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Assessment of the control measures of the category A diseases of Animal Health Law: Newcastle disease

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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 Newcastle disease (ND). 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. Several scenarios for which these control measures had to be assessed were designed and agreed prior to the start of the assessment. The monitoring period (21 days) was assessed as effective in non-vaccinated chicken and turkey flocks, although large uncertainty remains surrounding the effectiveness of this period in vaccinated galliform flocks and flocks of other bird species. It was also concluded that the protection (3 km radius) and the surveillance (10 km radius) zones contain 99% of the infections from an infectious 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 ND.

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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 this 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 restriction 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 covered in the Animal Health Law, was agreed on, and published in a separate technical report.

Newcastle disease (ND) is a highly contagious viral disease affecting domestic and wild birds caused by virulent strains of avian paramyxovirus (APMV-1) with an intracerebral pathogenicity index (ICPI) of 0.7 or greater in day-old-chicks (*Gallus gallus*); or with multiple basic amino acids at the C terminus of the F2 protein and phenylalanine at residue 117 (based on the OIE definition). APMV-1 strains can be classified based on their pathogenicity in chickens into low virulence (lentogenic), moderate (mesogenic) and highly virulent (velogenic) strains, the latter being subdivided into viscerotropic and neurotropic strains.

A qualitative assessment of the clinical examination procedures for Newcastle Disease (ND) was carried out. For assessing the effectiveness of the laboratory examination, a within flock compartmental (SEIR) model was designed using three different flock sizes. Furthermore, median time (days) to detection of a potential ND outbreak in a vaccinated or unvaccinated flock (and 95% prediction intervals) were calculated, given that a predefined number of samples were taken and tested using polymerase chain reaction (PCR). Different transmission rates, mean infectious periods and case fatality rates (no, medium or high mortality for unvaccinated birds; no, low or medium mortality for vaccinated birds) were used for unvaccinated and vaccinated flocks, respectively, to reflect the differences in the clinical manifestation of infection. The effectiveness of taking samples (mainly cloacal and oropharyngeal swabs to be analysed by PCR) for early detection (within 10 days post-infection) was assessed. For most scenarios assessed in unvaccinated or vaccinated birds, provided medium or high mortality occurs, taking these samples was sufficient to lead to the confirmation of the infection. However, in the event of a virus strain that causes low or no morbidity or low or no mortality in either unvaccinated or vaccinated flocks, the time to detection was greater, and therefore, 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. Due to the nature of some of the ND strains circulating in Europe at present, the sampling of 20 cloacal and 20 oropharyngeal swabs (or swabs from all birds if there are less than 20 in a flock) in each epidemiological unit, and the submission of five birds for necropsy, would not be considered sufficient to detect disease in a timely manner in all cases. If no individual clinical signs are obvious, but some abnormal production parameters are observed at the flock level, sampling 60 random birds in the flock is recommended.

To answer ToR 2 and to assess the minimum length of time that 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 an ND virus and the time to reporting a suspicion by the competent authority. The average time to reporting of suspicion was used then to assess the effectiveness of the length of the monitoring period. For most of the scenarios, the existing length of the monitoring period for ND (21 days) was considered sufficient for unvaccinated flocks of chickens, turkeys or pheasants; however, as clinical signs in vaccinated flocks (or with some strains in unvaccinated flocks) are not always obvious or present, this could result in longer periods to a suspicion report. For ducks and geese, only one study was available, and the panel concluded that it was not possible to assess with high certainty that the monitoring period was long enough, particularly in a region where disease had not already been reported. Some recommendations were given in this respect for some of the relevant

scenarios such as moving products from non-affected establishments in the protection zone and for repopulation.

To assess the effectiveness of the minimum length of time the measures are applied in the protection (PZ) and surveillance zones (SZ), the length of time between infection and the reporting of a suspicion as estimated in ToR 2 was used. The minimum length of time of the protection zone (21 days) and the surveillance zone (30 days) were considered effective in chicken and turkeys when the average and longest time assessed in ToR 2 for these species was considered (17 days for the PZ, and 23 days for the SZ for chickens and 17 days and 34 days for the PZ and SZ, respectively, in turkeys). Due to the lack of data available for infection in flocks in which vaccination had taken place, the time to detection, assuming a specific number of samples are submitted for analysis, was also used to assess the effectiveness of the length of these periods in vaccinated flocks.

To assess the effectiveness of the minimum radius to be implemented in the protection and surveillance zones (ToR 3.1), transmission kernels were used. As there were no kernels for ND itself, kernels built using data from outbreaks of Highly Pathogenic Avian Influenza (HPAI) were used, which was considered by the panel to have important similarities in terms of transmission dynamics within the same host population. These kernels represent the relative risk of transmission to each individual establishment from an infectious establishment. For HPAI, the probability of transmission beyond the protection zone (3 km) 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), dropped greatly to 0.05. For NDV, it was considered that the infection rate between birds was similar to that of low and medium transmission scenarios with HPAI, suggesting a lower between-farm transmission rate for NDV compared to HPAI. It was concluded with a 90–99% certainty that a 10-km radius will prevent transmission outside the surveillance zone in minimum 95% of all established zones. It is important to note that the transmission kernels presented cover only some of the risk pathways associated with spread from the index case and they do not take into 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), where these existed. However, certain disease control measures proposed in the Delegated Regulation, in particular in its Annexes, were considered as outdated, that is, 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 latter: 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) and 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, that is, 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 never been 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 establishment 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 the 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. The current manuscript is one of the 14 opinions covering ToRs 1, 2 and 3 for Newcastle disease (ND).
- 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 Regulation (EC) 2020/687 (hereinafter referred to as Delegated Regulation), for which the effectiveness of the sampling procedures will be assessed (Annex B). Although these scenarios will be assessed independently, some of these scenarios may be merged if the assessment processes are the same.
- d) To address ToR 2 (effectiveness of the monitoring period), seven scenarios previously agreed with the contractor were defined (Annex D). The assessment of the effectiveness of the monitoring period will be done 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 backwards or forwards from a specific date. If the length of the monitoring period estimated 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 (protection and surveillance 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; 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 ND, and therefore were not included in the assessment: (a) scenario 4 because no non-listed species are considered as epidemiologically relevant, (b) scenario 7 because the minimum radius of the protection zone for ND is 3 km and (c) scenarios 14 and 15 as they refer to ungulates.
- g) The duration of the monitoring period for ND 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 ND 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 ND as described in Annex X and XI of the Delegated Regulation are 21 and 30 days, respectively.
- j) For the purpose of this opinion, ND is defined in accordance with the Terrestrial Code of the OIE as 'an infection of poultry caused by Newcastle disease virus (NDV), which is an avian paramyxovirus serotype 1 (APMV-1) that meets one of the following criteria for virulence:
 - a) the virus has an intracerebral pathogenicity index (ICPI) in day-old-chicks (*Gallus gallus*) of 0.7 or greater; or
 - b) multiple basic amino acids have been demonstrated in the virus (either directly or by deduction) at the C-terminus of the F2 protein and phenylalanine at residue 117, which is the N-terminus of the F1 protein.'

2. Epidemiology and geographical distribution of ND

2.1. Epidemiology

Newcastle disease (ND) is a highly contagious viral disease affecting domestic and wild birds caused by virulent strains of an avian paramyxovirus (APMV-1) also called NDV (Newcastle Disease Virus), an RNA virus belonging to the genus *Orthoavulavirus* of family Paramyxoviridae. APMV-1 strains can be classified based on their pathogenicity in chickens: low virulence (lentogenic), moderate (mesogenic) and highly virulent (velogenic) strains, the latter being subdivided into viscerotropic and neurotropic strains. Pigeon paramyxovirus type 1 (PPMV-1) refers to certain NDV types predominantly infecting Columbiformes, but which can cause outbreaks of ND also in poultry (Spickler, 2016; OIE, 2013, 2018).

NDV infects more than 250 bird species including domestic and wild birds. Chickens are highly susceptible to ND compared to other species. Clinical signs are more likely to be observed in chickens, while turkeys are less severely affected; infection in geese and ducks is usually asymptomatic. Game birds (pheasants, partridges, peacocks, quail and guinea fowl) show a variable susceptibility. Ostriches and pigeons are susceptible. Infection has been reported in many species of wild birds such as waterfowl, gulls, pigeons and doves, passerines or psittacines, which could act as a potential reservoir. Most APMV-1 strains found in wild birds are lentogenic but could mutate to velogenic and cause ND if transmitted to domestic birds. NDV can infect humans and cause mild conjunctivitis (Alexander, 2000; Rauw et al., 2009; OIE, 2013, 2018; Spickler, 2016).

NDV is transmitted by inhalation or ingestion following close contact with infected animals or by aerosol, through faeces and respiratory secretions. Transmission also occurs through fomites (water, feed, beddings, eggshells, human clothing).

ND is endemic worldwide in poultry except in the US, Canada, Australia, New Zealand and in some countries of South America and Western and Central Europe. In these countries, prophylactic measures include strict biosecurity measures, vaccination of chicks and breeders (in countries where it is allowed) and depopulation in case of sporadic outbreak. Several types of vaccines are available to protect poultry. Live-attenuated vaccines produced from lentogenic or mesogenic strains of APMV-1 are usually administered in drinking water or by aerosol or by intraocular/intranasal instillation. Inactivated vaccines are injected individually; they are more expensive and provide lower immunity than live vaccines but are more thermostable. Recently, vaccines using recombinant technology (vector virus) have also been developed. Whatever the vaccines, regular boosters are needed to maintain protection. Vaccination prevents the disease but upon experimental/natural challenge vaccinated birds can become infected and excrete the virus (EFSA, 2007; Rauw et al., 2009; OIE, 2013, 2018; Spickler, 2016). Nonetheless, vaccination can reduce transmission in experiments (van Boven et al., 2008; Palya et al., 2014).

The severity of the disease depends on the host species, virus strain and immune status of the animals, which can be affected by poor environment and co-infections with other pathogens or by parasites.

The incubation period in poultry is usually 5–6 days (range 2–15 days) but can reach 3–4 weeks in some species. Infections with lentogenic strains of APMV-1 are typically asymptomatic or cause mild respiratory signs (coughing, sneezing, gasping) with no or low mortality.¹ Mesogenic strains are characterised by acute respiratory signs, decreased egg production and sometimes neurologic signs; the mortality rate is usually below 10% in adult chickens (unless co-infected) but can reach 50% in chicks. Infections with velogenic strains typically cause a severe disease in chickens and in some wild species, with morbidity and mortality up to 100% in non-vaccinated poultry. The first signs are lethargy, inappetence, ruffled feathers, and reddening and oedema of the conjunctiva. This is followed by greenish or white watery diarrhoea, dyspnoea and inflammation of the head and neck (cyanosis). Neurologic signs may appear at a later stage (tremors, spasms, wing/leg paralysis, torticollis, circling). Massive deaths usually occur 5–10 days post-infection in chickens depending on the strain. Viscerotrophic forms are currently the most prevalent strains and are characterised by severe diarrhoea and haemorrhagic intestinal lesions, while in neurotropic forms, mostly neurologic and respiratory signs are seen. Sometimes sudden death is observed without clinical signs. In laying hens, the disease causes a sharp drop in egg production; eggs contain a watery albumin and are misshapen with abnormally coloured, rough or thin shells. Surviving birds may develop neurologic sequelae and stop

¹ If the ICPI is below 0.7, these infections would not qualify as ND.

egg production. In pigeons, PPMV-1 infection is characterised by anorexia, polyuria/polydipsia and neuronal disorders; morbidity in adults is often below 10% and subclinical infections are common (EFSA, 2007; OIE, 2013, 2018; Spickler, 2016).

Detection of NDV is performed from oropharyngeal or cloacal swabs (live birds) or organs (spleen, lung, intestines, caecal tonsil, liver, kidneys, heart and brain) from dead birds (OIE, 2013). Conventional or real-time reverse transcriptase PCR (RT-PCR) tests can be used to identify the NDV genome directly in clinical specimens. Virus isolation is the prescribed test for international trade and is performed by inoculation of embryonated eggs and the recovery of a haemagglutinating virus that is inhibited with NDV antiserum (haemagglutination inhibition test) or by use of RT-PCR to detect NDV genome.

The pathogenicity of an APMV-1 strain is determined either through *in vivo* technique by inoculation of day-old-chicks (ICPI: intra-cerebral pathogenity index: range 0.0–2.0) or by *in vitro* techniques to determine the presence of a specific amino acids sequence at the Fusion protein precursor (F0) cleavage site. APMV-1 strains with ICPI ≥ 0.7 or with the presence of the specific sequence are considered as NDV and must be notified to OIE.

Serological tests include haemagglutination inhibition (HI) test, virus neutralisation test and enzyme-linked immunosorbent assays (ELISA). They are used in surveillance programmes or in assessment of post-vaccination antibody level (Alexander, 2000; EFSA, 2007; OIE, 2013, 2018; Spickler, 2016). IgG (referred to as IgY in birds) can be detected 7 days after infection (Miller and Koch, 2020).

2.2. Geographical distribution of ND

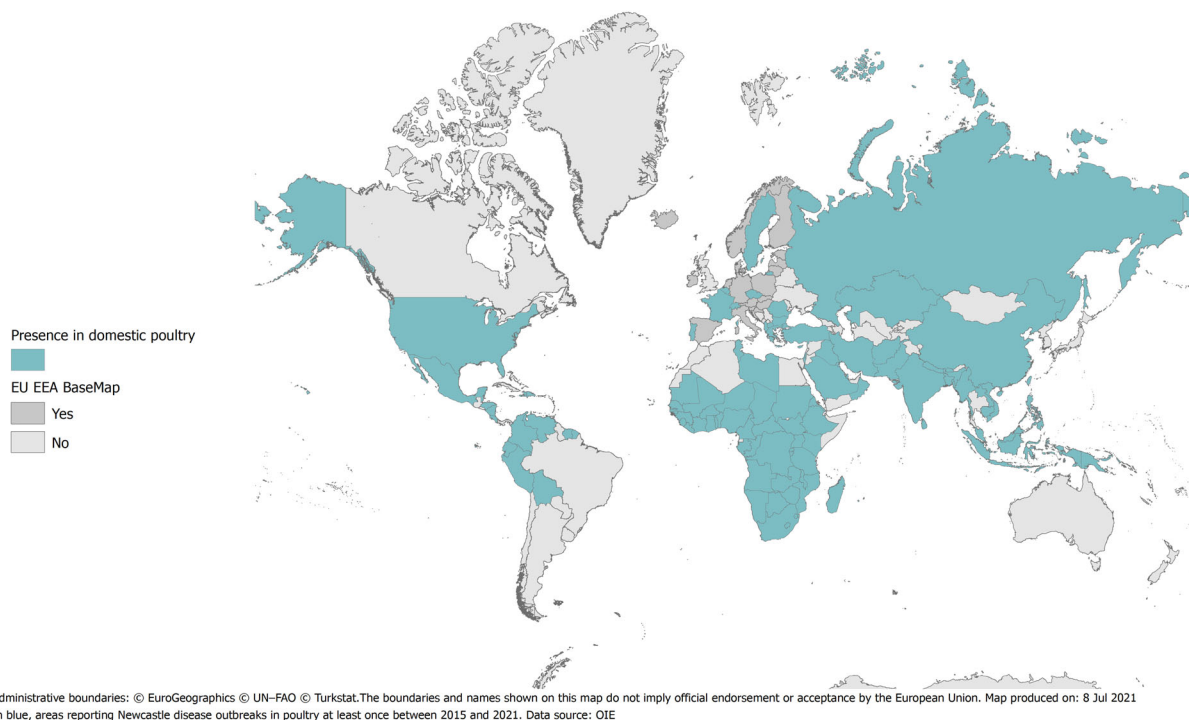


Figure 1: Map of countries with reported outbreaks of ND between 2015 and 2020 (Data sources: ADNS and OIE)

The map above illustrates the countries, which reported cases of ND in poultry between 2015 and 2020. What is clear is that there is a global distribution with all continents, except the Antarctic region, reporting cases. This is indicative of the presence of the virus in wild birds and the sporadic nature of spill-over into poultry. Some countries in the EU control infection through vaccination of poultry (particularly breeding and laying birds), while others rely on biosecurity and rapid disease control measures. However, the presence in wild birds means there is a constant, albeit variable, risk throughout the poultry keeping countries.

3. Data and methodologies

3.1. Methodology used in ToR 1

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, 2020), specific details of the methodology related to the ND opinion are presented below.

Model description

The within-flock dynamics of NDV were modelled using a stochastic *SEIR* epidemic model (Keeling and Rohani, 2008). The host population was divided into four classes: susceptible (i.e. uninfected), S; exposed (i.e. infected, but not yet infectious), E; infectious, I; and recovered, R.

The force of infection was 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 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). Disease-associated mortality was assumed to occur at a constant rate during the infectious period.

The number of birds in each class takes an integer value, while transitions between classes are stochastic processes. The number of transitions of each type during a small time interval δt was drawn from a binomial distribution with number of animals in the class, n , and transition probability, q (the appropriate per capita rate multiplied by δt) as parameters.

To capture a range of scenarios from small producers to large commercial producers, three initial flock sizes were considered: 100; 1000; and 10,000 birds. Transmission parameters were extracted from the published analyses of transmission experiments (van Boven et al., 2008; Tatár-Kis et al., 2020). Two scenarios were considered: transmission in an unvaccinated flock and transmission in a vaccinated flock (Table 1). The outcome of transmission experiments also suggested another scenario for vaccinated flocks in which $R_0 = 0.8$ (Tatár-Kis et al., 2020). However, in this scenario, $R_0 < 1$ and, hence, most outbreaks are very small (the 95th percentile for the proportion of birds infected during the outbreak was around 0.2%) and unlikely to be detected or have much impact. Accordingly, this scenario was not considered further.

In a systematic review of challenge experiments (Dórea et al., 2021), the median case fatality in unvaccinated chickens was 76% (25th percentile = 50%, 75th percentile = 100%). However, outbreaks in unvaccinated poultry with no or very limited observed mortality have also been reported (Danish Veterinary and Food Administration, 2003; Linde et al., 2010; SVA, 2014). In vaccinated birds, case fatality ranged from 0% to 40% (van Boven et al., 2008). Accordingly, no, medium and high mortality scenarios were considered for unvaccinated birds, while no, low and medium mortality scenarios were considered for vaccinated birds (Table 1). Based on the same systematic review (Dórea et al., 2021), the median proportion of unvaccinated birds with clinical signs was 100% (5th percentile = 50%, 25th percentile = 80%). For the no mortality scenarios, infected birds were assumed not to show clinical signs; for the low and high mortality scenario in unvaccinated birds, 80% and 100% were assumed to show signs, respectively (i.e. 25th percentile and median), and for the higher mortality (i.e. medium mortality) scenario in vaccinated birds, 50% were assumed to show clinical signs (assuming case fatality and proportion showing signs are correlated) (Table 1).

Table 1: Parameters used in the model for the transmission of Newcastle disease virus

Scenario		R_0	β^+	μ_E	k_E	μ_I	k_I	Case fatality (%)	Prop. clinical (%)
Unvaccinated	No mortality	3.2	0.67	2	10	4.8	10	0	0
	Medium mortality		0.96					50	80
	High mortality		1.32					75	100
Vaccinated	No mortality	1.6	0.47	3	10	3.4	10	0	0
	Low mortality		0.55					25	30
	Medium mortality		0.62					40	50

†: The transmission rate was calculated so that R_0 is the same in each scenario for mortality (see Annex G).

Within-flock dynamics of Newcastle disease virus

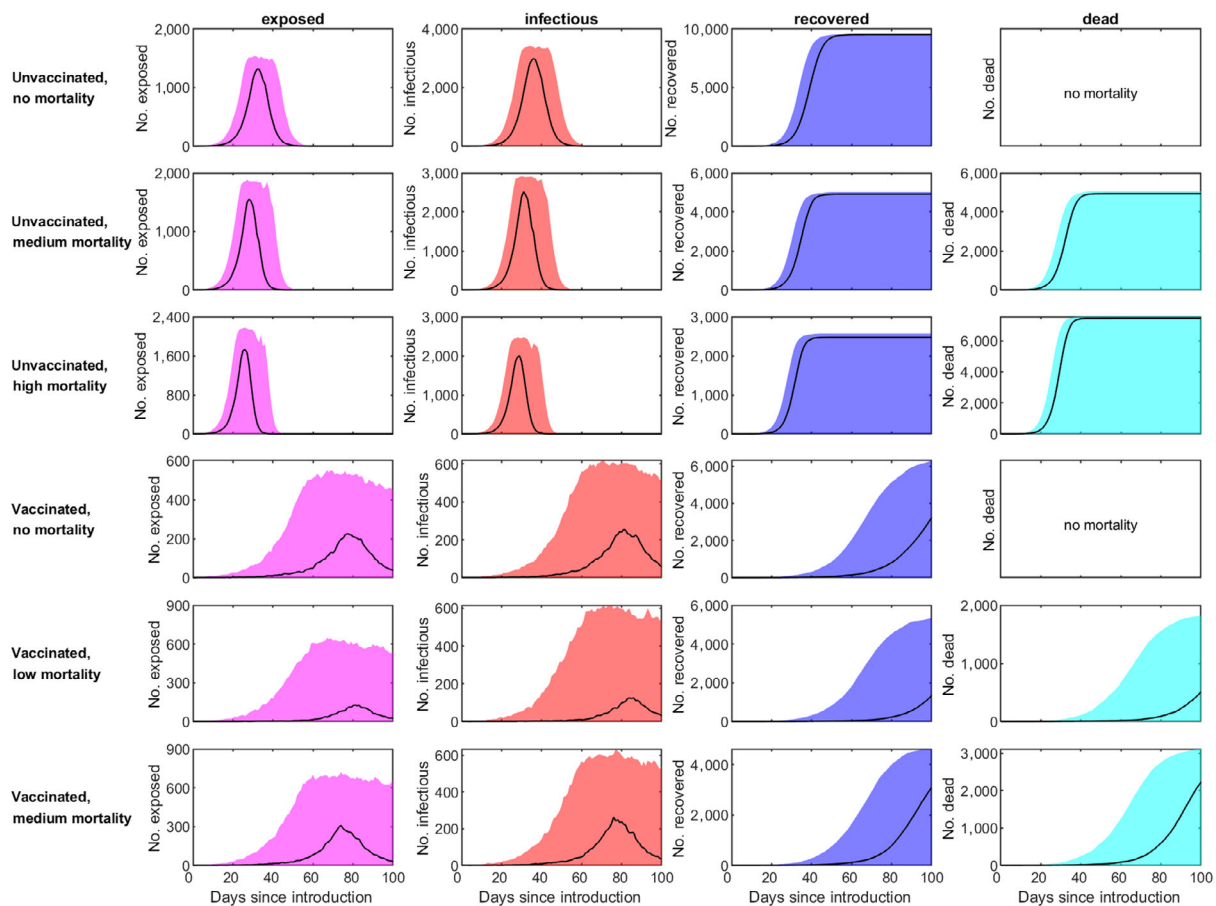


Figure 2: Within-flock dynamics of Newcastle disease virus in chickens. The plots show the median (solid line) and 95% prediction interval (shading) for the number of exposed birds (left-most column), infectious birds (second column), recovered birds (third column) and cumulative number of dead birds (right-most column) for the six scenarios in Table 1 (rows) assuming an initial flock size of 10,000 birds

Detection of Newcastle disease virus

Sampling live birds

For the model predictions, the prevalence of virus-positive birds was assumed to correspond to the prevalence of infectious birds, while the prevalence of seropositive birds was assumed to correspond to the prevalence of recovered birds. The prevalence is the proportion of live birds either virus-positive or seropositive, so the denominator in the calculations is the initial flock size minus the cumulative number of birds that have died of NDV.

Sampling dead or sick birds

The probability of detection, p_D , was computed using the hypergeometric distribution (i.e., sampling without replacement), so that

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

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 2, where m_B is the baseline mortality (proportion of birds dying as a result of non-ND reasons each day), v_B is the baseline morbidity (proportion of birds showing signs consistent with ND) and v_I is the proportion of infectious birds showing signs of ND (Table 1). Baseline morbidity and mortality were assumed to be 1% ($v_B = 0.01$) and 0.1% ($m_B = 0.001$), respectively.

The sensitivity and specificity of the diagnostic test used to confirm NDV were assumed to be 100%.

Table 2: Relationship between numbers of dead, sick and healthy birds and model variables

Sample	M	K	SS
Dead birds	$m_B(S(t) + E(t) + I(t) + R(t)) + D(t)$	$m_B I(t) + D(t)$	5
Sick birds	$v_B(S(t) + E(t) + R(t)) + v_I I(t)$	$v_I I(t)$	20
Healthy birds	$S(t) + E(t) + I(t) + R(t)$	$I(t)$	60

3.2. Methodology used in ToR 2

To estimate the time lag between infection and reporting of an ND suspicion (ToR 2), an extensive literature search (ELS) was outsourced by EFSA (OC/EFSA/ALPHA/2020/02 – LOT 2). The aim of this ELS was to answer the epidemiological question of: 'what is the average, shortest and longest period of time for an outbreak of ND to be reported by an official veterinarian (measured as the number of days from the earliest point of infection with ND virus, to the time of declaration of a suspicion by the competent authority)?'. To answer this question, an ELS on case reports, papers describing outbreaks or epidemics of ND 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. An ELS protocol similar to that shown in Annex 5 of the Methodology report (EFSA, 2020) was followed.

3.3. Methodology used in ToR 3

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

The assessment of radius size of restricted zones (ToR 3), to prevent further disease spread at a given probability, was performed by using disease transmission kernels (EFSA, 2020). However, for ND, no transmission kernel was found in the literature and no data was identified that would enable estimation of a kernel (available data included mostly hobby flocks). Consequently, it was decided to use the HPAI kernel as described in EFSA AHAW Panel (2021), for assessing the effectiveness of the minimum radius. Arguments to use this kernel are: (1) similarities between the viruses (single-stranded RNA and each carry a haemagglutinin and neuraminidase) with comparable tenacity outside the host, (2) same infection route (ingestion, inhalation) and excretion route of the virus (respiratory droplets, faeces) (Miller and Koch, 2020) and (3) similar hosts.

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 of non-vaccinated flocks, the outputs obtained from the ELS described in Section 3.2 were used. Further details can be

found in the Methodology report (EFSA, 2020). As no information was retrieved in terms of vaccinated flocks, the assessment of the length of the measures in these flocks was based on the time to detection results obtained in ToR 1 when using the SEIR compartmental model.

3.4. Uncertainty

A description of the methodology followed is provided in a Methodology report published by EFSA (EFSA, 2020). For this opinion, the impact of the uncertainties identified in the assessment of ToRs 1 (scenario 1), 2 and 3 was assessed collectively after transforming the objective of these ToRs into well-defined quantities of interest. Sources of uncertainty identified in the assessment are listed in Annex F.

For the scenario 1 in ToR1, aiming at the assessment of the effectiveness of existing sampling procedures to detect or rule out the presence of ND in kept animals in a suspected establishment based on clinical and laboratory examinations, it was agreed that a sampling strategy would be considered effective if it would allow the detection of the disease in at least 95% of the poultry establishments in which it was applied. Five different quantities of interest (QoI) were defined based on the vaccination status of the flocks and the reason triggering the suspicion (occurrence of clinical disease and mortality, drop in egg production and/or unexpected shell colour or other egg abnormalities in the absence of increased mortality and birds with clinical signs, or contact tracing with a previously infected holding) (Table 3):

- QoI 1.1: probability that, in 95% or more of all **non-vaccinated** poultry flocks **suspected due to the occurrence of clinical disease and mortality with signs resembling to ND**, the presence of the disease would be detected based on clinical examination and laboratory tests performed on **five dead birds and 20 birds with clinical signs**
- QoI 1.2: probability that, in 95% or more of all **vaccinated** poultry flocks **suspected due to the occurrence of clinical disease and mortality with signs resembling to ND**, the presence of the disease would be detected based on clinical examination and laboratory tests performed on **five dead birds and 20 birds with clinical signs**
- QoI 1.3: probability that, in 95% or more of all (**vaccinated or non-vaccinated**) poultry flocks **suspected due to a drop in egg production or unexpected shell colour or other egg abnormalities (in the absence of increased mortality or clinical signs)**, the presence of the disease would be detected based on clinical examination and laboratory tests performed on **60 birds selected at random**
- QoI 1.4: probability that, in 95% or more of all **non-vaccinated** poultry flocks **suspected due to contact tracing with a previously infected holding**, the presence of the disease would be detected **within 21 days** based on clinical examination and laboratory tests performed on **five dead birds and 20 birds with clinical signs if present, or on 60 birds selected at random if no clinical signs are observed**
- QoI 1.5: probability that, in 95% or more of all **vaccinated** poultry flocks **suspected due to contact tracing with a previously infected holding**, the presence of the disease would be detected **within 21 days** based on clinical examination and laboratory tests performed on **five dead birds and 20 birds with clinical signs if present, or on 60 birds selected at random if no clinical signs are observed**.

Table 3: Differences in the QoI defined to quantify the uncertainty regarding the effectiveness of sampling strategies considered for scenario 1 of ToR1

QoI	Vaccination status	Reason for suspicion	Sampling strategy
1.1	Non-vaccinated	Clinical disease and mortality	5 dead birds and 20 birds with clinical signs
1.2	Vaccinated	Clinical disease and mortality	5 dead birds and 20 birds with clinical signs
1.3	Vaccinated and non-vaccinated	Drop in egg production or egg abnormalities	60 birds selected at random
1.4	Non-vaccinated	Contact tracing	5 dead birds and 20 birds with clinical signs or 60 birds at random if no clinical disease
1.5	Vaccinated	Contact tracing	5 dead birds and 20 birds with clinical signs or 60 birds at random if no clinical disease

For ToR2, which aims at the assessment of the effectiveness of the length of the monitoring period under different scenarios, a given length was considered effective if it would serve its scenario-specific

purpose in at least 95% of the cases in which it was implemented. In this case, seven different QoI were defined based on the scenarios among those listed in Annex D, the poultry species in the establishment (Galliformes such as chicken, turkey and pheasants vs. Anseriformes such as ducks and geese) and whether the suspected establishment was the first case in a region or not (Table 4):

- QoI 2.1 (scenarios 1, 2 and 4): probability that, in 95% or more of all **chicken, turkey and pheasant non-vaccinated establishments** suspected and eventually confirmed **as the first cases in a previously unaffected region**, the initial infection would have occurred within 21 days before the date of notification of the suspicion.
- QoI 2.2 (scenarios 1, 2 and 4): probability that, in 95% or more of all **chicken, turkey and pheasant vaccinated establishments** suspected and eventually confirmed **as the first cases in a previously unaffected region**, the initial infection would have occurred within 21 days before the date of notification of the suspicion
- QoI 2.3 (scenarios 1, 2 and 4): probability that, in 95% or more of all **chicken, turkey and pheasant non-vaccinated establishments** suspected and eventually confirmed **in an already affected region**, the initial infection would have occurred within 21 days before the date of notification of the suspicion.
- QoI 2.4 (scenarios 1, 2 and 4): probability that, in 95% or more of all **chicken, turkey and pheasant vaccinated establishments** suspected and eventually confirmed **in an already affected**, the initial infection would have occurred within 21 days before the date of notification of the suspicion
- QoI 2.5 (scenarios 1, 2 and 4): probability that, in 95% or more of all **duck and geese establishments** suspected and eventually confirmed **as the first cases in a previously unaffected region**, the initial infection would have occurred within 21 days before the date of notification of the suspicion.
- QoI 2.6 (scenarios 1, 2 and 4): probability that, in 95% or more of all **duck and geese establishments** suspected and eventually confirmed **in an already affected region**, the initial infection would have occurred within 21 days before the date of notification of the suspicion.
- QoI 2.7 (scenario 3): probability that 95% or more of the **independent epidemiological units within chicken, turkey and pheasant non-vaccinated ND-affected establishments** that eventually become infected would have been infected within 21 days before the date of confirmation of the disease in the establishment.
- QoI 2.8 (scenario 3): probability that 95% or more of the **independent epidemiological units within chicken, turkey and pheasant vaccinated ND-affected establishments** that eventually become infected would have been infected within 21 days before the date of confirmation of the disease in the establishment.
- QoI 2.9 (scenario 3): probability that 95% or more of the **independent epidemiological units within duck and geese ND-affected establishments** that eventually become infected would have been infected within 21 days before the date of confirmation of the disease in the establishment.
- QoI 2.10 (scenario 6): probability that, in 95% or more of **chicken, turkey and pheasant ND-affected establishments that are depopulated and are in the vicinity of an unknowingly infected establishment**, the disease in the surrounding establishment is detected in the 21 days following the cleaning and disinfection of the ND-affected establishment.
- QoI 2.11 (scenario 6): probability that, in 95% or more of **duck and geese ND-affected establishments that are depopulated and are in the vicinity of an unknowingly infected establishment**, the disease in the surrounding establishment is detected in the 21 days following the cleaning and disinfection of the ND-affected establishment.

Table 4: Differences in the QoI defined to quantify the uncertainty regarding the effectiveness of monitoring periods considered for ToR2 (NA: non-applicable)

QoI	Scenario	Unit assessed	Poultry species	Vaccination status	First cases in a region
2.1	1, 2, 4	Suspected establishment	Chicken, turkey, pheasant	Non-vaccinated	Yes
2.2	1, 2, 4	Suspected establishment	Chicken, turkey, pheasant	Vaccinated	Yes

QoI	Scenario	Unit assessed	Poultry species	Vaccination status	First cases in a region
2.3	1, 2, 4	Suspected establishment	Chicken, turkey, pheasant	Non-vaccinated	No
2.4	1, 2, 4	Suspected establishment	Chicken, turkey, pheasant	Vaccinated	No
2.5	1, 2, 4	Suspected establishment	Duck and geese	Non-vaccinated	Yes
2.6	1, 2, 4	Suspected establishment	Duck and geese	Non-vaccinated	No
2.7	3	Independent unit in confirmed establishment	Chicken, turkey, pheasant	Non-vaccinated	NA
2.8	3	Independent unit in confirmed establishment	Chicken, turkey, pheasant	Vaccinated	NA
2.9	3	Independent unit in confirmed establishment	Duck and geese	Non-vaccinated	NA
2.9	6	Establishments surrounding depopulated units	Chicken, turkey, pheasant	Vaccinated and non-vaccinated	NA
2.10	6	Establishments surrounding depopulated units	Duck and geese	Non-vaccinated	NA

Finally, for ToR3, which aims at the assessment of the effectiveness of the minimum radii established in the protection and surveillance zones, a given radius was assumed to be effective if it would prevent transmission to outside of the zone in the 21 days (protection zone) or 30 days (surveillance zones) following the setting up of these zones. In this case, two QoI were defined:

- QoI 3.1: probability that in 95% or more of all protection zones with a radius of 3 km, there is no transmission to outside the zone in the 21 days following their establishment.
- QoI 3.2: probability that in 95% or more of all surveillance zones with a radius of 10 km, there is no transmission to outside the zone in the 21 days following their establishment.

Members of the WG provided their judgements individually for each of the QoI, along with the rationale supporting them, using the probability scale (Table 5) proposed in the EFSA uncertainty guidance (EFSA Scientific Committee, 2018).

Table 5: Approximate probability scale used for quantification of the uncertainty in the assessment

Probability term	Subjective probability range	Additional options	
Almost certain	99–100%	More likely than not: > 50%	Unable to give any probability: range is 0–100%
Extremely likely	95–99%		
Very likely	90–95%		
Likely	66–90%		
About as likely as not	33–66%		
Unlikely	10–33%	Report as 'inconclusive', 'cannot conclude' or 'unknown'	
Very unlikely	5–10%		
Extremely unlikely	1–5%		
Almost impossible	0–1%		

Individual judgements and rationales were discussed during a meeting in order to elicit a consensus group judgement for each QoI. The outputs of this assessment are provided in their respective sections.

4. Assessment

4.1. Assessment of sampling procedures (ToR 1)

4.1.1. Assessment of sampling procedures in the event of suspicion or confirmation of ND

4.1.1.1. In the event of a suspicion of ND 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 NDV in kept animals if the disease is present in that establishment, or to rule it out if not present (Art. 6 (2)). For further details, see Annexes B and C.

- **1st Scenario of sampling procedures.**
- ToR 1.1 and ToR 1.2 in accordance with Mandate.
- Article 6(2) of the Delegated Regulation (EU) 2020/687.
- Commission Implemented Regulation 2018/1882 on listed species.

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

- 1) It concerns an event of suspicion of ND in an establishment with kept animals of the listed species;
- 2) The listed species for ND as provided in Commission Implemented Regulation 2018/1882 are those belonging to the Aves class;
- 3) Subsequent to the suspicion, the competent authority shall immediately conduct an investigation to confirm or rule out the presence of the disease;
- 4) The official veterinarian must perform a clinical examination and collect samples for further laboratory examination (see Annex C for details on guidelines on how the clinical and laboratory examination must be carried out).

Summary of sampling procedures

There were no specific guidelines in the EU legislation in place prior to the implementation of AHL, for clinical examination of birds in flocks, where ND has been suspected. The only requirement enacted in the old Directive **92/66/EEC** Article 4 (a) was to record the number of birds in all categories of poultry on the holding which have died, which show clinical signs and which show no signs. The record had to be kept up to date to include birds born or dying during the period in which there was a suspicion.

In a manual published by Capua and Alexander (2009), more detailed guidelines are provided on how the clinical examination of birds should be conducted and what information must be recorded.

In the field guide for Australian veterinarians (Department of Agriculture and CSIRO, 2019), it is recommended in case of ND suspicion to submit whole birds for post-mortem examination.

In all cited guidelines, the confirmation of ND by means of laboratory testing is foreseen and recommendations for sample collection are provided.

In Annex III of the old EU Directive **92/66/EEC**, the tissues and excretions to be collected from sick and dead birds are listed and laboratory methods to be applied for testing and disease confirmation are described. No instructions on the number of samples to be collected are provided.

In the guidelines of the EU reference laboratory (IZSVE, 2015), it is recommended to collect, in the event of a suspected outbreak, blood serum samples from 20 birds if there are more than 20 birds in the flock and from all birds if the number is ≤ 20 .

In a manual of Capua and Alexander (2009), it is recommended to collect samples from sick as well as recently dead birds as follows:

- from birds showing signs of disease – 20 cloacal and 20 oropharyngeal swabs (or swabs from all birds if there are less than 20 in a flock) in each epidemiological unit.
- birds exhibiting overt clinical signs – a minimum of five birds for necropsy.
- From birds that are sick or apparently recovered – 20 blood samples or samples from all birds if less than 20 in flock, in each epidemiological unit.

In the guidelines of Western Australian Agricultural Authority (2018), collection of following samples is prescribed for submission to the diagnostic laboratory:

- choanal (mouth) and cloacal swabs from 10 birds.
- two millilitres of clotted blood samples from 15 birds.
- five dead birds or a range of tissues in formalin.

Assessment

In case the suspicion of ND in a flock has been raised due to the occurrence of the disease and mortality with signs resembling to ND or such birds are observed during the clinical examination of a suspected flock, the sick and dead birds in the flock should be targeted for sampling. An assessment of the effectiveness of the sampling described by Capua and Alexander (2009) is shown below.

Based on model analysis, in non-vaccinated flocks, and considering only scenarios where NDV is causing mortality, when testing five dead birds for NDV, the median time between introduction and detection (with 95% confidence) ranges from 10 to 14 days (depending on flock size and virulence of the virus strain causing the outbreak) (Table 6). The upper limit of this estimate is 22 days.

In vaccinated flocks, the median time to the detection of the virus in dead birds would be considerably delayed to 27–43 days post introduction and detection would be possible with 95% confidence only if the circulating strain is able to cause medium mortality. The upper limit for time to detection is 85 days.

Table 6: Median (M), lower (L) and upper (U) bounds of 95% prediction intervals, in days post-introduction, at which 95% confidence of detecting Newcastle disease virus is reached when testing five dead birds

scenario		Flock size								
		100			1,000			10,000		
		M	L	U	M	L	U	M	L	U
Unvaccinated	No mortality*	– [†]	– [†]	– [†]	– [†]	– [†]	– [†]	30	26	42
	Medium mortality	13	9	22	13	8	19	14	10	22
	High mortality	10	5	17	10	6	16	11	7	17
Vaccinated	No mortality	– [†]	– [†]	– [†]	– [†]	– [†]	– [†]	– [†]	– [†]	– [†]
	Low mortality	27	19	41	43	17	85	38	23	74
	Medium mortality	30	17	49	27	16	54	33	18	65

†: 95% confidence not reached in this scenario.

*: This refers to scenarios where the virus has an ICPI higher than 0.7, but no increased mortality is observed in the flock.

The effectiveness of sampling 20 sick birds (exhibiting clinical signs compatible with ND) was also assessed with the model. The results of the model analysis presented in Table 7 show that the infection would be detected in these birds with 95% confidence 9–13 days (median) post introduction of the virus into an unvaccinated herd. In vaccinated herds, the virus detection would be considerably delayed (median 30–34 days post introduction) and could be achieved with 95% confidence in larger flocks.

Table 7: Median (M), lower (L) and upper (U) bounds of 95% prediction intervals, in days post-introduction, at which 95% confidence of detecting Newcastle disease virus is reached when testing 20 sick birds

Scenario		Flock size								
		100			1,000			10,000		
		M	L	U	M	L	U	M	L	U
Unvaccinated	No mortality [†]	–	–	–	–	–	–	–	–	–
	Medium mortality	13	10	24	10	6	16	12	8	20
	High mortality	13	9	24	9	5	16	10	7	16

Scenario		Flock size								
		100			1,000			10,000		
		M	L	U	M	L	U	M	L	U
Vaccinated	No mortality [†]	–	–	–	–	–	–	–	–	–
	Low mortality	– [‡]	– [‡]	– [‡]	45	28	86	40	26	75
	Medium mortality	– [‡]	– [‡]	– [‡]	30	16	58	34	20	64

[†]: birds assumed not to show clinical signs, except for flock level signs such as egg drop and egg abnormalities.

[‡]: 95% confidence not reached in this scenario.

Table 8: Median (M), lower (L) and upper (U) bounds of 95% prediction intervals, in days post-introduction, at which 10% seroprevalence of Newcastle disease virus is reached

Scenario		Flock size								
		100			1,000			10,000		
		M	L	U	M	L	U	M	L	U
Unvaccinated	No mortality	14	11	21	23	18	29	30	26	42
	Medium mortality	14	11	22	22	18	28	30	25	38
	High mortality	15	11	24	21	18	28	28	26	35

As a conclusion, guidelines from Capua and Alexander (2009) could be considered effective for the detection of the infection in flocks with suspected ND if the circulating virus is causing mortality and/or overt clinical signs in unvaccinated or vaccinated flocks. However, in vaccinated flocks, the detection of the disease would be considerably delayed if only these guidelines were followed. Furthermore, the NDV strains circulating in the EU in recent years have been of relatively low virulence and have not caused mortality and clear clinical signs in affected flocks yet being classified as NDV based on the pathology index (see the epidemiology section). This sampling scheme cannot be considered sufficient for timely disease detection in these situations.

The results from the uncertainty analysis carried out indicated that a sampling strategy based on clinical examination and laboratory tests performed on five dead birds and 20 birds with clinical signs would be adequate to detect 95% or more of all non-vaccinated establishments suspected due to the occurrence of clinical disease and mortality with signs resembling to ND (QoI 1.1) with a 90–100% certainty (based on expert opinion). If the same strategy was applied in vaccinated establishments suspected due to the presence of clinical signs or mortality resembling to ND (QoI 1.2), the certainty that 95% or more of them would be detected was 66–99%. The wider uncertainty range was due to the increased difficulties to identify ND-affected animals in vaccinated flocks in which transmission of the disease would be slower and other non-ND-related morbidity and mortality could impair the effectiveness of the sampling strategy.

Development of new procedures

In flocks where the disease is suspected due to the drop in egg production, unexpected shell colour or other egg abnormalities and no increased mortality or birds with clear clinical signs could be identified, the following sampling scheme could be suggested:

Random sample of 60 birds of the flock for virus detection enabling the detection of the infection at a prevalence of 5% with 95% confidence (assuming 95% test sensitivity and 100% specificity).

The model analysis results (Table 9) show that the disease would be detected between 6 and 25 days (median) post introduction in unvaccinated flocks, and 13–71 days post introduction in vaccinated flocks with 5% prevalence of infectious birds. In order to achieve a similar time to detection in vaccinated flocks (to that in non-vaccinated flocks), the number of samples should be 80 for a 100 bird flock, and 500 and 2,400 for a flock of 1,000 and 10,000 birds, respectively; nonetheless, the range around the time to detection would be larger.

Overall, it was concluded with a 66–99% certainty that the application of a sampling strategy based on sampling 60 birds selected at random would be effective to detect 95% or more of the flocks suspected due to a drop in egg production or unexpected shell colour or other egg abnormalities and no increased mortality or birds with clear clinical signs (QoI 1.3). The uncertainty range reflects the

uncertainty regarding the degree of infection that would be needed in order to lead to a drop in egg production or the occurrence of egg abnormalities at a level sufficient to trigger a suspicion.

In case the suspicion is raised due to laboratory findings, the clinical examination of the flock as well as sampling should follow the guidelines described above, that would vary depending on the presence or lack of clinical signs at inspection.

Table 9: Median (M), lower (L) and upper (U) bounds of 95% prediction intervals, in days post-introduction, at which 95% confidence of detecting Newcastle disease virus is reached when testing 60 random birds

Scenario		Flock size								
		100			1,000			10,000		
		M	L	U	M	L	U	M	L	U
Unvaccinated	No mortality	6	3	14	17	12	24	25	20	36
Vaccinated		13	5	30	49	29	82	71	51	89

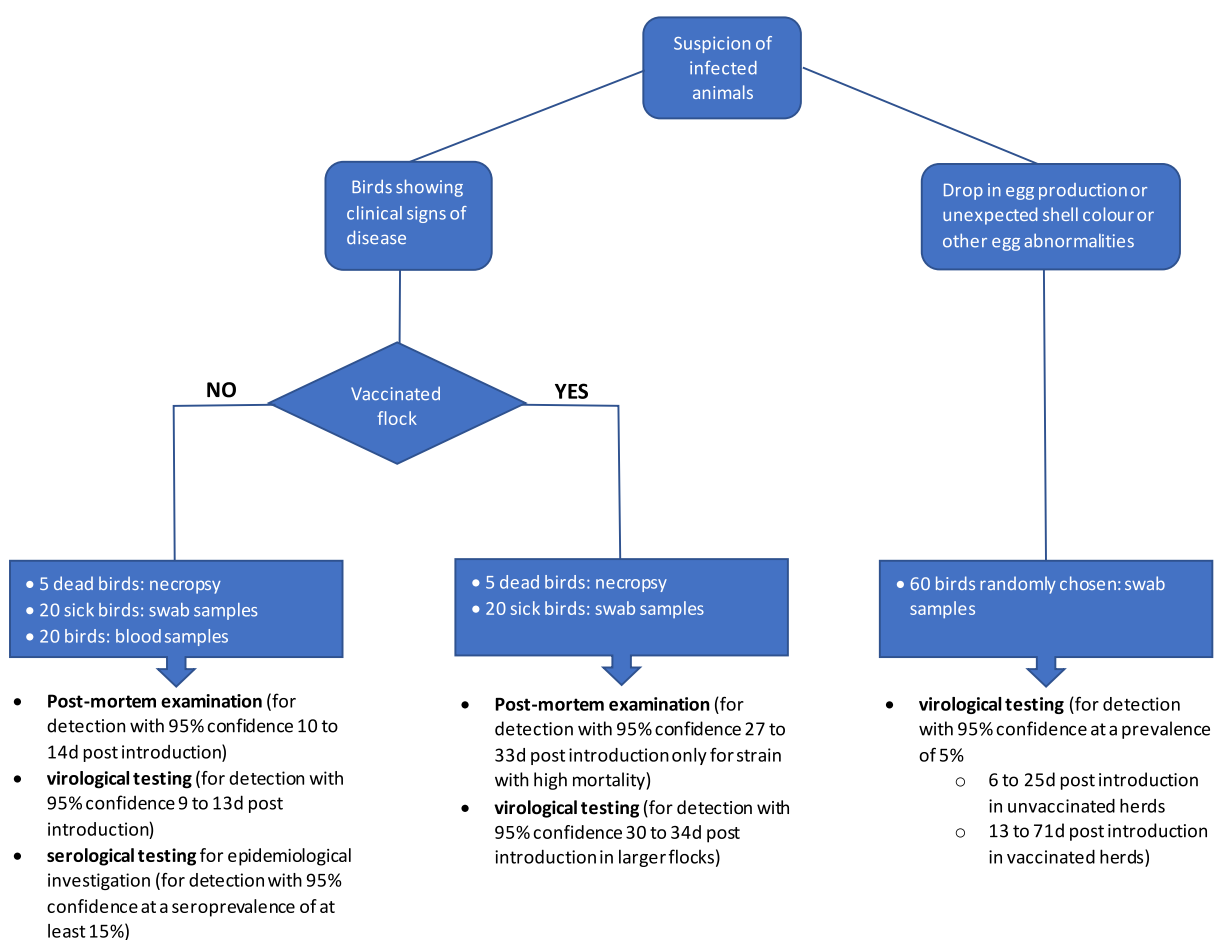


Figure 3: Decision tree for the suggested sampling procedure for NDV confirmation

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

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 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. For further details, see Annexes B and C.

- **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 (EU) 2020/687.
- Article 57 of the Regulation (EU) 2016/429.

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

- 1) It concerns an affected establishment officially confirmed with ND;
- 2) Kept animals of listed species found dead or before/when they are killed are sampled;
- 3) Competent authority collects 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.
 - b) confirming/ruling out disease in the event of preventive killing.

Summary of sampling procedures

No specific guidelines were found.

Assessment

Non-applicable as no specific guidelines were found.

Development of new procedures

1) Epidemiological inquiry

To establish the length of time ND may have been present in the establishment, it is important to carry out contact tracing, as it may reveal specific contacts in time with a previously infected establishment. In addition, in case of multiple flocks in the establishment, each of these flocks should be tested according to the protocol described in scenario 1, including dead birds, sick birds and sera. This will provide information on flock level prevalence of the infection across the establishment. Taking serological samples would help to assess the length of time the disease has been in the farm, as antibodies that are more widespread generally indicate a longer presence of the virus in the farm. Moreover, the presence of antibodies in birds of unvaccinated flocks also indicates a longer duration of the infection than the absence of antibodies. In case a holding was suspected based on egg drop or eggshell changes and no clinical signs are present, in addition to the above, the start of the egg drop/egg shell changes should be investigated in the production data of the establishment.

In case virus is detected, quick whole genome sequencing (WGS) is recommended in order to enable to help establishing the virus source and with it the possible moment of virus introduction and duration of the infection.

Regarding the effectiveness of the protocol described in scenario 1 to detect poultry flocks suspected due to contact tracing, when applied in non-vaccinated flocks, it was concluded with a 90–99% certainty that it would be effective for detecting 95% or more of all infected establishments (QoI 1.4). However, if the same strategy was applied in vaccinated flocks suspected due to contact tracing (QoI 1.5), the certainty regarding the effectiveness of the protocol was 33–99% due to the lack of knowledge regarding the stage of infection in which the flock could be and the potential for conducting the sampling when disease was only present at low levels, given that ND transmission would be much slower.

2) Preventive killing

In case of preventive killing, a clinical inspection and examination should take place in order to identify increased morbidity and mortality. In case these are present, dead and sick birds should be sampled and tested as described in scenario 1. In case no dead and sick birds are present, 60 randomly selected birds should be tested immediately for the presence of virus and antibodies. This

allows detection of a (sero)prevalence of 5% with 95% confidence. Further details are presented in scenario 1 above.

4.1.1.3. For granting a specific derogation from killing animals of the categories described in article 13.2 of the Delegated Regulation in an ND-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. For further details, see Annexes B and C.

- **3rd Scenario of sampling procedure.**
- ToR 1.1 and ToR 1.2 in accordance with Mandate.
- Article 13(3)c of the Delegated Regulation (EU) 2020/687.

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

- 5) It concerns an affected establishment where infection is officially confirmed;
- 6) In the establishment where there are kept animals of listed species of the following specific categories animal categories based on article 13(2):
 - animals kept in a confined establishment.
 - animals kept for scientific purposes or purposes related to conservation of protected or endangered species.
 - animals officially registered in advance as rare breeds.
 - animals with a duly justified high genetic, cultural or educational value.
- 7) 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;
- 8) The animals should be subjected to clinical surveillance, including laboratory examinations;
- 9) 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

According to the EU Reference Laboratory (IZSVE), given the similarity between Avian influenza and ND in terms of target species and tissue tropism, the collection of samples, tissue material to be examined and transport of samples are considered applicable in the management of both diseases. The diagnostic manual for AI recommends that 21 days after the last finding of HPAI (and therefore ND), 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.

In the old Directive 92/66/EEC, it is stated that a derogation from killing for carrier pigeons or other birds confirmed to be infected with ND can be applied, provided a ban on movement of the pigeons or birds kept in captivity outside the pigeon house or holding for at least 60 days after the clinical signs of Newcastle disease have disappeared is implemented. Birds of scientific interest can be derogated from being vaccinated provided serological surveillance is carried out, but there are no details.

Assessment

As determined in Section 4.2.2, 21 days is an effective monitoring period to determine whether an independent epidemiological unit within non-vaccinated ND-affected establishments would eventually become infected. This 21-day monitoring period is less likely to be effective in vaccinated establishments (see Table 6).

Development of new procedures

Where the affected establishment has vaccinated birds, a longer monitoring period should be used (see Table 6) during which time periodic virological surveillance can be used.

Where the establishment has unvaccinated birds, if no clinical signs are seen after a period of 21 days, a final serological sampling strategy can be applied. See Section 4.1.1 to apply the recommendations for sample sizes according to the mortality of the strain and the vaccination status of the birds.

4.1.1.4. For wild animals of the listed species within an ND-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. For further details, see Annexes B and C.

- 1) **5th scenario of sampling procedures.**
- 2) ToR 1.1 and ToR 1.2 in accordance with Article 14(1) of the Delegated Regulation (EU) 2020/687.
- 3) Article 57 of the Regulation (EU) 2016/429.
- 4) Commission Implemented Regulation 2018/1882 on listed species.

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

- a) It concerns a ND affected establishment (officially confirmed).
- b) It refers to wild animals of listed species within the establishment and in the surroundings of the establishment.
- c) As listed in Commission Implementing Regulation (EU) 2018/1882 for ND; the wild animals of listed species animals are those of Aves class.
- d) The competent authority may establish these sampling procedures in addition to other measures.
- e) 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 animals.

Summary of sampling procedures

No specific guidelines were found.

Assessment

Not applicable as there are no guidelines to be assessed.

Development of new procedures

Passive surveillance with testing of wild birds found dead in the surroundings of the affected establishment is recommended as a complement to the epidemiological investigation to help establishing the virus source.

4.1.1.5. 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. The purpose of the sampling procedures is to ensure the detection of the virus, if the virus is present in these animals. For further details, see Annexes B and C.

- **6th Scenario of sampling procedures.**
- ToR 1.1 and ToR 1.2 in accordance with Mandate.
- Article 26(2) of the Delegated Regulation (EU) 2020/687.

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

- 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, collection of samples for laboratory examination;
- 4) The purpose of sampling procedures is to confirm or rule out the presence of ND.

Summary of sampling procedures

No specific sampling procedures for the sampling of animals in establishments of the protection zone were found.

Below, guidelines for sampling animals in these establishments are provided under the development of new procedures.

Assessment

In Section 4.3.1, an assessment of the transmission taking place in the protection zone is carried out. Here, it can be observed how the majority of secondary infections are located in the protection zone. Nevertheless, results from outbreaks in the EU in the past two decades have shown that the proportion of secondary infections in commercial poultry holdings in the protection zone is low (ADIS, day of extraction 9/7/2021). The probability of secondary infection may depend on the poultry and flock density in the affected region (higher density associated with higher probability of transmission) and the vaccination status (vaccination reduces transmission in case of sufficiently high HI titre) (van Boven et al., 2008).

Development of new procedures

Clinical inspection and examination, complemented with systematic assessment of production records such as food and water intake and egg production, including proportion of egg abnormalities, should take place in all commercial establishments in the protection zone and sample collection as described in scenario 1 in the event of suspicion of the disease. Moreover, it is expected that in regions where vaccination is applied, a booster vaccination will be given to chickens to enhance immunity and reduce transmission. The clinical inspection and examination described above should precede vaccination. Vaccination should start from the periphery of the protection zone moving towards the centre to reduce as much as possible potential spread of the virus by vaccination teams.

4.1.1.6. 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. For further details, see Annexes B and C.

- **8th scenario of sampling procedures:**
- ToR 1.3 in accordance with Article 41 of the Delegated Regulation (EU) 2020/687.

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

- 1) It concerns the surveillance zone.
- 2) Sample of the establishments of kept animals of listed species in the surveillance zone.
- 3) 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.
- 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 sampling procedures

No specific guidelines are described in legislation.

Assessment

There are no specific guidelines to be assessed.

Development of new procedures

In case of a strain with medium or high mortality and morbidity, detection of outbreaks in the surveillance zone of an area where vaccination has not been applied could rely on passive surveillance. The reason is that the likelihood of observing a secondary outbreak is much higher in the protection zone than in the surveillance zone and, should outbreaks be detected in the protection zone, the latter is expanded as is the area where active surveillance takes place. For the commercial poultry keepers, these signs would also include production parameters such as egg drop, malformations of eggshells, reduced water and feed intake. In a vaccinated region, most likely a booster vaccination will be applied to gallinaceous poultry (at least in commercial flocks) and pigeons in the surveillance zone to enhance immunity and stop transmission. Consequently, in addition to the awareness campaign, a clinical inspection and examination should be performed by the veterinarian before the vaccination is applied (as in common practice because diseased animals should in general not be vaccinated) and in the event of suspected signs, they should be reported immediately. Visiting a random sample of farms for inspection and potential sample collection is not considered useful. The reason is (1) it is not considered necessary in the absence of secondary infections in the protection zone and the protection zone will be expanded in case secondary infections in that zone are detected, (2) the goal is to have the surveillance zone completely free from the virus, so establishing a design prevalence for the surveillance is not possible (instead this would require examining all holdings).

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). For further details, see Annexes B and C.

- **9th Scenario of sampling procedures.**
- ToR 1.4 in accordance with Article 28(5) of the Delegated Regulation (EU) 2020/687.
- Article 29 of the Delegated Regulation.

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

- 1) It concerns the protection zone.
- 2) Grant derogation for movement of kept animals of 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

No specific guidelines are available.

Assessment

There are no guidelines available to be assessed.

Development of new procedures

Non-vaccinated poultry: Clinical inspection should be complemented with systematic assessment of production records such as food and water intake and egg production, including proportion of egg abnormalities. In case clinical signs are observed on individual bird or flock level, the sampling scheme suggested under Section 4.1.1.1 should be followed.

Vaccinated poultry: as for non-vaccinated poultry.

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 (EU) 2020/687.

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

No guidelines are available.

Assessment

The risk of spreading NDV by DOC must be considered very unlikely (5–10% based on the probability range used for quantification of uncertainty shown in Table 5). Although vertical transmission of NDV has been reported, the significance of such transmission is not clear, as infection with virulent APMV-1 generally results in a dramatic drop in egg production and infected embryos die (EFSA AHAW Panel, 2017). In addition, the eggs are disinfected before hatching. Thus, infection will be noted in the hatchery. Moreover, as it takes at least 3 weeks between collecting the eggs and hatching, any infection would most likely be detected already in the infected parent flock before any DOC will have been moved. Thus, no clinical examination or testing is required.

Development of new procedures

Non applicable.

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 (EU) 2020/687.

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 regard 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

No guidelines are available.

Assessment

In this scenario, a higher risk of disease spread than in scenario 9 is assumed, as birds are not sent to slaughter but moved to another establishment.

Development of new procedures

Non-vaccinated: The recommendations would be similar to those in Section 4.1.2.1. In addition, follow-up investigation of the recipient farm, including systematic assessment of production records, morbidity and mortality would be recommended.

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). For further details, see Annexes B and C.

- **12th Scenario of sampling procedures.**
- ToR 1.4 in accordance with Mandate.
- Article 28(5) and article 37 of the Delegated Regulation (EU) 2020/687.

The following elements of the scenario were 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

No guidelines are available.

Assessment

There are no guidelines available to be assessed.

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. For further details, see Annexes B and C.

- **13th Scenario of sampling procedures.**
- ToR 1.4 in accordance with Mandate.
- Article 43(5) and article 44 of the Delegated Regulation (EU) 2020/687.

The following elements of the scenario were taken into consideration for the:

- 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

No guidelines are available.

Assessment

There are no guidelines available to be assessed.

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 (EU) 2020/687.

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

No guidelines are available.

Assessment

As per Section 4.1.2.2.

Development of new procedures

As per Section 4.1.2.2.

4.1.2.7. For ready-to-kill 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-kill 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 (EU) 2020/687.

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

- 1) It concerns the surveillance zone.
- 2) Ready-to-kill 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

No guidelines available.

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 move within the restricted zone, when restriction measures are maintained beyond the period set out in Annex XI of the Delegated Regulation. For further details, see Annexes B and C.

- **18th scenario of sampling procedures.**
- ToR 1.4 in accordance with article 56(1) of the Delegated Regulation (EU) 2020/687.

The following elements of the scenario were taken into consideration 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

No guidelines available.

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. For further details, see Annexes B and C.

- **19th scenario of sampling procedures.**
- ToR 1.5 in accordance with article 59(2) of the Delegated Regulation (EU) 2020/687.

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

- 1) It concerns the repopulation of a previous affected establishment.
- 2) Animals intended to 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

No specific guidelines are available for ND, but the EU Reference Laboratory states that collection of samples, choice of tissues and transport of samples can be used for both ND and HPAI diagnosis. The diagnostic manual states 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

There are no specific guidelines to be assessed.

Development of new procedures

Where the birds originate from NDV free regions, no testing before introduction is needed, where they originate from within the affected region, the procedures for movement within surveillance and protection zones should be followed (see scenarios 10, 11, 16, 17 and 18). Where birds originate from a region where vaccination is applied, the introduced birds should have been vaccinated prior to movement according to the scheme required by the local authorities.

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. For further details, see Annexes [B](#) and [C](#).

- **20th scenario of sampling procedures.**
- ToR 1.5 in accordance with article 59(9) of the Delegated Regulation (EU) 2020/687.

The following elements of the scenario were taken into consideration 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

No specific guidelines are available.

Assessment

There are no guidelines directly applicable to ND available to be assessed.

Development of new procedures

In the event of unusual mortalities and clinical signs during the repopulation, the procedures as described in Section [4.1.1.1](#) should be followed.

4.1.3.3. For animals that have been repopulated

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. For further details, see Annexes [B](#) and [C](#).

- **21st scenario of sampling procedures.**
- ToR 1.5 in accordance with article 59(5) of the Delegated Regulation (EU) 2020/687.

The following elements of the scenario were taken into consideration 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 sampling procedures

No specific guidelines are available.

Assessment

No guidelines directly applicable to ND are available to be assessed.

Development of new procedures

For vaccinated and non-vaccinated poultry, in the event that no clinical signs are seen, random sampling of 60 birds in each of the production units in question for virus detection (PCR), enabling the detection of the infection at a prevalence of 5% with 95% confidence (assuming 95% test sensitivity and 100% specificity), should be carried out.

4.2. Assessment of the length of the monitoring period

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 (EU) 2020/687 supplementing the rules laid down in Part III of Regulation (EU) 2016/429 (Animal Health Law).

The table in Annex D in this manuscript describes the seven scenarios for which an assessment of the length of the monitoring period for ND had been requested.

4.2.1. Results

A total of 1,350 references published after 1/1/2000 were retrieved. As only a limited number of references were available for outbreak data ($n = 4$) and from the EU/EEA ($n = 5$), the search was extended to simulation data and to data from the rest of the world. Among these references, 17 were selected to be included in the qualitative review. The full selection process is displayed in Figure 4.

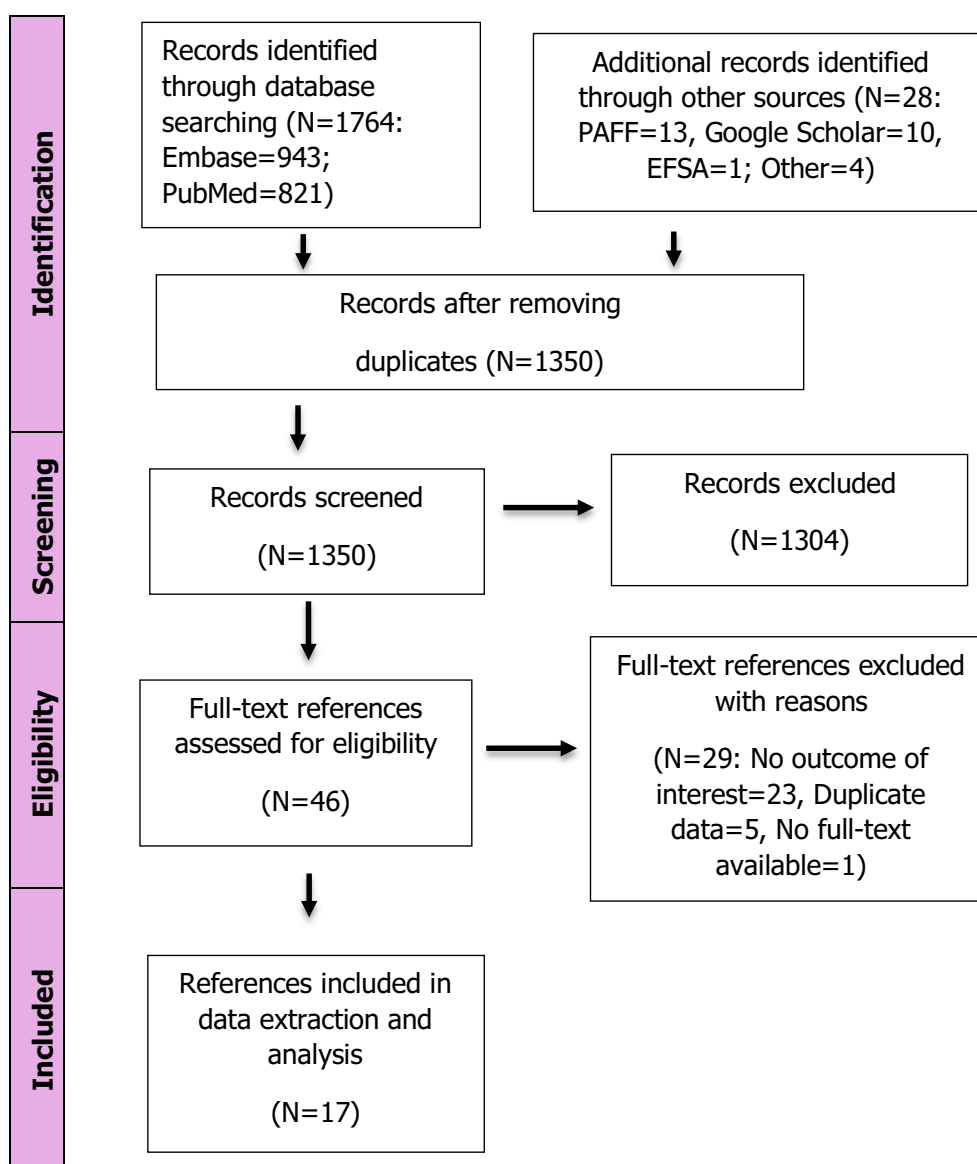


Figure 4: PRISMA diagram for the extensive literature search on monitoring period for Newcastle disease

Information retrieved by the ELS is summarised in Tables 10 and 11. Tables 10 and 11 provide an overview of the data that were extracted for the main outcome of interest, i.e. the period between the earliest point of infection and the suspicion report, for which five references were retrieved:

Table 10: Summary of the ND extraction for the period between earliest point of infection and suspicion report: outbreak data

Reference	Country	Year	Species/Farm type	Period (days)
ProMED (2005)	United Kingdom	2005	Pheasant/Game	20 ⁽¹⁾
PAFF (2015)	Romania	2015	Chicken/Indoor broiler	23 ⁽²⁾
PAFF (2018)	Belgium	2018	Chicken/Hobby	3 ⁽³⁾
PAFF (2019)	Romania	2019	Chicken/Rearing layer	21 ⁽⁴⁾

(1): Primary outbreak: Based on date of importation of pheasants from infected premise in France, birds were not vaccinated.

(2): Primary outbreak: Based on date of population of the farm with day-old-chicks, birds were vaccinated, but antibody titre testing showed low results of immunisation.

(3): Primary outbreak: Based on date of purchase of chickens at local exhibition, no information on the vaccination status of hobby flocks available.

(4): Primary outbreak: Based on date of population of the farm with 16-week-old hens, birds were vaccinated, but wrong applications of vaccination procedures were suspected.

Table 11: Summary of the ND extraction for the period between earliest point of infection and suspicion report: simulation data

Reference	Country	Year	Species/farm type	Period (days)
Sanchez-Vizcaino et al. (2010)	Spain	NA	Chicken and guinea fowl Turkey Duck and goose	Pert (3,14,30) ⁽¹⁾ Pert (6,17,34) ⁽¹⁾ Pert (6,25,40) ⁽¹⁾

(1): Pert distribution with minimum, most likely and maximum values; Model assumption based on the review of outbreak data from Europe reported to the OIE between 1992 and 2008.

As described in Table 10 (outbreak data), the shortest period between the earliest point of infection and the suspicion report found in the selected references was 3 days. This value was found in the context of an outbreak detected in 2018 in a hobby holding in Belgium (PAFF, 2018). From the epidemiological investigations, it was concluded that the most likely source of infection was the purchase of two ornamental birds (fancy chickens) at a local exhibition.

On the other hand, the longest period between the earliest point of infection and the suspicion report found in the selected references (Table 10) concerned the primary outbreak in 2015 in Romania in which 65,000 day-old-chicks were introduced in a commercial indoor broiler 23 days before a suspicion of ND was raised and reported (PAFF, 2015).

The results described in Table 10 indicate that the period of interest is likely to be shorter for chickens and guinea fowls than for turkeys, ducks and geese. This is in line with the literature that generally reports that the latter species are less prone to show severe clinical signs (EFSA, 2007).

Based on the ELS, the period between the earliest point of infection and the suspicion report was thus assessed as:

- 1.1 Based on the extracted values for chickens (n = 5) (Table 10):
 - 1.1.1. Average period = 17 days
 - 1.1.2. Shortest period = 3 days
 - 1.1.3. Longest period = 23 days
- 1.2 Based on the extracted values for pheasants (n = 1) (Table 10):
 - 1.2.1. Average/Shortest/Longest period = 20 days
- 1.3 Based on the extracted values for turkeys (n = 1) (Table 11):
 - 1.3.1. Average period = 17 days
 - 1.3.2. Shortest period = 6 days
 - 1.3.3. Longest period = 34 days
- 1.4 Based on the extracted values for ducks and geese (n = 1) (Table 11):
 - 1.4.1. Average period = 25 days
 - 1.4.2. Shortest period = 6 days
 - 1.4.3. Longest period = 40 days

4.2.2. Assessment

Considering the results presented above, an assessment of the effectiveness of the current monitoring period for ND, depending on the purpose of that period in the different scenarios shown in Annex D, was carried out. For ND, the length of the monitoring period as defined in Annex II of the Delegated Regulation 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 (EU) 2020/687.
- 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 an ND outbreak.

- 2nd scenario of monitoring period.
- ToR 2 in accordance with article 17(2) and Annex II of the Delegated Regulation (EU) 2020/687.
- 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 ND outbreak.

- 3rd scenario of monitoring period.
- ToR 2 in accordance with article 13(b) and Annex II of the Delegated Regulation (EU) 2020/687.
- 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 ND outbreak in an epidemiological unit in which the disease has not been confirmed, in order to provide derogations from killing the animals in this unit, if this unit has been completely separated, and handled by different personnel during this monitoring period.

For the first three 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 monitoring period is the time where the infection could have been present unknowingly 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 NDV into 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 ELS carried out and presented above, the average length of the time between infection and the suspicion report for chicken was estimated as 17 days, with a longest period of 23 days, based on references including an epidemiological investigation. The average length of the corresponding periods for turkeys and pheasants, respectively, was 17 and 20 days.

Considering the uncertainties related to the very limited evidence available, **in non-vaccinated chicken, turkey or pheasant flocks located in areas in which the disease was not previously present** (QoI 2.1), it was concluded with a 66–90% certainty that 95% or more of the ND-suspected establishments would have been infected within 21 days from the suspicion report (and therefore the length of the monitoring period of 21 days would be effective). This certainty was increased to 90–99% for **non-vaccinated chicken, turkey or pheasant flocks located in an already affected area** (QoI 2.3) given that there would be an increased awareness and enhanced sensitivity of sampling protocols.

For vaccinated chicken or turkey flocks located in areas where the disease had not been reported before (QoI 2.2), the uncertainty regarding infection occurring within 21 days from the suspicion report was very large (10–90%), due to the increased risk of infection going unnoticed when awareness is low. For **vaccinated chicken or turkey flocks located in already affected regions** (QoI 2.4), certainty increased to 33–99%.

For **independent epidemiological units within chicken, turkey or pheasant non-vaccinated ND-affected establishments** that eventually become infected (QoI 2.7), it was concluded with a 95–99% certainty that they would have been infected within 21 days from the date of suspicion, and therefore, the length of the monitoring period would be effective. The certainty range was reduced to 33–99% in case of epidemiological units in **chicken, turkey or pheasant vaccinated ND-affected establishments** (QoI 2.8) and of epidemiological units in ducks and geese ND-affected establishments (QoI 2.9).

In terms of the effectiveness of the length of the existing monitoring period for ducks and geese in relation to ND 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 average period for ducks and geese was estimated at 25 days, with a longest period of 40 days. Based on these results, the Panel Members concluded there was 10–90% certainty that ND **suspected duck and geese flocks** would have become infected within 21 days of the suspicion report if they were **located in a region where the disease was not known to be present** (QoI 2.5), which increased to 33–99% when located in an already affected region (QoI 2.6), thus reflecting a large uncertainty regarding the effectiveness of the existing length of the monitoring period. Nonetheless, this assessment should be taken cautiously due to the data being retrieved from a unique reference.

Scenario 4

- 4th scenario of monitoring period.
- ToR 2 in accordance with article 27(3)c and Annex II of the Delegated Regulation (EU) 2020/687.
- 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 ND outbreak in the protection zone. Products or other materials likely to spread the disease, must have 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 neither a suspect establishment nor 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).

Because the assessment of the effectiveness of the proposed alternative monitoring period is subjected to the same uncertainties described for Scenarios 1, 2 and 3, the same conclusion was reached for Scenario 4.

Scenarios 6 and 7

- 6th scenario of monitoring period.
- ToR 2 in accordance with article 57 (1) and Annex II of the Delegated Regulation (EU) 2020/687.
- 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 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 (EU) 2020/687.
- 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, 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 the repopulation process is not put at risk due to the disease still being present unknowingly in establishments within the surrounding area of the establishment to be repopulated (if an establishment tested positive to NDV 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, disinfection and disinfestation of the affected establishment.

In this regard, the number of days of the monitoring period for ND, 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 concluded there was a 95–100% certainty that any potentially chicken, turkey or pheasant infected establishment in the surrounding area would be detected in the 21 days following the cleaning, disinfection and disinfestation of the ND-affected establishment given the expected very high degree of awareness, in which case the existing length of the monitoring period (21 days) would be effective (QoI 2.10). In the case of duck and geese infected establishment in the surrounding area, this certainty was reduced to 33–99% (QoI 2.11)

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 ND 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 zone for ND should be of 3 and 10 km, respectively (see Annex E).

Results

The probability of transmission beyond given distances (if transmission were to occur from an infected establishment), including beyond the proposed radius for the protection and surveillance zones (3 km and 10 km, respectively) is shown in Figure 5. In addition, Figure 5 shows the distances at which a threshold probability of transmission beyond that distance is reached and the values are summarised in Tables 12 and 13.

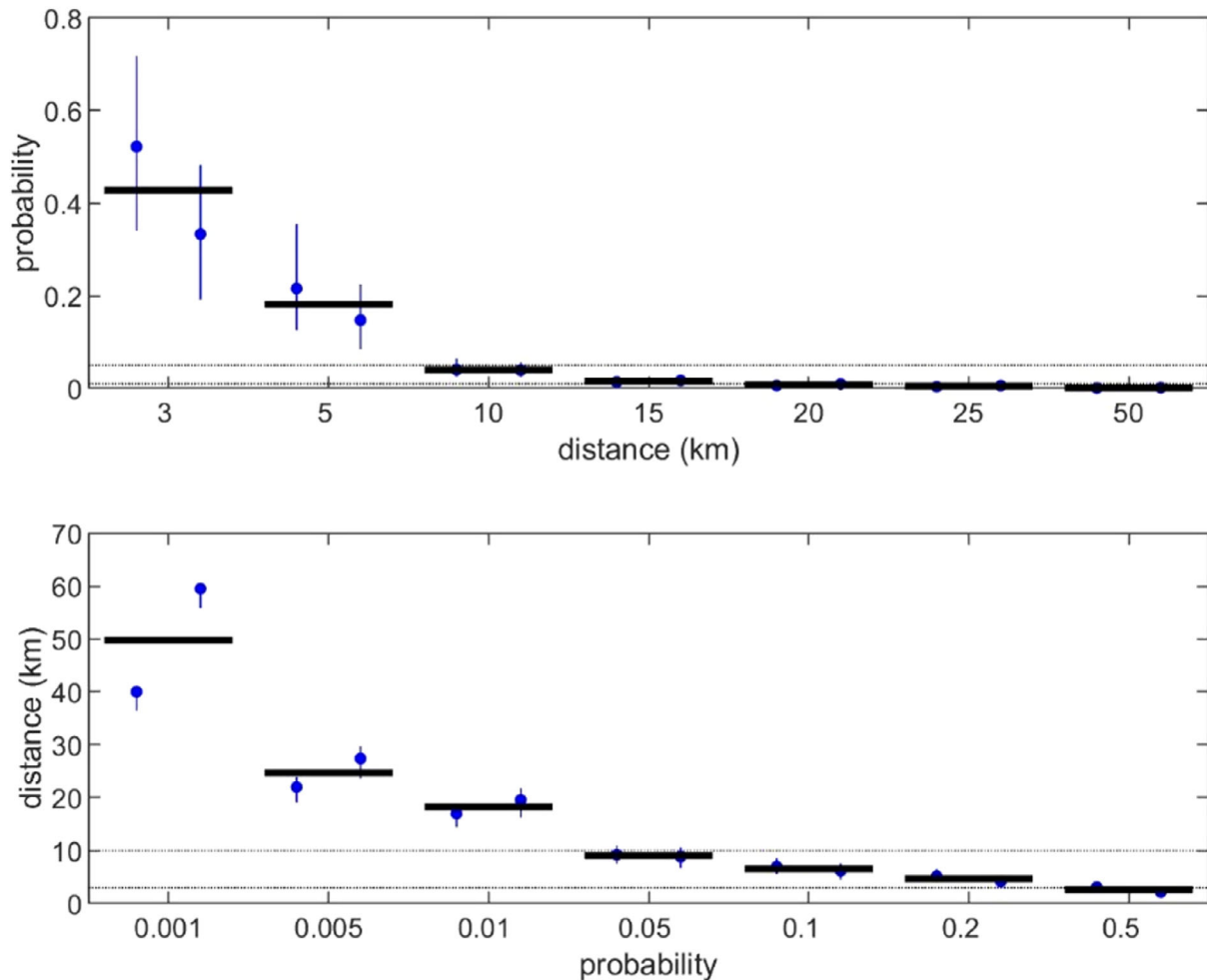


Figure 5: Assessment of the radius of the protection and surveillance zone for NDV assuming between farm transmission is similar to that of highly pathogenic avian influenza virus. The top panel shows the probability of transmission beyond a given distance (if transmission were to occur from an infected establishment) computed using the estimates (blue circles) and the lower and upper 95% confidence limits (error bars). Estimates were derived from outbreaks in the Netherlands and Italy. 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 and 10 km (i.e. the proposed radius of the protection and surveillance zones, respectively)

Table 12: Probability of transmission of NDV beyond different distances, assuming NDV transmission kernel is equal to the HPAIV kernel. Kernels were derived from HPAI outbreaks in the Netherlands (NL 2003) and Italy (IT 1999)

	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 13: Distances (km) at which the probability of transmission of NDV beyond that distance reaches a threshold level, assuming NDV transmission kernel is equal to the HPAIV kernel. Kernels were derived from HPAI outbreaks in the Netherlands (NL 2003) and Italy (IT 1999)

	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

It is important to note that kernels combine transmission routes, but do not include introductions from wild bird populations. 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, this is not considered necessary as such movements are followed up by tracing activities. 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.

Assessment

For this assessment, the transmission kernel of Highly Pathogenic Avian Influenza was selected to assess the minimum radius for the protection zone and the surveillance zone. Similar to HPAI we exclude virus spread by the movement of animals (should be detected by tracing contacts) and wild birds (distance cannot be estimated and become very large). Using a NDV specific transmission kernel was not possible, because no transmission kernel has been published and no suitable data sets were identified that could be used to estimate a NDV specific transmission kernel. NDV epidemics in Europe such as in Denmark in 2002 (ADIS, day of extraction 9/7/2021) and Belgium in 2018 (ADIS, day of extraction 9/7/2021) included hobby flocks mostly. The limited numbers of commercial flocks did not allow to estimate a transmission kernel. Both NDV and HPAIV are (single stranded enveloped) RNA viruses that are mainly excreted by respiratory and faecal routes and infection may occur through inhalation and ingestion. Consequently, transmission routes are similar for both infections. Outside their hosts both viruses are sensitive to inactivation by UV light and dehydration. Moreover, the infection rate parameters of between-bird transmission of NDV are similar to those of the low and medium transmission scenarios for HPAI, while a high infection rate parameter was not observed for NDV. Given the (non-linear) association between animal and between farm transmission (van Nes et al., 1998), this would suggest similar to lower between farm transmission for NDV compared to HPAI. Based on these similarities and on the absence of widespread between farm transmission in Sweden (where there is no vaccination) the HPAI transmission kernel could serve as a conservative estimate for the minimum radius to be used for ND, in particular in regions where vaccination is used to reduce within flock transmission.

Table 12 shows that the probability of transmission from an infected farm beyond the protection zone is 0.52 (the Netherlands) and 0.33 (Italy), respectively (if transmission were to occur). 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 (0.52 vs. 0.48, 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). In addition, we have to bear in mind that between farm transmission of NDV is most likely overestimated because the HPAI transmission kernel was used (see arguments above). 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.

Based on the available evidence, the Panel concluded with a 66–99% certainty that the minimum radius of 3 km would prevent transmission outside the protection zone in the 21 days following their setting up in 95% or more of all the zones that are established (QoI 3.1). For the surveillance zones, it was concluded with a 90–99% certainty that the minimum radius of 10 km would prevent transmission outside the zone in the 30 days following their setting up in 95% or more of the established zones (QoI 3.2). The uncertainty was derived from the limited evidence available and the difficulties extrapolating information from HPAI transmission kernels. These were considered an upper bound for NDV transmission given that HPAIV is more transmissible. The uncertainty was more limited in the case of the surveillance zone given that it was deemed very unlikely that transmission would occur outside the surveillance zone before occurring first beyond the protection zone, which would in turn result in the establishment of new surveillance zones.

4.3.2. Assessment of the minimum period

The purpose of this section is to assess the effectiveness to control ND spread 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.

The length of the minimum period of the protection zone and surveillance zone are 21 and 30 days, respectively (see Annex E). In the protection zone, all farms are visited for a clinical inspection. This aim is to quickly identify infected farms where infection has started before control measures were implemented. The movement control applies for 30 days, ensuring that possibly infected poultry in both protection and surveillance zones are not moved to uninfected farms.

The average and the longest time from infection to reporting of the suspicion, as assessed in ToR 2, was used to assess the effectiveness of the minimum length of the protection and surveillance zones. The minimum length of time of the protection zone (21 days) and the surveillance zone (30 days) were considered effective in non-vaccinated chicken and turkeys when the average and longest time, assessed in ToR 2 for these species, was considered (17 days for the PZ and 23 days for the SZ for chickens, and 17 days and 34 days for the PZ and SZ, respectively, in turkeys). It is concluded with a 90–99% certainty that these minimum periods would allow the detection of 95% or more of the new outbreaks due to infections starting before control measures were implemented.

Due to the lack of data retrieved in ToR 2 for flocks in which vaccination had taken place, the time to detection assessed in Section 4.1.1.1 (time to detection assuming a specific number of samples are submitted for analysis) was used instead to assess the effectiveness of the length of these periods in vaccinated flocks.

The upper limit of the period to detection in a flock of 10.000 birds includes 10–11 weeks indicating that the minimum periods may not be effective for that situation (33–99% certainty these periods would allow the detection of 95% or more of new outbreaks due to infections starting before control measures are implemented in vaccinated flocks). Testing 60 random birds does not reduce this period (Table 9). For the minimum period to be effective additional vaccination is needed to increase herd immunity in the flocks to the level $R_0 < 1$. In case of classical vaccines (not the vector vaccines), this can be measured by testing whether 80% of the flock has an HI titer > 3 (van Boven et al., 2008).

4.3.3. Uncertainty analysis

Several sources of uncertainty were identified during the scientific assessment (see Annex F), and their impact on the outputs of the assessment were quantified for scenario 1 in ToR1 and ToRs 2 and 3.

5. Conclusions and recommendations

Sampling procedure	Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise	Conclusions	Recommendations
ToR 1: In the event of suspicion or confirmation			
<p>1st scenario 4.1.1.1 In the event of a suspicion of ND in an establishment where animals of the listed species are kept</p>	<p><i>Annex III: Diagnostic procedures for the confirmation and differential diagnosis of Newcastle disease:</i></p> <p>The following procedures for the isolation and characterisation of Newcastle disease viruses should be regarded as guidelines and the minima to be applied in the diagnosis of the disease. 'Newcastle disease' means an infection of poultry caused by any avian strain of the paramyxovirus 1 with an intracerebral pathogenicity index (ICPI) in day-old-chicks greater than 0.7.</p> <p>Chapter 1: Sampling and treatment of samples: Cloacal swabs (or faeces) and tracheal swabs from sick birds; faeces or intestinal contents, brain tissue, trachea, lungs, liver, spleen and other obviously affected organs from recently dead birds. These organs and tissues may be pooled, but separate treatment of faecal material is essential.</p> <p><i>Avian influenza and Newcastle disease: a field and laboratory manual (Capua and Alexander, 2009):</i></p> <p>Chapter 4. Emergency Response on Suspicion of an Avian Influenza or Newcastle Disease Outbreak:</p> <p>Following the collection of preliminary data, a post-mortem examination of dead animals (or euthanised moribund animals) must be performed. Samples are then to be collected from organs exhibiting pathological lesions. Organs</p>	<p>The present guidelines for sampling of birds in flocks with suspected ND could be considered effective for the detection of the infection in case the circulating virus is causing mortality and/or overt clinical signs in vaccinated or unvaccinated flocks (90–100% certainty that 95% or more of them would be detected). In vaccinated flocks the detection of the disease would be considerably delayed (66–99% or 33–99% certainty that 95% or more of them would be detected if suspicion is due to clinical signs and mortality and due to contact tracing, respectively).</p> <p>The present guidelines for sampling could not be considered effective for the ND detection in case the circulating NDV strain does not cause mortality and clear clinical signs in affected flocks yet being classified as NDV based on the pathology index (66–99% certainty that 95% or more of them would be detected).</p>	<p>In flocks where the disease is suspected due to the drop in egg production or unexpected shell colour or other egg abnormalities and no increased mortality or birds with clear clinical signs could be identified the following sampling scheme could be suggested:</p> <p>Random sample of 60 birds of the flock for virus detection enabling the detection of the infection at a prevalence of 5% with 95% confidence (assuming 95% test sensitivity and 100% specificity)</p>

Sampling procedure	Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise	Conclusions	Recommendations
	<p>from different apparatuses should not be pooled, and if possible, cross-contamination due to excision of organs with single pair of scissors or pliers should be avoided.</p> <p>Chapter 5: Necropsy Techniques and Collection of Samples</p> <p>Ideally, sick as well as recently dead birds should be submitted to the laboratory for blood sampling, necropsy and diagnostic examination.</p> <p>In a suspected outbreak, 20 cloacal and 20 oropharyngeal swabs (or swabs from all birds if there are less than 20 in a flock) should be collected in each epidemiological unit. These should preferably be taken from birds showing signs of disease.</p> <p>A minimum of five birds should be collected for necropsy. Those birds exhibiting overt clinical signs should be selected for this purpose. 20 blood samples or samples from all birds if less than 20 in flock, should be collected in each epidemiological unit. Birds that are sick or apparently recovered should be targeted for blood sampling.</p> <p>This sampling scheme has been developed to ensure a 99% probability of detecting at least one positive serum if 25% or more of the flock is positive, regardless of flock size.</p> <p>Newcastle disease sampling method, Western Australia (Western Australian Agricultural Authority, 2018):</p> <p>The sampling method for events meeting the case definition of Newcastle disease are outlined below.</p>		

Sampling procedure	Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise	Conclusions	Recommendations
	<ul style="list-style-type: none"> – Collect choanal (mouth) and cloacal swabs from 10 birds. – Collect cloacal swabs from the same 10 birds. – Collect two millilitres (2 mL) clotted blood samples from each of 15 birds into tubes supplied by the Department of Primary Industries and Regional Development, Western Australia (DPIRD). – Collect five dead birds or a range of tissues in formalin. 		
<p>2nd scenario 4.1.1.2. For the purposes of the epidemiological enquiry as referred to Article 57 of Regulation (EU)2016/429 in an ND officially confirmed establishment</p>	No specific guidelines	Additional sampling may be useful to establish the length of duration of ND and to detect infection in case of preventive killing	<p>In case sick and dead birds are present, sampling according to 1st scenario is recommended.</p> <p>Examine change in production, and use serological testing and WGS to assist establishing the length of duration.</p> <p>In the absence of sick and dead birds, select 60 birds randomly for detection of virus and antibodies.</p>
<p>3rd scenario 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 ND-affected establishment</p>	No specific guidelines	As determined in Section 4.2.2, 21 days is an effective monitoring period to determine whether an independent epidemiological unit within non-vaccinated ND-affected establishments would eventually become infected. This 21 day monitoring period is less likely to be effective in vaccinated establishments.	<p>Where the affected establishment has vaccinated birds, a longer monitoring period should be used during which time periodic virological surveillance can be used where the derogation is for unvaccinated birds.</p> <p>Where the establishment has unvaccinated birds, if no clinical signs are seen after a period of 21 days, a final serological sampling strategy can be applied. See Section 4.1.1 to apply the recommendations for sample sizes according to the mortality of the strain and the vaccination status of the birds.</p>

Sampling procedure	Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise	Conclusions	Recommendations
<p>5th scenario 4.1.1.4. For wild animals of the listed species within the ND-affected establishment and its surroundings.</p>	<p>No specific guidelines</p>	<p>Passive surveillance of wild birds found dead or sick in the environment is considered useful to establish the source of infection.</p>	<p>Test birds found dead or sick within the (surroundings of) the infected holding for ND.</p>
<p>6th scenario 4.1.1.5. For animals of listed species in the non-affected establishments located in a protection zone</p>	<p>Holdings should be visited and sampled in case of clinical suspicion</p>	<p>Visiting all holdings in the protection zone and testing in case of clinical suspicion is considered effective.</p>	<p>In case of a clinical suspicion, sampling should be performed according to scenario 1. In case (booster) vaccination is applied, a clinical inspection should take place prior to starting vaccination. An awareness campaign among hobby/backyard keepers is recommended.</p>
<p>8th scenario 4.1.1.6. For non-affected establishments located in a surveillance zone</p>	<p>No specific guidelines</p>	<p>In case of a strain with high or medium morbidity/mortality in a region without vaccination, passive surveillance is considered effective.</p> <p>Visiting a random sample of holdings in the surveillance zone is not considered effective to eliminate the virus from the region.</p>	<p>An awareness campaign should be started in the surveillance zone. In regions with vaccination, thorough clinical inspection should proceed (booster) vaccination in a holding. If possible, records of production parameters should be used to identify early signs of infection.</p>
<p>ToR 1: To grant derogations for animal movements</p>			
<p>9th scenario 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</p>	<p>No specific guidelines</p>	<p>Clinical surveillance complemented with assessment of production records is considered effective in non-vaccinated poultry</p>	<p>In non-vaccinated poultry, clinical inspection should be complemented with systematic assessment of production records. In case clinical signs are observed on individual bird or flock level, the sampling scheme suggested under 4.1.1.1 should be followed.</p>

Sampling procedure	Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise	Conclusions	Recommendations
<p>10th scenario 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</p>	<p>No specific guidelines</p>	<p>The risk of spreading NDV by DOC must be considered very low.</p>	<p>No inspection or testing is required.</p>
<p>11th scenario 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</p>	<p>No specific guidelines</p>	<p>The consequences of missing the disease may be larger, compared to previous derogation scenarios, as birds are not sent to slaughter but moved to another establishment</p>	<p>Non-vaccinated: The recommendations would be similar to those in Section 4.1.2.1. In addition, follow up investigation of the recipient farm, including systematic assessment of production records would be recommended.</p>
<p>12th scenario 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</p>	<p>No specific guidelines</p>	<p>As per Section 4.1.2.1</p>	<p>As per Section 4.1.2.1</p>
<p>13th scenario 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</p>	<p>No specific guidelines</p>	<p>As per Section 4.1.2.1</p>	<p>As per Section 4.1.2.1</p>

Sampling procedure	Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise	Conclusions	Recommendations
16th scenario 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	As per Section 4.1.2.2	As per Section 4.1.2.2
17th scenario 4.1.2.7 For ready-to-lay poultry located in the surveillance zone to establishments located in the same MS	No specific guidelines	As per Section 4.1.2.3	As per Section 4.1.2.3
18th scenario 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	As per Section 4.1.2.3	As per Section 4.1.2.3
ToR 1: For repopulation purposes			
19th scenario 4.1.3.1 For the animals that are kept for the repopulation prior to their introduction	No specific guidelines	In case the birds originate from ND free regions, testing them prior to introduction is not needed.	In case of a vaccinated region, introduced birds should have been vaccinated by an approved protocol prior to introduction.
20th scenario 4.1.3.2 In the event of unusual mortalities or clinical signs being notified during the repopulation	No specific guidelines		In the event of unusual mortalities and clinical signs during the repopulation, the procedures as described in Section 4.1.1.1 should be followed.

Sampling procedure	Laboratory guidelines based on Council Directive 2003/85/EC if not stated otherwise	Conclusions	Recommendations
21st scenario 4.1.3.3 For animals that have been repopulated	No specific guidelines		For vaccinated birds, the sampling strategy as provided in Section 4.1.1.1 should be used. For unvaccinated birds, where no clinical signs are observed, a random sample as described in Section 4.1.1.1 should be used to detect presence of virus.
ToR 2			
Description	Conclusions	Recommendations	
4.2 Assessment of the length of the monitoring period of ND	Scenarios 1–3, 4 and 6 Based on available information retrieved through a literature review and expert opinion, the panel is 66–90% certain the monitoring period as defined in Annex II of the Delegated Regulation of 21 days is effective for non-vaccinated chicken, turkey and pheasant establishments located in a previously unaffected region, and 90–99% it is effective for non-vaccinated chicken, turkey and pheasant establishments in a previously affected region. For vaccinated chicken, turkey and pheasant establishments and duck and geese establishments, the monitoring period cannot be considered effective.	Scenarios 1–3, 4 and 6 For non-vaccinated chicken, turkey and pheasant flocks, it is recommended that the current monitoring period is maintained. For vaccinated establishments and duck and geese establishments, it is recommended to increase the monitoring period to 10 weeks, or, alternatively, subject all flocks to a booster vaccination that results in $R < 1$ (can be measured in classical vaccines as $> 80\%$ of flock $> HI$ titer 3).	
ToR 3			
Description	Conclusions	Recommendations	
4.3.1 Assessment of the minimum radius	Based on extrapolations made on available information on HPAI spatial spread and expert opinion, the panel is 66–99% certain the minimum radius of 3 km will prevent transmission outside the protection zone in 95% or more of the established zones. Regarding surveillance zones, the panel is 90–99% certain the minimum radius of 10 km will prevent transmission outside the surveillance zones in 95% or more of the established zones.	It is recommended that the current radii of the protection (3 km) and surveillance zones (10 km) are maintained.	
4.3.2 Assessment of the minimum period	The minimum periods of 21 (protection zone) and 30 (surveillance zone) are effective in the non-vaccinated situation (90–99% certainty will allow the detection of 95% or more of the new outbreaks starting before the control measures are implemented), but may not be effective (33–99%) in the vaccinated situation.	To be effective in the vaccinated situation booster vaccination should be applied in the flocks and at the time of lifting the restrictions, herd immunity should be reduced to $R_0 < 1$. In case of classical vaccines this implies $> 80\%$ of the birds have an HI titer > 3 .	

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Abbreviations

ASF	African swine fever
AHS	African horse sickness
CSF	Classical swine fever
CBPP	Contagious bovine pleuropneumonia
CCPP	Contagious caprine pleuropneumonia
dpi	days post inoculation
ELISA	enzyme-linked immunosorbent assay
ELS	extensive literature search
FMD	Foot and mouth disease
FMDV	Foot and mouth disease virus
HPAI	Highly Pathogenic Avian Influenza
LSD	Lumpy skin disease virus
NDV	Newcastle disease virus
OIE	World Organization for Animal Health
PCR	polymerase chain reaction
PZ	protection zone
RP	rinderpest virus

RT-PCR	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).

Terms	Definitions
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))
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

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
In the event of suspicion or confirmation				
ToR 1.1 ToR 1.2	6(2) of the 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)).	<ul style="list-style-type: none"> • event of suspicion of a category A disease • in an establishment • kept animals of listed species • 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) of the 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.	<ul style="list-style-type: none"> • affected establishment officially confirmed • kept animals of listed species found dead or before/when they are killed • competent authority collects samples for laboratory examination for the purposes of: <ul style="list-style-type: none"> a) supporting the epidemiological enquiry: <ul style="list-style-type: none"> - 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 1.1 ToR 1.2	Article 13(3)c of the 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	<ul style="list-style-type: none"> • affected establishment officially confirmed • kept animals of listed species of specific categories • animal categories based on article 13(2): <ul style="list-style-type: none"> 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

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
			ensuring that they do not pose a risk for the transmission of the disease.	<ul style="list-style-type: none"> 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 • sampling 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.	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 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	<ul style="list-style-type: none"> • 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

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
			sampling procedures is to ensure the detection of the virus, if the virus is present in these animals.	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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

ToRs	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)	<ul style="list-style-type: none"> • 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 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	<ul style="list-style-type: none"> • protection zone • grant 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.	<ul style="list-style-type: none"> • 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 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	<ul style="list-style-type: none"> • protection zone • kept animals of listed species • grant derogation for movement from a non-affected establishment in the protection zone

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
			for processing or disposal of animal by-products in which the kept animals are immediately killed (Art37)	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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 1.4	Article 43(5) and Article 46(1) of	16th scenario	To assess the effectiveness of disease-specific sampling procedures based on clinical and/or	<ul style="list-style-type: none"> surveillance zone kept birds of listed species

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenario
	the Delegated Regulation		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	<ul style="list-style-type: none"> 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.	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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.	<ul style="list-style-type: none"> 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

ToRs	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.	<ul style="list-style-type: none"> • 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.	<ul style="list-style-type: none"> • 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 – Existing sampling procedures for ND

Sampling scenarios for ND – Based on **Council Directive 92/66/EEC** if not stated otherwise

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
1st	To assess the effectiveness of disease-specific sampling procedures of animals of listed species in a suspected establishment, based on clinical examination (TOR1.1) and laboratory examination (TOR1.2), 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)).	<p>Article 4:</p> <p>1) When poultry in a holding are suspected of being infected or contaminated with Newcastle disease, Member States shall ensure that the official veterinarian immediately activates official investigation arrangements to confirm or rule out the presence of the disease and, in particular, must take or have taken the samples necessary for laboratory examination.</p> <p>2) As soon as the suspected infection is notified, the competent authority shall have the holding placed under official surveillance and shall in particular require that:</p> <p>a) A record be made of all categories of poultry on the holding showing in respect of each of the categories the numbers of poultry which have died, which show clinical signs, and which show no signs. The record shall be kept up-to-date to include birds born or dying during the period in which there is a suspicion. The data in the record shall be kept up-to-date and be produced on request, and may be checked at each visit;</p> <p>OIE Terrestrial Code, Chapter 10.9., Article 10.9.24. Surveillance strategies, 2. Clinical surveillance (OIE, 2019a):</p> <p>Monitoring of production parameters (e.g. a drop in feed or water consumption or egg production) is important for the early detection of NDV infection in some populations, as there may be no, or mild, clinical signs, particularly if they are vaccinated. A presumptive diagnosis of clinical ND in suspect infected populations should always be confirmed by virological testing in a laboratory.</p>	<p>Article 4:</p> <p>1) When poultry in a holding are suspected of being infected or contaminated with Newcastle disease, Member States shall ensure that the official veterinarian immediately activates official investigation arrangements to confirm or rule out the presence of the disease and, in particular, must take or have taken the samples necessary for laboratory examination.</p> <p>Article 12:</p> <p>Collection of samples and laboratory testing to detect the presence of Newcastle-disease virus shall be carried out in accordance with Annex III.</p> <p>Annex III: Diagnostic procedures for the confirmation and differential diagnosis of Newcastle disease:</p> <p>The following procedures for the isolation and characterisation of Newcastle disease viruses should be regarded as guidelines and the minima to be applied in the diagnosis of the disease.</p> <p>'Newcastle disease' means an infection of poultry caused by any avian strain of the paramyxovirus 1 with an intracerebral pathogenicity index (ICPI) in day-old-chicks greater than 0.7.</p> <p>Chapter 1: Sampling and treatment of samples: Cloacal swabs (or faeces) and tracheal swabs from sick birds; faeces or intestinal contents, brain tissue, trachea, lungs, liver, spleen and other obviously affected organs from recently dead birds. These organs and tissues may be pooled, but separate treatment of faecal material is essential.</p> <p>Chapter 2: Virus isolation: Virus isolation in embryonated fowls' eggs.</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
		<p><i>Avian influenza and Newcastle disease: a field and laboratory manual (Capua and Alexander, 2009):</i></p> <p>Chapter 4. Emergency Response on Suspicion of an Avian Influenza or Newcastle Disease Outbreak:</p> <p>In case of suspicion, an accurate clinical examination of the affected birds in order to establish the clinical condition of the susceptible animals, including sick and suspect birds. The clinical investigation must be performed on all susceptible species on site and it must begin from the clinically unaffected units.</p> <p>All the birds present, per species, must be identified, and for each species identified an outbreak report must be drafted, containing the number and location of birds in different pens and compartments of the farm, the date of onset of clinical signs, a description of clinical signs, reported percentage mortality. Particular attention must be paid to the vaccination history of the farm. All this information must be reported in the epidemiological inquiry. Following the collection of preliminary data, a post-mortem examination of dead animals (or euthanised moribund animals) must be performed.</p> <p><i>Emergency animal diseases: A field guide for Australian veterinarians; Chapter 3.15. (Department of Agriculture and CSIRO, 2019):</i></p> <p>Ideally, submit whole birds for post-mortem examination. In particular, examining whole birds may provide information on other differential diagnoses. Documenting gross lesions is also useful in determining the most appropriate samples for laboratory testing.</p>	<p>Chapter 3: Differential diagnosis: It is intended that all haemagglutinating viruses should be submitted to the national laboratory referred to in Annex II for full identification, characterisation and pathogenicity tests.</p> <p>The national laboratory should undertake full differential diagnosis of any haemagglutinating agent. Confirmation of Newcastle-disease virus would again be by inhibition in haemagglutination inhibition tests with monospecific chicken antisera. Intracerebral pathogenicity index tests as described in Chapter 7 should be carried out on all positive isolates. Pathogenicity indices of greater than 0.7 indicate the presence of virus requiring the full implementation of control measures.</p> <p>The Community reference laboratory should receive all haemagglutinating viruses from the national laboratories for further antigenic and genetic studies to enable a greater understanding of the epizootiology of the disease(s) within the Community in keeping with the functions and duties of the reference laboratory.</p> <p><i>OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 3.3.14 Newcastle disease, B.Diagnostic techniques (OIE, 2019b):</i></p> <p>The preferred method of diagnosis is virus isolation and subsequent characterisation.</p> <p>When investigations of ND are the result of severe disease and high mortality in poultry flocks, it is usual to attempt virus isolation from recently dead birds or moribund birds that have been killed humanely. Samples from dead birds should consist of oro-nasal swabs, as well as samples collected from lung, kidneys, intestine (including contents), caecal tonsils, spleen, brain, liver and heart tissues. These may be collected separately or as a pool, although brain and intestinal samples are usually processed separately from other samples.</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
			<p>Samples from live birds should include both tracheal or oropharyngeal and cloacal swabs, the latter should be visibly coated with faecal material. Swabbing may harm small, delicate birds, but the collection of fresh faeces may serve as an adequate alternative. Where opportunities for obtaining samples are limited, it is important that cloacal swabs (or faeces), tracheal (or oropharyngeal) swabs or tracheal tissue be examined as well as organs or tissues that are grossly affected or associated with the clinical disease. Samples should be taken in the early stages of the disease.</p> <p>Malattia di Newcastle. Procedure di intervento izz in caso di emergenza (IZSVE, 2015):</p> <p>1) Direct diagnosis: isolation and identification of the virus</p> <div data-bbox="1451 730 1973 1040" data-label="Diagram"> <pre> graph TD A[ORGANI - TAMPONI] --> B[Isolamento in uova embrionate SPF di pollo e/o colture cellulari (CEL)] A --> C[Real time RT-PCR] B --> D[CARATTERIZZAZIONE e PATOTIPIZZAZIONE con:] C --> D D --- E["• Inibizione dell'emoagglutinazione (HI) sul virus isolato • RT-PCR e sequenziamento (sito di clivaggio proteina F) • TEST DI PATOGENICITA' INTRACERBRALE (ICPI) sul virus isolato per capire se virus notificabile (solo se ICPI > 0.7)"] </pre> </div> <p>2) Indirect diagnosis: only possible in unvaccinated farms.</p> <p>Haemagglutination inhibition (HI) Samples to be collected in the event of a suspected outbreak:</p> <ul style="list-style-type: none"> – Blood serum: 20 samples if the animals present are > 20 and from all animals if the animals present are ≤ 20. – Cloacal and tracheal swabs, – tracts of the intestine as a whole, – Glandular stomach, liver, spleen, brain, trachea, lungs, whole animals.

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
			<p data-bbox="1447 248 2029 300"><i>Avian influenza and Newcastle disease: a field and laboratory manual (Capua and Alexander, 2009):</i></p> <p data-bbox="1447 336 2029 624">Chapter 4. Emergency Response on Suspicion of an Avian Influenza or Newcastle Disease Outbreak: Following the collection of preliminary data, a post-mortem examination of dead animals (or euthanised moribund animals) must be performed. Samples are then to be collected from organs exhibiting pathological lesions. Organs from different apparatuses should not be pooled, and if possible, cross-contamination due to excision of organs with single pair of scissors or pliers should be avoided.</p> <p data-bbox="1447 660 2029 711">Chapter 5: Necropsy Techniques and Collection of Samples</p> <p data-bbox="1447 748 2029 831">Ideally, sick as well as recently dead birds should be submitted to the laboratory for blood sampling, necropsy and diagnostic examination.</p> <p data-bbox="1447 868 2029 1007">In a suspected outbreak, 20 cloacal and 20 oropharyngeal swabs (or swabs from all birds if there are less than 20 in a flock) should be collected in each epidemiological unit. These should preferably be taken from birds showing signs of disease.</p> <p data-bbox="1447 1043 2029 1238">A minimum of five birds should be collected for necropsy. Those birds exhibiting overt clinical signs should be selected for this purpose. 20 blood samples or samples from all birds if less than 20 in flock, should be collected in each epidemiological unit. Birds that are sick or apparently recovered should be targeted for blood sampling.</p> <p data-bbox="1447 1275 2029 1386">This sampling scheme has been developed to ensure a 99% probability of detecting at least one positive serum if 25% or more of the flock is positive, regardless of flock size.</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
			<p data-bbox="1442 245 2000 331"><i>Australian Veterinary Emergency Plan, Disease Strategy Newcastle disease (Animal Health Australia, 2014):</i></p> <p data-bbox="1442 363 2033 624">Samples should be taken both from live, clinically affected birds and from recently dead birds. Serum, cloacal and tracheal swabs in virus transport medium or phosphate buffered glycerol saline, and/or fresh faeces should be taken from live birds. From dead birds, alimentary tract tissues (proventriculus, intestine, caecal tonsil), respiratory tissues (trachea, lung) and neurological tissues (brain), as well as heart and kidney, should be collected.</p> <p data-bbox="1442 655 2033 887">The usual approach to ND diagnosis is screening by real-time PCR. Any positives are further characterised by culture in eggs and by further molecular (genetic) analysis. Analysis of viral genetic sequence data allows assessment of pathogenicity as well as more detailed phylogenetic analysis. Isolates obtained from egg culture are identified antigenically by HI as well as with molecular tools.</p> <p data-bbox="1442 919 1993 1005"><i>Emergency animal diseases: A field guide for Australian veterinarians; Chapter 3.15 (Department of Agriculture and CSIRO, 2019):</i></p> <p data-bbox="1442 1037 2033 1241">Laboratory testing is required for the diagnosis of ND and is accomplished by one or more of these procedures: haemagglutination inhibition (more common), ELISA, virus isolation, qPCR, pathotyping PCR, histopathology, immunohistochemistry. Initial testing for AAvV-1 relies on qRT-PCR due the speed of testing and the high sensitivity and specificity of the test.</p> <p data-bbox="1442 1273 2000 1359">Samples to collect: – sera from live birds (to assess exposure to NDV or to determine vaccination titres)</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
			<p>– oral and cloacal swabs from sick and recently dead birds (for large numbers of samples, pools of three swabs may be submitted in viral transport media)</p> <p>– fresh tissue samples from the trachea, lungs, spleen, caecal tonsils, bursa of multiple dead birds (keep intestinal tract samples separate from other tissue samples to avoid contamination with bacteria during virus isolation).</p> <p>– fixed tissue, a full range of tissues (including the brain), in particular tissue showing gross pathological changes, fixed in 10% neutral buffered formalin.</p> <p>Newcastle disease sampling method, Western Australia (Western Australian Agricultural Authority, 2018):</p> <p>The sampling method for events meeting the case definition of Newcastle disease are outlined below.</p> <p>– Collect choanal (mouth) and cloacal swabs from 10 birds.</p> <p>– Collect cloacal swabs from the same 10 birds.</p> <p>– Collect two millilitres (2 mL) clotted blood samples from each of 15 birds into tubes supplied by the Department of Primary Industries and Regional Development, Western Australia (DPIRD).-Collect five dead birds or a range of tissues in formalin.</p>
2nd	<p>To assess the effectiveness of disease-specific sampling procedures, based on laboratory examination (ToR1.2), 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</p>	NA	<p>No specific guidelines described in legislation</p> <p>Article 7:</p> <p>1) The epizootiological inquiry shall deal with:</p> <p>– the length of time during which Newcastle disease may have existed on the holding or in the pigeon house;</p> <p>– the possible origin of the Newcastle disease on the holding or in the pigeon house and the identification of other holdings or pigeon houses in which there are poultry, pigeons or other birds kept in captivity which</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
	<p>are killed or found dead. The purposes of the epidemiological enquiry are described in Article 57 of Regulation (EU)2016/429.</p>		<p>may have become infected or contaminated from the same source;</p> <ul style="list-style-type: none"> – the movement of persons, poultry, pigeons or other birds kept in captivity or other animals, vehicles, eggs, meat and carcasses and any implement or substance likely to have carried Newcastle-disease virus to or from the holding or pigeon house in question. <p>Article 12: Collection of samples and laboratory testing to detect the presence of Newcastle-disease virus shall be carried out in accordance with Annex III (see 1st scenario).</p>
3rd	<p>To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR1.1) and laboratory (ToR1.2) 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.</p>	<p>No specific guidelines described in legislation</p> <p>Article 19:</p> <ol style="list-style-type: none"> 1) Where carrier pigeons or birds kept in captivity are suspected of being infected with Newcastle disease, Member States shall ensure that the official veterinarian immediately starts the official investigation procedures for confirming or notifying the presence of the disease; in particular, he shall take adequate samples for laboratory examination or see that they are taken. 2) As soon as it has been notified of the suspicion, the competent authority shall place the holding or pigeon house under official surveillance and order that no pigeon or bird kept in captivity, and nothing likely to transmit Newcastle disease, may leave the pigeon house or holding. 3) The measures provided for in paragraphs 1 and 2 shall not be withdrawn until the suspicion of Newcastle disease has been ruled out by the official veterinarian. 4) As soon as the presence of Newcastle disease has been officially confirmed, the competent authority shall, inter alia, order: <ol style="list-style-type: none"> a) application of the control and eradication measures provided for in Article 5 (1) (a), (b), (e) and (f) to the carrier pigeons or birds kept in captivity and pigeon houses infected with Newcastle disease; or 	<p>No specific guidelines described in legislation</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
		b) at least i) a ban on movement of the pigeons or birds kept in captivity outside the pigeon house or holding for at least 60 days after the clinical signs of Newcastle disease have disappeared; ii) destruction or treatment of any matter or waste likely to be contaminated. Treatment must guarantee the destruction of any Newcastle-disease virus present and all waste that has accumulated during the 60-day period referred to in (i); c) an epizootiological inquiry in accordance with Article 7.	
4th	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR1.1) and laboratory (ToR1.2) 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.	NA	NA
5th	To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR1.1) and laboratory (ToR1.2) 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 in legislation <i>Australian Veterinary Emergency Plan, Disease Strategy Newcastle disease (Animal Health Australia, 2014):</i> Wild birds appear to play little part in the spread of disease between flocks during an outbreak. The proposed OIE compartmentalisation of bird populations in countries and zones into domestic and free-living birds will enable wild birds to be treated in perspective unless clinical disease or infection is established; even if the virus is established in free-living birds, the infection status of commercial poultry would not be affected until infection occurred in that compartment.	No specific guidelines described in legislation

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
6th	<p>To assess the effectiveness of disease-specific sampling procedures based on clinical (ToR1.1) and laboratory (ToR1.2) 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.</p>	<p>Article 9:</p> <p>1) Once the diagnosis of Newcastle disease has been officially confirmed in poultry, the Member States shall ensure that the competent authority establishes around the infected holding a protection zone based on a minimum radius of three kilometres, itself contained in a surveillance zone based on a minimum radius of 10 kilometres. The establishment of the zones must take account of geographical, administrative, ecological and epizootiological factors relating to Newcastle disease, and of monitoring facilities.</p> <p>2) The measures applied in the protection zone shall include:</p> <p>a) the identification of all holdings having poultry within the zone;</p> <p>b) periodic visits to all the holdings having poultry, a clinical examination of those poultry including, if necessary, the collection of samples for laboratory examination; A record of visits and findings must be kept;</p> <p>3) The measures applied in the protection zone shall be maintained for at least 21 days after the carrying out of preliminary cleaning and disinfection operations on the infected holding in accordance with Article 11. The protection zone shall thereafter be part of the surveillance zone.</p> <p>Australian Veterinary Emergency Plan, Disease Strategy Newcastle disease (Animal Health Australia, 2014):</p> <p>In the initial stages, at least, samples should be taken of all species of birds that die in the Restricted Area (1–5 km around the Infected Premise) and they should be checked for ND lesions.</p> <p>Examinations need to be at least twice weekly by producers carrying out their own surveillance and reporting by telephone; and local disease control centre</p>	<p>Article 9:</p> <p>2) The measures applied in the protection zone shall include:</p> <p>The measures applied in the protection zone shall include:</p> <p>a) the identification of all holdings having poultry within the zone;</p> <p>b) periodic visits to all the holdings having poultry, a clinical examination of those poultry including, if necessary, the collection of samples for laboratory examination.(See Annex III in 1st scenario).</p> <p>Australian Veterinary Emergency Plan, Disease Strategy Newcastle disease (Animal Health Australia, 2014):</p> <p>In the initial stages, at least, samples should be taken of all species of birds that die in the Restricted Area (1–5 km around the Infected Premise) and they should be checked for ND lesions; specimens should be submitted to approved laboratories for virus isolation.</p> <p>Where vaccination is not being practised, serological sampling of flocks to provide a 95% level of confidence that virulent ND virus is not present at the 5% level (titres of > 210, or samples in which > 25% of the sample is > 25, should be viewed with suspicion).</p> <p>The Foreign Animal Disease Preparedness and Response Plan (FAD PRoP), Newcastle Disease Response Plan, The Red Book, Appendix D Updated ND Outbreak Surveillance Guidance and Rationale for Poultry (USDA-APHIS-VS, 2014):</p> <p>In at-risk premises in the Infected Zone (at least 3 km beyond perimeters of presumptive or confirmed Infected Premises):</p> <p>1) For commercial premises: Collect swabs for the</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
		<p>officers carrying out regular telephone surveillance of independent premises. Random visits to properties can be organised to discuss production performance.</p> <p>All reports of a decline in health status should be investigated further.</p>	<p>5-bird or 11-bird pool(s) on each premises once every 5 days for the duration of the quarantine.</p> <p>2) For backyard premises: Observe entire flock (swab if there are ND signs or epidemiological links) on each premises once every 5 days for the duration of the quarantine.</p>
7th	<p>To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR1.1) and laboratory (ToR1.2) 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.</p>	NA	NA
8th	<p>To assess the effectiveness of disease-specific sampling procedures, based on clinical (ToR1.1) and laboratory (ToR1.2) 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.</p>	<p>No specific guidelines described in legislation</p> <p>Article 9:</p> <p>4) The measures applied in the surveillance zone shall include:</p> <p>a) the identification of all holdings having poultry within the zone;</p> <p>5) The measures applied in the surveillance zone shall be maintained for at least 30 days after the carrying out of preliminary cleaning and disinfection operations on the infected holding in accordance with Article 11.</p> <p>Australian Veterinary Emergency Plan, Disease Strategy Newcastle disease (Animal Health Australia, 2014):</p> <p>Surveillance in the Controlled Area (2–10 km from borders of Restricted Area (RA)) will begin immediately the RA has been declared and will involve:</p> <ul style="list-style-type: none"> – Weekly telephone surveillance of susceptible flocks, 	<p>No specific guidelines described in legislation</p> <p>Australian Veterinary Emergency Plan, Disease Strategy Newcastle disease (Animal Health Australia, 2014):</p> <p>Surveillance in the Controlled Area (2–10 km from borders of Restricted Area (RA)) will begin immediately the RA has been declared and will involve:</p> <ul style="list-style-type: none"> – Swabbing dead birds (trachea and cloaca) for virus isolation at a level sufficient to determine infection with virulent virus in the highest priority commercial flocks, particularly those to be moved to slaughter; – Serological sampling of suspicious flocks and of a representative sample of commercial poultry flocks to provide a 95% level of confidence that the virulent ND virus is not present at the 5% level in the flock (titres of > 210, or samples in which > 25% of the sample is > 25, should be viewed with suspicion); meat chickens and spent hens can be sampled at the abattoir; <p>The Foreign Animal Disease Preparedness and</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
		including other species, with particular focus on commercial poultry. – Weekly reporting on flock health by producers and random visits to discuss flock performance and biosecurity measures. – Follow-up on any unusual disease conditions.	<i>Response Plan (FAD PRoP), Newcastle Disease Response Plan, The Red Book, Appendix D Updated ND Outbreak Surveillance Guidance and Rationale for Poultry (USDA-APHIS-VS, 2014):</i> In at-risk premises in the Buffer Zone (at least at least 7 km beyond the perimeter of the Infected Zone.): 1) For commercial premises: Collect swabs for the 5-bird or 11-bird pool(s) on each premises once every 5 days for the duration of the quarantine. 2) For backyard premises: Observe entire flock (swab if there are ND signs or epidemiological links) on each premises once every 5 days for the duration of the quarantine.
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).	Article 9: 2) (f) a prohibition on removing poultry and hatching eggs from the holding on which they are kept unless the competent authority has authorised the transport; i) of poultry for immediate slaughter to a slaughterhouse preferably located in the infected area or, if that is not possible, to a slaughterhouse designated by the competent authority outside the infected area. The special health mark provided for in Article 5 of Directive 91/494/EEC must be applied to this poultry meat; Movements allowed in (i), (ii) and (iii) shall be directly executed, under official control. They shall be authorised only after the official veterinarian has carried out a health inspection of the holding.	No specific guidelines described in legislation
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	Article 9: 2) (f) a prohibition on removing poultry and hatching eggs from the holding on which they are kept unless the competent authority has authorised the transport; ii) of day-old-chicks or ready-to-lay pullets to a holding	No specific guidelines described in legislation

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
	<p>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.</p>	<p>within the surveillance zone at which there are no other poultry. However, the Member States, that are not in a position to be able to provide transport of day-old-chicks or ready-to-lay pullets to a holding situated within the surveillance zone, shall be authorised, in accordance with the procedure laid down in Article 25, to have the said chicks and pullets transported to a holding outside the surveillance zone. The holdings referred to above must be placed under official control in accordance with Article 8 (2); Movements allowed in (i), (ii) and (iii) shall be directly executed, under official control. They shall be authorised only after the official veterinarian has carried out a health inspection of the holding.</p>	
11th	<p>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.</p>	<p>Article 9: 2) (f) a prohibition on removing poultry and hatching eggs from the holding on which they are kept unless the competent authority has authorised the transport; ii) of day-old-chicks or ready-to-lay pullets to a holding within the surveillance zone at which there are no other poultry. However, the Member States, that are not in a position to be able to provide transport of day-old-chicks or ready-to-lay pullets to a holding situated within the surveillance zone, shall be authorised, in accordance with the procedure laid down in Article 25, to have the said chicks and pullets transported to a holding outside the surveillance zone. The holdings referred to above must be placed under official control in accordance with Article 8 (2); Movements allowed in (i), (ii) and (iii) shall be directly executed, under official control. They shall be authorised only after the official veterinarian has carried out a health inspection of the holding.</p>	No specific guidelines described in legislation
12th	<p>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</p>	No specific guidelines described in legislation	No specific guidelines described in legislation

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
	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).		
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 in legislation Article 9: 4) The measures applied in the surveillance zone shall include: c) a prohibition on the movement of poultry out of the zone during the first 15 days, except for movement directly to a slaughterhouse outside the surveillance zone designated by the competent authority. The special health mark provided for in Article 5 of Directive 91/494/EEC must be applied to this poultry meat;	No specific guidelines described in legislation
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.	NA	NA
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	NA	NA

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
	zone, in order to complete the production cycle before slaughter.		
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 in legislation	No specific guidelines described in legislation
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 in legislation	No specific guidelines described in legislation
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 in legislation	
Repopulation			
19th	To assess the effectiveness of disease-specific sampling procedures based on laboratory examinations of the animals that are kept for the	NA	No specific guidelines described in legislation <i>The Foreign Animal Disease Preparedness and Response Plan (FAD PReP), Newcastle Disease</i>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
	repopulation prior to their introduction to rule out the presence of the disease.		<p>Response Plan, The Red Book, Appendix D Updated ND Outbreak Surveillance Guidance and Rationale for Poultry (USDA-APHIS-VS, 2014):</p> <p>6.2.3 Approved Sources of Poultry: Source flocks for all introduced poultry must test negative through rRT-PCR and other diagnostics, as determined by the Incident Command. A 24-h pre-movement clinical inspection is also required.</p>
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.	NA	No specific guidelines described in legislation
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.	NA	<p>No specific guidelines described in legislation</p> <p>Australian Veterinary Emergency Plan, (Animal Health Australia, 2014):</p> <p>Some serological surveillance will be required and it is recommended that this be performed on former infected premises, dangerous contact premises, trace premises and suspect premises at 30 days and at five months after restocking, in order to satisfy a 95% confidence of detecting infection at less than 5% prevalence. This would be supported on these premises by twice-weekly clinical examinations for 30 days and then fortnightly for five months. Virus isolation would be carried out on a sample of dead birds. Seropositive flocks will require further investigation and virus isolation attempts.</p> <p>The Foreign Animal Disease Preparedness and Response Plan (FAD PReP), Newcastle Disease Response Plan, The Red Book, Appendix D</p>

Scenario	Description of the Scenario	Clinical guidelines	Laboratory guidelines
			<p data-bbox="1447 247 2045 303"><i>Updated ND Outbreak Surveillance Guidance and Rationale for Poultry (USDA-APHIS-VS, 2014):</i></p> <p data-bbox="1447 335 2045 598">6.2.2. Testing Requirements for Restocking: Birds placed into previously infected houses or premises are subjected to weekly statistically valid testing by rRT-PCR for the presence of ND virus. The last test will be conducted at least 21 days after the birds are placed in the house: If the houses are left vacant for a period of 60 days after cleaning and disinfection is approved, there are no testing requirements.</p>

Annex D – Scenarios of ToR 2

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenarios
ToR 2	<p>Article 8 of the Delegated Regulation</p> <p>Article 57 of 2016/429 Regulation</p> <p>Annex II of the Delegated Regulation</p>	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.	<ul style="list-style-type: none"> • 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 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 enquiry is: <ol style="list-style-type: none"> 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	<p>Article 17(2) and Article 57 of 2016/429 Regulation</p> <p>Annex II of the Delegated Regulation</p>	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	<ul style="list-style-type: none"> • 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

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenarios
			of the epidemiological enquiry in the event of confirmation of the disease.	<ul style="list-style-type: none"> 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 enquiry is the same as above.
ToR 2	Article 13(b) of the 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.	<ul style="list-style-type: none"> 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 of the 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 have been obtained or produced, before this time period in order to be exempted from prohibitions of movements.	<ul style="list-style-type: none"> 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	<p>Article 32(c) of the Delegated Regulation</p> <p>Article 48(c) of the Delegated Regulation</p> <p>Annex II of the Delegated Regulation</p>	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.	<ul style="list-style-type: none"> • 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	<p>Article 57(1)b of the Delegated Regulation</p> <p>Annex II of the Delegated Regulation</p>	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.	<ul style="list-style-type: none"> • 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	<p>Article 59(4)b of the Delegated Regulation</p> <p>Annex II of the Delegated Regulation</p>	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	<ul style="list-style-type: none"> • repopulation of a previous affected establishment • kept animals of listed species to be repopulated • the animals may not be introduced at the same time

ToRs	Legislation	Scenario	Description of the Scenario	Elements of the Scenarios
			species intended for repopulation should be introduced.	<ul style="list-style-type: none"> 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

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 <i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> 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 <i>Burkholderia mallei</i> (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 (NDV)	3 km	10 km	21 days	9 days	30 days

Annex F – 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	1	<p>Uncertainties affecting the data used in the model: The model only considers three sample sizes which nevertheless cover the range of sizes of the majority of poultry flocks in Europe (with the exception of very large flocks with sizes > 10,000 birds).</p> <p>Data used for inferring the transmission parameters originated from experimental challenges using limited numbers of animals and specific viral and vaccine strains, and thus may not reflect the range of values that could occur in the field.</p>	<ul style="list-style-type: none"> • If very large flocks are affected the efficacy of the sampling strategies under certain circumstances (particularly in case of low virulence strains and/or vaccinated flocks) may be overestimated. • Circulation of strains with very different transmission dynamics compared with the ones used in the challenges from which data was extracted could result in an over or underestimation of the efficacy of the sampling strategy.
	2	<p>Uncertainties about model assumptions: model formulation assumes homogeneous mixing, what may be unrealistic in certain production types (e.g., laying hens in caged hens). Similarly, the model assumes frequency-dependent transmission, which better mimicked the transmission dynamics observed in HPAI experimental infections in turkeys (as opposed to density-dependent transmission, in which the number of contacts increases as the population size increases). Finally, model outputs are based on fixed and constant baseline non ND-related mortality (0.1%) and morbidity (1%) rates, which may not be reflective of the true situation particularly in certain production settings in which substandard performance indicators are common.</p>	<ul style="list-style-type: none"> • Transmission dynamics different from those assumed in the models could lead to over or underestimation of the efficacy of the sampling strategy, particularly in those scenarios in which transmission speed is more influential (e.g., low virulence strains and/or vaccinated flocks). • Increased baseline (non-ND related) morbidity and/or mortality could lead to an overestimation of the efficacy of the sampling strategy.
	3	<p>Uncertainties required to extrapolate from the model output to the parameter of interest: the model outputs provide information on the number of days from disease introduction in a flock required to reach a certain confidence level (95%) in the detection of ND through the analysis of dead, clinically affected or randomly selected animals. In contrast, the first three PoIs are already considering that the disease is already present at a certain (non-defined) threshold sufficient to trigger a suspicion due to the presence of clinical disease and dead birds in a non-vaccinated (PoI A) or non-vaccinated flock (PoI B), or due to the presence of a drop in egg production or unexpected shell colour or other egg abnormalities (PoI C). Thus, the higher that threshold is required to be, the more likely that by that point in time the number of days suggested by the model for achieving the target confidence in disease detection may have elapsed already.</p> <p>For PoI D, given that it is considering the probability of detection on a suspected flock due to contact tracing based on sampling sick, dead or randomly selected birds (if no sick and dead animals are present) within 21</p>	<ul style="list-style-type: none"> • If suspicion is raised with small changes in the baseline mortality (or with a small proportion of clinically affected animals with signs compatible with ND) the efficacy of the sampling strategy may be overestimated. Conversely, if suspicion is only raised once large increases in mortality (or large proportions of clinically affected animals with signs compatible with ND) the efficacy of the sampling strategy will be underestimated. • Similarly, the larger (or smaller) the change in baseline egg-production (or in the proportion of eggs showing abnormalities) needed to raise a suspicion, the higher the potential underestimation (or overestimation) of the true efficacy of the sampling strategy for farms without mortality or clinical signs. • In the case of farms suspected due to contact tracing, the number of days elapsed since they became infected at which

Source or location of the uncertainty	#	Nature or cause of uncertainty as described by the experts	Impact of the uncertainty on the assessment
		days, the question here would be whether 21 days may be enough for the disease to spread so that the 95% target confidence level is reached. In this case, the likely period between potential infection and eventual identification through tracing would have to be factored in for interpretation of the model outputs (e.g., if it would have taken one week for a contact farm to be identified after disease introduction, the disease would have been already circulating for seven days when the 21 days follow up period is set up).	they are identified will influence the effectiveness of the sampling strategy. This will be particularly important for low virulence strains and/or vaccinated flocks, in which the efficacy of the sampling strategy may be overestimated.
ToR 2	4	Only five references with relevant information were found in the ELR, mostly referring to ND in chicken (for different production purposes), and thus very limited information for other poultry species was available.	The true effectiveness of the monitoring period could have been over or underestimated.
	5	Because the date of introduction of the disease in an establishment cannot usually be determined with complete certainty, the time periods retrieved in the ELR references are based on different assumptions (typically introduction of new animals in a population/farm), identified as the most likely source of the infection	This could result in over/underestimations of the true length of the period of time between infection and notification.
ToR 3	6	Uncertainties affecting the data used in the models: data used to fit the two kernels available originated from two HPAI epidemics that occurred more than 15 years ago (Italy 1999–2000 and the Netherlands 2003). Due to changes in the poultry populations and management practices between regions (i.e. in different EU countries) and over time the results may not be representative of transmission dynamics expected now or in the future in other locations, particularly in areas where different (lower) farm densities are observed. Due to the unavailability of the exact dates in which farms were infected, the infection of poultry farms in the NL data set was assumed to occur six days before the day on which mortality first increased, and farms were assumed to become infectious after a two-day period of latent infection.	<ul style="list-style-type: none"> • Because a different (and generally considered more transmissible) virus was used to generate the kernels used in the assessment, kernel results may overestimate the probability of transmission at the distances assessed. • Differences between the populations considered in the studies from which the kernels originated and other outbreaks could result in an over or underestimation of the probability of transmission at the distances assessed. • Different virus strains may induce a shorter/larger latent period, what could lead to different (increased or decreased) estimates of transmission at increasing distances.
	7	Uncertainties about the choice of model and how realistic model assumptions are: The analyses of the Italian 1999–2000 epidemic only considered a single functional form for the kernel, so it is possible that another form could better capture the spread of ND. The analysis of the Netherlands 2003 outbreak considered five functional forms and only the best-fitting kernel was used in the zone size assessment. Furthermore, the default model from the Boender article assumed that both the infectious period at the farm level and the transmission kernel itself remained constant throughout the epidemic in spite of the implementation of more stringent control measures and increased awareness during the later weeks of the epidemic.	<ul style="list-style-type: none"> • The use of a different functional form could result in an over or underestimation of the probability of transmission at the distances assessed • An increased awareness and more stringent implementation of control measures could contribute to decrease the risk of transmission at the distances assessed.

Source or location of the uncertainty	#	Nature or cause of uncertainty as described by the experts	Impact of the uncertainty on the assessment
		<p>Kernels are a simple form of spatial model and incorporate all routes of transmission in a single description. This could have consequences for the probability of spread if different routes are of greater or lesser importance in different locations/epidemics.</p>	
	8	<p>Uncertainties required to extrapolate from the model output to the parameter of interest: the quantity of interest is the probability of transmission beyond the set thresholds occurs/is detected, while the output of the kernels is the probability of such transmission beyond the set thresholds occurs <i>assuming there is in fact some transmission</i></p>	<p>Since the output of the kernel can be interpreted as an <i>upper limit of the quantity of interest</i>, it will overestimate the true probability of disease transmission at increasing distances. How similar these two numbers will be however depends on how likely is that transmission from an affected establishment occurs, i.e. how likely is that there will be spread of viable ND virus from the affected establishment by any possible mean (aerosolisation, indirect contacts between farms, etc.), something that will vary depending on the biosecurity of the farms among other factors.</p>

Annex G – Additional information on the model calculation

Scenarios simulated using the transmission model are specified according to the basic reproduction number (R_0), the infectious period (mean, μ_I and variance, μ_I^2/k_I) and case fatality (C) (Table 1). The corresponding disease-associated mortality rate and transmission rate used in the simulations were calculated for each scenario as follows.

Disease-associated mortality rate

Assuming disease-associated mortality occurs at a constant rate, d , during the infectious period, the probability that an infected bird dies during its infectious period (i.e. the case fatality), C , is given by

$$C = \int_0^{\infty} (1 - \exp(-du))f(u)du,$$

where $f(u)$ is the probability density function for the gamma distribution for the infectious period. Evaluating this integral gives the case fatality as

$$C = 1 - \left(\frac{k_I/\mu_I}{k_I/\mu_I + d} \right)^{k_I}.$$

Hence, the disease-associated mortality rate for a scenario is given by

$$d = \frac{k_I}{\mu_I} \left(\frac{1}{(1 - C)^{1/k_I}} - 1 \right).$$

Transmission rate

In the transmission model, the basic reproduction number, R_0 , is given by

$$R_0 = \beta \times D,$$

where β is the transmission rate and D is the mean duration of infectiousness. If there is no disease-associated mortality (i.e. $C = 0$), the mean duration of infectiousness is the mean infectious period (i.e. $D = \mu_I$) and, hence, the transmission rate for the scenario is given by

$$\beta = \frac{R_0}{\mu_I}.$$

If there is disease-associated mortality (i.e. $C > 0$), the mean duration of infectiousness is given by

$$D = \frac{1}{d} \int_0^{\infty} (1 - \exp(-du))f(u)du = \frac{C}{d},$$

where C is the case fatality and d is the disease-associated mortality rate as given above. Hence, the transmission rate for the scenario is given by

$$\beta = \frac{1}{C} \frac{k_I}{\mu_I} \left(\frac{1}{(1 - C)^{1/k_I}} - 1 \right) R_0.$$