

The Swiss national program for the surveillance of influenza A viruses in pigs and humans: genetic variability and zoonotic transmissions from 2010 – 2022

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Das nationale Programm zur Überwachung von Influenza A Viren bei Schweinen und Menschen in der Schweiz: genetische Variabilität und zoonotische Übertragungen von 2010 bis 2022

Influenza-A-Viren (IAV) sind wahrscheinliche Kandidaten für Pandemien. Dieser Bericht fasst die Ergebnisse des nationalen Programms zur Überwachung von Influenzaviren bei Schweinen in der Schweiz zusammen und präsentiert Daten zu ihrer genetischen Vielfalt und zur Übertragung auf Menschen zwischen 2010 und 2022. Herausforderungen und Optimierungsmöglichkeiten des Programms werden diskutiert.

Nasentupfer oder Lungengewebe von Schweinen mit grippeartigen Anzeichen wurden mittels real-time RT-PCR auf Schweineinfluenzavirus (SIV) und saisonales humanes Influenzavirus A(H1N1)pdm09 untersucht. Positive Proben wurden für H1, N1, H3 und N2 subtypisiert. Parallel dazu wurden Menschen mit Grippe-symptomen und kurzlichem Kontakt zu erkrankten Schweinen gebeten, sich selbst mit einem Nasenabstrich zu beproben. Die Abstriche wurden auf IAV getestet, und positive Abstriche wurden weiter subtypisiert, um eine mögliche Übertragung zwischen Schweinen und Menschen zu identifizieren.

Bei den Schweinen wurde bei 375 von 674 Betriebsbesuchen SIV nachgewiesen. H1N1 ist der einzige Subtyp, der bisher bei Schweizer Schweinen nachgewiesen wurde. Der saisonale Humanstamm A(H1N1)pdm09 (Hämagglutinin (HA) Klade 1A) wurde bei sieben von 375 SIV-positiven Betriebsbesuchen nachgewiesen. Phylogenetische Analysen von partiellen Hämagglutinin (HA)- und Neuraminidase (NA)-Gensequenzen deuten darauf hin, dass die übrigen

Abstract

Influenza A viruses (IAV) are likely candidates for pandemics. This report summarizes the results of the Swiss national program for surveillance of influenza viruses in pigs by presenting data on their genetic diversity and on transmissions to humans between 2010 and 2022. Challenges and optimization options in the program are discussed.

Nasal swabs or lung tissue samples from pigs with influenza-like signs were screened by real-time RT-PCR for swine influenza virus (SIV) genomes and the human seasonal strain A(H1N1)pdm09; positive samples were subtyped for H1, N1, H3 and N2. In parallel, humans with influenza-like symptoms and recent contact with diseased pigs were asked to sample themselves with a nasal swab. Human swabs were tested for IAV, and positive swabs further subtyped to identify potential cross-species transmission between swine and humans.

In the pigs, SIV was detected in 375 of 674 farm visits. H1N1 was the only subtype detected in Swiss pigs so far. The human seasonal strain A(H1N1)pdm09 (Hemagglutinin (HA) clade 1A) was detected in seven out of 375 SIV positive farm visits. Phylogenetic analyses from partial HA and neuraminidase (NA) gene sequences indicate that the remaining pigs were infected with the Eurasian avian lineage (HA clade 1C), which is predominant in swine in Europe. The Swiss H1N1 strains form distinct phylogenetic clusters within HA clades 1C.2.1 and 1C.2.2 and seem to evolve comparably slowly. Infection of humans with SIV was identified in five cases. Sequence similarity analysis assigned the five viruses to the Eurasian avian lineage (C), clades 1C.2.1 and 1C.2.2. There was no evidence for sustained human-to-human transmission.

Continued surveillance of influenza viruses at the swine-human interface is of major importance to enable early detec-

Schweine mit dem eurasischen aviären Stamm (HA Klade 1C) infiziert waren, der bei Schweinen in Europa vorherrscht. Die Schweizer H1N1-Stämme bilden innerhalb der HA Klade 1C.2.1 und 1C.2.2 separate phylogenetische Cluster und scheinen sich vergleichsweise langsam zu entwickeln. In fünf Fällen wurden SIV-Infektionen bei Menschen festgestellt. Eine vergleichende Sequenzanalyse ordnete auch diese fünf Viren der eurasischen aviären Linie (C), Klade 1C.2.1 und 1C.2.2 zu. Es gab keine Hinweise auf eine anhaltende Übertragung von Mensch zu Mensch. Die weitere Überwachung der Influenzaviren an der Schnittstelle zwischen Schweinen und Menschen ist von grosser Bedeutung, um neue IAV-Subtypen und Veränderungen der epidemiologischen Situation frühzeitig zu erkennen.

Die Autorenschaft erachtet die Veröffentlichung von Daten aus dem Überwachungsprogramm als wesentlich, um das Bewusstsein für die Erkrankung sowohl in der Tierärzteschaft als auch in der breiten Öffentlichkeit zu stärken und die bestehenden Informationen zu zirkulierenden Influenzaviren in Europa zu ergänzen.

Schlüsselwörter: human, genetische Variabilität, Influenza, Überwachung, Schwein, Schweiz

tion of new IAV subtypes and changes in the epidemiological situation.

The authors consider the publication of data from the surveillance program to be essential in order to raise awareness of the disease among both veterinarians and the general public and to supplement existing information on circulating influenza viruses in Europe.

Keywords: human, genetic variability, influenza, surveillance, swine, Switzerland

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Introduction

Influenza A viruses (IAV) are highly diverse and classified into subtypes, whose nomenclature is based on the genetic and antigenic properties of the two major surface glycoproteins hemagglutinin (HA) and neuraminidase (NA).⁵³ Three characteristics contribute to the rapid evolution and high diversity of IAV subtypes making them likely candidates for pandemics:^{1,24,26,28}

- The structural proteins HA and NA, which are responsible for attachment to the cell via the cellular receptor sialic acid (HA) and for mucus degradation in the upper respiratory tract as well as release of virus progeny from the cell after replication (NA), face strong evolutionary pressure.
- The RNA polymerase of influenza viruses lacks proofreading ability, conferring a more rapid accumulation of point mutations in the genome. This process is referred to as *antigenic drift*.
- The segmented genome of IAV allows for reassortment, i.e. the reorganization of genome segments in cells co-infected with different IAVs, which may give rise to progeny viruses exhibiting markedly altered features. This phenomenon is known as *antigenic shift*.

In humans and pigs, IAVs establish a respiratory infection with fever, headache, myalgia, lethargy, dry cough, sneezing

and nasal discharge.^{28,50} In young and immunocompromised humans and animals severe illness with secondary bacterial infections and pneumonia can develop and eventually lead to death.^{1,26,33} The viruses are shed in nasal secretions and transmitted via direct contact between infected animals, indirect contact with virus-contaminated fomites, or through inhalation of aerosols.³⁴ In swine, influenza is considered one of the most important pathogens of the respiratory disease complex, causing high economic losses due to poor performance and prolonged fattening periods.³³

Continuous zoonotic and reverse zoonotic transmission of IAVs, especially between humans, swine and birds gives rise to endemic/enzootic strains but also to strains with pandemic potential.^{1,24,38} Endemic/enzootic IAV strains usually are the result of *antigenic drift* with subsequent minor changes in virus biology. The predominant endemic/enzootic IAVs currently circulating in pigs and humans are of the H1N1, H1N2 (swine only) and H3N2 subtypes. However, porcine and human strains are genetically and antigenically distinct from each other.^{26,28,30} The subtypes circulating in swine in Europe include three major H1 lineages of SIV which are distinct from their counterparts in North America and Asia:^{3,4,6,9,11,28,30,50}

- The Eurasian avian lineage (H1avN1, clade HA-1C), which was transmitted from wild ducks to pigs and appeared in 1979

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- The human-like H3N2 lineage, originating from the reassortment of human-like swine H3N2 and a H1avN1 in 1984
- The human seasonal lineage (H1huN2, clade HA-1B), which appeared in 1994 after reassortment of a human-like H3N2 and a seasonal human H1N1
- The classical swine lineage (H1N1pdm09, clade HA-1A), which circulates in the swine population since 2009, and is hypothesized to have arisen upon reassortment of H1avN1 and the North American triple reassortant H3N2

On farm level, H1avN1 is the dominant subtype in Europe, followed by other reassorted strains of H1N1 (including H1N1pdm09), H1N2 and H3N2.^{9,19}

Pandemic strains most often emerge upon *antigenic shift*, which is associated with abrupt changes in the antigenic properties of HA and NA. After infection with these strains, the host immune response is facing new epitopes, which are not or insufficiently covered by the immunological memory. Due to increasing immunity in the population and antigenic drift, pandemic strains may become endemic and replace the ones that previously circulated seasonally. The most recent example of strain replacement is the 2009 influenza H1N1 pandemic virus A(H1N1)pdm09 that replaced the former seasonal A(H1N1) in humans.⁴² This virus emerged as a result of viral reassortment between two influenza lineages that had been circulating in pigs for years, i.e. a triple-reassortant circulating in North America and the Eurasian avian-like lineage.⁴⁶ It was first detected in humans at the North American-Mexican border in March and April 2009 and, having acquired the ability for efficient human-to-human transmission, was declared a pandemic by the World Health Organization (WHO) by June 2009. By July the virus was reported from 122 countries including Switzerland, where the first human case was detected in April 2009.^{24,42} It rapidly became the predominant IAV variant circulating in humans, with death rates fortunately remaining low.⁴⁹ From humans, the virus made its way back into pigs via reverse zoonosis.

The cell receptor is a major determinant in host susceptibility to influenza viruses.^{1,10,32} Owing to the location and density of cell receptors and the tropism of IAV, swine are considered a key species of concern in the emergence of pandemic strains: human IAV strains have higher binding specificity to host glycoproteins containing sialic acid moieties in α 2,6-galactose-linkage, which are most abundantly expressed in epithelial cells of the human upper respiratory tract.^{6,10} In contrast, avian strains preferentially bind to α 2,3-galactose-linked sialic acid, which in avian species is present in the upper and the lower respiratory tract as well as the intestines, in humans, however, in the less accessible lower respiratory tract. Given the presence of both cell re-

ceptor types in pigs it is hypothesized that pigs can serve as intermediate hosts in which pre-adaptation of avian subtypes to humans occurs (i.e., a gradual shift in IAV binding specificity for α 2,3-gal to α 2,6-gal sialic acid) thereby favoring transmission to humans.^{6,10,28,31,47} Husbandry practices where pigs and wild birds or poultry are kept in close proximity to each other are therefore considered a risk factor for interspecies transmissions and emergence of novel influenza virus strains.³² The intensification of livestock farming, the invasion of wild animal habitats for agricultural purposes and the increasing transboundary movement of animals, humans and goods in recent decades have facilitated the global spread of such new virus variants.^{13,24,42}

Given its potential role as a transmitter of new, potentially pandemic strains, surveillance of influenza viruses in pigs is of high importance. In the context of the 2009 pandemic caused by the originally porcine influenza virus A(H1N1)pdm09, the influenza virus surveillance in humans and pigs launched in 2001 in Switzerland was intensified. In collaboration with the Federal Food Safety and Veterinary Office (FSVO), the Federal Office of Public Health (FOPH), the Institute of Virology at the Vetsuisse Faculty of the University of Zurich, the National Reference Center of Influenza (NRCI) at the University Hospital in Geneva and the Pig Health Service of SUISAG, a national program for passive surveillance of influenza viruses in pigs and in-contact humans was initiated, the aims of which were the following:

- Detection and molecular characterization of IAVs circulating in Swiss pigs and in-contact persons showing influenza-like symptoms, with a focus on HA and NA genes
- Where available, comparison of the genome sequences obtained from pigs and in-contact humans to identify potential cross-species transmission
- Early identification of the emergence of new IAV subtypes and changes in the epidemiologic situation in pigs

This study aims to present the results of the Swiss national program for the surveillance of influenza viruses in pigs and humans from 2010–2022, to discuss challenges in the success of national influenza virus surveillance and opportunities for optimization. The study does not claim to assess the prevalence of IAV in pigs.

Material and methods

Ethics statement

Porcine samples were taken by veterinarians (Pig Health Service, herd or private veterinarians) from clinically ill pigs with suspect SIV infection. Ethical approval was not sought as the national monitoring program only makes use of samples that are taken for specific diagnostic purposes.

The human samples from the target population (persons with influenza-like symptoms and recent contact with diseased pigs) were obtained by self-sampling. Based on an official letter from the FOPH stating that human data and samples were collected in accordance with the Swiss Epidemics Act (EpG SR 818.101), the cantonal ethics committee of Zurich waived ethical approval for this work (BASEC Req-2024-01630).

Sampling of pigs and humans

Within the scope of the monitoring program, pig farmers were asked to report pigs with influenza-like signs such as

cough and fever to the Pig Health Service, who organized the farm visit and sampling (Figure 1). Nasal swabs (FLOQSwab in UTM Universal transport medium; Copan, Brescia, Italy) were collected from acutely diseased pigs and contact animals by a veterinarian. Persons with influenza-like symptoms and recent contact to these pigs were asked to swab themselves. Consenting individuals were provided with flexible minitip FLOQSwabs (Copan) and 3 ml UTM tubes for self-sampling. In absence of recent influenza-like symptoms in humans, three (2010–2014) or two pigs (2015–2022) per farm were sampled. Three pigs were sampled if in-contact humans were symptomatic (Figure 1). In addi-

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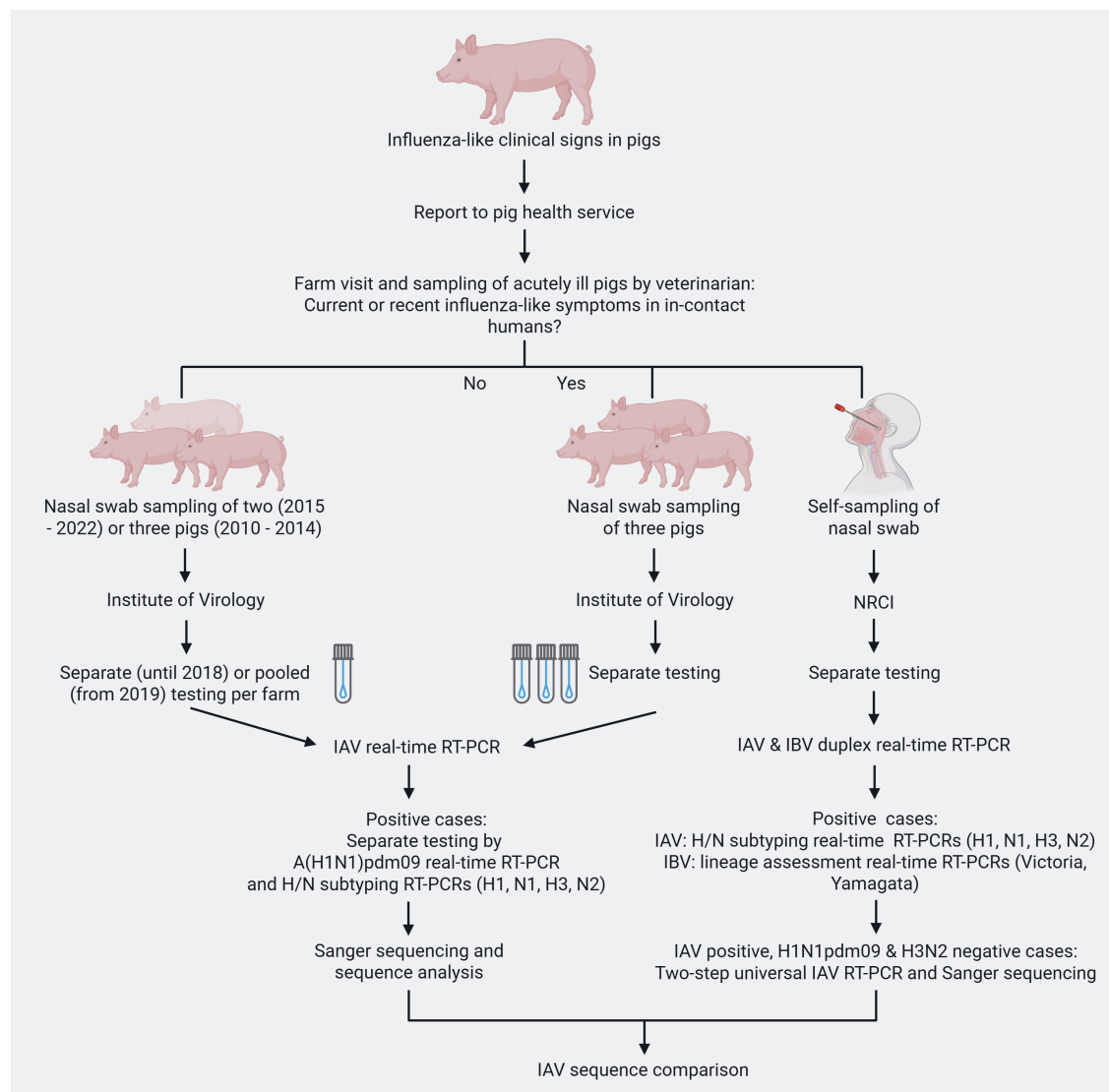


Figure 1: Workflow of sampling and testing porcine and human nasal swab samples for IAV in the context of the Swiss national SIV monitoring program. A part of the porcine samples was subjected to Illumina whole-genome sequencing, the results of which will be published elsewhere. Human samples were additionally tested for Influenza B virus (IBV). NRCI = National Reference Center for Influenza. (Figure created with BioRender.com)

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tion to the specimens collected on-site, nasal swabs (UTM, Copan), lung tissue or dry lung swabs from pigs with suspected influenza infection were collected by institutes of veterinary pathology upon necropsy. The samples were to be kept at 4°C until shipment (without cooling). Preferably, they should be sent to the responsible diagnostic laboratory on the same day with priority mail to arrive in the laboratory the next day. Porcine samples were analyzed at the Institute of Virology, University of Zurich. Human samples were analyzed at the National Reference Center for Influenza (NRCI) in Geneva. In the lab, the samples were kept at 4°C if analyzed within the next four days, otherwise at -20°C (Figure 1).

Testing of porcine samples

Until 2018, all porcine swabs were examined separately. From 2019, in case of absence of recent influenza-like symptoms in humans the porcine samples were pooled, yielding a single sample per farm and per visit. If human samples were collected due to suspect influenza cases, the porcine samples on the respective farm were analyzed individually (Figure 1). The nasal swabs were thoroughly vortexed and centrifuged at 2600 x g (Heraeus Multifuge 3S-R, Thermo Scientific) for 10 min at room temperature (RT). The supernatant of each swab was mixed with antibiotic and antimycotic solution (100X, Sigma-Aldrich, St. Louis, U.S.). For pooling, 80 – 100 µl of each supernatant were added to 560 µl AVL buffer containing 5,6 µl carrier RNA (QIAamp Viral RNA Mini Kit, Qiagen).

Lung tissue or lung swabs from institutes of veterinary pathology were pooled per farm. The dry swabs from lung tissue were immersed in 140 µl PBS, thoroughly vortexed and centrifuged for 5 min at 16000 x g. Lung tissue was subjected to three freeze-thaw cycles, centrifuged and 140 µl of supernatant were used for RNA extraction. The supernatants of lung tissue and lung swabs were mixed with 560 µl AVL buffer containing 5,6 µl carrier RNA. Extraction was carried out according to the manufacturer's instructions (QIAamp Viral RNA Mini Kit, Qiagen). If not processed immediately, the RNA was stored at -20°C if analyzed within the next four days, otherwise at -80°C.

Swine Influenza A real-time RT-PCR

For primary SIV detection, the extracted RNA was subjected to real-time RT-PCR targeting the matrix protein gene of IAV as well as the reference host gene GAPDH (Adiavet SIV Real Time, Adiagène-BioX Diagnostics, Ploufragan, France) (Figure 1; Supplementary Table 1).

Influenza A(H1N1)pdm09 real-time RT-PCR

All SIV positive samples were tested for the pandemic H1N1 strain from 2009 by a specific real-time RT-PCR (Figure 1; Supplementary Table 1).⁴³

H1N1 and H3N2 subtyping RT-PCRs and Sanger sequencing

For subtyping, SIV positive samples were subjected to conventional RT-PCR (Figure 1). Since H1N1 is the only SIV subtype detected in Switzerland so far, first, specific primers for H1 and N1 were used. All samples that tested negative against H1 or N1 were subsequently tested for H3 and/or N2.^{8,29} (Supplementary Table 1). PCR products were analyzed in a 1,5 % or 2 % agarose gel containing GelRed Nucleic Acid Stain (10'000X, Biotium, Hayward, CA, U.S.). Bands of the expected size were excised, and DNA extracted using the QIAquick Gel Extraction Kit (Qiagen). For Sanger sequencing (Microsynth, Balgach, Switzerland), the total DNA concentration was measured by spectrophotometry (Nanodrop, Thermo Fisher Scientific, Kloten, Switzerland), and approximately 45 ng DNA were mixed with 20 µM of the respective primer forward and reverse and topped-up with nuclease-free water to a final volume of 15 µl. Sequences were analyzed with NCBI nucleotide BLAST (<https://blast.ncbi.nlm.nih.gov>).²

Sequence analysis and generation of phylogenetic trees

Trimming of the ends resulted in HA and NA sequences of up to 392 and 457 nucleotides, respectively. Sanger sequencing traces were processed using *Tracy* (v0.7.6) with default parameters.⁴⁰ For samples with a single trace, the *basecall* module was used, whereas for samples with multiple traces and/or replicates, the *assemble* subcommand was applied. Subsequently, sequences for each segment were aligned using *MAFFT* (v7.310) with the options *--reorder --adjustdirection-accurately --op 5*.²² The resulting alignments were manually curated and trimmed. The phylogenetic relationships of the sequences were inferred using the maximum-likelihood nearest-neighbor interchange algorithm implemented in *FastTree2* (v2.1.10).³⁷ The trees were rerooted using the *root* command from the *ape* package (v5.8) with AB628080/JX138509 and AB628082 used as roots for the HA and NA trees, respectively.³⁵ Graphical representation of the trees was generated using *ggtree* (v3.12.0)⁵⁴ and *ggnewscale* (v0.5.0).⁷ Classification of HA sequences was performed using the online tool Subspecies Classification (with Swine Influenza H1 global classification, <https://www.bv-brc.org/app/SubspeciesClassification>, accessed August 4, 2024). To address limitations in NA segment classification, an in-house system was developed. Briefly, the trimmed alignments were used to calculate a phylogenetic distance matrix using *FAMSA* (v2.2.2) with the settings *-square_matrix -dist_export*.¹² The dimensionality of the resulting distance matrix was reduced using the classical multidimensional scaling (MDS) method, implemented via the *cmdscale* function in R (v4.4.0).⁴¹ The visible segregation of sequences was ultimately used as an approximation for NA segment classification. To assess whether the shift from HA clade C.2.1 to C.2.2 was statistically significant over time, a logistic regression with year as predictor and clade as target was implemented. All utilized scripts are available at <https://github.com/mwylerCH/>

SwissSIV. All assembled Sanger sequences were deposited on GenBank under the accession numbers PQ663270 – PQ663585 (Supplementary Table 2).

Testing of human samples

The human samples were tested in single approach. Prior to nucleic acid extraction, 200 – 400 µl of nasopharyngeal swab supernatant were spiked with 20 µl of cell culture supernatant of canine distemper virus (Onderstepoort vaccine strain), which served as extraction control and to assess the presence of PCR inhibitors in the samples. Viral RNA was then extracted using NucliSens easyMAG (2010 – 2017) and EMAG (since 2018) magnetic bead systems (BioMérieux, Italy) according to the manufacturer's instructions. RNA was recovered in 25 – 50 µl of NucliSens easyMAG Buffer 3. If not processed immediately, the RNA was stored at -80°C.

Influenza A and B duplex real-time RT-PCR

For primary Influenza A (IAV) and B (IBV) detection, the extracted RNA was subjected to duplex real-time RT-PCR targeting the matrix protein of IAV and the non-structural protein of IBV viruses (Supplementary Table 1).¹⁷

Influenza A subtyping and influenza B lineage assessment real-time RT-PCRs

Currently circulating human seasonal Influenza A subtypes are A(H1N1)pdm09 and A(H3N2). In parallel to IAV and IBV screening, all samples were subtyped using either H1pdm09 and H3 specific singleplex real-time RT-PCRs (2010 – 2017) or a quadruplex real-time RT-PCR targeting H1pdm09 and H3, as well as N1 and N2 genes (since 2018). Lineage (Yamagata and Victoria) was assessed for IBV positive samples (Figure 1; Supplementary Table 1).

RT-PCR and Sanger sequencing of samples suspected to be positive for zoonotic Influenza A

Human samples were suspect to be positive for SIV if they yielded a positive IAV real-time RT-PCR (pan A) result, but tested negative for human seasonal IAV strains, i.e. H1N1pdm09 and H3N2. In these cases, a conventional RT-PCR using universal IAV primers was performed, and positive samples were subjected to Sanger sequencing. In case of low yield for the universal PCR, a sequential nested PCR using type and segment-specific IAV primers was attempted. In detail, the extracted RNA was reverse transcribed into cDNA

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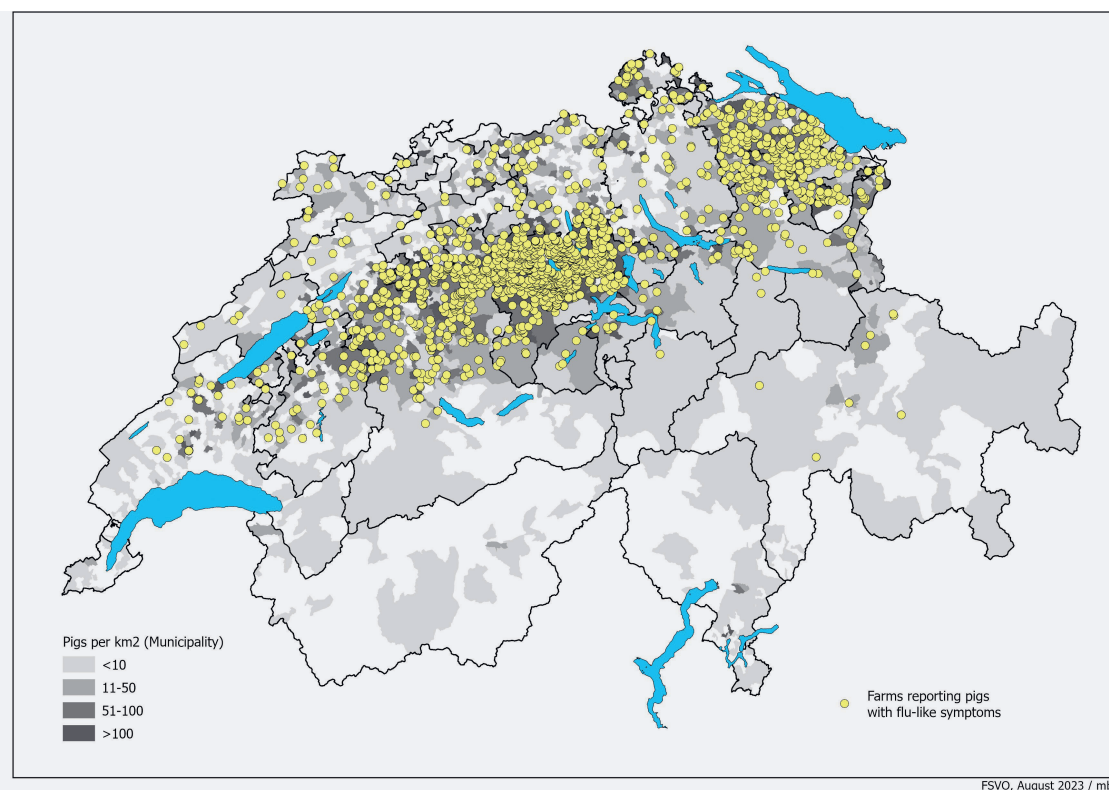


Figure 2: Regional distribution of farms reporting influenza-like signs in pigs from 2009 – 2022 (M. Binggeli, FSVO, 2023).

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using the Uni 12 primer (Supplementary Table 1).²¹ The cDNA tubes were placed on ice and were either immediately processed for PCR or stored at -20°C. The cDNA was used as template for amplification of the eight influenza virus ge-

nome segments in a single reaction using segment-specific primers (Supplementary Table 1).²¹ The porcine origin was confirmed, if by Sanger sequencing a partial or full sequence of at least one viral segment of SIV was obtained.

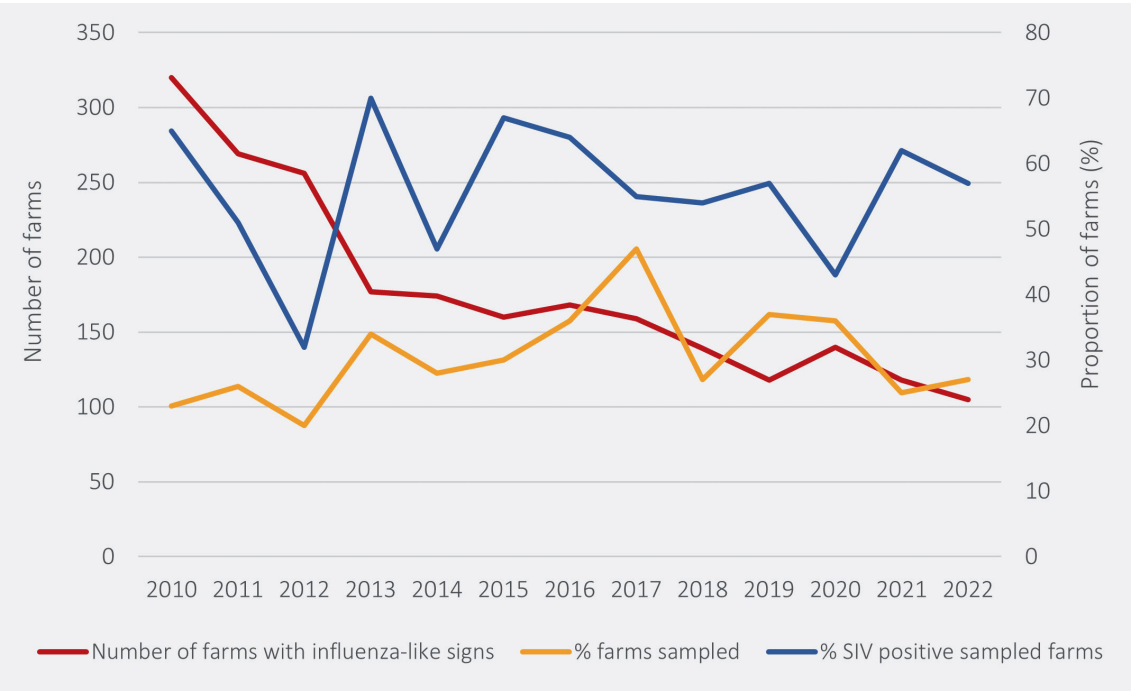


Figure 3: Number of farms reporting pigs with influenza-like signs (red line), proportion of farm visits with sampling (orange line) and proportion of sampled farms testing IAV positive (blue line) between 2010 and 2022.

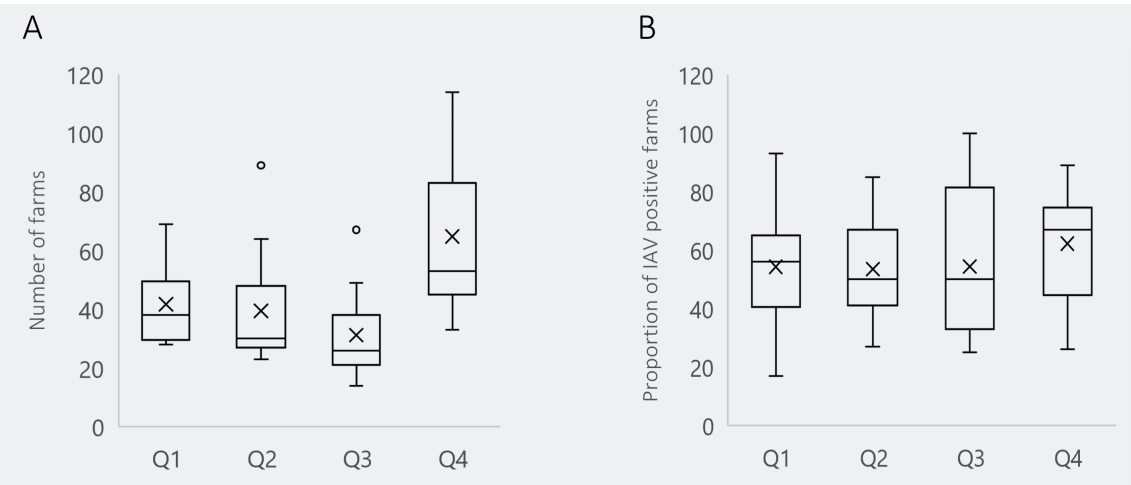


Figure 4: Number of farms reporting pigs with influenza-like signs (A), and percent of farm visits with IAV positive result by quarter (Q1 – Q4) (B) between 2010 and 2022. Lower/upper whisker = smallest/largest non-outlier values; box = interquartile range (Q1 – Q3), containing the middle 50% of the data; X in each box = mean value; line inside the box = median; circles = outliers.

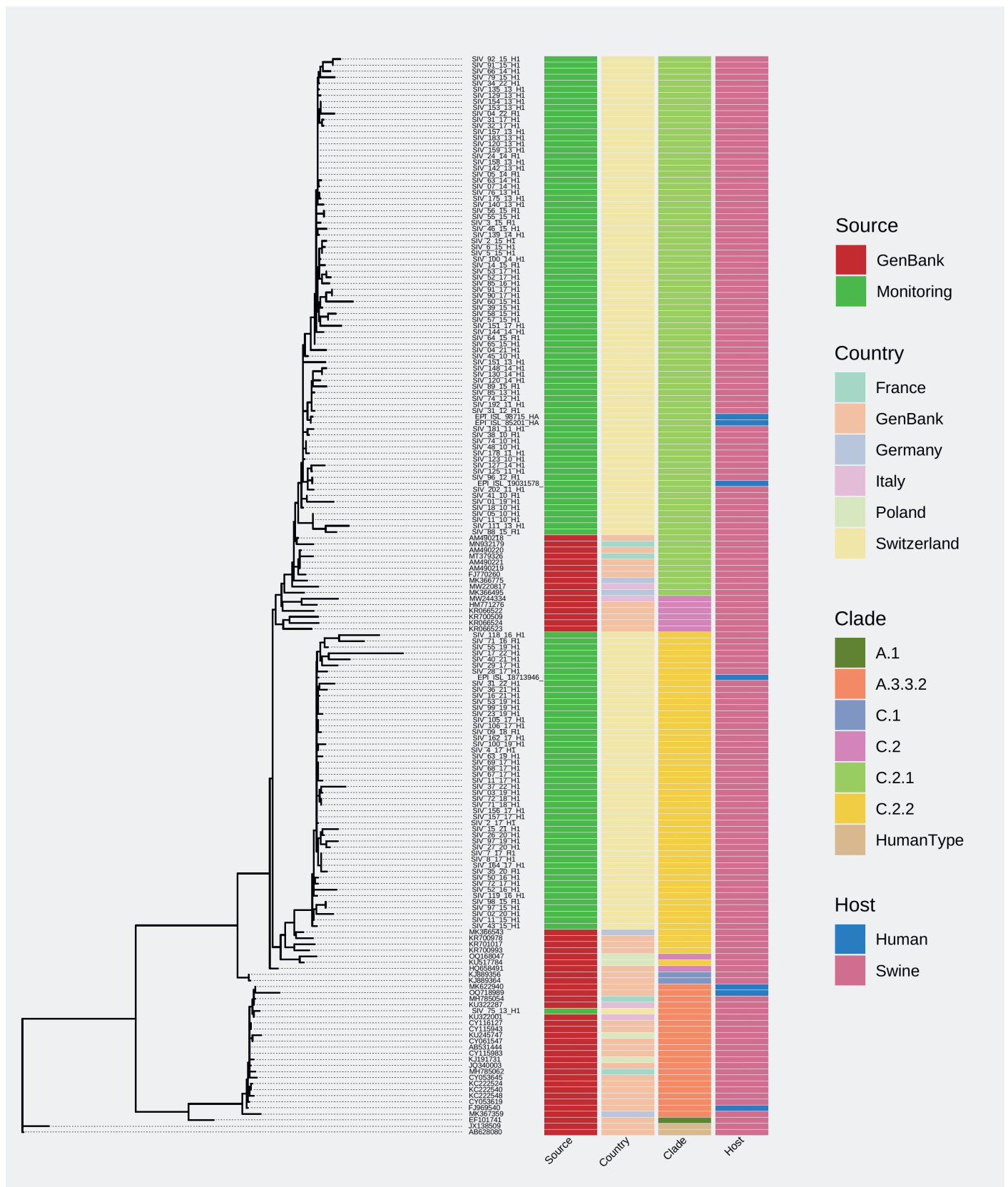


Figure 5: Maximum likelihood tree of the PCR-amplified region of the HA gene (approx. 450nt). The sequences obtained in the course of the Swiss national SIV monitoring program were labeled with 'SIV_number_year' and in the source section appear in green, whereas sequences from GenBank appear in red. Sequences were additionally characterized by country of origin, HA clade and host.

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For H1 and N1 genes, the resulting PCR products were sometimes subjected to additional segment-specific nested PCRs to increase amplification yield (Supplementary Table 1). Amplicons were purified using a MSB Spin PCRapace kit according to the manufacturer's instructions (Invitex Molecular GmbH, Berlin, Germany) and sequenced using strain-specific primers. Sanger sequencing was performed on an ABI 3500xL Genetic Analyzer (Applied Biosystems, Singapore). Sequences were processed and stored in SmartGene's Integrated Database Network System (IDNS) Influenza Module (<https://apps.idns-smartgene.com/apps/Apps.po>) and submitted to the global initiative on sharing all influenza data (GISAID) database.⁴⁵

Results

Porcine samples

From 2010 to 2022, a total of 2303 cases of pigs with influenza-like signs were reported from approximately 1400 farms in Switzerland (Figure 2). Over the years, the number of reports of farms with pigs showing influenza-like signs has continuously decreased from 320 in 2010 to 105 in 2022 (Figure 3).¹⁵ The number of reports was evenly distributed over the entire area with pig husbandry (Figure 2) and on average was highest in the last quarter of the year (Figure 4).

The proportion of farms sampled after reporting of influenza-like signs ranged from 20 – 47 % (Figure 3). On average 31 % of farms were sampled per year. In total, 674 program-related farm visits took place between 2010 and 2022, in which 1660 porcine nasal swab samples were collected. In the laboratory, 145 pooled nasal swabs and 1515 unpooled nasal swabs were analyzed. In addition, in 107 cases lung material was analyzed. The percentage of farm visits (total $n = 674$) with positive IAV testing per year ranged between 32 % and 70 % (Figure 3) and was highest in the last quarter of the year (Figure 4). IAV was detected in a total of 375 (= 56 %) farm visits. The IAV detection rate was 44,94 % ($n = 746$) in the nasal swab samples and 25,23 % ($n = 27$) in the lung samples.

According to subtyping RT-PCR, none of the SIV positive samples could be assigned to H3N2 or H1N2 strains. Influenza A(H1N1)pdm09 was detected by specific real-time RT-PCR in four nasal swab samples in 2011 and three nasal swab samples in 2013. For one of these samples (SIV_75_13_H1) a Sanger sequence was obtained, confirming its type (Figure 5). In the 375 IAV positive farm visits, the H1N1 subtype was detected on 196 occasions by subtyping RT-PCR and Sanger sequencing. In approximately 40 farm visits only H1, in approximately 80 farm visits only N1 and in approximately 60 farm visits no subtype could be identified.

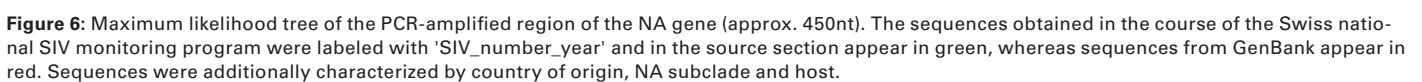
A total of 309 influenza sequences from PCR-amplified regions from pigs and humans were obtained after Sanger

sequencing: 125 sequences from the HA segment and 184 from the NA segment. Through sequence similarity analysis, the HA sequences could be assigned to clades C.2.1 and C.2.2. The only exception is the one sample (SIV_75_13_H1) from 2013 mentioned above, which belongs to HA clade A.3.3.2 (Figure 5 and 7). Although clades C.2.1 and C.2.2 are also present in various European countries, the Swiss samples form distinct clusters that differ from strains in neighboring countries. This distinction is observed in both the HA segment (Figure 5) and the NA segment (Figure 6). Given the long-term nature of the national surveillance program, the statistically significant temporal dynamics of the prevalence of SIV genotypes can be assessed (p val < 0.01; Figure 7). The beginning of the program was characterized by the predominance of C.2.1 genotypes. Starting from 2015, the occurrence of this clade narrows, and the clade is superseded by clade C.2.2, however, without fully disappearing. By sequencing both HA and NA segments, potential reassortments could be investigated. Sequences for both segments were available for 78 samples (75 porcine samples, 3 human samples). Since canonical SIV subtyping into clades is typically performed on the HA gene sequence only, a custom method was developed to categorize the circulating NA genotypes in Switzerland. Similar to previous observations in the HA segment, the Swiss NA sequences also form two distinct clusters, here referred to as subclade 1 and subclade 2 (Figure 6, Supplementary Figure 1). Interestingly, NA subclade 1 tends to be combined with HA clade C.2.1, while subclade 2 is typically associated with C.2.2. However, six possible reassortments were identified (Figure 7), in which a clade C.2.1 HA was combined with a subclade 2 NA and a clade C.2.2 HA with a subclade 1 NA.

Human samples

Between 2010 and 2022, in association with project-related farm visits ($n = 674$), a total of 150 humans on 118 farms reported influenza-like symptoms within ten days prior to the visit (Figure 8).¹⁵ In 64 cases the onset of clinical symptoms was reported 1 – 3 days, in 86 cases 4 – 10 days before the farm visit. On average, 33 % of humans with reported influenza-like symptoms were sampled per year. Over the years, the number of in-contact humans reporting symptoms has decreased from 22 in 2010 to 9 in 2022. In 455 project-related farm visits there were no reports of humans showing influenza-like symptoms in the 10 days prior to the farm visit, and in 103 visits, no information concerning illness in humans was available.¹⁵

Sixty-one individuals sent a self-collected nasal or nasopharyngeal swab to the NRCI (Figure 8). Of these, 29 persons reported symptom onset 1 – 3 days, 10 persons 4 – 10 days, and six persons more than 10 days prior to the farm visit. In 16 cases no information on symptom onset was available. The most common symptoms reported were cough (60,6 %), headache (36,1 %), myalgia (27,9 %), body temperature > 38°C, and rhinitis (18 %).



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Among the 61 sampled individuals, eleven tested positive for influenza virus. Three were positive for human influenza A (two A(H1N1)pdm09 and one A which could not be further characterized) and three for influenza B (two Yamagata and one Victoria) (Table 1). Five individuals were found positive for SIV (Table 2). Sequence analysis for HA and NA was successful in 4 and 3 cases, respectively, assigning the zoonotic viruses to the Eurasian avian lineage (clade HA-1C) (Figure 5 and 6; Table 2).

Discussion

Since its launch, the national surveillance program has functioned as a passive surveillance program funded by the government. It is relying on the reporting of flu-like signs in pigs by animal owners to the field veterinarians, which are then in charge of collecting samples from acutely diseased animals showing influenza-like signs like fever, nasal discharge and cough. The program is a risk-based initiative, in which samples are intended to be collected where the likelihood of SIV infection and detection is greatest, i.e. in animals exhibiting typical clinical signs of disease. Healthy animals and humans are thus not the primary target of the sampling strategy. SIV infections may have a mild or subclinical course and may be missed in these cases. It is not the aim of the program to assess the prevalence of SIV infections in pigs and humans, but to monitor the SIV strains circulating in Swiss pigs, allowing early identification of the emergence of new IAV subtypes and changes in the epidemiologic situation.

Here, we present the results of the Swiss surveillance program for SIV in the period of 2010 – 2022 and address various limitations and optimization options in order to improve passive surveillance in the future.

In the course of the Swiss national SIV monitoring program, reports of influenza-like signs in pigs and the detection of SIV in the respective farms occurred throughout the year, tending to be somewhat more frequent in the cold months. Nevertheless, as reported elsewhere, no seasonality of SIV can be inferred from these data (Figure 4).^{20,36,50}

In the present study, according to subtyping PCR none of the SIV positive samples could be assigned to H3N2 or H1N2 strains, making H1N1 the only subtype detected so far in the Swiss pig population (Figure 5 and 6).¹⁵ In a small number of SIV positive samples, we found evidence for reverse zoonosis in the Swiss pig population: influenza A(H1N1)pdm09 (HA-1A) was detected in a total of seven pig samples by specific real-time RT-PCR, four in 2011 and three in 2013. After having established itself in humans by 2009, the virus made its way back into pigs via reverse zoonosis, which is also the presumed route of transmission in the here described seven Swiss pig cases.^{38,42} Whereas formerly circulating human H1N1 strains were completely replaced by the pandemic variant, co-circulation of the pandemic strain with endemic viruses was observed in the pig population. However, at farm level H1avN1 (clade HA-1C) remained the predominant subtype in European pigs.^{9,36,55}

Table 1: Human samples positive for human influenza A (IAV) and B virus (IBV).

Date of collection	Symptom onset before sampling	Symptoms reported	Influenza Type
Dec 2012	1 – 3 days	Cough, headache, bronchitis	A(H1N1)pdm09
Feb 2013	4 – 10 days	Headache, myalgia	A
Mar 2013	1 – 3 days	Fever, cough, headache, rhinitis	B Yamagata
Feb 2018	1 – 3 days	Fever, cough, headache, myalgia	B Yamagata
Apr 2019	4 – 10 days	Cough, headache, myalgia	A(H1N1)pdm09
Jan 2020	4 – 10 days	Not reported	B Victoria

Table 2: Human samples positive for SIV. GISAID = Global Initiative on Sharing All Influenza Data; HA = hemagglutinin; NA = neuraminidase; n.d. = not determinable.

Date of collection	Symptoms reported	GISAID Accession	HA Clade	GenBank Accession HA	GenBank Accession NA
Oct 2010	Fever, myalgia, rhinitis	EPI_ISL_85201	C.2.1	PQ663335	PQ663568
Apr 2011	Cough, rhinitis	EPI_ISL_98715	C.2.1	PQ663336	PQ663567
Nov 2011	Cough, rhinitis	EPI_ISL_19031578	C.2.1	PQ663346	PQ663573
Dec 2016	Cough	EPI_ISL_242993	n.d.	–	–
Dec 2017	Cough, headache, myalgia, bronchitis	EPI_ISL_18713946	C.2.2	PQ663389	–

With the exception of the seven A(H1N1)pdm09 (HA-1A) positive samples as per specific real-time RT-PCR, all HA and NA RNA segments sequenced in the present study could be assigned to the Eurasian avian lineage (HA-1C), which is widespread in pigs in Europe (Figure 5 and 6).^{4,19} According to H1 and N1 sequence alignments, the Swiss H1N1 strains cluster into two HA clades, i.e. C.2.1 and C.2.2. Since 2015, the HA typing has shown a gradual shift from clade C.2.1 to C.2.2 (Figure 7). However, C.2.1 sequences are still detected. Despite their evolutive capacities, phylogenetic analyses indicate that compared to other countries there is little genetic diversity and that the genome composition of influenza viruses in Swiss pigs remained fairly stable over the years (Figure 5 – 7).^{9,19,30} Only six potential reassortments between H1 clades and N1 subclades were identified (Figure 7). The absence of H1N2 and H3N2 subtypes, which are circulating in the rest of Europe, the comparatively low diversity and distinct clustering of Swiss H1N1 strains (Figure 5 and 6) can be at least partly attributed to the Swiss pig production system, which is inherently isolated and influenced by low animal import lev-

els. Similar specific genetic clusters were observed for other viruses in Swiss pigs such as hepatitis E virus and atypical porcine pestivirus.^{23,51}

We also found evidence for transmissions of influenza virus from pigs to humans: among the 11 influenza virus positive swabs of in-contact humans, six tested positive for human strains and five (= 45 %) tested positive for SIV (Table 1 and 2). Sequence and phylogenetic analyses assigned the SIVs to the Eurasian avian lineage of H1N1 and to the HA clade which at that time was most frequently detected in the pigs, i.e. C.2.1 and C.2.2 (Figure 5 and 7). Given that the five individuals also had symptoms typical for influenza (Table 2), it is likely that pigs were the source of infection. Antibody testing of convalescent sera would have permitted more conclusive information but was not performed. Since the first well-documented reports in 1958 and 1974, human infections with SIVs (both H1N1 and H3N2) have continued to be reported sporadically worldwide. Usually, these infections caused only mild disease resembling human seasonal influenza.^{3,14,18,28,39} Sustained human-to-human

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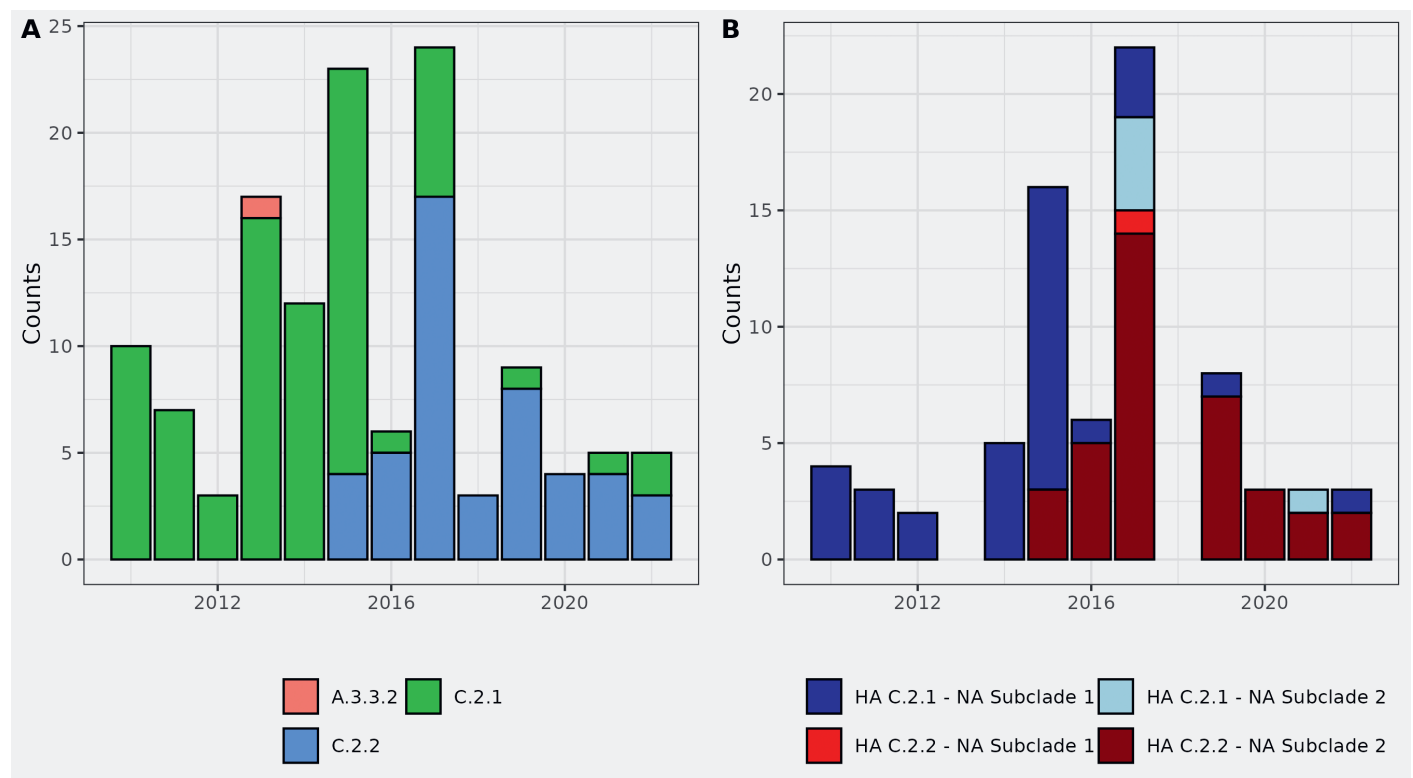


Figure 7: A) Temporal HA clade predominance of Swiss SIV strains from 2010 – 2022. B) Temporal assessment of HA clade and NA subclade combinations in Swiss SIV strains. Lighter-colored bars indicate putative reassortment events, representing viruses with non-canonical HA–NA pairings compared to the predominant clade combinations observed over time depicted with dark colors.

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transmission rarely followed and has not been observed in the present study either. Compared to the number of people directly or indirectly involved in pig production, the number of SIV infections detected in humans is low. Yet, considering that in the frame of the Swiss national SIV program 45 % of influenza positive human cases tested positive for SIV and that SIV infections are indistinguishable from seasonal human influenza, a high number of undetected cases must be expected. This hypothesis is supported by seroepidemiological studies in which higher seroprevalences of SIV were found in pig farmers and butchers than in people without occupational contacts to pigs.^{5,27,48}

It is undisputed that national and international monitoring programs in animals and humans with internationally coordinated early warning systems constitute an important pillar in the surveillance of influenza viruses worldwide. After more than 15 years since its launch, the Swiss monitoring program for SIV has brought to light various difficulties and hurdles that need to be addressed in the future: (1) The number of farms reporting pigs with influenza-like signs has continuously decreased from 320 in 2010 to 105 in 2022 (Figure 3).¹⁵ Likewise, the number of in-contact humans reporting influenza-like symptoms has decreased from 22 to 9 over the same period (Figure 8). In 103 cases,

no information concerning illness in humans was available. These reduced numbers most likely do not reflect a decrease in infections but rather a decline in disease awareness after the 2009 pandemic. For the future, all stakeholders including the farmers, veterinarians, meat producers and veterinary authorities should take measures to increase disease awareness. (2) After notification of influenza-like symptoms, the rate of farms and individuals sampled remained at a more or less constant low level of approximately 30 %. Well-orchestrated reporting, communication and cooperation between stakeholders is required in order to consistently sample pigs on reported farms. (3) In the IAV-positive farm visits, the rate of samples in which both HA and NA were successfully subtyped was rather low (196/375 = 52 %). In approximately 11 % only H1, in 21 % only N1 and in 16 % of cases no subtype was identified. For a large proportion of IAV positive samples, including human swabs, the Ct value after IAV real-time RT-PCR was rather high (data not shown), making successful subtyping and sequencing more difficult. High Ct values may be due to a naturally low viral load but may also be due to avoidable causes that need to be given greater attention in the future: sampling in the late stages of infection, suboptimal sampling, poor sample quality due to contamination with dirt and bacteria, improper sample handling and/or storage.

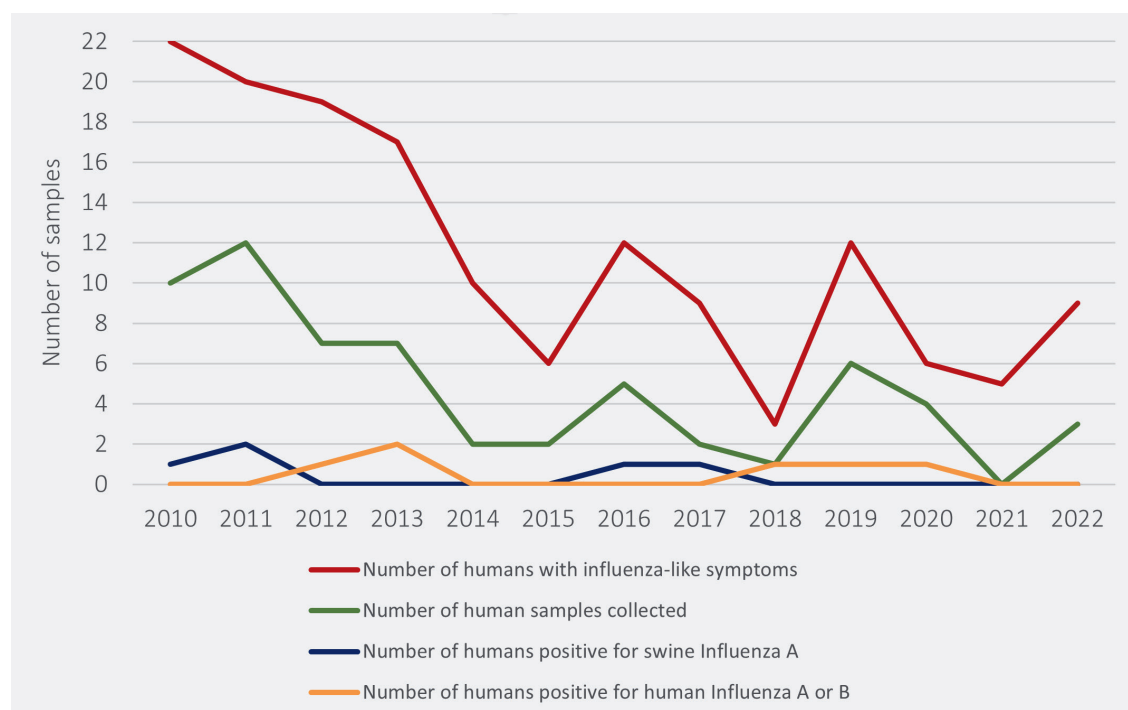


Figure 8: Number of humans reporting influenza-like symptoms in the context of the Swiss national SIV monitoring program (red line, n = 150), number of human samples collected (green line, n = 61), number of human samples positive for human Influenza A or B (orange line, n = 6) and number of human samples positive for SIV (blue line, n = 5) between 2010 and 2022.

Efficient SIV surveillance programs depend on sensitive and specific diagnostic methods which allow for cost-effective large-scale analysis. To detect circulating strains and new variants, currently several PCR assays are needed, which make the process expensive and time-consuming. Novel methods for high-throughput whole genome sequencing such as Illumina or Nanopore technologies, which have been extensively developed in recent years and have become more sensitive and affordable, could improve the surveillance of SIV and reduce the cost of virus subtyping in the long term.^{16,39} From a laboratory perspective, a future main goal of the national surveillance program is the implementation of a SIV MinION Nanopore sequencing protocol,²⁵ that allows for cost-efficient untargeted sequencing of all influenza virus genome segments present in a sample. This would also overcome the limitations of partial HA and NA sequencing and the restriction to H1, N1, H3 and N2 subtypes.

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Conflict of interest

The authors declare no conflict of interest.

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Programme national suisse de surveillance des virus de la grippe A chez les porcs et les humains: variabilité génétique et transmissions zoonotiques de 2010 à 2022

Les virus de la grippe A (IAV) sont susceptibles de provoquer des pandémies. Ce rapport résume les résultats du programme national suisse de surveillance des virus grippaux chez les porcs en présentant des données sur leur diversité génétique et sur les transmissions à l'homme entre 2010 et 2022. Les défis et les possibilités d'optimisation du programme sont également abordés.

Des prélèvements nasaux ou des échantillons de tissu pulmonaire provenant de porcs présentant des symptômes grippaux ont été analysés par RT-PCR en temps réel afin de détecter la présence du génome du virus de la grippe porcine (SIV) et de la souche saisonnière humaine A(H1N1)pdm09; les échantillons positifs ont été sous-typés pour H1, N1, H3 et N2. Parallèlement, les personnes présentant des symptômes grippaux et ayant récemment été en contact avec des porcs malades ont été invitées à effectuer elles-mêmes un prélèvement nasal. Les prélèvements humains ont été testés pour le virus IAV, et les prélèvements positifs ont été sous-typés afin d'identifier une éventuelle transmission inter-espèces entre les porcs et les humains.

Programma nazionale svizzero di sorveglianza dei virus influenzali A nei suini e negli esseri umani: variabilità genetica e trasmissioni zoonotiche (2010–2022)

I virus influenzali A (IAV) sono i principali candidati all'origine di future pandemie. Il presente rapporto sintetizza i risultati del programma nazionale svizzero di sorveglianza dei virus influenzali nei suini, con particolare attenzione alla variabilità genetica e alle trasmissioni zoonotiche verificatesi tra il 2010 e il 2022. Vengono inoltre discusse le principali sfide e le prospettive di ottimizzazione del programma.

Campioni nasali o di tessuto polmonare provenienti da suini con sintomatologia simil-influenzale sono stati analizzati mediante RT-PCR in tempo reale per la rilevazione dei genomi del virus dell'influenza suina (SIV) e del ceppo umano stagionale A(H1N1)pdm09. I campioni risultati positivi sono stati sottoposti a subtipizzazione per i segmenti H1, N1, H3 e N2. Parallelamente, soggetti umani con sintomi simil-influenzali e con contatto recente con suini infetti sono stati invitati ad effettuare un auto-prelievo nasale. I tamponi umani sono stati testati per la presenza di IAV e, in caso di positività, sottotipizzati per identificare potenziali eventi di trasmissione interspecie tra suini e uomo.

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Chez les porcs, le SIV a été détecté lors de 375 des 674 visites dans des exploitations. Le H1N1 est le seul sous-type détecté à ce jour chez les porcs suisses. La souche saisonnière humaine A(H1N1)pdm09 (clade 1A de l'hémagglutinine (HA)) a été détectée lors de sept des 375 visites dans des exploitations positives au SIV. Les analyses phylogénétiques des séquences partielles des gènes HA et neuraminidase (NA) indiquent que les autres porcs ont été infectés par la lignée aviaire eurasiennne (clade HA 1C), qui prédomine chez les porcs en Europe. Les souches H1N1 suisses forment des groupes phylogénétiques distincts au sein des clades HA 1C.2.1 et 1C.2.2 et semblent évoluer à un rythme relativement lent. Cinq cas d'infection humaine par le SIV ont été identifiés. L'analyse de similarité des séquences a permis de classer les cinq virus dans la lignée aviaire eurasiennne (C), clades 1C.2.1 et 1C.2.2. Il n'y avait aucune preuve d'une transmission interhumaine durable.

La surveillance continue des virus grippaux à l'interface entre les porcs et les humains est d'une importance capitale pour permettre la détection précoce de nouveaux sous-types de virus grippaux et de changements dans la situation épidémiologique.

Les auteurs considèrent que la publication des données issues du programme de surveillance est essentielle pour sensibiliser davantage les vétérinaires et le grand public à cette maladie et pour compléter les informations existantes sur les virus grippaux circulant en Europe.

Mots clés: Humain, variabilité génétique, grippe, surveillance, porc, Suisse

Nei suini, il SIV è stato rilevato in 375 delle 674 visite effettuate negli allevamenti. Finora, in Svizzera è stato riscontrato esclusivamente il sottotipo H1N1. Il ceppo umano stagionale A(H1N1)pdm09 (clade dell'emoagglutina HA 1A) è stato identificato in sette delle 375 visite positive per SIV. Le analisi filogenetiche delle sequenze parziali dei geni dell'emoagglutina (HA) e della neuraminidasi (NA) hanno indicato che i restanti suini erano infettati dal lignaggio aviario eurasiatico (clade HA 1C), predominante nei suini europei. I ceppi svizzeri H1N1 si raggruppano in cluster filogenetici distinti all'interno dei cladi HA 1C.2.1 e 1C.2.2, mostrando un'evoluzione relativamente lenta.

L'infezione umana da SIV è stata documentata in cinque casi. L'analisi di similarità delle sequenze ha attribuito i cinque virus al lignaggio aviario eurasiatico (clade 1C), con appartenenza ai cladi 1C.2.1 e 1C.2.2. Non sono emerse evidenze di trasmissione sostenuta da uomo a uomo.

La sorveglianza continua dei virus influenzali all'interfaccia suino-uomo riveste un'importanza cruciale per consentire l'individuazione precoce di nuovi sottotipi di IAV e per rilevare tempestivamente cambiamenti nel quadro epidemiologico.

Gli autori ritengono che la pubblicazione dei dati del programma di sorveglianza sia essenziale per sensibilizzare sia i veterinari che il pubblico in generale sulla malattia e per integrare le informazioni esistenti sui virus influenzali circolanti in Europa.

Parole chiave: Uomo; variabilità genetica; influenza; sorveglianza; suino; Svizzera

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