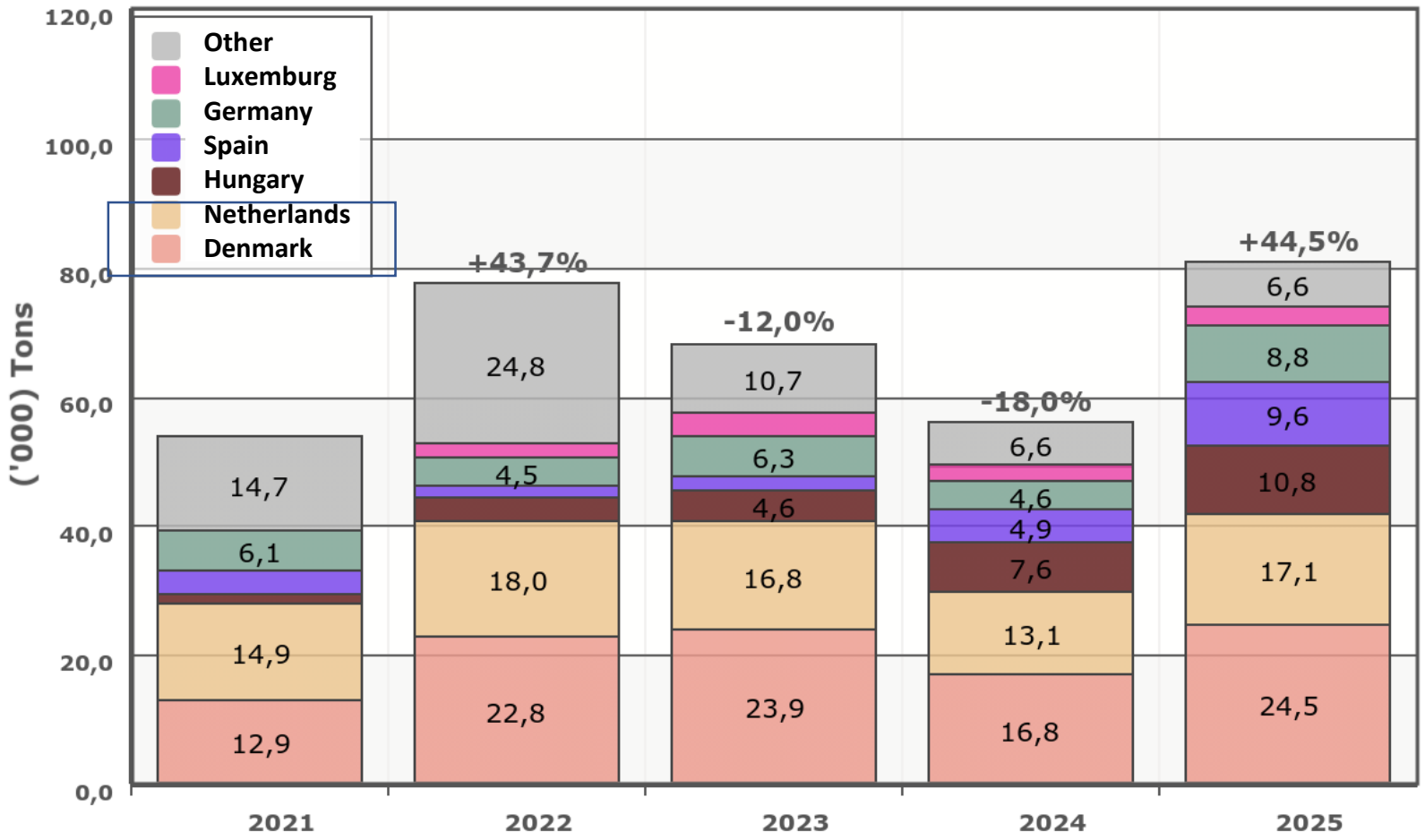
offlu
WOAH FAO
network of expertise on animal influenza

Veterinary diagnostic activity in Italy

The Italian Experimental Zooprophylactic Institutes (II.ZZ.SS) network, with their 10 headquarters and 90 peripheral diagnostic sections, are an important operational tool available to the National Health Service to ensure

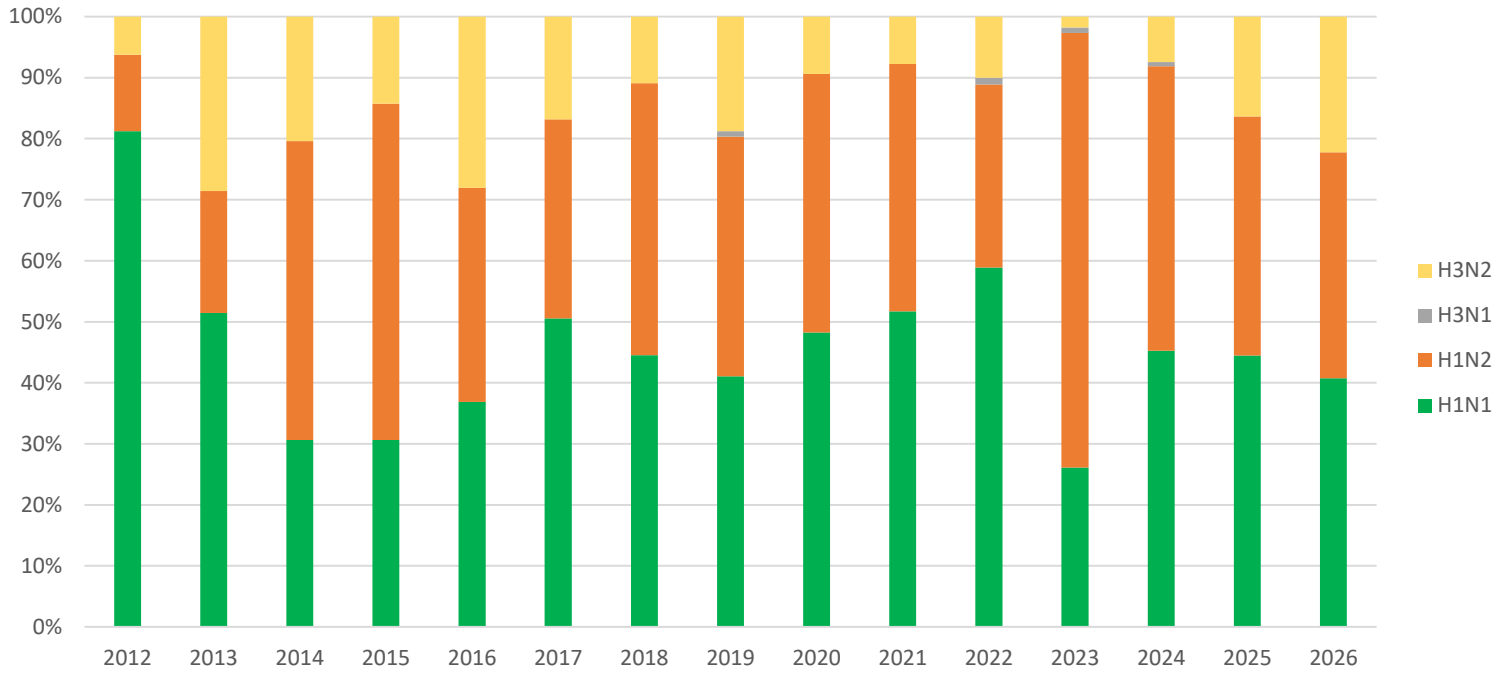
- epidemiological surveillance,
- experimental research,
- training of personnel,
- laboratory support diagnostics in the context of official control of foodstuffs

Live swine import in Italy



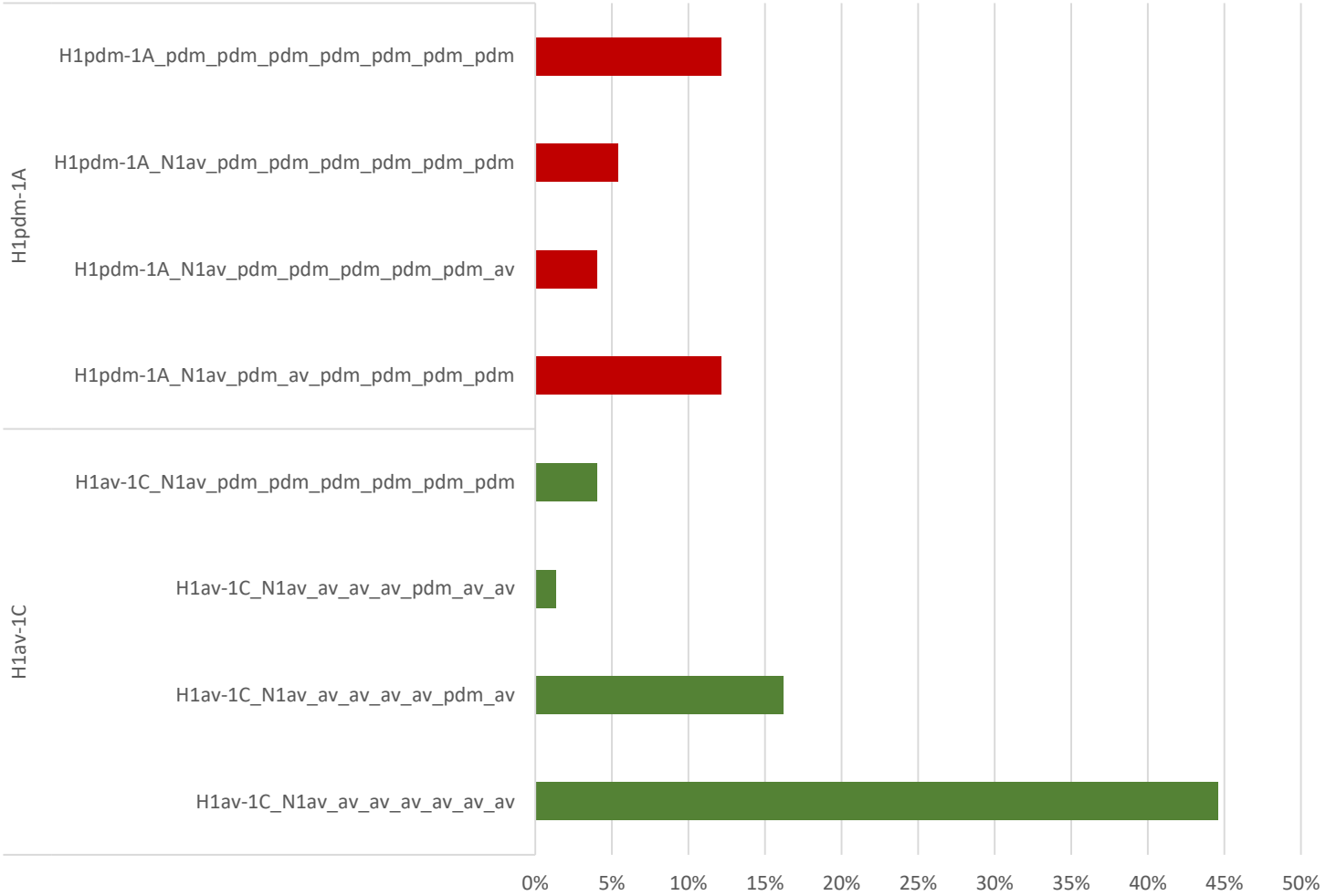
https://teseo.clal.it/?section=imp_exp_istat&cod=0103&mov=I

IAV-S subtypes circulating in Italy since 2012



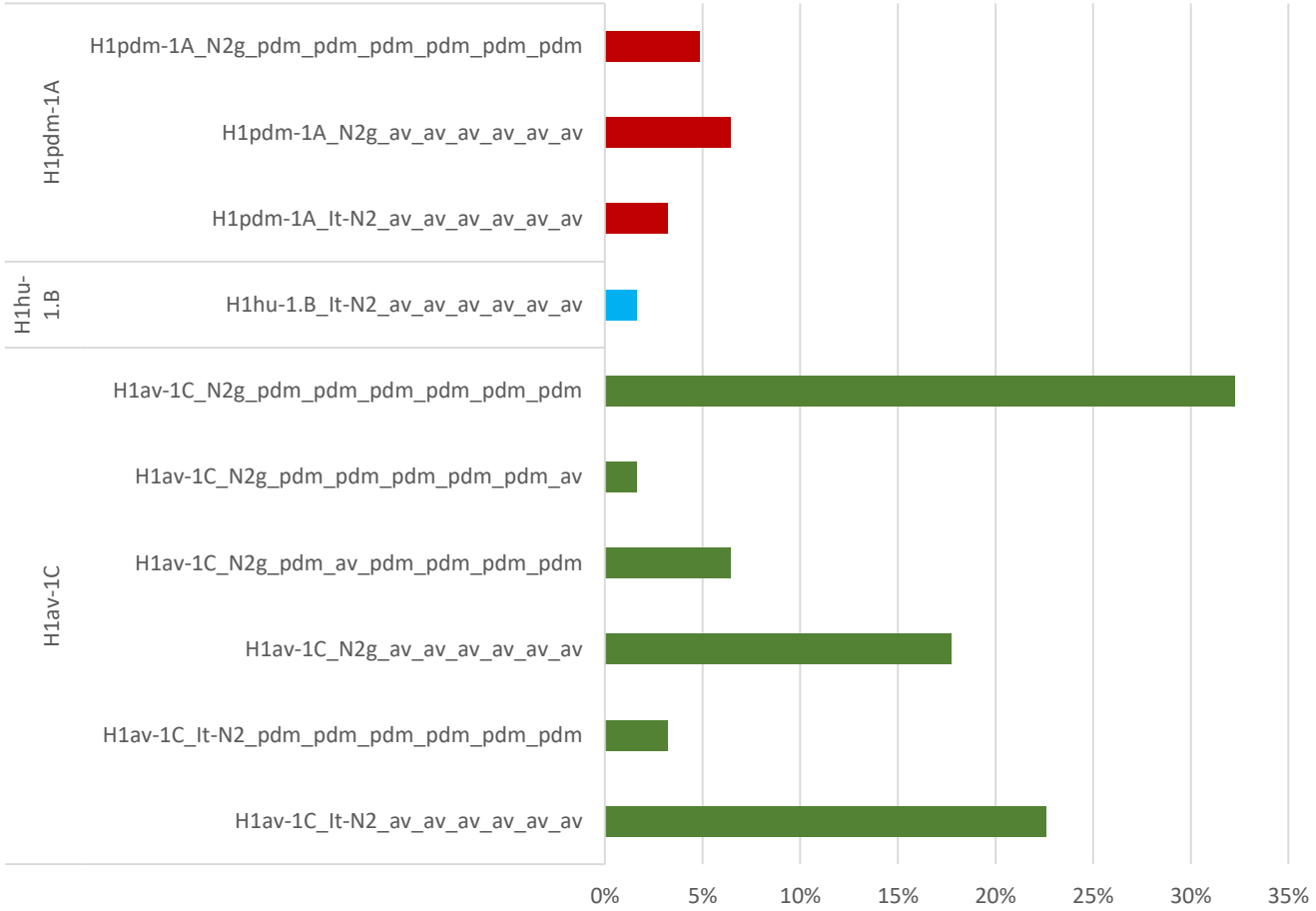
- H1N1 and H1N2 have been consistently predominant, while H3N2 has been detected with variable frequency

H1N1 genotypes since Jan-2025



74 strains

H1N2 genotypes since Jan-2025

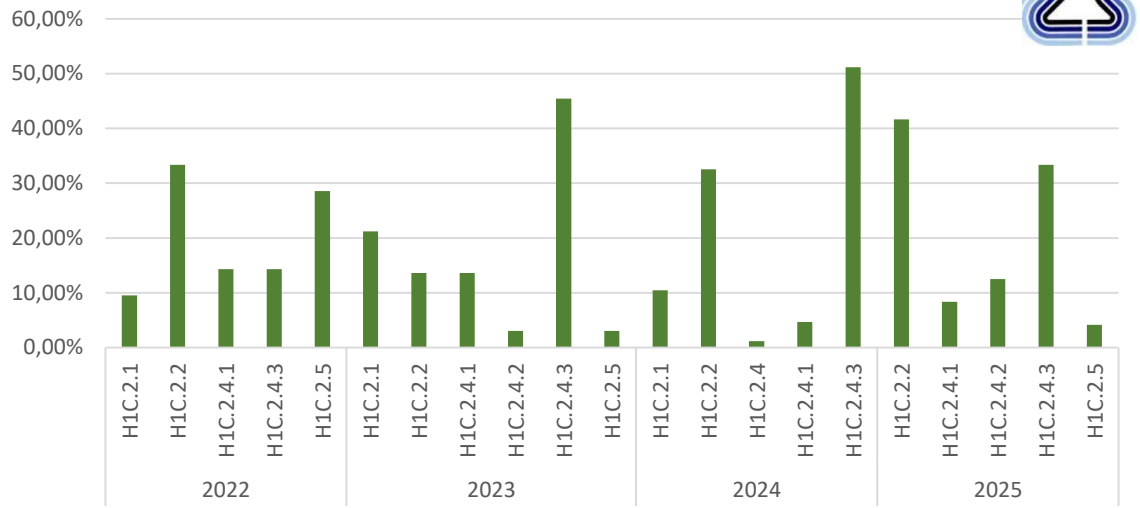


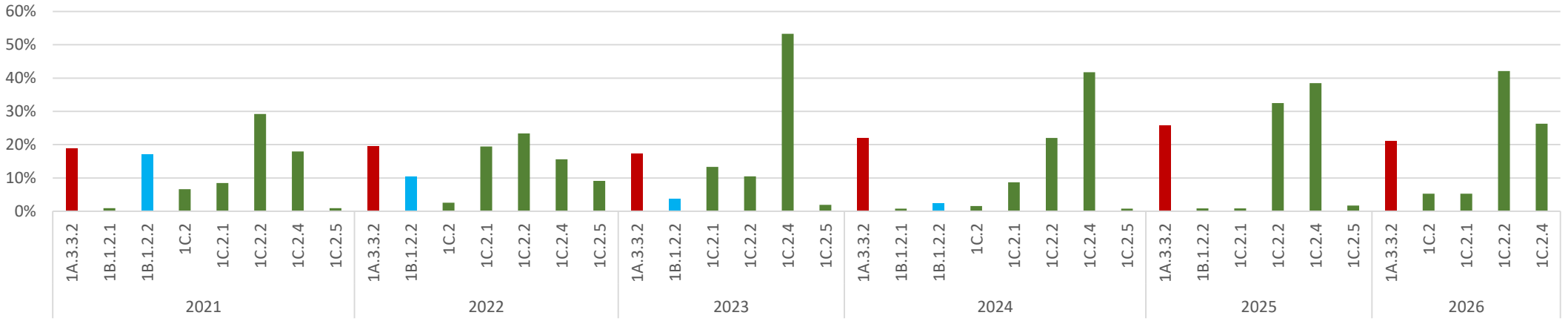
62 strains

H1 lineages and sub-lineages 2021-2025

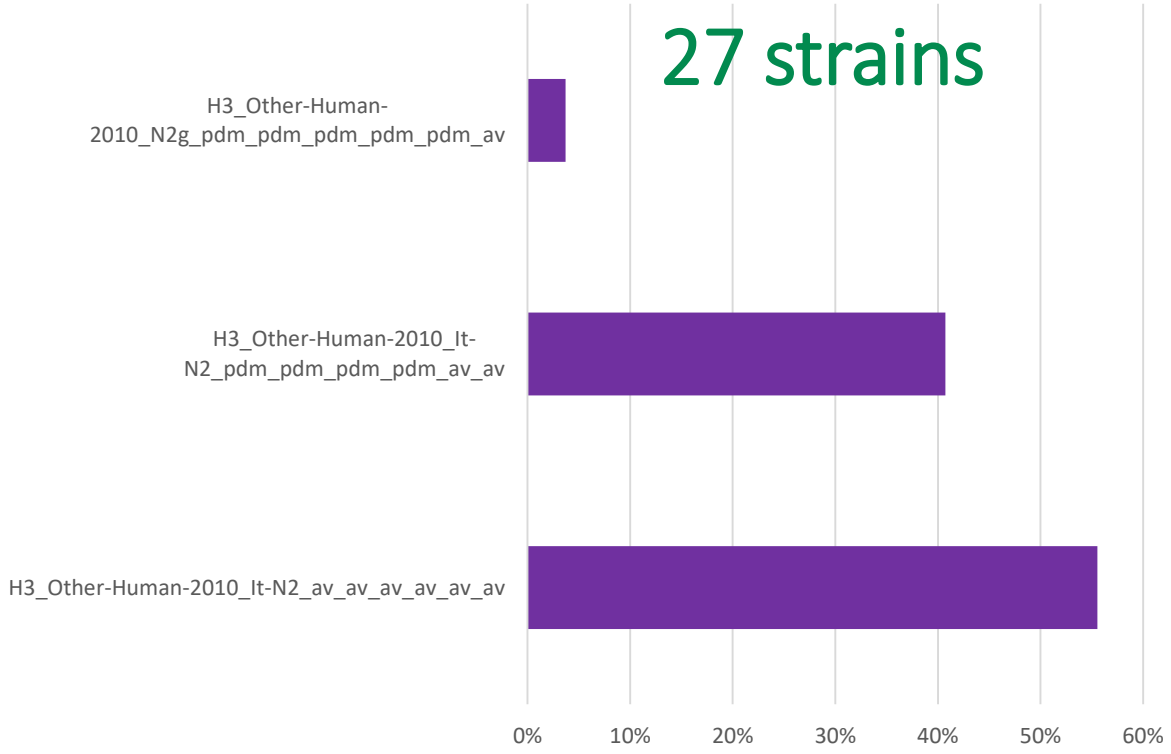
Great antigenic variability

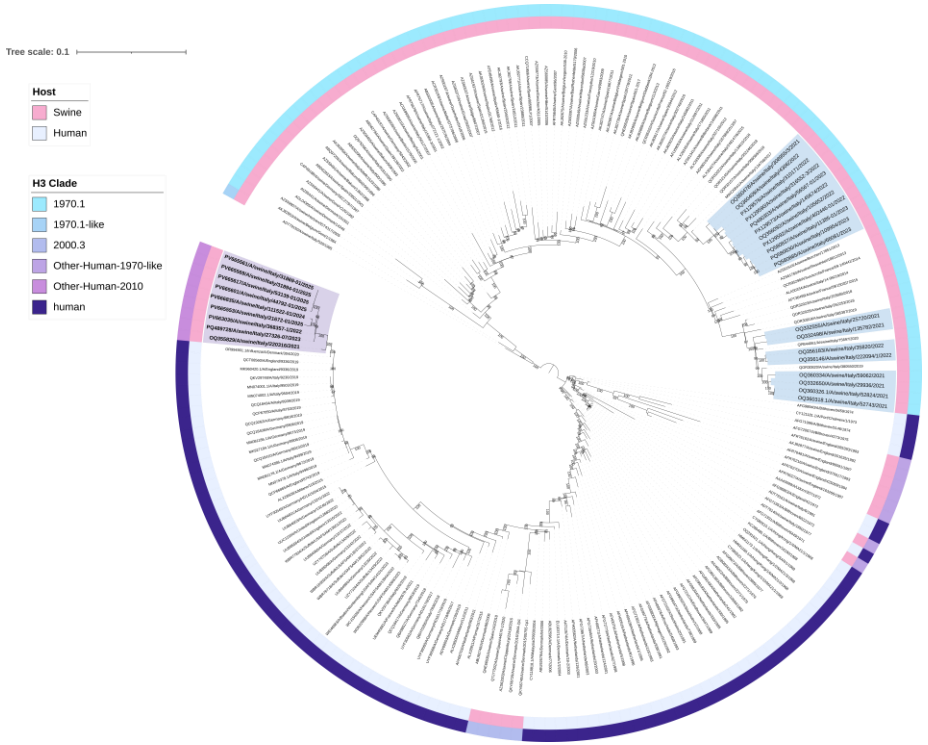
H1C.2





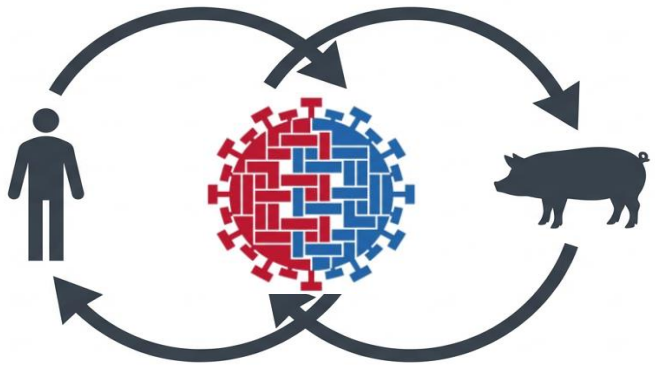
H3N2 genotypes (2010.3) since Jan-2025



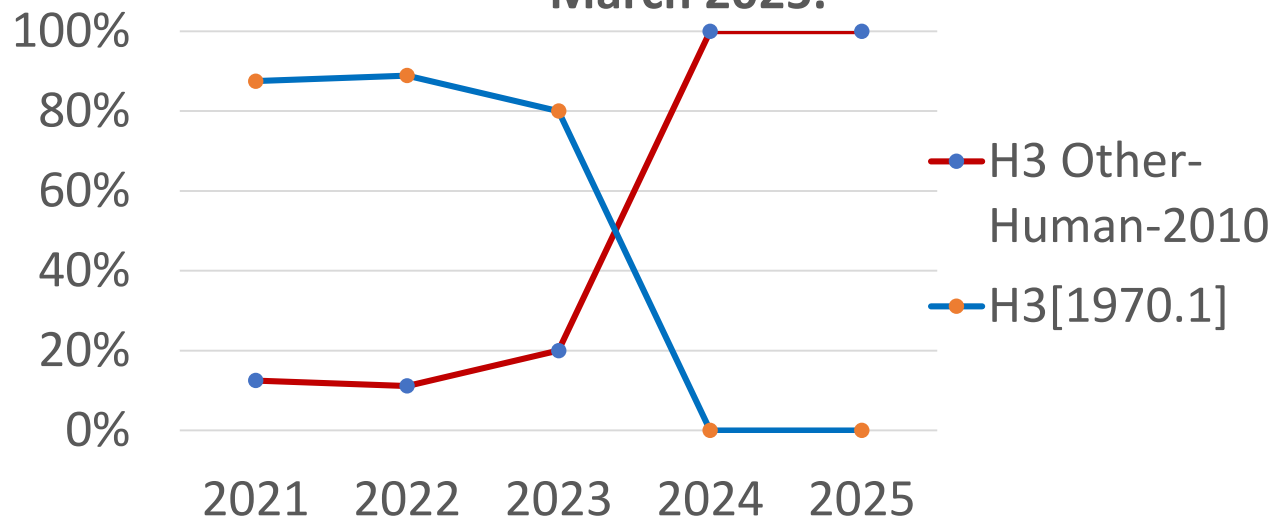


Soliani et al Viruses. 2025 Aug 27;17(9):1171. doi: 10.3390/v17091171.

H3N2 new genotype



Percentages of the two H3N2 lineages of IAV-S detected from January 2021 to March 2025.



HI test results on 88 sera from flu-vaccinated animals

Against the 1970.1 strain (swine)

93%
of vaccinated pigs is PROTECTED
(Titer >20).

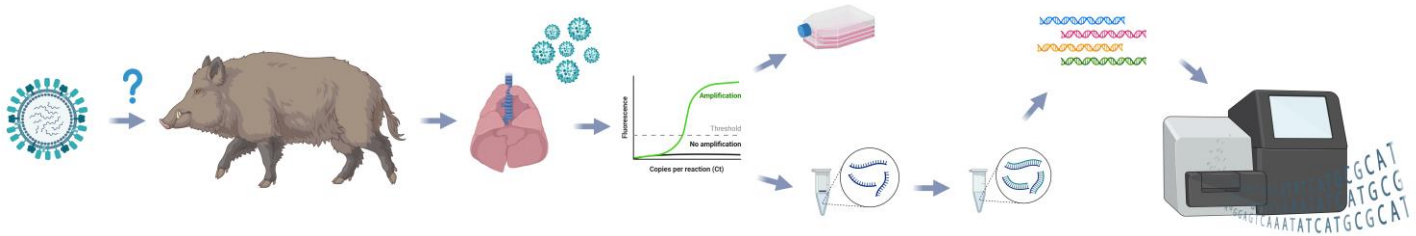
Hyperimmune reference serum:
High titer (1:640)

86%
of vaccinated pigs is VULNERABLE
(Titer <20, Negative).

Hyperimmune serum:
Almost no reaction (1:20)

Against the recent strain (other human-2010)

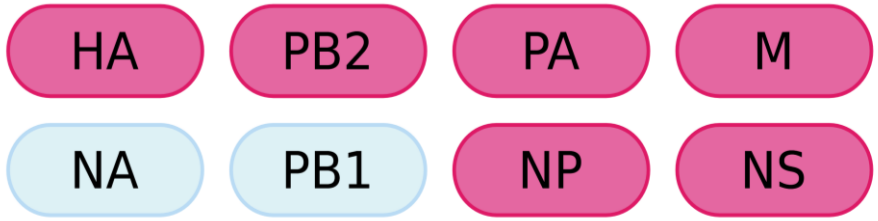
IAV-S in wild boar
(Emilia Romagna monitoring)



2023-2025
Screening 1400 samples

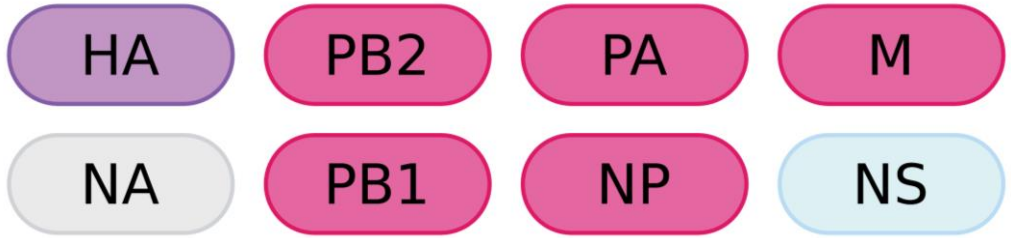
0.7% positivity

H1A3.3.3.2



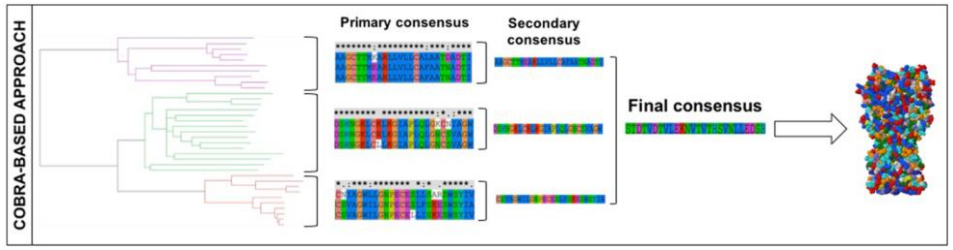
pink-colored segments -> pdm09-origin; light blue colored segments -> Eurasian-avian like origin

H3-Other human 2010



H3, pink-colored segments -> pdm09-origin;
light blue colored segments -> Eurasian-avian like origin.
Grey segment: non-typeable.

Improving diagnosis: Indirect ELISA test with computationally optimized broadly reactive antigens COBRA on NP-positive swine sera



cobra H1A pdm

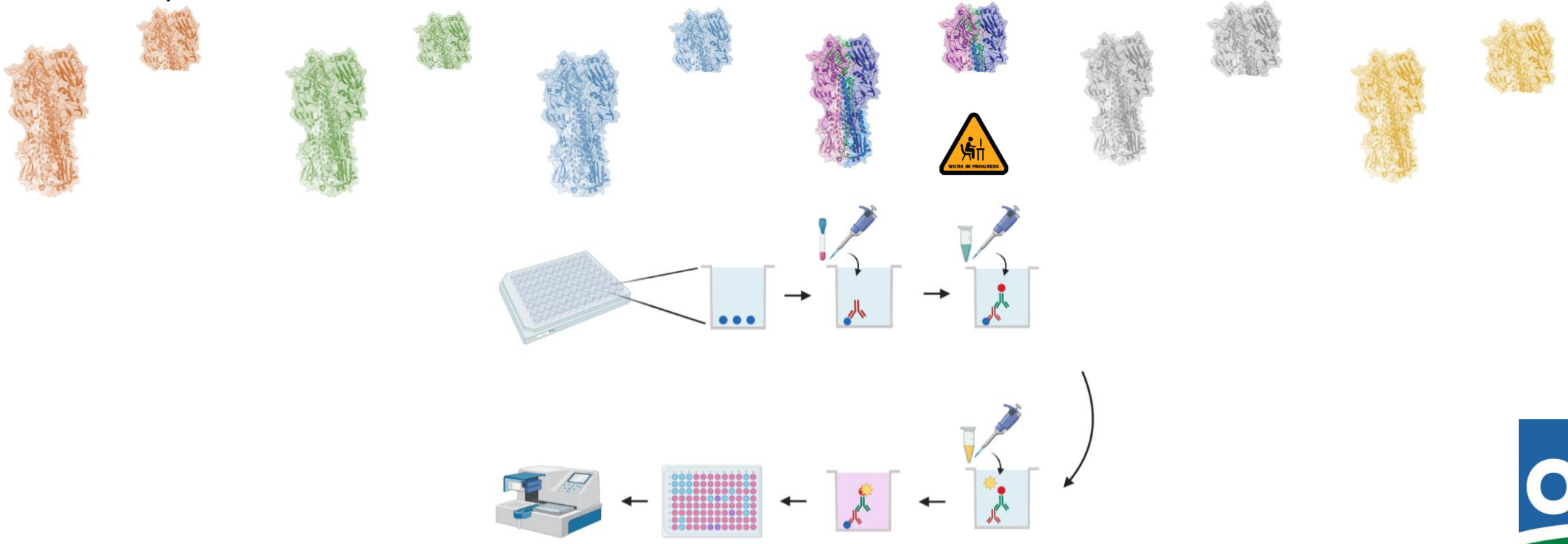
cobra H1B

wt H1C2.4 (Italy)

cobra H1C

cobra H3sw

wt H3human-like



10.7717/peerj.10180

WOAH FAO network of expertise on animal influenza

ESFLU CA21132

Role of the ESFLU Network 🤝

- 🏛 The European Swine Influenza Network (ESFLU) is an interdisciplinary consortium operating at European level.

- 🎯 The main objectives of ESFLU are:

– 🔗 Harmonisation of laboratory diagnostic methodologies
– 📊 Improvement of data comparability and sharing
– 🚨 Support to coordinated surveillance and risk assessment
– 🛡 Strengthening preparedness for animal and public health threats

ESFLU-WG1 output

. Scope of the Guidelines

- Provide harmonised laboratory guidance for the detection and monitoring of IAV-S.
- Cover the full diagnostic workflow, from sampling to virus characterisation.
- EU Specifically tailored to the European epidemiological and regulatory context.
- The methods described are indicative and do not endorse specific commercial products.

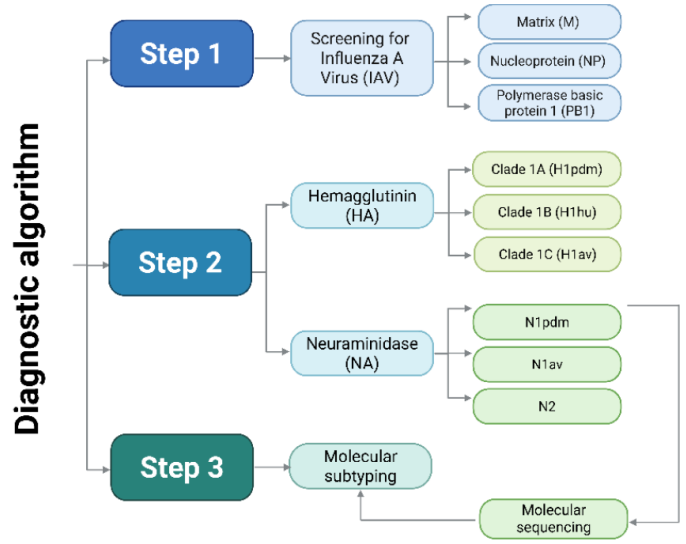
1° step collection of SOPs → 2° step Guidelines writing

WG1: Virus characterization: Leader and co-leader
Dr Sasan Fereidouni (Austria) and Dr Annika Graaf Rau (Germany)

ESFLU-WG1 output

Molecular Detection and Characterisation

- Real-time RT-qPCR is the primary diagnostic method for IAV-S detection.
- Initial screening targets the conserved matrix (M) gene.
- Positive samples undergo:
  - HA and NA subtyping by RT-qPCR
  - Further genetic characterisation when required
- Sequencing approaches include:
  - Sanger sequencing of HA and NA genes
  - Whole genome sequencing (Illumina or Oxford Nanopore platforms)
- ☑ Strict quality control and assay validation are required at all analytical steps.



Created in BioRender. Graaf Rau, A. (2025) https://BioRender.com/ngom3n7

ESFLU-WG1 output

Serological Testing

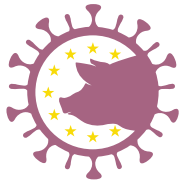
- Serological assays support surveillance and herd-level exposure assessment.
- Main approaches include:
  – ELISA targeting conserved viral antigens (e.g. nucleoprotein)
  – Hemagglutination inhibition (HI) assays for subtype-specific analysis
- ⚠ Interpretation requires caution due to cross-reactivity and antigenic diversity.

Virus Isolation and Titration

- Virus isolation enables antigenic and phenotypic characterisation of circulating strains.
- Isolation systems include:
  – Embryonated chicken eggs
  – Susceptible cell cultures (e.g. MDCK, Caco-2)
- Isolation must be confirmed by HA assays and/or RT-qPCR.
- Virus quantification is performed using $TCID_{50}$ or equivalent methods.
- All procedures require strict biosafety measures and full sample traceability.

Emerging Diagnostic Technologies

Thank you



ESFLU
European Swine
Influenza Network

Ministero della Salute