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Scientific Opinion on the assessment of the control measures of the category A diseases of Animal Health Law: Highly Pathogenic Avian Influenza

EFSA Panel on Animal Health and Welfare (EFSA AHAW Panel),
Søren Saxmose Nielsen, Julio Alvarez, Dominique Joseph Bicout, Paolo Calistri, Klaus Depner, Julian Ashley Drewe, Bruno Garin-Bastuji, José Luis Gonzales Rojas, Christian Gortázar Schmidt, Mette Herskin, Virginie Michel, Miguel Ángel Miranda Chueca, Paolo Pasquali, Helen Clare Roberts, Liisa Helena Sihvonen, Hans Spoolder, Karl Stahl, Antonio Velarde Calvo, Arvo Viltrop, Christoph Winckler, Kris De Clercq, Eyal Klement, Jan Arend Stegeman, Simon Gubbins, Sotiria-Eleni Antoniou, Alessandro Broglia, Yves Van der Stede, Gabriele Zancanaro and Inma Aznar

Abstract

EFSA received a mandate from the European Commission to assess the effectiveness of some of the control measures against diseases included in the Category A list according to Regulation (EU) 2016/429 on transmissible animal diseases ('Animal Health Law'). This opinion belongs to a series of opinions where these control measures will be assessed, with this opinion covering the assessment of control measures for Highly Pathogenic Avian Influenza (HPAI). In this opinion, EFSA and the AHAW Panel of experts review the effectiveness of: (i) clinical and laboratory sampling procedures, (ii) monitoring period and (iii) the minimum radius of the protection and surveillance zone, and the minimum length of time the measures should be applied in these zones. The general methodology used for this series of opinions has been published elsewhere; nonetheless, specific details of the model used for the assessment of the laboratory sampling procedures for HPAI are presented here. Here, also, the transmission kernels used for the assessment of the minimum radius of the protection and surveillance zones are shown. Several scenarios for which these control measures had to be assessed were designed and agreed prior to the start of the assessment. In summary, sampling procedures as described in the diagnostic manual for HPAI were considered efficient for gallinaceous poultry, whereas additional sampling is advised for Anseriformes. The monitoring period was assessed as effective, and it was demonstrated that the surveillance zone comprises 95% of the infections from an affected establishment. Recommendations provided for each of the scenarios assessed aim to support the European Commission in the drafting of further pieces of legislation, as well as for plausible ad hoc requests in relation to HPAI.

Keywords: Control measures, Avian Influenza, HPAI, sampling procedures, monitoring period, protection zone, surveillance zone

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Correspondence: alpha@efsa.europa.eu

Panel members: Søren Saxmose Nielsen, Julio Alvarez, Dominique Joseph Bicout, Paolo Calistri, Klaus Depner, Julian Ashley Drewe, Bruno Garin-Bastuji, José Luis Gonzales Rojas, Christian Gortázar Schmidt, Mette Herskin, Virginie Michel, Miguel Ángel Miranda Chueca, Paolo Pasquali, Helen Clare Roberts, Liisa Helena Sihvonen, Hans Spoolder, Karl Stahl, Antonio Velarde Calvo, Arvo Viltrop and Christoph Winckler.

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Summary

This opinion is part of a series of opinions, in which the three-first terms of reference (ToR) of a mandate received from the European Commission, have been considered. The background and specific details of this mandate can be found in the opinion. The ToRs in this mandate request an assessment of the effectiveness of:

- the clinical and laboratory examination in their capacity to detect disease (or estimate the disease prevalence within an establishment), either in suspect or confirmed animals in a single establishment or in establishments within restricted zones (ToR 1);
- the effectiveness of the duration of the monitoring period (for different scenarios) in the control of suspected and confirmed outbreaks (ToR 2);
- the size and duration of the restriction zones, in their capacity for mitigating disease spread (ToR 3).

In order to harmonise the approach to these assessments, the methodology used in this series of opinions, covering all Category A diseases, was agreed on, and published in a separate technical report (EFSA, 2020a,b).

A qualitative assessment of the clinical examination procedures for HPAI was carried out. For assessing the effectiveness of the laboratory examination, a within flock compartmental model was designed. Further, scripts were written that allowed the calculation of the median time (days) to detection of a potential HPAI outbreak in a flock (and 95% prediction intervals), given that 'standard samples', or other samples, were taken and tested using polymerase chain reaction (PCR). These scripts were run separately for Galliformes and Anseriformes, assuming different transmission rates and mean infectious periods, as well as different case fatality rates. The effectiveness of taking standard samples (to be analysed by PCR) for early detection (within 10 days post-infection) was assessed. For most scenarios tested, taking standard samples was sufficient to lead to the confirmation of the infection. Nonetheless, in the event of a virus strain that causes low transmission or low mortality, recommendations in terms of the most appropriate sampling strategy were given. Recommendations for the use of serological sampling were also provided for some of the scenarios.

To answer ToR 2, and to assess the minimum length of time measures should be implemented in the protection and surveillance zones (ToR 3.2), an extensive literature search (ELS) was carried out. This ELS aimed to assess the average, shortest and longest period between the earliest point of infection of a bird with a HPAI virus, and the time of reporting of a suspicion by the competent authority. The average time to the reporting of a suspicion report was used then to assess the effectiveness of the length monitoring period. For most of the scenarios, the existing length of the monitoring period for HPAI (21 days) was considered sufficient; nonetheless, as clinical signs in Anseriformes are not always obvious or present, this could result in longer periods to a suspicion report (compared to chickens and other species in the Galliformes order). Some recommendations were given in this respect for some of the relevant scenarios. To assess the effectiveness of the minimum length of time, the measures should be applied in the protection and surveillance zones, the average and the longest time assessed via the ELS were used, respectively. In this regard, the minimum length of time the protection zone (21 days) and the surveillance zone (30 days) must be in place for, when based on existing legislation, were considered effective.

To assess the effectiveness of the minimum radius to be implemented in the protection and surveillance zones (ToR 3.1), transmission kernels were used. These kernels had been built using data from previous outbreaks. These kernels represent the relative risk of transmission to each individual establishment from the affected establishment. For HPAI, it was observed that, assuming transmission from an affected establishment occurs, the probability of transmission beyond the protection zone was 0.52. Nonetheless, the probability of infection of an establishment located beyond 10 km (radius of the restriction zone including protection and surveillance zones), drops greatly to 5%. As not all establishments within the surveillance zone will be sampled, several recommendations were given in terms of how to mitigate the risk of further spread within the surveillance zone. It is important to note that the transmission kernels presented cover only some of the risk pathways associated with spread of the index case and they do not take account wildlife contact, or movements of live animals and products off the establishment prior to confirmation.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EU) 2016/429 on transmissible animal diseases ('Animal Health Law'), hereinafter referred to as AHL, requires the Commission to lay down detailed rules on the disease control measures against listed diseases as referred to in point (a), (b) and (c) of its Article 9 (category A, B and C diseases). The Commission is empowered to adopt delegated acts supplementing the rules laid down in Part III of Regulation (EU) 2016/429 on transmissible animal diseases (Animal Health Law) on disease control measures for listed diseases as referred to in point (a), (b) and (c) of its Article 9 (category A, B and C diseases). Therefore, the Commission has developed and adopted a Delegated Regulation laying down rules for the prevention and control of certain diseases ('the Delegated Regulation'). The rules laid down in the Delegated Regulation are in respect of terrestrial animals largely replicating the rules currently in force concerning the disease control measures in the event of animal diseases with serious effects on the livestock as they have proven to be effective in preventing the spread of those diseases within the Union. Consequently, many animal disease control measures laid down in existing Directives will be, to the extent that not already done by the Animal Health Law, replaced by the rules provided in the Delegated Regulation. At the same time, these rules have been aligned with the international standards from the World Organisation for Animal Health (OIE), wherever these existed. However, certain disease control measures proposed in the Delegated Regulation, in particular in its Annexes, were considered as outdated, i.e. possibly not based on most recent scientific evidence at the time of development. Their review is considered as necessary. Moreover, for those category A diseases for which rules were not established before or were not detailed enough, certain disease control and risk mitigating measures are, due to the lack of scientific basis, extrapolated from other diseases, for which rules existed in the past. Finally, for some other diseases the evidence and scientific knowledge, was not available to the Commission and to the Member States at the time of developing the Delegated Regulation due to the time constraints. The following diseases are examples of the later: infection with Rift Valley fever (RVF), infection with *Mycoplasma mycoides* subsp. *mycoides* SC (Contagious bovine pleuropneumonia) (CBPP), Contagious caprine pleuropneumonia (CCPP), Sheep pox and goat pox, infection with peste des petits ruminants virus (PPR), African horse sickness (AHS), Glanders. In this regard, the existing rules will cease to apply as from the date of application of the Animal Health Law and its complementing legislation including the Delegated Regulation, i.e. from 21 April 2021. Certain of the proposed measures for the prevention and control of category A diseases of terrestrial animals should therefore be assessed in order to ensure that they are effective and updated based on the latest scientific knowledge in this new set of legislation. This is particularly important in the case of those diseases that are less common or have been never reported in the Union.

1.1.1. ToR 1: Sampling of animals and establishments for the detection of *category A* diseases in terrestrial animals

Based on available scientific information, assess the effectiveness of existing sampling procedures to detect or rule out the presence of each category A disease of terrestrial animals and, in case of absence of effective procedures, develop them, in order to complete the rules provided for in Annex I to the Delegated Regulation. In particular, provide for disease-specific procedures for the sampling of:

ToR 1.1 Animals for clinical examinations to ensure the detection of the relevant category A disease during the performance of official investigations in establishments that are affected or suspected to be affected by category A diseases and visits in establishments located in restricted zones in accordance with Articles 6(2), 13(3)(c), 14(1) and 26(2) of the Delegated Regulation.

ToR 1.2 Animals for laboratory examinations to ensure the detection of the relevant category A disease during the performance of official investigations in establishments that are affected or suspected to be affected by category A diseases and visits in establishments located in restricted zones in accordance with Articles 6(2), 12(3), 13(3)(c), 14(1), 26(2) of the Delegated Regulation.

ToR 1.3 Establishments to ensure the detection of the relevant category A disease for the performance of visits in establishments located in protection zones larger than 3 km and establishments located in the surveillance zone in accordance with Articles 26(5) and 41 of the Delegated Regulation.

ToR 1.4 Animals for clinical and laboratory examinations to ensure the detection of the relevant category A disease for the movement of animals from restricted zones in accordance with Articles 28 (5), 43(5), 56(1)(c) of the Delegated Regulation.

ToR 1.5 Animals for laboratory examinations to ensure the detection of the relevant category A disease before and after being introduced in the affected for repopulation, in accordance with Article 59(2), (3) and (9) of the Delegated Regulation.

1.1.2. ToR 2: Monitoring period

ToR 2.1 Assess the effectiveness of the length of the monitoring periods set out in Annex II of the Delegated Regulation for each category A disease of terrestrial animals. In this regard, it is important to take into consideration that the monitoring period was introduced as a management tool, which represents a time frame of reference assigned to each category A disease for the competent authority to apply certain control measures and to carry out investigations in the event of suspicion and confirmation of category A diseases in terrestrial animals.

This assessment should be carried out with respect to the following situations:

a) the records analysis carried out by the competent authority in the framework of the epidemiological enquiry referred to in Article 57 of Regulation (EU) 2016/429, in the event of suspicion of a category A disease (Article 8(4) of the Delegated Regulation);
b) the derogation from killing in the event of an outbreak of a category A disease in establishments keeping animals of listed species in two or more epidemiological units (Article 13(1) of the Delegated Regulation);
c) the tracing carried out by the competent authority to identify establishments and other locations epidemiologically linked to an establishment affected by a category A disease (Article 17(2) of the Delegated Regulation);
d) the exemption applied to certain products from the prohibitions laid down in Annex VI taking into account the date they were produced (Article 27(3)(c) of the Delegated Regulation);
e) the specific conditions for authorising movements of semen from approved germinal product establishments in the protection and surveillance zones (Article 32(c) and 48(c) of the Delegated Regulation);
f) the repopulation of establishments affected by a category A disease (Article 57(1)(b) and 59(4)(b) of the Delegated Regulation).

ToR 2.2 Propose the length of what should be the monitoring period in those diseases for which the time is assessed as not effective.

1.1.3. ToR 3: Minimum radius of restricted zones and duration of the disease control measures in restricted zones

ToR 3.1 Assess the effectiveness to control the spread of the disease of the minimum radius of the protection and surveillance zones set out in Annex V of the Delegated Regulation for each category A disease of terrestrial animals.

ToR 3.2 Assess the effectiveness to control the spread of the disease of the minimum periods during which the competent authority should apply the restriction measures in the protection and surveillance zones as set out in Annex X and XI for each category A disease of terrestrial animals.

1.1.4. ToR 4: Prohibitions in restricted zones and risk-mitigating treatments for products of animal origin and other materials

ToR 4.1 Assess the effectiveness to control the spread of disease of prohibitions set out in Annex VI of the Delegated Regulation with respect to the risk associated for each category A disease, to the listed activities and commodities.

ToR 4.2 Review the available scientific information on risk-mitigating treatments that are effective to control the presence of category A disease agents in products of animal origin and other relevant materials. Based on this:

a) provide an opinion on the effectiveness of the risk-mitigating treatments for products of animal origin and other materials produced or processed in the restricted zone set out in Annex VII and VIII, and

b) if relevant, suggest new treatments or procedures that can be effective to mitigate or to eliminate such risk.

1.2. Interpretation of the Terms of Reference

To address the ToRs of this mandate, EFSA proposed and agreed with the European Commission the following:

a) The publication of 14 individual opinions, one per each of the diseases included in the list of category A diseases for terrestrial animals, with each of these opinions providing the answer to ToRs 1, 2 and 3. This document is one of the 14 opinions covering ToRs 1, 2 and 3 for Highly Pathogenic Avian Influenza (HPAI).
b) The publication of a unique opinion covering ToR 4 for all diseases listed (i.e. ToR 4 is not covered in this opinion).
c) To address ToR 1 (effectiveness of sampling procedures), EFSA agreed with the European Commission on 21 scenarios (based on different articles of the Delegated Act) for which the effectiveness of the sampling procedures will be assessed (Annex B). Although these scenarios will be assessed independently, some of them may be merged if the assessment results are the same.
d) To address ToR 2 (effectiveness of the monitoring period), seven scenarios previously agreed with the requestor were defined (Annex D). The assessment of the effectiveness of the monitoring period will be performed by assessing its ability to ensure that specific actions can be carried out without posing a risk of disease spread, if the monitoring period is calculated backward or forward from a specific date. If the length of the monitoring period as evaluated by EFSA is longer than the existing monitoring periods, the existing monitoring period will be considered non-effective. If the length of the monitoring period estimated by EFSA is shorter than the existing monitoring period, this existing monitoring period will be considered effective from a disease control point of view. No assessment of the plausible unnecessary economic burden that may be placed on the stakeholders as a result of an excessive length of the monitoring periods will be done by EFSA.
e) The assessment of the minimum duration and the length of the radius of the protection and surveillance zones (ToR 3) will be done independently. The setting of these two zones surrounding an affected establishment, and the control measures implemented in each one of the zones are based on the general principle that the probability of disease spread is larger the closer the establishment is to an affected establishment. The validity of this statement will not be assessed in this manuscript (e.g. transmission by wild birds will not follow this principle); nonetheless, the limitations that this assumption may have in the control of certain diseases will, when relevant, be discussed.
f) The following scenarios in ToR 1 (Annex B) were not relevant for HPAI, and therefore were not included in the assessment: a) scenario 7 because the minimum radius of the protection zone for HPAI is 3 km, and b) scenarios 14 and 15 as they refer to ungulates.
g) The duration of the monitoring period for HPAI as described in Annex II of the Delegated Regulation is 21 days.
h) The minimum length of the radius of the protection zone (PZ) and surveillance zone (SZ) for HPAI as described in Annex V of the Delegated regulation are 3 and 10 km, respectively.
i) The minimum duration of the measures in the PZ and SZ for HPAI as described in Annex X and XI of the Delegated Regulation is 21 and 30 days, respectively.

2. Epidemiology and geographical distribution of HPAI

2.1. Epidemiology

Highly pathogenic avian influenza (HPAI), also called 'fowl plague', is a contagious, multi-organ systemic viral disease of poultry, in contrast to low pathogenic avian influenza (LPAI) causing only mild or subclinical disease. HPAI is caused by infection with virulent strains of Type A influenza virus, belonging to the Orthomyxoviridae family, genus *Alphainfluenzavirus* (Swayne and Suarez, 2000). All HPAI virus strains belong to the H5 or H7 subtype (Spickler, 2015).

Avian influenza is a notifiable disease, with monitoring and surveillance being essential to quickly contain emerging outbreaks and reduce the risk of zoonotic transmission (Spickler, 2015). Aquatic

migratory birds, mostly within the orders of Anseriformes and Charadriiformes, are regarded as the reservoir for all genes associated with Influenza A viruses (USAHA 2008; Brouwer, et al., 2019). Although the involvement of wild birds in outbreaks is often difficult to prove, it is highly likely that domesticated poultry is infected through exposure to the faeces of wild birds (Richard et al., 2017). Other common sources of infection are both via the introduction of infected domestic poultry to a farm (followed by contamination through the faecal-oral route or by aerosol), and through the infection of domestic birds by contaminated fomites (Spickler, 2015). Airborne transmission between poultry farms is also likely in poultry-dense regions (Ypma et al., 2013). Existing national and international surveillance programmes consist of virological passive detection in dead birds, as well as active surveillance in living and hunted birds. In wild birds, both passive and active surveillance use PCR detection methods. In domestic birds, passive surveillance is used to detect all H5 and H7 subtypes, and is most effective at detecting HPAI, while active surveillance refers to the serological testing aimed mainly at LPAI detection (Brouwer et al., 2019).

HPAI can spread rapidly, causing severe disease and death in poultry species such as chicken, turkey, quail, guinea fowl, domestic duck as well as in wild birds species, and pet birds (OIE 2020). In a recent global seasonality analysis, it was shown how HPAI outbreaks in poultry peak in February and are lowest in September (Awada et al., 2018). Notably, Anseriformes, such as the Pekin duck, do not always exhibit severe clinical disease when infected with some HPAI strains (OIE 2019). Humans and other mammals (e.g. cats, pigs, ferrets) are occasionally cross-infected following close contact with, manipulation or ingestion of infected birds, as was observed in the past outbreaks of H5N1, H7N9 and H7N7 (Koopmans et al., 2004; Poovorawan et al., 2013).

Once infected, the incubation period for Influenza A in poultry species is between 1 and 7 days for an individual, and up to 21 days for a flock (USAHA, 2008). Symptoms of HPAI include coughing, sneezing, sinusitis, blood-tinged oral and nasal discharges, loss of feathers, diarrhoea, depression, inappetence and sudden death. Furthermore, egg production stops or decreases, producing deformed or shell-less eggs (Spickler, 2015). Necropsy findings include haemorrhages and necrosis of the respiratory and gastro-intestinal organs. Chicken and turkey species are most susceptible, with mortality rates being as high as 90–100% within 2–12 days after the first signs of illness. The differentiation between high and low pathogenicity is based on the mortality and severity of the disease in chickens; clinical manifestation and mortality in Anseriformes species, such as domestic ducks and geese, can vary depending on the virus strain, host species and the level of viral exposure, going from mild without mortality, to very severe with high mortality (USAHA, 2008; OIE, 2019; EFSA, 2020b; Swayne et al., 2020).

Diagnostic samples, collected by oropharyngeal and cloacal/faecal swabs from live birds and faecal or organ samples from dead birds, are tested using direct molecular techniques (real-time reverse transcription polymerase chain reaction (RT-PCR) (OIE 2019). For viral isolation (used as the reference standard), samples are inoculated and incubated in embryonated eggs, which are then tested for the presence of virus using RT-PCR, agar gel immunodiffusion (AGID), antigen-detection enzyme-linked immunosorbent assays (ELISAs), or other immunoassays. To distinguish between LPAI and HPAI viruses, genetic tests to detect patterns in the haemagglutinin (HA) and/or virulence tests are used (Intravenous Pathogenicity Index, IVPI). While antigen testing can produce results in as little as 15 min, the current recommendation by the World Organization for Animal Health (OIE) is that it should only be used to interpret the presence of influenza A at flock level and not for diagnosis in individual birds (OIE, 2020). Furthermore, future antigen tests should be species-specific and validated accordingly, as the most common test for chickens and turkey is not as sensitive in detecting disease in other avian species (Spickler, 2015).

2.2. Geographical distribution of the disease

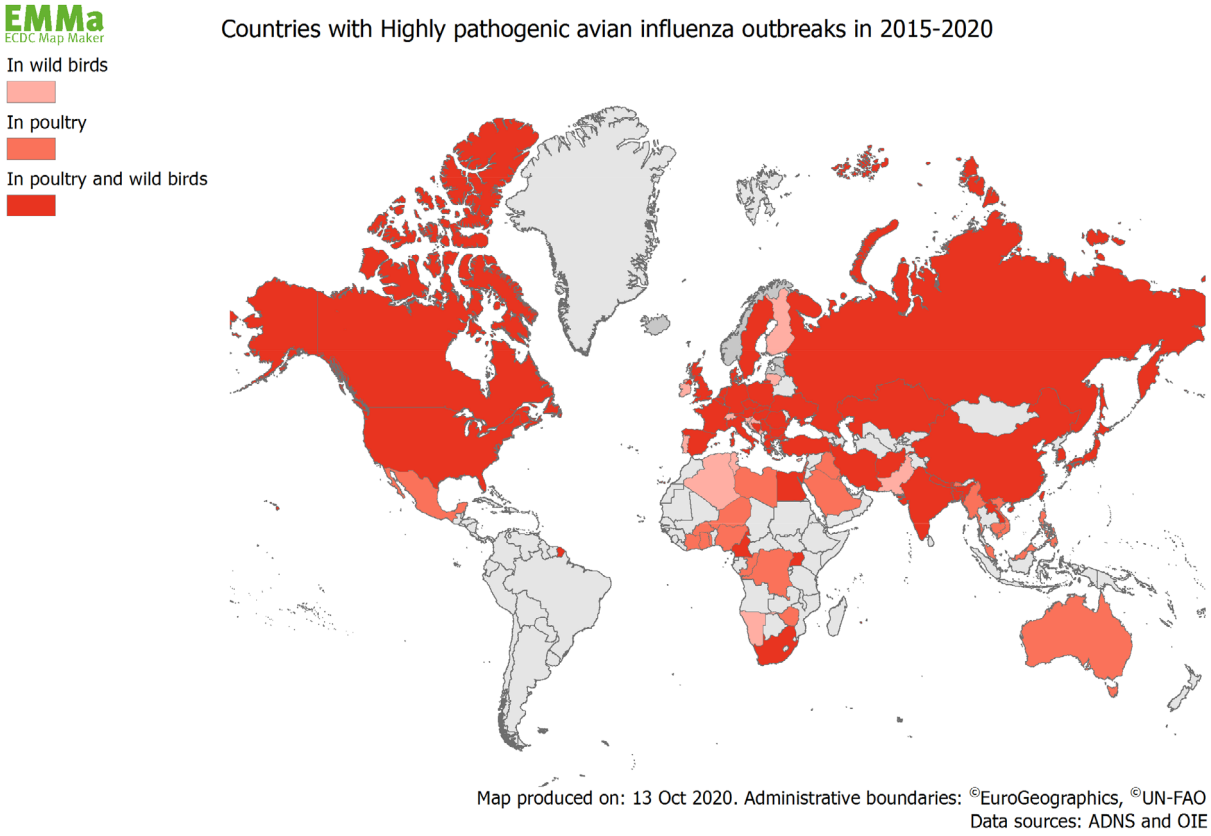

Figure 1: Map of countries where outbreaks of HPAI were reported in wild birds and/or poultry between 2015 and 2020 (Data sources: ADNS and OIE)

Using data from ADNS and OIE, Figure 1 above shows in a colour-coded manner the countries were HPAI outbreaks in poultry, and detections in wild birds, were worldwide reported between 2015 and 2020.

3. Data and methodologies

Although the general methodology applied to all opinions covering the assessment of control measures for the Category A diseases produced under this mandate has been published elsewhere (EFSA, 2020a), specific details of the methodology related to the HPAI opinion are presented below.

3.1. Methodology used in ToR 1

In order to assess in a quantitative manner, the effectiveness of the laboratory sampling procedures for HPAI (i.e. sample sizes described in existing legislation and presented in Annex C), a mathematical model for HPAI virus transmission was developed. The specifications of the model are described below together with the different transmission scenarios that were considered, and the parameters used. The model predictions for both, gallinaceous[1] poultry (Figure F.1), and for Anseriformes (Figure F.2), are shown in Annex F.

The number of birds in the different model compartments (susceptible, exposed, infectious, recovered and dead) resulting from this modelling exercise was used to estimate the number of birds in the different disease states over time (Table 1). The probability of detection, p_D, given a specific sample size was then computed using the hypergeometric distribution (assuming sampling without replacement), so that

[1] This term refers to the Galliformes order. This order includes turkey, grouse, chicken, New World quail and Old World quail, ptarmigan, partridge, pheasant, guineafowl, francolin, junglefowl, peafowl and the Cracidae.

$$p_D = 1 - \binom{K}{0}\binom{M-K}{SS}\Big/\binom{M}{SS}$$

where M is the total number of dead, sick or healthy birds, K is the number of dead, sick or healthy birds that are infected (and detectable) and SS is the number of dead, sick or healthy birds sampled.

The relationship between M and K and the model variables is given in Table 1, where m_B is the baseline mortality (proportion of birds dying each day due to reasons other than HPAI), ν_B is the baseline morbidity (proportion of birds showing signs consistent with HPAI) and ν_I is the proportion of infectious birds showing signs of HPAI. In this table, S, E, I, R and D are used to represent birds that are susceptible (i.e. uninfected), exposed (i.e. infected but not infectious), infectious (i.e. infected and infectious), recovered and dead (i.e. birds that have died due to HPAI, and so would be PCR positive), respectively.

Table 1: Relationship between numbers of dead, sick and healthy birds and model variables, and sample size (SS) of birds in the different disease states for which the probability was computed

Disease states	Total number of dead (M)	Total number of sick or healthy birds (K)	Sample size (SS)
Dead birds	$m_B(S(t) + E(t) + I(t) + R(t)) + D(t)$	$m_B(E(t) + I(t)) + D(t)$	5
Sick birds	$\nu_B(S(t) + E(t) + R(t)) + \nu_I I(t)$	$\nu_B E(t) + \nu_I I(t)$	20
Healthy birds	$(1-\nu_B)(S(t) + E(t) + R(t)) + (1-\nu_I)I(t)$	$(1-\nu_B)E(t) + (1-\nu_I)I(t)$	60

Baseline morbidity and mortality were assumed to be 1% and 0.1%, respectively (van Niekerk et al., 2012; Gonzales and Elbers, 2018).[2] For gallinaceous poultry, all infectious birds were assumed to show clinical signs. For Anseriformes, infectious birds were assumed to show clinical signs at baseline levels (i.e. 1%) in the no mortality scenarios, 10% of infectious birds were assumed to show clinical signs in the low mortality scenarios, while all (i.e. 100%) of infectious birds were assumed to show clinical signs in the high mortality scenarios. Birds were assumed to be tested by PCR, and a sensitivity and specificity of 100% was assumed.

Mathematical model and transmission scenarios considered

The within-flock dynamics of HPAI virus were modelled using a stochastic compartmental model (Keeling and Rohani, 2008). For gallinaceous poultry, the host population is divided into three classes: susceptible (i.e. uninfected), S; exposed (i.e. infected, but not yet infectious), E; and infectious (i.e. infected and infectious), I. All birds were assumed to die at the end of their infectious period. For Anseriformes, the host population is divided into four classes: susceptible (i.e. uninfected), S; exposed (i.e. infected, but not yet infectious), E; infectious, I; and recovered, R. Disease-associated mortality was assumed to occur at a constant rate during the infectious period.

The force of infection is given by,

$$\lambda(t) = \beta\frac{I(t)}{N(t)}$$

where β is the transmission rate, I(t) is the number of infectious birds and N(t) is the total number of birds at time t. This formulation assumes homogeneous mixing (i.e. individuals uniformly and randomly contact each other) and frequency-dependent transmission (i.e. the number of contacts is independent of the population size) (Keeling and Rohani, 2008). The durations of the latent (time between infection by HPAI and the birds becoming infectious) and infectious periods were assumed to follow gamma distributions with means μ_E and μ_I and shape parameters k_E and k_I, respectively (i.e. with variances μ_E^2/k_E and μ_I^2/k_I). This was incorporated in the model by subdividing the latent and infectious classes into k_E and k_I stages each of mean duration μ_E/k_E and μ_I/k_I, respectively (Anderson and Watson, 1980).

The number of birds in each class in the model takes integer values, while transitions between compartments are stochastic processes. The number of transitions of each type during a small-time

[2] Conservative estimates were selected.

interval δt was drawn from a binomial distribution with the number of birds in the appropriate class n and transition probability q (the appropriate per capita rate multiplied by δt) as parameters.

The initial flock size was assumed to be 10,000 birds for both gallinaceous poultry and Anseriformes. Parameter estimates are given in Table 2 for gallinaceous poultry and Table 3 for Anseriformes.

For gallinaceous poultry, the minimum, median and maximum transmission rates were obtained from a review and reanalysis of published transmission experiments (CVI and APHA, 2017). In the same transmission experiments, the mean infectious periods could be categorised as short (around 2 days) or long (around 6 days) (CVI and APHA, 2017), and the median for the experiments in the short and long categories was used in the scenarios. Thus, a total of six scenarios were considered for gallinaceous poultry.

For Anseriformes, there were two scenarios for the transmission rate and mean infectious period (CVI and APHA, 2017) and three scenarios for case fatality: none (0%), low (5%) or high (50%) to reflect the variability in manifestation (Swayne et al., 2020). Thus, six scenarios were considered for Anseriformes.

For gallinaceous poultry and Anseriformes, the mean latent period and the shape parameters for the latent and infectious periods were extracted from the published literature and were common to all scenarios.

Table 2: Parameters in the model for the transmission of highly pathogenic avian influenza virus in gallinaceous poultry

Scenario	β	μ_E	k_E	μ_I	k_I
Low transmission, short infectious period	0.76	0.24**	2**	1.65	5**
Medium transmission, short infectious period	1.43			1.65	
High transmission, short infectious period	4.50			1.65	
Low transmission, long infectious period	0.76			6.30	
Medium transmission, long infectious period	1.43			6.30	
High transmission, long infectious period	4.50			6.30	

**: Estimated by Bouma et al. (2009), see scenario B from their Table 4.
β – transmission rate.
μ_E – mean latent period.
k_E – shape parameter for gamma-distributed latent period.
μ_I – mean infectious period.
k_I – shape parameter for gamma-distributed infectious period.

Table 3: Parameters in the model for the transmission of highly pathogenic avian influenza virus in Anseriformes

Scenario	β	μ_E	k_E	μ_I	k_I	Case fatality
No mortality, transmission 1	1.6	1.0†	2‡	13.4	2‡	0
No mortality, transmission 2	4.7			4.3		0
Low mortality, transmission 1	1.6			13.4		0.05
Low mortality, transmission 2	4.7			4.3		0.05
High mortality, transmission 1	1.6			13.4		0.5
High mortality, transmission 2	4.7			4.3		0.5

†: Assumed by van der Goot et al. (2008) when analysing their data.
‡: Assumed value.
β – transmission rate.
μ_E – mean latent period.
k_E – shape parameter for gamma-distributed latent period.
μ_I – mean infectious period.
k_I – shape parameter for gamma-distributed infectious period.

3.2. Methodology used in ToR 2

To answer ToR 2, an extensive literature search (ELS) was outsourced by EFSA (OC/EFSA/ALPHA/2020/02 – LOT 2).[3] The aim of this ELS was to answer the epidemiological question: 'what is the average, shortest and longest period of time (measured as the number of days from the earliest point of infection with HPAI virus (HPAIV), to the time of declaration of a suspicion by the competent authority after the clinical investigation by an official veterinarian) for an outbreak of HPAI to be reported?'. To answer this question, an ELS on case reports, papers describing outbreaks or epidemics of HPAI, and any other relevant grey literature or data, was carried out. For the inclusion criteria in the ELS, the earliest point of infection had to have been estimated by carrying out an epidemiological investigation. Papers and other sources of data where the earliest point of infection was determined purely by subtracting a known incubation period from the date of the suspicion of the outbreak were excluded. The ELS was restricted to studies conducted in Europe or describing results obtained in Europe. If none or very few articles were retrieved (less or equal to 5) in the first search, the search was extended to the rest of the world. The general protocol used for the ELS is shown in Annex 5 of the Methodology report (EFSA, 2020a).

3.3. Methodology used in ToR 3

Methodology for assessing the effectiveness of the minimum radius of the protection and surveillance zones

Studies investigating the transmission of HPAI virus between farms using transmission kernels were identified in the published literature. The functional form, parameter estimates and the 95% confidence or credible intervals for the parameters (where provided) of the best-fitting kernels were extracted from each study.

For each kernel, the probability of transmission beyond given distances (if transmission were to occur from an infected establishment) was computed using the estimates and the lower and upper 95% confidence limits for the parameters. In addition, the distances at which a threshold probability of transmission beyond that distance is reached were also calculated for each kernel using the estimates, along with its lower and upper 95% confidence limits.

Methodology for assessing the effectiveness of the duration of the protection and surveillance zones

To estimate the duration of measures in the protection and surveillance zones, the outputs obtained from the ELS described in Section 3.2 were used. Further details can be found in the Methodology report (EFSA, 2020a).

3.4. Uncertainty

A description of the methodology followed to deal with uncertainty is provided in a Methodology report published by EFSA (EFSA, 2020a).

4. Assessment

4.1. Assessment of sampling procedures

4.1.1. Assessment of sampling procedures in the event of suspicion or confirmation of Highly Pathogenic Avian Influenza

4.1.1.1. In the event of a suspicion of HPAI in an establishment where animals of the listed species are kept

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures of animals of listed species in a suspected establishment, based on clinical examination (TOR 1.1) and laboratory examination (TOR 1.2), in their ability to detect HPAI in kept animals if the disease is present in that establishment, or to rule it out if not present (Art. 6 (2)).

[3] https://etendering.ted.europa.eu/cft/cft-display.html?cftId=6249

- **1st Scenario of sampling procedures**
- ToR 1.1 and ToR 1.2 in accordance with Mandate
- Article 6(2) of the Delegated Regulation
- Commission Implemented Regulation 2018/1882 on listed species

The following elements of the scenario should be taken into consideration during for the assessment (further details are shown in Annex B):

1) It concerns an event of suspicion of HPAI in an establishment of kept animals of the listed species
2) The listed species for HPAI, as provided in Commission Implemented Regulation 2018/1882, are species of the Class Aves
3) In the event of a suspicion of HPAI, the competent authority shall immediately conduct an investigation to confirm or rule out the presence of the disease

During the visit, the official veterinarian must perform clinical examinations and collect samples for laboratory examinations.

Summary of sampling procedures as described in the diagnostic manual

A diagnostic manual for Avian Influenza is set out in the Annex of Commission Decision 2006/437/EC[4] (hereinafter referred to as 'diagnostic manual'); here, the clinical and laboratory guidelines for HPAI are described in detail. A summary of the guidelines for the different scenarios to be assessed, are shown in Annex C of this opinion.

In the diagnostic manual, it is recommended that, in the case of a suspicion of HPAI, production and health records (daily mortality, egg production and feed and/or water intake) are verified by the official veterinarian at arrival to the suspect establishment, with the records going back one week before the commencement of clinical signs. The inspection of these records is part of the epidemiological investigation that the official veterinarian must carry out in a suspect establishment in order to identify the most likely point of entry in time, plausible epidemiological links, etc. (this point will be discussed further in the assessment of ToR 2). Aside, the inspection of the production and health records is recommended in order to identify flocks (or other form of larger or smaller epidemiological units) where further clinical inspection should be prioritised, and where samples may be taken.

The official inspection should also include a clinical inspection of each production unit with a clinical examination of poultry or other captive birds, in particular those that appear sick. The main clinical signs are variable depending on the virulence of the virus, the species and other epidemiological factors. Detailed description of the clinical signs as well as post-mortem lesions observed in the different species is also presented in the diagnostic manual. As described in this manual, unless the competent authority is satisfied that a suspected outbreak may be excluded on the basis of the clinical inspection, the 'standard samples' must be taken from each production unit. The number of samples to be taken in what it is denominated as 'standard samples' is described below. Subsequent to receiving the results from the laboratory examination, and independently of the results, a final clinical inspection of the poultry must be carried out prior to the lifting of the official surveillance.

The standard samples to be taken are:

For virological testing:

Any flock showing clinical signs must be targeted with at least five sick/dead birds and/or at least 20 tracheal/oropharyngeal and 20 cloacal swabs (or from all birds if less than 20 birds are present) taken from these birds.

For serological testing:

Birds appearing sick or that have apparently recovered must be targeted with at least 20 blood samples (or from all birds if less than 20 birds are present) being taken.

[4] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006D0437&from=EN

Assessment

The existing diagnostic manual for avian influenza is very comprehensive, with very precise details on how the clinical and laboratory examinations for HPAI should be carried out. In terms of the guidelines for clinical examination, and although already mentioned in the diagnostic manual, it is deemed primordial that the clinical inspection is carried out in the full establishment, and not just in some flocks or production units. A clinical inspection of the entire establishment should lead to the selection of birds (dead or sick) for further examination and/or laboratory sampling.

In the diagnostic manual, samples from dead birds, or birds showing clinical signs should be taken, and sent for laboratory analysis. According to Gonzales and Elbers (2018), and Schreuder et al. (2020), mortality is the most sensitive parameter for early detection of HPAI in chicken flocks. In the latter publication, it is suggested that an increased mortality ratio may be the most objective parameter to detect HPAI infection at an early stage on both chicken, and Pekin duck establishments; nonetheless, observation of clinical signs may influence sometimes the early notification of a suspicion.

Using the methodology described in Section 3.1, the number of days (median and 95% prediction interval) from the introduction to the detection of HPAI with a 95% confidence was calculated when a specific set of samples (i.e. samples from 5 dead birds, 20 sick birds, and 60 healthy birds) was submitted for laboratory analysis (under different transmission scenarios assumptions). The model predictions are also shown in Annex F.

The results are shown below in Tables 4 and 5 for Galliformes and Anseriformes transmission scenarios, respectively. A figure showing the probability of detection in Galliformes and Anseriformes for the different transmission scenarios is also shown below (Figure 2).

Table 4: Median (95% prediction interval) time (days post introduction) to 95% confidence for detection of highly pathogenic avian influenza virus in gallinaceous poultry

Scenario	Sample size for laboratory examination		
	5 dead birds	20 sick birds	60 healthy birds
Low transmission, short infectious period	6 (3, 13)	9 (3, 27)	43 (33, 62)
Medium transmission, short infectious period	4 (3, 6)	4 (2, 6)	10 (8, 12)
High transmission, short infectious period	3 (2, 3)	2 (1,2)	3 (3, 4)
Low transmission, long infectious period	9 (6, 14)	5 (3, 10)	14 (12, 19)
Medium transmission, long infectious period	7 (5, 9)	3 (2, 5)	8 (6, 10)
High transmission, long infectious period	4 (3, 4)	2 (1, 3)	3 (3, 4)

Table 5: Median (95% prediction interval) time (days post introduction) to 95% confidence for detection of highly pathogenic avian influenza virus in Anseriformes

Scenario*	Sample		
	5 dead birds	20 sick birds	60 healthy birds
No mortality, transmission 1	11 (10, 12)	10 (8, 11)	8 (7, 10)
No mortality, transmission 2	6 (5, 7)	5 (5, 7)	5 (4, 6)
Low mortality, transmission 1	10 (8, 12)	7 (6, 10)	8 (7, 11)
Low mortality, transmission 2	5 (4,7)	4 (2, 5)	5 (4, 6)
High mortality, transmission 1	8 (6, 11)	5 (3, 8)	10 (8, 13)
High mortality, transmission 2	4 (3, 6)	3 (2, 5)	5 (4, 7)

*: Transmission 1: low transmission, long infectious period (IP); transmission 2: high transmission, short IP.

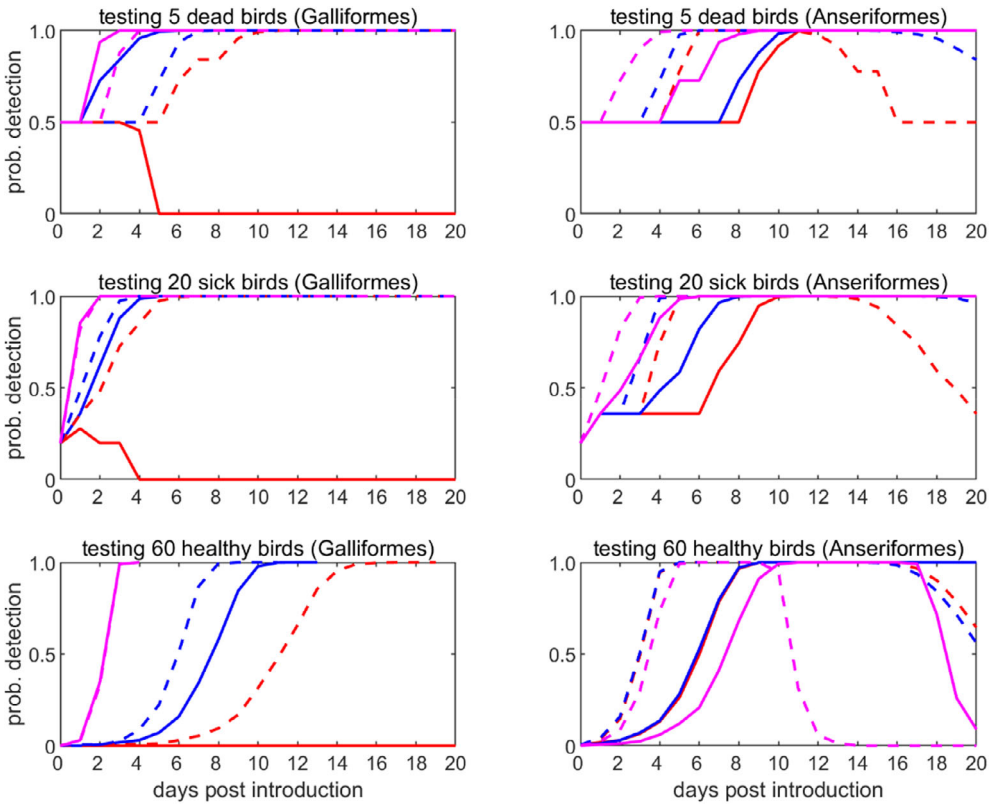

Figure 2: Probability of detection of highly pathogenic avian influenza virus in gallinaceous poultry (left column) or Anseriformes (right column). Plots show the median probability of detection for different scenarios. For gallinaceous poultry, scenarios are short (solid lines) or long (dashed lines) infectious periods and low (red), median (blue) or high (magenta) transmission rates (see Table 2 in Section 3.1). For Anseriformes, scenarios are no (red), low (blue) or high (magenta) mortality with different transmission rates/infectious periods (solid or dashed lines) (see Table 3 in Section 3.1)

Table 4 shows the median time to detection of HPAI in Gallinaceous poultry. The table shows that testing 5 dead birds or swabs from 20 sick birds would result in median times between virus introduction to detection ranging from 3 to 9 days, indicating that collecting and testing these samples on those days would result in the HPAI diagnosis 95 out of 100 times. The upper limits of the 95% prediction intervals are 10 days or lower for all but the scenarios with the low transmission rate (scenarios 1 and 4). In those scenarios, the upper limit could be up to 14 days when testing 5 dead birds, or 27 days when testing swabs of 20 sick birds. However, the combination of testing 5 dead birds and 20 tracheal/oropharyngeal and 20 cloacal swabs would result in an increase of the detection probabilities, and a decrease in the time to detection. Table 5 shows the results obtained for the different transmission scenarios considered for Anseriformes. In this case, if virus strains are associated with clinical disease and mortality, testing 5 dead birds, or samples of 20 sick birds result in a median time between introduction and 95% probability of detection of 4–10, and 3–7 days, respectively. Among all scenarios considered for Anseriformes, the upper limits of the 95% prediction intervals are 10 and 12 days, respectively. The median time is generally shorter when collecting 20 samples from sick birds than when collecting 5 dead birds, the reasons being the higher sample size (5 vs 20), the higher case morbidity compared to the case fatality and the longer infectious period/generation time. Remarkably, for the scenarios in this table where we assume no case morbidity and case fatality (two-first scenarios in Table 5), where only background mortality and morbidity are considered, 60 healthy birds have a comparable sensitivity to testing 5 dead birds or 20 sick birds in the scenarios 3–6, with median detection times of 8 and 5 days (60 birds). However, unlike the other scenarios, in the absence of a clinical manifestation and disease associated mortality, there is no information on the onset of the disease, so it is unknown at what stage of the disease the samples are collected. In that case serological testing has added value, although outbreaks will be detected by the serological testing at a late stage, due to the time between infection and seroconversion. The required sample size detects

15% seroprevalence with 95% confidence, which is sufficient to detect a major outbreak at the level of a production unit (flock). Nonetheless, serological testing would not be of use in terms of detecting HPAI in gallinaceous poultry, as most of the flock will have died before seroconversion takes place. In both Anseriformes and Galliformes, confirmation should always be based on the identification of the virus, and not antibodies.

Development of new procedures

In summary, the sample sizes of 5 dead birds or samples of 20 sick birds for HPAI investigation are sufficient to detect HPAI in gallinaceous poultry, because outbreaks are generally detectable within 10 days after introduction (see Section 4.2). A combination of 5 dead birds and 20 sick birds is recommended to prevent relatively late detection that could occur when dealing with a strain with a low transmission rate parameter. In case we are dealing with a virus strain that is associated with clinical disease in Anseriformes, sampling five dead birds, or 20 sick birds are also sufficient, although testing 20 sick birds would achieve an earlier detection compared to testing five dead birds. In case of a virus that does not induce mortality, or clear clinical signs, testing 60 healthy birds, either swabs for PCR or blood samples for serology (assuming 100% sensitivity of the serological test), per production unit is recommended. Serological testing of gallinaceous poultry can be omitted.

4.1.1.2. For the purposes of the epidemiological enquiry as referred to Article 57 of Regulation (EU)2016/429 in an HPAI officially confirmed establishment

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures, based on laboratory examination (ToR 1.2), in their ability to detect the disease in the event of preventive killing, and in their ability to support with the epidemiological investigation (disease detection, prevalence estimation, virus identification, etc.) in kept animals of listed species in an affected establishment, before or when they are killed or found dead. The purposes of the epidemiological enquiry are described in Article 57 of Regulation (EU)2016/429. See Annex B.

- **2nd Scenario of sampling procedures**
- ToR 1.2 in accordance with Mandate
- Article 12(3) and the Art. 7 (4) (Preventive killing) of the Delegated Regulation
- Article 57 of the Regulation (EU) 2016/429

The following elements of the scenario should be taken into consideration during for the assessment:

1) It concerns to the sampling carried out in a HPAI confirmed establishment (Art. 12(3)), or to a suspect establishment where preventive killing is carried out (Art. 7(4))
2) It refers to kept animals of listed species found dead or when they are being killed
3) The competent authority must collect samples for laboratory examination
4) The purposes of the sampling are:
    a) supporting the epidemiological enquiry to:
        i) identify the likely origin of the disease;
        ii) calculate the likely length of time that the disease is present;
        iii) identify establishments where the animals could have contracted the disease and movements from the affected establishment that could have led to the spread of the disease; and
        iv) obtain information on the likely spread of the listed disease in the surrounding environment, including the presence and distribution of disease vectors
5) confirming/ruling out disease in the event of preventive killing.

Summary of the laboratory sampling procedures as described in the diagnostic manual

No clinical examination is required, standard samples (5 dead birds and/or swabs from 20 sick birds) must be taken according to the guidelines presented in the diagnostic manual for HPAI in each production unit.

Assessment

Standard sampling is of limited use to address the objectives of this scenario (other than in the event of preventive killing) as the farm has been confirmed using the standard sampling; it is therefore

unclear how any additional sampling on the same farm would help with the epidemiological investigation. This sampling is, however, useful to establish how widespread the infection is across the farm in case of multiple production units. In that case the assessment of Section 4.1.1.1 applies. Similarly, in the event of preventive killing, the assessment for Section 4.1.1.1 should apply.

Development of new procedures

Information should be gathered during the epidemiological investigation of the affected establishment regarding contacts with other farms in order to establish the most likely source of introduction, and its spread to other farms. For that purpose, whole genome sequencing (WGS) is important to link outbreaks, and the officially collected samples can be used for that purpose. Additional sampling in production units previously not sampled may contribute to establish the extent of spread on the farm. Sampling in each production unit should be carried out as advocated in Section 4.1.1.1. To establish the length of the infectious period in gallinaceous poultry, the temporal pattern of daily mortality prior to detection can be used to estimate the most likely time window of introduction (Bos et al., 2007). In the future, it may be useful to have samples of dead birds across the establishment/flock for WGS as the genetic variation of the viruses, in combination with molecular clock, may help estimating the introduction time. Blood samples for serology are useful in production units without apparent signs of the infection, as this may help differentiate between a primary incursion of HPAI (e.g. from wild birds) and a mutation from LPAI to HPAI. In the latter case, seropositive birds might be detected. Twenty samples per production unit allows for detection of a 15% seroprevalence with 95% confidence (RiBESS+ tool[5] was used for this calculation).

In Anseriformes, mortality will only be a good indicator in case we are dealing with a strain associated with a high case fatality rate (Schreuder et al., 2020). Given that it takes 2 weeks for an animal to seroconvert, and some extra days (depending on the infection rate parameter) for the infection to spread across the flock, estimating the seroprevalence in such a farm would provide helpful information to test the length of the infectious period. For example, assuming a 50% a priori seroprevalence, taking 43 samples in production units of the affected epidemiological unit would be enough to estimate the prevalence with a 10% precision and 95% confidence (RiBESS+ tool[5] was used for this calculation).

4.1.1.3. For granting a specific derogation from killing animals of the categories of article 13.2 of the Delegated Regulation in an HPAI affected establishment

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species belonging to the categories described in article 13(2) of an affected establishment, in order to grant a specific derogation from killing these animals, while ensuring that they do not pose a risk for the transmission of the disease.

[5] https://efsa.openanalytics.eu/app/ribess

- **3rd Scenario of sampling procedures**
- ToR 1.1 and ToR 1.2 in accordance with Mandate
- Article 13(3)c of the Delegated Regulation

The following elements of the scenario should be taken into consideration for the assessment:

1) It concerns an affected establishment where infection is officially confirmed
2) In the establishment there are kept animals of listed species of the following specific categories animal categories based on article 13(2):
    a) animals kept in a confined establishment
    b) animals kept for scientific purposes or purposes related to conservation of protected or endangered species
    c) animals officially registered in advance as rare breeds
    d) animals with a duly justified high genetic, cultural or educational value
3) the competent authority may grant specific derogation from killing all the animals of listed species belonging to any of the above categories in an affected establishment, provided that specific conditions are fulfilled
4) The animals should be subjected to clinical surveillance, including laboratory examinations
5) Sampling procedures should ensure that the animals do not pose a risk of transmission of the category A disease if left alive

Summary of sampling procedures as described in the diagnostic manual

Twenty-one days after the last finding of HPAI, a clinical inspection and examination must be performed, and samples for laboratory testing must be taken from each production unit. Samples include any dead poultry or other captive birds present at the time of sampling and, where practical, tracheal/oropharyngeal and cloacal swabs from at least 60 poultry or other captive birds or from all such poultry or other captive birds where less than 60 are present on the holding; or, if the birds are small, exotic and not used to being handled or handling them would be dangerous for people, samples of fresh faeces must be collected. Derogations for sampling may be granted based on the risk assessment. The sampling and laboratory testing of such samples must continue until two consecutive negative laboratory results are obtained which must be at least 21 days apart.

Assessment

Given that a HPAI outbreak is usually associated with increased mortality, collecting and testing samples of any dead poultry or captive bird is appropriate. This will most likely be sufficient for gallinaceous poultry (see Section 4.1.1.1), but for many other species, the case fatality rate of HPAI might be quite low. Consequently, additional testing is useful in these instances.

The current procedure aims at detecting a 5% prevalence with 95% confidence. There is no information available to know whether the prevalence of virus positive birds will exceed the 5% level at the establishment level, should infection and transmission occur. The population dynamics of the infection are unknown in most captive birds and in particular when the HPAI infection behaves like an LPAI infection and there is no random mixing of the birds, as a result the testing of swabs for viral RNA may be insufficient. Serology could add in confidence of freedom in those cases, which is also increased by requiring two subsequent negative tests with 21 days interval as the diagnostic manual establishes.

Development of new procedures

It is recommended to maintain the current procedure, although serological testing could be added when possible.

4.1.1.4. For the animals of non-listed species kept in an HPAI affected establishment

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of non-listed species kept in an affected establishment, in their ability to ensure the detection of the virus if the virus is present in these species.

- **4th Scenario of sampling procedures**
- TOR 1.1 and TOR 1.2 in accordance with Mandate
- Article 14(1) of the Delegated Regulation
- Article 57 of the Regulation (EU) 2016/429
- Commission Implemented Regulation 2018/1882 on listed species

The following elements of the scenario should be taken into consideration for the assessment:

1) It concerns an affected establishment officially confirmed
2) In the affected establishment there are kept animals of non-listed species of epidemiological relevance for the control of the disease
3) Animals of non-listed species are those animals that are not listed in Commission Implementing Regulation (EU) 2018/1882 for each of the category A diseases
4) The animal species acting purely as mechanical carriers of the virus will not be covered
5) The competent authority is not obliged to carry out the sampling of non-listed species, but they may establish it in addition to other measures
6) The purpose of the sampling procedures is to ensure detection of the virus in these species

Summary of sampling procedures as described in the diagnostic manual

This scenario is in particular targeted at pigs, because they are considered the domesticated mammalian host most likely to become infected by an avian influenza virus. Moreover, reassortment with swine influenza viruses could take place (Bourret, 2018). The guidelines prescribe an inspection of the production records and clinical inspection of the animals on the establishments. Given the similarities of avian influenza viruses to swine influenza viruses, respiratory problems and fever are the most likely signs, should the infection be clinically manifest. For sampling, it is prescribed that 60 pigs are tested for presence of the virus, and at least 60 blood samples must be collected from the pigs for serology, 2–4 weeks from the date of culling. Similar procedures are described for the movement of pigs.

Where the official veterinarian has a suspicion that other domestic mammals on the holdings, in particular those with identified susceptibility to infection with avian influenza viruses of H5 and H7 subtypes, may have been in contact with the infected poultry or other captive birds (or the products thereof), samples for laboratory tests must be taken. Of the other common species, equines and minks are also relevant (Parrish et al., 2015; Short et al., 2015). Given observations that cats may be infected with H5N1 avian influenza virus and can transmit it to other cats experimentally (Harder and Vahlenkamp, 2010), sampling dead cats is also prescribed.

Assessment

The current procedure aims at detecting a 5% prevalence with 95% confidence. There is no information available to assess whether a 5% prevalence of virus positive pigs will be reached should infection and transmission occur, as the population dynamics of the infection in pigs are unknown (as it is an avian strain and not swine influenza). However, the serological testing may be useful as a proxy of the cumulative incidence in addition to testing for virus, which would allow assessing whether extensive transmission has occurred. In the existing guidelines, pigs must be serologically sampled between 2 and 4 weeks from the date of culling. Nonetheless, should infection have occurred at the culling day, it is highly unlikely that 14 days later a 5% seroprevalence will have been reached in a reasonably sized pig herd, unless massive virus introduction would have taken place. The period of 14 days after the date of cull seems therefore too short.

Restricting testing of cats to dead cats seems justified given that infection in cats has only been detected in dead cats associated with outbreaks in poultry (Harder and Vahlenkamp, 2010). This implies that they act as a spill over host and not a reservoir host. Similarly, testing of other potentially susceptible species like mink and equines should be restricted to animals showing clinical signs or that have died.

Development of new procedures

A risk assessment should be made on the farm to assess the exposure of the non-listed species. Swab sampling for virus detection should be focused at pigs that are clinically ill (respiratory signs, fever), and those most intensively exposed to infected poultry (e.g. located near the poultry, fed with broken eggs etc.). This will increase the likelihood of detecting the virus. Serological testing should also focus on those animals that have been most intensively exposed to infected poultry (e.g. close vicinity or feeding of broken eggs). Serological testing of pigs should not be done before 4 weeks after culling of the poultry. Should infection have been introduced in the pig herd around the time of culling, this would allow for a few generations of infected pigs and their time to seroconversion to reach a detectable level of 5%. Clinically affected mink and equines on affected poultry establishments should be sampled in a similar way as cats provided the risk assessment would indicate possible exposure.

4.1.1.5. For wild animals of the listed species within the HPAI affected establishment and its surroundings

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the wild animals of listed species within the affected establishment and in its surroundings. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these wild species.

- **5th Scenario of sampling procedures**
- TOR 1.1 and TOR 1.2 in accordance with Mandate
- Article 14(1) of the Delegated Regulation
- Article 57 of the Regulation (EU) 2016/429
- Commission Implemented Regulation 2018/1882 on listed species

The following elements of the scenario should be taken into consideration during for the assessment:

1) It concerns an affected establishment officially confirmed
2) They may exist wild animals of listed species within the establishment and in the surroundings of the establishment
3) The competent authority may establish these sampling procedures in addition to other measures
4) The purpose of the sampling procedures in wild animals of listed species is to ensure the detection of the virus, if the virus is present in these wild species

Summary of the sampling procedures as described in the diagnostic manual

No guidelines available in the diagnostic manual.

Development of new procedures

Testing any dead animals of the listed species would be useful. Guidelines for carrying out passive surveillance in wild birds should be followed. Birds included in the list of target species of interest for HPAI defined by EFSA should be prioritised for sampling (EFSA, 2017).

4.1.1.6. For animals of listed species in the non-affected establishments located in a protection zone

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species in establishments located in the protection zone up to 3 km radius. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these animals.

- **6th Scenario of sampling procedures**
- TOR 1.1 and TOR 1.2 in accordance with Mandate
- Article 26(2) of the Delegated Regulation

The following elements of the scenario should be taken into consideration during for the assessment (See also the table in Annex B):

1) It concerns the protection zone with radius up to 3 km
2) Official veterinarians must visit at least once all the non-affected establishments with kept animals of listed species located in the protection zone
3) Among others, they must perform a clinical examination of kept animals of listed species and if necessary, a collection of samples for laboratory examination
4) The purpose of sampling procedures is to confirm or rule out the presence of a category A disease

Summary of sampling procedures as described in the diagnostic manual

Clinical inspection in the protection zone is prescribed as in Section 4.1.1.1 and given suspicion is raised, standard sampling is applied. If there are no clinical signs, the standard samples must be taken 21 days following the date of the last suspected contact with an infected holding or when the poultry or other captive birds are killed.

Assessment

In case of the presence of clinical signs, the assessment described in Section 4.1.1.1 applies.

In case of no signs, standard sampling in gallinaceous poultry is considered non-informative, as the case fatality of HPAI is always very high, so the likelihood of detecting the virus in apparently healthy birds in a flock where no dead birds or sick birds are present is extremely low. Clinical investigation will be sufficient to confirm the presence of HPAI.

However, HPAI virus may induce mild or subclinical infection in Anseriformes; in this case sampling of healthy birds is recommended similar to Section 4.1.1.1.

Development of new procedures

As per Section 4.1.1.1.

4.1.1.7. For non-affected establishments located in a surveillance zone

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species, for the sampling of the establishments located within the surveillance zone. The purpose of the sampling procedure is to ensure disease detection if the virus is present in establishments within the surveillance zone.

- **8th Scenario of sampling procedures**
- ToR 1.3 in accordance with Mandate
- Article 41 of the Delegated Regulation

The following elements of the scenario should be taken into consideration during for the assessment (See also the table in Annex B):

1) It concerns the surveillance zone
2) Refers to the sampling of establishments with kept animals of the listed species in the surveillance zone
3) Official veterinarians carry out visits to a sample of the establishments among others to perform clinical examination of kept animals of listed species and if necessary, collection of samples for laboratory examination
4) The purpose of sampling procedure is to ensure the detection of the disease if the disease is present in any of the establishments

Summary of the sampling procedures as described in the diagnostic manual

The establishments to be visited are those in which increased morbidity, mortality or where change in production data has been reported.

After investigating the production records and carrying out a clinical inspection, standard samples must be taken from each production unit on those establishments that report increased mortality, morbidity or change in production.

Assessment

The number of establishments that should be sampled within a surveillance zone may be limited to those, where an increased morbidity, mortality or where a change in production data has been reported. Because of this, the assessment would be similar to Section 4.1.1.1.

Development of new procedures

For Galliformes, no new procedures are needed. Due to the quick development of the disease, and the extremely high mortality usually observed, HPAI can be easily detected by farmers; raising awareness about the importance of early reporting of suspicions among poultry owners and keepers in the zone would be primordial. The management of a suspicion would therefore be similar to Section 4.1.1.1. For Anseriformes, relying on increased awareness might not be sufficient in case of a virus strain that causes mild or no signs of infection in these species. In view of the objective to find any infected establishment in the surveillance zone, establishments with Anseriformes in the surveillance zone should be visited and sampled similar to those in the protection zone (Section 4.1.1.6). Alternatively, farmers can be encouraged to collect dead birds to be pooled and tested weekly (bucket sampling). Table 5 in Section 4.1.1.1 shows that even in cases where there is no case fatality due to the virus, testing dead animals (irrespective of the cause of death) would lead to HPAI detection if the virus is present in the flock with a median time between introduction and detection of 6–11 days. Bucket sampling might even be more sensitive than shown in Table 5 as comorbidity of HPAI will likely reduce the survival probability to other diseases. In addition, Anseriformes farms could be tested serologically before the surveillance zone is lifted (60 samples per farm, 3–4 weeks after the last outbreak).

4.1.2. Assessment of sampling procedures to grant derogations for animal movements

4.1.2.1. From non-affected establishments located in the protection zone to slaughterhouses located within the protection zone or in the surveillance zone or outside the restricted zone

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant a derogation from prohibitions in the movement of animals, and allow for the animals to be moved to a slaughterhouse located within the protection zone or in the surveillance zone or outside the restricted zone (Art29).

- **9th Scenario of sampling procedures**
- ToR 1.4 in accordance with Mandate
- Article 28(5) of the Delegated Regulation
- Article 29 of the Delegated Regulation

The following elements of the scenario should be taken into consideration for the assessment:

1) It concerns the protection zone
2) It regards to the granting of a derogation for the movement of kept animals of the listed species from a non-affected establishment in the protection zone
3) Animals to be moved to a slaughterhouse located within the protection zone or in the surveillance zone or outside the restricted zone
4) Clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved

Summary of sampling procedures as described in the diagnostic manual

An investigation of production records and a clinical investigation should take place before moving the animals to the slaughterhouse. Moreover, upon arrival at the slaughterhouse, a detailed investigation should take place by an official veterinarian. The number of samples to be taken from each production unit, less than 48 h prior to the time of departure of the poultry, should be based on the outcome of a risk assessment, but at least 60 tracheal/oropharyngeal and/or 60 cloacal swabs must be sampled.

Assessment

The procedure ensures that clinical signs of HPAI infection will be detected, and the sampling procedure ensures that a 5% prevalence will be detected with 95% confidence. In case of gallinaceous poultry, the testing will add little to the clinical inspection of the farm, which is much more informative due to the rapid onset and severe nature of the disease in those species.

Development of new procedures

For gallinaceous poultry, clinical inspection on the day of the movement (and not at 48 h as required in the diagnostic manual) would reduce the probability of virus transmission as HPAI might develop very rapidly in such flocks (see Table 4 in Section 4.1.1.1). In the event that no clinical signs are seen in gallinaceous poultry and provided production records indicate no issues at the time of movement, sampling could be omitted. In contrast, in Anseriformes, clinical manifestation may be mild or even absent, so the sampling as per diagnostic manual is recommended. In addition to the sampling prior to the movement, the probability of moving infected birds could be further reduced by weekly bucket sampling and testing (collecting dead birds in Anseriformes farms, pooling them and testing them weekly (EFSA AHAW Panel et al., 2017)) (see Section 4.1.1.7).

4.1.2.2. For day-old-chicks (DOC) from a non-affected establishment located in the protection zone, hatched from eggs originating in or outside the restricted zone to an establishment located in the same Member State but if possible, outside the restricted zone

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of day-old-chicks (DOC) located in the protection zone and hatched from eggs originating in the restricted zone or outside the restricted zone. The sampling procedures should ensure the movement of these day-old-chicks to an establishment located in the same Member State but if possible, outside the restricted zone.

- **10th Scenario of sampling procedures**
- ToR 1.4 in accordance with Mandate
- Article 28(5) and article 30(1) of the Delegated Regulation

The following elements of the scenario should be taken into consideration during for the assessment:

1) It concerns the protection zone
2) It regards to the granting of a derogation for the movement of day-old-chicks from a non-affected establishment in the protection zone hatched from eggs originating in or outside the restricted zone
3) Day-old-chicks to be moved to an establishment located in the same Member State but if possible, outside the restricted zone

Summary of sampling procedures as described in the diagnostic manual

For the movement of day-old-chicks (DOC), no inspection or testing is required. For the movement of hatching eggs, inspection of the parent flock and standard sampling is required.

Assessment

The risk of spreading HPAI by DOC is considered very low (probability range) (EFSA AHAW Panel, 2017), because the virus is highly lethal for the embryo (preventing hatching), and the eggs are disinfected before hatching. Infection will be noted in the hatchery and, moreover, as it takes at least 3 weeks between collecting the eggs and hatching, in gallinaceous poultry from the infected parent

flock will have been detected before any DOC will have been moved. However, this may be different for Anseriformes if the course of infection is mild.

Development of new procedures

In the absence of clinical signs during clinical inspection, standard sampling of gallinaceous parent flocks could be omitted. In addition, in Anseriformes, parent flocks testing could be expanded by bucket sampling and serological testing (EFSA AHAW Panel, 2017).

4.1.2.3. For ready-to-lay poultry from a non-affected establishment located in the protection zone to establishments located in the same MS and if possible, within the restricted zone

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the protection zone to establishments located in the same MS and if possible within the restricted zone.

- **11th Scenario of sampling procedures**
- ToR 1.4 in accordance with Mandate
- Article 28(5) and article 30(2) of the Delegated Regulation

The following elements of the scenario should be taken into consideration during for the assessment:

1) It concerns ready-to-lay poultry in non-affected establishments in the protection zone
2) It regards to the granting of a derogation for the movement of ready-to-lay poultry from a non-affected establishment in the protection zone
3) Ready-to-lay poultry to be moved to an establishment located in the same Member State and if possible, within the restricted zone

Summary of sampling procedures as described in the diagnostic manual

As per Section 4.1.2.1.

Assessment

In this scenario, the consequences of missing the disease may be larger as birds are not sent to slaughter but moved to another establishment.

Development of new procedures

The recommendations would be similar to those in Section 4.1.2.1. In addition, follow-up investigation of the recipient farm would be useful. In gallinaceous poultry that would require collecting information of any clinical manifestation. For Anseriformes that could include weekly bucket sampling and serological testing 4 weeks after moving the birds.

4.1.2.4. From non-affected establishments located in the protection zone to a plant approved for processing or disposal of animal by-products in which the animals are immediately killed

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant derogation from prohibitions in the movement of these animals to a plant approved for processing or disposal of animal by-products in which the kept animals are immediately killed (Art37).

- **12th Scenario of sampling procedures**
- ToR 1.4 in accordance with Mandate
- Article 28(5) and article 37 of the Delegated Regulation

The following elements of the scenario should be taken into consideration for the assessment:

1) It concerns the protection zone
2) To grant derogation for movement of kept animals of listed species from a non-affected establishment in the protection zone
3) The animals to be moved to a plant approved for processing or disposal of animal by-products in which the kept animals are immediately killed
4) Clinical examinations and laboratory examinations of animals kept in the establishment, including those animals to be moved

Summary of sampling procedures as described in the diagnostic manual

No specific sampling procedures are present in the diagnostic manual.

Assessment

As per Section 4.1.2.1.

Development of new procedures

As per Section 4.1.2.1.

4.1.2.5. From an establishment in a surveillance zone to a slaughterhouse located within or outside the restricted zone and from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of listed species in order to grant derogation from prohibitions and allow for these animals to be moved: (a) from an establishment in a surveillance zone to a slaughterhouse located within or outside the restricted zone, (b) from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone.

- **13th Scenario of sampling procedures**
- ToR 1.4 in accordance with Mandate
- Article 43(5) and article 44 of the Delegated Regulation

The following elements of the scenario should be taken into consideration during for the assessment:

1) It concerns kept animals of listed species of the establishments in the surveillance zone
2) To grant derogation for movement from an establishment in the surveillance zone to be moved to a slaughterhouse within the restricted zone or outside the restricted zone
3) To grant derogation for movement from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone
4) Clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved

Summary of sampling procedures as described in the diagnostic manual

No specific sampling procedures are present in the diagnostic manual.

Assessment

Although the a priori risk of infection in the surveillance zone should be lower than in the protection zone (this is the case under the assumption of transmission increasing with decreased distance to an outbreak); nonetheless, the transmission kernel results shown in Table 9 Section 4.3.1 indicate that the probability of transmission of HPAI beyond 3 km is 52–33% (depending on the Kernel selected). Because of this, the assessment would be the same as the assessment for the movement of birds from establishments located in the protection zone (Section 4.1.2.1). For farms outside the surveillance zone moving animals into the surveillance zone, no added inspection or testing would be needed.

Development of new procedures

As per Section 4.1.2.1.

4.1.2.6. For day-old-chicks from a non-affected establishment located in the surveillance zone, to an establishment located in the same Member State where they were hatched

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations to grant derogation of movements of day-old-chicks hatched from establishment located in the surveillance zone, from eggs originating within the surveillance zone and eggs originating outside the restricted zone, to an establishment located in the same Member State where they were hatched.

- **16th Scenario of sampling procedures**
- ToR 1.4 in accordance with Mandate
- Article 43(5) and article 46(1) of the Delegated Regulation

The following elements of the scenario should be taken into consideration during for the assessment:

1) It concerns the surveillance zone
2) To grant derogation for movement of day-old-chicks hatched from establishment located in the surveillance zone, from eggs originating from establishment within the surveillance zone or eggs originating from outside the restricted zone
3) To be moved to an establishment located in the same Member State
4) Clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved

Summary of sampling procedures as described in the diagnostic manual

No specific sampling procedures are present in the diagnostic manual.

Assessment

As per Section 4.1.1.2.

Development of new procedures

As per Section 4.1.1.2.

4.1.2.7. For ready-to-lay poultry located in the surveillance zone to establishments located in the same MS

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the surveillance zone to establishments located in the same MS.

- **17th Scenario of sampling procedures**
- ToR 1.4 in accordance with Mandate
- Article 43(5) and article 46(2) of the Delegated Regulation

The following elements of the scenario should be taken into consideration during for the assessment:

1) It concerns the surveillance zone
2) Ready-to-lay poultry of an establishment in the surveillance zone
3) To be moved to an establishment located in the same Member State
4) Clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved

Summary of sampling procedures as described in the diagnostic manual

No specific sampling procedures are present in the diagnostic manual.

Assessment

As per Section 4.1.2.3.

Development of new procedures

As per Section 4.1.2.3.

4.1.2.8. From an establishment located in the restricted zone to move within the restricted zone when restriction measures are maintained beyond the period set out in Annex XI of the Delegated Regulation

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment located in the restricted zone of an outbreak in order to allow their movement within the restricted zone, when restriction measures are maintained beyond the period set out in Annex XI of the Delegated Regulation.

- **18th Scenario of sampling procedures**
- ToR 1.4 in accordance with Mandate
- Article 56(1) of the Delegated Regulation

The following elements of the scenario should be taken into consideration during for the assessment:

1) It concerns the restricted zone when restriction measures are maintained beyond the period set out in Annex XI
2) To grant derogation for movement of kept animals of listed species from an establishment within the restricted zone
3) Clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved

Summary of sampling procedures as described in the diagnostic manual

No specific sampling procedures are described in the diagnostic manual

Assessment

As per Section 4.1.2.3.

Development of new procedures

As per Section 4.1.2.3.

4.1.3. Assessment of sampling procedures for repopulation purposes

4.1.3.1. For the animals that are kept for the repopulation prior to their introduction

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that are kept for the repopulation prior to their introduction to rule out the presence of the disease.

- **19th Scenario of sampling procedures**
- ToR 1.5 in accordance with Mandate
- Article 59(2) of the Delegated Regulation

The following elements of the scenario should be taken into consideration during for the assessment:

1) It concerns the repopulation of a previous affected establishment
2) Animals intended for repopulation shall be sampled prior to their introduction into the establishment of destination
3) The samples shall be collected from a representative number of animals to be introduced of each consignment from each establishment or from a representative number of animals of each consignment (if animals are all to be introduced at different times or from different establishments of origin)
4) Laboratory examinations
5) The purpose sampling procedures is to rule out the presence of the disease

Summary of sampling procedures as described in the diagnostic manual

The diagnostic manual estates that at least 20 blood samples should be tested as soon as the poultry have been placed in the holding except in the case of day-old chicks; if appropriate, such sampling may be performed on the holding of origin of the poultry before movement to the holding for repopulation.

According to the Delegated Act (Art. 59 (2), (3) and (9), samples shall be collected from:

- a representative number of all the animals to be introduced in the establishment, if they are all introduced at the same time and from the same establishment of origin; or
- a representative number of animals of each consignment, if animals are all to be introduced at different times or from different establishments of origin.

In the case of day-old-chicks, the competent authority may decide not to perform the sampling for laboratory examination.

Assessment

If the birds originate from a disease-free area, sampling would not be needed. However, collecting samples of 20 birds could be useful to detect antibodies induced by an LPAI infection that may not be detected based on clinical signs. Such antibodies could prevent a clinical manifestation of infection of the birds in case of ongoing infection. Because of this, the assessment remains the same as per Section 4.1.1.5.

Development of new procedures

Not needed

4.1.3.2. In the event of unusual mortalities or clinical signs being notified during the repopulation

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, in the event of unusual mortalities or clinical signs being notified during the repopulation, to rule out the presence of the disease.

- **20th Scenario of sampling procedures**
- ToR 1.5 in accordance with Mandate
- Article 59(9) of the Delegated Regulation

The following elements of the scenario should be taken into consideration during for the assessment:

1) It concerns the repopulated establishment
2) Unusual mortalities or clinical signs during the repopulation
3) The official veterinarians shall without delay collect samples for laboratory examination
4) The purpose of sampling procedures is to rule out the presence of the disease

Summary of sampling procedures as described in the diagnostic manual

Samples of dead poultry or swabs taken from their carcasses from a maximum of 10 dead birds per week during the 21-day period from the date of the re-population.

Assessment

Testing 10 dead birds will detect HPAI infection with a high probability (see Tables 4 and 5 in Section 4.1.1.1). In addition, testing tracheal and cloacal swabs of 20 birds results in a high probability of detection (See Figure 2 in Section 4.1.1.1). When dealing with a strain with a very mild infection, additional samples may be required.

Development of new procedures

The protocol described in the diagnostic manual may be kept, but further testing is recommended in waterfowl when dealing with a virus strain that induces mild infection. In this situation, it would be recommended to test 60 healthy birds (tracheal and cloacal swabs) within the last week of the 21-day period.

4.1.3.3. For repopulated animals

The purpose of this section is to assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, on the last day of the monitoring period calculated forward from the date on which the animals were placed in the repopulated establishment. In case the repopulation takes place in several days, the monitoring period will be calculated forward from the last day in which the last animal is introduced in the establishment.

- **21st Scenario of sampling procedures**
- ToR 1.5 in accordance with Mandate
- Article 59(5) of the Delegated Regulation

The following elements of the scenario should be taken into consideration during for the assessment:

1) It concerns the repopulated establishment
2) Animals that have been used for repopulation
3) Laboratory examinations
4) Sampling procedures to rule out the presence of the disease

Summary of the sampling procedures as described in the diagnostic manual

Samples (or swabs) of 10 dead birds must be tested, and in waterfowl 20 tracheal and 20 cloacal swabs should be taken within the last week of the 21-day period.

Assessment

Same as per Section 4.1.3.2.

Development of new procedures

Same as per Section 4.1.3.2.

4.2. Assessment of the length of the monitoring period for HPAI

The concept of the monitoring period was introduced as a management tool for the investigation and control of suspected and confirmed outbreaks of Category A diseases in terrestrial animals. This tool aimed to standardise the methodology by which relevant authorities responded to suspected and confirmed cases of these diseases. In this regard, a disease-specific monitoring period was set for each of the 14 diseases included in the Category A list. Throughout the EU legislation, the monitoring period is used as an aid in the control of these diseases, although the specific purpose in which the monitoring period is used varies depending on the articles of the legislation.

The length of the monitoring period for each disease is set out in Annex II of the Commission Delegated Regulation supplementing the rules laid down in Part III of Regulation (EU) 2016/429 (Animal Health Law).

Annex D in this Opinion describes the seven scenarios for which an assessment of the length of the monitoring period had been requested. Only six scenarios were assessed, as scenario number 5 (related to the movement of frozen semen) was considered not relevant in relation to HPAI.

For the assessment of ToR 2, the methodology described in Section 2.3 of the Technical Report published by EFSA was followed (EFSA, 2020a). Some details of the methodology are presented in this opinion in Section 3.2. In order to assess the length of the monitoring period, the purpose of this monitoring period for each of the scenarios was ascertained.

To answer the ToR, an ELS on the average, shortest and longest period of time between the earliest point of infection of a bird with a HPAI virus, and the time of reporting of a suspicion by the competent authority, was carried out. The time period between reporting of a suspicion and the notification of the disease was also assessed. Several outcomes were designed for the ELS, and the results are presented below.

4.2.1. Results

A total of 1,090 references published after 01/01/2000 were retrieved. Among these references, 48 were selected to be included in the qualitative review. The full selection process is displayed in Figure 3.

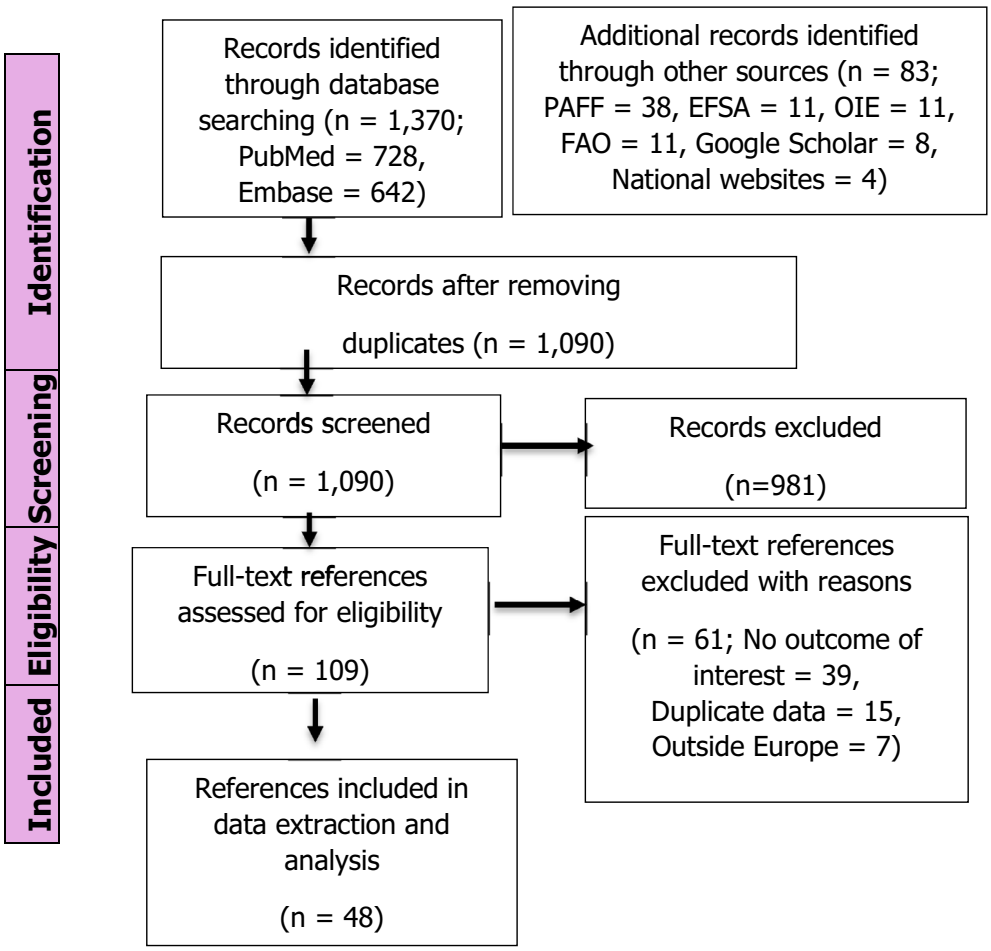

Figure 3: PRISMA diagram for the extensive literature search on monitoring period for highly pathogenic avian influenza

Information retrieved by the ELS is summarised in Tables 6 and 7. Table 6 shows the information retrieved for the main outcome of interest (period between the earliest point of infection and the reporting of a suspicion).

Table 6: Summary of HPAI literature extraction for the periods between earliest point of infection and suspicion report/confirmation

Reference	Country	Outbreak year	Species	Period between earliest point of infection and suspicion report (days)
Bos et al. (2007)	Netherlands	2003	Chickens	12(1)
Hobbelen et al. (2020)	Netherlands	2014 2016 2016	Chickens Chickens Ducks	9.8; 11.8; 14.8(2) 5.9; 7.4(2) 9.5; 14.5; 18.8(2)
APHA (Animal & Plant Health Agency) (2015)	United Kingdom	2015	Chickens	11(3)

(1): The mode.
(2): The mean of the period were estimated using SEIR models and within-flock mortality data, and based on the following detection rule: ≥ 0.5% mortality on each of two consecutive days.
(3): Strong suspicion of first infection in an outbreak following contact with wild birds infected with LPAI followed by a mutation event in poultry to HPAI (based on molecular epidemiology studies).

For HPAI outbreaks in chickens, the shortest period between the earliest point of infection and the suspicion report was estimated at 5.9 days by Hobbelen et al. (2020) using within-flock mortality data from the 2016 H5N8 outbreak in a layer farm in the Netherlands. On the other hand, the longest period, 14.8 days, was estimated by the same authors using within-flock mortality data from the 2014 outbreak.

For HPAI outbreaks in ducks, the only data retrieved were from Hobbelen et al. (2020) based on mortality data from the 2016 H5N8 outbreaks in three meat duck farms. These results suggest that the period between the earliest point of infection and the suspicion report is longer in duck than in chicken HPAI outbreaks. Indeed, the mean period for ducks was estimated between 9.5 and 18.8 days. Based on these data, we calculated that the period between the earliest point of infection and the suspicion report in duck HPAI outbreaks was on average 14.3 days (median = 14.5 days).

In summary and based on the data shown in Table 6:

- Average period = 10.3 days (chickens) and 14.3 days (ducks)
- Shortest period = 5.9 days (chickens) and 9.5 days (ducks)
- Longest period = 14.8 days (chickens) and 18.8 days (ducks)

In addition, other periods reported in the literature are described in Table 7. In Table 7, 10 values of periods between the earliest point of infection and confirmation (AH - RC Animal Health - Regulatory Committee, 2017c; APHA, 2017) were also extracted, the values ranging from 8 to 25 days. As this period includes the period between suspicion report and confirmation (median/mean = 2 days, as calculated from the articles retrieved), an average delay between the earliest point of infection and suspicion report of 14 days (1–25 days) was estimated.

Table 7: Summary of the HPAI extraction for the delays in the outbreak reporting process References (Ref.), number of extracted values (n), median, mean, minimal (min) and maximal (max) values

Delay (days)	Ref.	n	Median	Mean	Min	Max
Earliest point of infection and confirmation	Animal Health - Regulatory Committee (2017c); APHA (2017)	10	16	16	8	25
Suspicion report and confirmation	Animal Health - Regulatory Committee (2010, 2014a,b); OIE (World Organisation for Animal Health) (2014); Animal Health - Regulatory Committee (2015a, 2015b, 2015c, 2015d, 2015e); APHA (2015a, 2015b); Animal Health - Regulatory Committee (2016a, 2016b, 2016c, 2016d, 2016e, 2016f, 2016g, 2017a, 2017b, 2017d, 2017e, 2017f, 2017g, 2017h, 2018a, 2018b, 2020a, 2020b, 2020c)	29	2	2	0	7

4.2.2. Assessment

Considering the results presented above, an assessment of the effectiveness of the monitoring period for HPAI, depending on the purpose of that period in the different scenarios shown in Annex D, was carried out. For HPAI, the length of the existing monitoring period is 21 days.

Scenarios 1, 2 and 3

- **1st Scenario of monitoring period**
- ToR 2 in accordance with article 8 and Annex II of the Delegated Regulation
- Article 57 of the Regulation (EU) 2016/429
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of the notification of the suspicion of a category A disease in an establishment with kept animals of listed species, for the purposes of the epidemiological enquiry in the event of a suspicion of a HPAI outbreak

- **2nd Scenario of monitoring period**
- ToR 2 in accordance with article 17(2) and Annex II of the Delegated Regulation
- Article 57 of the Regulation (EU) 2016/429
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of notification of the suspicion of a category A disease in an establishment with kept animals of listed species, for the purposes of the epidemiological enquiry in the event of confirmation of a HPAI outbreak

- **3rd Scenario of monitoring period**
- ToR 2 in accordance with article 13(b) and Annex II of the Delegated Regulation
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of confirmation of a HPAI outbreak in an epidemiological unit in which the disease has not been confirmed, in order to provide derogations from killing the animals in these unit, if this unit has been completely separated, and handled by different personnel during this monitoring period

For the first third scenarios, the main purpose of the use of the monitoring period is to be able to carry a full epidemiological investigation (i.e. in Scenarios 1 and 2, at the time of the suspicion and confirmation, respectively), or part of the epidemiological investigation (i.e. scenario 3, where the aim is to identify any possible epidemiological links between the affected establishment and any separated non-affected epidemiological units). The length of the monitoring period should then dictate how far back or forward the activities related to tracing (and other activities needed during an epidemiological investigation) should go (checks for production records, animal movement records, etc.). This period of time is the time where the infection could have been present undetected in an establishment, and due to the regular activities carried out in this establishment, could have spread to other epidemiological units. In the case of scenario 3, if no epidemiological links between the establishment that has been confirmed positive and the other epidemiological units are found during the investigation, and only if other conditions described in the legislation are met, a derogation from killing the animals in the separated non-affected epidemiological units could be granted.

The period of time when the disease could have been present unknowingly in an establishment, equates then to the time period between the entry of the HPAI virus in the establishment and the reporting of the suspicion. Once the suspicion has been officially reported, control measures are implemented, and further spread is in this way prevented.

Based on the results from Table 6, the period between the earliest point of infection and the suspicion report in chicken HPAI outbreaks was on average 10.3 days with a maximum of 14.8 days.

In terms of the effectiveness of the length of the existing monitoring period for chicken HPAI outbreaks, the Panel Members consider the existing length of the monitoring period (21 days) effective.

This assessment remains for scenario 3 even if the start of the monitoring period is counted from the date of confirmation (instead of date in which the suspicion is reported) of the outbreak, as the

average time between suspicion and confirmation is 2 days based on our results. This will lead to an average length between the first entry and the confirmation of 12.3 days for chickens.

In terms of the effectiveness of the length of the existing monitoring period for ducks, and in general for Anseriformes HPAI outbreaks, the ELS carried out reflects the lack of literature addressing the time between disease entry and the reporting of the suspicion. From the available data, the mean period for ducks was estimated between 9.5 and 18.8 days, with an average of 14.3 days. Based on these results, the Panel Members consider the existing length of the monitoring period effective. Nonetheless, this assessment should be taken cautiously due to the data being retrieved from a unique reference. Also, it must be noted that clinical signs in Anseriformes are not always obvious or present. This would likely result in longer periods (compared to chickens and other species in the Galliformes order), where the disease could have been unknowingly present in the establishment. Serological investigation could provide more information in those cases (see Section 4.1.1.2), but a precise length of this period cannot be established (see H5N1 in France 2015/16).

To reduce the uncertainty in the length of the period between introduction and the suspicion report for Anseriformes, it is recommended to collect further information during future outbreaks.

Scenario 4

- **4th Scenario of monitoring period**
- ToR 2 in accordance with article 27(3)c and Annex II of the Delegated Regulation
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of notification of the suspicion of the latest HPAI outbreak in the protection zone. Products or other materials likely to spread the disease, must had been obtained or produced, before this time period in order to be exempted from prohibitions of movements

The main purpose of the monitoring period in scenario 4 is to ensure that certain products or materials, likely to spread the disease that have been produced in a non-affected establishment located in the protection zone of an affected establishment, can be moved safely and without posing a risk of disease spread. In this scenario, and in contrast with the previous three scenarios, the establishment of concern is not either a suspect establishment or an affected establishment. For the assessment of this scenario, we assume that the earliest plausible point of infection of these products or materials in the establishment of concern would be the earliest plausible point of infection of the establishment that originated the protection zone. If these products have been obtained or produced before the earliest point of infection of the affected establishment, then they could be exempted from prohibitions to be moved, as long as other conditions specified in the legislation are met (e.g. the products must have been clearly separated during the production process, storage and transport, from products not eligible for dispatch outside the restricted zone).

Considering the average length of time between the earliest point of entry and suspicion in establishments with chickens of 10.3 days, and the maximum period of 14.8 days extracted from Table 6 above, the Panel Members consider the existing length of the monitoring period (21 days) effective; the same assessment applies for establishments containing species of the Anseriformes order (average of 14.3 days with a longest period of 18.8 days). As discussed for Scenarios 1, 2 and 3, it is recommended to collect further information during future outbreaks in order to better assess the earliest point of infection in Anseriformes establishments.

Scenarios 6 and 7

- **6th Scenario of monitoring period**
- ToR 2 in accordance with article 57 (1) and Annex II of the Delegated Regulation
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated forward from the date of the final cleaning and disinfection in an HPAI affected establishment, after which the repopulation of the establishment may be allowed by the competent authority (assuming relevant control of insects and rodents was carried out)

- **7th Scenario of monitoring period**
- ToR 2 in accordance with article 59 (4) and Annex II of the Delegated Regulation
- Aim: to assess the effectiveness of the length of the Monitoring Period, as the time period calculated forward from the date the first animal was introduced for the purpose of repopulation after an HPAI outbreak, during this monitoring period, all animals of the listed species intended for repopulation should be introduced

In Scenarios 6 and 7, the monitoring period is used in the context of repopulation. In Scenario 6, the monitoring period is used to ensure that repopulation is not put at risk due to the disease still being present unknowingly in poultry establishments within the surrounding area of the establishment to be repopulated (if an establishment tested positive to HPAI virus within a distance equal or lower to the radius of the surveillance zone, the repopulation process could not take place). Repopulation can only take place after a number of days equal to the monitoring period have elapsed since the final cleaning and disinfection of the affected establishment.

In this regard the number of days of the monitoring period for HPAI, counted from the day of the final cleaning and disinfection must ensure enough time for any potentially infected surrounding establishment to be reported as a suspicion. Considering the results presented above, the Panel Members consider the existing length of the monitoring period (21 days) effective, as it would allow for the identification of any potentially infected establishment in the surrounding area prior to the repopulation taking place.

In Scenario 7, the monitoring period must be counted forwards from the date in which the first animal is introduced into the establishment to be repopulated, with all the animals intended for repopulation of this establishment being introduced within the length of time of this monitoring period.

The aim of the monitoring period in this scenario is to ensure the early detection of any potentially recently infected animal intended for repopulation once they have been moved into the repopulated establishment. Although the preferred option is that all animals or flocks are introduced into the establishment to be repopulated at the same time, this is not always feasible. The first clinical and laboratory sampling of the repopulated animals takes place once all the animals are in situ. By restricting the period of time animals may be introduced into the establishment, the period of time the disease could be unknowingly spreading within the establishment is reduced. Assuming that the latest point of infection of the first poultry batch introduced into the repopulated establishment is the day when the birds are moved, clinically ill birds would be observed at the first visit, if this visit is carried out a number of days equal to the incubation period, or more precisely after the incubation plus notification period (as a minimum prevalence may be needed in order to detect the presence of the disease in the flock). The Panel Members consider the existing length of the monitoring period (21 days) effective, as it would allow for early detection at the first visit following re-stocking of potentially infected birds.

4.3. Assessment of the minimum radius and time periods of the protection and surveillance zones set in place subsequent to a disease outbreak

4.3.1. Assessment of the minimum radius

The purpose of this section is to assess the effectiveness to control the spread of HPAI by implementing a protection and surveillance zones of a minimum radius, as set out in Annex V of the Delegated Regulation, surrounding the establishment where the disease has been confirmed. Based on this regulation, the minimum radius of the protection and surveillance zones for HPAI should be of 3 and 10 km, respectively.

Results

To answer this ToR, transmission kernels have been used to analyse outbreak data for two epidemics of highly pathogenic avian influenza in Europe (Table 8). Transmission kernels show the probability of infection of a farm as a function of the distance to an infected farm. The same functional form for the kernel was used in both cases, namely,

$$k(r) = (1 + (r/d_0)^{\alpha})^{-1}$$

where k is the kernel, r is the distance to an infected farm, d_0 is the distance at which the kernel is reduced by 50%, and α is the parameter controlling how rapidly the kernel declines with distance. The

fitted kernels vary in shape between the epidemics (Figure 4). Of the four kernels fitted to the The Netherland 2003 data, the extended kernel was used for the zone size assessment. In this case, the kernel parameters were estimated allowing for (uninfected) farms in neighbouring countries and so was deemed to be most relevant for the European situation.

Table 8: Transmission kernels for highly pathogenic avian influenza virus

Epidemic	Parameters*		Reference
	d_0 (km)	α	
The Netherlands 2003, default	1.9 (1.1, 2.9)	2.1 (1.8, 2.4)	Boender et al. (2007)
The Netherlands 2003, extended	3.1 (2.3, 4.1)	2.7 (2.5, 3.0)	
The Netherlands 2003, HRP	1.8 (0.7, 2.9)	2.7 (2.4, 6.9)	
The Netherlands 2003, LRP	5.4 (2.8, 9.2)	2.5 (2.0, 3.1)	
Italy 1999–2000	2.2 (1.4, 2.9)	2.1 (1.9, 2.3)	Dorigatti et al. (2010)

*: 95% confidence or credible intervals are shown in brackets.

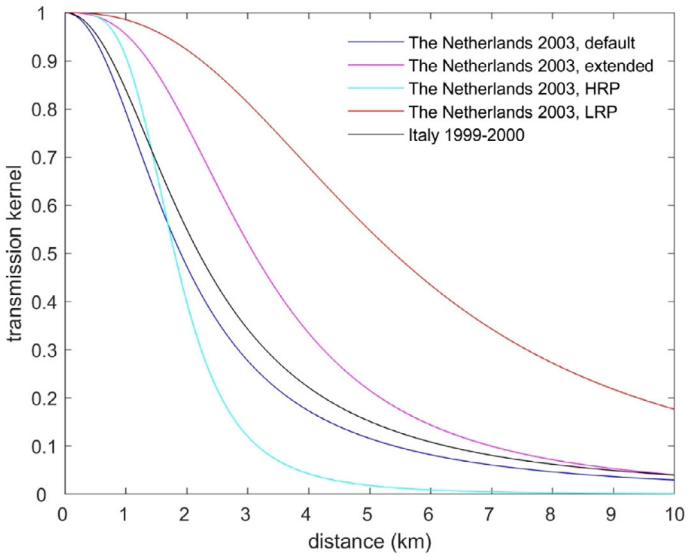

Figure 4: Transmission kernels for highly pathogenic avian influenza virus

For the extended Netherlands and Italian kernels, the probability of transmission beyond given distances (if transmission were to occur from an infected establishment) was computed using the estimates, lower 95% confidence limits and upper 95% confidence limits, including beyond the proposed radius for the protection and surveillance zones (3 km and 10 km, respectively) (Figure 5) (as mentioned above the rest of the kernels from the Netherlands were not used further). In addition, the distances at which a threshold probability of transmission beyond that distance is reached were also calculated for each kernel using the estimates, lower 95% confidence limits and upper 95% confidence limits (Figure 5). The corresponding values computed using the estimates are summarised in Tables 9 and 10.

As expected, we see a clear decrease in infection probability as the distance to an infected farm increases. It is important to note that kernels combine all transmission routes for a virus into a single description. The kernels presented here have been estimated for H7 viruses in epidemics where wild birds did not play an important role. This has the advantage that the kernels result from between farm transmission and are useful for establishing a zone around an infected farm aiming to reduce the risk of spread associated with that particular infected farm. As movement of animals can take place over much larger distances, if included in the kernel estimates, these animal movements will extend the kernel tails. Obviously, the kernels cannot predict the risk of farms to become infected by infected wild birds as their habitat is not restricted to the affected farm.

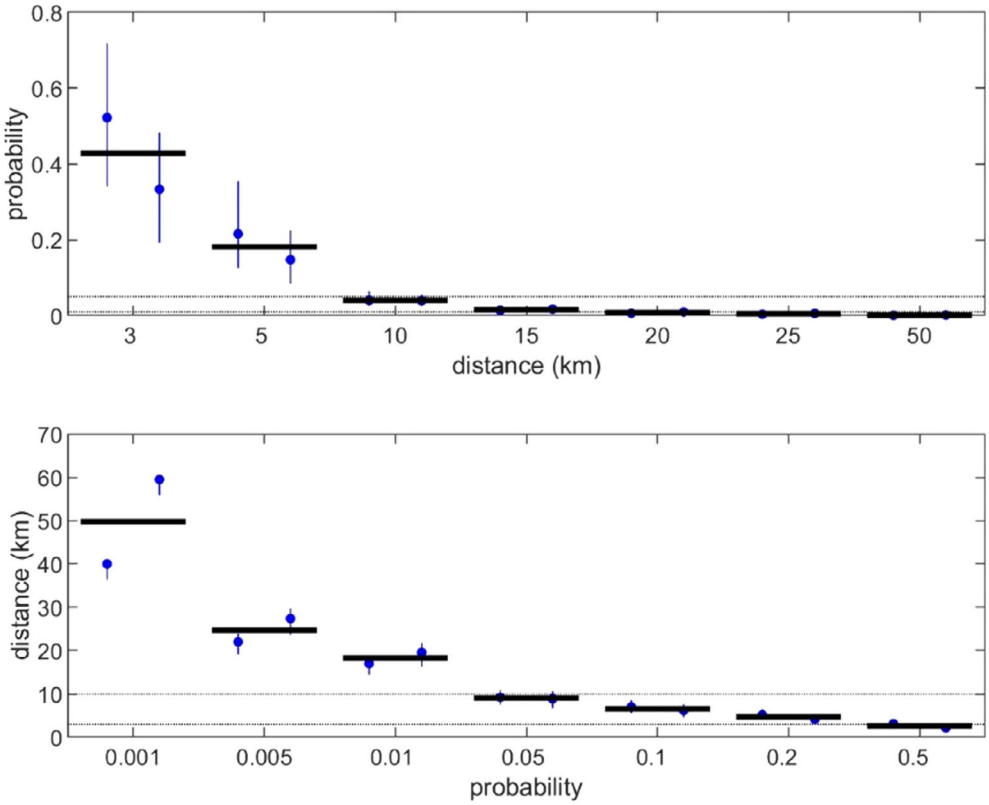

Figure 5: Assessment of the radius of the protection and surveillance zone for highly pathogenic avian influenza virus. The top panel shows the probability of transmission beyond a given distance (if transmission were to occur from an infected establishment) computed using the estimates (blue circles) and the lower and upper 95% confidence limits (error bars) for the extended Netherlands and Italian kernels in Table 8. The thick black line indicates the median probability for all kernels. The black dotted lines indicate threshold probabilities of 0.05 and 0.01. The bottom panel shows the distances at which a threshold probability of transmission beyond that distance is reached calculated using the estimates (circles) and lower and upper 95% confidence limits (error bars) for each kernel. The thick black line indicates the median distance for the two kernels that were assessed. The black dotted lines indicate distances of 3 km and 10 km (i.e. the proposed radius of the protection and surveillance zones, respectively)

Table 9: Probability of transmission of highly pathogenic avian influenza virus beyond different distances

	Distance (km)						
	3	**5**	**10**	**15**	**20**	**25**	**50**
NL 2003	0.52	0.22	0.04	0.01	0.007	0.004	< 0.001
IT 1999	0.33	0.15	0.04	0.02	0.01	0.006	0.001

Table 10: Distances (km) at which the probability of transmission of highly pathogenic avian influenza virus beyond that distance reaches a threshold level

	Threshold probability of transmission						
	0.001	**0.005**	**0.01**	**0.05**	**0.1**	**0.2**	**0.5**
NL 2003	40.0	22.0	17.0	9.2	7.0	5.2	3.1
IT 1999	59.5	27.4	19.6	8.9	6.2	4.2	2.2

Assessment

Table 9 shows that, if transmission occurs, the probability of transmission from an infected farm beyond the protection zone is 0.52 (the Netherlands) and 0.33 (Italy), respectively. Likewise, the probability of transmission beyond the surveillance zone is 0.04 for both the Dutch and Italian estimates. Considering the probability of transmission (0.04) outside the surveillance zone, and the fact that subsequent to the lifting of the protection zone, the control measures applied to the surveillance zone remain in the protection zone, it is concluded that the probability of transmission outside the surveillance zone is below 5%, which is aligned with the 95% probability mentioned in some articles of the AHL. Nonetheless, when assessing independently the probability of transmission beyond the protection zone, we observed that the estimates are high using the 3 km radius. For the Netherlands, the proportion of transmission events inside and outside the protection zone is very similar (0.48 vs 0.52, respectively). To reduce the transmission probability beyond the protection zone to 0.1, the radius should be increased to 7 km. The cost for this is that many more farms will have to be visited (assuming equal distribution across the region, the number of farms in the protection zone would increase by a factor 5.4). Given that HPAI in gallinaceous poultry inevitably causes high mortality it will almost certainly be noted and reported. This may be different for Anseriformes in case of a virus strain with limited mortality. Weekly testing of dead birds (bucket sampling) would be an appropriate strategy to take this into account (see Section 4.1.1.7). Aside, the 95% probability described above to be used as a threshold is an aleatory figure and depends on a manager willingness to accept risk (as having a zero-risk policy is not an option). If the aim is to reduce the probability of transmission beyond the surveillance zone to 0.01 (and not 0.05 as assumed above), the radius should be increased to 17 (the Netherlands) or 20 km (Italy). This, nonetheless, would on average increase the number of farms in the surveillance zone (affected by movement restrictions) fourfold.

It is important to note that these probabilities do not take into account the risk of transmission imposed by wild birds, as very few wild birds were involved in the epidemics from which these data were extracted.

4.3.2. Assessment of the minimum period

The purpose of this section is to assess the effectiveness to control the spread of disease of the minimum periods during which the competent authority should apply the restriction measures in the protection and surveillance zones as set out in Annex X and XI of the Delegated Regulation supplementing the rules laid down in Part III of Regulation (EU) 2016/429 (AHL).

The length of the minimum period of the protection zone and surveillance zone are 15 and 30 days, respectively. In the protection zone, all farms are visited for a clinical inspection. This will aim at detecting infections that have started before control measures were implemented. The aim is to quickly identify infected farms. The movement control applies for 30 days, ensuring that possibly infected poultry in both protection zone and surveillance zone is not moved to uninfected farms.

From Table 6 in Section 4.2.1, it follows that the median time between introduction and suspicion is 10.3 days in chickens and 14.4 days in ducks. The maximum period between introduction and suspicion is 18.8 days. Moreover, the periods in this table refer to new outbreaks in previously unaffected regions and will likely be shorter in case of actively looking for the infection. Consequently, the duration of 30 days movement ban is effective to detect infected poultry farms and to prevent the movement of infected poultry from the surveillance zone. However, this should be accompanied by active surveillance in farms with Anseriformes in the surveillance zone as recommended in Section 4.1.1.7.

4.3.3. Uncertainty analysis

Although several sources of uncertainty were identified during the scientific assessment (see Annex G), their impact on the outputs of the assessment could not be quantified.

5. Conclusions and Recommendations

A summary of the conclusions and recommendations made for each of the scenarios assessed within each of three ToRs is presented below. For ToR 1, the recommendations were based on the sections with the development of new sampling procedures.

ToR 1			
Sampling procedure	**Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC**	**Conclusions**	**Recommendations**
4.1.1.1 In the event of a suspicion of HPAI in an establishment where animals of the listed species are kept	Standard samples to be taken: For virological testing: – at least 5 sick/dead birds and/or – at least 20 tracheal/oropharyngeal and 20 cloacal swabs (or from all birds if a small number of birds present) Birds showing clinical signs must be targeted For serological testing: – at least 20 blood samples (or from all birds if small number of birds present) Birds appearing sick or that have apparently recovered must be targeted	Taking the standard sample sizes of 5 dead birds or samples of 20 sick birds for the investigation of a suspicion is sufficient to detect HPAI in gallinaceous poultry in general within 10 days after introduction In case of a virus strain that is associated with clinical disease in Anseriformes, sampling 5 dead birds, or 20 sick birds is sufficient to detect HPAI in Anseriformes poultry In case of a virus strain causing low transmission either in Gallinaceous or Anseriformes, taking 5 samples from dead birds or 20 samples from sick birds would not lead to the detection with 95% confidence if samples are taken 10 or less days subsequent to the viral introduction	A combination of testing 5 dead birds and 20 sick birds is recommended in Gallinaceous and Anseriformes poultry to prevent relatively late detection that could occur when dealing with a strain with a low transmission rate parameter In case of a virus that does not induce mortality, or clear clinical signs, confirmation of a suspicion could be done by testing 20 sick birds (if available), or 60 healthy birds, for PCR or serology, per production unit
4.1.1.2 For the purposes of the epidemiological enquiry as referred to Article 57 of Regulation (EU) 2016/429 in an HPAI officially confirmed establishment	Standard samples must be taken from poultry and other captive birds which are killed, in each production unit	Standard sampling (Section 4.1.1.1) is useful to establish how widespread the infection is across the farm in case of multiple production units and in case of preventive killing	To establish the length of the infectious period in gallinaceous poultry, the temporal pattern of daily mortality prior to detection can be used to estimate the most likely time window of introduction, in Anseriformes serological testing will be helpful for that purpose (43 samples per production unit)

ToR 1			
Sampling procedure	**Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC**	**Conclusions**	**Recommendations**
4.1.1.3 For granting a specific derogation from killing animals of the categories of article 13.2 of the Delegated Regulation in an HPAI affected establishment	Samples must be taken for laboratory testing, 21 days following the date of the last positive finding of HPAI from each production unit and at 21 days intervals Samples to be taken: i) samples of any dead poultry or other captive birds present at the time of sampling; ii) where practical, tracheal/oropharyngeal and cloacal swabs from at least 60 poultry or other captive birds or from all such poultry or other captive birds where less than 60 are present on the holding; or if the birds are small, exotic and not used to being handled or handling them would be dangerous for people, samples of fresh faeces must be collected However, the competent authority may grant derogations from the sample size referred to in i) and ii), based on the outcome of a risk assessment The sampling and laboratory testing of such samples must continue until two consecutive negative laboratory results are obtained which must be at least 21 days apart	Collecting and testing of any dead bird will most likely be sufficient in gallinaceous poultry	It is recommended to maintain the current procedure, although serological testing could be added when possible

ToR 1			
Sampling procedure	**Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC**	**Conclusions**	**Recommendations**
4.1.1.4 For the animals of non-listed species kept in an HPAI affected establishment	Samples to be taken: i) Nasal/oropharyngeal swabs from at least 60 pigs from each production unit or from all pigs where less than 60 pigs are present in the production unit, must be taken before or on the day the infected poultry or other captive birds are culled ii) At least 60 blood samples must be collected from the pigs, two to four weeks from the date of the cull. Samples must be collected in such a way that at least one sample is obtained from groups of pigs that are in direct contact with each other Samples to be taken before pigs can be moved out of the establishment: i) The movement of pigs to other holdings may be authorised if at least 60 nasal/oropharyngeal swabs and 60 blood samples from pigs, from each production unit, 14 days following the date of the positive findings of the presence of AI have given negative results ii) The movement of pigs to a slaughterhouse may be authorised if at least 60 nasal/oropharyngeal swabs, from each production unit, 14 days following the date of the positive findings of the presence of AI have given negative results iii) In the case of inconclusive or positive laboratory results, any further investigations required to exclude the infection or transmission of AI amongst pigs	In non-listed species a risk assessment should be made on the farm to assess the exposure of the non-listed species Swab sampling for virus detection should be focused at pigs that are clinically ill, and those most intensively exposed to infected poultry. Serological testing is also focused on those animals that have been most intensively exposed to infected poultry	Serological testing of pigs should not be done before 4 weeks after culling of the poultry Clinically affected mink and equines on an affected poultry farm should be sampled in a similar way as cats provided the risk assessment would indicate possible exposure

ToR 1			
Sampling procedure	**Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC**	**Conclusions**	**Recommendations**
	Others: Where the official veterinarian has a suspicion that other domestic mammals on the holdings, in particular those with identified susceptibility to infection with AI viruses of H5 and H7 subtypes, may have been in contact with the infected poultry or other captive birds, samples for laboratory tests must be taken. Investigations in other mammals other than pigs which are susceptible to AI including cats must be undertaken With specific reference to HPAI H5N1, the following must be carried out for testing cats: gross pathological lesions, associated with viral replication, concentrate on the lungs and liver, therefore samples for virological investigations must preferably be taken from these organs of dead animals. In living animals, preferably tracheal/oropharyngeal swabs must be taken for virus detection. In addition, faecal swabs can be taken separately		
4.1.1.5 For wild animals of the listed species within the HPAI affected establishment and its surroundings	No specific guidelines described	No guidelines	Testing any dead animals of the listed species would be useful. Guidelines for carrying out passive surveillance in wild birds should be followed. Birds included in the list of target species of interest for HPAI defined by EFSA should be prioritised for sampling

ToR 1			
Sampling procedure	**Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC**	**Conclusions**	**Recommendations**
4.1.1.6 For animals of listed species in the non-affected establishments located in a protection zone	If there are clinical signs present in poultry or other captive birds or indications of an increase in daily mortality (> 3 times normal mortality rate of the flock) or a depression in daily egg production (> 5%) or a decrease in daily feed and/or water intake (> 5%): the standard samples must be taken immediately from each production unit If there are no signs, the standard samples must be taken 21 days following the date of the last suspected contact with an infected holding or when the poultry or other captive birds are killed If there are no signs but the species of poultry or other captive birds are not expected to clearly express clinical signs of disease, or in the case of vaccinated birds, the competent authority may decide, based on the outcome of a risk assessment that the standard samples must be taken from each production unit	In case of clinical signs, standard sampling is sufficient (Section 4.1.1.1)	In the absence of clinical signs, clinical inspection is sufficient in gallinaceous poultry, but sampling 20 health birds is recommended in Anseriformes
4.1.1.7 For non-affected establishments located in a surveillance zone	Standard samples must be taken from each production unit	For gallinaceous poultry sampling only establishments where an increased morbidity, mortality or where a change in production data have been reported is sufficient, for Anseriformes, this may be insufficient in case of a virus strain with mild virulence to Anseriformes	In view of the objective to find any infected establishment in the surveillance zone, establishments with Anseriformes in the surveillance zone should be visited and sampled similar to those in the protection zone (Section 4.1.1.6). Alternatively, farmers can be encouraged to collect dead birds to be pooled and tested weekly (bucket sampling)
4.1.2.1 From non-affected establishments located in the protection zone to slaughterhouses located within the protection zone or in the surveillance zone or outside the restricted zone	Samples to be taken based on the outcome of a risk assessment: at least 60 tracheal/oropharyngeal and/or 60 cloacal swabs must be taken from poultry from each production unit to be sent to slaughter less than 48 h prior to the time of departure of the poultry	In case of gallinaceous poultry, this sampling will add little to the clinical inspection of the farm, which is much more informative due to the rapid onset and severe nature of the disease in those species	A clinical inspection on the day of the movement is recommended for gallinaceous poultry. In Anseriformes, weekly testing of dead birds (bucket sampling) is recommended to reduce the probability of moving infected birds

ToR 1			
Sampling procedure	**Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC**	**Conclusions**	**Recommendations**
4.1.2.2 For day-old-chicks (DOC) from a non-affected establishment located in the protection zone, hatched from eggs originating in or outside the restricted zone to an establishment located in the same Member State but if possible, outside the restricted zone	Guidelines do not require any clinical or laboratory examination of the day-old-chicks but the holding of destination must be placed under surveillance (see Section 4.1.1.6) Standard samples must be taken from each production unit of a parent flock holding prior to the movement of hatching eggs	The risk of spreading HPAI by DOC is considered very low	In the absence of clinical signs during clinical inspection, standard sampling of gallinaceous parent flocks could be omitted In Anseriformes, parent flocks testing could be expanded by bucket sampling and serological testing
4.1.2.3 For ready-to-lay poultry from a non-affected establishment located in the protection zone to establishments located in the same MS and if possible, within the restricted zone	Samples to be taken based on the outcome of a risk assessment: at least 60 tracheal/oropharyngeal and/or 60 cloacal swabs must be taken from poultry from each production unit to be sent to slaughter less than 48 h prior to the time of departure of the poultry	In case of gallinaceous poultry, this sampling will add little to the clinical inspection of the farm, which is much more informative due to the rapid onset and severe nature of the disease in those species	A clinical inspection on the day of the movement is recommended for gallinaceous poultry. In Anseriformes, weekly testing of dead birds (bucket sampling) is recommended to reduce the probability of moving infected birds Follow-up testing in the recipient flock is recommended. In Galliformes, this implies collecting information of any clinical manifestation, in Anseriformes that could be complemented by bucket sampling and serological testing 4 weeks after moving the birds
4.1.2.4 From non-affected establishments located in the protection zone to a plant approved for processing or disposal of animal by-products in which the animals are immediately killed	No specific guidelines described	4.1.2.1	4.1.2.1

ToR 1			
Sampling procedure	**Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC**	**Conclusions**	**Recommendations**
4.1.2.5 From an establishment in a surveillance zone to a slaughterhouse located within or outside the restricted zone and from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone	No specific guidelines described	Because there is a considerable risk of an infected farm in the surveillance zone, a similar procedure to the protection zone would be advisable	A clinical inspection on the day of the movement is recommended for gallinaceous poultry. In Anseriformes, weekly testing of dead birds (bucket sampling) is recommended to reduce the probability of moving infected birds
4.1.2.6 For day-old-chicks from a non-affected establishment located in the surveillance zone, to an establishment located in the same Member State where they were hatched	No specific guidelines described	4.1.1.2	4.1.1.2
4.1.2.7 For ready-to-lay poultry located in the surveillance zone to establishments located in the same MS	No specific guidelines described	4.1.2.3	4.1.2.3
4.1.2.8 From an establishment located in the restricted zone to move within the restricted zone when restriction measures are maintained beyond the period set out in Annex XI of the Delegated Regulation	No specific guidelines described	4.1.2.3	4.1.2.3

ToR 1			
Sampling procedure	**Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC**	**Conclusions**	**Recommendations**
4.1.3.1 For the animals that are kept for the repopulation prior to their introduction	The diagnostic manual, Article 49(3)(b) and (c) — Re-population of holdings, specifies that 'if appropriate' at least 20 blood samples should be taken as soon as the poultry have been placed in the holding except in the case of day-old chicks; if appropriate such sampling may be performed on the holding of origin of the poultry before movement to the holding for re-population The Delegated Regulation (Art. 59 (2), (3) and (9)), supplementing Regulation (EU) 2016/429 estates that: Samples shall be collected from: i) A representative number of all the animals to be introduced in the establishment, if they are all introduced at the same time and from the same establishment of origin; or ii) A representative number of animals of each consignment, if animals are all to be introduced at different times or from different establishments of origin In the case of day-old-chicks, the competent authority may decide not to perform the sampling for laboratory examination	If the birds originate from a disease-free area, sampling would not be needed for HPAI Collecting samples of 20 birds could be useful to detect antibodies induced by an LPAI infection that may not be detected based on clinical signs	

ToR 1

Sampling procedure	Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC	Conclusions	Recommendations
4.1.3.2 In the event of unusual mortalities or clinical signs being notified during the repopulation 4.1.3.3 For animals that have been repopulated	Samples to be taken during routine surveillance of repopulated establishments from each production unit: i) At least 20 blood samples as soon as the poultry have been placed in the holding except in the case of day-old chicks; if appropriate such sampling may be performed on the holding of origin of the poultry before movement to the holding for re-population ii) Samples of dead poultry or swabs taken from their carcasses from a maximum of 10 dead birds per week during the 21-day period from the date of the re-population iii) Where the holding has previously been infected with HPAI 20 tracheal/oropharyngeal and 20 cloacal swabs must also be taken from waterfowl (ducks/geese) from each production unit, if appropriate, within the last week of the 21-day period from the date of re-population Nonetheless, no specific sampling procedures are described in the event of unusual mortalities or clinical signs being notified during the repopulation	Testing 10 dead birds will detect HPAI infection with a high probability. Testing tracheal and cloacal swabs of 20 birds results in a high probability of detection. When dealing with a strain with a very mild infection additional samples may be required	When dealing with a virus strain with mild virulence in waterfowl, it would be recommended to test 60 healthy birds (tracheal and cloacal swabs) within the last week of the 21 day period

ToR 2

Description	Conclusions	Recommendations
4.2 Assessment of the length of the monitoring period of HPAI	Based on the time between virus introduction and outbreaks confirmation from literature, the existing length of the monitoring period are considered effective for gallinaceous poultry and for Anseriformes	It is recommended to keep the length of the monitoring period To reduce the uncertainty in the length of the period between introduction and the suspicion report for Anseriformes, it is recommended to collect further information during future outbreaks

ToR 3		
Description	**Conclusions**	**Recommendations**
4.3.1 Assessment of the minimum radius	It is concluded that the probability of transmission outside the surveillance zone is below 5%. The probability of transmission beyond the protection zone is approximately 50%. It is important to note that these probabilities do not take into account the risk of transmission imposed by wild birds. To reduce the transmission probability beyond the protection zone to 0.1, the radius should be increased to 7 km, but this would increase the number of farms in the protection zone by a factor 5.4	It is recommended to keep the minimum radius of the protection and surveillance zones For Anseriformes poultry, in order to increase the probability of detecting outbreaks in the surveillance zone, weekly bucket sampling or carrying out serological sampling prior to the lifting of the restrictions in the surveillance zone is recommended
4.3.2 Assessment of the minimum period	The duration of 30 days movement ban is effective to detect infected poultry farms and to prevent the movement of infected poultry from the surveillance zone	It is recommended to keep the duration of restriction zones During the surveillance period, active surveillance in farms with Anseriformes in the surveillance zone is recommended

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Abbreviations

ASF	African swine fever
AHS	African horse sickness
AGID	agar gel immunodiffusion
CSF	Classical swine fever
CBPP	Contagious bovine pleuropneumonia
CCPP	Contagious caprine pleuropneumonia
DOC	day-old-chicks
ELISAs	enzyme-linked immunosorbent assays
ELS	extensive literature search
FMD	Foot and mouth disease
HPAI	Highly Pathogenic Avian Influenza
IVPI	Intravenous Pathogenicity Index
LPAI	low pathogenic avian influenza
LSD	lumpy skin disease virus
NCD	Newcastle disease virus
OIE	World Organization for Animal Health
PZ	protection zone
RP	rinderpest virus
RT-PCR	real-time reverse transcription polymerase chain reaction
RVFV	Rift Valley fever virus
SPGP	Sheep pox and goat pox
SZ	surveillance zone
ToR	Terms of Reference

Annex A – Definitions in EU legislation

Terms	Definitions
Clinical examination	The clinical examination comprises: (i) an initial general evaluation of the animal health status of the establishment which comprises all the animals of listed species kept in the establishment; and (ii) an individual examination of the animals included in the sample referred to in point (a). The sampling of animals for clinical examination is carried out in accordance with point A.1 of Annex I for terrestrial animals; (Delegated Regulation article 3)
Confined establishment	Means any permanent, geographically limited establishment, created on a voluntary basis and approved for the purpose of movements, where the animals are: (a) kept or bred for the purposes of exhibitions, education, the conservation of species or research; (b) confined and separated from the surrounding environment; and (c) subject to animal health surveillance and biosecurity measures; (AHL: Regulation 2016/429 article 4(48))
Epidemiological unit	Means a group of animals with the same likelihood of exposure to a disease agent; (AHL: Regulation 2016/429 article 4(39))
Establishment	Means 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: (a) households where pet animals are kept; (b) veterinary practices or clinics; (AHL: Regulation 2016/429 article 4(27))
Health status	Means the disease status as regards the listed diseases relevant for a particular listed species with respect to: (a) an animal; (b) animals within: (i) an epidemiological unit; (ii) an establishment; (iii) a zone; (iv) a compartment; (v) a Member State; (vi) a third country or territory; (AHL: Regulation 2016/429 article 4(34))
Infected zone	Means a zone in which restrictions on the movements of kept and wild animals or products and other disease control and biosecurity measures may be applied with the view to preventing the spread of a category A disease in the event of official confirmation of the disease in wild animals; (Delegated Regulation article 2(15))
Kept animals	Means animals which are kept by humans, including, in the case of aquatic animals, aquaculture animals; (AHL: Regulation 2016/429 article 4(5))
Outbreak	Means the officially confirmed occurrence of a listed disease or an emerging disease in one or more animals in an establishment or other place where animals are kept or located; (AHL: Regulation 2016/429 article 4 (40)
Protection zone	Means a zone around and including the location of an outbreak, where disease control measures are applied in order to prevent the spread of the disease from that zone; (AHL: Regulation 2016/429 article 4(42))
Listed diseases	Means diseases listed in accordance with Article 5(1); (AHL: Regulation 2016/429 article 4 (18)) List of the diseases (AHL: Regulation 2016/429, Annex II)
Listed species	Means an animal species or group of animal species listed in accordance with Article 8(2), or, in the case of emerging diseases, an animal species or group of animal species which meets the criteria for listed species laid down in Article 8(2); (AHL: Regulation 2016/429 article 4(20)) List of species and groups of species (Commission Implemented Regulation 2018/1882)
Monitoring periods	It is appropriate to follow a single approach for the measures to apply in the event of a category A disease. However, the epidemiology of diseases should be taken into account to establish the appropriate moment for the competent authority to apply control measures and to carry out investigations if there is suspicion or confirmation of those diseases. Therefore 'monitoring periods' should be provided, as reference time frames for each category A disease affecting terrestrial animals based on incubation periods and other relevant elements that may affect the spread of the disease; (Delegated Regulation whereas 10)
Restricted zone	Means a zone in which restrictions on the movements of certain animals or products and other disease control measures are applied, with a view to preventing the spread of a particular disease into areas where no restrictions are applied; a restricted zone may, when relevant, include protection and surveillance zones; (AHL: Regulation 2016/429 article 4(41))

Terms	Definitions
Surveillance zone	Means a zone which is established around the protection zone, and where disease control measures are applied in order to prevent the spread of the disease from the protection zone; (AHL: Regulation 2016/429 article 4(43))
Wild animals	Means animals which are not kept animals; (AHL: Regulation 2016/429 article 4(8))
Zone	Means: (a) for terrestrial animals, an area of a Member State, third country or territory with a precise geographical delimitation, containing an animal subpopulation with a distinct health status with respect to a specific disease or specific diseases subject to appropriate surveillance, disease control and biosecurity measures; (AHL: Regulation 2016/429 article 4 (35))

Annex B – Scenarios of ToR 1

ToR	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
ToR 1.1 ToR 1.2	6(2) Delegated Regulation	1st Scenario	To assess the effectiveness of disease-specific sampling procedures of animals of listed species in a suspected establishment, based on clinical examination (TOR 1.1) and laboratory examination (TOR 1.2), in their ability to detect a category A disease in kept animals if the disease is present in that establishment, or to rule it out if not present (Art. 6 (2))	• Event of suspicion of HPAI outbreak • in an establishment • kept animals of listed species • the listed species for HPAI as provided in Commission Implemented Regulation 2018/1882 are species of the Class Aves • the competent authority shall immediately conduct an investigation to confirm or rule out the presence of the suspected listed disease • official veterinarians perform clinical examinations and collect samples for laboratory examinations
ToR 1.2	Art. 12(3), Art. 7 (4) (Preventive killing) Delegated Regulation, and Art. 57 Reg.2016/429	2nd Scenario	To assess the effectiveness of disease-specific sampling procedures, based on laboratory examination (ToR 1.2), in their ability to detect the disease in the event of preventive killing, and in their ability to support with the epidemiological investigation (disease detection, prevalence estimation, virus identification, etc.) in kept animals of listed species in an affected establishment, before or when they are killed or found dead. The purposes of the epidemiological enquiry are described in Article 57 of Regulation (EU)2016/429	• affected establishment officially confirmed • kept animals of listed species found dead or before/when they are killed • competent authority collects samples for laboratory examinationfor the purposes of: a) supporting the epidemiological enquiry: – to identify the likely origin of the disease – to calculate the likely length of time that the disease is present – to identify establishments where the animals could have contracted the disease and movements from the affected establishment that could have led to the spread of the disease – to obtain information on the likely spread of the listed disease in the surrounding environment, including the presence and distribution of disease vectors b) confirming/ruling out disease in the event of preventive killing

ToR	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
ToR 1.1 ToR 1.2	Article 13(3)c Delegated Regulation	3rd Scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species belonging to the categories described in article 13(2)) of an affected establishment, in order to grant a specific derogation from killing these animals, while ensuring that they do not pose a risk for the transmission of the disease	• affected establishment officially confirmed • kept animals of listed species of specific categories • animal categories based on article 13(2): (a) animals kept in a confined establishment (b) animals kept for scientific purposes or purposes related to conservation of protected or endangered species (c) animals officially registered in advance as rare breeds (d) animals with a duly justified high genetic, cultural or educational value • the competent authority may grant specific derogation from killing all the animals of listed species belonging to any of the above categories in an affected establishment, provided that specific conditions are fulfilled • the animals should be subjected to clinical surveillance, including laboratory examinations • vsampling procedures should ensure that the animals do not pose a risk of transmission of the category A disease if left alive
ToR 1.1 ToR 1.2	Article 14(1) of the Delegated Regulation Art. 57 Reg.2016/429	4th Scenario	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of non-listed species kept in an affected establishment, in their ability to ensure the detection of the virus if the virus is present in these species	• kept animals of non-listed species of epidemiological relevance for the control of the disease • animals of non-listed species are those animals that are not listed in Commission Implementing Regulation (EU) 2018/1882 for each of the category A diseases • animal species acting purely as mechanical carriers of the virus will not be covered • The competent authority is not obliged to carry out the sampling of non-listed species, but they may establish it in addition to other measures • sampling procedures to ensure detection of the virus in these species
ToR 1.1 ToR 1.2	Article 14(1) of the Delegated Regulation Art. 57 Reg.2016/429	5th Scenario	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the wild animals of listed species within the affected establishment and in its surroundings. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these wild species	• affected establishment officially confirmed • wild animals of listed species within the establishment and in the surroundings of the establishment • the competent authority may establish these sampling procedures in addition to other measures • sampling procedures in wild animals of listed species to ensure the detection of the virus, if the virus is present in these wild species

ToR	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
ToR 1.1 ToR 1.2	Article 26(2) of the Delegated Regulation	6th Scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species in establishments located in the protection zone. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these animals	• protection zone with radius up to 3 km • non-affected establishments with kept animals of listed species • all the non-affected establishments within the protection zone • official veterinarians must visit at least once all the establishments • among others, they must perform a clinical examination of kept animals of listed species and if necessary, collection of samples for laboratory examination • sampling procedures to confirm or rule out the presence of a category A disease
ToR 1.3	Article 26(5) of the Delegated Regulation point A.3 of Annex I	7th Scenario	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species, for the sampling of establishments located in a protection zone when the radius is larger than 3 km. The purpose of the sampling procedure is to ensure disease detection of the virus if the virus is present in establishments within the protection zone	• protection zone with radius larger than 3 km • non-affected establishments of kept animals of listed species • sample of the non-affected establishments in the protection zone • in a protection zone with a radius equal to 3 km, official veterinarians must carry inspections in all establishments within the 3 km • In case of a radius larger than 3 km, official veterinarians may not visit all establishments, but a sample of those. EFSA is requested to assess how many of these establishments should be inspected, in order to ensure the detection of the virus, if the virus is present in animals in these establishments • among others perform clinical examination of kept animals of listed species and if necessary, collection of samples for laboratory examination • sampling procedure to ensure the detection of the disease if the disease is present in any of these establishments
ToR 1.3	Article 41 of the Delegated Regulation	8th Scenario	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species, for the sampling of the establishments located within the surveillance zone. The purpose of the sampling procedure is to ensure disease detection if the virus is present in establishments within the surveillance zone	• surveillance zone • establishments of kept animals of listed species • sample of the establishments in the surveillance zone • official veterinarians carry out visits to a sample of the establishments • among others perform clinical examination of kept animals of listed species and if necessary, collection of samples for laboratory examination • sampling procedure to ensure the detection of the disease if the disease is present in any of the establishments

ToR	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
Derogations to allow animal movements				
ToR 1.4	Article 28(5) of the Delegated Regulation Article 29 of the Delegated Regulation	9th Scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant a derogation from prohibitions in the movement of animals, and allow for the animals to be moved to a slaughterhouse located within the protection zone or in the surveillance zone or outside the restricted zone (Art29)	• protection zone • kept animals of listed species • grant derogation for movement from a non-affected establishment in the protection zone • be moved to a slaughterhouse located within the protection zone or in the surveillance zone or outside the restricted zone • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 28(5) and Article 30(1) of the Delegated Regulation	10th Scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of day-old-chicks located in the protection zone and hatched from eggs originating in the restricted zone or outside the restricted zone. The sampling procedures should ensure that the movement of these day-old-chicks to an establishment located in the same Member State but if possible, outside the restricted zone	• protection zone • vgrant derogation for movement from a non-affected establishment in the protection zone • day-old-chicks from non-affected establishment located in the protection zone, hatched from eggs originating in or outside the restricted zone • to be moved to an establishment located in the same Member State but if possible, outside the restricted zone • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 28(5) and Article 30(2) of the Delegated Regulation	11th Scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the protection zone to establishments located in the same MS and if possible within the restricted zone	• protection zone • ready-to-lay poultry • grant derogation for movement from a non-affected establishment in the protection zone • to be moved to an establishment located in the same Member State and if possible, within the restricted zone • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved

ToR	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
ToR 1.4	Article 28(5) and Article 37 of the Delegated Regulation	12th Scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant derogation from prohibitions in the movement of these animals to a plant approved for processing or disposal of animal by-products in which the kept animals are immediately killed (Art37)	• protection zone • kept animals of listed species • grant derogation for movement from a non-affected establishment in the protection zone • to be moved to a plant approved for processing or disposal of animal by-products in which the kept animals are immediately killed • clinical examinations and laboratory examinations of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 43(5) and Article 44 of the Delegated Regulation	13th Scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of listed species in order to grant derogation from prohibitions and allow for these animals to be moved : a) from an establishment in a surveillance zone to a slaughterhouse located within or outside the restricted zone, b)from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone	• surveillance zone • kept animals of listed species • grant derogation for movement from an establishment in the surveillance zone to be moved to a slaughterhouse within the restricted zone or outside the restricted zone • grant derogation for movement from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 43(5) and Article 45(1) of the Delegated Regulation	14th Scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of kept ungulates of listed species in order to grant a derogation and allow for the animals to be moved from an establishment in the surveillance zone to pastures situated within the surveillance zone	• surveillance zone • kept ungulates of listed species • grant derogation for movement from an establishment in the surveillance zone • to be moved to pastures situated within the surveillance zone • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 43(5) and Article 45(2) of the Delegated Regulation	15th Scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of kept ungulates of listed species in order to grant derogation and allow to be moved from an establishment in the surveillance zone to an establishment belonging to the same supply chain, located in or outside the surveillance zone, in order to complete the production cycle before slaughter	• surveillance zone • kept animals of listed species • grant derogation for movement from the surveillance zone • to be moved to an establishment belonging to the same supply chain, located in or outside the surveillance zone, to complete the production cycle before slaughter • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved

ToR	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
ToR 1.4	Article 43(5) and Article 46(1) of the Delegated Regulation	16th Scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations to grant derogation of movements of day-old-chicks hatched from establishment located in the surveillance zone, from eggs originating within the surveillance zone and eggs originating outside the restricted zone, to an establishment located in the same Member State where they were hatched	• surveillance zone • kept birds of listed species • grant derogation for movement of day-old-chicks hatched from establishment located in the surveillance zone, from eggs originating from establishment within the surveillance zone or eggs originating from outside the restricted zone • to be moved to an establishment located in the same Member State • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 43(5) and Article 46(2) of the Delegated Regulation	17th Scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the surveillance zone to establishments located in the same MS	• surveillance zone • ready-to-lay poultry • to be moved to an establishment located in the same Member State • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
ToR 1.4	Article 56(1)c of the Delegated Regulation	18th Scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment located in the restricted zone of an outbreak in order to allow their move within the restricted zone, when restriction measures are maintained beyond the period set out in Annex XI	• restricted zone when restriction measures are maintained beyond the period set out in Annex XI • kept animals of listed species • grant derogation for movement from an establishment within the restricted zone • clinical examinations and laboratory examination of animals kept in the establishment, including those animals to be moved
Repopulation				
ToR 1.5	Article 59(2), (3) of the Delegated Regulation	19th Scenario	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that are kept for the repopulation prior to their introduction to rule out the presence of the disease	• repopulation of a previous affected establishment • kept animals of listed species • Animals intended to repopulation shall be sampled prior to their introduction into the establishment of destination • samples shall be collected from a representative number of animals to be introduced of each consignment from each establishment or from a representative number of animals of each consignment (if animals are all to be introduced at different times or from different establishments of origin) • laboratory examinations • sampling procedures to rule out the presence of the disease

ToR	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
ToR 1.5	Article 59(9) of the Delegated Regulation	20th Scenario	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, in the event of unusual mortalities or clinical signs being notified during the repopulation; to rule out the presence of the disease	• repopulated establishment • unusual mortalities or clinical signs during the repopulation • the official veterinarians shall without delay collect samples for laboratory examination • sampling procedures to rule out the presence of the disease
ToR 1.5	Article 59(5) of the Delegated Regulation	21st Scenario	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, on the last day of the monitoring period calculated forward from the date on which the animals were placed in the repopulated establishment. In case the repopulation takes place in several days, the monitoring period will be calculated forward from the last day in which the last animal is introduced in the establishment	• repopulated establishment • kept animals of listed species • Animals that have been used for repopulation • Laboratory examinations • Sampling procedures to rule out the presence of the disease

Annex C – Sampling procedures for HPAI

Scenario	Description of the Scenario	Clinical guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC	Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC
1st	To assess the effectiveness of disease-specific sampling procedures of animals of listed species in a suspected establishment, based on clinical examination (TOR 1.1) and laboratory examination (TOR 1.2), in their ability to detect a category A disease in kept animals if the disease is present in that establishment, or to rule it out if not present (Art. 6 (2))	Commission Decision (2006/437/EC) approving a Diagnostic manual for avian influenza (hereinafter called 'Diagnostic manual') in accordance with Article 7 of Directive 2005/94/EC, requires the: – inspection of production and health records (daily mortality, egg-production and feed-and/or water intake) from one week before the commencement of clinical signs, – clinical inspection in each production unit: evaluation of clinical history and clinical examination of poultry or other captive birds, in particular those that appear sick, – if competent authority is not satisfied with clinical inspection, standard samples must be taken, – final clinical inspection of the poultry independent of the results of the laboratory examination prior to the lifting of the official surveillance	Standard samples to be taken: For virological testing: o at least 5 sick/dead birds and/or o at least 20 tracheal/oropharyngeal and 20 cloacal swabs (or from all birds if a small number of birds present) Birds showing clinical signs must be targeted For serological testing: o at least 20 blood samples (or from all birds if small number of birds present). Birds appearing sick or that have apparently recovered must be targeted

Scenario	Description of the Scenario	Clinical guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC	Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC
2nd	To assess the effectiveness of disease-specific sampling procedures, based on laboratory examination (ToR 1.2), in their ability to detect the disease in the event of preventive killing, and in their ability to support with the epidemiological investigation (disease detection, prevalence estimation, virus identification, etc.) in kept animals of listed species in an affected establishment, before or when they are killed or found dead. The purposes of the epidemiological enquiry are described in Article 57 of Regulation (EU)2016/429	No specific guidelines described	Standard samples must be taken from poultry and other captive birds which are killed, in each production unit
3rd	To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species belonging to the categories described in article 13(2)) of an affected establishment, in order to grant a specific derogation from killing these animals, while ensuring that they do not pose a risk for the transmission of the disease	Check of the production and health records of the holding, if such records exist. Clinical inspection in each production unit, including an evaluation of its clinical history and clinical examinations of poultry or other captive birds, in particular those that appear sick	Samples must be taken for laboratory testing, 21 days following the date of the last positive finding of HPAI from each production unit and at 21 days intervals Samples to be taken: (i) samples of any dead poultry or other captive birds present at the time of sampling, (ii) where practical, tracheal/oropharyngeal and cloacal swabs from at least 60 poultry or other captive birds or from all such poultry or other captive birds where less than 60 are present on the holding; or if the birds are small, exotic and not used to being handled or handling them would be dangerous for people, samples of fresh faeces must be collected. However, the competent authority may grant derogations from the sample size referred to in (i) and (ii), based on the outcome of a risk assessment The sampling and laboratory testing of such samples must continue until two consecutive negative laboratory results are obtained which must be at least 21 days apart

Scenario	Description of the Scenario	Clinical guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC	Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC
4th	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of non-listed species kept in an affected establishment, in their ability to ensure the detection of the virus if the virus is present in these species. Species: PIGS and others (cats in the case of H5N1)	A check of the production and health records of the holding, if such records exist. A clinical inspection in each production unit, including an evaluation of its clinical history. Clinical examinations of the pigs, in particular those that appear sick	Samples to be taken: o Nasal/oropharyngeal swabs from at least 60 pigs from each production unit or from all pigs where less than 60 pigs are present in the production unit, must be taken before or on the day the infected poultry or other captive birds are culled o At least 60 blood samples must be collected from the pigs, 2–4 weeks from the date of the cull. Samples must be collected in such a way that at least one sample is obtained from groups of pigs that are in direct contact with each other <u>Samples to be taken before pigs can be moved out of the establishment:</u> o The movement of pigs to other holdings may be authorised if at least 60 nasal/oropharyngeal swabs and 60 blood samples from pigs, from each production unit, 14 days following the date of the positive findings of the presence of AI have given negative results o The movement of pigs to a slaughterhouse may be authorised if at least 60 nasal/oropharyngeal swabs, from each production unit, 14 days following the date of the positive findings of the presence of AI have given negative results o In the case of inconclusive or positive laboratory results, any further investigations required to exclude the infection or transmission of AI amongst pigs

Scenario	Description of the Scenario	Clinical guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC	Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC
			Others: Where the official veterinarian has a suspicion that other domestic mammals on the holdings, in particular those with identified susceptibility to infection with AI viruses of H5 and H7 subtypes, may have been in contact with the infected poultry or other captive birds, samples for laboratory tests must be taken. Investigations in other mammals other than pigs which are susceptible to AI including cats must be undertaken With specific reference to HPAI H5N1, the following must be carried out for testing cats: gross pathological lesions, associated with viral replication, concentrate on the lungs and liver, therefore samples for virological investigations must preferably be taken from these organs of dead animals. In living animals, preferably tracheal/oropharyngeal swabs must be taken for virus detection. In addition, faecal swabs can be taken separately
5th	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the wild animals of listed species within the affected establishment and in its surroundings. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these wild species	No specific guidelines described	No specific guidelines described

Scenario	Description of the Scenario	Clinical guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC	Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC
6th	To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species in establishments located in the protection zone. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these animals	a) b) c) A check of the production and health records of the holding, if such records exist The daily mortality data and daily data on feed- and/or water intake must be checked if available, for the period beginning one week before the date of contact with the flock suspected of being infected with AI until the date of the inspection of the holding. A clinical inspection in each production unit, including an evaluation of its clinical history and clinical examinations of poultry or other captive birds, in particular those that appear sick.	a)b)c) If there are clinical signs present in poultry or other captive birds or indications of an increase in daily mortality (> 3 times normal mortality rate of the flock) or a depression in daily egg production (> 5%) or a decrease in daily feed and/or water intake (> 5%) : the standard samples must be taken immediately from each production unit a) If there are no signs, the standard samples must be taken 21 days following the date of the last suspected contact with an infected holding or when the poultry or other captive birds are killed b) If there are no signs but the species of poultry or other captive birds are not expected to clearly express clinical signs of disease, or in the case of vaccinated birds, the competent authority may decide, based on the outcome of a risk assessment that the standard samples must be taken from each production unit
7th	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species, for the sampling of establishments located in a protection zone when the radius is larger than 3 km. The purpose of the sampling procedure is to ensure disease detection of the virus if the virus is present in establishments within the protection zone	N/A (current protection zone radius is 3 km)	N/A (current protection zone radius is 3 km)

Scenario	Description of the Scenario	Clinical guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC	Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC
8th	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR 1.1) and laboratory (ToR 1.2) examinations of the animals of listed species, for the sampling of the establishments located within the surveillance zone. The purpose of the sampling procedure is to ensure disease detection if the virus is present in establishments within the surveillance zone	A check of the production and health records of the holding A clinical inspection in each production unit, including an evaluation of its clinical history Clinical examinations of poultry or other captive birds, in particular those that appear sick	Standard samples must be taken from each production unit
Derogations to allow animal movements			
9th	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant a derogation from prohibitions in the movement of animals, and allow for the animals to be moved to a slaughterhouse located within the protection zone or in the surveillance zone or outside the restricted zone (Art29)	A check of the production and health records of the holding A clinical inspection in each production unit, including an evaluation of its clinical history Clinical examinations of any poultry, in particular those that appear sick less than 24 h prior to the time of departure of the poultry The official veterinarian shall ensure that a detailed examination of the poultry is carried out at the designated slaughterhouse when the poultry arrive and after they are slaughtered	Samples to be taken based on the outcome of a risk assessment: at least 60 tracheal/oropharyngeal and/or 60 cloacal swabs must be taken from poultry from each production unit to be sent to slaughter less than 48 h prior to the time of departure of the poultry

Scenario	Description of the Scenario	Clinical guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC	Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC
10th	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of day-old-chicks located in the protection zone and hatched from eggs originating in the restricted zone or outside the restricted zone. The sampling procedures should ensure that the movement of these day-old-chicks to an establishment located in the same Member State but if possible, outside the restricted zone	Current guidelines do not require any clinical or laboratory examination of the day-old-chicks but the holding of destination must be placed under surveillance (see scenario 6). The guidelines for inspecting a parent flock holding prior to the movement of hatching eggs are: a) a check of the production and health records of the holding, and b) a clinical inspection in each production unit every 15 days.	Guidelines do not require any clinical or laboratory examination of the day-old-chicks but the holding of destination must be placed under surveillance (see scenario 6). Standard samples must be taken from each production unit of a parent flock holding prior to the movement of hatching eggs
11th	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the protection zone, to establishments located in the same Member State and if possible within the restricted zone	The guidelines include: a) check of the production and health records of the holding, b) a clinical inspection in each production unit, including an evaluation of its clinical history and, c) clinical examinations of the poultry, in particular those that appear sick less than 24 h prior to the time of departure of the poultry.	Samples to be taken based on the outcome of a risk assessment: at least 60 tracheal/oropharyngeal and/or 60 cloacal swabs must be taken from poultry from each production unit to be sent to slaughter less than 48 h prior to the time of departure of the poultry
12th	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment in a protection zone, in order to grant derogation from prohibitions in the movement of these animals to a plant approved for processing or disposal of animal by-products in which the kept animals are immediately killed (Art37)	No specific guidelines described	No specific guidelines described

Scenario	Description of the Scenario	Clinical guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC	Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC
13th	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of listed species in order to grant derogation from prohibitions and allow for these animals to be moved: a) from an establishment in a surveillance zone to a slaughterhouse located within or outside the restricted zone, b) from an establishment outside the surveillance zone to a slaughterhouse situated in the surveillance zone	No specific guidelines described	No specific guidelines described
14th	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of kept ungulates of listed species in order to grant a derogation and allow for the animals to be moved from an establishment in the surveillance zone to pastures situated within the surveillance zone	N/A	N/A
15th	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of kept ungulates of listed species in order to grant derogation and allow for them to be moved from an establishment in the surveillance zone to an establishment belonging to the same supply chain, located in or outside the surveillance zone, in order to complete the production cycle before slaughter	N/A	N/A

Scenario	Description of the Scenario	Clinical guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC	Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC
16th	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations to grant derogation of movements of day-old-chicks hatched from establishment located in the surveillance zone, from eggs originating within the surveillance zone and eggs originating outside the restricted zone, to an establishment located in the same Member State where they were hatched	No specific guidelines described	No specific guidelines described
17th	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations, to grant a derogation from prohibitions in the movement of ready-to-lay poultry located in the surveillance zone to establishments located in the same Member State	No specific guidelines described	No specific guidelines described
18th	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or laboratory examinations of the animals of an establishment located in the restricted zone of an outbreak in order to allow their move within the restricted zone, when restriction measures are maintained beyond the period set out in Annex XI	No specific guidelines described	No specific guidelines described

Scenario	Description of the Scenario	Clinical guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC	Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC
Repopulation			
19th	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that are kept for the repopulation prior to their introduction to rule out the presence of the disease	Only an assessment of laboratory guidelines is requested	The diagnostic manual, Article 49(3)(b) and (c) — Re-population of holdings, specifies that 'if appropriate' at least 20 blood samples should be taken as soon as the poultry have been placed in the holding except in the case of day-old chicks; if appropriate such sampling may be performed on the holding of origin of the poultry before movement to the holding for re-population The Delegated Regulation (Art. 59 (2), (3) and (9)), supplementing Regulation (EU) 2016/429 estates that: Samples shall be collected from: o A representative number of all the animals to be introduced in the establishment, if they are all introduced at the same time and from the same establishment of origin; or o A representative number of animals of each consignment, if animals are all to be introduced at different times or from different establishments of origin o In the case of day-old-chicks, the competent authority may decide not to perform the sampling for laboratory examination

Scenario	Description of the Scenario	Clinical guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC	Laboratory guidelines based on Commission Decision (2006/437/EC) in accordance with Article 7 of Directive 2005/94/EC
20th	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, in the event of unusual mortalities or clinical signs being notified during the repopulation; to rule out the presence of the disease	Only an assessment of laboratory guidelines is requested	Samples to be taken during routine surveillance of repopulated establishments from each production unit: o At least 20 blood samples as soon as the poultry have been placed in the holding except in the case of day-old chicks; if appropriate such sampling may be performed on the holding of origin of the poultry before movement to the holding for re-population o Samples of dead poultry or swabs taken from their carcasses from a maximum of 10 dead birds per week during the 21-day period from the date of the re-population o Where the holding has previously been infected with HPAI 20 tracheal/oropharyngeal and 20 cloacal swabs must also be taken from waterfowl (ducks/geese) from each production unit, if appropriate, within the last week of the 21-day period from the date of re-population. onetheless, no specific sampling procedures are described in the event of unusual mortalities or clinical signs being notified during the repopulation
21st	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that have been repopulated, on the last day of the monitoring period calculated forward from the date on which the animals were placed in the repopulated establishment. In case the repopulation takes place in several days, the monitoring period will be calculated forward from the last day in which the last animal is introduced in the establishment	Only an assessment of laboratory guidelines is requested	Samples to be taken during routine surveillance of repopulated establishments from each production unit: o At least 20 blood samples as soon as the poultry have been placed in the holding except in the case of day-old chicks; if appropriate such sampling may be performed on the holding of origin of the poultry before movement to the holding for re-population o Samples of dead poultry or swabs taken from their carcasses from a maximum of 10 dead birds per week during the 21-day period from the date of the re-population o Where the holding has previously been infected with HPAI 20 tracheal/oropharyngeal and 20 cloacal swabs must also be taken from waterfowl (ducks/geese) from each production unit, if appropriate, within the last week of the 21-day period from the date of re-population.

Annex D – Scenarios of ToR 2

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenarios
ToR 2	Article 8 Delegated Regulation Article 57 of 2016/429 Regulation Annex II of the Delegated Regulation	1st Scenario	To assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of the notification of the suspicion of a category A disease in an establishment with kept animals of listed species, for the purposes of the epidemiological enquiry in the event of a suspicion	• event of suspicion of a category A disease • in an establishment with kept animals of listed species • time period calculated backwards from the date of the of the notification of the suspicion • time period before the suspicion, during which the pathogenic agent may have been introduced in the establishment and may have spread outside the establishment. • the aim of the epidemiological enquire is: (a) identify the likely origin of the listed disease in question and the means of its spread (b) calculate the likely length of time that the listed disease has been present (c) identify establishments and epidemiological units therein, food and feed businesses or animal by-products establishments, or other locations, where animals of listed species for the suspected listed disease may have become infected, infested or contaminated (d) obtain information on the movements of kept animals, persons, products, vehicles, any material or other means by which the disease agent could have been spread during the relevant period preceding the notification of the suspicion or confirmation of the listed disease (e) obtain information on the likely spread of the listed disease in the surrounding environment, including the presence and distribution of disease vectors
ToR 2	Article 17(2) Article 57 of 2016/429 Regulation, Annex II of the Delegated Regulation,	2nd Scenario	To assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of notification of the suspicion of a category A disease in an establishment with kept animals of listed species, for the purposes of the epidemiological enquiry in the event of confirmation of the disease	• event of confirmation of a category A disease • in an establishment with kept animals of listed species • time period calculated backwards from the date of the notification of the suspicion • time period before the suspicion, during which the pathogenic agent was introduced in the establishment and during which it could have spread outside the establishment. • The aim of the epidemiological enquire is the same as above.

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenarios
ToR 2	Article 13(b) Delegated Regulation Annex II of the Delegated Regulation	3rd Scenario	To assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of confirmation of a category A disease in an establishment with kept animals of listed species, during which the epidemiological units in which the disease has not been confirmed were kept completely separated and handled by different personnel, in order to provide derogations from killing	• event of confirmation of a category A disease • in an affected establishment with kept animals of listed species • non-affected epidemiological units kept separated • to provide derogation from killing for animals in non-affected separated epidemiological units • to exclude any possible contact between the affected establishment and the separated epidemiological units as per the epidemiological enquiry • time period calculated backwards from the date of the confirmation • time period before the confirmation, during which the pathogenic agent may have been introduced in the separated non-affected epidemiological units of the affected establishment
ToR 2	Article 27(3)c Delegated Regulation Annex II of the Delegated Regulation	4th Scenario	To assess the effectiveness of the length of the Monitoring Period, as the time period calculated backwards from the date of notification of the suspicion of the latest outbreak of a category A disease in the protection zone. Products or other materials likely to spread the disease, must had been obtained or produced, before this time period in order to be exempted from prohibitions of movements	• protection zone • non-affected establishments • Products or other materials likely to spread the disease, obtained or produced, before the start of the monitoring period of the affected establishment that originated the protection zone • time period calculated backwards from the date of suspicion of the latest outbreak in the protection zone • time period before the notification of the suspicion, during which the products and materials produced in the non-affected establishments of a protection zone may have been contaminated by the pathogenic agent of the disease

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenarios
ToR 2	Article 32(c) Delegated Regulation Article 48(c) Delegated Regulation Annex II of the Delegated Regulation Annex II of the Delegated Regulation	5th Scenario	To assess the effectiveness of the length of the Monitoring Period, as the time period calculated forwards from the date of semen collection from animals of listed species kept in approved germinal product establishments in the protection or in the surveillance zone, to prove that the donor animal has tested favourable on a sample taken not earlier than 7 days after the monitoring period	• protection or surveillance zone • non-affected approved germinal establishments • semen from kept animals (donor) of listed species • semen collected after the estimated date of the earliest infection of the earliest affected establishment that originated the protection zone/surveillance zone (if belonging to more than one protection or surveillance zones). • to take samples from the donor for laboratory analysis at least 7 days after the end of the monitoring period • to authorise movements of semen from approved germinal product establishments located in the protection or surveillance zones in case of favourable laboratory results • time period calculated forwards from the date of semen collection • time period after the semen collection, during which the animal donor if infected could be detected by the relevant diagnostic test
ToR 2	Article 57(1)b Delegated Regulation Annex II of the Delegated Regulation	6th Scenario	To assess the effectiveness of the length of the Monitoring Period, as the appropriate time period calculated forwards from the date after the final cleaning and disinfection and when relevant control of insects and rodents was carried out in an affected establishment, after which the repopulation of the establishment may be allowed by the competent authority	• repopulation of a previous affected establishment • kept animals of listed species • to allow the repopulation of an affected establishment • time period calculated forwards from the date of the final cleaning and disinfection of the establishment • time period to ensure that the repopulation exercise is not put at risk due to the disease being unknowingly present in an establishment in the surrounding area
ToR 2	Article 59(4)b Delegated Regulation Annex II of the Delegated Regulation	7th Scenario	To assess the effectiveness of the length of the Monitoring Period, as the appropriate time period calculated forwards the date when the first animal was introduced, during which all the animals of listed species intended for repopulation should be introduced	• repopulation of a previous affected establishment • kept animals of listed species to be repopulated • the animals may not be introduced at the same time • time period calculated forwards from the date when the first animal was introduced • time period during which animals intended for repopulation, should be introduced and the process of repopulation be completed

Annex E – Minimum radius and minimum period of duration of protection and surveillance zones according to the legislation in place (Regulation (EU) 2016/429)

Category A diseases	Minimum radius of protection zone Annex V	Minimum radius of Surveillance zone Annex V	Minimum period of duration of measures in the protection zone (Article 39(1)) Annex X	Additional period of duration of surveillance measures in the protection zone (Article 39(3)) Annex X	Minimum period of duration of measures in the surveillance zone (as referred to in Articles 55 and 56 of this Regulation) Annex XI
Foot and mouth disease (FMD)	3 km	10 km	15 days	15 days	30 days
Infection with rinderpest virus (RP)	3 km	10 km	21 days	9 days	30 days
Infection with Rift Valley fever virus (RVFV)	20 km	50 km	30 days	15 days	45 days
Infection with lumpy skin disease virus (LSD)	20 km	50 km	28 days	17 days	45 days
Infection with *Mycoplasma mycoides* subsp. mycoides SC (Contagious bovine pleuropneumonia) (CBPP)	Establishment	3 km	45 days	Not applicable	45 days
Sheep pox and goat pox (SPGP)	3 km	10 km	21 days	9 days	30 days
Infection with peste des petits ruminant virus (PPR)	3 km	10 km	21 days	9 days	30 days
Contagious caprine pleuropneumonia (CCPP)	Establishment	3 km	45 days	Not applicable	45 days
African horse sickness (AHS)	100 km	150 km	12 months	Not applicable	12 months
Infection with *Burkholderia mallei* (Glanders)	Establishment	Establishment	6 months	Not applicable	Not applicable
Classical swine fever (CSF)	3 km	10 km	15 days	15 days	30 days
African swine fever (ASF)	3 km	10 km	15 days	15 days	30 days
Highly pathogenic avian influenza (HPAI)	3 km	10 km	21 day	9 days	30 days
Infection with Newcastle disease virus (NCD)	3 km	10 km	21 days	9 days	30 days

Annex F – Within-flock dynamics of HPAIV in Galliformes and Anseriformes

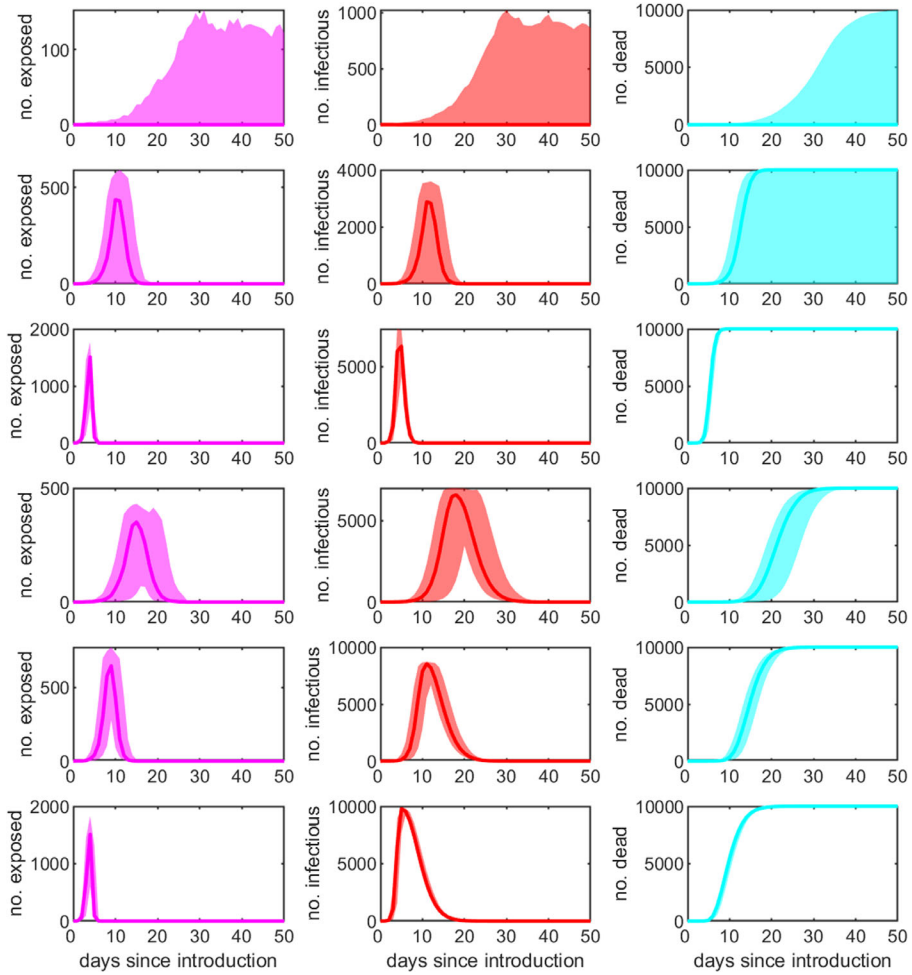

Figure F.1: Within-flock dynamics of highly pathogenic avian influenza virus in gallinaceous poultry. The plots show the median (solid line) and 95% prediction interval (shading) for the number of exposed birds (magenta, left column), infectious birds (red, middle column) and cumulative number of dead birds (cyan, right column) for six scenarios (rows; see Table 2 for details)

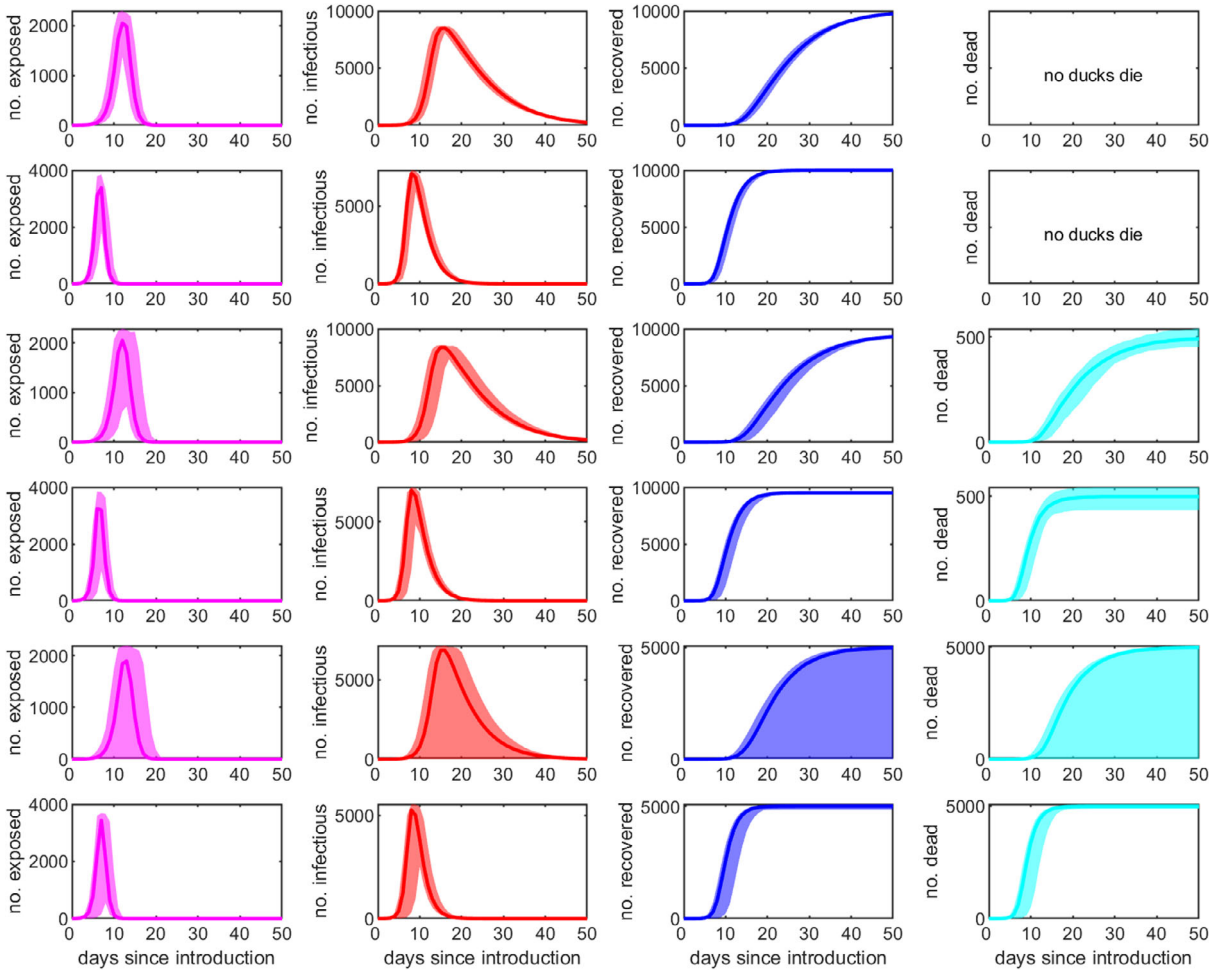

Figure F.2: Within-flock dynamics of highly pathogenic avian influenza virus in Anseriformes. The plots show the median (solid line) and 95% prediction interval (shading) for the number of exposed birds (magenta, first column), infectious birds (red, second column), recovered birds (blue, third column) and cumulative number of dead birds (cyan, fourth column) for six scenarios (rows; see Table 3 for details)

Annex G – Uncertainty

Source or location of the uncertainty	Nature or cause of uncertainty as described by the experts	Impact of the uncertainty on the assessment
ToR 1	Estimates of transmission parameters and infectious period mostly originate from experimental settings. Moreover, they are restricted to chicken and ducks	It is unclear what effect in the assessment this uncertainty would have (in the event of transmission taking place in other species)
ToR 1	Assumptions of transmission model (homogeneous mixing, frequency dependent transmission)	It is unclear what the effect of this uncertainty would be in the assessment
ToR 1	Sensitivity of the diagnostic tests is assumed to be 100%	The effectiveness of the proposed sampling strategy may be overestimated if the tests failed to detect correctly all infected animals
ToR 2	Information on the period elapsed between the earliest point of infection and the suspicion report could only be retrieved from a limited number of references that were mostly describing outbreaks in chicken (only one paper for ducks) and originated from only two countries in the EU	The effectiveness of the proposed monitoring period could be underestimated, in particular for certain Anseriformes that show limited clinical manifestation
ToR 3	The kernel estimates are based on only two epidemics (Italy and the Netherlands) and are based on H7 viruses, not H5	It is unclear what the effect in the assessment would be. In Europe no kernels are available based on H5 viruses. The reason is that the kernels are based on between farm transmission and involvement of wild bird infections makes it (in the absence of WGS data of the viruses) difficult to distinguish new introductions from between farm spread
ToR 3	Minimum period of the protection and surveillance zone: Similar to ToR 2	Similar to ToR 2