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OPERATIONAL GUIDANCE

Testing and detection of zoonotic influenza virus infections in humans in the EU/EEA, and occupational safety and health measures for those exposed at work

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Operational guidance

This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Cornelia Adlhoch.

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Cover picture: NIAID (Colorized transmission electron micrograph of a cross-section of SW31 (swine strain) influenza virus particles)

Contents

Abbreviations

BSL	Biological Safety Level
cDNA	complementary Deoxyribonucleic Acid
US CDC	United States Centers for Disease Control and Prevention
DFA	Direct Immuno Fluorescence assay
DNA	Deoxyribonucleic Acid
ECDC	European Centre for Disease Prevention and Control
ECMO	Extracorporeal Membrane Oxygenation
EFSA	European Food Safety Authority
EISN	European Influenza Surveillance Network
ELISA	Enzyme-Linked Immunosorbent Assay
ERLI-Net	European Reference laboratories for influenza network
EU-OSHA	European Agency for Safety and Health at Work
EURL	European Union Reference Laboratory
EWRS	Early Warning and Response System
FAO	Food and Agriculture Organization of the United Nations
GISAID	Global Initiative on Sharing All Influenza Data
GISRS	Global Influenza Surveillance and Response System
HA	Haemagglutinin
HI	Haemagglutination Inhibition
HPAI	Highly Pathogenic Avian Influenza
IHR	International Health Regulations
ICTV	International Committee for Taxonomy of Viruses
IPC	Infection Prevention and Control
LPAI	Low Pathogenic Avian Influenza
LPM	Live Poultry Market
LRTI	Lower Respiratory Tract Infection
NIC	National Influenza Centre
RADT	Rapid Antigen Diagnostic Test
RBCs	Red Blood Cells
RNA	Ribonucleic Acid
OSH	Occupational Safety and Health
POCT	Point of Care Test
PPE	Personal Protective Equipment
RT-LAMP	Reverse transcription Loop-Mediated Isothermal Amplification
RT-PCR	Reverse transcription polymerase chain reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Corona Virus 2
SIV	Swine Influenza Virus
Variant virus	Zoonotic transmission of a swine influenza virus to a human e.g. A(H1N1) variant virus = A(H1N1)v
VTM	Virus transport media
WGS	Whole Genome Sequencing
WHO	World Health Organization
WOAH	World Organisation for Animal Health
WHO-CC	World Health Organization Collaborative Centre

Glossary

Establishment: as defined by Regulation (EU) 2016/429 on transmissible animal diseases 'any premises, structure, or, in the case of open-air farming, any environment or place, where animals or germinal products are kept, on a temporary or permanent basis, except for households where pet animals are kept, and veterinary practices or clinics'.

Avian influenza: as defined in the Commission Delegated Regulation (EU) 2020/689 is a disease classified into two distinct categories based on the pathotype of the influenza A virus (now called *Alphainfluenzavirus influenza*):

- **Highly pathogenic avian influenza (HPAI)** means an infection of poultry or other captive birds, or wild birds caused by a) an influenza A virus of H5 and H7 subtypes or any influenza A virus with an intravenous pathogenicity index (IVPI) greater than 1.2 or b) avian influenza viruses of the subtypes H5 or H7 with genome sequences codifying for multiple basic amino acids at the cleavage site of the haemagglutinin molecule, similar to that observed for other HPAI viruses, indicating that the haemagglutinin molecule can be cleaved by a host ubiquitous protease.
- **Low pathogenic avian influenza (LPAI)** means an infection of poultry or other captive birds or wild birds caused by avian influenza viruses of subtypes H5 or H7 that do not come within the HPAI definition.

An infection of birds caused by avian influenza viruses of non-H5 or H7 subtypes is also commonly defined as LPAI.

Reassortment: ability of influenza and other segmented viruses to exchange genome segments between different influenza virus strains when co-infecting the same cell in an individual, thus creating new viruses with different characteristics based on a new composition of segments from the different viruses.

Executive summary

Zoonotic influenza viruses remain a concern for human health in Europe. Influenza viruses circulating in animal species can sporadically be transmitted to humans, causing mild to very severe disease. These viruses have the potential to cause pandemics, such as the 2009 influenza pandemic caused by a triple-reassorted virus spreading from pigs to human, or to severely affect public health, such as during the epidemics of H5N1 in Egypt or H7N9 in China [1].

Viral evolutionary processes, such as reassortment events in influenza viruses, are driving factors for the emergence of new viruses with pandemic potential. The rapid identification of animal influenza viruses being transmitted from animal species to humans and between humans is the key to implementing control and follow-up measures as early as possible. The monitoring of influenza viruses in animal and human populations provides baseline information for any epidemiological situation assessment.

Given the ongoing widespread circulation, evolution and reassortment of influenza viruses in mainly pig and avian populations in Europe and globally, and the threat of increasing transmissibility to mammals including humans, this document aims to provide guidance for public health and laboratory experts in identifying human infections with animal influenza viruses as early as possible to provide early warning and inform risk assessments and public health measures. Regular quarterly reports on the European and global situation concerning avian influenza, together with monthly updates on zoonotic influenza in the Communicable Disease Threats Reports (CDTR), contribute to situational awareness and early warning [2,3].

There are groups of people with occupations that may expose them to animals with possible or confirmed zoonotic influenza virus infection. If people have been exposed to animals that may be infected, and if they present with respiratory symptoms or conjunctivitis, they should be tested. Similarly, patients with a severe acute respiratory disease of unknown aetiology, including those testing positive with an influenza virus that cannot be sub-typed, and severely ill patients with prior animal exposure should be considered for zoonotic influenza virus testing. Recent findings of encephalitis/meningoencephalitis with high viral loads in the brain samples of mammals (seals, foxes, porpoises, dolphins, black bears, etc.) infected with A(H5N1) or A(H5N8) viruses suggest the possibility of avian influenza infection in patients presenting with atypical symptoms such as encephalitis, neurological affection and unclear aetiology. Testing and detection of zoonotic influenza can be carried out with polymerase chain reaction assays, enabling a highly sensitive and rapid direct molecular detection of zoonotic influenza viral genomes. The approach to diagnosis using real-time RT-PCR adopted in most laboratories is based primarily on targeting the M1 matrix gene, which is a standard target for the differentiation of type A and type B influenza viruses. Since genetic sequences differ among various subtypes of zoonotic influenza viruses, it is necessary to obtain or design PCR primers and probes that will specifically detect the influenza subtype of interest.

Serological tests should not be used for initial detection and characterisation of a potential zoonotic event. However, serological methods include simple and inexpensive techniques utilising standard laboratory equipment that can identify zoonotic influenza virus subtypes and measure HA-specific antibodies to the virus to answer different questions related to previous exposure or prevalence in specific populations. Nevertheless, serological tests have several limitations that need to be considered, such as the fact that cross-reactions can occur between different lineages within one subtype, or even among different subtypes. Moreover, the results obtained only provide information on historical exposure to zoonotic influenza viruses and do not provide viral genetic information which is vital for evaluating the potential pandemic threat of strains.

Due to the broad diversity of zoonotic influenza viruses, surveillance by genomic evaluation has become indispensable. The nucleotide-level resolution of in-depth whole genome sequencing permits phylogenetic analysis and molecular epidemiological studies for a detailed understanding of an outbreak. Countries with capacity and resources available should use whole-genome sequencing to identify emerging zoonotic influenza viruses. Sequencing is the most robust method for distinguishing between zoonotic and seasonal strains. The availability and increasing use of whole genome sequencing for routine diagnostics can support the identification of zoonotic transmission events and laboratories are strongly encouraged to apply these methods when influenza viruses cannot be typed or subtyped using the classical methods. Phenotypic tests (e.g. airborne transmission capacity in mammals) on viruses with genetic characteristics indicating zoonotic potential should also be performed in laboratories with an adequate level of biosafety (BSL-3 or higher).

Since transmission is likely in workplaces where animal contact cannot be avoided and workers will probably be at the forefront of outbreaks, occupational safety and health measures should be taken at such premises and enhanced at those where occupational cases have been identified. There is a comprehensive body of legislation defining the obligations of employers and framing the measures to be taken. Employers should periodically revise their workplace risk assessment and ensure that all necessary technical, organisational, maintenance and hygiene measures are taken to prevent the infection of workers. These measures include the avoidance of aerosol and dust, ventilation, separation of work and personal clothing, and measures to prevent contamination of worker accommodation. Employers must keep a record of any workers that may have been exposed to the viruses and offer health surveillance as appropriate. The workplaces concerned include animal breeding centres, farms, zoos and slaughterhouses, however workers in laboratories, healthcare facilities and waste management could also be exposed.

Preparedness plans covering zoonotic influenza updates and regular training courses are important measures, and simulation exercises in a One Health context also make it possible to test readiness. Public health professionals and clinicians should be aware of the need to test for zoonotic influenza virus infection in patients with respiratory illnesses and recent exposure to animals potentially infected with influenza virus, and to perform virus characterisation or whole genome sequencing of severe cases. This is vital for identifying transmission events, initiating follow-up investigations and detecting human-to-human spread. Recent data from infected mammals identified the highest viral load in brain samples which indicates that exposed humans could present with non-respiratory atypical symptoms, such as encephalitis/meningoencephalitis.

1. Background

Influenza viruses of animal origin circulating in different animal species have been transmitted to humans, causing sporadic isolated zoonotic infections [4]. In rare circumstances, newly emerging reassorted influenza viruses have caused pandemics. The ability of influenza viruses to exchange genome segments with another influenza virus in co-infected individuals, creating new viruses with different characteristics, can enable transmission to and between humans. Other evolutionary adaptation mechanisms are related to the silent or functional mutations within the genome altering protein structures (e.g. of the virion or other functional mechanisms).

The animal influenza viruses relevant to human health belong to type A influenza viruses and can also be identified through generic detection systems of type A influenza viruses, now designated as *Alphainfluenzavirus influenzae* by the International Committee for the Taxonomy of Viruses (ICTV) [5]. Viruses formerly designated as influenza types B, C, and D do not have pandemic potential and thus are not specifically addressed in this report: influenza B causes epidemics in humans and has been shown to be able to infect seals, but no transmission from animals to humans has been reported [6]. Influenza C causes mild disease in humans but not generally seasonal epidemics; the virus has been detected in pigs but is not relevant for the animal-human interface [7]. Influenza D has been mostly detected in ruminants and to a lesser extent in pigs [8], with a minor zoonotic potential to transmit to humans [9]. Nevertheless, the pathway described in this report is valid for identifying human infections with any animal-derived influenza virus type.

Wild aquatic birds are the main influenza A virus reservoir in nature, harbouring 16 of 18 hemagglutinin (HA) and nine of 11 recognised neuraminidase (NAs) subtypes. Occasionally, avian influenza viruses are transmitted directly or indirectly from wild birds into the poultry population, where the H5 and H7 subtypes can evolve from a low pathogenic into a highly pathogenic form. Prior to 2002, highly-pathogenic avian influenza (HPAI) viruses were limited almost exclusively to poultry. Following the emergence in 1996 of a highly-pathogenic avian influenza A(H5N1) virus, namely A/goose/Guangdong/1/1996 (Gs/GD), there have been increasing detections of HPAI viruses in wild birds, often associated with mortality in poultry when introduced in poultry establishments.

Since 2005, highly-pathogenic avian influenza (HPAI) A(H5Nx) viruses of the Gs/GD lineage have been periodically introduced into Europe through the autumn migrations of birds, causing several small and large seasonal winter epidemics with high mortality in wild birds, transmission to caged/domestic birds (e.g. in zoos or backyards), and outbreaks in poultry establishments. Recently, HPAI A(H5Nx) viruses of clade 2.3.4.4, a descendant of the Gs/GD lineage, have been responsible for three large epidemics during the 2016−17, 2020−21 and 2021−22 winter seasons. Distinct genotypes belonging to five different subtypes within clade 2.3.4.4b (H5N1, H5N2, H5N3, H5N4, H5N5 and H5N8) have been described recently [2].

Transmission events to different mammal species, such foxes, seals, pigs and other carnivores, as well as humans and the identification of viruses with mutations associated with mammalian adaptation and high diversification and reassortment might point to an increased zoonotic risk related to currently circulating clade 2.3.4.4b HPAI A(H5Nx) viruses. Persistent detection in poultry and constant findings of different viral subtypes in wild birds, even during the summer months in northern European countries, are signals of the potential endemicity of these HPAI viruses in Europe, posing a constant threat to birds and mammals.

Over the last decade, several avian or swine influenza virus subtypes have been reported to have caused human infections such as influenza type A swine H1N1 or H1N2, and H3N2 viruses, as well as avian H5Nx, H7N9 and H9N2 viruses [10-13]. Nevertheless, more unusual avian influenza viruses such as A(H3N8), A(H6N2), A(H7N4) and A(H10N3) or A(H10N8) have also caused human infections [10-13]. Avian influenza H5 or H7 viruses in birds are classified as high or low pathogenic for chickens, based on their cleavage site and disease progression (ability to cause systemic infection in chickens, resulting in high fatality). However, there is no correlation between the pathogenicity of avian influenza viruses in birds and the infectious and pathogenic potential in humans [14,15].

With regard to swine influenza, the main factors for susceptibility are vaccination status or level of immunity of the pig herds, animal and farming management practices, climatic conditions and intercurrent infections with other respiratory agents. Pigs can be infected with swine influenza as well as human and avian influenza viruses and can therefore become intermediate hosts for the transmission of avian influenza viruses to humans and act as a 'mixing vessel' for reassortant influenza viruses [16].

In Europe, different swine influenza viruses have incorporated different gene segments from seasonal human A(H1N1)pdm09 influenza viruses in retro-zoonotic events and are continuously circulating within the pig population. Over time, different reassortments have led to a high variability of swine influenza viruses that have caused sporadic human infections in those who have close contact with pigs or their environment [10-13,17].

Since the occurrence of human cases due to avian influenza or swine influenza virus cannot be excluded, and transmission events to humans (as well as increased potential for zoonotic transmission) have been observed, adequate preparedness is essential. Activities for the early identification, monitoring, and control of outbreaks in animal populations, together with the application of appropriate measures, especially in an occupational context where the first contact is likely to occur, might reduce and limit the risk of human infections. However, such activities cannot prevent them completely. It is therefore important to be prepared to identify possible human cases, detect and confirm the virus, start investigations and implement measures rapidly.

2. Methods

This guidance document has been developed using different sources of information, such as epidemic intelligence data, peer-reviewed publications, reports, and guidance documents from national or international agencies. This work has not been based on systematic reviews of the literature available for all of the different aspects covered in this document. The draft version was shared with the Disease Network Coordination Committee (DNCC) for review, as well as with the experts at the European Agency for Safety and Health at Work (EU-OSHA), the European Union Reference Laboratory (EURL) for Avian Influenza and Newcastle Disease and the European Food Safety Authority (EFSA).

3.Who to monitor and test for zoonotic influenza viruses?

Exposure

Transmission of zoonotic (avian or swine) influenza viruses to humans is a rare event and the human cases reported have been sporadic [18,19]. People and other mammals are usually infected through a mix of different transmission routes and exposure - e.g. direct or close contact with infected animals or their body fluids, tissues, feathers, droppings, ingestion and inhalation of aerosolised virus, and exposure to a contaminated environment [20]. The probability of infection with zoonotic influenza viruses varies with the type of interaction between human and infected animal (e.g. culling, bird ringing) and depends on the contact type (duration and route) and viral dose.

Primary infection occurs mostly in an occupational context and effective measures need to be taken at workplaces to avoid further spread, as outlined in the respective section. Unprotected exposure to avian or swine influenza virus may occur in different ways: direct contact with infected animals, such as pigs, or birds. This may be either as a result of handling, slaughtering, de-feathering, butchering, preparation for consumption, or disposal of carcasses in an industrial/agricultural/domestic setting, or following the ringing or swabbing of wild birds, or the collection of dead animals in the wild. Infection can also occur as a result of contact with environments/fomites/surfaces contaminated with faeces or animal parts (carcasses, organs, fluids) - e.g. during clean up and disposal activities or in backyard holding areas which are accessible to wild birds - or prolonged exposure to birds - e.g. farmers and their families.

Risk settings and population groups at risk

Occupational or other population groups working or otherwise carrying out activities in the settings described below are at increased risk of being exposed to zoonotic influenza viruses by having close, unprotected contact with potentially infected animals. As such, they should be made aware of the risk and apply the measures set out in Section 4. This includes backyard farmers, hunters or forest workers, rangers or others with possible exposure to infected swine or wild boar. These groups should be made aware of the potential risk of transmission and reminded to take preventive measures, including use of protective equipment when in direct contact with animals (see Table 1 for examples).

Occupational and other groups in contact with wild birds

Wild birds, especially aquatic migratory birds, play a very important role in the global spread of influenza A virus and have been severely affected during the latest 2020–2021 and 2021–2022 epidemics, with mass mortality events in Europe [21]. During these epidemics, A(H5N8), A(H5N1) and other viral genotypes have been detected in a large number of different wild bird species, including non-migrating resident birds. Wild birds are presumed to be one of the most likely vectors for the introduction of avian influenza into resident European bird populations and poultry establishments [22]. Wild birds infected with avian influenza virus that are found dead or sick represent a risk of exposure to the general population who might find them (e.g. on beaches) and touch them. Those working with wild birds, such as bird ringers, ornithologists, and staff at wild bird, animal or nature conservation centres, or carrying out other activities where they are likely to occasionally have contact with potentially infected wild birds (e.g. bird hunters) are at higher risk of exposure to avian influenza viruses and should consider wearing personal protective equipment (PPE).

Commercial poultry establishments and backyard farms, zoos and other settings for captive birds

The route of transmission from wild birds into poultry establishments is believed to be exposure via direct contact with wild birds, or indirectly as a result of contaminated environments or material brought into the establishments [23]. Other common sources of infection are via the introduction of infected poultry into an establishment or the introduction of contaminated fomites or material (e.g. equipment, vehicles, feed, cages or clothing) [24]. Highly pathogenic avian influenza viruses cause a high mortality rate in affected poultry and when outbreaks in commercial poultry establishments or backyard farms are identified, surveillance and control measures must be immediately implemented in accordance with legislation. During these activities, farmers, veterinarians, those responsible for culling the birds and other workers are exposed to a large number of highly infected chickens, turkeys or other farmed bird species.

Avian influenza outbreaks have also been reported in smaller establishments of captive birds (e.g. birds kept for breeding, backyard farms and zoos). Organisers of exhibitions or shows with live birds should also be aware of the possibility of exposure and the risk of infection [25].

Previous analyses have investigated the exposure events during avian influenza epidemics [26]. With larger culling operations organised during outbreaks, trained and experienced staff might be more likely to wear PPE at establishments, while hobby farmers or people exposed to infected backyard animals might take less protective precautions and therefore be directly exposed to infected birds.

Live bird markets

In Asia, live bird markets (LBMs) play a critical role in maintaining, amplifying and disseminating avian influenza among poultry and from poultry to humans. Previous epidemiological studies have demonstrated the transmission potential of avian influenza virus via contact, droplets and airborne routes at LBMs [27-29]. In those countries where avian influenza virus is enzootic, surveillance studies have demonstrated that genetically diverse avian influenza viruses are highly prevalent among poultry at LBMs [27,30]. LBMs are widely distributed in both urban and suburban areas across China and South-East Asia and pose a significant threat for neighbouring residents, poultry market/shop workers and visitors. The high density of live poultry markets, dense populations, and extensive live poultry transportation network in these regions could favour large-scale and trans-border avian influenza spread in poultry, thereby increasing the risk of human infection [31]. LBMs play a very minor role in the EU/EEA and are only relevant in the sense that those travelling outside Europe and visiting live bird or animal markets need to be aware of the potential risk associated with the exposure to animals and the environment. Returning travellers with respiratory symptoms reporting exposure to live bird or animal markets 10−14 days before symptoms onset should undergo further testing and inform healthcare establishments of any potential exposure.

Pig farms and shows

Swine influenza is common among pig populations worldwide and different, genetically-distinct lineages circulate globally all year round [32,33]. Transmission events have mainly been reported in occupational settings, as a result of close, direct, unprotected contact with pigs or contaminated environments. Those most at risk of swine influenza are farmers and workers, swine farmers and swine farm workers, people involved in the breeding and feeding of pigs or the cleaning of pig farms, those involved in culling, slaughterhouse workers, and veterinarians [19]. Transmission has been observed among farmers, their families or people in close contact with pig farms in Europe. In the US, swine influenza virus transmission events have been reported in visitors to traditional agricultural fairs where contact with various farm animals, including pigs, is encouraged [34-37]. However, fairs of this kind with large numbers of participants are not frequently held in the EU/EEA. Organisers of exhibitions or shows with live birds or pigs also organised on a smaller scale in EU/EEA countries should also be aware of the possible exposure and infection risk for visitors and workers and take appropriate measures [25].

Wild boar

As with commercial pigs, wild boar can be infected with swine influenza viruses and people who have close, unprotected contact with wild boar, such as hunters or forest workers, can be at risk of infection.

Healthcare workers and contacts of confirmed cases

Healthcare workers treating patients with confirmed zoonotic influenza virus infection and close contacts of such patients can also be at increased risk.

Table 1. Examples of populations at risk of being occupationally or recreationally exposed to avian or swine influenza viruses

Possible exposure to:	
Avian influenza virus	**Swine influenza virus**
Poultry farmers, bird breeders and farm workers	Swine farmers and farm workers
Backyard farmers	Backyard farmers
People involved in the breeding and feeding of birds or poultry and the cleaning of farms	People involved in the breeding and feeding of pigs and the cleaning of pig farms
Workers involved in culling and waste disposal at poultry establishments or other bird farms	Workers at pig slaughterhouses and those involved in waste disposal
Wild bird hunters	Hunters exposed to wild boar
Bird ringers	
Workers at wildlife conservation, recovery centres or zoos	Workers at wildlife conservation, recovery centres or zoos
Veterinarians	Veterinarians
People visiting live bird or animal markets and animal shows	People visiting animal markets, agricultural fairs and animals shows
Healthcare workers dealing with infected patients	Healthcare workers dealing with infected patients
People in close contact with dead or sick birds potentially infected with avian influenza without wearing protective equipment	People who are in close contact with pigs without wearing protective equipment
Health care or laboratory workers taking or analysing specimens.	Health care or laboratory workers taking or analysing specimens.

Clinical symptoms

The effects of avian influenza virus infections in humans range from asymptomatic infection to mild-to-severe disease progressions. In Europe, human infections with avian influenza viruses have mainly involved mild respiratory illness or influenza-like symptoms of the upper respiratory tract (fever, sore throat and cough). Conjunctivitis has been commonly reported in patients, as well as gastrointestinal symptoms. In avian influenza A(H5N1) transmission events to different mammal species (e.g. dolphins, porpoises, bears), the highest viral loads in severely ill or deceased animals have been identified in brain samples, showing signs of encephalitis [38]. Therefore, atypical non-respiratory symptoms, such as encephalitis or meningoencephalitis, might also occur in exposed humans.

In more severe cases, a rapid progression to severe pneumonia, sepsis with shock, acute respiratory distress syndrome, encephalitis, varying degrees of encephalopathy and even deaths have been reported [39,40].

Common initial symptoms for patients from China infected with A(H5Nx) or A(H7N9) avian influenza viruses were high fever (above or equal to 38°C) and a cough, followed by more severe symptoms involving the lower respiratory tract, including dyspnoea or difficulty breathing. Other symptoms such as diarrhoea, vomiting, abdominal pain, bleeding from the nose or gums, encephalitis, neurological symptoms [41] and chest pain were also reported in some of these patients [42].

Recent infections with highly pathogenic avian influenza (HPAI) viruses of clade 2.3.4.4b have been observed in mammals, such as foxes and seals, with neurological effects that progressed to death (mainly in carnivore species) [43]. Therefore, neurological symptoms could also be related to avian influenza infection in mammals, including humans, and influenza diagnosis should be considered as a differential diagnosis in patients with severe neurological affection of unknown aetiology.

Unlike the severe respiratory disease frequently detected in most human cases of A(H7N9) infection, human infection associated with other influenza viruses within the H7 subtype has typically consisted of eye infection: between 1996 and 2014 over 80% of the confirmed or presumed H7 human infections presented with conjunctivitis [19,44].

The case fatality rate for A(H5) and A(H7N9) subtype virus infections among humans is much higher than that for seasonal influenza infections with up to 40% [45]. Human infections with avian influenza A(H7N7) viruses seem less severe, but fatal outcomes have been reported [18]. However, A(H9N2) viruses are more likely to be detected in children and have caused a milder disease in most of the reported cases. These A(H9N2) viruses are not present in the poultry or bird population in Europe and therefore do not currently represent a risk to humans in the EU/EEA countries.

For human infections with swine influenza viruses, most cases have been mild, resembling seasonal influenza, mainly presenting with fever, cough, pharyngitis, rhinorrhoea and myalgia, and only a few cases have required hospitalisation. Health conditions such as asthma or other lung diseases, diabetes, obesity, auto-immune disorders, immunosuppressive therapy, neurological or cardiovascular disorders or pregnancy are predisposing factors for hospitalisation. A few cases have been reported with serious illness presenting with severe acute respiratory infection and requiring extracorporeal membrane oxygenation (ECMO) [17,46].

Monitoring

Clinical symptoms usually occur within 10–14 days of exposure and the treating physician should ask about any suspected animal exposure prior to disease onset. Similarly, practitioners should ask patients about any exposure to potentially infected animals before onset of symptoms.

The monitoring and follow-up of people or workers after outbreaks of avian influenza or confirmation of swine influenza cases is managed by different authorities within the Member States - e.g. the animal, occupational safety and health, public health, agricultural or environmental sector – and there are different areas of responsibility and levels of collaboration across the sectors.

In an outbreak setting (for example, during culling activities at an establishment where avian influenza has been confirmed), public health authorities should consider active (daily or frequent feedback) or passive (self-monitoring and reporting of symptoms) monitoring of those exposed for up to 10 days following exposure. A concerted approach is recommended, involving the national occupational safety and health authorities and the occupational safety and health preventive services of the establishment.

Healthcare workers treating patients with confirmed zoonotic influenza virus infection and close contacts of these patients should be monitored closely for signs of illness for up to 10 days following exposure and managed as a possible case if they develop compatible symptoms during this period.

People who experience symptoms after exposure should self-isolate until influenza has been ruled out. Patients with severe respiratory diseases should be tested for influenza and, if positive for influenza, the virus should be subtyped. It is good practice to share specimens with the national influenza centre or national reference laboratory for avian influenza or swine influenza, particularly if an infection of animal origin is suspected and the subtype cannot be determined.

Sentinel systems for the monitoring of influenza are in place across Europe and zoonotic influenza viruses are sometimes identified through the routine testing of patients with influenza-like illness or acute respiratory illness in primary or secondary care. However, sentinel systems usually cover less than 5% of the population in most countries and are not suitable for the early and comprehensive detection of zoonotic infections [47].

ECDC monitors outbreaks in animals and reports of potential human cases of zoonotic influenza, including avian and swine influenza, on a regular basis through its epidemic intelligence activities. Detailed information is exchanged among experts from the animal and public health sector, as well as the European Influenza Surveillance Network (EISN) and other international organisations (e.g. World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), World Organisation for Animal Health (WOAH)) to better assess the situation. WOAH publishes regular updates on influenza viruses with zoonotic potential and also displays the officially reported notifications through the World Animal Health Information System [48]. An overview of the outbreaks in Europe can also be found on the websites of the EU Reference Laboratory for Avian Influenza and Newcastle Disease [49] and the European Commission [50].

Considerations for the identification of a potential or suspected human case

During an outbreak, additional information is required to perform a situational risk assessment and decide on the next steps if a person with compatible symptoms and exposure to animals or contact with a confirmed human case has been identified. The following information and criteria may support decision-making:

Circumstances of potential exposure:

- exposure setting (commercial establishment, backyard, environment, market, slaughterhouse, etc.)
- epidemiological information about the outbreak setting (animal species, number of animals, onset of outbreaks, etc.)
- nature of exposure (farming, animal breeding, culling, slaughtering, de-feathering, etc.)
- level and quality of exposure (occupational, direct/indirect)
- duration of exposure (minutes, hours, several days)
- time since exposure (time between exposure and onset of symptoms)
- number of co-workers
- contacts of potential case.

Circumstances that may increase the likelihood of infections:

- avian influenza confirmed in birds/swine influenza confirmed in pigs/wild boar
- contact with a possible or confirmed case (co-worker, healthcare worker, family member)
- zoonotic influenza confirmed in contact
- presence of symptoms in the patient (acute lower respiratory illness with fever (>38C) and cough, conjunctivitis, and gastroenteritis)
- Positive result of a rapid antigen detection test (RADT) for influenza A in an outbreak situation.

Measures applied:

- level and quality of prevention and protection measures including PPE (no protection, FFP2, respirator, eye protection, gloves, etc.)
- possible PPE breaches where PPE should have been worn
- other conditions that might increase potential PPE breaches, such as hot weather conditions.

Additional measures already initiated:

- medical treatment (antivirals)
- laboratory investigations started
- isolation after exposure and self-monitoring for symptoms for a period of 10–14 days.

Collection, storage and transport of specimens

Successful influenza virus diagnosis depends largely on the quality of the specimen and the conditions under which it is stored and transported prior to laboratory processing. Specimens for the isolation of influenza viruses in cell culture and the direct detection of viral antigens or nucleic acids should ideally be collected within three days of the onset of clinical symptoms. A variety of specimens are suitable for the diagnosis of avian and swine influenza human infections. The best specimens are material collected with oropharyngeal swabs, bronchioalveolar washes, conjunctival washes or tracheal aspirates. Specimens from nasopharyngeal swabs are acceptable, but they may contain a low quantity of the virus in patients with severe disease with lower respiratory tract infection (LRT) [40]. LRT specimens may be the most suitable in cases of pneumonia. In the case of mechanically-ventilated patients, the best specimens from the respiratory tract are throat, nasal-cavity, bronchioalveolar lavage and endo-tracheal aspirates. When swabs are used, the preferred choice is swabs with a plastic shaft (i.e. not a cotton swab with a wooden shaft) [51]. The use of virus transport media (VTM) is recommended to prolong the viability of the viruses.

For serological testing, an acute-phase serum specimen (3–5 ml whole blood) should be taken promptly after the onset of clinical symptoms – and no later than seven days afterwards. A convalescent phase serum specimen should be subsequently be collected after 2–4 weeks [52].

Specimens for virus isolation should be stored at 4°C immediately after collection and promptly transported to the laboratory. Processed specimens should be inoculated into susceptible cell cultures as soon as possible. If the specimens cannot be processed within 48 hours, they should be kept frozen at or below -70 °C, ideally in liquid nitrogen. To prevent loss of infectivity, repeated freezing and thawing must be avoided.

Samples from patients meeting clinical and epidemiological criteria that suggest possible infection with highly pathogenic avian influenza virus should be manipulated using BSL-2 containment and BSL-3 practices as a minimum. All manipulations of live virus samples must be performed within a class-II (or higher) biosafety cabinet.

ECDC organises annual wet lab training courses with the European Reference laboratories for influenza network (ERLI-Net) laboratories, focusing on virus detection, virus characterisation, virus isolation and bioinformatics. Online training can also be provided for interested experts [53].

4. Occupational safety and health measures for those exposed at work

Infections with zoonotic influenza have been observed in certain occupational settings [54]. There is an extensive set of regulations to protect workers from health and safety risks and in accordance with occupational health and safety legislation, employers have responsibilities. At EU level, the minimum requirements are set out in the Biological Agents Directive [56] and have to be transposed into national legislation in every Member State [54,55,57]. Employers' responsibilities include the implementation of appropriate preventive measures after a workplace risk assessment, taking into account all risks, including the risks from unintentional exposure to biological agents, in agreement with the health or safety committee or with workers' representatives. This includes prioritising technical and organisational measures over personal measures, the obligation to consult, inform and train workers and the need to provide appropriate health surveillance. Appropriate records also need to be kept and it may be mandatory to notify public health authorities in the event of specific infections.

In case of a potential exposure to avian or swine influenza, workers should be protected following an updated workplace risk assessment and prevention measures implemented accordingly [56]. These measures include avoiding dust and aerosols (for example when cleaning and handling litter), enhanced ventilation and the use of appropriate PPE when other technical and organisational protective measures offer insufficient protection to workers. Work clothing and street clothing should be stored separately. It should be ensured that potentially contaminated areas are separated from clean areas (black/white areas) and appropriate hygiene measures applied. In agricultural settings, including poultry establishments, care should be taken to avoid contamination of domestic areas, for example through contaminated work clothing, in order to avoid exposing family workers and relatives to additional risk. Appropriate PPE should be provided by employers and properly stored and disposed of, and workers should be trained in its use and disposal. The employer should provide the possibility for storing work clothing and street clothing separately. Specific measures should be set out for culling operations and handling dead animals and waste.

When there is a suspected outbreak, the workplace risk assessment should be revised, taking into account all occupational risks, including the increased physical load on workers from applying additional measures and wearing PPE. Appropriate measures should then be taken. To reduce the risk of infection with zoonotic influenza viruses, workers should be protected from direct physical contact with sick or dead animals or their remains (e.g. sick birds, poultry carcasses, poultry faeces or litter, surfaces and water that might be contaminated with poultry excretions in the case of infected or dead birds, or pigs, bodily fluids and faeces or litter in the case of pigs or wild boar). Preventive measures include limiting the number of workers potentially in contact with infected animals, areas and objects; physically separating and limiting access to contaminated areas; strict avoiding the production of aerosols and dust; enhanced ventilation; specific cleaning and disinfection procedures and, where technical and organisational measures are insufficient, appropriate personal protective equipment (PPE). It must be ensured that potentially contaminated areas are separated from clean areas (black-white areas) [41,54,56]. Working and living areas should be strictly separated. For example, if seasonal workers live at the farm premises, it should be possible for them to decontaminate themselves before entering the accommodation. Good hand hygiene practice (proper use of gloves, hand-washing, and waterless hand sanitisers) is important and helps prevent infections and keep the virus from spreading. The employer should provide facilities for hand washing and appropriate disinfection products and ensure decontamination where there is a risk of infection. Showering at the worksite or at a nearby decontamination station at the end of the work shift and leaving all contaminated clothing and equipment at work should be recommended and facilitated by the employer. Workers must receive training and instructions on appropriate working practices, the application of the technical, organisational and decontamination measures, the use of PPE, and how to put on, use, take off, and dispose of it. Employers should ensure that there are proper waste storage and disposal facilities in closed containers. Workers should be informed of any emergency measures and know to whom they should report any incident that could cause the spread of the virus. Workers should follow the employer's health and safety rules, employ preventive measures and wear or use all the required equipment.

If self-isolation is advised, employers should set out clear procedures clarifying how workers should self-isolate, the duration of isolation and any health surveillance they should undergo. Employers should seek advice from the occupational health services and/or occupational physician on any measures and also recommend health surveillance for any other potentially infected workers. Workers should be able to follow public health recommendations for self-isolation and subsequently return to work safely. The confidentiality of sensitive medical data should be respected and maintained. If a worker is found to be suffering from an infection or illness as a result of exposure, health surveillance should be offered to other workers.

Employers of workers exposed to biological agents must keep a list of workers exposed to group 3 and/or 4 biological agents for a minimum of 10 years following exposure (or 40 years following exposure resulting in an infection), indicating the type of work done and the biological agent to which they were exposed [56]. Highly pathogenic avian influenza viruses HPAIV (H5), e.g. H5N1, highly pathogenic avian influenza viruses (H7), e.g. H7N7, H7N9, Influenza A virus A/New York/1/18 (H1N1) (Spanish flu 1918), Influenza A virus A/Singapore/1/57 (H2N2), and Low Pathogenic Avian Influenza Virus (LPAI) H7N9 are currently classified in group 3. Laboratories carrying out work involving group 2, 3 or 4 biological agents for research (e.g. those carrying out sequencing of the virus) must determine the relevant containment measures in order to minimise the risk of infection.

Specific measures apply to workers in other professions at risk, for instance those working in abattoirs or involved in culling, those working at veterinary practices, and healthcare professionals dealing with infected patients. The principles of occupational health and safety prevention also apply to these professionals and similar preventive measures should be implemented by their employers. In the event of an outbreak, the occupational safety and health authorities may be consulted on the appropriate preventive measures to take. Ideally, occupational safety and health authorities should be notified of any such outbreaks – e.g. by the public health authorities.

5. Testing and detection systems for zoonotic influenza virus infections in humans

Molecular/nucleic acid-based tests

Reverse Transcription Polymerase chain reaction (RT-PCR) enables a highly sensitive and rapid molecular detection of zoonotic influenza viral genomes. The RT-PCR enables template viral RNA to be reverse transcribed, producing complementary DNA (cDNA), before being amplified for detection. However, the two-step approach has now been largely replaced by the preferred molecular detection tests for influenza A virus using real-time RT-PCR. This is a modification to the RT-PCR that reduces the time required to identify both virus subtype and pathotypes [58]. This method can be used directly on clinical samples, reducing the risk of contamination. It also shortens the laboratory turn-around time, providing rapid results that can greatly facilitate the investigation of respiratory illness outbreaks [52].

The approach to diagnosis using real-time RT-PCR adopted in most laboratories has been based on initial generic detection of influenza viruses in clinical samples, primarily by targeting internal virus proteins such as the M1 matrix protein. These proteins are highly conserved in influenza viruses and are therefore useful targets for the differentiation of type A and type B. Influenza A subtypes are defined by their surface HA and NA proteins, which is why primers and probes that specifically target the corresponding genes are effective for determining the subtype of these viruses.

Loop-mediated isothermal amplification is an excellent, specific and sensitive one-step amplification method for detection of influenza. Its fundamental property is that it does not require a specific machine for processing, as is the case for real-time PCR and conventional PCR for amplification. An RT-LAMP method has been developed for specific evaluation and subtyping of influenza viruses, using two sets of forward primer and two sets of inner primers. One set of primers recognises the outer region and the other set recognises the inner region, giving a result with 93.8% specificity [59].

Since genetic sequences differ among the various subtypes of zoonotic influenza viruses, it is necessary to obtain or design PCR primers and probes that will specifically detect the influenza subtype of interest.

If a zoonotic influenza virus is suspected, and regular subtyping is inconclusive for seasonal influenza viruses (despite low Ct values in the M-gene PCR confirming type A virus), specific testing should be initiated. In general, influenza viruses with inconclusive or unknown virus subtype should be shared with national influenza centres (NICs) and the WHO Collaborating Centres (WHO-CC) for further virus characterisation analysis.

In an outbreak setting, specimens should be directly and specifically tested for relevant zoonotic influenza viruses, particularly if preliminary rapid tests show type A virus infection.

Avian influenza

Specific primer pairs and probes are designed based on the known HA and NA sequences of avian influenza viruses (H5N1, H7N9, H7N7, etc.) and will specifically amplify the targeted sequence of only one subtype. The two zoonotic H9 lineages, Y280 and G1, responsible for several human cases in Asia and Africa, are not currently circulating in the poultry population in Europe and therefore do not represent a risk to humans in the EU at present. H9 is relevant in countries where H9 is present in the poultry population in both commercial and non-commercial settings.

Newer rapid methods have been developed that enable simultaneous detection and subtyping, allowing rapid identification of an influenza A virus using arrays [60] or microchip [61] technologies. The validated Eurasian real-time RT-PCR tests have proven valuable in the investigation of many H5Nx HPAI clinical samples and other subtypes submitted to international reference laboratories from Europe, Africa, Asia and North America since 2005 [62,63].

The loop-mediated isothermal amplification (LAMP) system for H5 and H7 detection has shown high sensitivity and reliable specificity but may have limited application because of susceptibility to viral mutations, affecting the target regions and reducing virus detection [64].

Results of PCRs for avian influenza can be obtained within under three hours in a functioning diagnostic laboratory and hence the technique is extremely useful for rapid diagnosis, especially during an outbreak. The PCR can detect the presence of avian influenza in both live and dead viruses, which is why its sensitivity is 2−13% higher than that of cell culture since cell culture can only detect live viruses [51].

Swine influenza

Swine influenza viruses that circulate in pig populations and sporadically transmit to and infect humans are called 'variant' viruses and a 'v' is added to the virus subtype to identify this as a zoonotic virus (H1N1v, H1N2v, H3N2v). Subtyping is carried out targeting the HA gene and using specific primers and probes that are designed based on the known HA sequences of swine influenza viruses. These primers and probes amplify the targeted sequence of one specific subtype. The challenge in determining a swine influenza virus infection is to integrate seasonal influenza virus gene segments into the circulating swine influenza viruses. PCR systems may detect seasonal A(H1N1)pdm09 components (e.g. real-time PCR assays developed for detection of the A(H1N1)pdm09 and swine H1 viruses, targeting a conserved region in the HA gene of the viruses can discriminate the (H1N1)pdm09 and swine H1 viruses from other subtypes and seasonal viruses). However they do not differentiate between (H1N1)pdm09 and swine H1 viruses [65]. Diagnostic real-time RT-PCR for generic influenza A virus (targeting the M-gene) will detect swine-origin triple reassortant A(H3N2) viruses as human influenza A. However, the subtyping reaction would probably have a decreased sensitivity or would not detect the triple reassortant SIV A(H3N2) viruses when using primers and probes for H3 or N2 of human influenza A viruses. Probes directed against other genes - e.g. the nucleoprotein gene - could enable preliminary differentiation between human seasonal H3N2 viruses and the zoonotic H3N2 viruses.

Distinguishing between swine-specific and seasonal A(H1N1)pdm09 and A(H3N2) viruses is extremely challenging, and not always possible. Depending on the origin of the surface genes HA or NA, seasonal A(H1N1)pdm09 and A(H3N2) primers/probes either cannot distinguish between swine and seasonal influenza, or have a decreased sensitivity and are unable to detect them. If there is a suspicion of swine influenza as a result of exposure and/or symptomatology, primary diagnostic laboratories that do not have specific primers/probes for subtyping should send the sample to the NIC for typing or sequencing. The HA and NA gene sequencing would provide more robust information, particularly for swine influenza differentiation. If a case is confirmed as positive for swine influenza, samples should be shared with WHO-CC for further antigenic and genetic characterisation.

Rapid antigenic diagnostic test (RADT) or point-of-care test (POCT)

Rapid virological diagnosis is important for early case identification, initiation of specific antiviral therapy and implementation of infection control measures for patients suffering from influenza. Rapid antigenic diagnostic tests (RADT) or point-of-care tests (POCT) have been designed to be performed under field conditions, in outpatient settings by non-laboratory-trained persons, without requiring BSL-2 or BSL-3 facilities. Most POC test kits provide results within 15 to 30 minutes and are based on antibodies that specifically bind to the influenza virus nucleoprotein [66]. Usually these test kits consist of a colorimetric strip which, upon incubation with a colour-generating substrate, displays the presence of the viral antigens as a specific colour band.

Commercially available RADTs can either a) detect and distinguish between influenza A and B viruses without distinguishing the subtype ((H1N1)pdm09 or H3N2 for seasonal viruses) or lineage (B/Victoria or B/Yamagata); b) detect both influenza A and B but not distinguish between influenza A and B viruses or c) detect only influenza A viruses. None of these tests can differentiate between human or zoonotic influenza virus subtypes.

Few comparisons of RADTs with RT-PCR for the detection of influenza A virus have been published. In a study with a cohort of 1 023 patients, it was observed that the sensitivity and specificity of the RADT were 79.4% and 97.2%, respectively [67]. In another study performed during the 2009 influenza A(H1N1)pdm09 outbreak, a comparison was made between RADT, rapid chromatographic immunoassay and Direct Immuno-Fluorescence assay (DFA) test with real-time PCR for detection of swine origin influenza virus (S-OIV). The sensitivities of the rapid influenza tests, the DFA test and RT-PCR [68]were 9.7%, 20.6%, and 32.35% respectively and the specificities were 98.2%, 99% and 99% respectively [68]. Although limited by small numbers, when published side-by-side, comparisons of RADTs to detect influenza A (H1N1) and seasonal influenza A viruses suggest that the sensitivity of RADTs to detect novel influenza A (H1N1) virus is equal to or lower than the sensitivity to detect seasonal influenza viruses [69,70]. Although RADT can differentiate between influenza A and influenza B, they cannot differentiate between subtypes of influenza A which makes them less favourable, since further testing is required for confirmation. Therefore, it is very important to understand the limitations of RADTs in order to appropriately interpret results for clinical management [71].

The rapid testing methods for avian influenza only have a limited validity for the detection of human cases of avian influenza and may result in false positive and false negative results, which is why they are not recommended for the routine detection of avian influenza viruses [51].

However, RADTs could be useful in an outbreak setting for rapid diagnostics, and initial rapid assessment to initiate treatment and isolation of suspected cases, and also to trigger additional control and diagnostic measures in outbreaks [51].

Serology

Serological tests for zoonotic influenza have limitations: they only provide information on historical exposure to influenza viruses rather than viral genetic information which is vital for evaluating the potential pandemic threat of strains, and cross-reactions can occur between different lineages within one subtype, or even among different subtypes [72]. The haemagglutination inhibition (HI) assay is widely regarded as a reliable method for the detection of antibodies to influenza viruses [73]. In mechanical terms, the assay relies on the inhibition of the interaction between the viral hemagglutinin (HA) glycoprotein and sialic acid receptors on the surface of red blood cells (RBCs) by antibodies which are directed against the HA receptor binding pocket [74].

The HI assay is used to check the virus antigenic type, the subtype classification specificity of antibodies for hemagglutinin subtypes and to confirm an influenza infection [75,76]. HI antibody titres in serum peak 2–3 weeks after an infection and begin to decline after 3–6 months. Paired sera, collected at the time of the presumed infection (acute serum) and approximately three weeks later (convalescent serum), are needed for the serological diagnosis of influenza virus infection. If there has been a recent infection with a given influenza virus subtype, the convalescent sera will show rising antibody titres to that subtype.

Serological testing can be applied in the context of outbreaks and follow-up studies to evaluate the seroconversion upon exposure and support overall risk assessment for zoonotic transmission.

Avian influenza

Serological tests are a simple and inexpensive technique using standard laboratory equipment. The haemagglutination inhibition (HI) test can be used to identify avian influenza virus subtypes, as well as measuring HA-specific antibodies to the virus [77]. The HI test can therefore be used to type the patient antibodies to avian influenza virus when a standard homologous or antigenically correlate avian influenza antigen is available to be used as reference material. The sensitivity of the HI test for the detection of antibodies against avian influenza viruses in human sera can be improved by replacing avian RBCs with equine RBCs in the test [78,79]. In an outbreak situation, information on seropositivity is important for managing the epidemiological situation [51]. The main objective for using the HI test is to detect antibodies and confirm seroconversion following exposure. Additionally, the HI can be used to determine the antigenic characteristics of influenza viral isolates and is a standard technique in the animal health sector for surveillance and monitoring purposes, although it is not routinely applied in public health.

The immunofluorescence test is a rapid and sensitive method for directly detecting the presence of avian influenza antigens in clinical samples. The infected cells from the clinical samples are fixed to a slide and the viral antigen is revealed by combining them with avian-specific monoclonal antibodies that may be either directly tagged with a fluorescent dye (direct test) or made to interact with a second anti-antibodies tagged with the fluorescent dye (indirect test). The fluorescence is visualised under a microscope [51]. Compared to cell culture, the immunofluorescence test has a sensitivity of 70–100% and a specificity of between 80 and 100%. Immunofluorescence is the test of choice when fairly rapid results are required as the results can be available within one day [51].

The microneutralisation assay is a highly sensitive and specific assay for detecting virus-specific neutralising antibodies to influenza viruses in human and animal sera, including the detection of virus-specific antibodies to avian influenza A(H5N1) virus in human serum, and the potential detection of antibodies to other avian subtypes [52]. Under field conditions, the micro-neutralisation test has detected an H5-specific antibody in human serum at titres that could not be detected by the conventional hemagglutination inhibition assay [51]. The results of neutralisation may be obtained within three days. However, because of the live nature of the influenza virus used, only BSL-3 laboratories with cell culture facilities can undertake such testing [51]. The microneutralisation assay has several advantages for detecting antibodies to influenza virus. First of all, it primarily detects antibodies to the influenza viral HA protein and thus can identify functional strain-specific antibodies in human and animal sera. Secondly, since an infectious virus is used, the assay can be carried out quickly once the emergence of a novel virus is recognised [52].

Swine influenza

The haemagglutination inhibition (HI) test is the most widely used serological assay to detect anti-influenza antibodies in swine sera in the animal health sector.

The HI test is highly subtype-specific and separate tests must be performed with H1N1, H3N2 and H1N2 strains as antigens. ELISA (enzyme-linked immunosorbent assay) is a very specific and sensitive method for influenza detection. A sandwich ELISA assay was developed during the 2009 A(H1N1)pdm09 influenza pandemic, using specific monoclonal antibodies and horseradish-peroxidase-linked rabbit anti-HA polyclonal antibodies. The overall sensitivity of the ELISA (0.57) was higher than the RADT test (0.43) [80]. In a different study, 1 086 sera were analysed from 43 swine herds for different reference strains (H1N1, H3N2, H1N2, H1N1pdm) using ELISA and the HI test and it was found that ELISA sensitivity and specificity was higher than HI: 72.65% and 63.01%, respectively [81]. However, ELISA cannot detect infection at an early stage, which is why a new ELISA-based immunosensor was developed using AuNP (gold nanoparticles) for improved sensitivity.

One study that investigated the seroprevalence of swine influenza viruses among people with occupational exposure to pigs found serological evidence of swH1N1 transmission from pigs to swine workers, and suggested that anti-swH1N1 antibodies have induced cross-protective immunity against the H1N1pdm virus [82].

Due to retro-zoonotic events of A(H1N1)pdm09 viruses transmitting from humans to swine since 2009 and the reassortment of seasonal and swine influenza viruses with highly-diverse reassorted influenza viruses circulating in the pig population in Europe, it is nearly impossible to distinguish between swine-specific and seasonal influenza viruses using serological assays.

Sequencing including Whole Genome Sequencing (WGS)

Over the last decade, the data from WGS of pathogenic microorganisms has played a key role in detecting and managing epidemics of infectious diseases, as well as supporting the development of diagnostic tests and vaccines. Given that unit costs are being reduced with improvements in technology, greater use of sequencing technologies should offer powerful opportunities to simultaneously detect and sequence from clinical samples in a laboratory or field setting (for example by applying nanopore technology) [83]. Sanger sequencing methodology, which typically enables the rapid determination of a single (HA) target gene in 24–36 hours to define virus pathogenicity, has been widely used for decades, and still has widespread utility. However, as genomic data can be rapidly determined using next generation sequencing technology, this allows a broader analysis using a range of bioinformatics tools [84]. The nucleotide-level resolution of in-depth WGS permits phylogenetic analysis and molecular epidemiology studies to achieve a detailed understanding of the outbreak [85]. Translation of nucleotide sequences of all genomic segments into amino acid sequences enables data mining for other virus characteristics, or traits such as tropism and host range markers, including zoonotic and predicted antiviral drug susceptibility. Such data are invaluable for informing outbreak management [58].

The genomes of influenza viruses frequently undergo genetic changes and have a high level of genetic diversity, which may lead to the emergence of zoonotic influenza viruses. To cope with the emergence of new influenza virus strains and to select the appropriate vaccine strains or establish preventive measures, considerable effort is being devoted to monitoring the genetic rearrangements of these viruses [86]. WGS technology is used in a wide range of applications, including population surveillance for emerging strains and the subsequent creation of new vaccines and outbreak research in communities [87]. WGS second-generation devices produce high-quality data but are cost intensive, based on stationary technology, requiring time and resources. Amplicon sequencing using NGS platforms, such as the Illumina platform and Ion Torrent technology, has high initial and running costs and is time-consuming, however the establishment of a rapid, portable, more affordable, real-time third-generation sequencing approach can improve outbreak response and WGS availability.

Due to the broad diversity of zoonotic influenza viruses, surveillance using full genomic evaluation has become indispensable. In an outbreak scenario WGS should be used to identify potentially emerging zoonotic influenza viruses [88]. Those countries with available capacities and resources should use WGS to identify emerging zoonotic influenza viruses as sequencing is the most reliable way of distinguishing between swine and seasonal strains. The availability and increasing use of WGS for routine diagnostics may support the identification of zoonotic transmission events. Laboratories are strongly encouraged to apply these methods when influenza viruses cannot be typed or subtyped with the usual methods.

It is of the utmost importance that clinicians are aware of the need to test patients with respiratory illnesses for influenza virus infection and perform virus characterisation or whole genome sequencing, at least of severe cases, in order to identify clinical cases, initiate follow-up investigations and detect human-to-human transmission.

All generated sequence information should be shared and uploaded to the GenBank public database or GISAID sequence platform in a timely manner, and possibly also sent to the European Nucleotide Archive (ENA).

Laboratory confirmation

Suspected cases of zoonotic influenza virus infection require laboratory confirmation by specific PCR and, if possible, sequence generation. Specimens should be shared with NICs and WHO-CC for further characterisation.

If a human infection with a zoonotic or novel influenza virus is detected, it is mandatory to notify EWRS and WHO within 24 hours in accordance with the Implementing Regulation (EU) 2020/690 [89] and the International Health Regulations (IHR). Case definitions for avian influenza virus H5 and H7 infections in humans have been developed [90].

According to WHO guidelines[1], all laboratory-confirmed positive results for influenza A/H5, H7 or H9 should be further tested and confirmed by a WHO H5 Reference Laboratory or a WHO-recommended laboratory. Influenza A/H5, H7 or H9 -positive materials, including human specimens, RNA extracts from human specimens, and influenza A/H5, H7 or H9 virus in cell-culture fluid or egg allantoic fluid should be forwarded to a WHO H5 Reference Laboratory or a WHO-recommended laboratory. However, it should be noted that zoonotic influenza viruses are not limited to these subtypes. If a laboratory lacks the capacity to perform specific influenza A subtype identification procedures, it should forward the specimens/virus isolates to a national influenza centre[2] or a WHO H5 Reference Laboratory. Information about the sequence should be provided to the GISAID sequence platform, GenBank or ENA.

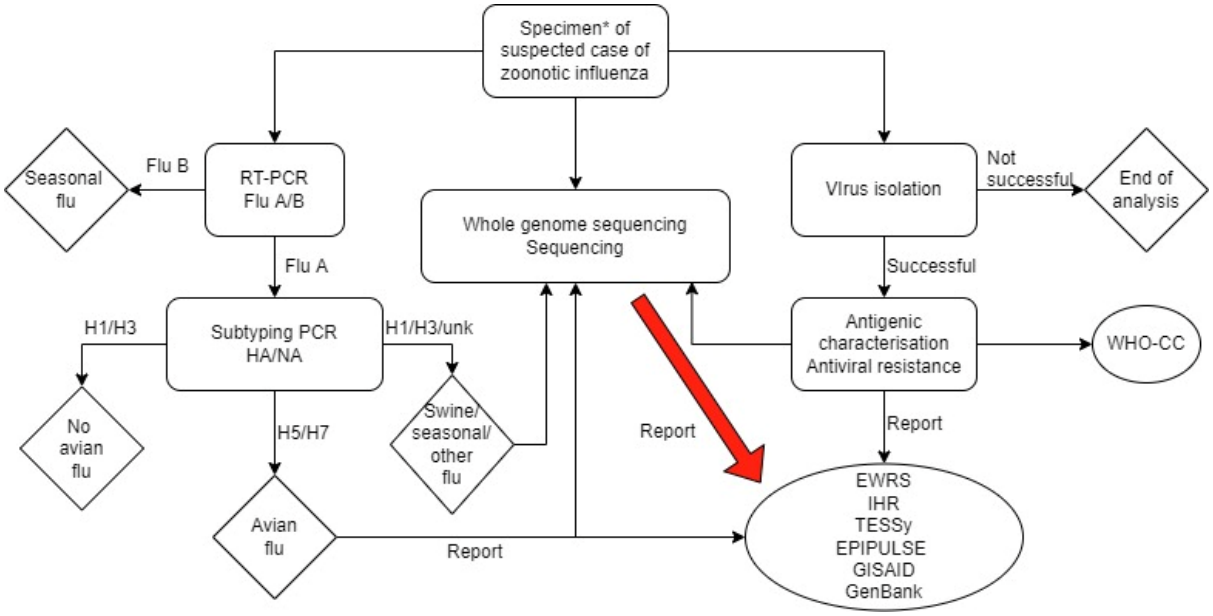
WHO has produced guidance describing the communication and publication of analysis results on how to share influenza viruses/specimens with potential to cause human influenza pandemics[3].

[1] WHO Global Influenza Preparedness Plan

[2] National influenza centres: https://www.who.int/initiatives/global-influenza-surveillance-and-response-system/national-influenza-centres

[3] http://www.who.int/csr/disease/avian_influenza/guidelines/Guidance_sharing_viruses_specimens/en/index.html Virus sharing (who.int) and Operational Guidance to National Influenza Centres and H5 Reference Laboratories of the Global Influenza Surveillance and Response System (GISRS) and other Nationally Authorized Laboratories for Sharing Influenza Viruses with Pandemic Potential (IVPP) with WHO Collaborating Centres of GISRS under the Pandemic Influenza Preparedness Framework

Figure 1. Flow chart displaying the testing procedures to confirm a case of zoonotic influenza



** Specimen:*

- *oropharyngeal swabs, bronchoalveolar washes, conjunctival washes or tracheal aspirates for molecular detection and cell culture*
- *acute-phase serum specimen and convalescent serum specimen for serology*
- *Suspected case see considerations for the identification above.*

6. Other zoonotic influenza viruses

Influenza viruses have a large host spectrum and are able to infect, circulate among and transmit between many different species such as seals, bats, horses, mink, dogs, cats, tigers, lions, foxes and other mammals.

Equine influenza is caused by two different subtypes of influenza type A viruses, H7N7 commonly known as equine 1, and H3N8 known as equine 2 [91]. Genetic analysis has indicated that the H3N8 strain is more closely related to avian influenza viruses than to other equine influenza viruses [92]. Moreover, interspecies transmission of the equine influenza has been reported in an unusual variety of hosts. Until 2004, when a canine influenza outbreak emerged, canines were not considered as a possible host for equine influenza [93]. The outbreak was caused by an evolved A(H3N8) strain, known for causing mild clinical symptoms in equines [90]. Furthermore, equine influenza virus has also been isolated from racing greyhounds in the USA [94] and Bactrian camels in Mongolia [95]. Although, there are very few reports of equine influenza infection in humans, it is considered that the most probable cause for the human pandemic in 1889 was due to the involvement of H3N8 equine influenza [96].

Influenza type A viruses of multiple subtypes including H3N3, H4N5, H4N6, H7N7 and H10N7 have been isolated from seals, H1N3 and H13N2 from whales and H10N7 from mink [97,98]. These infections cause mortality in the infected animals, notably H7N7 in seals, and have also caused conjunctivitis in humans in close contact with the seals. The available evidence indicates that these influenza viruses originate from aquatic birds, and may cause localised disease outbreaks, but have not established permanent lineages in seals, whales or mink. A study has shown that harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) can also be infected with influenza B [6]. As yet, no evidence has been found of influenza B virus being transmitted from seals to humans [6].

Avian influenza virus infections in cats have rarely been documented [99,100]. In 2016, an A(H7N2) outbreak in cats at a New York city shelter was reported [101]. More importantly, the first cat-to-human transmission was detected, with the patient having mild symptoms (sore throat, myalgia and cough).

Mink have previously been known to be susceptible to influenza virus infection [102,103]. Since 1984, several influenza virus subtypes, such as H10N4, H3N2, swH3N2/pH1N1, H1N2 and H9N2 have been isolated from mink [104-109]. Since both alpha-2,3 sialic acid receptors (SAα2,3-Gal) and alpha-2,6 sialic acid receptors (SAα2,6-Gal) are detected in the respiratory tract of mink, mink could serve as intermediary influenza virus hosts between poultry and humans [108]. Sun et al. found that farmed mink in China were commonly infected with avian and human influenza viruses, of which human A (H1N1)09pdm and avian H9N2 influenza virus infections were the most prevalent [110]. Furthermore, in 2015 two H5N1 outbreaks were reported in mink establishments, 200 km apart, in

north-east China [111]. The high seroprevalence of combined avian and human influenza viruses suggests a strong likelihood of co-infections and thus, farmed mink could serve as 'mixing vessels' for the generation of novel reassortant viruses, as is the case for pigs [110]. Several studies have shown that mink are susceptible to circulating avian (H7N9, H5N6, and H9N2), human (H3N2 and H1N1/09pdm) and equine influenza A viruses [102,105,110,112,113].

7. Reporting human cases with zoonotic influenza virus infection

Early Warning Response System

Under EU legislation, human infections with avian influenza viruses are notifiable within 24 hours through the Early Warning and Response System (EWRS) in accordance with EU Decision 1082/2013/EU [114].

International Health Regulations

Human cases of zoonotic influenza virus infection must be reported through the International Health Regulations (IHR) notification system [115] 'Each State Party shall notify WHO, by the most efficient means of communication available, by way of the National IHR Focal Point, and within 24 hours of assessment of public health information, of all events that may constitute a public health emergency of international concern within its territory according to the decision instrument, and any health measure implemented in response to those events.'

The European Surveillance System

ECDC has indicator-based surveillance reporting and countries should report to the European Surveillance System (TESSy) the number of tested individuals and the number of confirmed zoonotic influenza virus cases by different subtype in aggregated and standardised case-based format.

EpiPulse

EpiPulse allows countries to share and update epidemiological, microbiological and any additional information on influenza strains. Although confirmed and unconfirmed cases, as well as the number of confirmed deaths, can be reported, the sharing of these data in EpiPulse does not replace the official reporting to TESSy and the mandatory notification through EWRS.

Sharing of sequences

The timely characterisation of viruses and the sharing of sequence information are crucial for the monitoring of virus evolution and for virus vaccine development. The sharing of sequence data through the GISAID EpiFlu (GISAID, online), or other sequence databases, and of virus isolates with WHO Collaborating Centres is important for public health assessment, improvement of diagnostics and the development of candidate vaccines. Influenza viruses can be shared through the Global Influenza Surveillance and Response System (GISRS) or GenBank. Sharing of sequences with ENA is also encouraged.

8. Monitoring of influenza virus infections in animals

Avian influenza

Surveillance programmes for avian influenza have been compulsory in all EU Member States since 2003 and are essential for the early detection of avian influenza viruses that may pose a risk to animal and/or human health.

These surveillance programmes must comply with guidelines laid down by the European Commission in accordance with the relevant EU legislative frameworks. In particular, Council Directive 2005/94/EC [116] and Commission Decision 2010/367/EU [117] were recently unified under the umbrella of Regulation (EU) 2016/429 on transmissible animal diseases (so-called Animal Health Law) and related implementation acts, representing the first unified European framework for animal diseases that can be transmitted to other animals or humans. To supplement the rules laid down in the Animal Health Law, the Commission has developed and adopted a Delegated Regulation, laying down rules for the prevention and control of certain diseases, including HPAI.

According to Annex II to Delegated Regulation (EU) 2020/689, the primary objectives of surveillance programmes for AI are a) the early detection of HPAI viruses infections in poultry and wild birds, b) the detection of HPAI in poultry species which generally do not show significant clinical signs, c) the detection of LPAI viruses infections that have the potential to easily spread in areas with a high density of poultry, possibly leading to mutation into highly pathogenic forms, and d) the increase of knowledge on HPAI and LPAI viruses posing a potential zoonotic risk. The Regulation (EU) 2020/689 also lays down the general rules for the sampling and laboratory testing methods of the surveillance programmes for AI. An overview of the AI surveillance programmes in poultry and wild birds and the related sampling and laboratory testing methods set out in Regulation (EU) 2020/689 are described below.

Surveillance in poultry

The early detection system shall as a minimum include the investigation of any clinical sign or post-mortem lesion suggesting HPAI and any change in normal production and health parameters (e.g. increase in mortality rate, drop in feed and water intake or drop in egg production) at poultry establishments, particularly those located in areas identified as being at high risk of HPAI introduction and spread. In high-risk areas, regular testing of samples collected from dead or sick poultry may also be relevant when an increased risk has been identified at national, EU or regional level due to HPAIV having been detected in poultry and/or wild birds. These measures must apply to all poultry populations. Complementary surveillance activities must apply to poultry species that generally do not display significant clinical signs (e.g. ducks, geese and quails) and these must be risk-based. This means that they should account for all the relevant factors that could increase the likelihood of HPAI introduction, including the geographical area and the wildlife present in the area (e.g. proximity of the establishment to water bodies and places where migratory birds may gather, or a period of the year when there is an increase in the migratory flow) and the characteristics of the local production systems (e.g. establishment biosecurity level, trade and movements of poultry, density of establishments). Risk-based surveillance has to be applied to 'poultry establishments for which the competent authority has assessed that clusters of infection with LPAIV have repeatedly occurred in the past or are deemed more likely to occur'. In this case, risk factors to consider are primary related to the housing conditions, species and production type of the farmed birds, and the list of relevant species, in addition to duck, geese and quails, is extended to the *Galliformes* species.

Surveillance in wild birds

The early detection of HPAI in wild birds is based on sampling and testing of birds found dead, injured, sick, or hunted with clinical signs, always following a risk-based approach that considers the relevant information on ornithology, virology, epidemiology, and the environment, to identify 'high-risk areas'. In addition, all suspected episodes of mortality in wild birds must be investigated to exclude HPAI. This risk-based passive surveillance of wild birds is essential to allow the detection of HPAI viruses before viral transmission occurs at poultry establishments and to implement the appropriate control measures. In addition, the risk-based surveillance may include an active component involving the testing of healthy birds that are trapped or hunted and of sentinel birds at key sites (in particular those where wild bird species are entering the European Union during their migratory movements, at least from north-easterly and eastern routes).

A list of the relevant bird species subject to surveillance should as a minimum include wild bird species that have been shown to be at greater risk of being infected and transmitting HPAIV, in particular migratory water birds. This list of 'wild bird targeted species' is available on the EURL website. Additional wild bird species may be included depending on their specific epidemiological relevance in the territory of the Member State under assessment.

Sampling and laboratory testing methods

The number of poultry establishments to be sampled and the number of birds to be tested per establishment has to be defined based on a statistically valid sampling method, where the frequency for sampling and testing is based on risk assessments. The time period for sampling must ideally coincide with seasonal production for each production category and must take into account the identified high risk period for HPAIV.

With regard to testing methods, virological methods must be used when the aim is a) the early detection of HPAI in poultry and wild birds, b) the follow up of sero-positive findings in case of detection of LPAIV clusters in poultry establishments and c) complementary surveillance for HPAI in poultry species which generally do not show significant clinical signs of HPAI. In this last case, serological methods can also be applied as relevant. Serological methods must be applied when aiming to detect clusters of LPAIV-infected establishments, except where there are justified reasons to opt for virological testing.

EURL for Avian Influenza

Since January 2019, the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) in Italy has become the European Union Reference Laboratory (EURL) for Avian Influenza [118]. Its aim is to actively assist the National Reference Laboratories (NRLs) in the diagnosis of HPAI influenza outbreaks, by carrying out confirmatory diagnosis, and to ensure that NRLs are correctly implementing harmonised and up-to-date diagnostic protocols for AI.

The diagnostic procedures for testing the samples collected within the surveillance programmes are outlined in the Diagnostic Manual for Avian Influenza, as set out in Decision 2006/437/EC, and this remains technically valid even since the entry into force of the 'Animal Health Law' [119]. A recent EFSA opinion [120] reviewed the effectiveness of clinical and laboratory sampling procedures, and evaluated the sampling procedures described in the Diagnostic Manual. It outlines that while sampling and diagnostic procedures can be considered efficient for gallinaceous poultry, additional sampling is advised for *Anseriform.* Specific guidelines are provided for the application of a weekly pool sampling (known as 'bucket sampling') in poultry species that may not manifest clinical signs when infected with HPAI. On the EURL IZSVe website, a document is available summarising information on the collection of samples, tissue material to be examined and transport of samples, as well as detailed procedures for carrying out the diagnostic methods recommended by the EURL IZSVe [119].

Reporting and notification to the European Commission

According to Article 9 of the Implementing Regulation (EU) 2020/2002 [121], the national competent authorities must submit their surveillance programme to the European Commission by 31 May of the year preceding the year in which they start an application for Union funding. Moreover, according to Article 6, data on the results of implementing the European Union's surveillance programmes shall be reported by 15 March each year. Since January 2019, EFSA has been collating all data related to the avian influenza surveillance activities taking place in the Member States in a harmonised manner.

Irrespective of the planned surveillance programmes, each primary outbreak/detection of HPAI in poultry and non-poultry bird species must be notified to the Animal Disease Information System (ADIS) (ex ADNS), an online tool designed to help national competent authorities to register the evolution of the situation concerning relevant infectious animal diseases [122] within 24 hours of confirmation. Secondary outbreaks should be notified at the latest on the first working day of each week covering the previous week. According to Article 14 of the Delegated Regulation (EU) 2020/687, in the event of an HPAI outbreak at a poultry establishment, sampling procedures may be established by the competent authority for the animal species being kept that differ to the procedures for birds. The minimum information to be provided in ADIS is listed in Annex II of the Implementing Regulation (EU) 2020/2002, and includes details on the region and geographical location of the outbreak, the type of outbreak (primary/secondary), date of suspicion and confirmation, diagnostic method used, control measures taken, origin of the disease, affected bird population (poultry or non-poultry, and in case of wild birds the bird species is also requested).

Surveillance and monitoring reports on avian influenza

The European Food Safety Authority (EFSA) supports Member States in data collection and surveillance activities on avian influenza and produces annual reports on surveillance for avian influenza in poultry and wild birds in the Member States, as well as quarterly overviews of avian influenza virus detections in poultry, captive birds, wild birds and humans, in collaboration with ECDC and the EURL for Avian Influenza.

Annual reports on avian influenza surveillance

Since January 2019, EFSA has been requested by the European Commission to 'collate, validate, analyse and summarise in an annual report the results from avian influenza surveillance carried out by Member States in poultry and wild birds' [122]. In its annual reports, EFSA summarises the results of (a) serological surveys to monitor the circulation of avian influenza virus subtypes H5 and H7 in poultry (i.e. active surveillance in poultry), (b) virological detection of avian influenza in wild birds found dead or moribund (i.e. passive surveillance in wild birds) and, for Member States providing these data on a voluntary basis, (c) active surveillance on live and hunted wild birds.

Quarterly reports on avian influenza monitoring

Quarterly reports are produced by EFSA in collaboration with ECDC and the EURL, providing an overview of the development of the avian influenza situation in the EU and worldwide. The reports describe HPAI detections in poultry, captive and wild birds, the relevant outbreaks of LPAI virus in poultry and captive birds, the genetic characterisation of avian influenza viruses, and the human cases reported in the period. The description of the detection of avian influenza in poultry and wild birds is based on the data submitted to ADIS by Member States, data submitted to the WOAH-WAHIS database for non-EU countries, and additional data on poultry outbreaks collected by EFSA (e.g. poultry animal species, production type, type of farming, presence of outdoor access, type of surveillance through which the case has been identified and signs identified in the species involved). EURL provides information on the genetic characteristics of avian influenza viruses based on the virus genomic sequences available from GISAID, which are provided by the National Reference Laboratories (NRL) or directly generated by EURL. ECDC provides information on cases reported in humans. In addition, information available on HPAI cases detected in other mammals is retrieved and reported. EFSA's working group of experts on avian influenza uses the data collected to assess the status of risk for the poultry industry, based on the circulation of HPAI viruses in migratory birds, the domestic sector and the environment, and suggests appropriate options for response. Meanwhile, ECDC assesses the risk for the general public and for those exposed to HPAI viruses, occupationally or otherwise, in the EU/EEA.

Swine influenza

There is no routine surveillance in place for swine influenza in pig populations in Europe. Surveillance is solely based on individual national or supranational initiatives to review the situation and assess available swine influenza vaccines [123-125].

9. 'One-Health' approach

Human sector laboratories are encouraged to work more closely with animal health laboratories, as they could profit from their experience and knowledge in diagnosing zoonotic influenza viruses. This would guarantee a more efficient system, while increasing collaboration in a 'One-Health' framework.

The strengthening of the 'One-Health' approach and sustainable development goals to link environment, animal and human health are crucial for the early prevention of health risks.

10. Conclusions and potential implications

Zoonotic influenza viruses remain a concern for human health in Europe. Reassortment events relating to swine, avian and human viruses should be monitored carefully and any transmission to humans should be identified as early as possible to prevent further spread. It is of the utmost important that public health experts and clinicians are aware of the need to test symptomatic patients with occupational exposure or contact with a confirmed case of zoonotic influenza in order to identify clinical cases and detect human-to-human transmission early on.

During an outbreak of zoonotic influenza, workers are likely to be in the front line, along with those working in professions involving animal contact. However, laboratory workers, healthcare personnel or those involved in the culling and disposal of animal bodies are also at risk. It should be noted that there is a legal framework governing occupational risks, setting out the obligations of employers in order to prevent such infection, irrespective of whether there is an outbreak. These obligations include technical, organisational, maintenance and hygiene measures, such as the avoidance of aerosol and dust, the separation of work and street clothing, ventilation and, as a last resort, if the other measures are insufficient, the wearing of PPE. Occupational safety and health measures need to be taken if workers could be exposed to zoonotic influenza viruses and specific measures must be applied when cases are identified in an occupational setting.

Employers should periodically revise their workplace risk assessment and ensure that all necessary technical and organisational measures are taken to avoid the infection of workers. Workers should be offered health surveillance, which should be enhanced following potential exposure and the offer of surveillance should also be extended to other workers who may be exposed. Employers should also consult their occupational health services or health and safety committee and set and adapt the measures in consultation with workers or their representatives. In the event of an infection, work sites need to be decontaminated, and procedures should be set out for this in advance. As the zoonotic influenza virus subtypes described here are classified as Group 3 biological agents, employers need to keep a record of exposed workers in accordance with occupational safety and health regulations. Occupational safety and health authorities may be consulted on the preventive and control measures, including those taken in the event of an outbreak, and the measures may be checked by the occupational health and safety authorities in collaboration with animal and public health authorities. Specific measures should be implemented for the treatment of patients, analysis of specimens, culling of animals or disposal of waste. Workers who may have been exposed and infected should inform their healthcare providers, so that measures can be taken to protect them from infection.

Successful influenza virus diagnosis depends largely upon the quality of the specimen and its timely collection. Specimens for isolation of influenza viruses in cell culture and direct molecular detection should ideally be collected within three days of the onset of clinical symptoms. If the specimens are collected later than seven days after symptom onset, the result may be a false negative or the material may not be suitable for further genetic or antigenic characterisation. In order to maintain the viability of the viruses for longer periods, it is recommended that virus transport media be used. A similar challenge arises when performing virus isolation since it only detects the presence of whole infectious virus, so specimens that have not been properly stored or shipped may yield a false negative result due to loss of viability of the virus. Another challenging aspect for performing virus isolation is that it requires a BSL3 diagnostic laboratory, specialised equipment and trained personnel. For serological testing, an acute-phase serum specimen should be taken promptly after the onset of clinical symptoms, while a convalescent phase serum specimen should be collected 2–4 weeks later.

General influenza detection systems will most probably identify un-subtypeable influenza A viruses in patients with zoonotic influenza if no specific assays are performed. Such specimens need to be shared with reference laboratories (NICs or/and WHO Collaborating Centres) for further analysis and confirmation. Rapid antigen tests used in clinical settings can only detect type A influenza, if suitable at all for the detection of avian influenza virus infections in humans. This adds to the risk of missing a human case if no additional confirmatory test, including subtyping, is performed. In addition, when working with swine influenza, it is very challenging to distinguish between swine-specific and seasonal A(H1N1)pdm09 and A(H3N2) viruses. Therefore, depending on the origin of the surface genes HA or NA, seasonal A(H1N1)pdm09 and A(H3N2) primers/probes either cannot distinguish between swine and seasonal influenza, or have a decreased sensitivity and are unable to detect. The HA and NA gene sequencing or whole genome sequencing can provide more robust information for swine influenza differentiation.

To cope with the emergence of new influenza virus strains and select appropriate vaccine strains or establish preventive measures, considerable effort is being devoted to monitoring the genetic rearrangements of these viruses through WGS. Countries with available capacities and resources should use WGS to identify emerging zoonotic influenza viruses as sequencing is the most robust way to distinguish between zoonotic and seasonal strains. The availability and increasing use of WGS for routine diagnostics may support the identification of zoonotic transmission events. Laboratories are therefore highly encouraged to apply these methods when influenza viruses cannot be typed or subtyped with the usual methods.

In addition, clinicians should take into consideration the possibility of zoonotic influenza in severe cases with an un-typeable influenza A infection and should request virus characterisation and whole genome sequencing of the specimen collected. The recent transmission event of avian H5N1 influenza to humans in the UK in January 2022 and the detection of an H5N1 human case in the US in April 2022 are a strong reminder of the importance of establishing monitoring activities, testing protocols and initiating follow-up investigations to identify human transmission and investigate any possible human-to-human spread though contact-tracing [126,127].

Links to resources and previously published reports

Quarterly joint EFSA, ECDC, EURL avian influenza overview reports: Latest situation update of the avian influenza situation in EU/EEA
Estimation of the number of exposed people during highly pathogenic avian influenza virus outbreaks in EU/EEA countries, October 2016-September 2018.
Protective Measures for Humans against Avian Influenza A(H5N8) Outbreaks in 22 European Union/European Economic Area Countries and Israel, 2016-17.
Highly pathogenic avian influenza A(H5N8) outbreaks: protection and management of exposed people in Europe, 2014–15 and 2016.
ECDC Avian Influenza Preparedness Workshop: slides
ECDC Rapid risk assessment page for avian influenza
ECDC Threats and outbreaks of zoonotic influenza diseases
ECDC Annual Epidemiological Reports on avian influenza
ECDC Zoonotic influenza webpages
ECDC Preparedness information and country preparedness plans
ECDC Facts, factsheets and Q&A

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Disclaimer

All data published in this Guidance are correct to the best of our knowledge at the time of publication. Maps and figures published do not represent a statement on the part of ECDC or its partners on the legal or border status of the countries and territories shown.

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